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# THE EFFECT OF LOADING MAGNITUDE ON CHONDROCYTE CALCIUM SIGNALING IN SITU

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## SUMMARY
Chondrocyte metabolic activity is stimulated by mechanical loading and has been linked to the adaptive/degenerative changes in cartilage associated with osteoarthritis. Calcium signaling has been identified as an initial step in the process by which chondrocytes respond biologically to mechanical loading. In order to investigate the effect of loading magnitude on chondrocyte calcium signaling, a custom indentation system that allows for imaging chondrocytes in intact cartilage attached to its native bone was used. Femoral condyle and patellar samples were incubated in ratiometric calcium-sensitive dyes and imaged continuously under compressive mechanical loads of 10-40% strain. Chondrocyte calcium signaling increased with increasing load for both joint regions and appeared to correlate with local extracellular matrix strains. These results provide new insight into the mechanisms underlying possible signaling pathways for cartilage homeostasis and the onset and progression of osteoarthritis.

## INTRODUCTION
Osteoarthritis (OA) is a joint disease characterized by a breakdown of the cartilage extracellular matrix (ECM). Chondrocyte metabolic activity is stimulated by mechanical deformation and is associated with structural changes in the ECM [1], suggesting that these cells play a key role in the onset and progression of OA. Calcium is a ubiquitous intracellular signal that is an initial step in chondrocyte mechanotransduction [2,3]. Loading magnitude has been identified as a possible key factor in chondrocyte calcium signaling in *in vitro* studies [4], however recent work has shown that the $Ca^{2+}$ signaling behavior of chondrocytes differs in the intact cartilage [5]. Chondrocyte deformation has also been shown to differ between joint regions [6]. Therefore, the purpose of this study was to investigate the effects of compressive loading magnitude on chondrocyte calcium signaling *in situ*. We hypothesized that the percentage of cells exhibiting calcium signal(s) increases with increasing compressive loads.

## METHODS
Femoral condyles (COND, N=10) and patellae (PAT, N=4) were harvested from 6-8 month old New Zealand White rabbits. Intact cartilage-on-bone samples were fluorescently labeled with Fura Red and Fluo-4 for 1 hr at 37°C. Tissue specimens were then mounted in a custom indentation system [7] and loaded to compressive strains of 10, 20, 30 and 40% for 3 minutes each. Starting 60 s prior to loading, confocal image scans were recorded at 0.25 Hz for the duration of mechanical testing. All experiments were conducted at 37°C. The ratio of fluorescence intensity between dyes was normalized to the moving average for each cell. Calcium signals were defined as an increase over baseline greater than 10 times the minimum of the standard deviation of the normalized ratio (Fig. 1) [5]. Amplitude and duration of the calcium signals were also determined. Results are presented as means ± 1 standard error.

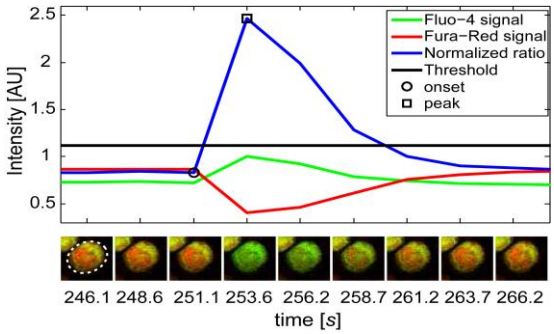


**Figure 1:** Calcium signal of an exemplar chondrocyte showing signal characteristics. Figure adapted from [5] with permission.

## RESULTS AND DISCUSSION
Compressive mechanical loading resulted in 32±10% and 60±21% of cells responding with at least one calcium signal for femoral condyle and patella samples, respectively. Most calcium signaling activity occurred during or immediately following the transient loading phase rather than during the static holding phase (Fig. 2). The number of responding cells increased with increasing compressive load for both joint regions (Fig 2, 3).

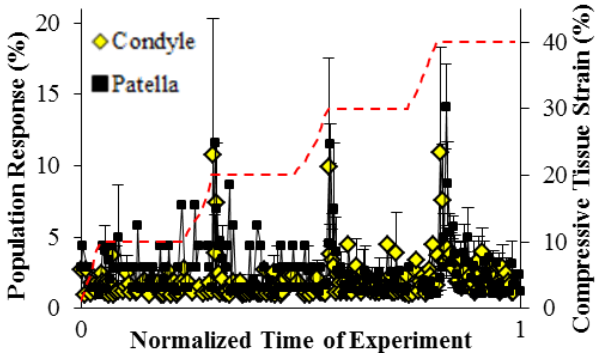


**Figure 2:** Average population response of condyles (yellow diamonds) and patellar (black squares) samples shown with applied loading (red line).

Calcium signal amplitude remained consistent from 10-30% tissue strain before decreasing at 40%; this was true for both joint regions. The signal amplitude in femoral condyles was greater than in patellae for all tissue loads (Table 1). Calcium signal duration increased from 10-30% and was unchanged from 30-40% tissue strain for femoral condyle chondrocytes, whereas for patellar cells signal duration increased consistently from 10-40% (Table 1).

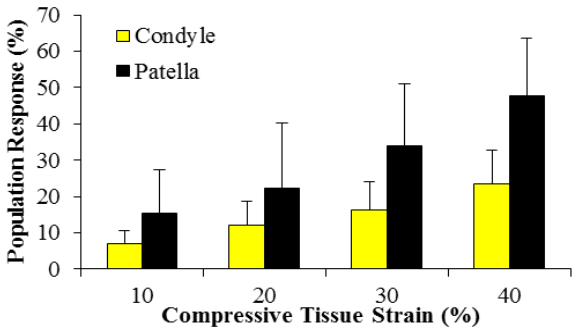


**Figure 3:** Percentage of *in situ* chondrocytes responding with calcium signaling activity from condyles (yellow bars) and patellae (black bars).

There was a trend towards an increasing population of *in situ* chondrocytes exhibiting calcium signals with increasing loading magnitude. Previous studies have reported decreased $Ca^{2+}$ sensitivity for repeated mechanical loads [5], suggesting that the trend may be underestimated in the current study. In general, patellar samples exhibited greater population response than femoral condyles (Fig. 3). This result correlates well with observed differences in local ECM strain between these joint regions (Fig. 4) [6].

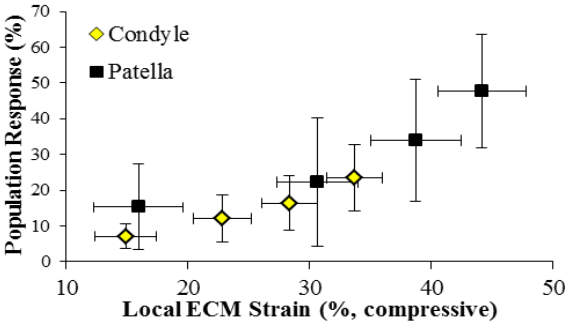


**Figure 4:** Population response of *in* situ chondrocytes as a function of local ECM strain in the superficial zone for femoral condyles (yellow diamonds) and patellar (black squares) samples (ECM strain data from [6]).

The changes in signal characteristics (duration, amplitude) with respect to load may indicate a shift in purpose of the calcium signals, as duration is associated with the distance to the effector system of the signal [2]. Differences between joint regions may also be related to different local tissue deformation (Fig. 4) and may indicate differences in the purpose of the calcium signals between joint regions.

## CONCLUSIONS

The current results support previous observations that mechanical loading stimulates calcium signaling in articular cartilage chondrocytes. Our hypothesis that calcium signaling activity increases with increasing load magnitude was confirmed. Furthermore, this work suggests that calcium signaling behavior may be related to local ECM strain for superficial zone cells. Other factors, such as strain-rate or frequency may be more critical in modulating the calcium signaling response of chondrocytes under dynamic conditions. Currently, OA is a prevalent and irreversible joint disease in which clinical treatment options are limited. These results present an important step towards understanding the mechanisms underlying possible signaling pathways for the onset and progression of OA.

## ACKNOWLEDGEMENTS

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**Table 1:** Calcium response data, signal characteristics, and ECM deformation (for condyles: 946 signals from n=281 responders/822 cells, N=10 condyles; for patella: 672 signals from n=200 responders/354 cells, N=4 patellae). Local ECM strain data measured in our previous work [6].

| | Joint Region | Applied Compressive Tissue Strain (%) | | | |
|---|---|---|---|---|---|
| | | 10 | 20 | 30 | 40 |
| **Population Response (%)** | COND | 7.1 ± 3.3 | 12.1 ± 6.5 | 16.4 ± 7.6 | 23.5 ± 9.2 |
| | PAT | 15.3 ± 12.0 | 22.3 ± 17.9 | 34.0 ± 17.1 | 47.7 ± 15.9 |
| **Signal Amplitude (arbitrary units)** | COND | 0.68 ± 0.04 | 0.69 ± 0.03 | 0.69 ± 0.03 | 0.61 ± 0.02 |
| | PAT | 0.60 ± 0.03 | 0.60 ± 0.02 | 0.62 ± 0.02 | 0.57 ± 0.02 |
| **Signal Duration (s)** | COND | 30.5 ± 1.1 | 33.0 ± 0.9 | 34.8 ± 0.9 | 33.4 ± 0.8 |
| | PAT | 27.7 ± 1.2 | 32.2 ± 1.3 | 36.3 ± 1.0 | 37.3 ± 0.9 |
| **Local ECM Strain (% compressive) [6]** | COND | 15 ± 2 | 23 ± 2 | 28 ± 2 | 34 ± 2 |
| | PAT | 16 ± 4 | 31 ± 3 | 39 ± 4 | 44 ± 4 |