**GROWTH PLATE CHONDROCYTE VOLUME INCREASE: THE PROPORTION OF CELL ENLARGEMENT ATTRIBUTABLE TO OSMOTICALLY ACTIVE SPACE.**

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INTRODUCTION: Long bone growth is driven by a sequence of events comprising growth plate chondrocyte proliferation and enlargement, in conjunction with matrix remodelling (1). Hypertrophic cell enlargement is a rapid process, with cell volume increasing by up to 800µm3·hour⁻¹ (2) and is the major determinant of bone lengthening (3). Although important, the mechanisms that underlie cell enlargement are poorly understood. Cell volume may be driven by an increase in both osmotically active (swelling) and inactive components (hypertrophy). Previous stereological analysis of ‘fixed’ growth plate tissue indicated cytoplasmic space and nucleoplasm increased from 35% to 85% of the cell volume between proliferative and hypertrophic cells respectively (1, 2). They therefore suggest the vast majority of cell volume increase being the result of fluid accumulation (swelling). If true, the osmotic active/inactive proportion of cells should change with position down the growth plate. In order to test this hypothesis and further clarify the characteristics of growth plate chondrocyte enlargement, live cell osmotic sensitivity studies were performed. In situ cells from 7-day rat proximal tibia growth plates were imaged in 3-dimensions using a two-photon laser scanning microscope (2PLSM) following osmotic challenge (80-580mOsm). Subsequent cell volume measurements were made using 3-dimensional image reconstruction software, and the osmotic sensitivity of cells from proliferative and hypertrophic zones of the growth plate analysed using a Boyle Van’t Hoff plot. These preliminary studies suggest the proportion of proliferative zone cell osmotically active space, and hence the cell volume attributable to water accumulation in hypertrophic zone cells previously may have been over-estimated.

METHODS: Hind leg tibia were removed under aseptic conditions from 3 Sprague-Dawley rat pups (7 days old) and cleaned of soft tissue, halved by sagital bisection, and placed into DMEM (280mOsm). The gradient of the best fit line (linear regression) describes osmotically active/inactive fraction, 25.3±6.28% and 21.8%±7.00% respectively.

**RESULTS:** The average cell volume for PZ and HZ cells was 1505 µm³ and 1752 µm³, respectively (n=3, total number of cells = 19) respectively, a significant increase (P<0.05; two-tailed t-test) between the gradient of the best fit line (linear regression) describes osmotically active/inactive fraction, 25.3±6.28% and 21.8%±7.00% respectively.

**DISCUSSION:** Previous histological studies have suggested that the majority of cell volume increase was due to cell swelling. Using 2PLSM we were able to visualise these cells in situ in 3-dimensions, allowing us to determine cell volumes after osmotic challenge. The preliminary data suggests the osmotically inactive fraction, of PZ and HZ cells is the same. Therefore cells have increased their volume with the relative proportions of osmotic fractions remaining unchanged. Cell volume increase was therefore the result of a proportionate increase in cell hypertrophy and swelling. The difference in results is due to an under representation of osmotically active space of PZ cells assumed from stereological analysis.

The osmotically inactive fraction reported here (~20%) for both PZ and HZ cells is likely to be an underestimation. This is because the extra-cellular osmolality will be higher due the nature of the extra-cellular matrix. Therefore the Boyle Van’t Hoff plot would be shifted to the left, the y-intercept being nearer to 30-40%, an osmotically active fraction of 60-70%, for both PZ and HZ cells. Whilst cell swelling may previously have been over-estimated, the contribution it makes to cell enlargement is still substantial. Mechanisms which drive fluid accumulation (osmolyte accumulation) are therefore still immensely worthy of further study (4).


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**Figure 1.** In situ hypertrophic and proliferative cell volume (µm³) changes in response to osmotic challenge. Data points show the means with standard error bars. * Denotes significant volume difference (P<0.05; one-tailed t-test) from cell volume at 280mOsm medium osmolarity.

**Figure 2.** Boyle Van’t Hoff plot of in situ PZ and HZ chondrocytes. Lines show best fit linear regression.