Response of Growth Plate Chondrocytes to Hydrostatic Loading In Vitro Shao, YY; Welter, JF; Wang, L; and Ballock, RT The Cleveland Clinic Foundation, Cleveland, OH, Department of Biology, Case Western Reserve University, Cleveland, OH. Ballocr@ccf.org

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Introduction: INTRODUCTION
Although it is well established that mechanical forces can influence the behavior of growth plate chondrocytes (the Hueter-Volkman principal of physeal growth), little is known about the response of growth plate chondrocytes to these mechanical forces at the molecular level. Parathyroid hormone-related peptide (PTHrP) and Indian hedgehog (Ihh) are known to be important regulators of the pace of hypertrophic differentiation at the growth plate. In this study, we investigated the effects of hydrostatic compression forces on growth plate chondrocytes, focusing on how these mechanical forces modulate elements of the PThrP-Ihh feedback loop.

Materials and Methods: METHODS
Epiphyseal chondrocytes were harvested from the distal femoral growth regions of two day-old Sprague Dawley rats and cultured as three-dimensional cell pellets (3x10^5 cells/pellet) for five days in serum-free DMEM:F12 medium supplemented with ITS+. The pellets were then either subjected to hydrostatic compression forces for periods up to 48 hours or cultured as unloaded controls. A custom-designed mechanical loading system was used to apply an intermittent 1 MPa hydrostatic compression force (one hour on, one hour off) to the cell pellets for the designated testing time. The hydrostatic loading system consists of a sterile bioreactor chamber which houses up to 9 pellets in a perforated holder. The reactor chamber is clamped into the hydraulic section of the system, and is separated from it by a gas-permeable flexible Teflon membrane. Computer-controlled tandem high-speed micro-gear pumps force water (maintained at 37°C and equilibrated with 7.5% CO2 in air) through the hydraulic section, thereby pressurizing the reactor. Heat- and gas-exchange occurs through the Teflon membrane. Half of the culture medium is replaced every 12 hours during loading. Pellets, tissue samples and tissue-engineered constructs have been grown and loaded in this system for up to three weeks.

Total RNA was collected from the cell pellets and quantitative real-time PCR was performed. Signaling through the Ihh pathway was also examined by measuring activity of a transiently transfected Gli-dependent luciferase reporter. Cellular proliferation was measured by using CellTiter 96AQueous Assay Kit (Promega) immediately after the compression.

Results: RESULTS
Growth plate chondrocytes subjected to hydrostatic compression forces demonstrated transiently increased cellular proliferation compared to the unloaded controls after 24 hours of compression. Following 48 hours of compression, proliferation returned to control levels, however quantitative RT-PCR analysis demonstrated that expression of Ihh, Patched (ptc), and the PTH-PTHrP receptor (pthR) were all markedly increased (Figure 1). In addition, Ihh expression was examined after shorter periods of compression (0.5, 1, 2, and 4 hours). Expression of Ihh increased 1.5 fold in response to hydrostatic compression as early as 30 minutes after initiation of loading (Figure 2).

Hydrostatic compression also significantly increased Gli-luciferase reporter activity (Figure 3). This compression-induced increase in Gli-luciferase reporter activity was almost completely inhibited by the Ihh antagonist cyclopamine.

Discussion: CONCLUSIONS
Intermittent hydrostatic compression loading of growth plate chondrocytes markedly increases expression and activity of key elements of the PTHrP-Ihh signaling pathway. These effects can be observed as early as 30 minutes after the load is applied. These data implicate the Ihh signaling pathway in particular as a critical transducer of mechanical forces into biological signals in growth plate chondrocytes.