Correlating Biomechanics and Biological Signals in Subjects Undergoing High Tibial Osteotomy

Catherine Holt1, Gemma Whatling1, Cleo Bonnet1, Karen Brakspear2, Christopher Wilson3, Rhys Williams3, Hatice Ozturk3, Deborah Mason4.
1Cardiff University, Cardiff, United Kingdom, 2Bristol University, Bristol, United Kingdom, 3Cardiff and Vale Trust, Cardiff, United Kingdom, 4Cardiff University, CARDIFF, United Kingdom.


Introduction: Whilst studies have revealed how biomechanics vary in normal individuals and change in specific disease states, mechanisms that cause abnormal biomechanics to induce joint degeneration are not well understood. Knee osteoarthritis predominantly affects the medial compartment. For many surgical and rehabilitation interventions based on restoring joint biomechanics to protect against joint degeneration, evidence is lacking about how and when to intervene. We assess functional and biological responses to altered loading in a human model of joint realignment; high tibial osteotomy (HTO) surgery. Loading abnormalities are measured using the peak-external-knee-adduction-moment (EKAM) (1) indicating medial compartment loading and knee-adduction-angular-impulse (KAAI) reflecting joint wear (2). HTO realigns the joint by introducing a tibial wedge, potentially preventing further degeneration. We are modelling gait dynamics to determine subject specific load-distribution in tibial subchondral bone, cartilage and meniscus, pre- and post- HTO and relating this to mechanically regulated signals in subchondral bone.

The neurotransmitter, glutamate, is thought to transmit mechanical signals in bone. Glutamate activates various glutamate receptors and transporters, which are present and function in a range of musculoskeletal tissues, including bone, cartilage, meniscus and synovium (3). Activation of these receptors influences processes such as inflammation and destruction of cartilage and bone, which contribute to arthritic joint degeneration (4). Glutamate also acts on nerves within the joint to mediate peripheral nociceptive pathways to modulate the pain experienced by patients with arthritis. Thus glutamate signaling represents a mechanism whereby mechanical load through the joint can directly influence joint pathology and pain. We have investigated mRNA expression of glutamate signalling components across the subchondral of patients undergoing HTO surgery to determine whether this varies in anatomical regions subject to different loads.

Methods: Joint function is investigated using 3D motion analysis (8-camera Qualysis, Sweden), force-plates (2, Bertec Inc.), and imaging. External knee adduction moments, EKAMs, were calculated for 10 patients, pre HTO surgery using a Visual3D (Cmotion Inc) pipeline. Using instrumented prosthetic knee loading data from patients performing level walking from the Orthoload dataset (K1L, K2L, K3R, K4R, K5R; Bergmann, G. (ed.), Charité Universitaetsmedizin Berlin (2008) "OrthoLoad". Retrieved July 2013 from http://www.Orthoload.com) (5), force components were used as inputs to a tibia model to evaluate the maximum equivalent stress (ANSYS, Inc.), in the four joint quadrants (medial and lateral, anterior and posterior), 15 mm below the tibial plateau. Trend curves relating Orthoload patient EKAM to their associated strains in the four quadrants were then developed to predict strains in HTO patients based on subject specific EKAMs. Gene expression was quantified in sub-chondral bone cores taken at HTO surgery. After snap freezing, RNA was extracted, reverse transcribed and specific genes quantified using absolute quantitative polymerase chain reaction. Strain distribution patterns across all four joint quadrants were related to gene expression patterns.

Results: For 15 recruited patients (52.8 ± 6.61yrs; 89.3 ± 16.84kg; 1.74 ± 0.12m), knee loading changed significantly (p<0.05, ANOVA, n=10 pre and 6 post-HTO) following opening wedge HTO. This is associated with significant (P<0.05) pain reduction and improved function [Oxford Knee Score/Knee Outcome Survey]. Patients demonstrate a range of Peak-EKAM pre-surgery. Peak-EKAM and KAAI decrease from pre- to post-surgery by 49.77% and 65% respectively. Musculoskeletal models simulate joint loading (Gait2392, OpenSim); e.g., in one patient the Joint Reaction Force increased by 15% at 2 years post-HTO, with a 9% reduction in the forwards force component and 15% and 5 % increase in the upwards and medial forces respectively, indicating more confident joint loading and increased lateral loading.

We have quantified housekeeping genes (18S, GAPDH, HPRT1), glutamate transporters implicated in mechanotransduction in bone (EAATs 1 and 3) and glutamate receptors implicated in arthritis (NR2D, KA1) in 10 patients. Five patients expressed equivalent amounts of EAAT1 and NR2D in medial and lateral subchondral bone, although the total amount varies up to 5 fold across patients. The remaining patients show up to 6 fold changes in EAAT1/NR2D in medial v lateral sub-chondral bone. In one patient, KA1 GluR mRNA switched on and EAAT1ex9skip switched off 6 months after surgery. Comparisons of 3 patients EKAM revealed highest EAAT1 mRNA expression in the patient with highest EKAM values.

When orthoload data was used to predict strain patterns, all HTO patients showed a similar pattern of strain prior to surgery
although absolute strain values varied (fig 1). Gene expression data also showed consistent patterns across patients. For example, EAAT3 and NR2D tended to be lower in medial and anterior aspects of the joint (fig 2). In the four patients where both strain and molecular data were available, highest strains in the medial posterior quadrant were associated with increased KA1 mRNA expression (Fig 3).

**Discussion:** Patients undergoing HTO surgery have similar strain patterns across the joint, with increased strain medially and posteriorly. However, the amplitude of strain differences varies across patients. HTO patients show gene specific mRNA expression patterns across the joint quadrants. In a pilot study of four patients, KA1 mRNA expression was increased in regions where strain was highest, whereas NR2D and EAAT3 tended to be increased laterally. These differences in mRNA expression may reflect mechanical regulation of glutamatergic signalling in sub-chondral bone that may influence pain, inflammation and pathology.

**Significance:** We have constructed a pipeline to relate biomechanical changes pre- and post- HTO to gene expression changes in the bone. These data are the first to link biological signals to calculated strain data in humans undergoing HTO surgery. This provides an opportunity to determine whether signals known to influence pain, inflammation and pathology from preclinical models, are important in human arthritis. Comparisons in medial and lateral expression patterns at surgery reveal patient specific differences, which may reflect abnormal joint biomechanics. Longitudinal analysis in the same patients will enable us to determine whether HTO surgery alters these signals. These unique studies will reveal new disease mechanisms that can be targeted with drugs, surgery or rehabilitation to reduce the symptoms of osteoarthritis.

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**References:**
4. Flood et al 2007, Arth and Rheum