How does high tibial osteotomy affect osteoarthritis in the adult human knee?

An analysis of biological and biomechanical factors.

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Abstract

Introduction: Knee osteoarthritis (OA) is an extremely common, debilitating condition but there remain significant gaps of understanding regarding its pathogenesis and management. For medial compartment OA, high tibial osteotomy (HTO) offers joint preservation with good clinical outcomes. However little is known about how its clinical effects are achieved. This cross-disciplinary project aimed to link bioscientific, mechanical and clinical data to investigate how HTO influences knee OA. The first strand investigated the biological environment (including glutaminergic pathways potentially related to OA). The second, analysed changes in the mechanical environment resultant from HTO and the third looked for potential links between the two.

Methods: Firstly, samples of subchondral bone were taken from the tibial plateau at time of HTO surgery and plate removal. These were analysed by reverse transcription PCR (RT-qPCR), looking for proportional changes in expression pre- to post-operatively in selected markers related to OA (EAAT1, EAAT3, NR2D, GRIK4, SOST, IL-6).
Secondly, clinical radiographs were measured to examine the relationship between proximal tibial morphology, limb alignment and potential compensatory changes at the hip, ankle and subtalar joints.
Finally, principal component analysis was employed to investigate potential correlations between the biological, radiological and mechanical data.

Results: Results showed potential downregulation in expression of NR2D, GRIK4 and SOST within the quadrant of the tibial plateau where loading is most reduced by HTO.
Radiographic analysis suggested that although change of proximal tibial shape by HTO is related to change in limb alignment, these measures are

not as closely related as expected and there are changes in subtalar alignment and stance width which may modulate the effect.
PCA and correlation analysis identified potential phenotypic groups, markers of outcome and links between the biological and mechanical changes seen in HTO.

Conclusion: This thesis has identified novel biological and mechanical changes resultant from HTO surgery, in addition to revealing potential linking mechanisms between them. It supports the theory that HTO causes measurable change to the pathology underpinning OA; potentially halting, slowing or reversing progression. By enhancing understanding of both HTO and OA, and by providing promising avenues for further research, it will enable clinicians to better counsel patients and therefore have a direct positive impact on patient care.

Acknowledgements

There are so many people to whom I owe thanks for their help and support with this project. It has not been without its challenges (a global pandemic shutting the university for example) and I could not have completed it without the backing of so many excellent people.

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On the clinical side, I cannot of course omit to mention Mr Chris Wilson (an osteotomy master and legend in his own lifetime) whose faith and expertise has been invaluable. Also the CAVOC research team (led by Mat Williams) whose hard work behind the scenes keeps the show on the road, and of course the patients who have given up their time (and samples) for the benefit of others.

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Last but never least, a heartfelt thank you to my husband and family. You know I couldn't do it without you.

Summary of publications, presentations and awards associated with this study to date

Publications:

Patients have a wider stance following valgus high tibial osteotomy.
Kinghorn AF, Bowd J, Whatling G, Wilson C, Mason D, Holt C. Osteoarthritis and Cartilage. 2020 Apr; 28(Supplement 1): S248 (Published abstract)

Oral Presentations:

"Patients have a wider stance following high tibial osteotomy"
British Orthopaedic Research Society Conference (2020)

"Glutamate receptor expression changes after high tibial osteotomy"
British Orthopaedic Association Annual Congress, "Best of the Best" and Basic Science sections (2019)

Poster Presentations:

Glutamate receptor expression changes after High Tibial Osteotomy
British Orthopaedic Research Society/Bone Research Society Conference (2019)

Patients have a wider stance following valgus High Tibial Osteotomy
Accepted for OARSI World Congress on Osteoarthritis, Vienna – event cancelled due to COVID pandemic. (2020)

Awards/Prizes:

Andrew Sprowson Award for Translational Research
British Orthopaedic Research Society Conference (2020)

Best Presentation
Wales Deanery Orthopaedic Registrars' Day (2019)

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List of Abbreviations

ADAM	A Distintegrin And Metalloproteinase
AL	Anterolateral
ALP	Alkaline Phosphatase
AM	Anteromedial
ARUK	Arthritis Research UK
BBRCVA	Biomechanics and Bioengineering Research Centre Versus Arthritis
BMI	Body Mass Index
BML	Bone Marrow Lesions
CAVOC	Cardiff and Vale Orthopaedic Centre
COMP	Cartilage Oligomeric Matrix Protein
CTX	C-terminal Telopeptide of Collagen Type
dGEMRIC	Delayed Gadolinium-Enhanced Magnetic Resonance Imaging of Cartilage
DLST	Double Limb Support Time
DNA	Deoxyribonulceic Acid
EAAT	Excitory Amino Acid Transporter
EKAM	External Knee Adduction Moment
EMG	Electromyography
ERCC	External RNA Controls Consortium
FNS	Femoral Neck-Shaft Angle
GFN	Ground-Femoral Neck Angle

Glu	Glutamate
GRF	Ground Reaction Force
GRIK4	Glutamate Ionotropic Receptor Kainate type subunit 4
GT	Ground-Talus Angle
HTO	High Tibial Osteotomy
IQR	Interquartile Range
KAM	Knee Adduction Moment
KL	Kellgren-Lawrence
IL	Interleukin
IR	Infrared
JLCA	Joint Line Convergence Angle
LDFA	Lateral Distal Femoral Angle
LDTA	Lateral Distal Tibial Angle
Mik	Mikulicz Point
MOW	Medial Opening Wedge
MP	Mikulicz Point
MPTA	Medial Proximal tibial Angle
MRI	Magnetic Resonance Imaging
mRNA	Messenger Ribonucleic Acid
mTFA	Mechanical Tibio-Femoral Angle
NHS	National Health Service
NICE	National Institute of Clinical Excellence
NMDA	N-methyl-d-aspartate
NR2D	NMDA receptor 2D

NTC	No Template Control
OA	Osteoarthritis
OKS	Oxford Knee Score
OPG	Osteoprotegerin
PACS	Pain Audit Collection System Brief Pain Inventory
PCA	Principal Component Analysis
PCR	Polymerase Chain Reaction
PL	Posterolateral
PM	Posteromedial
qPCR	Quantitative Polymerase Chain Reaction
RANKL	Receptor Activator of Nuclear factor-κB ligand
RNA	Ribonucleic Acid
ROM	Range Of Movement
RT	Reverse Transcription
SF	Synovial Fluid
SOST	Sclerostin
SW	Stance Width
TKR	Total Knee Replacement
TNF	Tumour Necrosis Factor
UKR	Unicondylar Knee Replacement
Wnt	Wingless-related intergration site

Chapter 1:

Introduction and Background

1.1 MOTIVATION

Osteoarthritis of the knee represents a significant and growing burden of disease in the UK. ARUK estimates approximately 17% of adults aged over 45 in England and Scotland (data not currently available for Wales) are affected by OA of the knee, with approximately 5% suffering severe symptoms (Arthritis Research UK, 2018). It is estimated that OA costs the UK economy approximately 1% of annual gross national product (NICE, 2012) and as risk of knee OA rises with both age and obesity, prevalence is expected to increase over the coming years.

In the 1970s, treatment of knee OA was revolutionised by the development of total knee replacements (Ranawat, 2002) and they have remained a staple of treatment, with over 106, 000 patients undergoing some form of knee arthroplasty in England, Wales or Northern Ireland in 2017 (National Joint Registry, 2018). For many patients, arthroplasty remains the most appropriate option; however, there exist significant treatment gaps for some subgroups of patients.

One such group are active patients with symptomatic OA, localised to the medial compartment (London, Miller and Block, 2011). These patients are often younger, with higher functional demands and as such, conservative measures such as analgesia, joint injections, lifestyle modification or walking aids do not provide sufficient relief for these patients to continue in employment and a normal active life (London, Miller and Block, 2011). Equally, at the other end of the treatment spectrum, arthroplasty procedures (be that total knee replacement (TKR) or unicompartmental (UKR)) can be too invasive and restrictive for this group. Although arthroplasty surgery might relieve pain, issues include restriction on activities (due to the risk of wear, loosening or fracture), change in knee function and the problem of likely multiple future revisions, as all prostheses have a finite life span

(London, Miller and Block, 2011; National Joint Registry, 2018). Studies have suggested that among younger patients or those with higher activity expectations, satisfaction with knee arthroplasty is lower (Williams *et al.*, 2013; Choi and Ra, 2016) and the revision rate is, as one might expect, higher (Bayliss *et al.*, 2017).

High tibial osteotomy could provide a solution for some of these patients. The aim of this procedure (which will be explained in more detail later in this chapter) is to realign the weight bearing axis of the leg via cutting and reshaping the tibia, just below the knee. By shifting the distribution of load within the knee (offloading affected portions) it has been proposed that HTO can slow or halt the progression of osteoarthritis of the knee. Cochrane reviews of the current literature suggest that clinically it is a worthwhile operation for patients with medial compartment arthritis as in all eight included studies, patients reported less pain and improved function (Brouwer *et al.*, 2014). However there is no evidence to support the relative effectiveness of HTO versus UKR (Brouwer *et al.*, 2014). Most of the studies have focussed on clinical outcomes rather than biological/biomechanical effects but there are some which have looked specifically at joint cartilage post procedure and some show evidence of cartilage regeneration (i.e. a reversal of osteoarthritic changes) after HTO (Kanamiya *et al.*, 2002; Koshino *et al.*, 2003; Parker *et al.*, 2011).

Without a thorough understanding of how HTO can work, it is difficult to select which patients may benefit from HTO and to counsel them appropriately. It is hoped therefore that the findings of this project will add to the understanding of the scientific basis of HTO and this would have a direct positive impact on patient care. It may also be able to reveal mechanisms underlying mechanically-induced joint degeneration.

1.2 AIMS AND OBJECTIVES

The aim of this MD thesis is to shed further light on the scientific basis of high tibial osteotomy surgery. This will involve investigating the biological, mechanical, clinical and radiological factors as well as the interplay between them.

The overarching hypothesis of the Biomechanics and Bioengineering Research Centre Versus Arthritis (BBRCVA) HTO project is that HTO can slow, halt or reverse the progression of OA of the knee. This can be broken down into a network of cumulative hypotheses as shown in Figure 1.

Within this can be found the areas on which this MD project will focus. These hypotheses are:

Hypothesis 1: (Chapter 3) Medial opening wedge HTO causes measurable changes in molecular markers of osteoarthritis in subchondral bone.

Samples of subchondral bone have been taken from study participants at the time of HTO and again at the time of plate removal. These provide a longitudinal pre and post operative comparison within each patient.
The samples will then be subject to RNA extraction and reverse-transcriptase linked quantitative polymerase chain reaction (RTqPCR) analysis of the glutamate signalling pathway and markers of bone turnover. Results will be analysed by a variety of statistical methods. Trends will be looked for by comparing pre to post means and longitudinal changes.

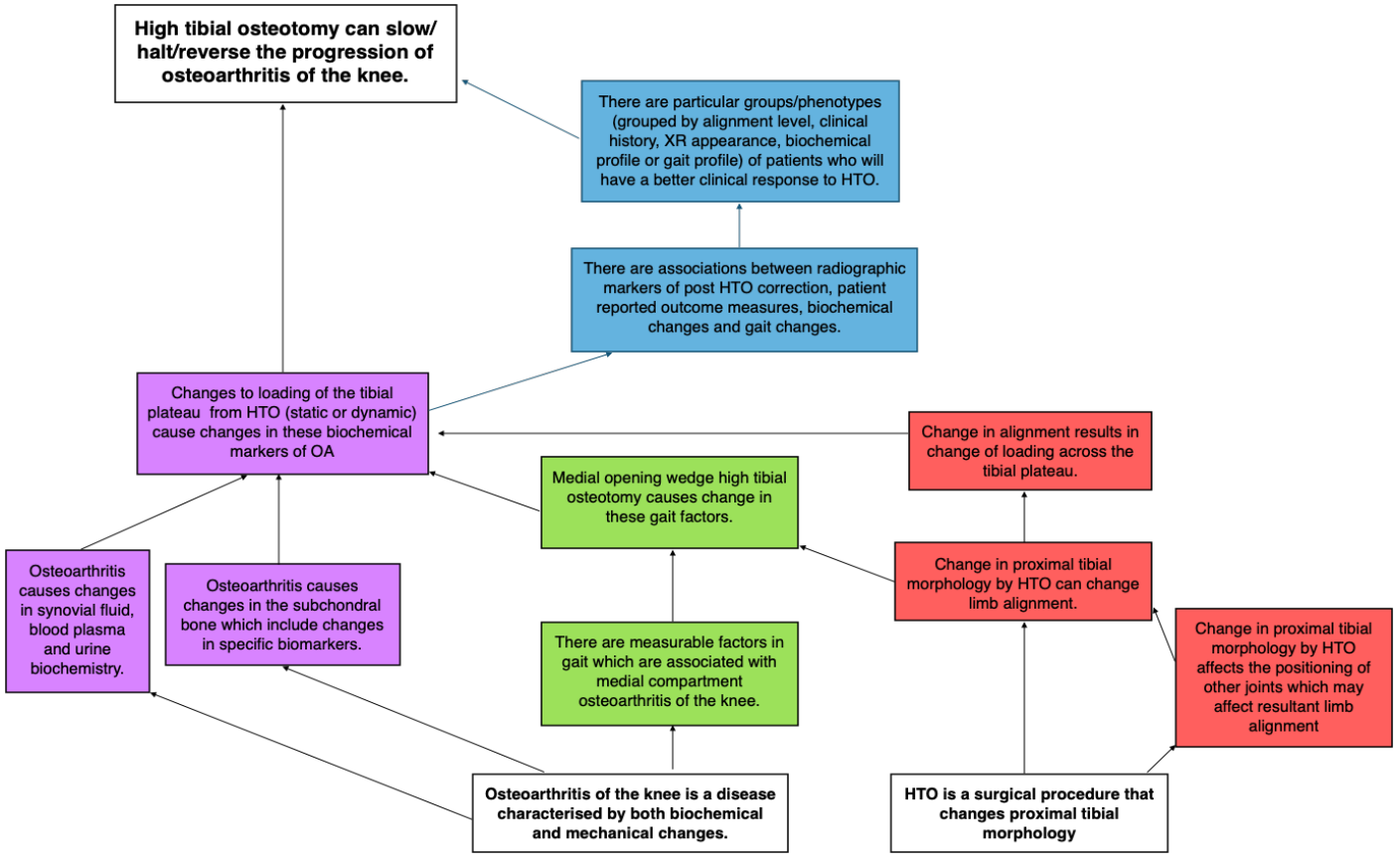

Figure 1: Hypothesis Network. Demonstrating interlinked domains of investigation (purple=biological, green=gait, red=radiological, blue=linkages)

Hypothesis 2: (Chapter 4) Medial opening wedge HTO causes measurable changes in the radiological alignment of the lower limb beyond those anticipated as a result of the surgical correction.

Patients have undergone full length weightbearing radiographs of both lower limbs both before and after their HTO. These will be analysed to look at the relationship between known alignment measures but also to look for any compensatory changes occurring at other joints in the lower limb.

Hypothesis 3: (Chapter 5) Biological and biomechanical changes seen following medial opening wedge HTO are related.

Data from biological samples, clinical history, radiological and gait analysis will be examined looking for trends between variables following HTO. Results will be analysed by a variety of statistical methods. Trends will be looked for by comparing pre to post means and longitudinal changes. Secondly, multivariate analysis will be used to reveal subgroups of patients with specific phenotypes and factors that commonly associate. This may identify different phenotypes of patient who are more or less likely to respond successfully to HTO surgery.

The network of hypotheses were developed over a series of discussions with my supervisor and reflect both my own scientific journey and the adaptations which occurred both as a result of COVID challenges but also as observations informed further investigation. The aim was for me to provide a clinical and multidisciplinary perspective for the overall project but also to learn some of the underlying science and procedures required within the different areas of investigation (biosciences and bioengineering). The first focus was on increasing understanding of the biological processes

underlying both OA and HTO. Part of the personal objective here was also to learn the procedure for RNA extraction and reverse-transcriptase linked quantitative polymerase chain reaction (RTqPCR). Initial plans had been to follow this up with ELISA analysis of synovial fluid/blood/urine. However, due to the shutdown of the university in March 2020 this was not possible and it was necessary for me to pursue investigations that could be done remotely from the laboratory. However, during this time I had made anecdotal observations that there were changes occurring in the patients' radiographs which were not previously described. I felt that not only was this of scientific importance but that incorporating this investigation into the MD would strengthen the original multidisciplinary aim for both my project and the centre as whole. Therefore although the COVID restriction made a change in direction unavoidable, this was not to the detriment of either the thesis or my own scientific journey.

1.3 BACKGROUND

1.3.1 The Knee Joint

1.3.1.1 Structure of the knee joint

The knee is a synovial joint which encompasses three compartments; the medial and lateral compartments between the distal femur and proximal tibia and the patellofemoral joint anteriorly.

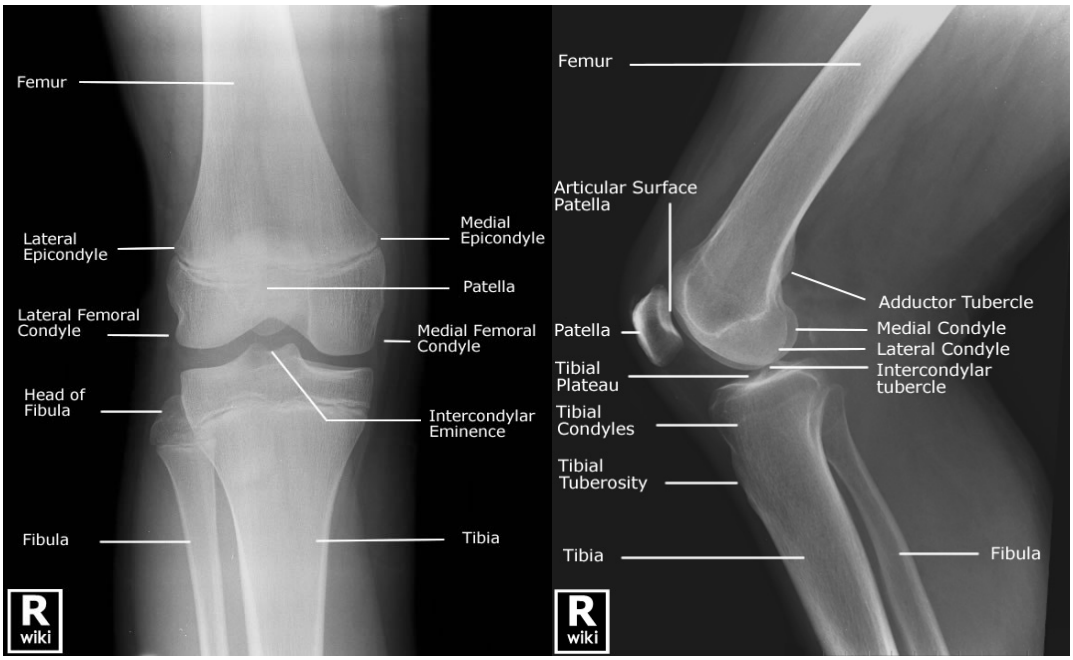

Figure 2: AP and lateral radiographs of a normal knee. (Wikiradiography)

The bone surfaces are lined with articular hyaline cartilage. This provides a surface which is very low friction (ideal for a bearing surface) but which can also distribute force across its surface area (important for a weight and impact bearing joint). It is composed of chondrocytes within an extracellular matrix of water, collagen (predominantly type II), proteoglycans, glycosaminoglycans and glycoproteins (Ramachandran, 2006). These components are arranged in a layered structure as shown in figure 3.

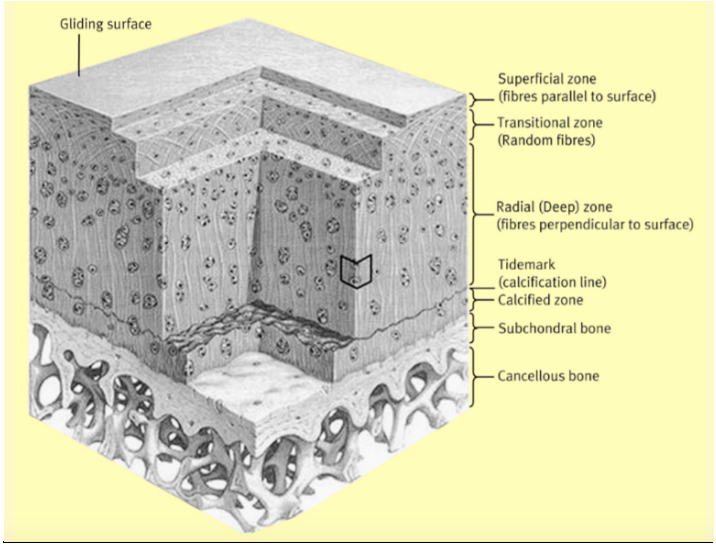

Figure 3: The layered structure of hyaline cartilage (Kheir 2009)

The hyaline cartilage has no vascular, neural or lymphatic supply so relies on diffusion from the synovial fluid (Ramachandran, 2006; Karuppal, 2017). However there is increasing evidence of a complex signaling interplay between the cartilage and the underlying subchondral bone (Sharma *et al.*, 2013).

The femoral condyles have a convex curvature much greater than the concave curvature of the relatively flat tibial plateau. The menisci (two crescentic pieces of fibrocartilage) deepen the “cups” of the tibial plateau, allowing greater area for force distribution and improving stability. Ligamentous stability is added via the medial and lateral collateral ligaments (preventing varus/valgus displacement) and the anterior and posterior cruciate ligaments (preventing anterior/posterior or rotatory displacement). The surrounding musculature also plays a role in stabilising the joint.

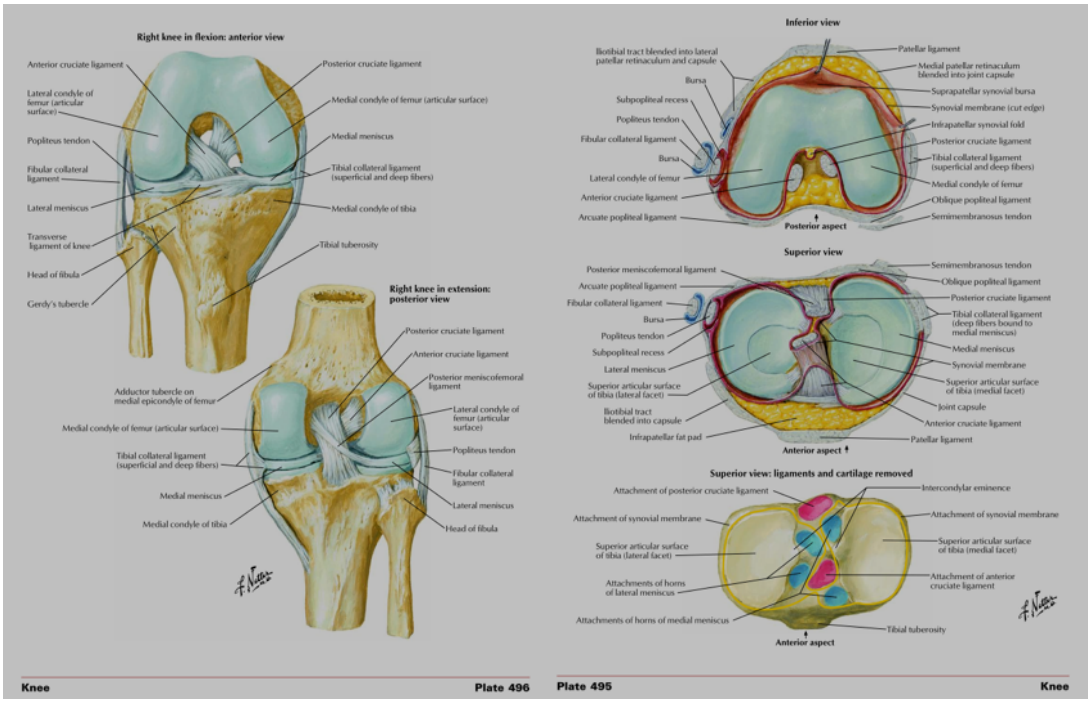

Figure 4: Knee anatomy (Netter's Anatomy Atlas)

1.3.1.2 Biomechanics of the knee joint

The knee consists of two separate joints but in this thesis, it is primarily the tibiofemoral joint (rather than the patellofemoral joint) that is of interest. At its most simple, it can be thought of as a hinge as the primary motion is flexion/extension, with a range of motion of approximately 0-130° (Ramachandran, 2006; Masouros, Bull and Amis, 2010). However, in reality, the motion of the knee joint is far more complex. Firstly it includes a "roll-back" mechanism by which the femur rolls back on the tibia during flexion, thus avoiding any trapping of the soft tissues (including neurovascular structures) in the popliteal fossa (Figure 5) (Ramachandran, 2006; Masouros, Bull and Amis, 2010). Secondly, there is also a rotational component. In full extension, the tibia rotates externally relative to the femur by up to 30° in the "screw-home mechanism" thought to allow locking of the knee to reduce energy expenditure when standing (Ramachandran, 2006; Masouros, Bull and Amis, 2010). During flexion however, the opposite occurs with the tibia internally rotating. However, due to the asymmetric shape of the

tibiofemoral joint – with the medial tibial plateau providing a much deeper cup – the axis of this rotation is within the medial compartment (Ramachandran, 2006; Masouros, Bull and Amis, 2010).

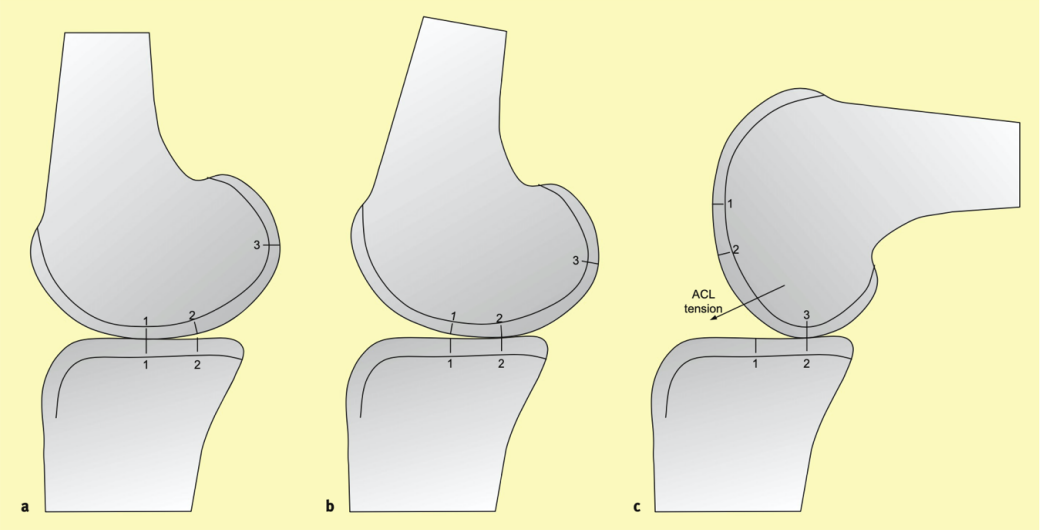

Figure 5: Knee joint kinematics in the sagittal plane during gait. **a** Extension: contact is located centrally. **b** Early flexion: posterior rolling; contact continuously moves posteriorly. **c** Deep flexion: femoral sliding; contact is located posteriorly; the unlocking of the ACL prevents further femoral roll back (Masouros, 2010)

These movements are combined within the gait cycle and also results in adduction/abduction motion as shown in figures 6-7 with maximal abduction in the stance phase and maximal adduction in swing phase (Fukuchi, Fukuchi and Duarte, 2018).

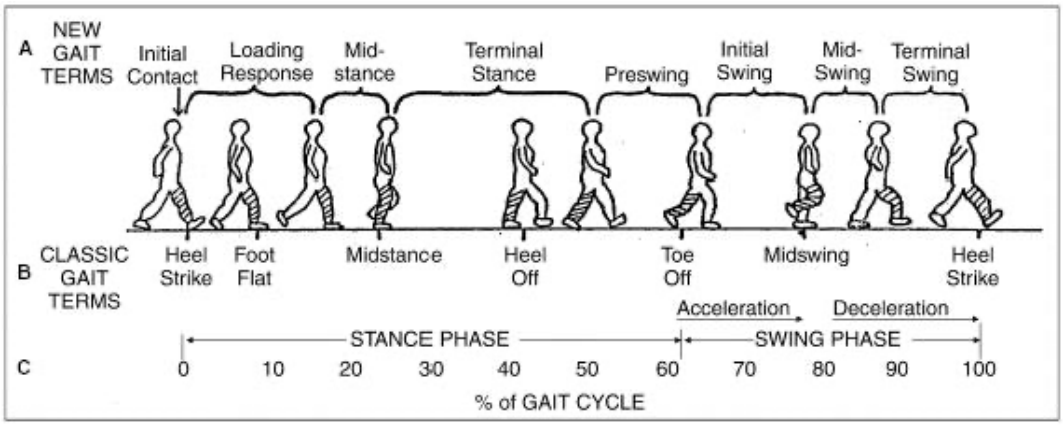

Figure 6: Gait cycle. Stages of walking gait (with traditional and updated terminology) (Orthobullets)

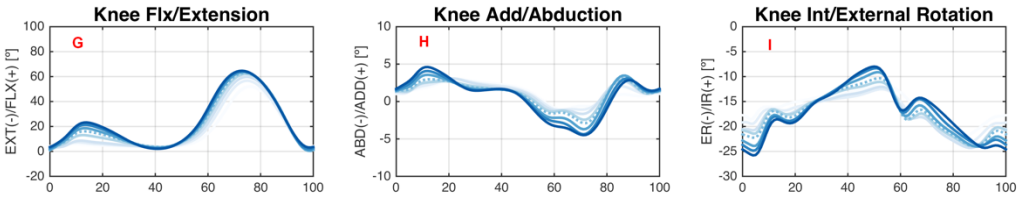

Figure 7: Angular kinematics of knee during treadmill walking. G) Sagittal plane, H) Coronal plane, I) Axial plane (Fukuchi 2018).

1.3.2 Osteoarthritis of the Knee

1.3.2.1 Overview

Osteoarthritis is a degenerative disease of synovial joints which causes symptoms of pain and stiffness. Although OA can occur in any joint, knees are a very common site. ARUK estimates approximately 17% of adults aged over 45 in England and Scotland (data not currently available for Wales) are affected by OA of the knee, with approximately 5% suffering severe symptoms (Arthritis Research UK, 2018). As risk of knee OA rises with both age and obesity (Arthritis Research UK, 2018), prevalence is expected to increase over the coming years. On plain radiographs OA is characterised by loss of joint space, subchondral sclerosis, subchondral bone cysts and osteophyte formation (Bulstrode *et al.*, 2011). However, these are relatively late stage changes and earlier changes can be seen on MRI scanning, including bone marrow lesions (BMLs), cartilage loss or changes in joint tissue metabolism (Shapiro *et al.*, 2014; Barr *et al.*, 2015). In some cases there is a clear predisposing factor (e.g. post traumatic osteoarthritis in approximately 12% of cases (Kornah *et al.*, 2019)) but the majority of cases are considered idiopathic/primary osteoarthritis and tend to occur in older people. Perhaps for this reason, many people including some doctors still refer to it as “wear and tear” arthritis (Berenbaum, 2013) and characterise it as a loss of joint hyaline cartilage (from being worn away through use) causing painful and debilitating “bone on bone” contact.

However, this rather basic model does not appear to explain all the clinical findings. For example, patients commonly report or present with “flares” where the affected joint develops a transient but painful effusion (Bonnet and Walsh, 2005). In addition, there is increasing evidence of an inflammatory process occurring in osteoarthritis joints (Berenbaum, 2013) and altered metabolism (Mobasheri *et al.*, 2017). But it does not appear to be cartilage which is driving this inflammatory process as the chondrocytes themselves

have low metabolic activity, as well as the cartilage being avascular and aneural (Ramachandran, 2006; Berenbaum, 2013). Instead, investigating the inflammatory pathways has revealed that OA (like rheumatoid arthritis) is a whole joint disease with mediators released by cartilage, bone and synovium (Berenbaum, 2013) and a range of poorly understood potential risk factors and biomarkers (Mahmoudian *et al.*, 2021).

1.3.2.2 Biochemical signaling in knee OA

A variety of mediators are produced by or have been isolated in particular joint tissues in the osteoarthritic knee (subchondral bone, cartilage, synovium, synovial fluid). There is also evidence of “cross-talk” between these different tissues, for example subchondral bone and cartilage, via these mediators which appears upregulated in OA (Burr and Gallant, 2012; Pan *et al.*, 2012; G. Li *et al.*, 2013). The knowledge of these pathways involved remains incomplete but some of the major component groups have been isolated.

The first category are inflammatory mediators. These are related not just to pain but also to cartilage degradation (Berenbaum, 2013; Bonnet *et al.*, 2015). This group includes both pro-inflammatory cytokines (interleukin-1β (IL-1β), tumour necrosis factor-α (TNFα), IL-6, IL-15, IL-17, IL-18) but also their anti-inflammatory counterparts (IL-4, IL-10, IL-13) (Wojdasiewicz, Poniatowski and Szukiewicz, 2014).

The second category are markers of bone or cartilage turnover. Bone remodelling is a continuous reparative process throughout the skeleton, including in the subchondral bone, and is controlled by a balance of osteoclastic (bone resorbing) and osteoblastic (bone forming) activity (Ramachandran, 2006; Bulstrode *et al.*, 2011). Changes within the subchondral bone seen on MRI and plain radiographs show that this process is affected in OA (Burr and Gallant, 2012) and biochemical markers have

been identified within the involved pathways which may allow this process to be tracked (Watt, 2018). Alkaline phosphatase (ALP) is a marker of osteoblastic activity (Marcus, R *et al.*, 2013) whereas C-terminal telopeptide of collagen type I (CTX-I) is a marker of osteoclastic activity (Bilezikian, J, Raisz, L, and Martin, T, 2008). Again there is a delicate balance between these two processes and this is mediated by the opposing actions on the wingless-related integration site (Wnt) pathway of receptor activator of nuclear factor-κB ligand (RANKL) and osteoprotegerin (OPG) as seen in Figure 8 (Mitchell and Streeten, 2013).

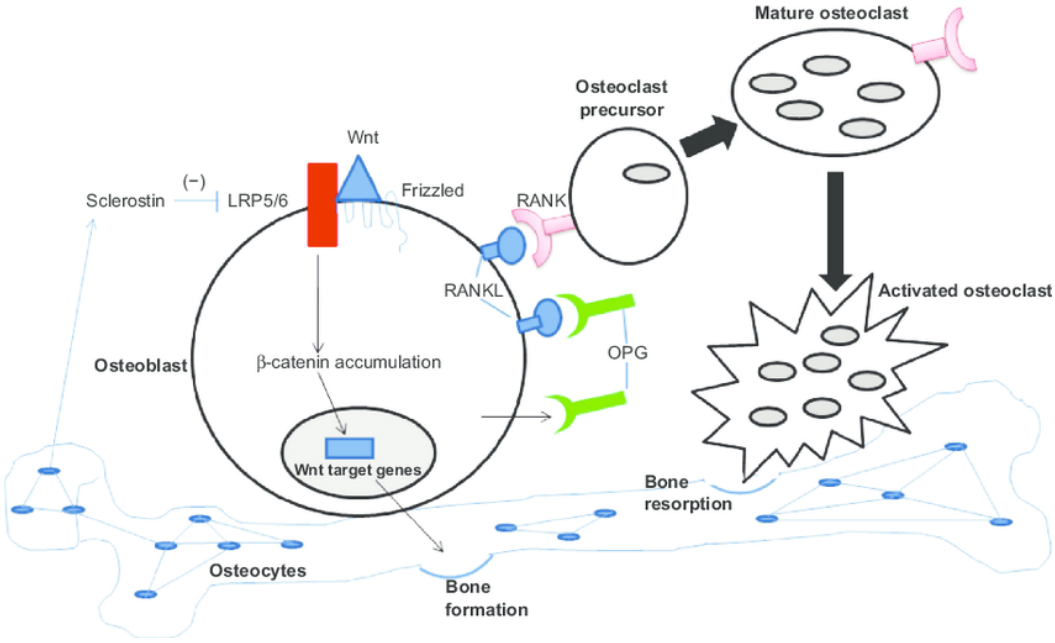

Figure 8: RANK/RANKL/OPG pathway in bone remodelling. The balance between bone formation and resorption is largely regulated by the Wnt pathway (bone formation), the RANK (pink symbols)/RANKL (blue symbols) pathway (osteoclast activation), and sclerostin (negative regulation of bone formation). Osteoblasts express the cell surface receptors RANKL and Wnt and also secrete a soluble decoy receptor, OPG (green symbols). Wnt protein binds coreceptors Fizzle-Fz and LRP5/6, leading to stabilization of β-catenin and its translocation to the nucleus to regulate target genes, resulting in increased bone formation. In the absence of OPG, RANKL on the osteoblast surface is available to bind RANK present on osteoclast precursors. Binding of RANK/RANKL leads to osteoclast maturation and resorption of bone. Sclerostin, secreted by osteocytes, inhibits Wnt from binding LRP5. Abbreviations: RANK, receptor activator of nuclear factor-kappa B; RANKL, receptor activator of nuclear factor-kappa B ligand; OPG, osteoprotegerin; Wnt, winglessrelated integration site; LRP, low-density lipoprotein receptor protein. (Mitchell and Streeten, 2013)

In addition, high bone turnover is also associated with increased vascularity and production of catabolic enzymes such as those of the A disintegrin and metalloproteinase (ADAM) family (Burr and Gallant, 2012). These in turn lead to increased cartilage breakdown products such as C-telopeptide of collagen type II (CTX-II) and cartilage oligomeric matrix protein (COMP) (Tseng, Reddi and Di Cesare, 2009; Duclos *et al.*, 2010).

Key to understanding OA however, is understanding how these biochemical processes within the knee are related to load. One component of this pathway appears to be sclerostin which appears to be downregulated by load and has an inhibitory effect on the Wnt signalling pathway (fig. 8) (Mitchell and Streeten, 2013; Galea, Lanyon and Price, 2017). The sclerostin pathway is one mechanism by which osteophytes act as the mechanoreceptive orchestrators of bone remodelling. This can be seen dramatically in high bone mass conditions such as Van Buchem diesase and sclerosteosis in which there is pathologically low sclerostin expression (Delgado-Calle, Sato and Bellido, 2017).

Another signalling pathway involving glutamate also appears to have a key role in the load-dependent bone remodelling process. Glutamate is an extracellular signalling molecule which is released by multiple cell types in the body. It is the major excitatory neurotransmitter in the brain (Meldrum, 2000), however there is also evidence that it is released by synoviocytes,

osteoblasts, osteoclasts and chondrocytes (Skerry, 2008; Piepoli *et al.*, 2009; McNearney *et al.*, 2010); and glutamate receptors have been identified in multiple cell types regulating pain, cytokine/inflammatory mediator release and cell proliferation (Parada-Turska *et al.*, 2006; Flood *et al.*, 2007; Miller *et al.*, 2011; Lindblad *et al.*, 2012). In addition, synovial fluid levels of glutamate are increased in animal models of osteoarthritis (Lawand, McNearney and Westlund, 2000; Jean *et al.*, 2005; Bonnet *et al.*, 2020).

Evidence from loading experiments on rat ulnas first suggested that the glutamate pathway in bone was responsive to load (Mason *et al.*, 1997) and a potential mechanism for this action was suggested by Brakspear and Mason in 2012 (Figure 9). They posited that glutamate was a key signalling molecule between components of the bone turnover pathway, being released by osteoblasts and affecting both osteoclasts, osteoblasts and their respective progenitor cells via a range of receptors and transporters. The terminology varies across papers but the suggested pathways by which glutamate triggers and intracellular response (as detailed in Figure 9) involves combinations of excitatory amino acid transporters (EAATs) which appear in bone to be regulated by load (Mason *et al.*, 1997) alongside three types of ionotropic glutamate receptors; N-methyl-D-aspartate (NMDA), a-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and kainite (KA).

Activation of these glutamate signalling pathways have been identified as contributing to inflammation, bone remodelling, pain and cartilage degradation, the hallmarks of OA (Bonnet *et al.*, 2015, 2020). The evidence suggests therefore that glutamate mediated signalling pathways may be the linchpin of OA pathogenesis, linking as they do, mechanical load to OA symptomatology and outcomes.

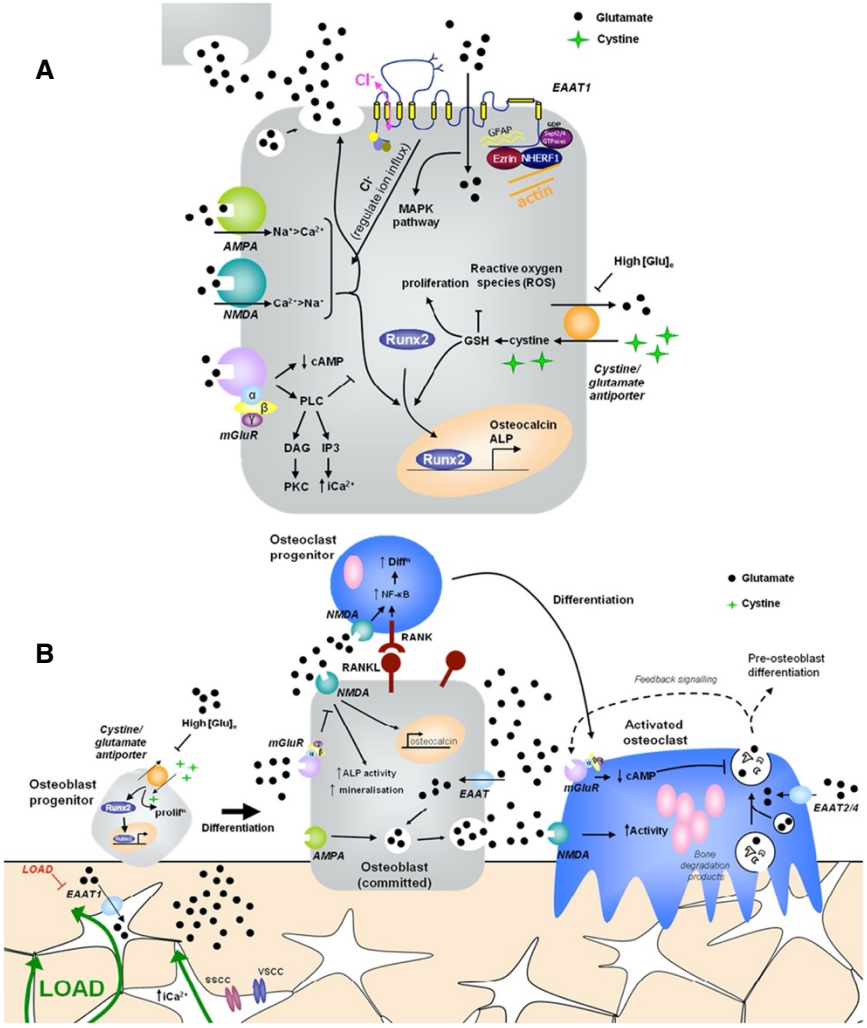

Figure 9: Hypothetical model of glutamate signalling in **(A)** osteoblasts and **(B)** bone. **(A)** Osteoblasts release glutamate to activate glutamate receptors in an autocrine and paracrine manner and express functional ionotropic and metabotropic glutamate receptors. iGluR activation leads to glutamate release and increased Runx2 activity, regulating osteocalcin expression, ALP activity and mineralization (Hinoi et al., 2002c, 2003; Ho et al., 2005). mGluR activation inhibits NMDA receptor signals in osteoblasts via PLC activated pathways (Gu and Publicover, 2000). High extracellular [glutamate] inhibits the cystine/glutamate antiporter, suppressing proliferation and reducing Runx2 activity due to depletion of GSH (Uno et al., 2007; Takarada-Iemata et al., 2010). EAATs transport glutamate into osteoblasts, modulating localized receptor responses, but also activating a chloride flux, which may function as a voltage clamp, act directly as a receptor, or modulate GluR activation by regulating ion influx (Danbolt, 2001; Huggett et al., 2002). Various proteins interact with the intracellular domains of EAAT1 and glutamate transport through EAATs activates MAPK (Abe and Saito, 2001). **(B)** Opening of stretch- and voltage-sensitive calcium channels (SSCC, VSCC) in osteocytes in response to mechanical load increases intracellular [Ca^{2+}] to induce glutamate release into junctions with neighboring osteocytes (Mason, 2004). Down-regulation of EAAT1 in mechanically loaded osteocytes (Mason et al., 1997) would increase extracellular [glutamate] which could regulate osteoblast differentiation and activity as described above. Osteoclasts are also likely to be regulated by released glutamate. Osteoclasts express EAATs 2 and 4 (Hinoi et al., 2007; Takarada and Yoneda, 2008) and NMDA receptor activation promotes NF-κB stimulated osteoclast differentiation (Peet et al., 1999; Merle et al., 2003) and increases mature osteoclast activity (Chenu et al., 1998; Itzstein et al., 2000; Mentaverri et al., 2003). Mature osteoclasts release glutamate in conjunction with bone degradation products, which can act on autoregulatory mGluRs, preventing further glutamate release (Morimoto et al., 2006). Therefore, glutamate signals may contribute to mechanical cues and coupling of bone remodeling. PLC, phospholipase C; DAG, diacylglycerol; cAMP, cyclic adenosine monophosphate; PKC, protein kinase C; IP3, inositol triphosphate; ALP, alkaline phosphatase; ROS, reactive oxygen species; MAPK, mitogen-activated protein kinase; EAAT, excitatory amino acid transporter; GSH, glutathione. (Brakspear and Mason, 2012)

1.3.2.3 Gait changes in knee OA

As would be expected, OA changes how the knee functions during gait. Studies have shown patients with OA of the knee to have slower walking speeds with a slower cadence, shorter stride and a pattern that minimises the length of time spent loading the affected leg (Mills, Hunt and Ferber, 2013; Favre and Jolles, 2016; DeFrate *et al.*, 2019). In addition, likely due to increased stiffness of the joint, a reduction in knee flexion (both in terms of angle and moment) is seen (Astephen *et al.*, 2008; Favre and Jolles, 2016).

However, in keeping with the knowledge that knee movement occurs in all three planes, frontal plane changes are also seen. The knee adduction moment (KAM) appears critical, particularly in medial compartment OA and it has been suggested that it acts as a surrogate marker for load distribution between the medial and lateral compartments (Baliunas *et al.*, 2002; Favre and Jolles, 2016).

As gait is a whole body movement, compensatory changes are also seen at other joints including the ankle, hip and trunk (Astephen *et al.*, 2008; Favre and Jolles, 2016). There is also of course much interest in ascertaining which of these mechanical changes is best linked to early OA (so could have potential as a diagnostic tool) (DeFrate *et al.*, 2019) and whether changing gait could have an impact on the progression of OA in the knee (Whelton *et al.*, 2017).

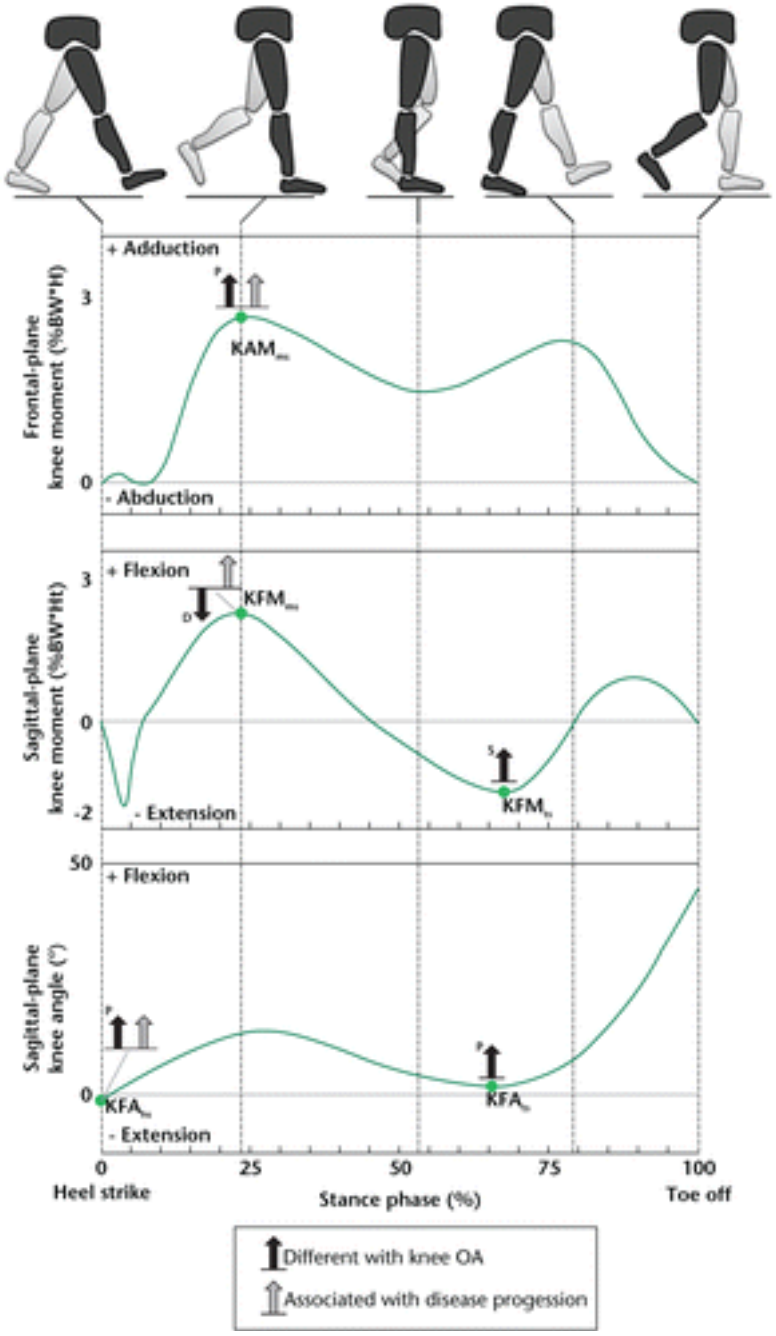

Figure 10: Overview of gait alterations consistently reported with medial knee OA. The black arrows indicate differences between groups of individuals at diverse stages of the disease, and the gray arrows indicate gait parameters which have been associated with OA progression in longitudinal studies. P, progressive differences from non-OA subjects to moderate and severe OA patients; D, differences between non-OA and OA individuals; S, differences between patients with severe knee OA and both non-OA individuals and moderate OA patients; KAM, knee adduction moment; KFM, knee flexion moment; KFA, knee flexion angle; hs, heel-strike; ms, mid-stance; ts, terminal stance. (Favre and Jolles, 2016)

1.3.2.4 Surgical options for treatment of OA of the knee

As discussed in the introduction, there remains a treatment gap for active patients with symptomatic OA, localised to the medial compartment (London, Miller and Block, 2011; C. S. Li *et al.*, 2013). They are served neither by conservative measures nor by arthroplasty so a joint conserving option such as high tibial osteotomy is a potentially attractive option.

1.3.3 High tibial osteotomy

1.3.3.1 Background to HTO

An osteotomy is the procedure of making a cut to the bone and this can be used as a method to change the angle of a bone/joint surface and thus the alignment of the limb (Bulstrode *et al.*, 2011; Merriam-Webster Dictionary, 2019). Around the knee, this could be a high tibial osteotomy or a distal femoral osteotomy and these can be performed on the medial or lateral sides of the bone depending on level of malalignment, desired realignment goal and anatomical restrictions (Bulstrode *et al.*, 2011). Equally, osteotomies can be described as “opening wedge” (where a cut is made and a wedge-shaped gap is then opened up) or “closing wedge” (where two converging cuts are made and a wedge of bone removed before the gap is closed). Whichever process is used, a metal plate and screws is used for support whilst the bone heals (Bulstrode *et al.*, 2011; Sabzevari *et al.*, 2016).

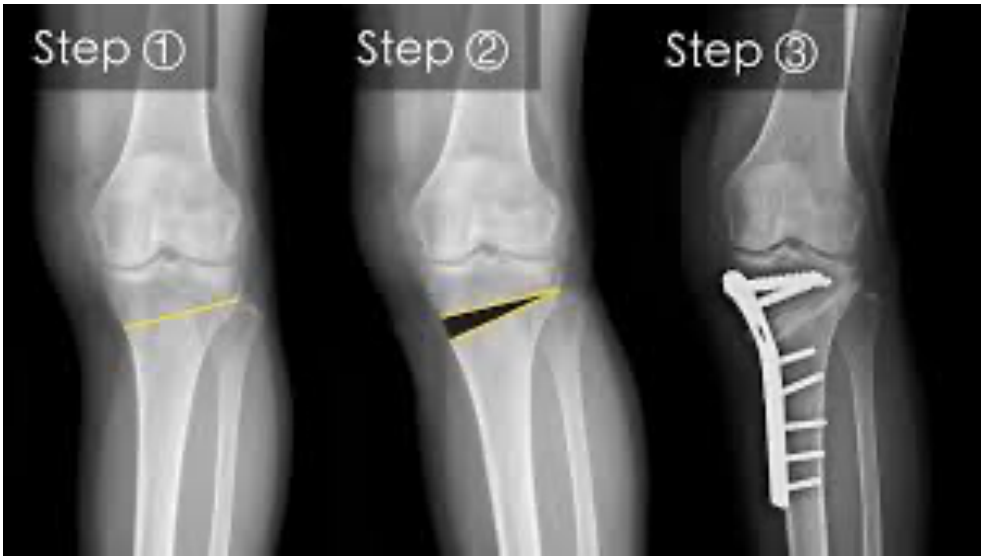

Figure 11: Medial opening wedge high tibial osteotomy. Demonstrating the opening wedge osteotomy and stabilisation with locking plate.

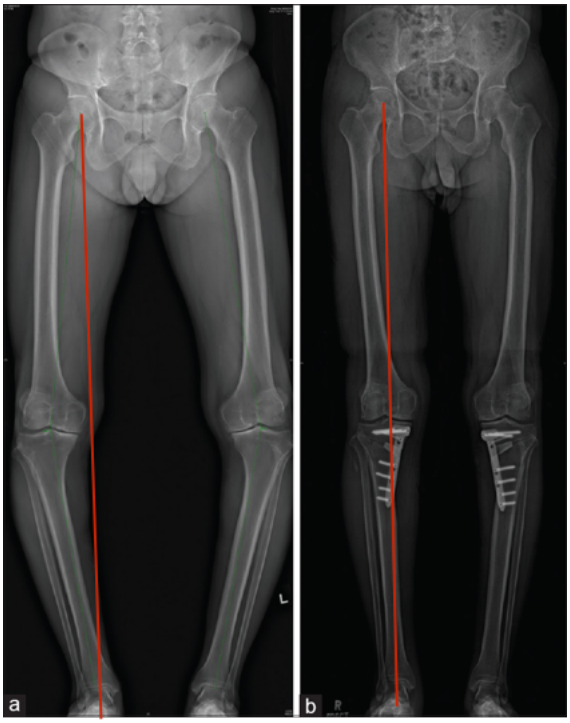

Figure 12: Realignment of the weightbearing axis in a varus knee following medial opening wedge HTO. a) Before surgery, weightbearing axis (red line) falls medial to knee joint. b) Post surgery, weightbearing axis now falls through the centre of the knee.

In this study, the focus is on medial opening wedge high tibial osteotomy (MOW-HTO). By increasing the medial length of the tibia compared to the lateral side of the bone, a valgus angulation is made at the knee. This means that the mechanical axis of the limb moves laterally at the knee; thus off-loading the medial compartment whilst increasing loading of the lateral compartment (Figure 12). Therefore this is suitable for varus malaligned knees and/or those with medial compartment osteoarthritis. This was first reported in 1958 but was popularized after further promising results in the 1970s, in an era in which arthroplasty was not yet a commonly performed/successful procedure (Sabzevari *et al.*, 2016). With the rise of arthroplasty (TKR/UKR), osteotomy fell out of favour, partially because fixation methods were very rudimentary leading to poor outcomes (Sabzevari *et al.*, 2016; Wilson, 2016). However, with advances in technology and angular stable plates, these issues became less problematic as patients

were able to weightbear early and the modern era of HTO was able to progress (Wilson, 2016).

By shifting the distribution of load within the knee (offloading affected portions) it has been proposed that HTO can slow or halt the progression of osteoarthritis of the knee. Cochrane reviews of the current literature suggest that clinically it is a worthwhile operation for patients with medial compartment arthritis as all eight studies, reported less pain and improved function (Brouwer *et al.*, 2014). However there is no evidence to support the relative effective of HTO versus UKR (Brouwer *et al.*, 2014). Most of the studies have focussed on clinical outcomes rather than biological/biomechanical effects but there are some which have looked specifically at joint cartilage post procedure and some show evidence of cartilage regeneration (i.e. a reversal of osteoarthritic changes) after HTO (Kanamiya *et al.*, 2002; Koshino *et al.*, 2003; Parker *et al.*, 2011).

There are also extended indications for HTO as it may also be used to mitigate knee instability after ligamentous injury. This may be combined with a procedure for medial OA as it is not uncommon for patients with a sports injury background to have both an anterior cruciate ligament deficient knee as well as medial compartment OA secondary to a medial meniscal injury and/or debridement.

Without a thorough understanding of how HTO can work, it is difficult to select which patients may benefit from HTO and to counsel them appropriately. It is hoped therefore that the findings of this project will add to the understanding of the scientific basis of HTO and this would have a direct positive impact on patient care. It may also be able to reveal mechanisms underlying mechanically-induced joint degeneration. This may help inform a multimodal approach to knee OA; considering correcting the mechanical alignment as a precursor/adjunct to cartilage regeneration procedures.

1.3.3.2 How does HTO affect osteoarthritis of the knee?

To assess what is known about the mechanism by which HTO has its impact on OA of the knee, a review of the current literature was undertaken. The online databases Pubmed, EMBASE and NHS Wales e-library were searched from 1990 to 2022 using the terms “high tibial osteotomy” and “osteoarthritis” (MeSH terms included). 1,581 papers were identified by the initial search and following title review, 81 were identified as relating to the mechanism of action of HTO on OA in the human, adult knee. Following review of abstracts, 68 papers were included. These were analysed based on their focus (e.g. mechanical effects, effect on cartilage, subchondral bone). The results of this analysis were combined into a proposed hypothetical model of action for HTO as described below.

Effect of HTO on cartilage

For many years, the established wisdom was that hyaline joint cartilage could not regenerate (Mandelbaum *et al.*, 1998; Ramachandran, 2006). Damaged joint surfaces were therefore doomed to osteoarthritic progression towards an eburnated, sclerotic subchondral surface and the clinical symptoms of pain, stiffness and restricted quality of life that this brings. If this were true than the best that could be hoped for from HTO would be to reduce patients’ pain via offloading the affected compartment or maybe at most to slow the progression of OA.

However, there are several researchers who have studied the joint surface post HTO, observing evidence of cartilage regeneration (i.e. a reversal of osteoarthritic changes) (Odenbring *et al.*, 1992; Schultz and Gobel, 1999; Kanamiya *et al.*, 2002; Koshino *et al.*, 2003; Spahn *et al.*, 2012; Jung *et al.*, 2014; C.-W. Kim *et al.*, 2017; Tsukada and Wakui, 2017). These studies have primarily reported cartilage regeneration observed via arthroscopy, therefore there is some discrepancy in how they identify the type of cartilage

cover. Some do not specify, some refer to increases in fibrocartilage cover (Kanamiya *et al.*, 2002; Jung *et al.*, 2014), others describe repair of cartilage lesions in the offloaded compartment (Spahn *et al.*, 2012; C.-W. Kim *et al.*, 2017; Tsukada and Wakui, 2017). Koshino et al. (2003) however, states specifically that in 47 out of 146 knees studies, full coverage by “hyaline-like” cartilage was observed at arthroscopy, with an increase to partial fibrocartilage coverage in a further 86 (Koshino *et al.*, 2003). They report that this regeneration occurs even in areas of eburnated bone (Koshino *et al.*, 2003). A single study using arthroscopic observation reported no signs of repair induced by HTO (Wakabayashi *et al.*, 2002).

In addition to observing the cartilage surface arthroscopically, other methods have been employed. Three studies have assessed cartilage health using delayed gadolinium-enhanced magnetic resonance imaging of cartilage (dGEMRIC) (Parker *et al.*, 2011; Rutgers *et al.*, 2012; d’Entremont *et al.*, 2015). Results from this method appear mixed. Rutgers et al. (2012) reported no change related to HTO (Rutgers *et al.*, 2012), d’Entremont et al. (2015) reported no overall change following HTO but improvements in a specific subgroup with shared kinematics (d’Entremont *et al.*, 2015) and Parker et al. (2011) reported positive change in the medial tibial plateau but not across the compartment as a whole (Parker *et al.*, 2011). A further study reported improved cartilage health in the medial compartment based on MRI T2 changes without compensatory deterioration laterally but did not find correlation with alignment measures(Atkinson *et al.*, 2022). One study biopsied the cartilage and reported both growth of fibrocartilage and improved health of hyaline cartilage with areas of repair (Odenbring *et al.*, 1992). One further study looked at radiographic surrogate markers of cartilage such as Kellgren-Lawrence grading and joint space narrowing (Huizinga *et al.*, 2017). They reported that progression of these OA signs post-HTO appeared slowed relative to population norms, but there was no

control group within the study and the researchers were not blinded to the treatment (Huizinga *et al.*, 2017).

Further evidence for this effect of HTO on cartilage regenerative capacity could come from studies looking at the success rates of cartilage repair procedures when combined with HTO. However, although there are multiple studies looking at pairing HTO with a range of cartilage repair procedures (microfracture, autologous cartilage implantation, stem cell therapies etc.) all but two of the studies are designed to determine how adding the cartilage repair procedure changes the success rate of the HTO (Schultz and Gobel, 1999; Sterett and Steadman, 2004; Matsunaga *et al.*, 2007; Bauer *et al.*, 2012; Ferruzzi *et al.*, 2014; Lee *et al.*, 2018). As the adjunct procedures and their quoted success rates are so varied, it is impossible to establish from this, whether the converse could be true, i.e. whether adding HTO to these cartilage repair procedures could improve their success. There is however some evidence to support this hypothesis from a paper showed untreated malalignment to be the leading cause of cartilage repair failure (Krych *et al.*, 2018). In addition, the study by Bode et al. (2003) suggested that survival rates for autologous chondrocyte implantation were higher if patients underwent a concomitant HTO, if their initial alignment had been more than 5 degrees varus (Bode *et al.*, 2013).

Biomechanical model of HTO effects

The question then posed is, if cartilage repair/regeneration is occurring, what is it about HTO that promotes or allows this? In other tissues of the musculoskeletal system, healing is dependent on the correct interplay of biological and mechanical factors. This can be seen in fracture healing for example, where deficiencies in either the biological (e.g. poor blood supply) or mechanical (e.g. too much movement at the fracture site) environment can lead to non-union (Ramachandran, 2006). It is reasonable to suspect the same might be true for the cartilage of the human knee. In a varus limb for

example, with an overloaded and osteoarthritic medial compartment, one could hypothesise that the medial joint surface could not heal under those conditions in the same way as animal models where destabilisation of the joint causes quick progression to severe osteoarthritis. However, a medial opening wedge HTO, by lateralising the mechanical axis of the limb, changes the mechanical loading of the medial compartment. As all joint tissues are reactive to mechanical changes (Skerry, 2008; McBride and Silva, 2012; Poulet, 2015), this will also cause changes in the biological environment, thus potentially creating the appropriate conditions for cartilage regeneration.

To investigate this, both the mechanical and biological effects of HTO must be investigated concurrently within individual patients to illuminate the underlying mechanism.

Mechanical Effects of HTO

Studies have been performed looking at mechanical effects of HTO both in static and dynamic circumstances. Several studies confirmed that by shifting the mechanical axis laterally, load within the medial compartment of the knee was decreased (Agneskirchner *et al.*, 2007; Ogden *et al.*, 2009; Suero *et al.*, 2015; Trad *et al.*, 2018). Some of these used cadaveric simulation (Agneskirchner *et al.*, 2007; Ogden *et al.*, 2009; Suero *et al.*, 2015) and others used computational models (Trad *et al.*, 2018). Three studies looked at the clinical effects of HTO related to mechanical axis change. Two of these studies concluded that HTO improved clinical scores but that these improvements were diminished by either under or over correction (Briem *et al.*, 2007; El-Azab *et al.*, 2011). However W-Dahl et al. did not find any correlation between degree of change in alignment and improvement in pain scores (W-Dahl, Toksvig-Larsen and Roos, 2009). The relationship between mechanical axis correction and joint compartment load is not a simple one however. For example, El-Azab et al. noted that ligament or soft tissue laxity can affect the degree of correction (El-Azab *et al.*, 2011), Suero et al.

showed that rotation of the distal tibial fragment can negate any offloading effect (Suero *et al.*, 2017) and Nakayama et al. demonstrated how if correction results in joint line obliquity, this can result in excessive shear stress on tibial articular cartilage (Nakayama *et al.*, 2018).

Numerous studies have also been performed looking at dynamic measures during gait, with the most common measure studied being the external knee adduction moment (KAM). This is used as a surrogate marker for medial compartment loading and has been implicated in medial compartment osteoarthritis (Amis, 2013; Whelton *et al.*, 2017). There is also a strong body of evidence that a valgus high tibial osteotomy reduces KAM (Wang *et al.*, 1990; Wada *et al.*, 1998; Ramsey *et al.*, 2007; Bhatnagar and Jenkyn, 2010; Lind *et al.*, 2013; Leitch *et al.*, 2015; Marriott *et al.*, 2015, 2019; Birmingham *et al.*, 2017; Whelton *et al.*, 2017, 2017; Badie, Katouzian and Rostami, 2018; da Silva *et al.*, 2018; Whatling *et al.*, 2020). The majority of this evidence comes from in vivo gait analysis of HTO patients comparing pre- to post-operative measures with or without comparison to healthy controls (Wang *et al.*, 1990; Wada *et al.*, 1998; Ramsey *et al.*, 2007; Lind *et al.*, 2013; Marriott *et al.*, 2015, 2019; Birmingham *et al.*, 2017; Whelton *et al.*, 2017; da Silva *et al.*, 2018; Whatling *et al.*, 2020). Other studies using computational models, developed and optimised from subject specific gait data have shown similar findings (Bhatnagar and Jenkyn, 2010; Badie, Katouzian and Rostami, 2018).

What is less clear is the optimal level or reduction in KAM that would give the best patient outcomes. Briem et al. found that correction levels far from the mean had higher KAM at 12 months post-surgery (Briem *et al.*, 2007) and Birmingham et al. suggested that those with KAM in the middle quartiles had the greatest improvement in clinical scores (Birmingham *et al.*, 2017). This may mean that those with only mildly abnormal KAM gain relatively little improvement from surgery whereas, possibly, for those with very severely

abnormal KAM, the effects may not be as reversible. A meta-analysis by Lee et al. also suggested that although HTO appeared to reduce KAM in a reproducible way, the relationship between change in KAM and change in mechanical axis remained controversial (Lee *et al.*, 2017).

In addition to changes in KAM, other changes in gait have been identified following HTO including an increase in walking speed (Lind *et al.*, 2013), decrease in varus thrust (Takemae *et al.*, 2006; Lind *et al.*, 2013; Lee *et al.*, 2017; da Silva *et al.*, 2018) and a reduction in "adaptive mechanisms" to the malalignment such as trunk lean and short stride length (Lee *et al.*, 2017; Whatling *et al.*, 2020). There is also evidence of the importance of considering the three-dimensional impact of osteotomy surgery. Several studies report changes in the sagittal plane (Lind *et al.*, 2013; da Silva *et al.*, 2018; Whatling *et al.*, 2020) as well as evidence that a change in foot progression angle (i.e. a rotational change) can affect KAM/medial compartment loading (Wang *et al.*, 1990; Whatling *et al.*, 2020). It must also not be forgotten that HTO has the potential to affect not just the knee but the whole limb and beyond that the whole body. There is already evidence that changes in coronal or transverse plane alignment at the proximal tibia can cause changes in contact pressure at the ankle (Suero *et al.*, 2015, 2017).

Effect of HTO on joint biology

It is clear that HTO causes measurable changes in the mechanics of the joint (both static and dynamic) and also that it is associated with improved knee function, reduced osteoarthritis symptoms and potential cartilage recovery. What is less clear is the biological mechanisms that link these domains. It is known that bone biology is highly responsive to mechanical loading both in healthy individuals where site specific bone gain is observed to be activity dependent, and clinically where it underlies modes of fracture healing, remodelling and phenomena such as stress shielding (Skerry, 2008; McBride and Silva, 2012). This is also true of the subchondral bone which displays

load related changes in osteoarthritis ranging from bone marrow lesions to subchondral sclerosis (Poulet, 2015; Donell, 2019). However, little is known about how HTO may interact with these processes.

Some studies have suggested that following HTO, subchondral bone density in the medial compartment decreases relative to the lateral compartment and this appears to correlate with functional outcome (Akamatsu *et al.*, 1997; Takahashi, Tomihisa and Saito, 2003; Gersing *et al.*, 2018). Further evidence for the effect of mechanical off-loading on subchondral bone come from MRI based studies which also show reduction in bone marrow lesions (BMLs)/subchondral bone oedema in the medial compartment following HTO, although it remains unclear to what extent this correlates with clinical knee scores (Kroner *et al.*, 2007; Kim *et al.*, 2019; Wang *et al.*, 2021).

Further evidence for the resultant biological effects of mechanical changes come from the synovium and synovial fluid with studies demonstrating reduced inflammatory changes in the synovium (Nakashima, Koshino and Saito, 1998) and reduced inflammatory cytokine levels within the knee post HTO (Bai *et al.*, 2017).

Discussion

It seems at present that we have pieces of the puzzle regarding how HTO works but as yet, how they fit together remains largely unknown. Particularly, how these domains of mechanical and biological changes are linked remains a gap in knowledge. The early appearance of BMLs in the pathogenesis of OA compared to cartilage lesions, coupled with the mechanoreceptive properties of subchondral bone and its posited role in the nutrition of cartilage (Donell, 2019), suggests that it may be subchondral bone health which is driving OA rather than cartilage (Mason, 2004b; Vazquez *et al.*, 2014; Watt *et al.*, 2019). Glutamate pathways within the subchondral bone have been suggested as a keystone to this process as there is evidence they

provide a link between the mechanical environment of the bone and inflammatory pathways involved in pain, inflammation and cartilage destruction (Mason, 2004b; Brakspear and Mason, 2012; Bonnet *et al.*, 2015; Whatling *et al.*, 2018).

There are also at present large gaps in our understanding of the pathogenesis of OA, particularly in its early stages. Currently the clinical diagnosis of OA is made based on radiographic features of loss of joint space (i.e. cartilage loss), subchondral sclerosis, subchondral cysts and osteophytes (Bulstrode *et al.*, 2011); all arguably late changes and signs of a joint being pushed beyond its threshold for repair into a salvage mode. If this is true then it may be that by this stage it is too late for HTO to be effective (Donell, 2019). Where this “point of no return” lies however is unknown and may depend on multiple other patient factors.

There is therefore evidence for a linking hypothesis for the efficacy of HTO. There is little doubt that surgical realignment of the proximal tibia causes both changes in the mechanical axis of the limb and consequent changes in joint compartment pressures and gait. This change in the mechanical environment appears to coincide with measurable changes in the biology of several joint tissues (including cartilage, subchondral bone and synovial fluid) although the mechanism of this mechano-biological link remains unclear. In turn, these observed biological changes include downregulation of the pathways of pain, inflammation and cartilage destruction that represent the hallmarks of OA.

Chapter 2:

Methodology

Methodology general to all subsequent chapters will be discussed below. Further methods will be discussed within the relevant chapters where required. This thesis is based within a collaborative research project, as such the author has taken part to varied degrees in all the described methods and data gathering alongside other members of the team. The respective contributions will be made clear within each section.

Approval for this work was granted by the Wales Research Ethics Committee 3 (10/MRE09/28) and Cardiff and Vale University Health Board and informed consent was collected from each participant.

2.1 PATIENT SELECTION AND RECRUITMENT

All patients who were listed for a medial opening wedge HTO (MOW-HTO) were identified and approached by the research team at Cardiff and Vale Orthopaedic Centre (CAVOC). They were given information about the study in both verbal and written form before consenting for involvement at a later date (Appendix 1). Inclusion criteria were as follows:

- Aged 18-80
- Undergoing medial opening wedge high tibial osteotomy
- Operative indication includes medial osteoarthritis
- Able to understand the study information sheets and give informed consent
- Able to walk 10 metres unaided (required for gait analysis)
- No neurological or musculoskeletal condition which affects gait/activities of daily living other than that under study (knee OA)

Some clinical and radiological data was collected from patients at the time of their clinic visits to CAVOC. Bone and synovial fluid samples were taken at two time points; pre-HTO (on the day of surgery/at the start of the operation)

and post-HTO (at the time of plate removal, approximately 12 months later). Patients were also asked to attend the gait lab at the BBRCVA centre in the Department of Engineering. Here a clinical history and any blood/urine samples were taken and they underwent gait analysis (section 2.6). These visits also occurred at set time points post operatively (3, 6 and 12 months).

The data gathered from participants consisted of biological samples, clinical data, radiological measurements and gait analysis. The biological samples consisted of four needle biopsies of the subchondral bone (from the four quadrants; anteromedial/anterolateral/posteromedial/posterolateral), synovial fluid, urine and blood.

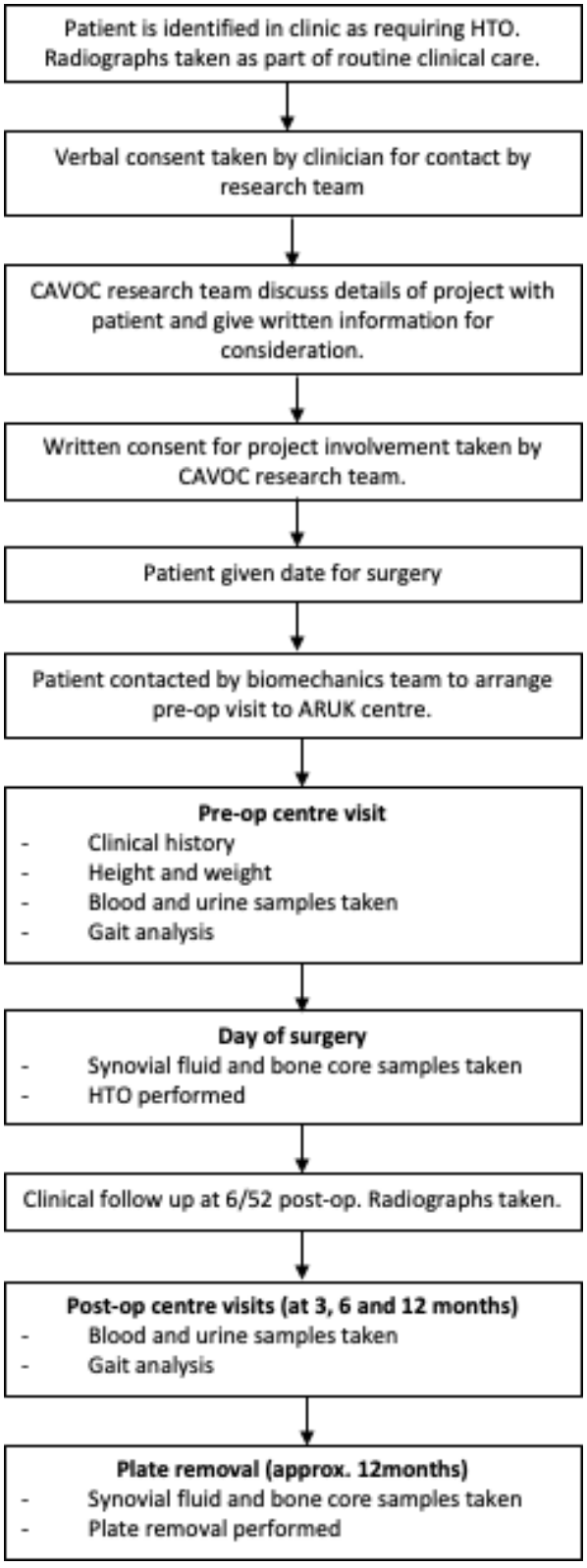

Figure 13: Pathway of involvement for study participants

2.2 MEDIAL OPENING WEDGE HTO AND SAMPLE ACQUISITION

The surgical procedures were carried out by members of the Orthopaedic team at CAVOC, including in some cases by the author (under consultant supervision).

The intended correction was first calculated using the Miniaci method on the IMPAX software system (Elson, Petheram and Dawson, 2015).

The patient was anaesthetised, set up in theatre with the image intensifier and tourniquet (which was not inflated until after samples had been taken) and the limb prepared and draped to establish sterility.

Samples were then taken. First the knee joint is aspirated to collect synovial fluid and then subchondral bone cores are taken by needle biopsies from 1cm below the joint line in the anteromedial, posteromedial, posteromedial and posterolateral quadrants (Figure 14). The image intensifier was used to visualise the needle position.

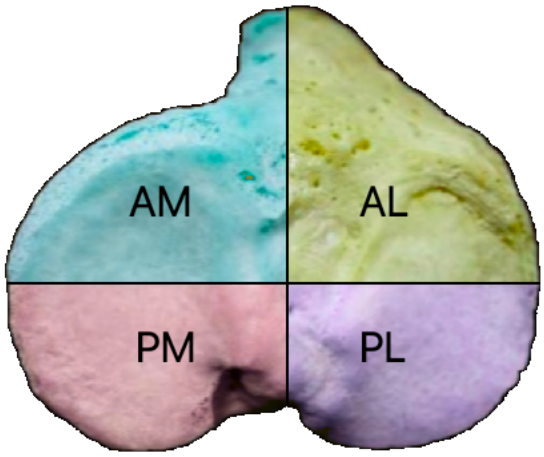

Figure 14: Quadrants of the tibial plateau (AM=anteromedial, AL=anterolateral, PM=posteromedial, PL=posterolateral)

The bone samples were immediately placed into labelled cryovials and placed in a liquid nitrogen carrier before transport to the labs where they were stored in freezers at -80degrees centigrade. The synovial fluid aspirates were transferred to a 5ml falcon tube, cool packed and sent to the laboratory for processing within 30 minutes. The fluid was centrifuged at 5000G for 15 minutes to remove cells and then the supernatant was aliquoted and stored at -80degrees centigrade.

Following sample collection, the tourniquet was inflated and the surgery begun. An anteromedial incision was made medial to border of patellar tendon and then extended down to tibial surface, peeling back a superficial portion of the medial collateral ligament.

Two parallel Kirshner wires were inserted under image intensifier guidance to mark the position and angle of the intended cut. A precision saw was used to make a biplane cut, under image intensifier guidance, leaving a lateral hinge of bone intact. Osteotomes were used to complete cut where necessary, whilst preserving the lateral hinge. Stacked osteotomes and laminar spreaders were used to slowly stretch open the wedge to the required size as determined by the preoperative planning. The space could be packed with cancellous bone chips or a custom cut wedge of donated femoral head if required but this was not the standard practice of the lead surgeon. A plate (usually Tomofix (DuPuySynthes)) was applied and fixed with locking screws.

The wound was closed in layers, dressed and the patient recovered. The post-operative instructions were for six weeks toe-touch (less than 10kg pressure) weightbearing with crutches and this was assisted by physiotherapists. Once the patient was safely mobile on crutches, they were discharged home and reviewed in clinic at six weeks post operatively.

All patients were offered removal of the plate at approximately 12 months post HTO once the bone is healed. This is because the plate is often prominent/irritant to the soft tissues and may also have a longer-term effect on the metaphyseal bone due to its stiffness. The procedure for this involves sample acquisition via the same method described above and then removal of the plate by opening of the original incision. Patients returned to full activities/weightbearing post operatively.

2.3 RADIOGRAPHIC MEASUREMENTS

Measurements were made on plain radiographs (long leg alignment films and knee joint films) using the IMPAX software (AGFA). The majority of the radiographic assessment was carried out by the author but an early cohort of patients had been analysed by a previous centre team member. The measurements taken include mechanical tibio-femoral angle (mTFA), Mikulicz point, medial proximal tibial angle (MPTA), lateral distal femoral ankle (LDFA) and lateral distal tibial angle (LDTA) (Figure 15).

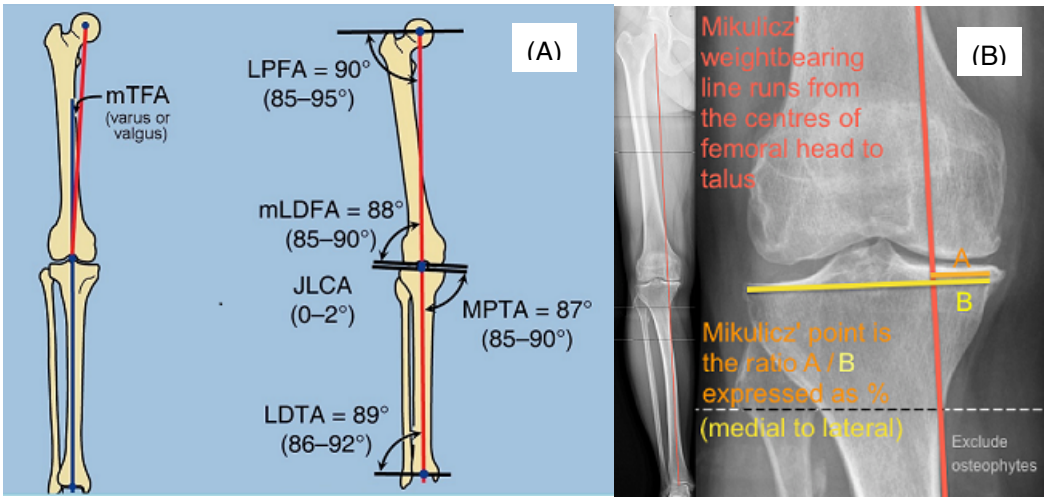

Figure 15: Standard radiographic measures for HTO. (A) Measurements made on long leg alignment and knee joint radiographs, with normal measures (and ranges). mTFA=mechanical tibiofemoral angle, LPFA=lateral proximal femoral angle, mLDFA=mechanical lateral distal femoral angle, JLCA=joint line convergence angle, MPTA=medial proximal tibial angle, LDTA=lateral distal tibial angle. (B) Mikulicz point measurement method. (Elson *et al.*, 2015)

These measurements were taken as follows:

- Mechanical tibio-femoral angle (mTFA) is drawn from the centre point of the femoral head to the centre of the talus. This is expressed as degrees of varus (a valgus angle will be expressed as a negative)
- Mikulicz point is the ratio between the medial tibial edge to the weightbearing axis and the width of the proximal tibial joint line. This is calculated by first drawing the weightbearing axis of the limb (a line from the centre of the femoral head to the centre of the talus). Where this intersects the tibial plateau, the distance from the intersection to the medial plateau edge is taken and divided by the total width of the tibial plateau. This maybe expressed as a percentage. If the weightbearing axis falls entirely medial to the tibial plateau, the figure will be negative.
- Medial proximal tibial angle (MPTA) is the angle between a line from the centre of the tibial spines to the centre of the talus and the proximal tibial joint line. It is a measure of any deformity within the proximal tibia and is what is physically changed during HTO surgery.
- (Mechanical) Lateral distal femoral angle (LDFA or mLDFA) is the angle between the mechanical axis of the femur (a line from the centre of the femoral head to the centre of the intercondylar notch) and the femoral joint line (a line drawn across the joint surface of the femoral condyles. It is a measure of any deformity within the distal femur and should not change following HTO surgery as is a measure inherent to the bone.
- Lateral distal tibial angle (LDTA) is the angle between the mechanical axis of the tibia (a line drawn from the interspinous notch to the centre of the talus) and the distal tibial joint surface. It is a measure of any deformity within the distal tibia and should not change following HTO surgery as is a measure inherent to the bone.

The severity of OA was assessed from radiographs of the knee using the Kellgren-Lawrence grade (Kellgren and Lawrence, 1957) (Table 1).

Grade	Radiologic Findings
0	No radiological findings of osteoarthritis
I	Doubtful narrowing of joint space and possible osteophytic lipping
II	Definite osteophytes and possible narrowing of joint space
III	Moderate multiple osteophytes, definite narrowing of joint space, small pseudocystic areas with sclerotic walls and possible deformity of bone contour
IV	Large osteophytes, marked narrowing of joint space, severe sclerosis and definite deformity of bone contour

Table 1: Kellgren-Lawrence grading system. (Kellgren and Lawrence, 1957)

2.4 BONE CORE ANALYSIS

The bone core samples have been obtained as part of a large, longitudinal study led by Professor Deborah Mason since 2009, funded by the Biomechanics and Bioengineering Research Centre Versus Arthritis (BBRCVA). Bone cores have been obtained from CAVOC with oversight from Mrs Cheryl Cleary and Dr Paul White. The majority of RNA extraction and RTqPCR has been performed by Mrs Carole Elford. Having demonstrated competency with basic laboratory procedures (e.g. pipette accuracy), the author was been taught the procedure by Ms Elford and performed all stages of the technique independently on repeated samples and under supervision for a small number original samples (approximately 5 samples). This was due to the very small volume of sample for each patient. All analysis of the resulting data for all samples was performed by the author after discussion of strategy with Prof. Mason and training via online statistics/software tutorials.

2.4.1 RNA extraction

Bone samples were taken from the -80°C freezer and placed on dry ice to keep them frozen. A dewer was filled ¾ full with liquid nitrogen for cooling the dismembrator chambers. The chambers were cleaned by washing in 1M NaOH then one wash with distilled water and two washes with 75% ethanol. When they were dry, the chambers (with ball bearing in the larger half of the chambers) were placed in the liquid nitrogen using long forceps and left to cool.

One of the larger halves of the chambers was removed from the liquid nitrogen, keeping it full. One HTO bone sample was then transferred into this chamber and the liquid nitrogen allowed to evaporate. Then the lid of the chamber was removed from the liquid nitrogen and placed on top of the larger, lower chamber half containing the bone. The chamber was then

placed in the dismembrator (mikro-dismembrator-U, Sartorius) at 2000rpm for 1 minute.
The chamber was then opened and 500μl of TRIzol (Invitrogen) added to each side of the chamber. As the TRIzol began to thaw, it was mixed by pipetting until the mixture was homogenous. The mixture was then transferred to an RNase/DNase free Eppendorf and kept on dry ice or stored at -80°C until ready to proceed with the next stage.
If chambers were to be reused, they were cleaned as described above.

Once ready to proceed, the samples were thawed at room temperature and incubated at room temperature for 5 minutes. At this point, the samples were spiked with 20μl of an ERCC (External RNA Controls Consortium) mix (Invitrogen) which contains a number of synthetic RNA oligonucleotides in defined amounts and ratios to allow the efficiency of the extraction to be investigated (Munro *et al.*, 2014). Successful RNA extraction should result in a maintained ERCC amount and ratio. Variability in ERCC standards after RNA extraction and RTqPCR would allow variance in the molecular measurement process to be defined. The mix was a proprietary blend of 92 transcripts (250 – 2000nt in length) that mimic natural eukaryotic mRNAs. It is sold at a 100X concentration and was diluted to 1X for use in this process.

Following this, 0.2ml of chloroform (Invitrogen, >99.5% chloroform) was added and each tube shaken vigorously for 15 seconds, then incubated at room temperature for 2-3 minutes. They were then centrifuged at 12,000g for 15 minutes at 4°C. The aqueous layer (containing the RNA) was transferred to a clean tube, making sure not to take any of the interface (but storing the remainder of the sample at -80°C in case needed for DNA or protein extraction at a later date). 0.5ml of isopropanol was added, mixing gently by inversion and incubated at room temperature for 15 minutes before centrifuging at 12,000g for 30 minutes at 4°C. The supernatant was discarded and the resulting RNA pellet washed in 1ml of 75% ethanol. The

samples were vortexed and centrifuged at 7500g for 15 minutes at 4°C. Again, the supernatant was discarded and the pellet allowed to air dry at room temperature (approximately 5-10 minutes). The pellet was resuspended in 44μl molecular biology water, placing it into a heating block at 56°C for 10 minutes.

2.4.2 DNAse treatment

To avoid DNA contamination of the RNA samples, they were treated with DNAse and then reprecipitated and again checked by NanoDrop.
The 44μl sample was placed into a 1.5ml tube with 5μl 10X buffer, 1μl DNase 1 (Invitrogen, 1U/μl) and incubated at 37°C for 30 minutes. The tube was centrifuged briefly before adding 10μl of inactivation reagent (Invitrogen), vortexing well and incubating at room temperature for 2 minutes (mixing the tube 3 times within this time). Following this, the samples were centrifuged at 10000g for 1.5 minutes and the supernatant transferred to a clean 1.5ml tube. If necessary, at this point the samples could be returned to storage at -80°C.
If proceeding, 50μl of the sample was mixed with 5μl 3M sodium acetate pH 5.5 (0.1 vol.), 150μl 100% ethanol (3 vol.) and 1μl glycogen then incubated at -80°C for a minimum of 45 minutes or overnight at -20°C. After either option, the mixture was centrifuged at 12,000rpm for 30 minutes at 4°C. The supernatant was discarded and the pellet washed with 1ml 75% ethanol, vortexed and centrifuged at 7,500rpm for 15 minutes at 4°C. Again, the supernatant was discarded, the pellet allowed to air dry for 10 minutes and then resuspended in 20μl RNAse and DNAse free water.
Following this precipitation, the samples were read by NanoDrop, recording ng/μl as well as 260/280 and 260/230 ratios. Samples were then diluted in RNAse and DNAse free water to make each sample up to 50ng/μl in 4μl aliquots.
Samples were then stored at -80°C ready for reverse transcription.

2.4.3 Reverse transcription

The RNA was then reverse transcribed to cDNA.

First, the PCR work station and PCR pipettes were cleaned with 70% ethanol. The dNTP mix (Promega, total concentration 40mM) and Oligo dT (Promega, 500 μg/ml) reagents were thawed on ice along with the samples, 5X buffer (Life Technologies) and DTT (Life Technologies, 100mM). These were mixed gently by hand and briefly centrifuged prior to use. The enzyme reagents (RNasin (Promega, 40U/μl), Superscript 3 (Life Technologies, 200U/μl)) were kept at -20°C until required and kept on ice at all stages.

Using filter pipette tips, the following were pipetted into sterile 0.2ml tubes on ice; 3μl sample, 0.5μl random primer mix, 1μl dNTP mix (Promega), 8.5μl RNase free water. The tubes were mixed gently, centrifuged briefly and then placed in the PCR machine (Techne TC-312) at 65°C for 5 minutes. When finished, the samples were cooled on ice and centrifuged briefly before adding 4μl 5X buffer (Life Technologies), 1μl DTT (Life Technologies, 100mM), 1μl RNasin (Promega, 40U/μl) and 1μl Superscript 3 (Life Technologies, 200U/μl). After gently mixing and centrifuging briefly the sample was again placed in the PCR machine (Techne) at 25°C for 5 minutes, 50°C for 50 minutes and 70°C for 15 minutes.
At this point, the samples could be stored at -20°C or qPCR was performed.

2.4.4 qPCR

The amount of cDNA present was measured using quantitative polymerase chain reaction (qPCR).
Mrs Carole Elford had cloned each gene of interest into pGEMT, and the sequenced PCR product had been purified and used to establish a standard curve from concentrations of 10^7 copies down to 10^1 copies, with herring sperm (315mg/μl) added as a blocking agent. The cloned template DNA standards were added to the qPCR plate after all other cDNA samples in a

separate laboratory area to prevent contamination. The standard curve was used to calculate the efficiency of the reaction (which must be between 90-110% for accurate quantitation) and allow accurate quantification (Thermo Fisher, 2022).

Within a 96-well plate, the first two rows were saved for the standard curve for the gene of interest, two wells used as No Template Controls (NTC) where water is substituted for cDNA and the remainder were used for analysis of bone core cDNA. All sample wells ("unknowns") contained a mix of 1μl sample, 1μl specific primer probe, 8μl RNAse-free water and 10μl Taqman master mix (Thermo Fisher Scientific) containing buffer, dNTPs (Promega, total concentration 40mM), reference dye and DNA polymerase. Wells for the standard curve contained 1μl primer probe, 10μl Taqman master mix (Thermo Fisher Scientific), 7μl RNase free water, 1μl herring sperm and 1μl of plasmid at each specified concentration. The NTC wells contained the same except for an extra 1μl RNase free water in place of the plasmid. PCR was then carried on a Stratagene Mx300p machine (Thermo Fisher Scientific). The cycling conditions were and initial step of 50°C/2mins, 95°C/10mins followed by 40 cycles of 95°C/15secs denaturation with 60°C/1min annealing and extension.

2.5 ANALYSIS OF BONE CORE DATA

The readings for the genes of interest from the PCR process were first compared against the geometric mean of two reference genes which had been previously identified as consistently stable in the bone samples when analysed by Genorm. These were the genes HPRT1 and YWHAZ. This controlled for difference in count number due to different efficiencies in the RNA extraction and RTqPCR process.

The data were analysed either as absolute count numbers for each quadrant, or each quadrant expressed as a percentage of the total RNA expression of the tibial surface (i.e. RNA expression in posteromedial quadrant divided by the sum of RNA expression in all four quadrants).

The difference between the pre-HTO time point and the post-HTO time point (samples taken at plate removal, approximately 12 months following HTO) were analysed using SPSS software (details of analysis in Chapter 3).

2.6 GAIT ANALYSIS METHODOLOGY

The gait analysis has been carried out by the biomechanics and bioengineering team led by Professor Cathy Holt; including Dr Paul Biggs, Dr Gemma Whatling and Dr Jake Bowd. Prior to cessation of activitiy due to COVID, the author took part in a small number of patient sessions in order to learn the process of data collection and the different aspect of gait studied. The procedure involved is explained below. The data has been collected and provided by the Biomechanics and Bioengineering Research Centre Versus Arthritis team as part of the collaborative project. This was supplemented by reading the papers and theses from previous project team members as well as multi-disciplinary discussion meetings which provided a better understanding of the data components to allow the author's independent analysis.

All gait analysis was performed within the gait laboratory at the BBRCVA in the School of Engineering, Cardiff University. The laboratory was set up to allow measurement of kinetic, kinematic, temporo-spatial and muscle activity parameters. The perimeter of the laboratory features nine Qualisys Oqus 7 optoelectric infrared (IR) cameras (Qualisys, Sweden) to allow motion capture. There were six force plates hidden within the 10m walkway as well as an instrumented staircase which also has force plates embedded (Bertec Corporation, Ohio, USA) which allow measurement of ground reaction force (GRF) through a range of activities. In addition, 14 wireless Trigno™ electromyography (EMG) electrodes (Delsys Inc, Massachusetts, USA) were available to measure muscle activity. The resultant data was analysed using Visual 3D software (C-motion, USA).

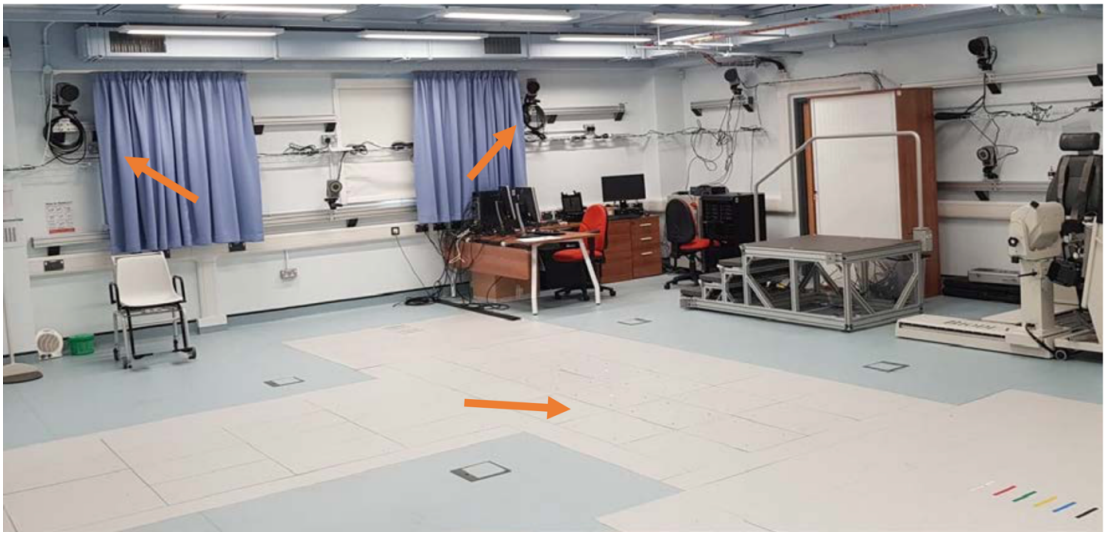

Figure 16: The BBRCVA motion analysis laboratory, Cardiff University. Orange arrows highlight IR cameras and integral force plates (photo courtesy of Dr Nidal Khatib)

Patients were requested to wear non-restrictive and non-reflective clothing, preferably just shorts for men and shorts plus a sports bra for women. This was to prevent any obscuring of sensors from the cameras. Height and weight were taken on arrival and body mass index (BMI) calculated as these were used for calibration and to normalise the results between patients.

Measurement of knee range of motion were taken and patients were also requested to complete patient reported outcome scores including the Oxford knee score (OKS) and the pain audit collection system brief pain inventory (PACS).

Retro-reflective markers were affixed to the patient (using hypoallergenic tape) in the positions shown in figure 17. These allow the IR cameras to track the body's movement in 3D by subdividing the motion into separate segments; each defined by bony landmarks and a centre of rotation.

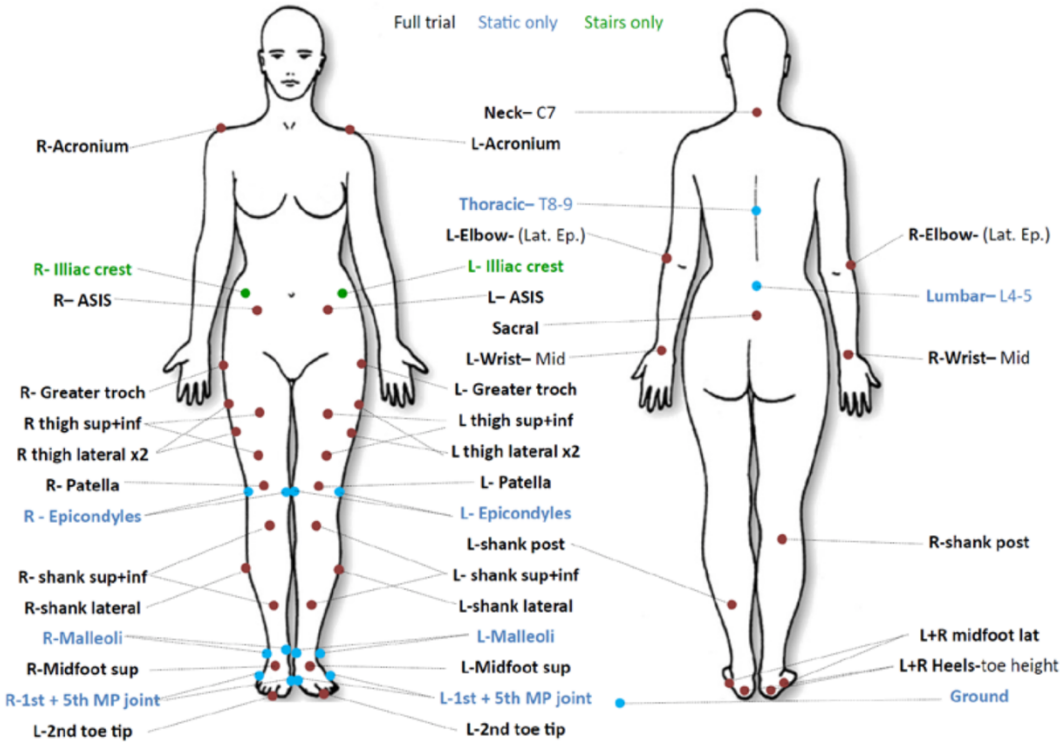

Figure 17: Motion capture reflective marker placement (Khatib, 2018)

Following this, the EMG sensors were also secured to the patient in the positions shown in figure 18 to measure the contractions/co-contractions in the specified lower limb muscle groups.

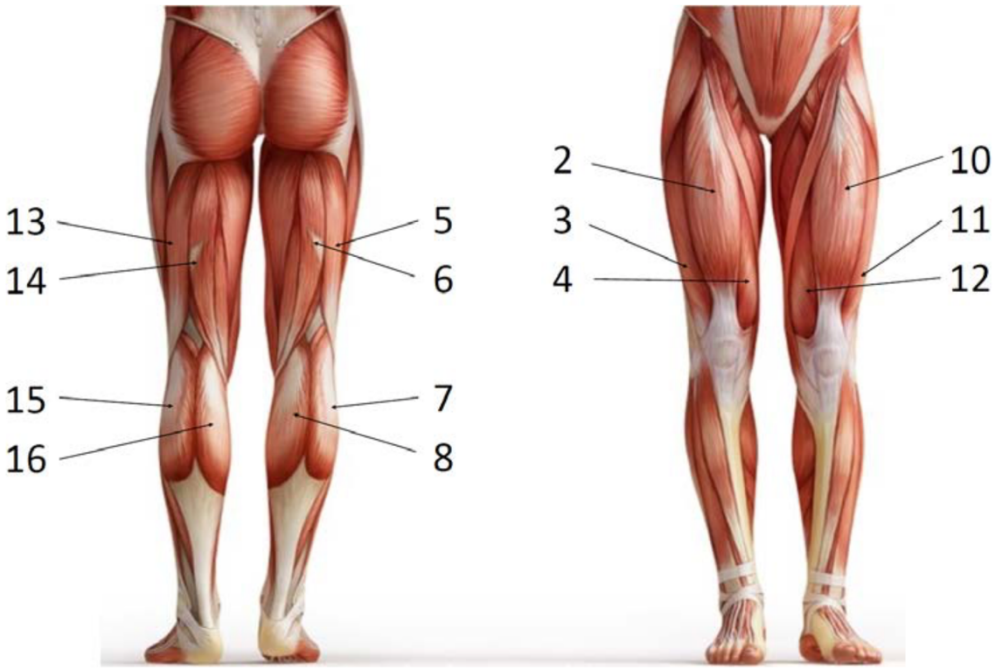

Figure 18: Positioning of EMG sensors. Rectus femoris (2, 10); vastus lateralis (3,11); vastus medialis (4,12); biceps femoris (5, 13); semitendinosus (6,14); gastrocnemius lateral (7,15); gastrocnemius medial (8, 16) (Khatib, 2018)

Once all the markers were secured and the system calibrated, the patient was asked to perform a series of activities which include:

- Walking at a comfortable speed along the flat walkway
- Sitting and rising from a chair
- Walking up and down stairs

There were many potential biomechanical and tempo-spatial measurements generated from this technique but those relevant to this thesis include:

- Cycle time (a measure of walking speed)
- Double stance support time
- External knee adduction moment (EKAM) – a surrogate marker for medial compartment knee loading (Figure 19)

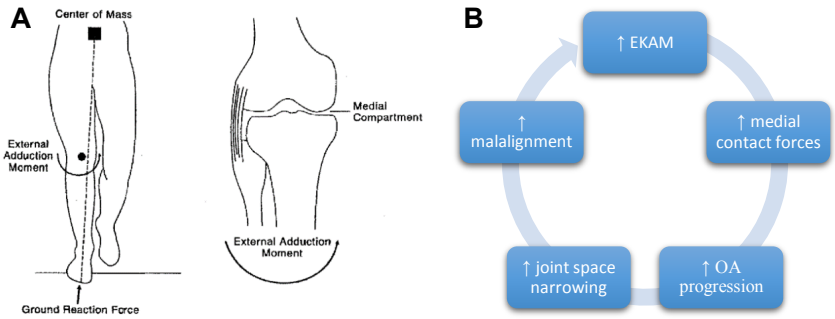

Figure 19: EKAM during gait. (A) The GRF passes medially to the centre of the knee, causing a frontal plane moment. This acts anticlockwise at the tibia, potentially causing increased contract forces. (B) Cause and effect relationship between EKAM, OA and knee malalignment (Biggs, 2016)

Chapter 3:

Effects of HTO on subchondral bone

3.1 INTRODUCTION

Subchondral bone is a biologically active tissue that is responsive to changes in load (Skerry, 2008; McBride and Silva, 2012). Pathological load related changes such as bone marrow lesions and subchondral sclerosis occur in OA (Poulet, 2015; Donell, 2019) and are related to joint structure, joint loading, pain and function (Felson *et al.*, 2001, 2003; Yusuf *et al.*, 2011; Alliston *et al.*, 2018). There also appears to be evidence that these OA features in the subchondral bone are altered by HTO (Akamatsu *et al.*, 1997; Takahashi, Tomihisa and Saito, 2003; Kroner *et al.*, 2007; Gersing *et al.*, 2018; Kim *et al.*, 2019). Therefore, this tissue represents a good target within which to analyse the biological effects of HTO.

Although much is known about how bone cells, in particular osteocytes, respond to mechanical load (Klein-Nulend *et al.*, 2013; Hemmatian *et al.*, 2017), less is known about mechanically induced bone changes in OA. However glutamate appears to be a key mediator in the OA pathway. Glutamate is released by both osteoblasts and osteoclasts (Skerry, 2008) and greater synovial fluid concentrations of glutamate are found in animal and human models of OA, showing correlation with concentrations of OA related cytokines (Lawand, McNearney and Westlund, 2000; Jean *et al.*, 2005; Bonnet *et al.*, 2015, 2020). Glutamate receptors have been identified in pathways regulating pain, cytokine/inflammatory mediator release and cell proliferation (Mason, 2004b; Parada-Turska *et al.*, 2006; Flood *et al.*, 2007; Miller *et al.*, 2011; Lindblad *et al.*, 2012; Bonnet *et al.*, 2015, 2020) and within the bone, the glutamate pathway is responsive to load (Mason *et al.*, 1997; Brakspear and Mason, 2012). Therefore four components of the glutamate pathway were selected for analysis; two glutamate transporters (EAAT1 and EAAT3) and subunits of two glutamate receptors (NR2D, a subunit of an NMDA receptor and GRIK4, a subunit of a kainate receptor) previously reported to be expressed and functional in human osteoblasts/cytes/clasts

(Mason *et al.*, 1997; Huggett *et al.*, 2002; Mason, 2004b; Brakspear and Mason, 2012; Bonnet *et al.*, 2015).

In addition to the markers of glutamate pathway activity, two further markers were chosen; SOST to look at bone turnover (due to the previously discussed role of mechanoreceptive osteocytes in OA) and IL-6 to look for evidence inflammation.

3.2 AIM

To address Hypothesis 1 (see Introduction) that:
"Medial opening wedge HTO causes measurable changes in molecular markers of osteoarthritis in subchondral bone."
This chapter therefore aims to identify if any of the chosen biological markers show significant changes in expression across the tibial surface following HTO.

The **null hypothesis** is that change in mechanical loading via HTO has no effect on the mRNA expression of EAAT1 and 3, NR2D, GRIK4, SOST and IL-6 within individual patients across four joint quadrants.

3.3 METHODOLOGY

Patient recruitment, surgical procedure, sample acquisition, transport, and storage RNA extraction, RT-QPCR and bone core analysis were performed as described in Chapter 2. Approval for this work was granted by the Wales Research Ethics Committee 3 (10/MRE09/28) and Cardiff and Vale University Health Board and informed consent was collected from each participant.

Cohort 1: The initial cohort of patients were analysed in Autumn 2018 and included 21 patients who had bone cores taken and analysed for both the pre-operative and post-operative time points. A complete set of eight results (four quadrants pre-operatively plus four quadrants post-operatively) was not obtained for all patients and all markers (e.g. due to insufficient samples etc.). Therefore the participant number (n) differs for the analysis of each marker but is clearly stated for each.

Cohort 2: By Summer 2019, data for a further 5 patients were available. This second cohort was added to the first (Cohort 1+2 n=26) and the analysis repeated.

3.3.1 Statistical analysis

Data analysis was undertaken using Excel (Microsoft® Excel for Mac, Version 16.45; Redmond, WA, USA) and SPSS (IBM SPSS Statistics for Macintosh, Version 26.0; Armonk, NY, USA).

The analysis was undertaken to look for change in proportional expression of a quadrant from pre- to post-HTO. This was specified after work by Mrs

Carole Elford identified that absolute RNA copy numbers for samples appeared to be inconsistent when the RT-PCR was repeated for the same sample, even if the repeats were performed in parallel. The results of these tests are shown in Figure 20 and Table 2.

The ratio calculations show that within each run, the results are relatively consistent but across the repeats (even when performed in parallel) there is a pronounced difference; with counts in the first repeat being approximately 40% lower than original but approximately 40% higher in the second repeat. Interestingly there appears to be a higher percentage error when different PCRs were performed on cDNA derived from the same RT then when different RT-PCRs were performed on the same sample. Further work is planned to assess the extent of this discrepancy and the point in the process which causes it. However, it appears inherent to the method and means that comparing a patient's pre-HTO to post-HTO RNA levels in terms of absolute count number appears to be invalid. What does appear to stay constant in repeated tests however is the ratio of mRNA expression between different quadrants (i.e. if 50% of all the NR2D RNA for example was found in the posteromedial compartment on one test, this proportion will remain at approximately 50% when retested, even though the absolute copy number may differ).

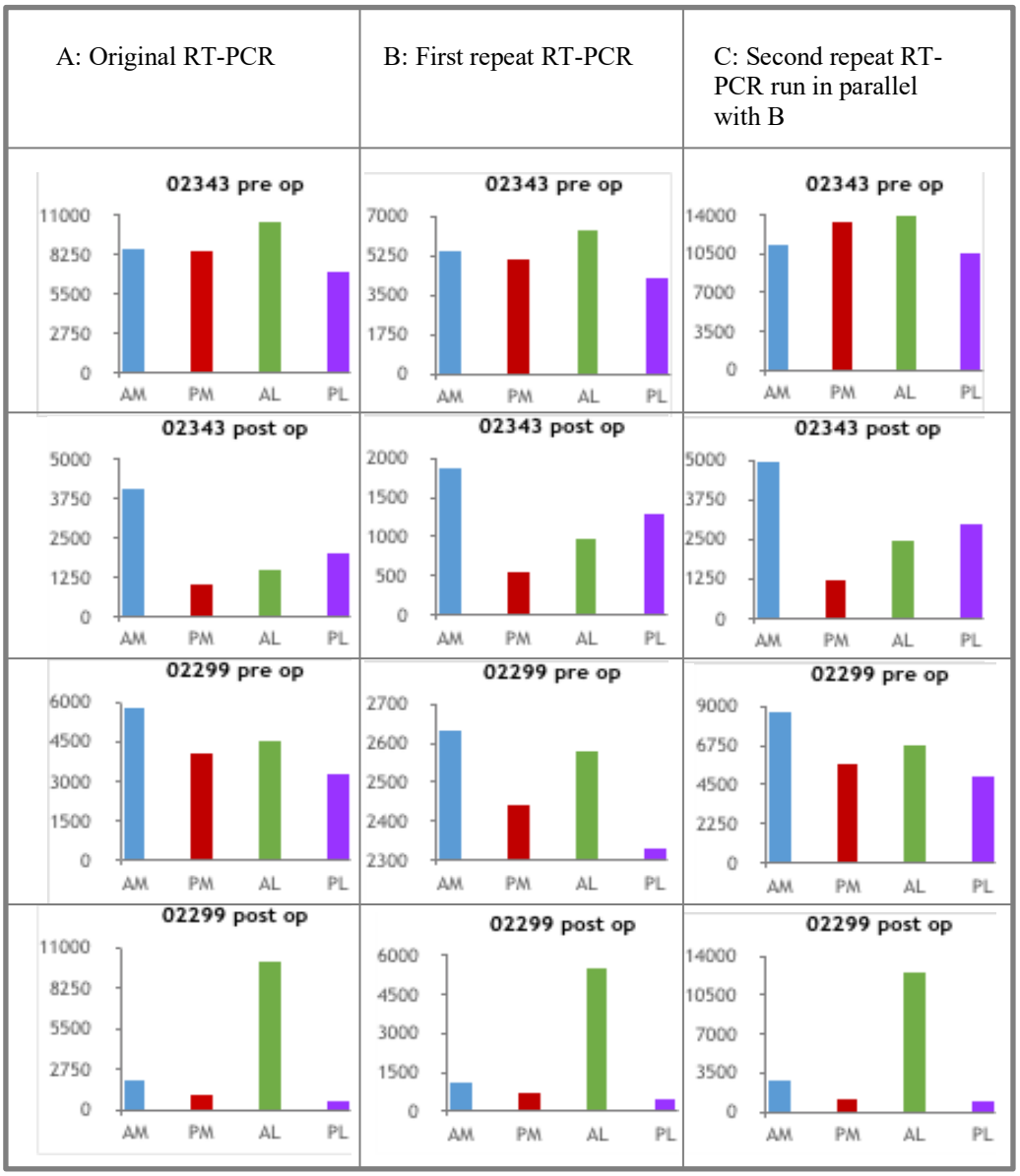

Figure 20: Results of repeated RT-PCR for YWHZ RNA (housekeeping gene) for pre and post op samples of patients 02343 and 02299. Column A shows the initial results. Columns B and C show two repeated RT-PCR for the same samples which were run in parallel with each other. (Results courtesy of Ms Carole Elford)

Sample	1st repeat/original		2nd repeat/original		2nd repeat/1st repeat	
	Ratio	% Error	Ratio	% Error	Ratio	% Error
02343_pre_AM	0.63	-37.3%	1.30	30.5%	2.08	108.1%
02343_pre_PM	0.61	-39.3%	1.58	58.0%	2.60	160.5%
02343_pre_AL	0.61	-39.2%	1.31	31.4%	2.16	116.3%
02343_pre_PL	0.60	-39.9%	1.49	49.3%	2.48	148.3%
02343_post_AM	0.46	-54.3%	1.21	21.0%	2.65	164.7%
02343_post_PM	0.54	-45.6%	1.21	20.9%	2.22	122.5%
02343_post_AL	0.67	-33.2%	1.69	69.1%	2.53	153.0%
02343_post_PL	0.65	-34.7%	1.50	50.1%	2.30	129.8%
02299_pre_AM	0.46	-54.4%	1.51	51.1%	3.31	231.1%
02299_pre_PM	0.60	-40.2%	1.40	39.5%	2.33	133.2%
02299_pre_AL	0.57	-43.0%	1.48	48.1%	2.60	159.8%
02299_pre_PL	0.71	-28.5%	1.52	51.6%	2.12	112.1%
02299_post_AM	0.53	-46.7%	1.39	39.5%	2.62	161.8%
02299_post_PM	0.64	-36.3%	1.05	4.9%	1.65	64.6%
02299_post_AL	0.55	-45.0%	1.25	25.1%	2.28	127.7%
02299_post_PL	0.83	-17.4%	1.88	88.5%	2.28	128.2%
MEAN	**0.60**	**-39.7%**	**1.42**	**42.4%**	**2.39**	**138.8%**
RANGE	**0.46 - 0.83**		**1.05 - 1.88**		**1.65 - 2.65**	

Table 2: Ratios and percentage error of repeated RT-PCR for YWHZ RNA (housekeeping gene) for pre and post op samples of patients 02343 and 02299.

When these repeated results are analysed using the absolute copy numbers, it can be seen graphically (Figure 21) that the patterns across the quadrants are similar but the scale of the variation across the repeats (as shown by the error calculations in Table 3 and error bars in Figure 22) brings into question the validity of comparing one patient to another, or one patient's pre- and post-operative scores.

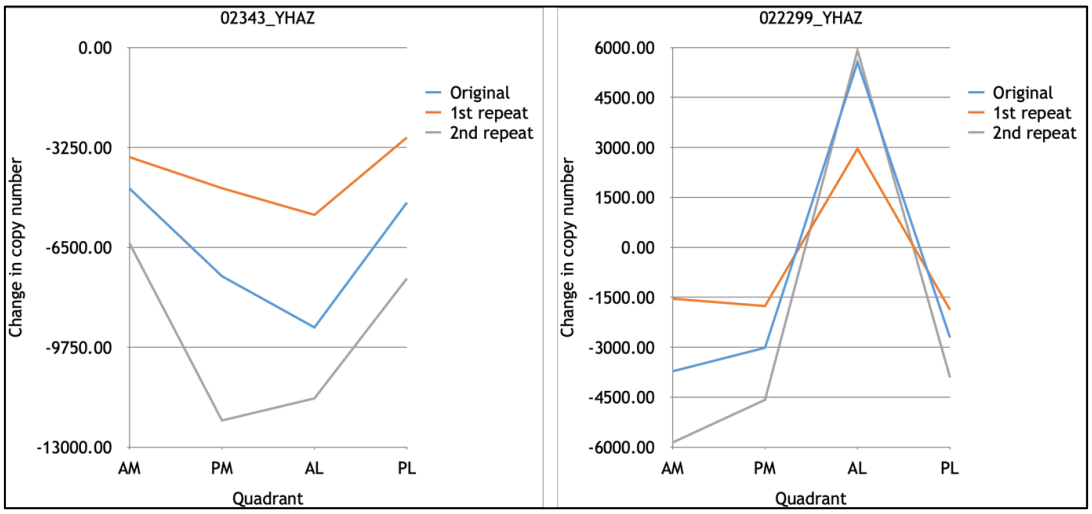

Figure 21: Repeated RT-PCR for YWHZ RNA (housekeeping gene) raw copy number data for patients 02343 and 02299 showing pattern across quadrants of pre to post op difference. Patterns similar but wide variation in absolute figures.

	A: Original	B: 1st repeat	C: 2nd repeat	Mean	Standard Error	Standard Dev.	95% Confidence Interval	
02343_pre_AM	8654	5426	11290	8457	1696	2937	1161	15753
02343_pre_PM	8461	5133	13370	8988	2392	4144	-1306	19282
02343_pre_AL	10570	6423	13890	10294	2160	3741	1001	19588
02343_pre_PL	7033	4228	10500	7254	1814	3142	-551	15058
02343_post_AM	4067	1860	4923	3617	912	1580	-309	7543
02343_post_PM	1022	556	1236	938	201	348	74	1802
02343_post_AL	1463	977.9	2474	1638	441	763	-258	3534
02343_post_PL	1990	1300	2987	2092	490	848	-15	4199
02299_pre_AM	5763	2630	8707	5700	1755	3039	-1849	13249
02299_pre_PM	4085	2444	5699	4076	940	1628	33	8119
02299_pre_AL	4525	2579	6700	4601	1190	2062	-520	9723
02299_pre_PL	3259	2330	4941	3510	764	1323	222	6798
02299_post_AM	2038	1086	2843	1989	508	880	-196	4174
02299_post_PM	1067	680	1119	955	138	240	359	1551
02299_post_AL	10080	5539	12610	9410	2069	3583	509	18310
02299_post_PL	548	452	1032	677	179	311	-95	1450

Table 3: Error calculations and 95% confidence intervals for raw copy number data. Repeated RT-PCR for YWHZ RNA (housekeeping gene) for pre and post op samples of patients 02343 and 02299.

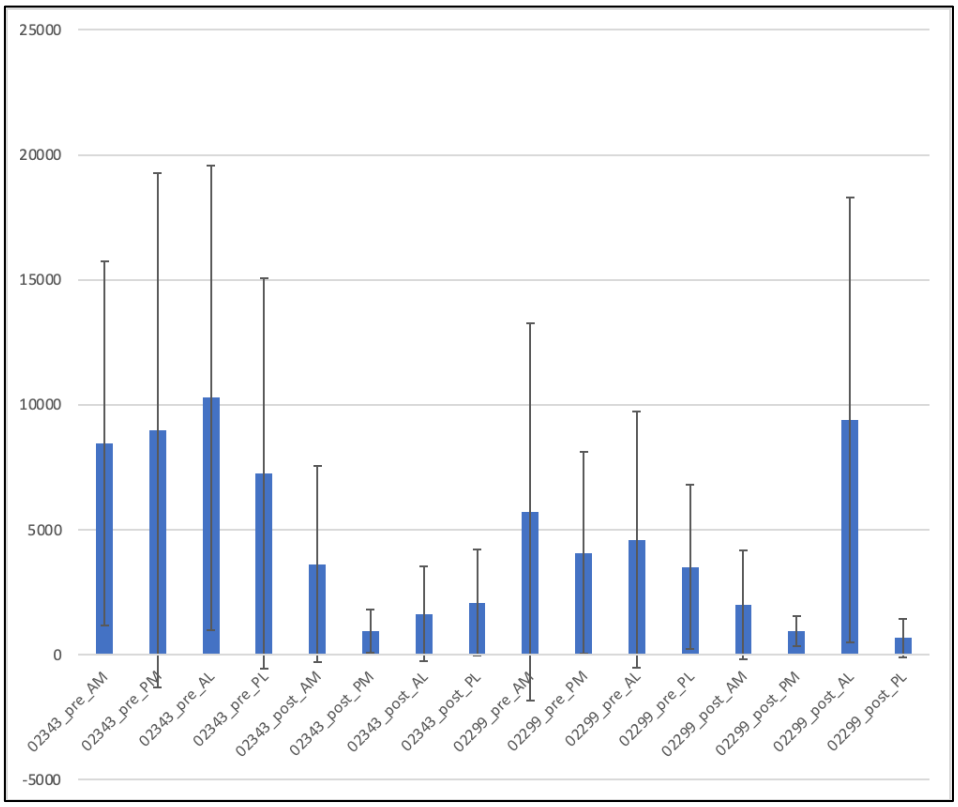

Figure 22: Mean copy number with 95% confidence intervals for raw copy number data. Repeated RT-PCR for YWHZ RNA (housekeeping gene) for pre and post op samples of patients 02343 and 02299.

However, when the same data is analysed to look at percentage change in each quadrant compared to the total across the tibial plateau (percentage change in expression), the three repeated data sets show almost identical patterns (figure 23) and much reduced error values (table 4 and figure 24).

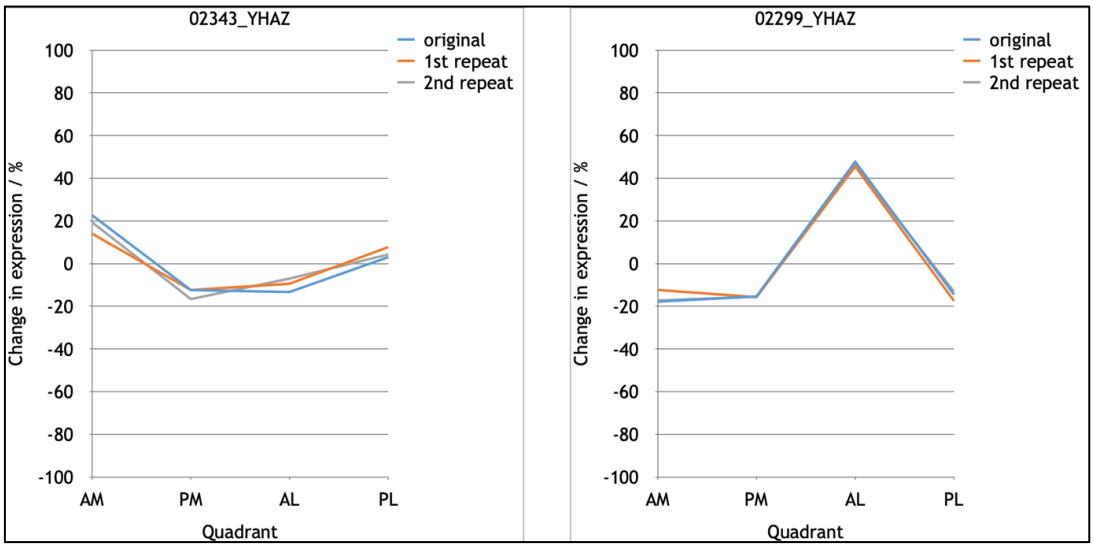

Figure 23: Repeated RT-PCR for YWHZ RNA (housekeeping gene) percentage change for patients 02343 and 02299 showing pattern across quadrants of pre to post op difference.

	A: Original	B: 1st repeat	C: 2nd repeat	Mean	Standard Error	Standard Dev.	95% Confidence Interval	
02343_AM	**22.69**	14.05	19.35	18.70	2.52	4.36	7.87	23.05
02343_PM	**-12.41**	-12.36	-16.62	-13.80	1.41	2.45	-19.87	-11.35
02343_AL	**-13.32**	-9.45	-7.03	-9.93	1.83	3.17	-17.81	-6.76
02343_PL	**3.04**	7.76	4.3	5.03	1.41	2.44	-1.04	7.48
02299_AM	-17.84	-12.34	-17.28	-15.82	1.75	3.03	-23.34	-12.79
02299_PM	-15.4	-15.72	-15.52	-15.55	0.09	0.16	-15.95	-15.39
02299_AL	47.74	45.57	45.91	46.41	0.67	1.17	43.51	47.57
02299_PL	-14.5	-17.51	-13.11	-15.04	1.30	2.25	-20.63	-12.79

Table 4: Error calculations and 95% confidence intervals for percentage change in expression. Repeated RT-PCR for YWHZ RNA (housekeeping gene) for pre and post op samples of patients 02343 and 02299.

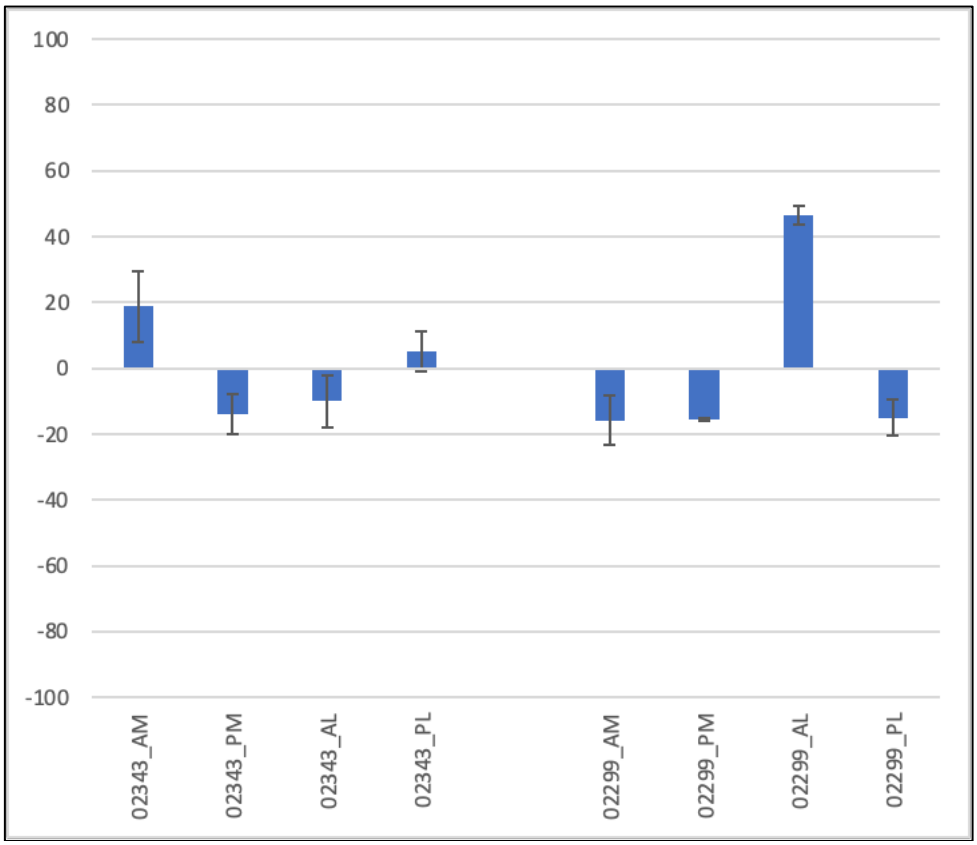

Figure 24: Mean percentage change with 95% confidence intervals. Repeated RT-PCR for YWHZ RNA (housekeeping gene) for pre and post op samples of patients 02343 and 02299.

Having decided therefore to calculate proportional changes in expression across the tibial plateau surface, the mRNA copy numbers for each quadrant, normalised to reference genes YWHAZ and HPRT1 (see Methodology), were analysed as a percentage of the total RNA expression of the tibial surface (i.e. RNA expression in posteromedial quadrant divided by the sum of RNA expression in all four quadrants). The difference between the pre-HTO time point and the post-HTO time point (samples taken at plate removal, approximately 12 months following HTO) were then calculated. A positive value suggests more relative expression in that quadrant (as a proportion of total joint surface expression) following HTO (i.e. upregulation).

The data was first analysed via box plots to identify any extreme outlying results (judged as those greater than three times the interquartile range (IQR) about the third quartile or greater than three times the IQR below the first quartile). The raw data of any extreme outliers was then examined to check for any discrepancies that may require exclusion (e.g. large proportion of “no count” readings in calculation).

The data was then checked for normal distribution using the Shapiro-Wilk test ($p>0.05$ representing sufficiently normal distribution). If these assumptions were met, the paired Student T-test was used to judge the significance of any difference (significance level $p<0.05$). If the data was not normally distributed, it was analysed using a non-parametric test, the Wilcoxon signed-rank test (significance level $p<0.05$) as data transformation using either a square root or logarithmic method was prohibited by the presence of negative or zero results.

A Chi-square test was also employed to examine if there is any relationship between quadrant and up/down regulation of mRNA expression of the target genes post-HTO. If the sample data was insufficient for a Chi-square test, Fisher’s exact test was used instead.

Any significant results were examined via Spearman’s rank to see if they correlated with each other (i.e. were occurring in the same patients).

Following addition of Cohort 2, the two cohorts were also compared against each other using an independent sample T-test to assess if there were any significant differences between the two cohorts which might explain differing mRNA expression results.

Further analysis of correlations between this biological data and further mechanical/patient data will be subsequently explored in Chapter 5.

3.4 RESULTS

3.4.1 Initial analysis of Cohort 1 (n=21)

The first cohort included 21 knees within 20 patients (one patient (02299) had bilateral sequential osteotomies). 20 knees were male, 1 was female. Mean age at time of surgery of 49.6 years (range 33 – 61).

3.4.1.1 EAAT1, Cohort 1 (n=20)

The graphs in figure 25 show change in expression of EAAT1 between pre- and post-HTO for each of the four quadrants. A positive value suggests more expression in that quadrant (as a proportion of total joint surface expression) following HTO (i.e. proportional upregulation).

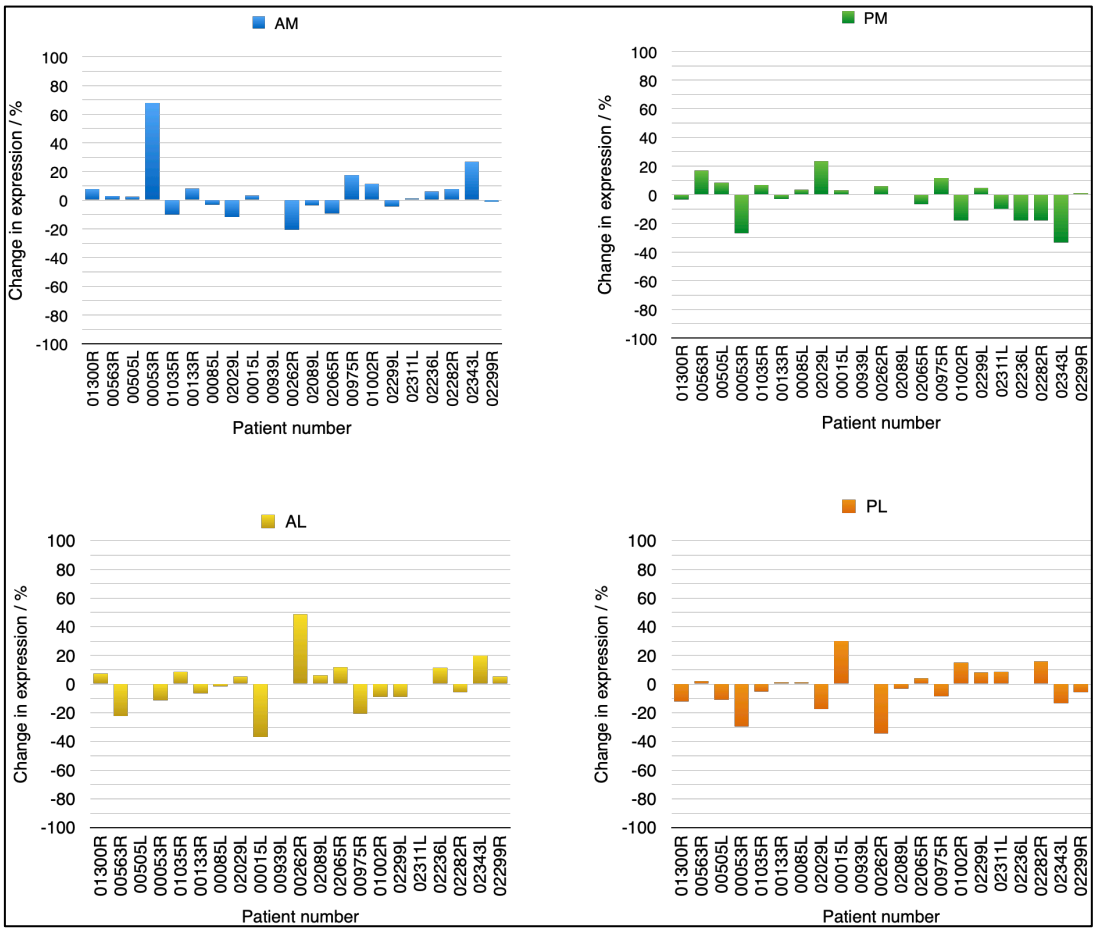

Figure 25: Change in proportional mRNA expression of EAAT1 (Cohort 1) pre- to post-HTO across four quadrants of the tibial plateau.

	AM	PM	AL	PL
Increased expression	11	11	10	10
Decreased expression	8	9	10	10
Exclusions	1 (00053R)	0	0	0
Shapiro-Wilk's p value	0.976	0.694	0.269	0.938
Mean / %	5.02	-2.50	0.14	-2.65
Range / %	-20.59 - 26.86	-33.18 - 23.54	-36.76 - 48.74	-34.21 - 30.16
Standard Error / %	4.08	3.25	3.94	3.38
T-test p value	0.234	0.450	0.973	0.443
Wilcoxon signed-rank p value	N/A	N/A	N/A	N/A

Table 5: Summarised statistical analysis of change in proportional mRNA expression of EAAT1 (Cohort 1) pre- to post-HTO across four quadrants of the tibial plateau.

Across the 20 knees, EAAT1 mRNA expression was increased medially in slightly more patients (11/20 for both AM and PM), whereas equal numbers of patients showed increased/decreased EAAT1 MRNA expression in either of the lateral quadrants. Fisher's exact test did not reveal any statistically significant differences in up/down regulation of EAAT1 mRNA expression based on quadrant (p=0.959).

One patient (00053R) was excluded (having been identified as an outlier) as analysis of the original data revealed multiple "no count" readings across the quadrants resulting in poor data quality for the analysis.

Across all quadrants, changes in mean EAAT1 mRNA expression were very small and not statistically significant (increased by 5.02% (+/- 4.08%) in AM (p=0.234), decreased by 2.50% (+/-3.25%) in PM (p=0.450), increased by 0.14% (+/- 3.94%) in AL (p=0.973), decreased by 2.65% (+/- 3.38%) in PL (p=0.443)).

Analysis of the pattern of change across quadrants (Figure 26) showed a variety of different patterns across the samples including several with minimal change (Pattern 3).

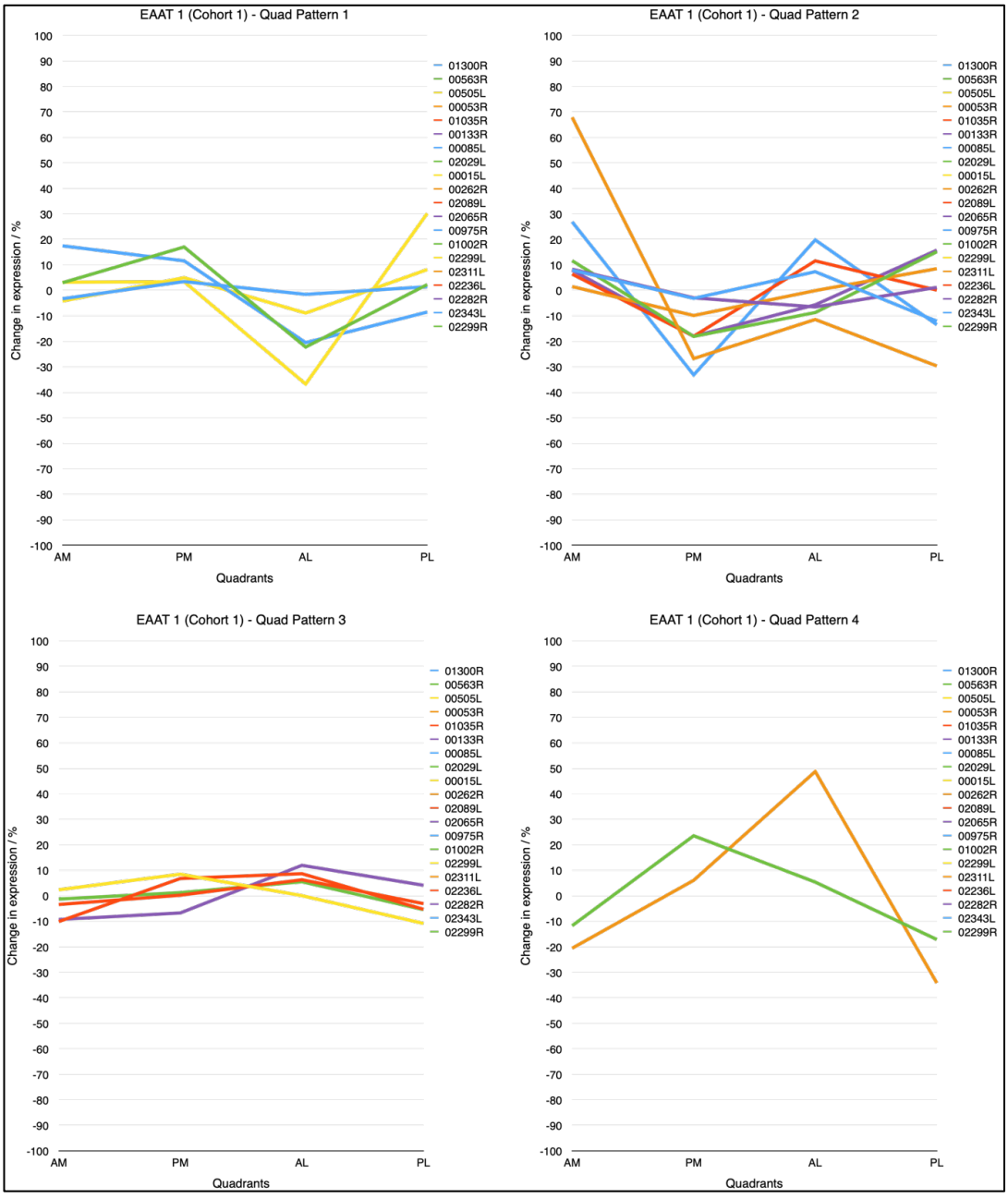

Figure 26: Patterns of change in proportional mRNA expression of EAAT1 across quadrants of the tibial plateau (Cohort 1) pre- to post-HTO.

3.4.1.2 EAAT3, Cohort 1 (n=19)

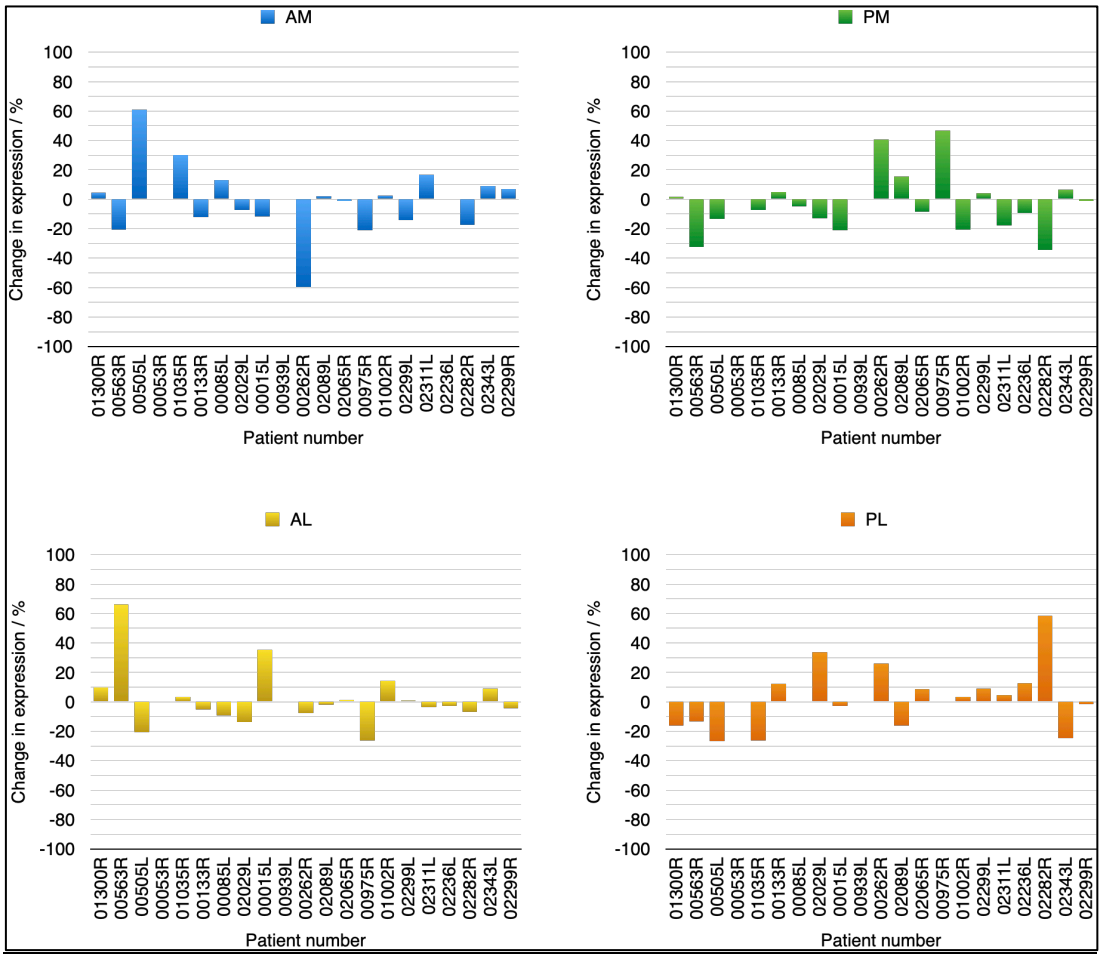

Figure 27: Change in proportional mRNA expression of EAAT3 (Cohort 1) pre- to post-HTO across four quadrants of the tibial plateau.

	AM	PM	AL	PL
Increased expression	9	7	8	11
Decreased expression	10	12	11	8
Exclusions	0	0	0	0
Shapiro-Wilk's p value	0.183	0.110	0.003	0.186
Mean / %	-0.98	-3.29	[Median -0.04]	2.24
Range / %	-59.34 - 60.78	-34.47 - 46.65	-26.43 - 66.07	-26.80 - 58.61
Standard Error / %	5.52	4.82	N/A	4.95
T-test p value	0.861	0.503	N/A	0.656
Wilcoxon signed-rank p value	N/A	N/A	0.687	N/A

Table 6: Summarised statistical analysis of change in proportional mRNA expression of EAAT3 (Cohort 1) pre- to post-HTO across four quadrants of the tibial plateau.

Across the 19 knees, EAAT3 mRNA expression decreased in slightly more patients in all quadrants except PL (10/19 for AM, 12/19 for PM, 11/19 for AL but 8/19 for PL). A Chi-square test did not reveal any statistically significant differences in up/down regulation of EAAT3 mRNA expression based on quadrant (p=0.603).

Across all quadrants, changes in mean EAAT3 mRNA expression were very small and not statistically significant (decreased by 0.98% (+/- 5.52%) in AM (p=0.861), decreased by 3.29% (+/-4.82%) in PM (p=0.503), increased by 2.24% (+/- 4.95%) in PL (p=0.656)). Data for the AL quadrant was not normally distributed (as assessed by the Shapiro-Wilk's test (p=0.003)) so the Wilcoxon signed-rank test was used. This showed a very small median decrease (-0.04%) but this was also not significant (p=0.687).

Again, a variety of different patterns of expression were seen (Figure 28).

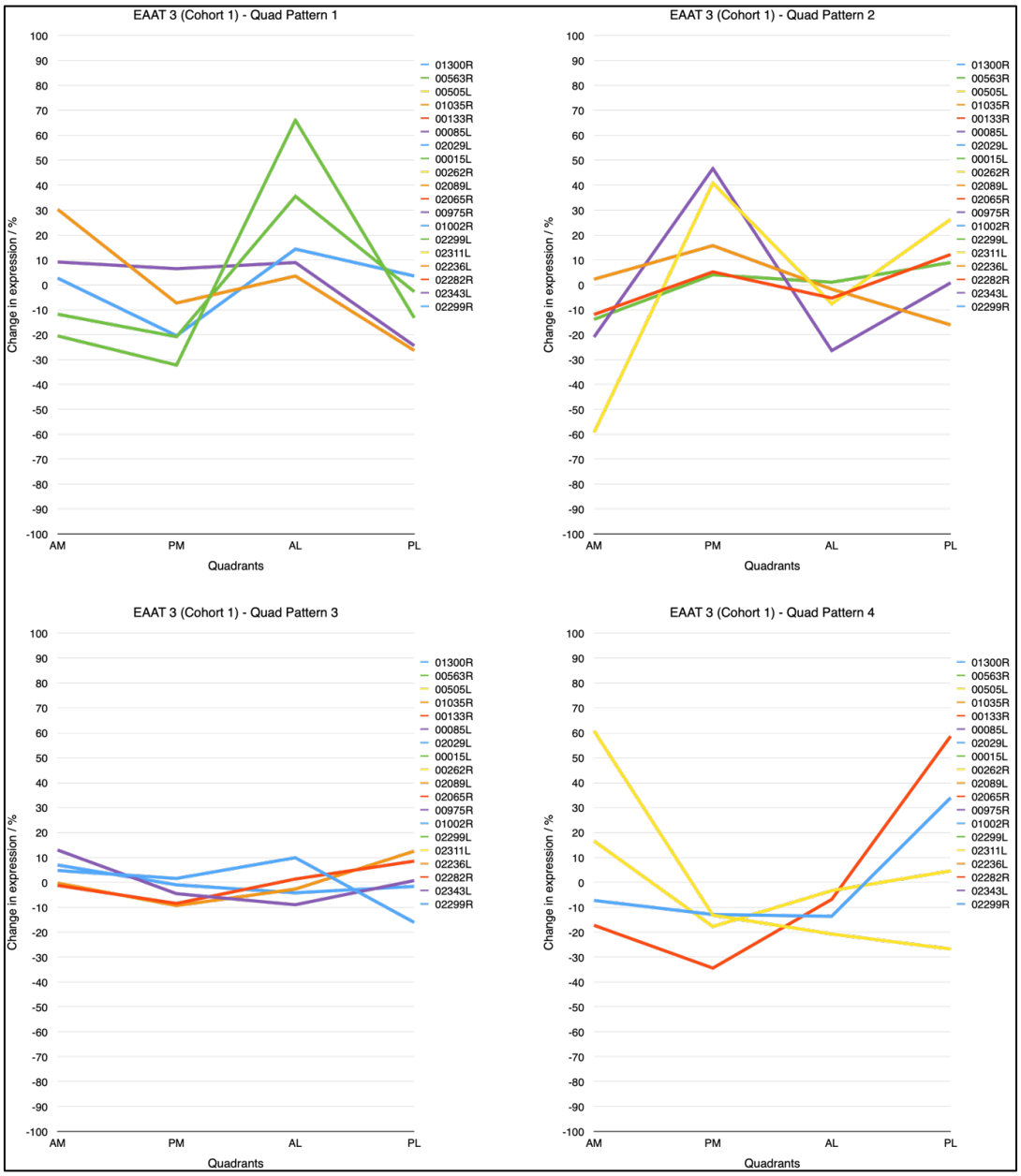

Figure 28: Patterns of change in proportional mRNA expression of EAAT3 across quadrants of the tibial plateau (Cohort 1) pre- to post-HTO.

3.4.1.3 NR2D, Cohort 1 (n=18)

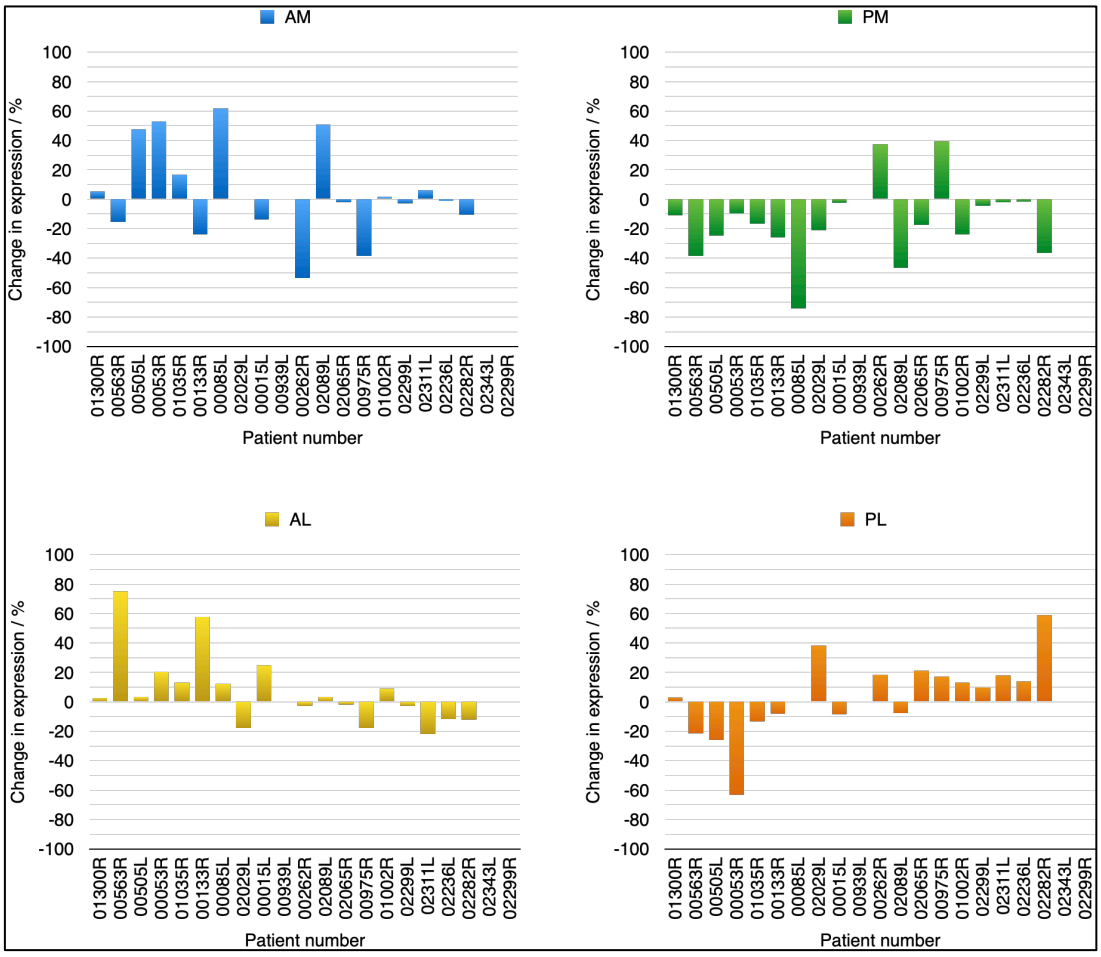

Figure 29: Change in proportional mRNA expression of NR2D (Cohort 1) pre- to post-HTO across four quadrants of the tibial plateau.

	AM	PM	AL	PL
Increased expression	9	**2**	8	11
Decreased expression	9	**16**	10	7
Exclusions	0	**0**	0	0
Shapiro-Wilk's p value	0.224	**0.276**	0.010	0.548
Mean / %	4.55	**-15.51**	-3.05	3.55
Range / %	-53.44 - 61.77	**-46.69 - 39.37**	-21.67 - 57.87	-63.17 - 59.1
Standard Error / %	7.42	**6.33**	5 N/A	6.29
T-test p value	0.548	**0.025**	N/A	0.580
Wilcoxon signed-rank p value	N/A	N/A	0.349	N/A

Table 7: Summarised statistical analysis of change in proportional mRNA expression of NR2D (Cohort 1) pre- to post-HTO across four quadrants of the tibial plateau. (Statistically significant results shown in bold)

Across the 18 knees, NR2D mRNA expression in the PM quadrant decreased in the majority of patients (16/18) and **the change in mean expression for this quadrant was statistically significant (decreased by 15.5% +/- 6.33% (p=0.025)).**

Across the other quadrants, the changes in mean NR2D mRNA expression were small and not statistically significant (increased by 4.55% (+/- 7.42%) in AM (p=0.548), increased by 3.55% (+/- 6.29%) in PL (p=0.580)) with equal numbers increasing/decreasing for AM, a small majority (10/18) decreasing for AL and a small majority (11/18) increasing for PL. Data for the AL quadrant was not normally distributed (as assessed by the Shapiro-Wilk's test (p=0.010)) so the Wilcoxon signed-rank test was used. This showed a small median decrease (-3.05%) but this was also not significant (p=0.349).

A Chi-square test did reveal a **statistically significant difference in up/down regulation of NR2D mRNA expression based on quadrant (p=0.010).** Post hoc analysis involved pairwise comparisons using the z-test of two proportions with a Bonferroni correction. This revealed **significant differences between the PM quadrant and both of the lateral quadrants** (AL or PL) but not with the other medial quadrant (AM). The lateral quadrants (AL and PL) were not significantly different from each other.

There was less variability noted in the patterns of change across the quadrants with the majority showing downregulation in PM and corresponding upregulation in AM and AL (Figure 30).

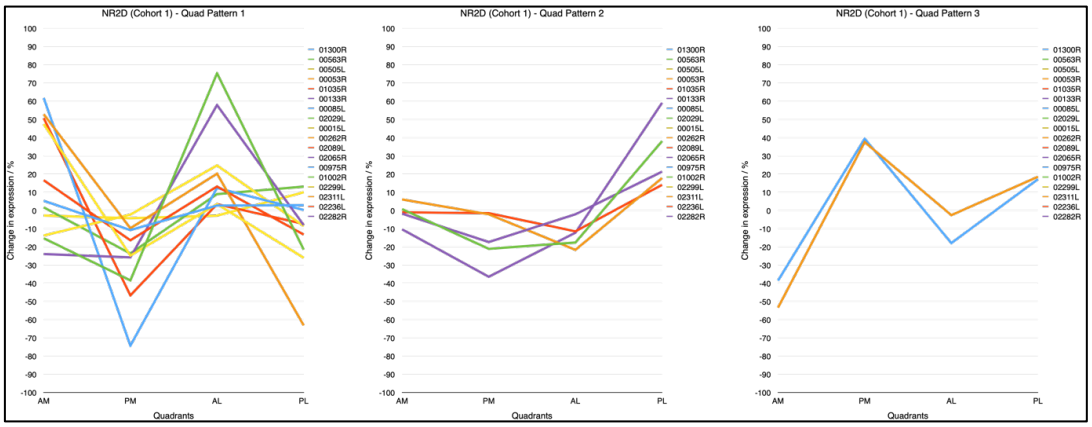

Figure 30: Patterns of change in proportional mRNA expression of NR2D across quadrants of the tibial plateau (Cohort 1) pre- to post-HTO.

3.4.1.4 GRIK4, Cohort 1 (n=17)

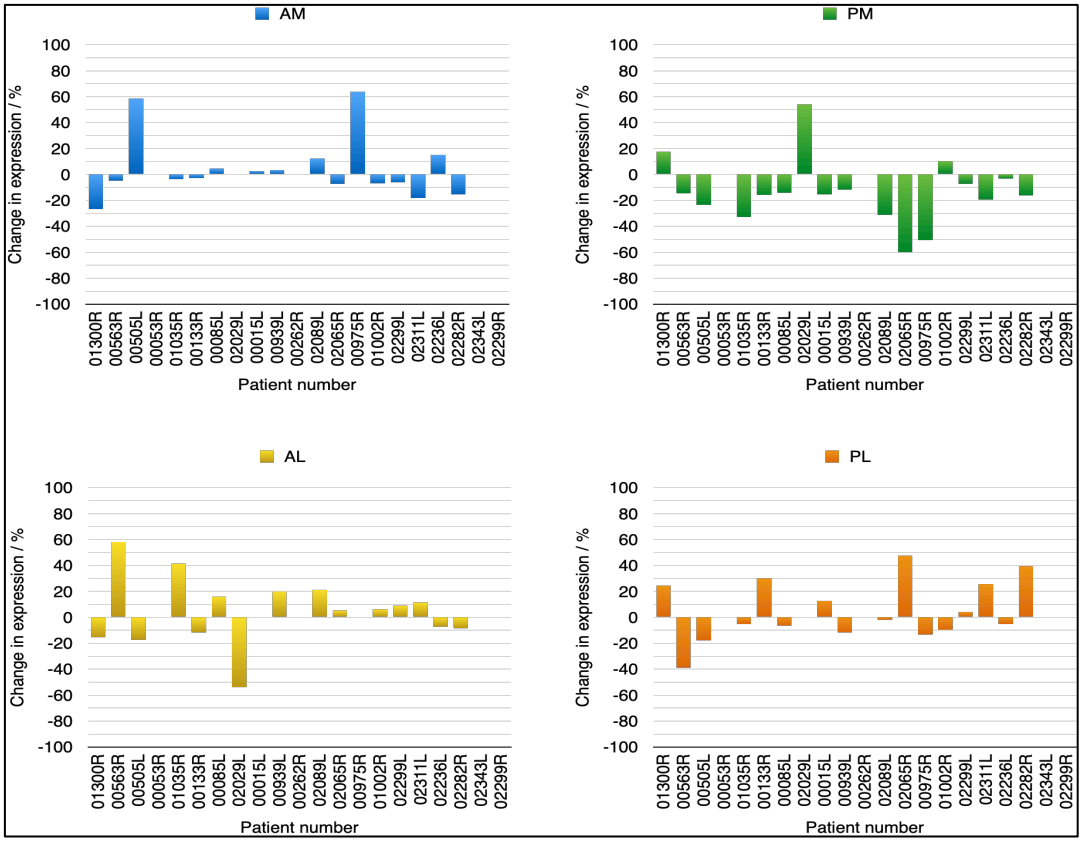

Figure 31: Change in proportional mRNA expression of GRIK4 (Cohort 1) pre- to post-HTO across four quadrants of the tibial plateau.

	AM	PM	AL	PL
Increased expression	8	**2**	10	7
Decreased expression	8	**14**	6	9
Exclusions	1 (00975R)	**1 (02029L)**	0	0
Shapiro-Wilk's p value	0.015	**0.166**	0.725	0.540
Mean / %	[Median -2.93]	**-17.91**	4.68	4.65
Range / %	-26.66 - 58.55	**-59.86 - 17.59**	-53.94 - 58.1	-38.82 - 47.55
Standard Error / %	N/A	**4.89**	6.02	5.49
T-test p value	N/A	**0.002**	0.476	0.439
Wilcoxon signed-rank p value	0.776	N/A	N/A	N/A

Table 8: Summarised statistical analysis of change in proportional mRNA expression of GRIK4 (Cohort 1) pre- to post-HTO across four quadrants of the tibial plateau. (Statistically significant results shown in bold)

GRIK4 mRNA expression in the PM quadrant decreased in the majority of patients (14/17) and **the change in mean expression for this quadrant was statistically significant (decreased by 17.9% +/- 4.89% (p=0.002)).**

In the lateral compartments, the changes in mean GRIK4 mRNA expression were small and not statistically significant (increased by 4.68% (+/- 6.02%) in AL (p=0.476), increased by 0.540% (+/- 4.65%) in PL (p=0.439)) with 10/17 increasing for AL and 9/17 decreasing for PL. Equal numbers of patients increased/decreased expression in the AM quadrant but the data was not normally distributed (as assessed by the Shapiro-Wilk's test (p=0.015)) so the Wilcoxon signed-rank test was used. This showed a small median decrease (-2.93 %) but this was also not significant (p=0.776).

Fisher's exact test did not reveal any statistically significant differences in up/down regulation of GRIK4 mRNA expression based on quadrant (p=0.128).

Again there was clear dominance of patterns showing downregulation in PM with corresponding upregulation in AM and AL (Figure 32).

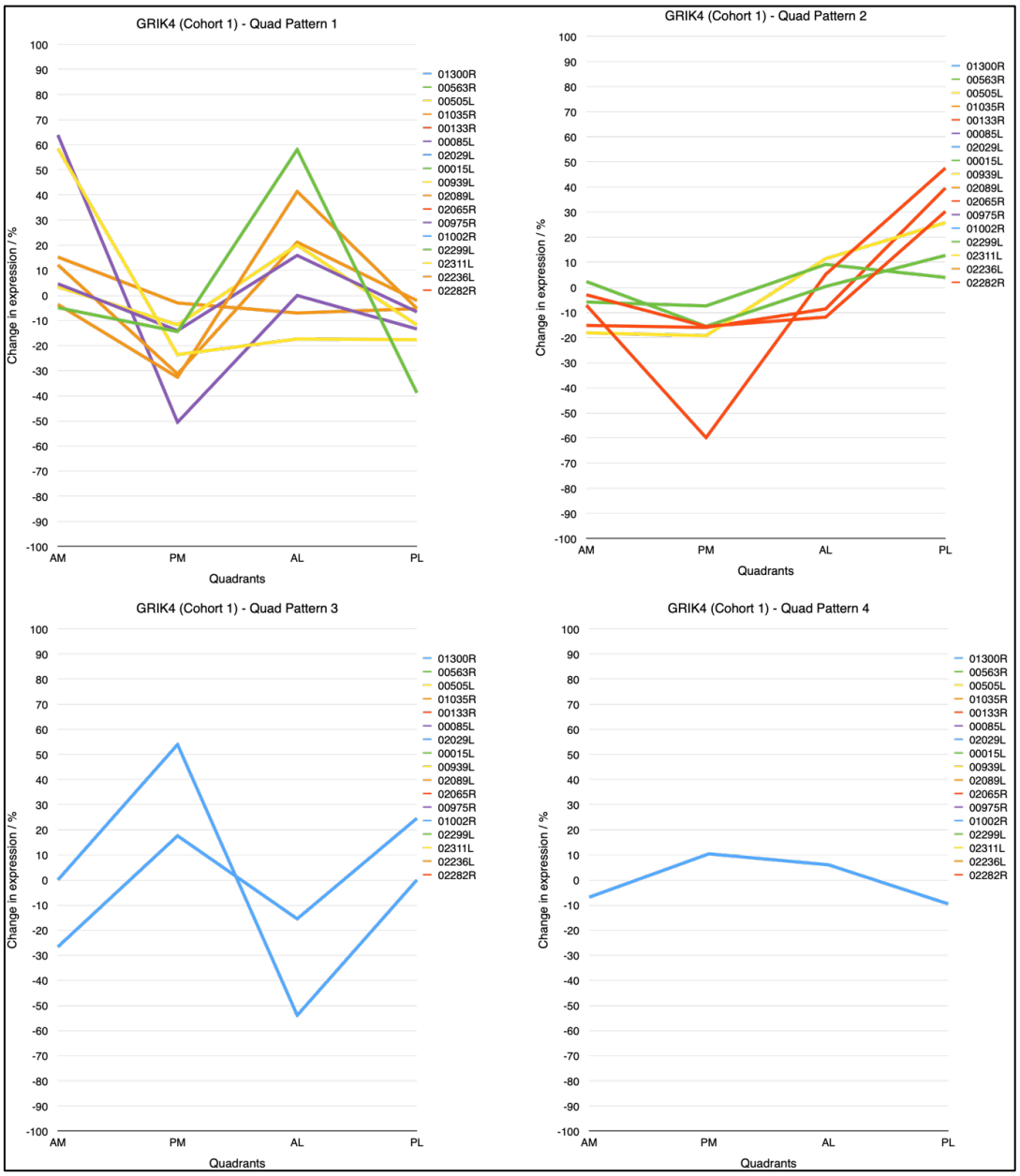

Figure 32: Patterns of change in proportional mRNA expression of GRIK4 across quadrants of the tibial plateau (Cohort 1) pre- to post-HTO.

3.4.1.5 Sclerostin Cohort 1 (n=20)

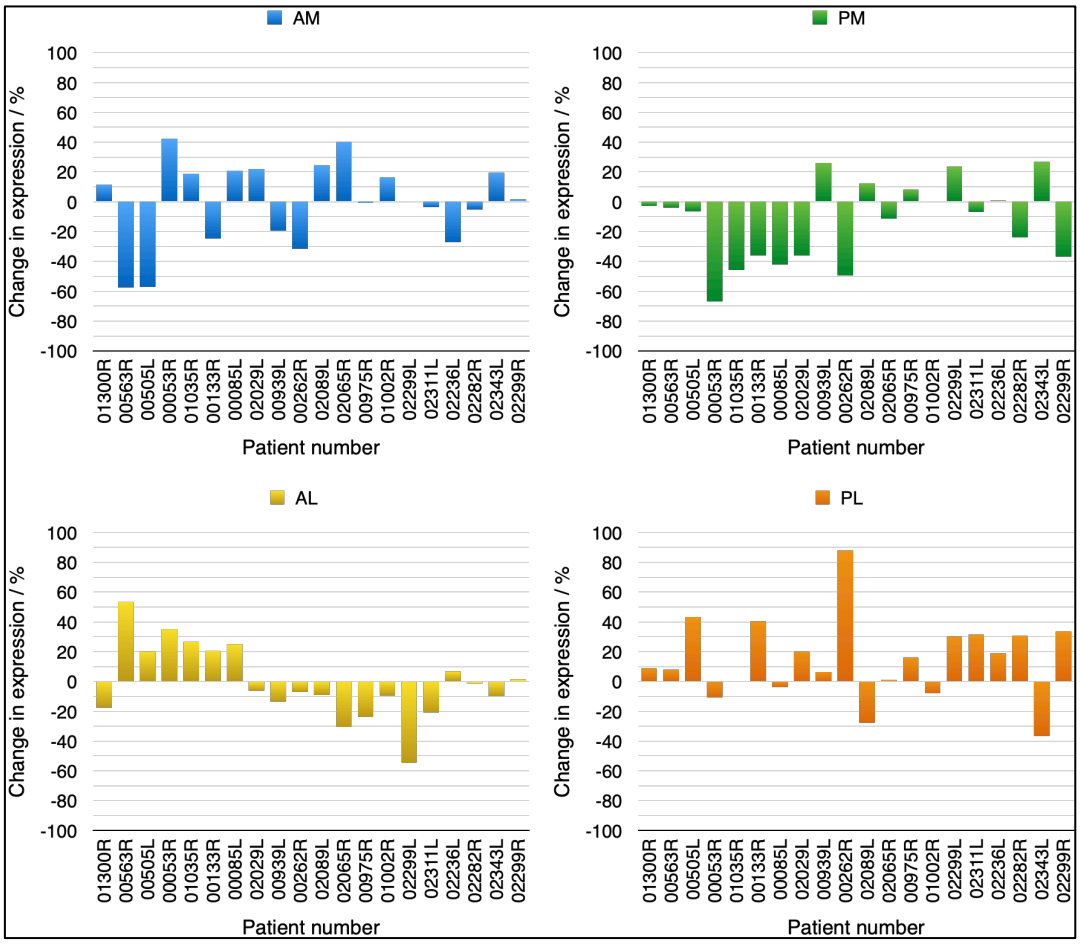

Figure 33: Change in proportional mRNA expression of SOST (Cohort 1) pre- to post-HTO across four quadrants of the tibial plateau.

	AM	PM	AL	PL
Increased expression	11	**7**	8	**14**
Decreased expression	9	**13**	12	**6**
Exclusions	0	**0**	0	**0**
Shapiro-Wilk's p value	0.254	**0.401**	0.868	**0.569**
Mean / %	-0.44	**-13.42**	-0.68	**14.54**
Range / %	-57.34 - 42.26	**-66.81 - 26.92**	-54.68 - 53.39	**-36.70 - 87.89**
Standard Error / %	6.37	**6.09**	5.64	**6.17**
T-test p value	0.946	**0.040**	0.905	**0.029**
Wilcoxon signed-rank p value	N/A	**N/A**	N/A	**N/A**

Table 9: Summarised statistical analysis of change in proportional mRNA expression of SOST (Cohort 1) pre- to post-HTO across four quadrants of the tibial plateau. (Statistically significant results shown in bold)

Statistically significant changes were seen in proportional change in mean SOST mRNA expression in the posterior quadrants post-HTO. The majority of patients (13/20) showed decreased expression posteromedially (decreased by 13.4% +/- 6.09% (p=0.040)) whereas the majority of patients (14/20) showed increased expression posterolaterally (increased by 14.5% +/- 6.17% (p=0.029)).

In the anterior quadrants, the changes in mean SOST mRNA expression were small and not statistically significant (decreased by 0.44% (+/- 6.37%) in AM (p=0.946), decreased by 0.68% (+/- 5.64%) in AL (p=0.905)) with 11/20 increasing for AM and 8/20 increasing for AL.

A Chi-square test did not reveal any statistically significant differences in up/down regulation of SOST mRNA expression based on quadrant (p=0.112).

The pattern of change across the quadrants was very variable but patients did appear to fall into one of the six patterns shown in Figure 34. The most common pattern (Quad Pattern 2) shows proportional down regulation in PM but upregulation in the lateral quadrants, particularly PL.

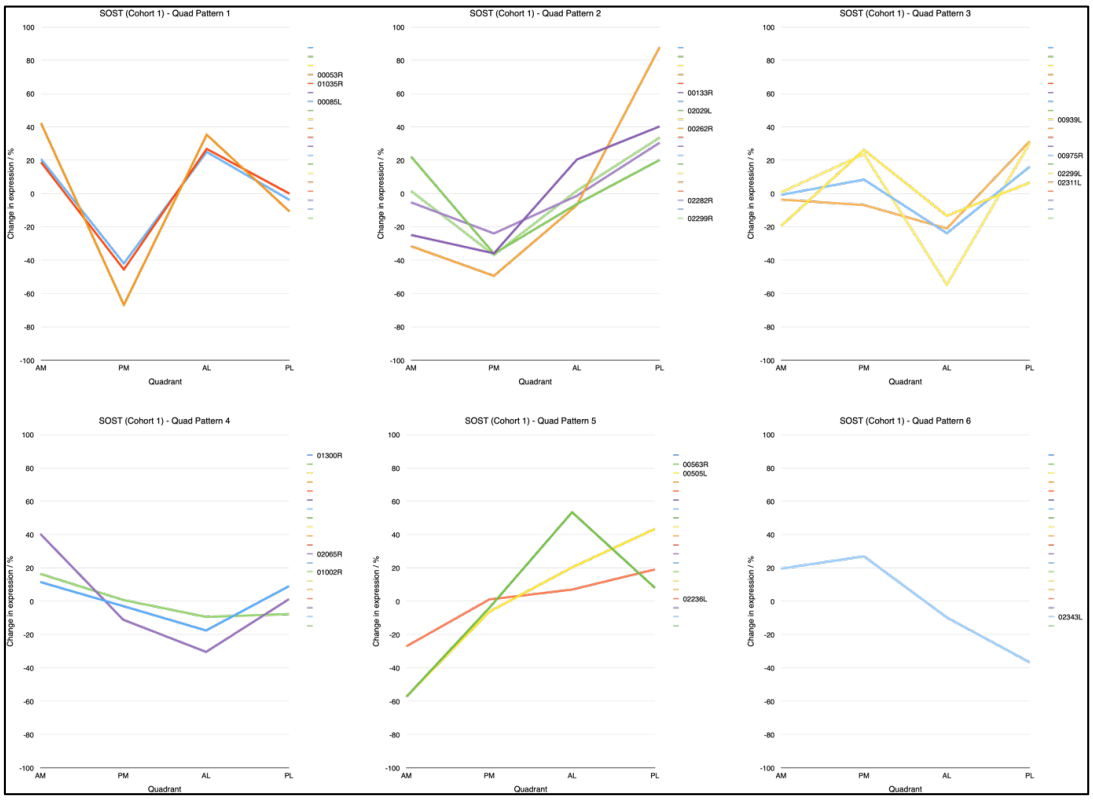

Figure 34: Patterns of change in proportional mRNA expression of SOST across quadrants of the tibial plateau (Cohort 1) pre- to post-HTO.

3.4.1.6 IL-6, Cohort 1 (n=18)

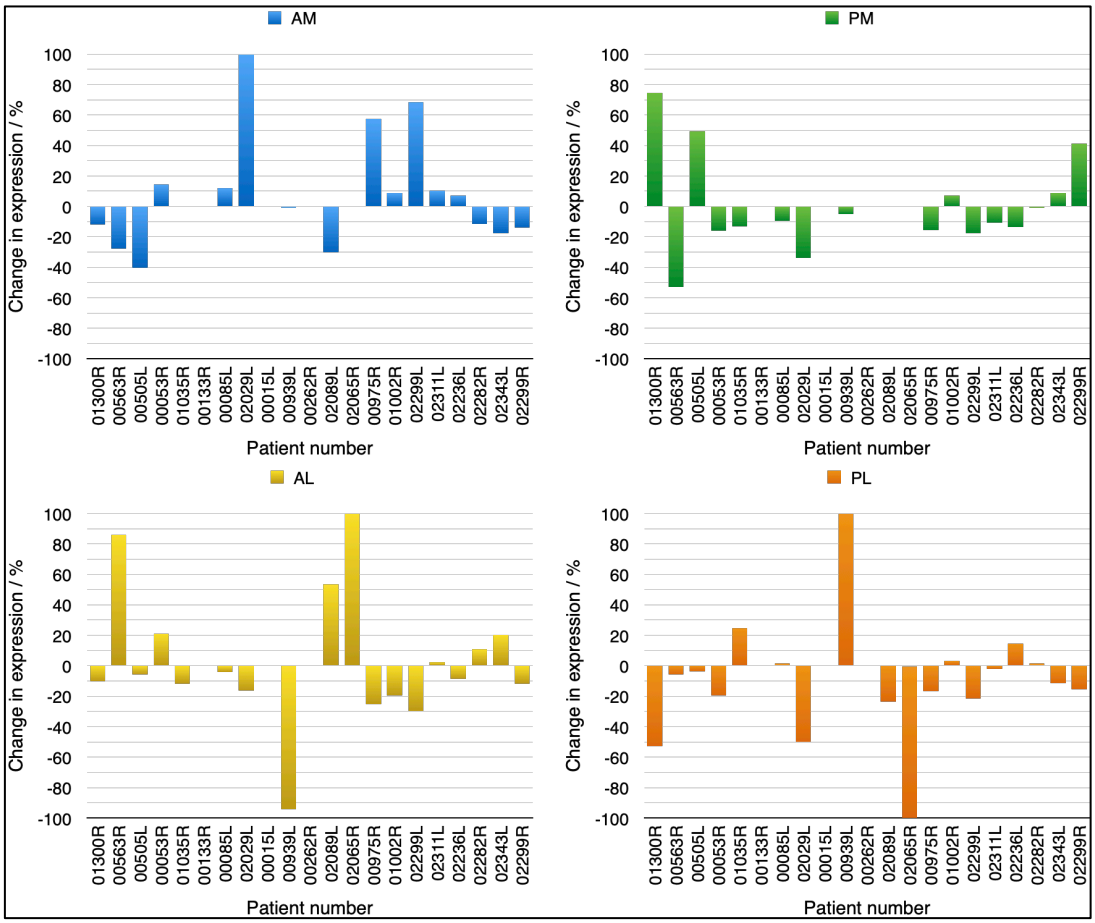

Figure 35: Change in proportional mRNA expression of IL6 (Cohort 1) pre- to post-HTO across four quadrants of the tibial plateau.

	AM	PM	AL	PL
Increased expression	8	5	7	5
Decreased expression	8	10	10	10
Exclusions	1 (02029L)	0	0	2 (00939L, 02065R)
Shapiro-Wilk's p value	0.073	0.022	0.089	0.602
Mean / %	[Median 0.00]	[Median -4.88]	4.38	-8.43
Range / %	-40.31 - 68.34	-52.76 - 74.68	-94.01 - 86.02	-52.59 - 24.57
Standard Error / %	N/A	N/A	10.31	5.10
T-test p value	N/A	N/A	0.692	0.094
Wilcoxon signed-rank p value	0.756	0.460	N/A	N/A

Table 10: Summarised statistical analysis of change in proportional mRNA expression of IL6 (Cohort 1) pre- to post-HTO across four quadrants of the tibial plateau.

A proportional decrease in IL6 mRNA expression was seen for the majority of patients in all quadrants except AM (decreased in 8/17 for AM, 10/18 for PM, 10/18 for AL and 10/16 for PL). Fisher's exact test did not reveal any statistically significant differences in up/down regulation of IL6 mRNA expression based on quadrant (p=0.501).

The lateral quadrants showed a normal distribution of data but the changes seen in mean IL6 mRNA expression were very small and not statistically significant (increased by 4.38% (+/- 10.31%) in AL (p=0.692), decreased by -8.43% (+/-9.30%) in PL (p=0.094). Data for the medial quadrants was not normally distributed (as assessed by the Shapiro-Wilk's test) but neither showed significant changes in median expression on the Wilcoxon signed-rank test. AM showed no median change at all (p=0.756) and PM showed a decrease of 4.88% but again this was not significant (p=0.460).

There was a lot of variability in patterns of expression as shown in Figure 36. There were three main patterns in addition to some unique/anomalous patterns.

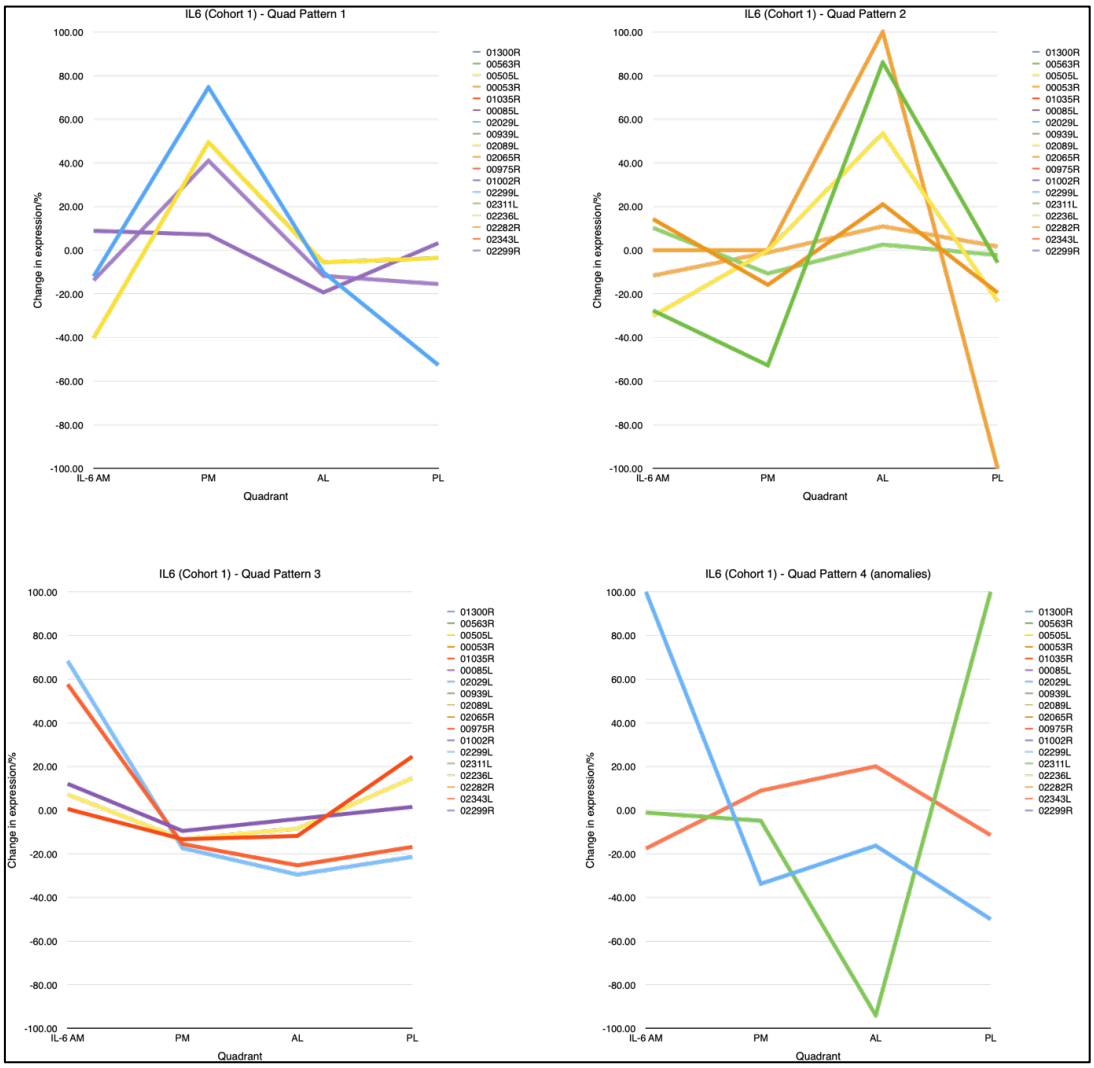

Figure 36: Patterns of change in proportional mRNA expression of IL-6 across quadrants of the tibial plateau (Cohort 1) pre- to post-HTO.

3.4.1.7 Correlations between significant variables (Cohort 1)

For the three biological variables where a statistically significant trend was seen in the PM quadrant (NR2D, GRIK4, SOST), analysis was undertaken to see whether these variables were related i.e. whether the changes were seen in the same patients. Each pair of variables was plotted on a scatter plot and a Spearman's rank-order correlation performed.

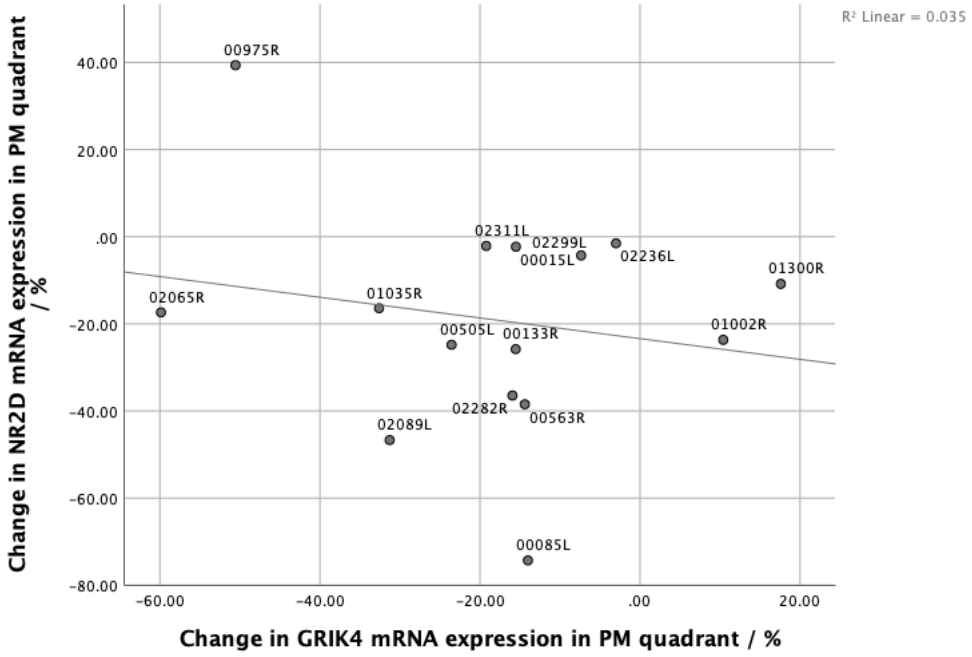

Figure 37: Comparison of proportional change in NR2D and GRIK4 mRNA expression (Cohort 1) pre- to post-HTO within the PM quadrant.

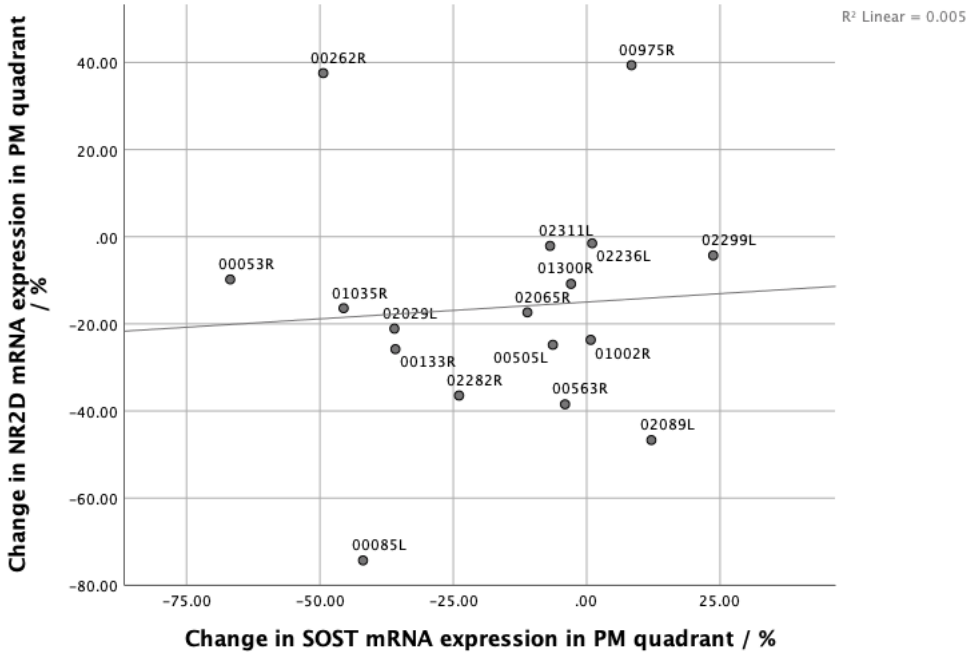

Figure 38: Comparison of proportional change in NR2D and SOST mRNA expression (Cohort 1) pre- to post-HTO within the PM quadrant.

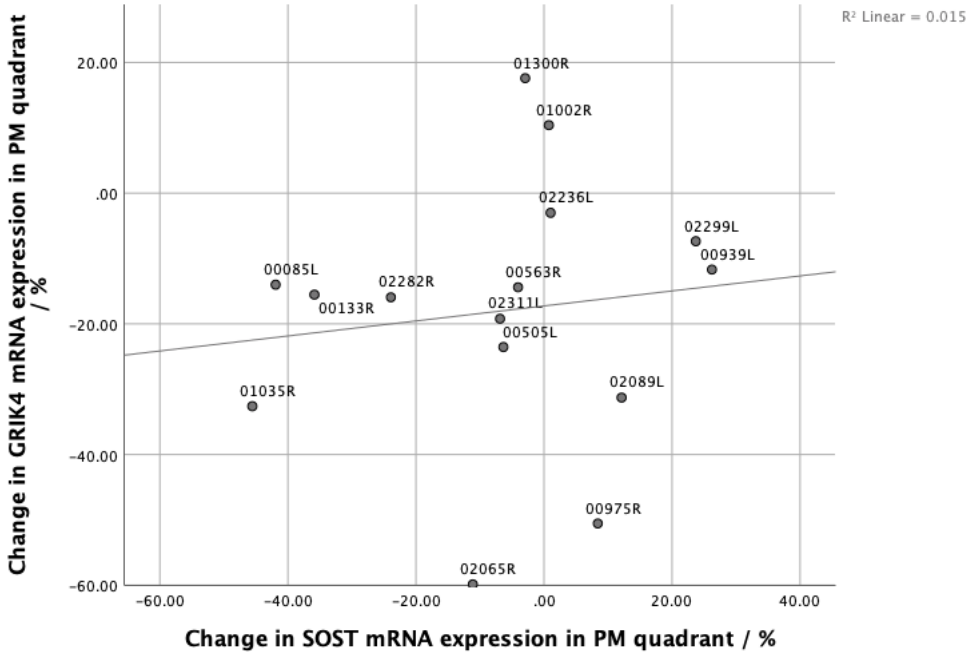

Figure 39: Comparison of proportional change in GRIK4 and SOST mRNA expression (Cohort 1) pre- to post-HTO within the PM quadrant.

			NR2D_PM	GRIK4_PM	SOST_PM
Spearman's rho	**NR2D_PM**	**Correlation Coefficient**		0.000	0.076
		p value		1.000	0.772
		N		15	17
	GRIK4_PM	**Correlation Coefficient**	0.000		0.321
		p value	1.000		0.243
		N	15		15
	SOST_PM	**Correlation Coefficient**	0.076	0.321	
		p value	0.772	0.243	
		N	17	15	

Table 11: Spearman's rank-order correlation of proportional change in NR2D, GRIK4 and SOST mRNA expression (Cohort 1) pre- to post-HTO within the PM quadrant.

No significant correlation was seen between any of the three variables.

3.4.2 Repeat analysis including Cohorts 1+2 (n=26)

Cohort 1+2 included 26 knees within 24 patients (two patients (02299 and 00975) had bilateral sequential osteotomies). 24 knees were male, 2 were female. Mean age at time of surgery of 50.3 years (range 33 – 64).

3.4.2.1 EAAT1, Cohort 1+2 (n=25)

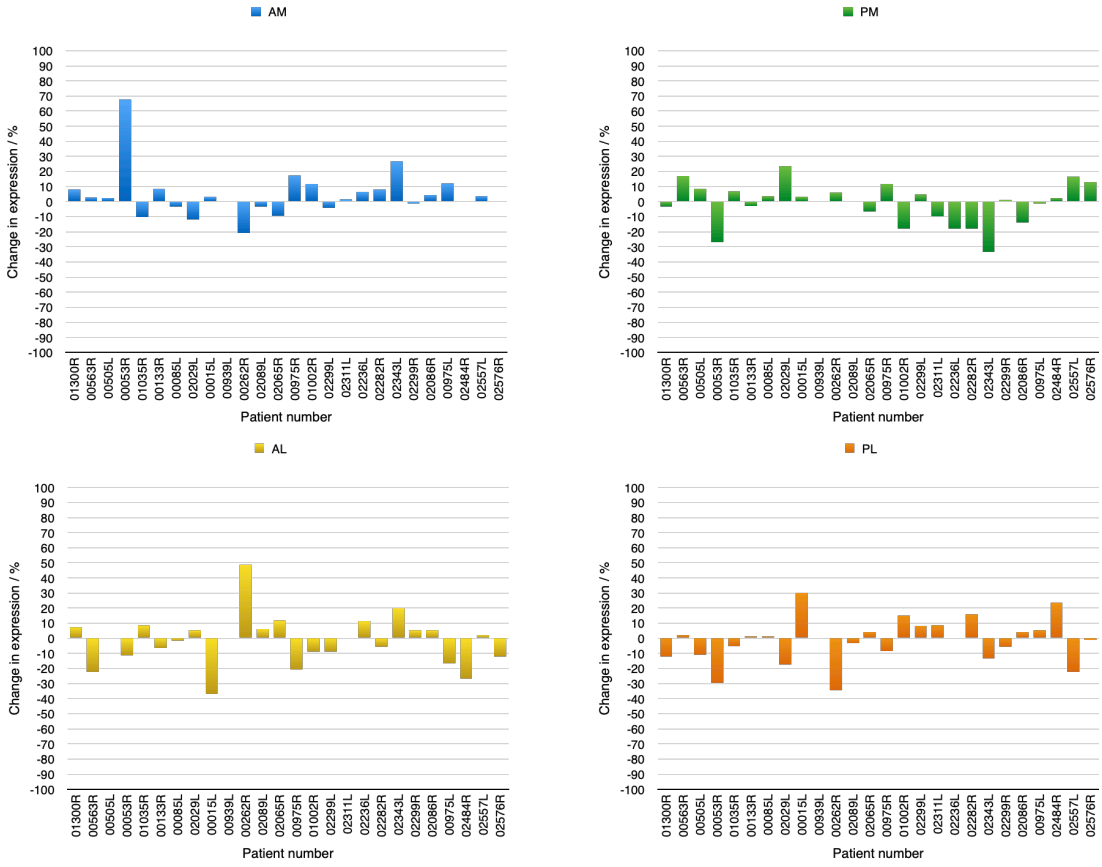

Figure 40: Change in proportional mRNA expression of EAAT1 (Cohort 1+2) pre- to post-HTO across four quadrants of the tibial plateau.

	AM	PM	AL	PL
Increased expression	15	14	12	13
Decreased expression	8	11	13	12
Exclusions	0	0	0	0
Shapiro-Wilk's p value	0.980	0.641	0.242	0.960
Mean / %	4.84	-1.32	-1.80	-1.71
Range / %	-20.59 - 67.88	-33.18 - 23.54	-26.66 - 48.74	-34.21 - 23.74
Standard Error / %	3.27	2.81	3.41	3.03
T-test p value	0.152	0.641	0.602	0.577
Wilcoxon signed-rank p value	N/A	N/A	N/A	N/A

Table 12: Summarised statistical analysis of change in proportional mRNA expression of EAAT1 (Cohort 1+2) pre- to post-HTO across four quadrants of the tibial plateau.

Across the broader cohort, EAAT1 mRNA expression was continued to be increased medially in slightly more patients (15/25 for AM, 14/25 for PM), with approximately equal numbers of patients showing increased/decreased EAAT1 MRNA expression in either of the lateral quadrants. Fisher's exact test did not reveal any statistically significant differences in up/down regulation of EAAT1 mRNA expression based on quadrant (p=0.912).

As with the initial cohort, across all patients, changes in mean EAAT1 mRNA expression were very small and not statistically significant (increased by 4.84% (+/- 73.2%) in AM (p=0.152), decreased by 1.32% (+/-2.81%) in PM (p=0.641), decreased by 1.80% (+/- 3.41%) in AL (p=0.602), decreased by 1.71% (+/- 3.03%) in PL (p=0.577)).

The patterns of change across the quadrants remained variable (Figure 41).

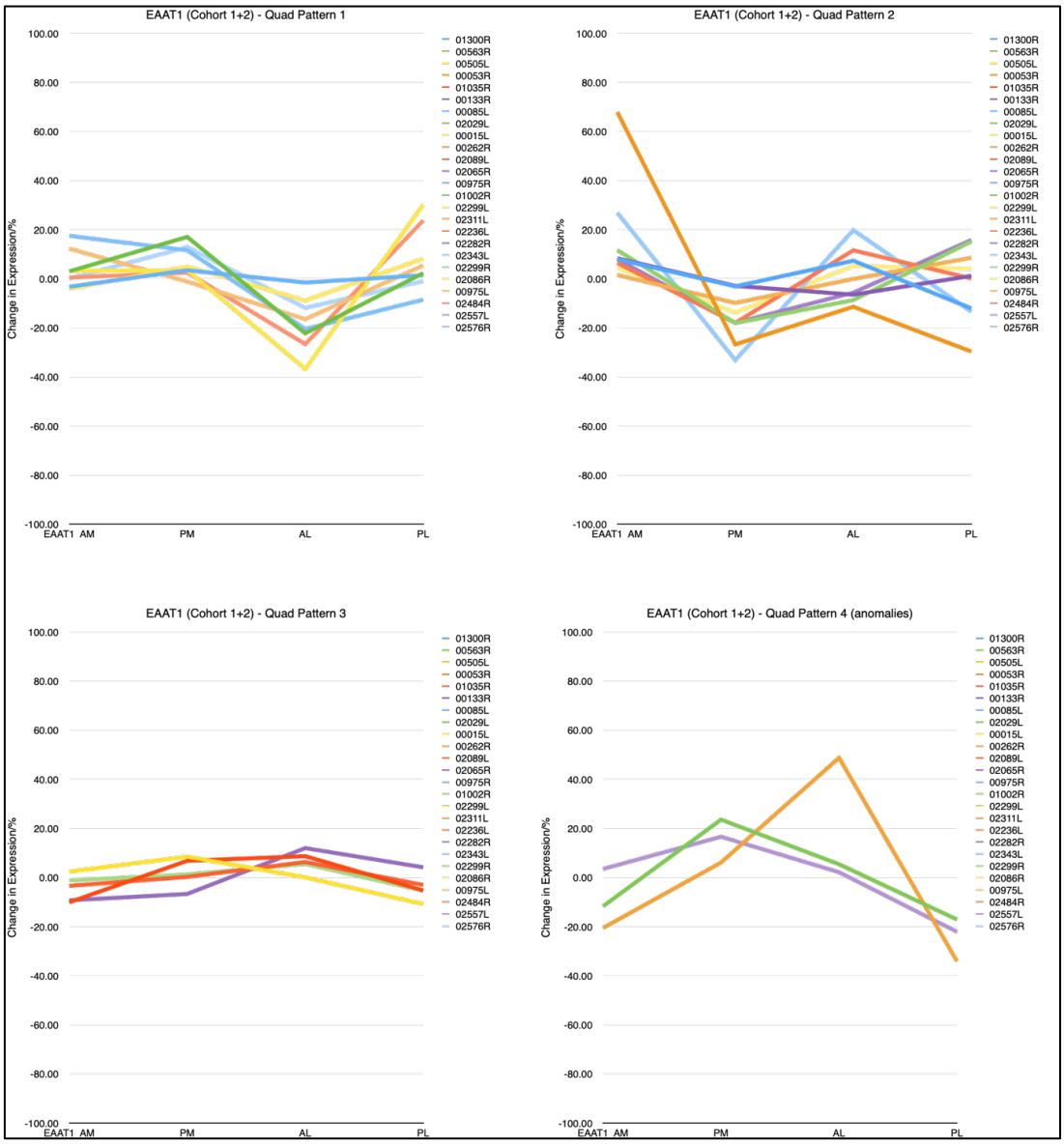

Figure 41: Patterns of change in proportional mRNA expression of EAAT1 across quadrants of the tibial plateau (Cohort 1+2) pre- to post-HTO.

3.4.2.2 EAAT3, Cohort 1+2 (n=24)

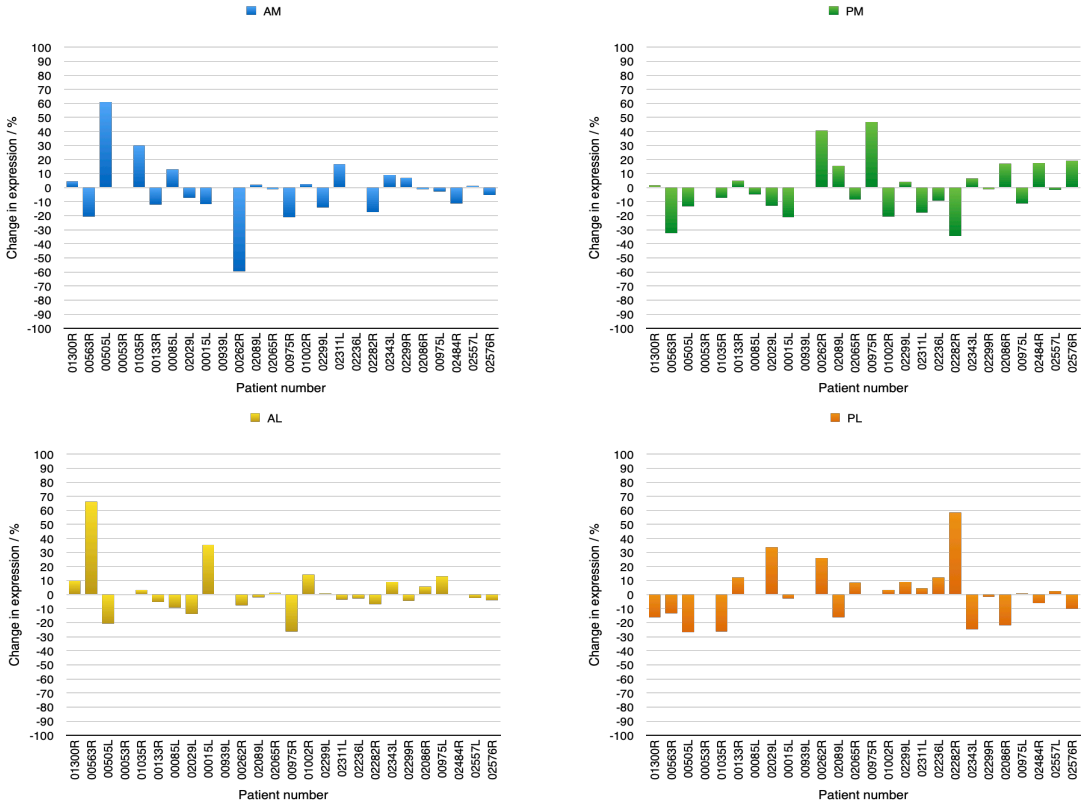

Figure 42: Change in proportional mRNA expression of EAAT3 (Cohort 1+2) pre- to post-HTO across four quadrants of the tibial plateau.

	AM	PM	AL	PL
Increased expression	10	10	10	13
Decreased expression	14	14	14	11
Exclusions	0	0	0	0
Shapiro-Wilk's p value	0.021	0.410	0.001	0.060
Mean / %	[Median -1.19]	-0.90	[Median -2.04]	0.35
Range / %	-59.34 - 60.78	-34.47 - 46.65	-26.43 - 66.07	-26.39 - 58.61
Standard Error / %	N/A	4.09	N/A	4.06
T-test p value	N/A	0.828	N/A	0.931
Wilcoxon signed-rank p value	0.475	N/A	0.819	N/A

Table 13: Summarised statistical analysis of change in proportional mRNA expression of EAAT3 (Cohort 1+2) pre- to post-HTO across four quadrants of the tibial plateau.

Similarly to with Cohort 1, in the larger cohort, EAAT3 mRNA expression decreased in more patients in all quadrants except PL (14/24 for AM, 14/24 for PM, 14/24 for AL but 11/24for PL). A Chi-square test did not reveal any statistically significant differences in up/down regulation of EAAT3 mRNA expression based on quadrant (p=0.768).

Across all quadrants, changes in EAAT3 mRNA expression were very small and not statistically significant. Data for the anterior quadrants was not normally distributed but showed small, non-significant median decreases (decreased by 1.19% in AM (p=0.475), decreased by 2.04 in AL (p=0.819). In the posterior quadrants, data was normally distributed but again, changes in means were very small and not significant (decreased by 0.90% (+/-4.09%) in PM (p=0.828) and increased by 0.35% (+/-4.06%) in PL (p=0.931)).

Patterns of expression remained variable (Figure 43).

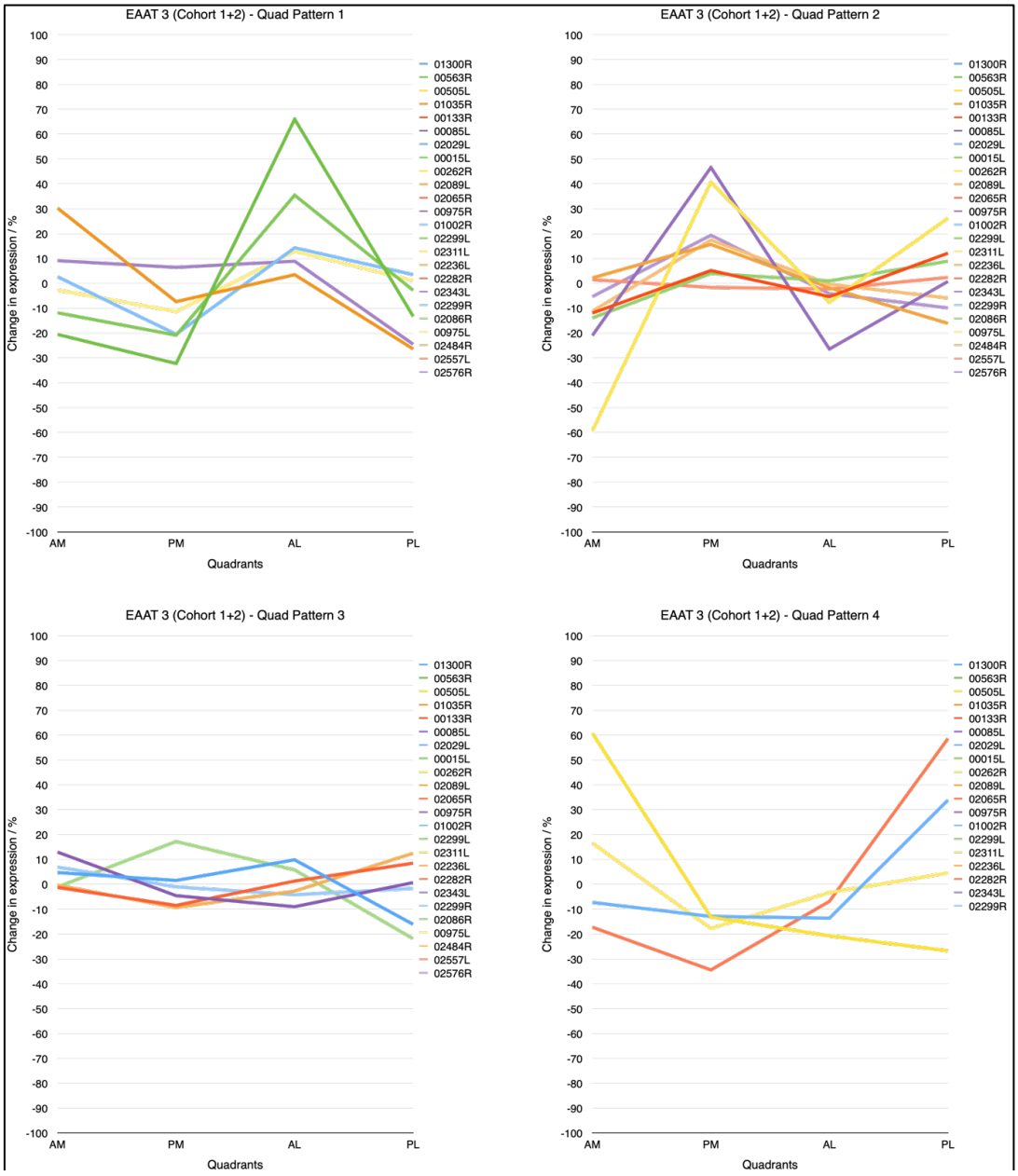

Figure 43: Patterns of change in proportional mRNA expression of EAAT3 across quadrants of the tibial plateau (Cohort 1+2) pre- to post-HTO.

3.4.2.3 NR2D, Cohort 1+2 (n=23)

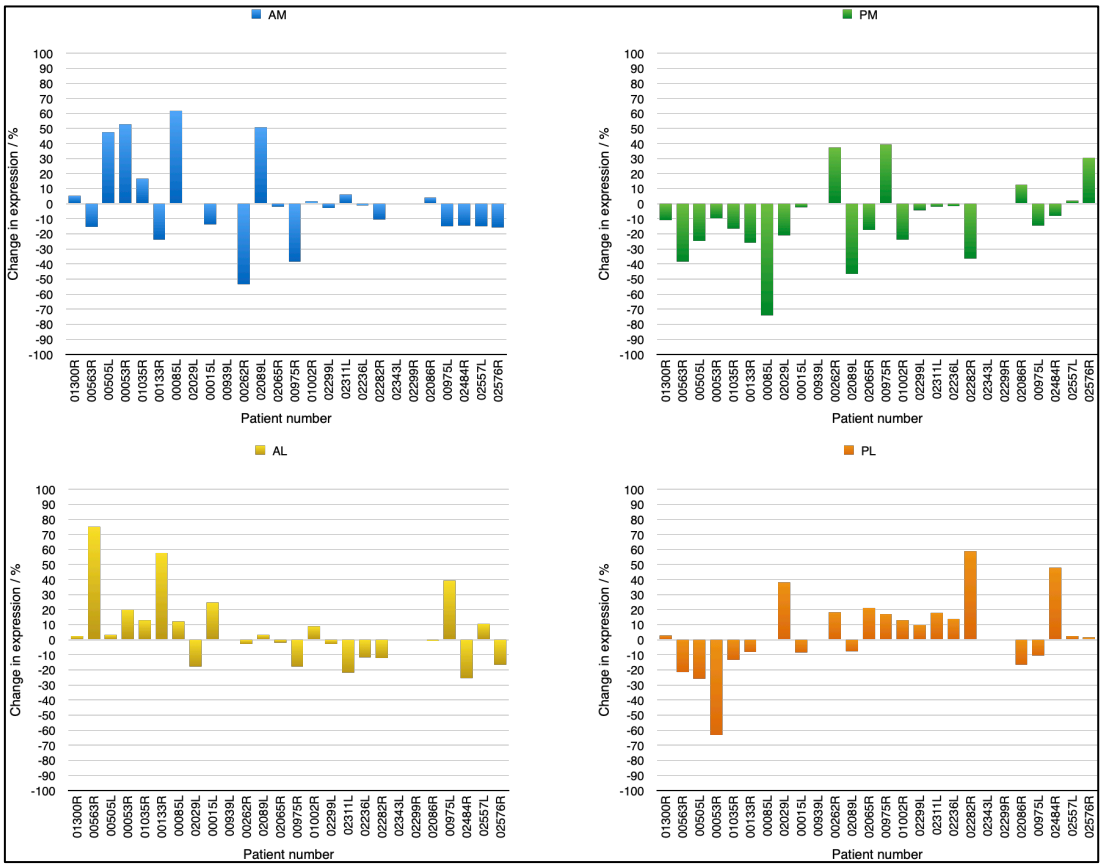

Figure 44: Change in proportional mRNA expression of NR2D (Cohort 1+2) pre- to post-HTO across four quadrants of the tibial plateau.

	AM	PM	AL	PL
Increased expression	10	5	12	14
Decreased expression	13	18	11	9
Exclusions	0	0	0	0
Shapiro-Wilk's p value	0.035	0.469	0.015	0.557
Mean / %	[Median -1.78]	-11.14	[Median 2.68]	3.88
Range / %	-53.44 - 50.69	-74.30 - 39.37	-25.33 - 75.30	-63.17 - 47.96
Standard Error / %	N/A	5.46	N/A	5.38
T-test p value	N/A	0.053	N/A	0.478
Wilcoxon signed-rank p value	0.670	N/A	0.484	N/A

Table 14: Summarised statistical analysis of change in proportional mRNA expression of NR2D (Cohort 1+2) pre- to post-HTO across four quadrants of the tibial plateau.

Following the addition of the additional 5 knees to the cohort, NR2D mRNA expression in the PM quadrant still decreased in the majority of patients (13/23) but the magnitude of the change in mean expression for the quadrant was smaller and no longer statistically significant to the $p<0.05$ threshold (decreased by 11.14% +/- 5.46% ($p=0.053$).

A Chi-square test did reveal a **statistically significant difference in up/down regulation of NR2D mRNA expression based on quadrant ($p=0.049$).** Post hoc analysis involved pairwise comparisons using the z-test of two proportions with a Bonferroni correction. This revealed **significant differences between the PM and PL quadrants.** The anterior quadrants did not show any significant differences with any other quadrant.

Posterolaterally the change in mean NR2D mRNA expression was small and not statistically significant (increased 3.88% +/- 5.38% ($p=0.478$). Data for the anterior quadrants were not normally distributed as assessed by the Shapiro-Wilk's test so the Wilcoxon signed-rank test was used. This showed a small, non-significant changes in median expression (decreased 1.78% in AM ($p=0.670$), increased 2.68% in AL ($p=0.484$)).

The addition of Cohort 2 also increased the variability of patterns seen across the quadrants (Figure 45).

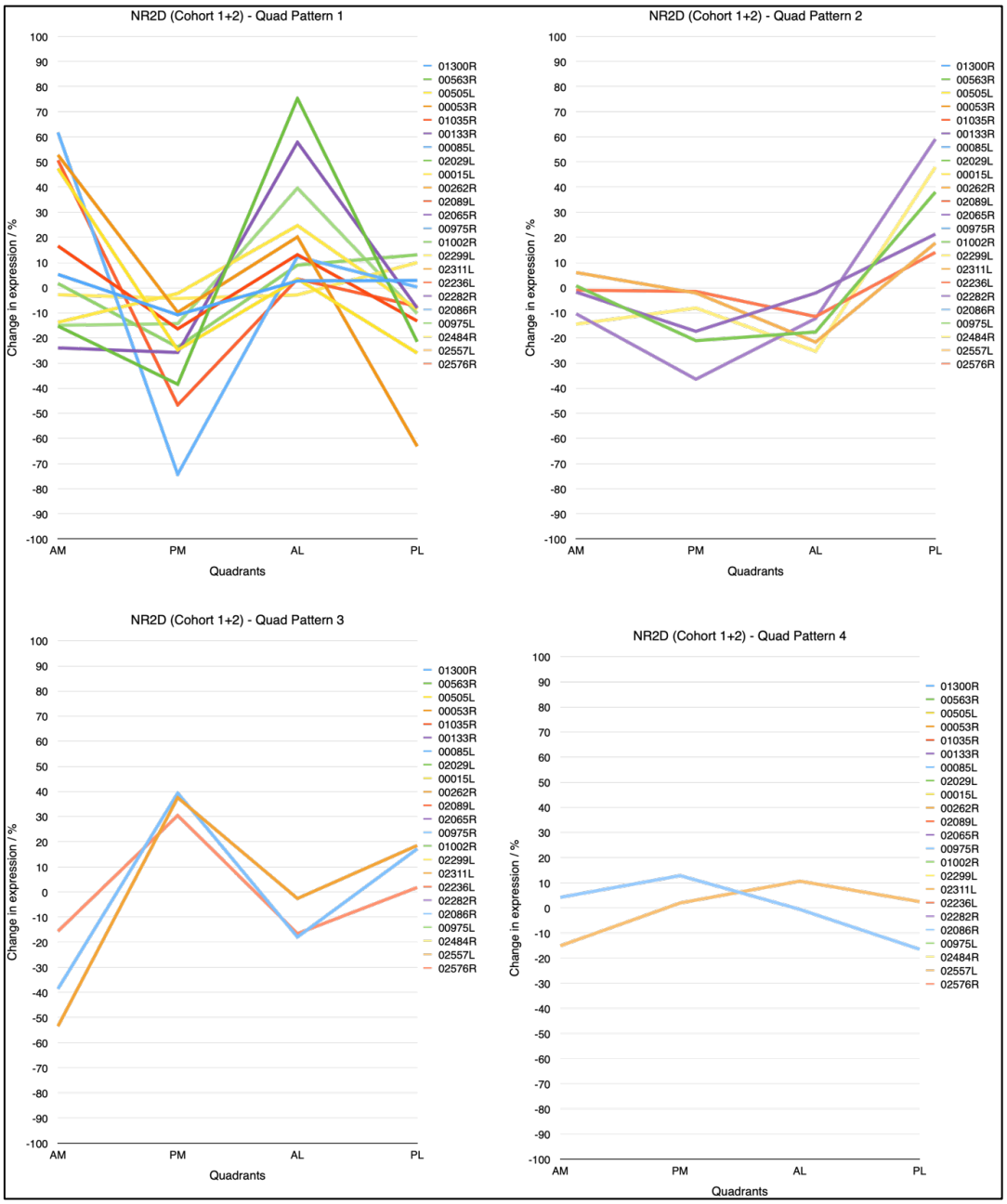

Figure 45: Patterns of change in proportional mRNA expression of NR2D across quadrants of the tibial plateau (Cohort 1+2) pre- to post-HTO.

3.4.2.4 GRIK4, Cohort 1+2 (n=22)

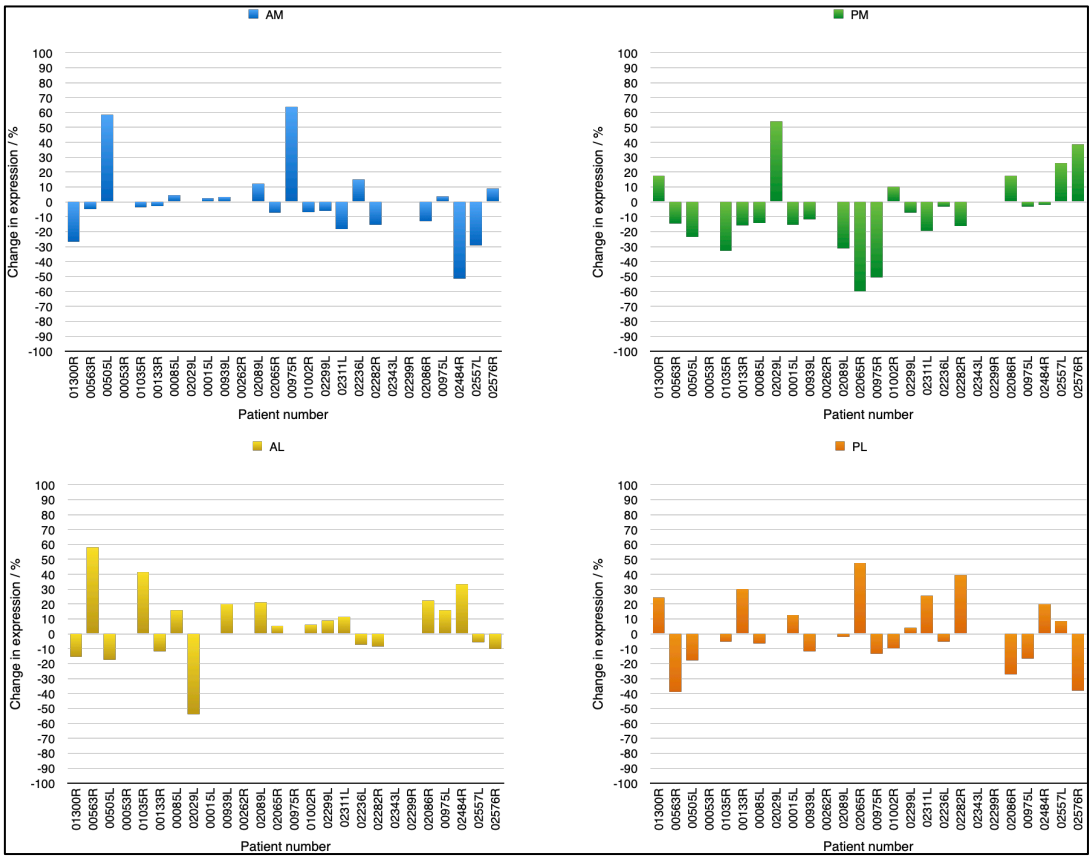

Figure 46: Change in proportional mRNA expression of GRIK4 (Cohort 1+20.637) pre- to post-HTO across four quadrants of the tibial plateau.

	AM	PM	AL	PL
Increased expression	9	6	13	9
Decreased expression	12	16	8	12
Exclusions	0	0	0	0
Shapiro-Wilk's p value	0.081	0.637	0.713	0.772
Mean / %	-3.02	-7.94	6.24	1.01
Range / %	-51.52 - 58.54	-59.86 - 38.80	-53.94 - 58.11	-38.82 - 47.55
Standard Error / %	4.83	5.01	5.20	5.22
T-test p value	0.536	0.130	0.245	0.848
Wilcoxon signed-rank p value	N/A	N/A	N/A	N/A

Table 15: Summarised statistical analysis of change in proportional mRNA expression of GRIK4 (Cohort 1+2) pre- to post-HTO across four quadrants of the tibial plateau.

GRIK4 mRNA expression in the PM quadrant decreased in the majority of patients (16/22) but the change in mean expression for this quadrant was no longer statistically significant after addition of Cohort 2 (decreased by 7.94% +/- 5.01% (p=0.130)).

In the other three quadrants, changes in mean GRIK4 mRNA expression were likewise small and not statistically significant (decreased by 3.01% (+/- 4.83%) in AM (p=0.536), increased by 6.24% (+/- 5.02%) in AL (p=0.245), increased by 1.01% (+/- 5.22%) in PL (p=0.848)) with 12/22 decreasing for AM, 13/22 increasing for AL and 12/22 decreasing for PL.

Fisher's exact test did not reveal any statistically significant differences in up/down regulation of GRIK4 mRNA expression based on quadrant (p=0.264).

Again the addition of Cohort 2, increased the variability of the patterns across quadrants (Figure 47).

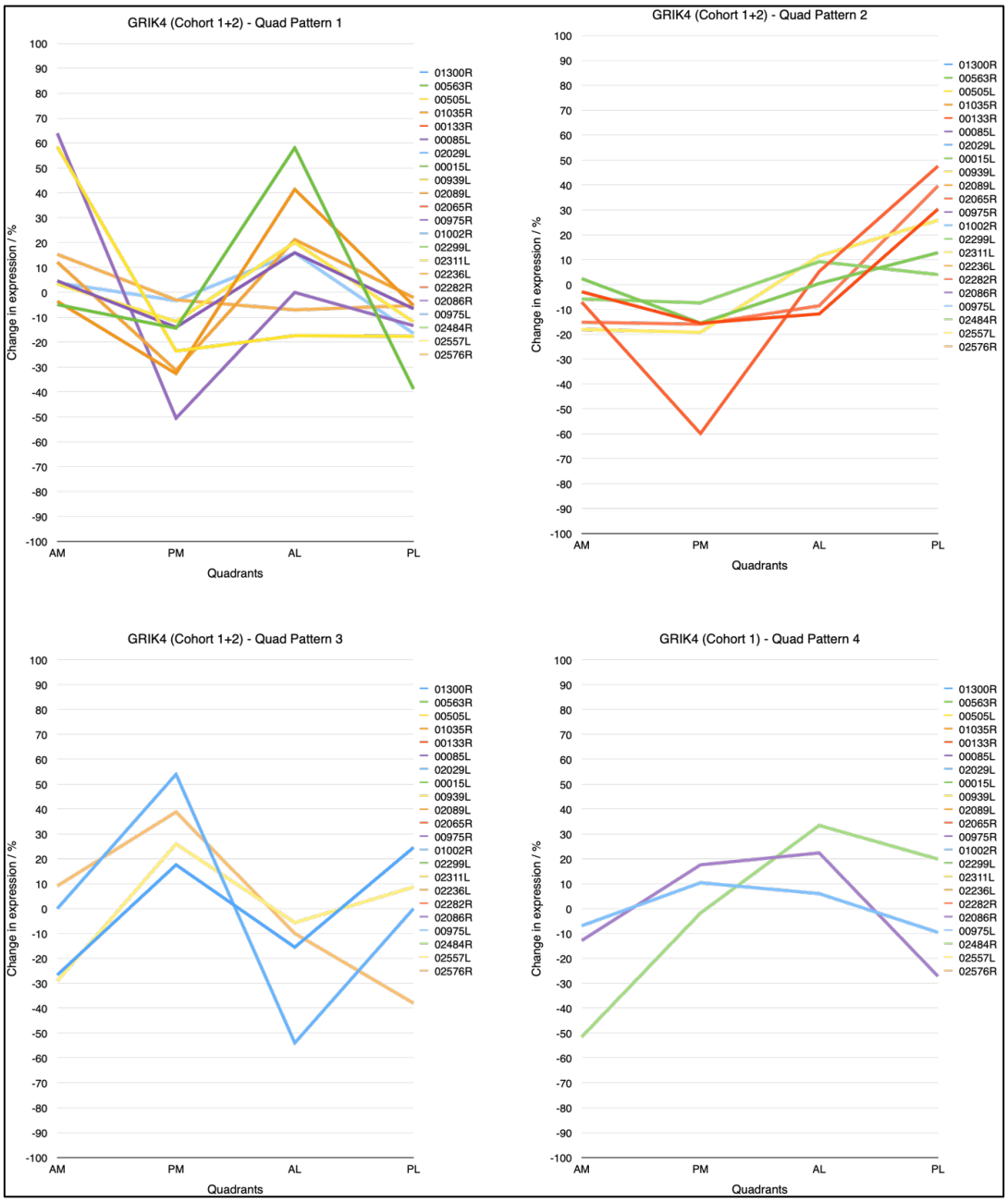

Figure 47: Patterns of change in proportional mRNA expression of NR2D across quadrants of the tibial plateau (Cohort 1+2) pre- to post-HTO.

3.4.2.5 Sclerostin, Cohort 1+2 (n=25)

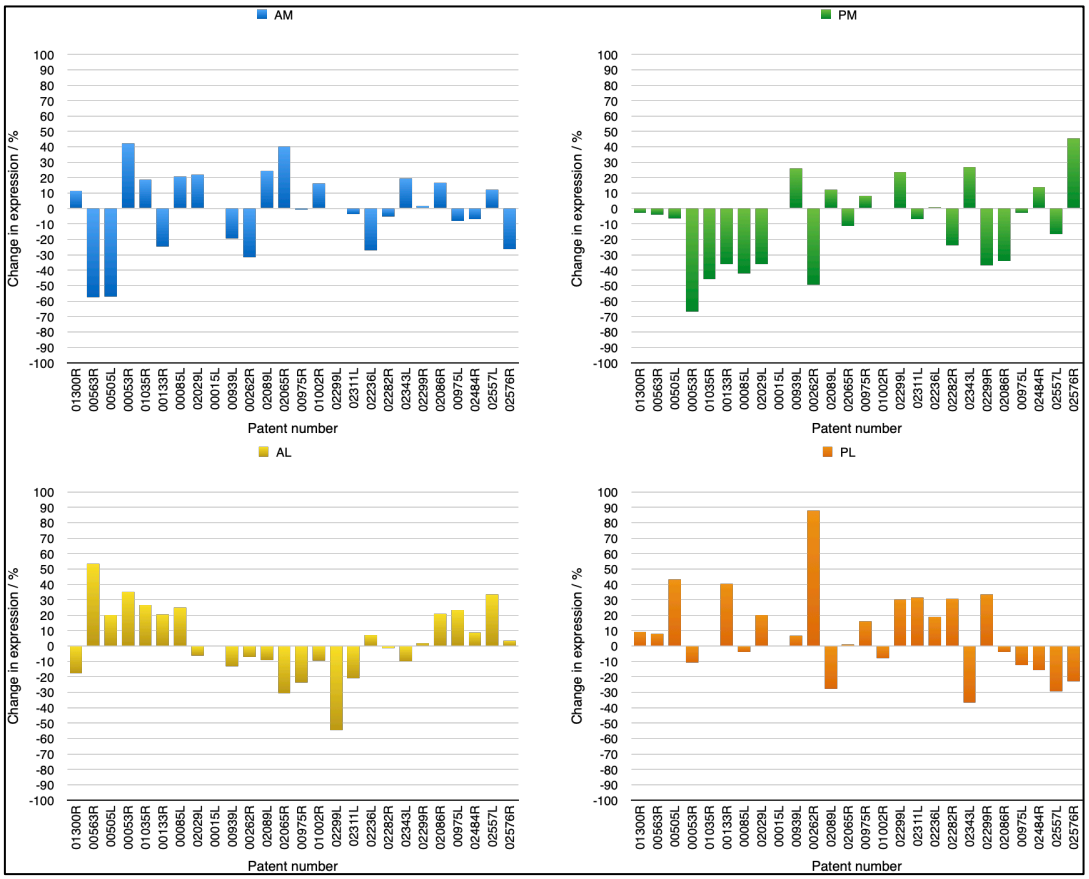

Figure 48: Change in proportional mRNA expression of SOST (Cohort 1+2) pre- to post-HTO across four quadrants of the tibial plateau.

	AM	PM	AL	PL
Increased expression	13	9	13	14
Decreased expression	12	16	12	11
Exclusions	0	0	0	0
Shapiro-Wilk's p value	0.272	0.861	0.941	0.349
Mean / %	-0.83	-10.51	3.07	8.27
Range / %	-57.34 - 42.26	-66.81 - 45.52	-54.68 - 53.39	-36.70 - 87.89
Standard Error / %	5.26	5.57	4.84	5.60
T-test p value	0.876	0.071	0.532	0.153
Wilcoxon signed-rank p value	N/A	N/A	N/A	N/A

Table 16: Summarised statistical analysis of change in proportional mRNA expression of SOST (Cohort 1+2) pre- to post-HTO across four quadrants of the tibial plateau.

The most marked changes were seen again in the posterior quadrants but these changes in mean SOST mRNA expression no longer show statistical significance after the addition of Cohort 2. The majority of patients (16/25) showed decreased expression posteromedially (decreased by 10.51% +/- 5.57% (p=0.071)) whereas the majority of patients (14/25) showed increased expression posterolaterally (increased by 8.27% +/- 5.60% (p=0.153)).

In the anterior compartments, the changes in mean SOST mRNA expression were small and also not statistically significant (decreased by 0.83% (+/- 5.26%) in AM (p=0.876), increased by 3.07% (+/- 4.84%) in AL (p=0.532)) with 13/25 increasing for AM and 13/25 increasing for AL.

A Chi-square test did not reveal any statistically significant differences in up/down regulation of SOST mRNA expression based on quadrant (p=0.501).

After the addition of Cohort 2, the two most frequent patterns of change across the quadrants (Quad Patterns 1 and 2) both showed noticeable downregulation in PM (Figure 49). The most common pattern was now Quad Pattern 1 which appears to show that it is more the AL quadrant which is proportionally upregulated rather than PL.

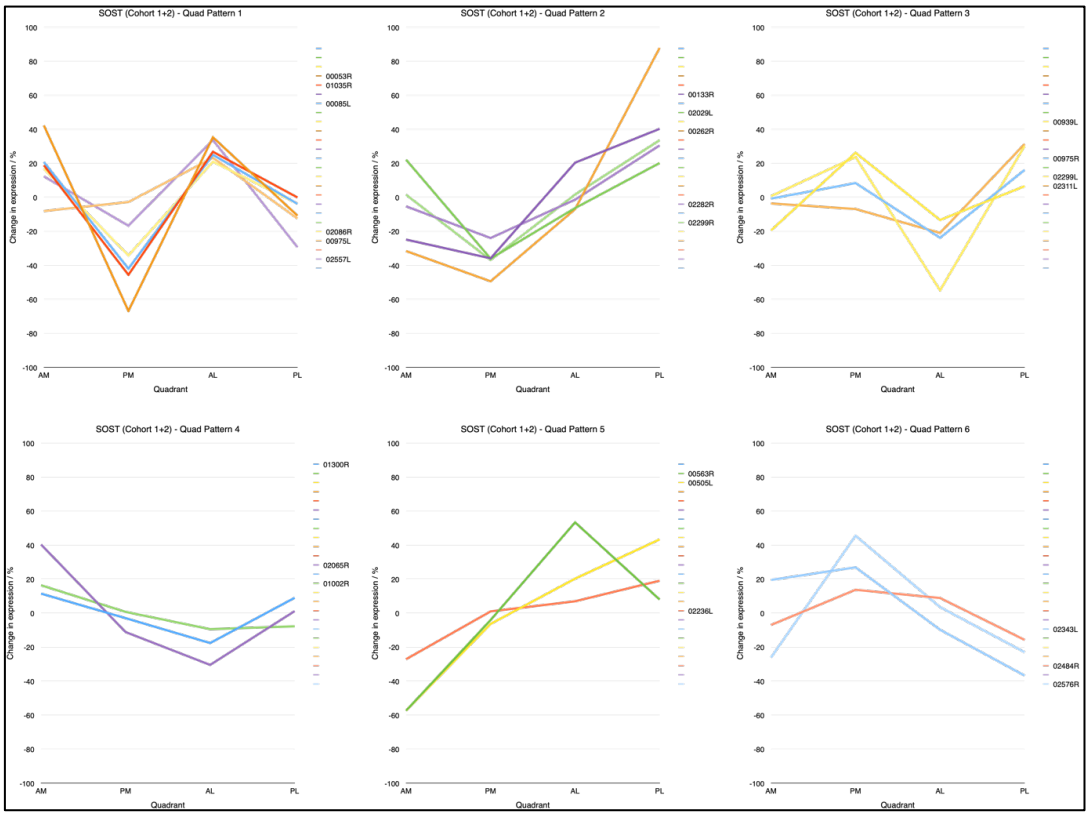

Figure 49: Patterns of change in proportional mRNA expression of SOST across quadrants of the tibial plateau (Cohort 1+2) pre- to post-HTO.

3.4.2.6 IL-6, Cohort 1+2 (n=23)

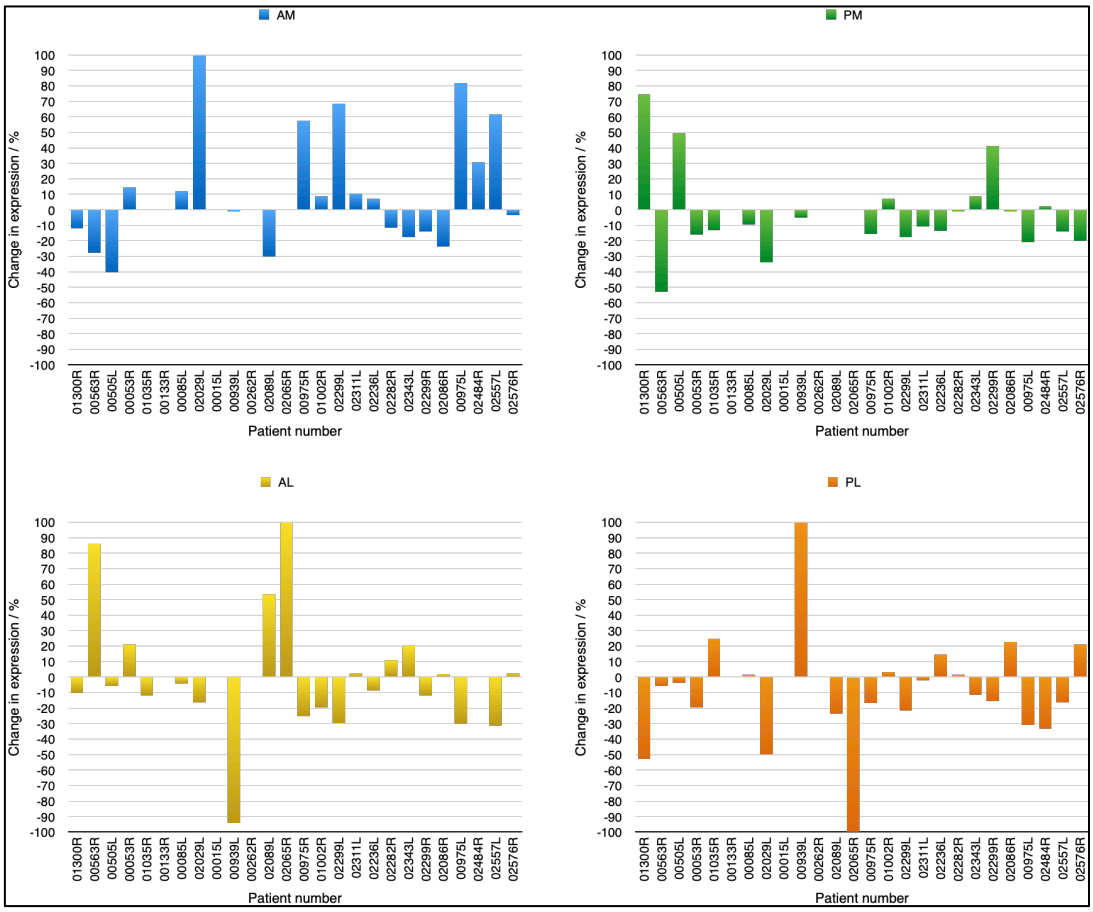

Figure 50: Change in proportional mRNA expression of IL6 (Cohort 1+2) pre- to post-HTO across four quadrants of the tibial plateau.

	AM	PM	AL	PL
Increased expression	11	6	9	8
Decreased expression	10	15	14	15
Exclusions	1 (02029L)	0	0	0
Shapiro-Wilk's p value	0.049	0.003	0.019	0.784
Mean / %	[Median 0.27]	[Median -7.21]	[Median -4.79]	-8.15
Range / %	-40.31 - 81.67	-52.76 - 74.68	-94.01 - 100	-100 - 100
Standard Error / %	N/A	N/A	N/A	4.48
T-test p value	N/A	N/A	N/A	0.085
Wilcoxon signed-rank p value	0.614	0.179	0.506	N/A

Table 17: Summarised statistical analysis of change in proportional mRNA expression of IL6 (Cohort 1+2) pre- to post-HTO across four quadrants of the tibial plateau.

Again, a proportional decrease in IL6 mRNA expression was seen for the majority of patients in all quadrants except AM (decreased in 10/22 for AM, 15/23 for PM, 14/23 for AL and 15/23 for PL). Fisher's exact test did not reveal any statistically significant differences in up/down regulation of IL6 mRNA expression based on quadrant (p=0.317).

Only the PL quadrant showed a normal distribution of data but the mean decrease in expression shown did not meet the threshold for statistical significance (-8.15% +/- 4.48% (p=0.085)).

Data for the other three quadrants was not normally distributed (as assessed by the Shapiro-Wilk's test) and none showed significant changes in median expression on the Wilcoxon signed-rank test.

The addition of Cohort 2 caused increased variability in the patterns of expression change seen across the quadrants (Figure 51).

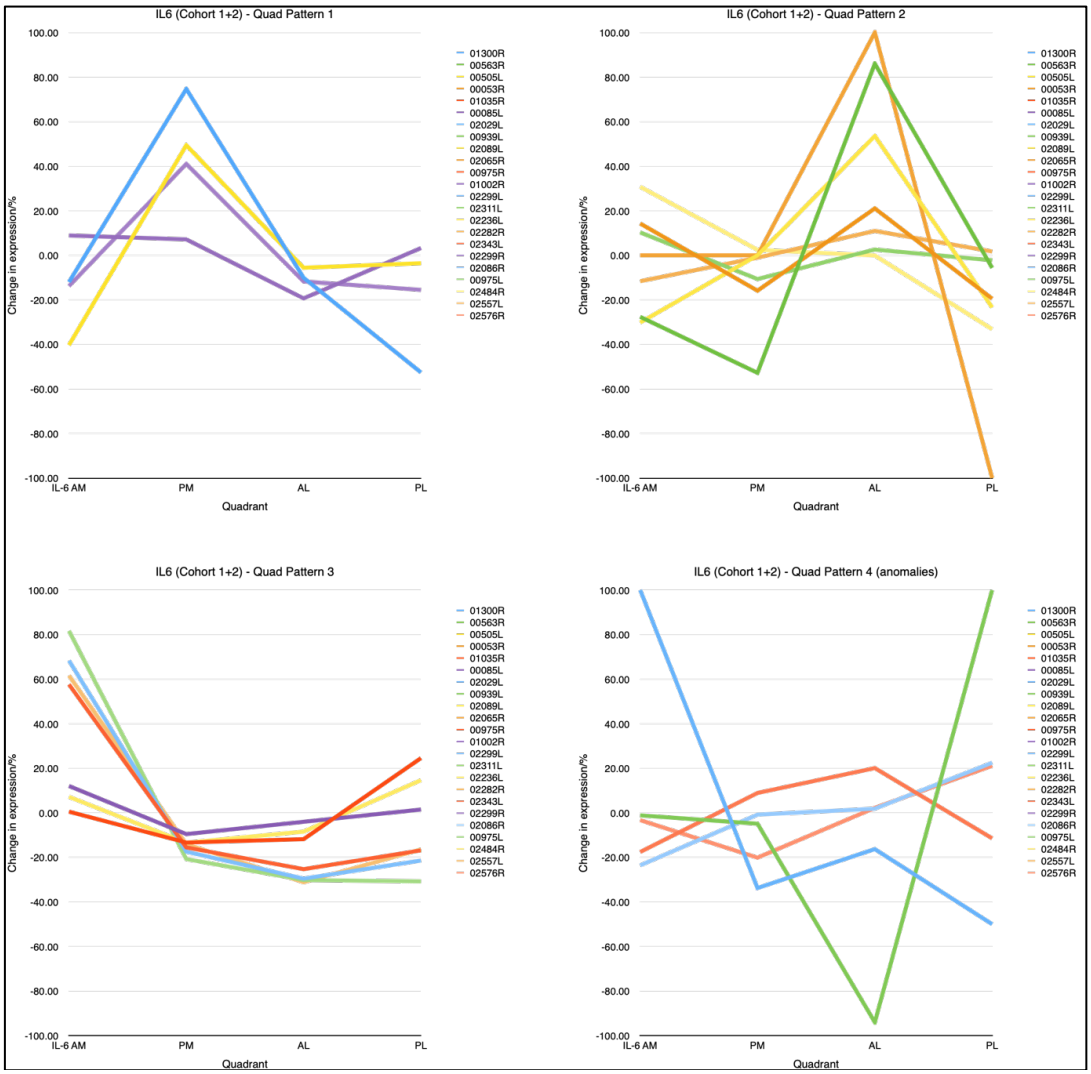

Figure 51: Patterns of change in proportional mRNA expression of SOST across quadrants of the tibial plateau (Cohort 1+2) pre- to post-HTO.

3.4.3 Summary of Results

		Cohort 1 Change in expression (%)	Cohort 1 p-value	Cohort 1+2 Change in expression (%)	Cohort 1+2 p-value
EAAT1	**AM**	5.02	0.234	4.84	0.152
	PM	-2.50	0.450	-1.32	0.641
	AL	*-0.03*	*0.881*	-1.80	0.602
	PL	-2.65	0.443	-1.71	0.577
EAAT3	**AM**	-0.98	0.861	*-1.19*	*0.475*
	PM	-3.29	0.503	-0.90	0.828
	AL	*-0.04*	*0.687*	*-2.04*	*0.819*
	PL	2.24	0.656	*0.73*	*0.797*
NR2D	**AM**	4.55	0.548	*-1.78*	*0.670*
	PM	**-15.51**	**0.025**	-11.14	0.053
	AL	*-3.05*	*0.349*	2.68	0.484
	PL	3.55	0.580	3.88	0.478
GRIK4	**AM**	*-2.93*	*0.776*	-3.02	0.536
	PM	**-17.91**	**0.002**	-7.94	0.130
	AL	4.68	0.476	6.24	0.245
	PL	4.65	0.439	1.01	0.848
SOST	**AM**	-0.44	0.946	-0.83	0.876
	PM	**-13.43**	**0.040**	-10.51	0.071
	AL	-0.68	0.905	3.07	0.532
	PL	**14.54**	**0.029**	8.27	0.153
IL-6	**AM**	*0.00*	*0.756*	*0.27*	*0.614*
	PM	*-4.88*	*0.460*	*-7.21*	*0.179*
	AL	4.38	0.692	*-4.79*	*0.506*
	PL	-8.43	0.094	-8.15	0.085

Table 18: Summary of proportional change in expression results. Figures shown in **bold** represent statistically significant results ($p<0.05$). Figures shown in *italic* represent non-parametric data analysed with Wilcoxon signed-rank rather than t-test.

	Chi-squared test p-value	
	Cohort 1	**Cohort 1+2**
EAAT1	*0.959*	*0.912*
EAAT3	0.603	0.768
NR2D	**0.010***	**0.049****
GRIK4	*0.128*	*0.264*
SOST	0.112	0.501
IL6	*0.501*	*0.317*

* Statistically significant difference seen between PM quadrant and both lateral quadrants on pairwise comparison

** Statistically significant difference seen between PM and PL quadrants on pairwise comparison

Table 19: Summary of homogeneity test results. Figures shown in **bold** represent statistically significant results ($p<0.05$). Figures shown in *italic* represent Fisher's exact test results rather than Chi-square test.

3.4.4 Analysis of differences between Cohorts 1 and 2

As shown in the summary of results, following the inclusion of Cohort 2, the magnitude of the mean changes seen for NR2D, GRIK4 and SOST were diminished and their statistical significance was lost (although some of the results remain close to the $p<0.05$ threshold).

The clinical data from these five patients was analysed to look for any obvious causes for their effect on the overall results. Of the five, two had significant post-operative complications (one with a readmission to hospital due to severe pain and swelling, another with problems of delayed union requiring a bone stimulator and ongoing symptoms of instability). It is difficult to say to what extent these issues may have an impact on the results.

The next stage was to look across all the available clinical, biological and radiological data to see if there were any differences between the two cohorts. This was done using independent T-tests (see table 20). This showed the following statistically significant ($p<0.05$) differences between the cohorts:

- Pre-operative EAAT3 in the AL quadrant (lower in Cohort 2)
- Pre-operative (and difference pre-to post-operative) GRIK4 in the PM quadrant (lower in Cohort 2 pre-operatively therefore greater pre to post difference).
- Pre-operative (and difference pre- to post-operative) SOST in the PL quadrant (higher in Cohort 2 pre-operatively therefore resultant reduced difference)
- Post-operative (and difference pre- to post-operative) MPTA (lower in Cohort 2 post-operatively and resultant smaller difference).

Table 20: Independent T-tests comparing variables between Cohort 1 (n=21) and Cohort 2 (n=5). Mean difference of Cohort1 – Cohort 2. Statistically significant results shown in **bold**.

	t	df	Sig. (2-tailed)	Mean Diff	Std. Error Diff	95% Confidence Interval Diff.	
						Lower	Upper
EAAT1_AM_pre	0.25	23	0.81	0.78	3.15	-5.73	7.30
EAAT1_AM_post	0.22	23	0.83	1.67	7.48	-13.82	17.15
EAAT1_AM_diff	0.11	23	0.92	0.88	8.35	-16.40	18.16
EAAT1_PM_pre	-0.25	23	0.81	-0.89	3.58	-8.29	6.51
EAAT1_PM_post	-1.34	23	0.19	-6.78	5.05	-17.23	3.68
EAAT1_PM_diff	-0.83	23	0.41	-5.89	7.06	-20.50	8.73
EAAT1_AL_pre	-0.49	23	0.63	-1.59	3.27	-8.36	5.18
EAAT1_AL_post	1.20	23	0.24	8.09	6.73	-5.84	22.02
EAAT1_AL_diff	1.14	23	0.27	9.69	8.47	-7.83	27.21
EAAT1_PL_pre	0.51	23	0.62	1.70	3.37	-5.27	8.67
EAAT1_PL_post	-0.53	23	0.60	-2.98	5.65	-14.67	8.70
EAAT1_PL_diff	-0.61	23	0.55	-4.69	7.68	-20.57	11.20
EAAT3_AM_pre	-0.83	22	0.42	-4.71	5.67	-16.47	7.06
EAAT3_AM_post	-0.22	22	0.83	-1.93	8.67	-19.91	16.06
EAAT3_AM_diff	0.25	22	0.80	2.78	10.98	-19.99	25.56
EAAT3_PM_pre	0.88	22	0.39	4.40	4.99	-5.94	14.75
EAAT3_PM_post	-0.88	22	0.39	-7.10	8.05	-23.80	9.60
EAAT3_PM_diff	-1.15	22	0.26	-11.50	10.00	-32.24	9.24
EAAT3_AL_pre	**1.77**	**22**	**0.09**	**5.21**	**2.95**	**-0.91**	**11.33**
EAAT3_AL_post	0.49	22	0.63	4.85	9.98	-15.85	25.56
EAAT3_AL_diff	-0.04	22	0.97	-0.36	9.41	-19.87	19.15
EAAT3_PL_pre	-0.95	22	0.35	-4.91	5.18	-15.66	5.85
EAAT3_PL_post	0.52	22	0.61	4.17	8.06	-12.55	20.89
EAAT3_PL_diff	0.90	22	0.38	9.08	10.04	-11.74	29.89
NR2D_AM_pre	-0.81	21	0.43	-5.38	6.66	-19.22	8.47
NR2D_AM_post	0.98	21	0.34	10.38	10.57	-11.60	32.37
NR2D_AM_diff	1.09	21	0.29	15.76	14.45	-14.29	45.80
NR2D_PM_pre	1.13	21	0.27	9.92	8.80	-8.38	28.22
NR2D_PM_post	-1.37	21	0.19	-10.15	7.41	-25.56	5.26
NR2D_PM_diff	-1.57	21	0.13	-20.07	12.82	-46.73	6.60
NR2D_AL_pre	-0.15	21	0.89	-0.81	5.57	-12.39	10.77

NR2D_AL_post	0.44	21	0.67	5.04	11.55	-18.98	29.06
NR2D_AL_diff	0.46	21	0.65	5.85	12.81	-20.78	32.49
NR2D_PL_pre	-0.57	21	0.58	-3.73	6.56	-17.37	9.91
NR2D_PL_post	-0.48	21	0.64	-5.28	11.08	-28.32	17.77
NR2D_PL_diff	-0.12	21	0.91	-1.54	13.35	-29.31	26.23
GRIK4_AM_pre	-1.33	20	0.20	-11.05	8.29	-28.34	6.25
GRIK4_AM_post	0.90	20	0.38	9.92	10.97	-12.97	32.81
GRIK4_AM_diff	1.72	20	0.10	20.97	12.16	-4.41	46.34
GRIK4_PM_pre	**1.87**	**20**	**0.08**	**14.41**	**7.71**	**-1.67**	**30.49**
GRIK4_PM_post	-1.29	20	0.21	-14.73	11.38	-38.47	9.01
GRIK4_PM_diff	**-2.35**	**20**	**0.03**	**-29.14**	**12.41**	**-55.03**	**-3.26**
GRIK4_AL_pre	0.78	20	0.44	5.99	7.64	-9.95	21.93
GRIK4_AL_post	-0.07	20	0.94	-0.82	11.34	-24.48	22.84
GRIK4_AL_diff	-0.57	20	0.58	-6.81	12.06	-31.95	18.34
GRIK4_PL_pre	-0.97	20	0.35	-9.36	9.68	-29.56	10.84
GRIK4_PL_post	0.61	20	0.55	5.63	9.30	-13.78	25.04
GRIK4_PL_diff	1.28	20	0.22	14.98	11.69	-9.41	39.37
SOST_AM_pre	-0.43	22	0.67	-3.67	8.55	-21.39	14.06
SOST_AM_post	-0.51	22	0.61	-3.97	7.75	-20.03	12.09
SOST_AM_diff	-0.02	22	0.98	-0.30	12.99	-27.24	26.63
SOST_PM_pre	0.62	22	0.54	5.89	9.55	-13.90	25.69
SOST_PM_post	-0.70	22	0.49	-5.86	8.36	-23.19	11.46
SOST_PM_diff	-0.90	22	0.38	-11.76	13.03	-38.77	15.26
SOST_AL_pre	1.65	22	0.11	12.32	7.46	-3.15	27.79
SOST_AL_post	-1.00	22	0.33	-8.32	8.34	-25.62	8.98
SOST_AL_diff	-1.81	22	0.08	-20.65	11.38	-44.25	2.96
SOST_PL_pre	**-2.41**	**22**	**0.03**	**-14.55**	**6.03**	**-27.05**	**-2.05**
SOST_PL_post	1.74	22	0.10	18.15	10.43	-3.48	39.78
SOST_PL_diff	**2.56**	**22**	**0.02**	**32.70**	**12.77**	**6.22**	**59.17**
KL	0.71	24	0.48	0.21	0.30	-0.40	0.82
mTFA_pre	0.11	24	0.92	0.18	1.66	-3.25	3.61
mTFA_post	-0.70	23	0.49	-0.78	1.10	-3.06	1.51
mTFA_diff	-0.57	23	0.57	-0.75	1.30	-3.44	1.95
Mik_pre	-0.14	24	0.89	-0.99	7.08	-15.62	13.63
Mik_post	0.81	24	0.43	3.90	4.80	-6.01	13.81
Mik_diff	0.83	24	0.41	4.89	5.87	-7.22	17.01

MPTA_pre	-0.92	24	0.37	-1.17	1.27	-3.79	1.45
MPTA_post	**1.77**	**23**	**0.09**	**2.07**	**1.17**	**-0.35**	**4.48**
MPTA_diff	**2.27**	**23**	**0.03**	**2.91**	**1.28**	**0.25**	**5.56**
LDTA_pre	-0.85	20	0.40	-3.07	3.59	-10.56	4.43
LDFA_pre	-1.15	24	0.26	-1.32	1.15	-3.69	1.04
TT_pre	-0.29	17	0.77	-0.88	3.00	-7.21	5.45
TT_post	0.78	15	0.45	2.60	3.32	-4.47	9.67
TT_diff	1.10	15	0.29	2.93	2.67	-2.76	8.63
SW_pre	0.25	18	0.81	0.41	1.64	-3.05	3.86
SW_post	-0.02	18	0.98	-0.03	1.30	-2.76	2.70
SW_diff	-0.20	18	0.85	-0.44	2.22	-5.10	4.23
Age	-0.60	20	0.56	-2.35	3.94	-10.58	5.87
Height	0.49	5	0.64	0.05	0.11	-0.22	0.33
Weight	0.36	5	0.73	5.15	14.15	-31.22	41.52

3.5 DISCUSSION

In the initial cohort of 21 patients, there was a statistically significant decrease in expression of NR2D, GRIK4 and SOST in the PM quadrant following HTO. In addition to the decrease in the PM quadrant there was a corresponding statistically significant increase in SOST expression in the PL quadrant.

Looking first at the changes in SOST, the reciprocal changes seen in the PM and PL quadrants do support a theory of response to loading. This is in keeping with the established literature which suggests that sclerostin expression by osteocytes is down-regulated by load, which removes its inhibition of bone formation via the Wnt/ β-catenin (Lin *et al.*, 2009; Delgado-Calle, Sato and Bellido, 2017; Weivoda, Youssef and Oursler, 2017). However, the SOST changes shown in this chapter secondary to HTO appear to be contrary to initial expectations. Previous studies have suggested that sclerostin expression is decreased in osteoarthritis (Power *et al.*, 2010; Chan *et al.*, 2011; Weivoda, Youssef and Oursler, 2017) and suggested that this reduced inhibition of bone formation is responsible for the subchondral sclerosis and osteophyte formation which is characteristic of osteoarthritis. Therefore, it would be reasonable to expect that when load is reduced in an osteoarthritic PM quadrant (and applied to the PL) via a valgising HTO, that sclerostin expression would increase medially and decrease laterally. However in the results outlined above, the inverse has been observed (although it is worth noting that due to the requirement to normalise across quadrants, the scale of this response is unknown).

It is the author's hypothesis that these seemingly counterintuitive results could be explained by considering the chronicity and severity of OA in different circumstances. In established, chronic OA with radiographic changes and widespread cartilage degradation, the subchondral bone is

being subjected to long term abnormal loading and the existing damage is irreparable. Therefore the end stage compensation mechanism is to decrease sclerostin expression, sclerose the subchondral bone and stiffen the joint with osteophyte formation. At this point, it is arguably too late for an HTO to have any effect beyond symptomatic relief. However, if the OA is at an early stage, the joint is suffering from a short-term abnormal load and an acute, potentially healable injury. Therefore what is needed is controlled inflammation and bone turnover (starting with bone breakdown) to repair the lesions. This requires an increase in sclerostin, potentially resulting in the oedematous bone marrow lesions seen in early OA.

Although this mechanism is the author's conjecture, there is support within the literature for considering these two circumstances as distinct and therefore explain some of the seemingly contradictory existing evidence about sclerostin. Firstly, sclerostin is not just expressed by osteocytes. There is evidence that it is expressed by chondrocytes but only in the presence of OA, leading Chan et al. to hypothesise that sclerostin has a protective effect against cartilage degradation (Chan *et al.*, 2011; Weivoda, Youssef and Oursler, 2017). Crucially however, in these animal studies, the mode of OA examined was post-traumatic, i.e. an acute injury rather than chronic degradation and Chan et al. also hypothesised that disease chronicity may explain some of the conflicting findings in sclerostin research (Chan *et al.*, 2011). Secondly, in human studies, both serum and synovial sclerostin levels have been shown to be inversely proportional to OA severity (as measured by KL grade), supporting the idea that it may be only in the late, radiographically apparent stages of OA that the decreases in sclerostin are seen (Mabey *et al.*, 2014). Thirdly there is evidence that sclerostin may have a negative feedback role in modulating inflammation (Rauch and Adachi, 2016), again giving weight to the hypothesis that increased sclerostin is needed in the early inflammatory stage of OA in order to allow controlled repair rather than inflammatory destruction.

The other two biomarkers which in Cohort 1 showed significant change were NR2D and GRIK4. Again both of these showed downregulation in the PM quadrant with NR2D also showing statistically significant differences in regulation of PM vs AL or PL on the Chi-square test (maintained in PM vs PL after addition of Cohort 2). NR2D is a subunit of an N-methyl-D-aspartate (NMDA) type glutamate receptor and GRIK4 is a subunit of a kainate type glutamate receptor. Both of these are known to be expressed in bone (in osteocytes, osteoblasts and osteoclast precursors) as well as other tissue types within the synovial joint as part of the glutamate signalling pathway (Lin *et al.*, 2008; Brakspear and Mason, 2012; Bonnet *et al.*, 2015). Glutamate is found in higher concentrations in osteoarthritic joints and even higher in inflammatory arthropathies and has been shown to act via receptors to cause pain, inflammation and cartilage degradation (Mason, 2004b; Flood *et al.*, 2007; Brakspear and Mason, 2012; Bonnet *et al.*, 2015, 2020). However, there is also evidence to suggest this pathway is mechanically regulated and has an anabolic effect on bone mass (Mason *et al.*, 1997; Lin *et al.*, 2008; Brakspear and Mason, 2012). Therefore, downregulation of NR2D or GRIK4 in the PM quadrant post HTO would suggest there is again a downregulation of bone formation/subchondral sclerosis.

It is of interest that the changes seen in SOST, NR2D and GRIK4 did not appear to be occurring in the same patients. This suggests these may be part of independent load related mechanisms and, along with the high degree of variability in the results, that each patient's response to a change in load is bespoke to the biological and mechanical environment of their knee at that time. The high degree of heterogeneity across the patients, coupled with the relatively small numbers does make it challenging to assess if these trends are significant, as witnessed by the change in overall results when the second cohort of five patients was added to the data set. However, the fact that three separate markers all initially showed changes all within the same

(PM) quadrant does support the hypothesis that HTO causes measurable changes in the biology of the knee joint. Modulation of these pathways could represent evidence that the progression of OA is being slowed or halted but it remains possible (given the previously discussed papers describing cartilage regrowth) that this could be creating an environment suitable for cartilage regrowth (potential reversal of osteoarthritic changes) (Kanamiya *et al.*, 2002; Koshino *et al.*, 2003; Parker *et al.*, 2011). The changes described in the PM quadrant represent novel findings as at the time of writing the author is unaware of any published literature showing changes in subchondral bone gene expression following HTO.

It is interesting that all these markers showed significance in the PM quadrant specifically. Although MOW-HTO offloads the knee in a medial to lateral direction, the question is then raised as to why the changes occur in the PM rather than AM quadrant. The answer lies in the mechanical action of the knee joint. During gait, the contact points of the tibiofemoral joint are located progressively posteriorly as the knee flexes and engages the "roll-back" mechanism previously described (Masouros, Bull and Amis, 2010).

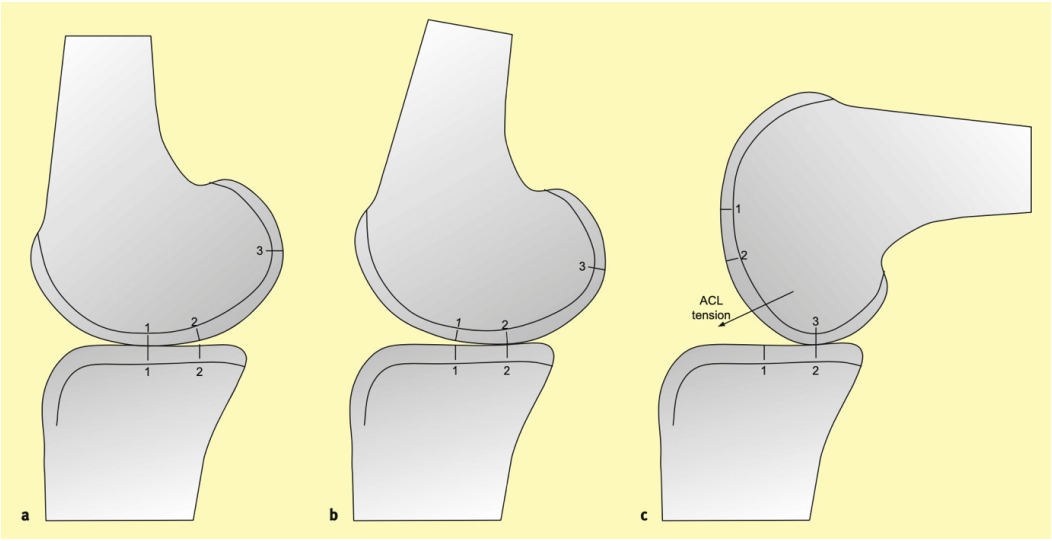

Figure 52: Knee joint kinematics in the sagittal plane during gait (reproduced from Masouros, Bull and Amis 2010). **a** Extension: contact is located centrally. **b** Early flexion: posterior rolling; contact continuously moves posteriorly. **c** Deep flexion:

femoral sliding; contact is located posteriorly; the unlocking of the ACL prevents further femoral roll back.

In addition, the more the knee is flexed, the further posterior the body weight is situated relative the knee and therefore the longer the moment arm and the greater the force transmitted through the joint. Previous studies have suggested walking produces forces of 3.4 body weight (BW), increasing to 4.3 BW when climbing stairs and 8.5 when walking downhill or getting up from a chair (Masouros, Bull and Amis, 2010). These increased forces are occurring during flexion and therefore as shown above are being disproportionately transmitted into the PM quadrant of the tibial plateau.

In addition, there is some early evidence that the site of knee contact force is also more posterior in patients with OA than controls (Meireles *et al.*, 2017).

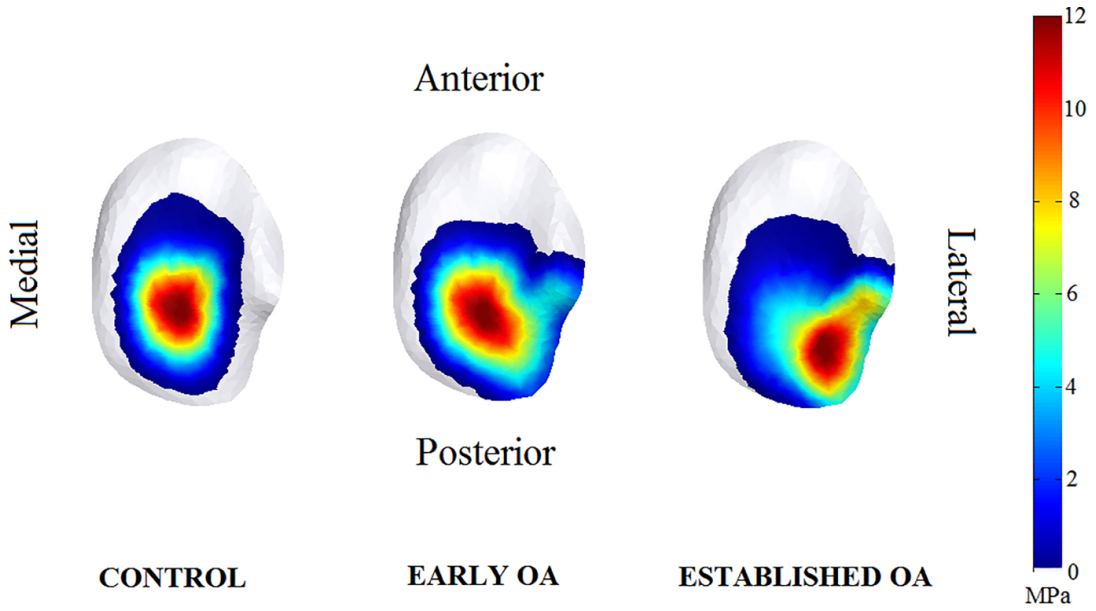

Figure 53: Group-averaged contact pressure distributions on the articular surfaces of medial tibial plateau at the time instant of the first peak medial knee contact force. Reproduced from Meireles et al. 2017.

Following the inclusion of Cohort 2, the magnitude of the changes seen for NR2D, GRIK4 and SOST were diminished and their statistical significance was lost (although some of the results remain close to the $p<0.05$ threshold). The analysis of differences between the two cohorts suggested some possible contributing factors to this. The significant difference in MPTA is particularly interesting as this suggests that Cohort 2 underwent an on average smaller correction than Cohort 1 which may explain why the effect of adding this Cohort into the biological data reduced the magnitude of any biological changes seen. This is also further evidence to support the hypothesis of interlinked biological and mechanical variables and will be explored further in Chapter 5.

It is however important to remember that the associations identified in this chapter are not necessarily causal and that with small numbers and a very heterogenous data set, the validity of these findings are far from certain. The heterogeneity of the data, combined with the small numbers of patients with complete data remains the main limitation in the study as a whole. Whilst all data represent within patient longitudinal changes in the proportion of gene expression in each quadrant, this does not allow the overall extent of gene expression to be quantified, only the extent of change in gene expression. For example, expression within a quadrant could go from 50% pre-operatively to 25% post-operatively, suggesting down regulation. However, the expression of that gene was increased across all quadrants, pre- to post-operatively, this could be erroneous.

There are also some methodological concerns regarding the lack of repeatability in count numbers after RT-PCR (Taylor *et al.*, 2019) and whether the adjustment of these to proportions is a valid mathematical analysis.

3.6 CONCLUSION

As hypothesised, there does appear to be evidence of measurable biological change in the subchondral bone of the tibial plateau following HTO. These changes seem to be seen specifically in the PM quadrant with some possible reciprocal changes in the PL quadrant for some markers. The changes seen suggest potential downregulation of pathways involved in pain, inflammation, cartilage degradation and bone turnover; all features of osteoarthritis. The fact this downregulation is seen across multiple markers, specifically in the quadrant most offloaded by MOW-HTO lends weight to the theory that mechanical changes cause biological change within the knee following HTO that may slow, halt or even reverse OA (by providing conditions favourable to cartilage regrowth) and is some of the first evidence identified that links these two domains.

The effect of the addition of Cohort 2 to the data however throws the validity of the initial findings into some doubt as the statistical significance of the results dissipated. The investigation of the differences between the cohorts suggests this may reflect the complex interplay of variables in a very heterogenous patient group where response is patient specific. It is of particular interest that the degree of correction appears to have an influence on the data and this will be explored in greater detail in a later chapter.

Chapter 4:

Radiological measurement of the effect of HTO on limb alignment

4.1 INTRODUCTION

HTO surgery is predicated on the idea that changing the shape of the proximal tibia reliably and predictably affects the alignment of the limb by shifting the mechanical weightbearing axis; a line drawn from the centre of the femoral head to the centre of the talus, across the knee joint. In medial opening wedge HTO (MOW-HTO), the technique directly increases the medial proximal tibial angle (MPTA) by opening a wedge on the medial side (Figure 54), thus creating more valgus limb alignment at the knee joint and reducing the mechanical tibiofemoral angle (mTFA). This is intended to create a lateral shift in the point at which the mechanical axis intersects the knee joint (i.e. the Mikulicz point). (Figure 55).

Figure 54: Medial opening wedge high tibial osteotomy

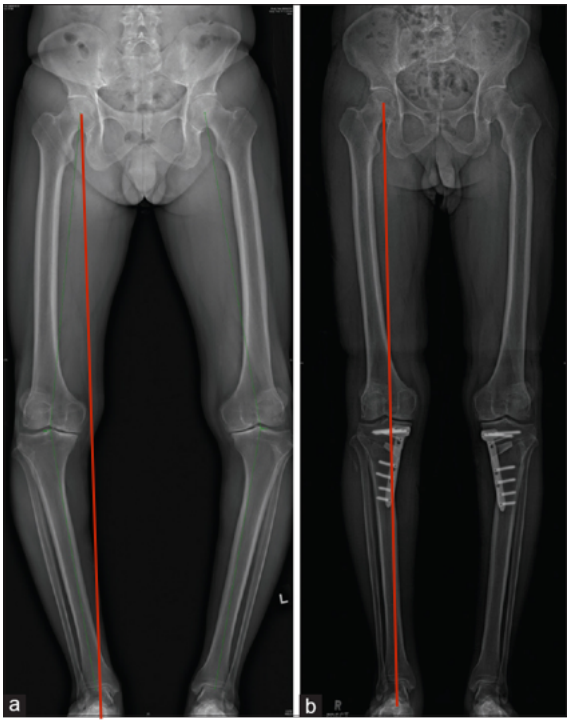

Figure 55: Increase in MPTA via MOW-HTO causes limb alignment to become more valgus with a lateralised Mikulicz point.

Current evidence, including a Cochrane review (Brouwer *et al.*, 2014), suggests HTO is clinically effective for unicompartmental knee OA however there remains much debate around the most accurate surgical methodology. The optimal corrected alignment originally posited by Fujisawa was to aim for a mechanical axis intercepting the tibial plateau at approximately 62% of the medial to lateral distance (Fujisawa, Masuhara and Shiomi, 1979). However, subsequent studies have suggested that the risks of overcorrection beyond 65% (lateral compartment pain and degeneration) are just as significant as the risks of undercorrection below 50% (recurrence of varus malalignment and continuing medial compartment symptoms) and suggest a compromise aim of 55% (Sabzevari *et al.*, 2016; Martay *et al.*, 2018).

Key to being able to evaluate this further is the facility to predict and produce the desired post-operative alignment in an accurate and repeatable way. However, this has proved challenging, with multiple studies investigating changes to surgical technique or preoperative planning that could increase

correction accuracy, e.g. involving computer assisted navigation, templating software, patient specific instrumentation (Marti *et al.*, 2004; Cerciello *et al.*, 2020; He *et al.*, 2020; Kim *et al.*, 2020; Miller, Maddox and El-Daccache, 2020; Alemayehu *et al.*, 2021).

All these methodological changes concentrate on increasing the accuracy of the MPTA correction (i.e. wedge size) intraoperatively and are therefore based on the premise that a change to MPTA causes a predictable change in mechanical axis (as measured by mTFA or MP). This is the basis of the widely used Miniaci method of preoperative planning, where either digitally or manually, a proximal tibial wedge angle and height is computed from geometric analysis of the limb alignment (Miniaci *et al.*, 1989; Elson, Petheram and Dawson, 2015). However, there is increasing evidence that despite seemingly accurate pre-operative planning, discrepancies are found between the planned and achieved correction angles, suggesting that confounding factors, such as ligamentous laxity or degree of correction may be at work (Dugdale, Noyes and Styer, 1992; Amis, 2013; S. H. Kim *et al.*, 2017; Miller, Maddox and El-Daccache, 2020; So *et al.*, 2020).

The premise of this chapter is that the relationship between MPTA and lower limb alignment (as measured by mTFA or MP) is not as simple as previously supposed and that the change in mechanical axis resultant from a change in MPTA is translated and modulated via a range of compensatory mechanisms within the limb, specific to the individual.

The previous chapter demonstrated how varied the biological response is to HTO within the knee joint. It is hoped that by close analysis of the radiographs, some sources of that variability may be identified.

4.2 AIM

To address Hypothesis 2 (see Introduction) that:
"*Medial opening wedge HTO causes measurable changes in the radiological alignment of the lower limb beyond those anticipated as a result of the surgical correction.*"

This chapter aims to first investigate the relationship between MPTA and mechanical axis measures within the study population to assess the degree of correlation. This will then be followed by an investigation of other resultant alignment changes seen within the limbs on clinical radiographs. These will include measures of hip, ankle and subtalar joint alignment as well as ligamentous change within the knee (joint line convergence angle) as defined in the methodology.

4.3 METHODOLOGY

Patient recruitment and surgical procedure were performed as described in Chapter 2.

64 patients underwent medial opening wedge HTO surgery for medial compartment OA. Of these 20 did not have pre- and postoperative full-length radiographs of both legs and 9 had radiographs that were inadequate to take all measurements (e.g. incomplete inclusion of both hips and both ankles). Therefore, the number of participants (n) included at each stage of the analysis varied from 35 to 63 depending on availability of suitable radiographs (63 patients had pre-operative and post-operative radiographs sufficient to allow MPTA/mTFA/MP analysis. 35 had pre- and post-operative radiographs sufficient to allow full analysis of all hip, knee and ankle measures).

Patients underwent full length radiographs of the lower limbs as part of their routine clinical care in order to assess their alignment pre-operatively, both for diagnosis and for operative planning. This was then repeated at their first follow up appointment (six weeks postoperatively) to assess the resultant correction, providing the patient was comfortable to bear weight equally across both legs. X-ray images were taken by clinical radiographers at the Cardiff and Vale Orthopaedics Centre (CAVOC), University Hospital Llandough. Patients were instructed to stand comfortably with equal weight on both legs. Rotation at the knee was controlled by ensuring patellae are facing forward but foot position is at patient discretion. Radiographs were viewed using SYNAPSE (PACS) software (Fujifilm, Tokyo, Japan) and measurements were taken with digital rulers using this platform by the author.

The standard radiographic measures used clinically to assess and plan HTO (mTFA, MP and MPTA) were measured using the standard landmarks as defined below (Staubli and Jacob, 2010; Elson, Petheram and Dawson, 2015). In order to assess the coronal plane effect of HTO on the hip and ankle/subtalar joint several additional radiographic measures (including some designed by the author) were utilised and will be explained below. The novel measures are not yet validated but are undergoing analysis of inter and intraobserver reliability as part of a post-thesis project.

Whole lower-limb alignment was assessed using the mechanical tibiofemoral angle (mTFA). As shown in Figure 56, this is the angle between the mechanical axis of the femur (measured from centre of femoral head to midpoint of the intercondylar notch) and the mechanical axis of the tibia (centre of the tibial plateau to centre of the tibial plafond) and expressed in degrees of varus.

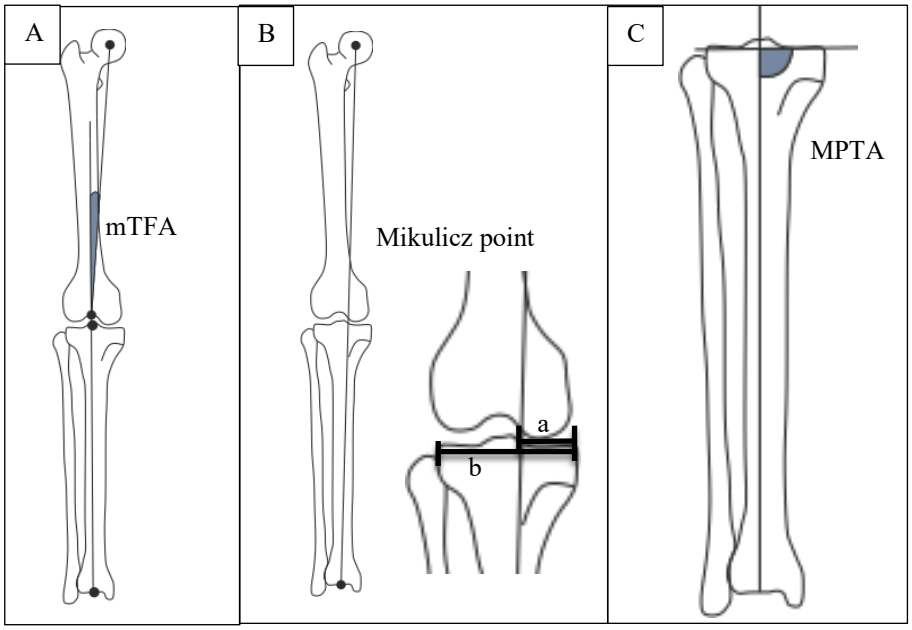

Figure 56: A: Mechanical tibiofemoral angle (mTFA). B: Mikulicz point (MP) (=a/b). C: Medial proximal tibial angle (MPTA)

The effect of this angle on the weightbearing axis at the knee was expressed using the Mikulicz point (MP), defined as the percentage of the tibial plateau from medial to lateral intersected by the mechanical axis of the limb (Staubli and Jacob, 2010; Elson, Petheram and Dawson, 2015) (Figure 56).

The degree of proximal tibial deformity was measured via the medial proximal tibial angle (MPTA), measured as the angle between the tibial plateau and the axis of the tibia (Figure 56).

The degree of angulation within the knee joint itself was measured using the joint line convergence angle (JLCA knee), measured as the medial angle between a line tangential to the femoral condyles and a line tangential to the tibial plateau concavities (Figure 57).

These measurements were chosen to give markers of severity of deformity and, when compared pre- to postoperatively, to quantify the magnitude of the alignment change following the surgery. These measurements are routinely

used for the planning of HTO surgery (Staubli and Jacob, 2010; Amis, 2013; Elson *et al.*, 2015; Elson, Petheram and Dawson, 2015; Lee *et al.*, 2015; Sabzevari *et al.*, 2016).

The coronal alignment of the hip was assessed using the femoral neck-shaft angle (FNS) to measure the intrinsic varus/valgus of the proximal femur, and the ground-femoral neck (GFN) angle as a measure of hip position (abduction/adduction) (Figure 57). GFN angle is a new measure proposed by the author. It is proposed that one method of compensating for a change in coronal alignment at the knee would be to abduct or adduct at the hip joint. As the hip rotates, this would result in a change of angle between the femoral neck and the ground (i.e. the horizontal of the coronal plane radiograph as the image is taken parallel to the floor). This could be confounded if there were rotation in the axial plane hence the use of FNS as a control measure. FNS is intrinsic to the bony architecture of the proximal femur so if this remains constant, it suggests any change in GFN is true abduction/adduction.

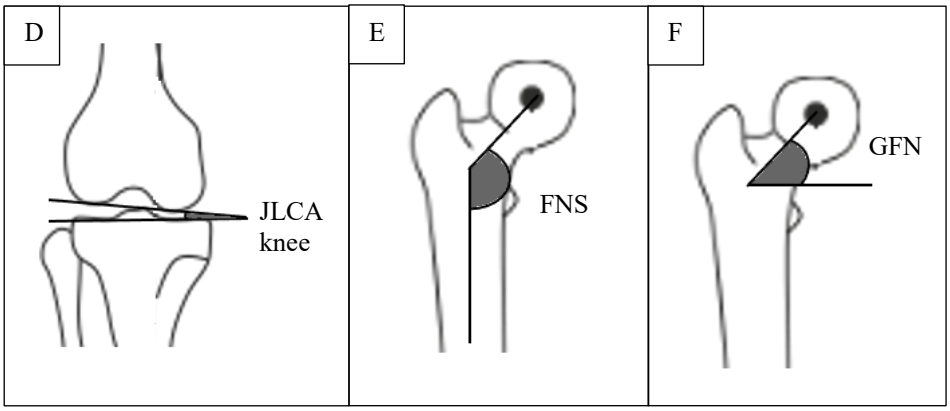

Figure 57: D: Joint line convergence angle (JLCA) of the knee. E: Femoral neck-shaft angle (FNS). F: Ground-femoral neck angle (GFN).

Similarly, compensation in the coronal plane may occur distally, either through the ankle joint or through adaptation at the subtalar joint. The alignment of the subtalar joint was measured using the ground-talus angle

(GT), a measure of the angle between the proximal surface of the talus and the ground (i.e. the horizontal of the radiograph as the image is taken parallel to the floor), as shown in Figure 58. The GT represents the degree of compensation at the subtalar joints of the foot in order to maintain a plantigrade sole in the presence of a varus lower limb. This angle has variable terminology in the existing literature (talar inclination, ankle joint line orientation, talar tilt) but in line with the most prevalent definitions, taluses tilted laterally (medial side higher) had positive values whereas those tilted to the medial side were negative (Lee *et al.*, 2015; Xie *et al.*, 2019). The terminology used has been chosen by the author to be descriptive and therefore give maximum clarity of meaning.

The lateral distal tibial angle (LDTA), the angle between the tibial axis and the tibial plafond, was also measured and the difference between the two, the joint line convergence angle (JLCA) of the ankle was calculated (Figure 58).

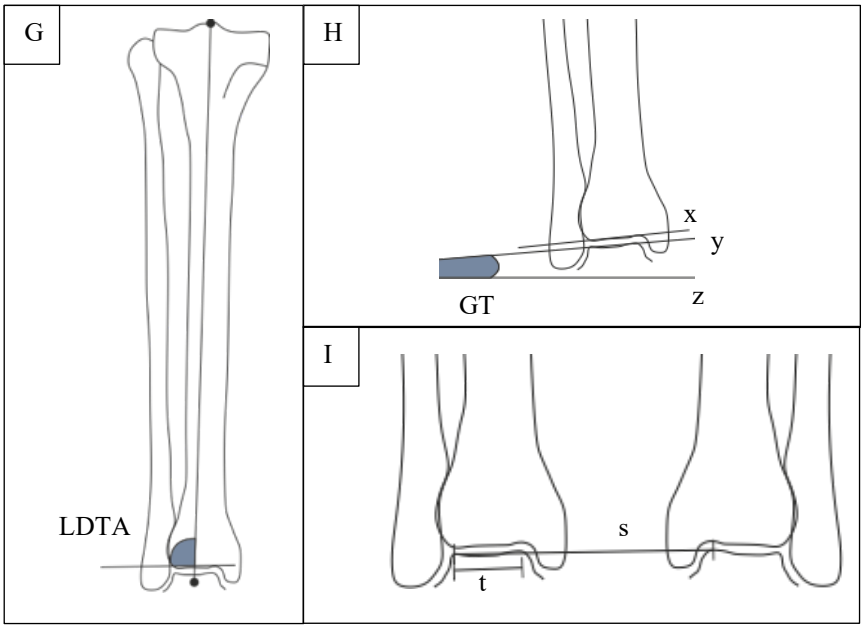

Figure 58: G: Lateral distal tibial angle (LDTA). H: Ground-talus angle (GT) calculated as the angle between y and z. Joint line convergence angle (JLCA) of the ankle calculated as the angle between x and y. I: Stance width (SW), calculated as s/t.

An increased JLCA would suggest adaptation by way of joint laxity within the ankle (tibiotalar) joint itself.

The LDTA should not change in the short-term following HTO as it is inherent to the distal tibia so therefore also acts a control variable to identify any inter-radiograph error (e.g. from a rotated film).

In addition to the measurements within the operated limb, anecdotal evidence from early viewings of radiographs suggested that some patients may adapt to a change in alignment at the knee by a change in stance. Therefore, to explore this, the stance width (SW) was also measured from a fixed point on one talus to the corresponding point on the opposite side (Figure 58). By dividing this by the width of the talus, (measured from the highest points medially and laterally) this gives a standardised measure across patients/radiographs for SW in the unit of talar-widths, independent of radiograph magnification or patient size. A change in stance following HTO has not been reported in previous literature and no standard measurement exists at the time of writing. Therefore, this method of assessing stance width represents a new measure as proposed by the author, standardised across patients and radiographs (although as mentioned previously, inter/intraobserver reliability analysis is pending).

Results were analysed using SPSS software (IBM SPSS Statistics for Macintosh, Version 26.0; Armonk, NY, USA).

To investigate the relationship between MPTA and mechanical axis measures, a two-tailed Pearson's correlation co-efficient was applied to the pre-operative, post-operative and the pre- to post-operative differences, with a significance level set at 0.05. The Pearson's correlation co-efficient was chosen after confirming the data met the assumptions for this test (linearity, lack of outliers and normality as assessed by Shapiro-Wilk's test).

To investigate the influence of additional alignment variables, first a paired sample T-test was used to identify which measures showed significant change from pre- to postoperative (with a significance level of $p < 0.05$). Following this, a two-tailed Pearson's correlation coefficient was applied to the pre-operative measurements and the pre- to postoperative differences, again with a significance level set at 0.05.

There are variations in how Pearson's correlation coefficient results are interpreted in the literature with a variety of largely arbitrary cut offs and varied use of language (Schober, Boer and Schwarte, 2018; *11. Correlation and regression | The BMJ*, 2020; Laerd statistics, 2022; www.andrews.edu, 2022). For the purposes of this chapter, the coefficient results will be described as shown in table 21.

Pearson Correlation Coefficient	Interpretation
0.00 - 0.10	Negligible
0.10 - 0.39	Weak
0.40 - 0.69	Moderate
0.70 - 0.89	Strong
0.90 - 1.00	Very strong

Table 21: Pearson Correlation Coefficient Interpretation.

4.4 RESULTS

4.4.1 MPTA vs mechanical axis

There were 63 operated limbs in this cohort evenly split right and left (54% right, 46% left). The mean age at operation was 51 (range 33 to 65) and 94% were men.

Looking first at the preoperative values (n=63) (Table 22) as expected there are significant correlations ($p<0.05$) between the three variables.

	MPTA_pre (°)	mTFA_pre (°)	MP_pre (%)
MPTA_pre (°)		-0.557 (p<0.001)	0.546 (p<0.001)
mTFA_pre (°)	-0.557 (p<0.001)		-0.958 (p<0.001)
MP_pre (%)	0.546 (p<0.001)	-0.958 (p<0.001)	

Table 22: Pearson's correlation coefficient for pre-operative MPTA, mTFA and MP.

MPTA correlates negatively with mTFA and positively with MP as would be expected (showing lower levels of varus in patients with a higher/more normal MPTA). The two mechanical axis measures are also negatively associated as expected (increased varus means a more medial and therefore lower MP).

However, it is the strength of correlation which is most interesting. The correlation between the two mechanical axis measures is close to -1 (-0.958) which is what would be expected given these are measuring the same factor in alignment but via different methods. The very strong degree of correlation between the two independently measured variables suggests good levels of accuracy in the measurement process.

The correlation between MPTA and the mechanical axis measures (mTFA and MP) however is only moderate (-0.557 mTFA and 0.546 MP respectively).

The relative variability in the correlations can be seen in the following scatter plots (Figures 59-61):

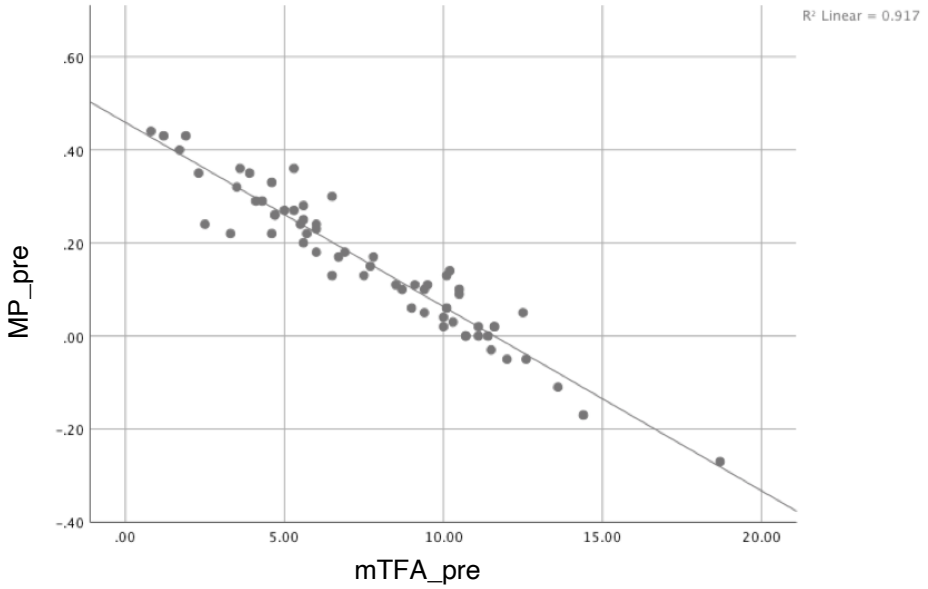

Figure 59: Scatter plot of pre-operative MP versus mTFA

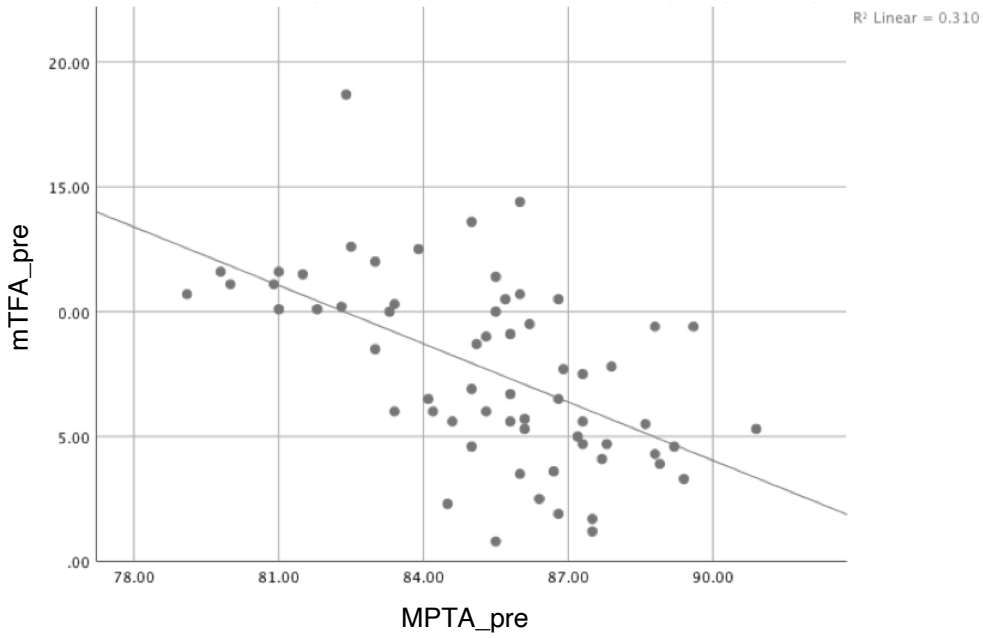

Figure 60: Scatter plot of pre-operative mTFA versus MPTA

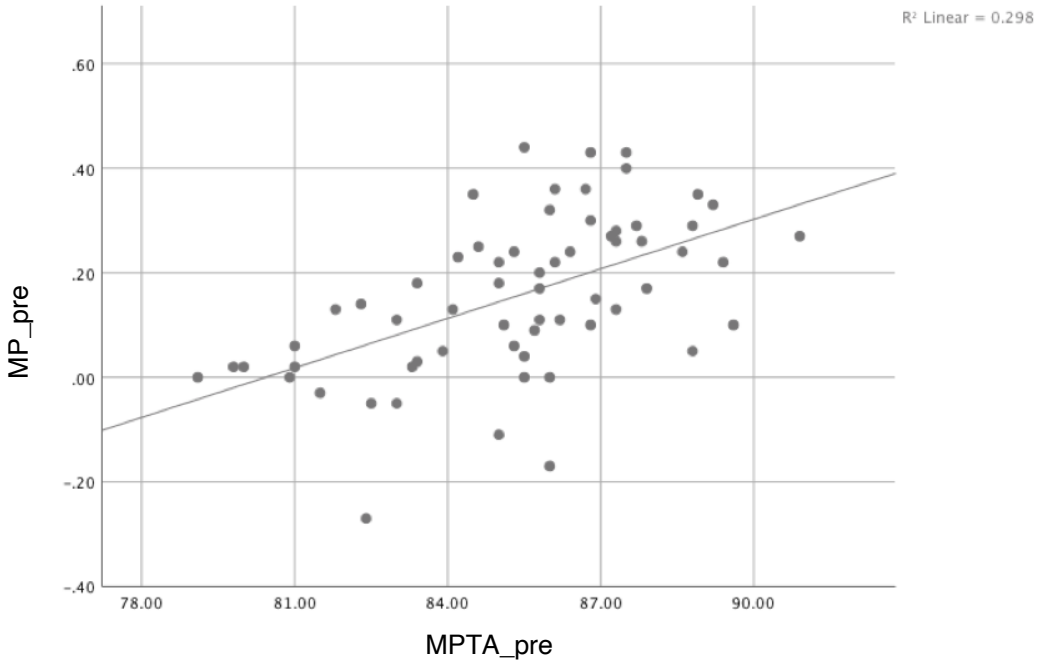

Figure 61: Scatter plot of pre-operative MP versus MPTA

Moving to the post-operative values (n=55) (Table 23), a similar picture is seen with the expected significant correlations ($p<0.05$) between the three variables.

	MPTA_post (°)	**mTFA_post (°)**	**MP_post (%)**
MPTA_post (°)		-0.522 (p<0.001)	0.518 (p<0.001)
mTFA_post (°)	-0.522 (p<0.001)		-0.969 (p<0.001)
MP_post (%)	0.518 (p<0.001)	-0.969 (p<0.001)	

Table 23: Pearson's correlation coefficient for post-operative MPTA, mTFA and MP.

The correlations also show the same pattern as the pre-operative data with a very strong correlation between mTFA and MP (-0.969) and a moderate correlation between MPTA and mTFA (-0.522) or MP (0.518).

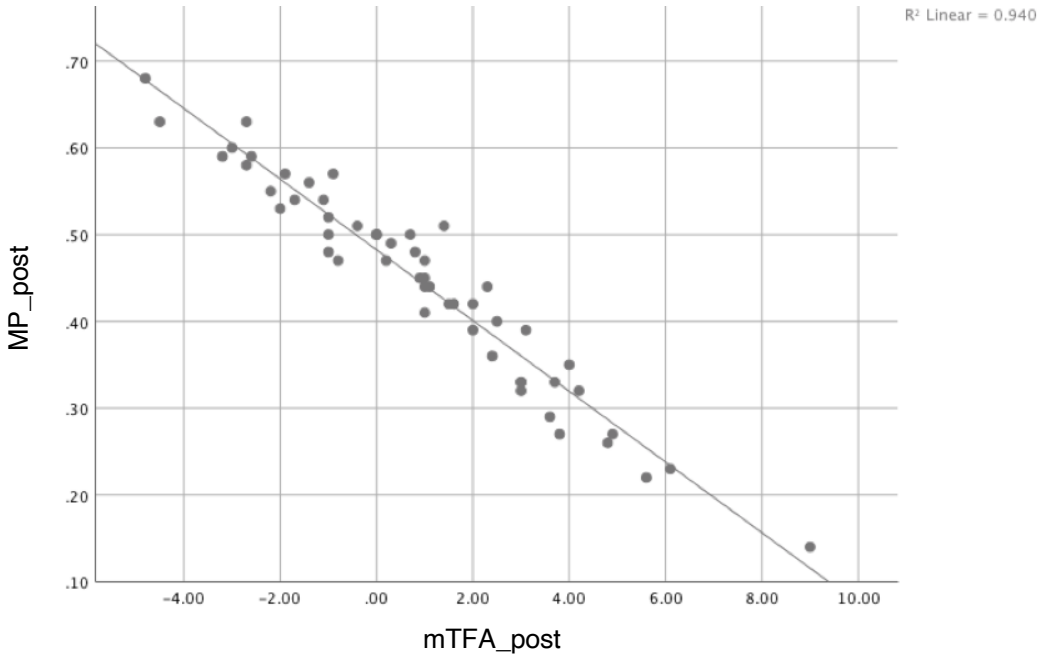

Figure 62: Scatter plot of post-operative MP versus mTFA

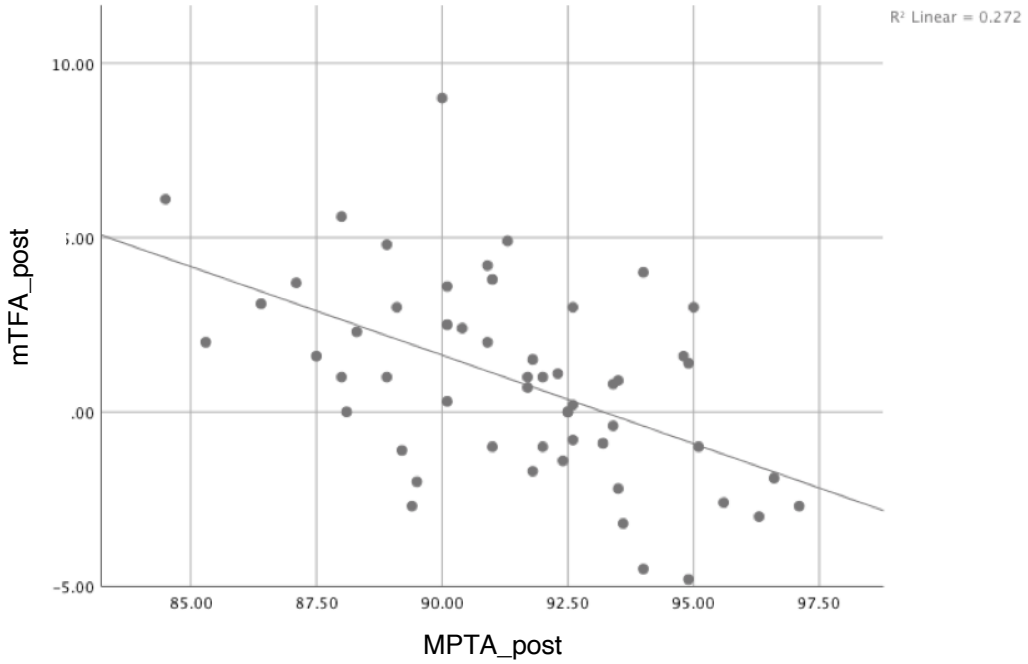

Figure 63: Scatter plot of post-operative mTFA versus MPTA

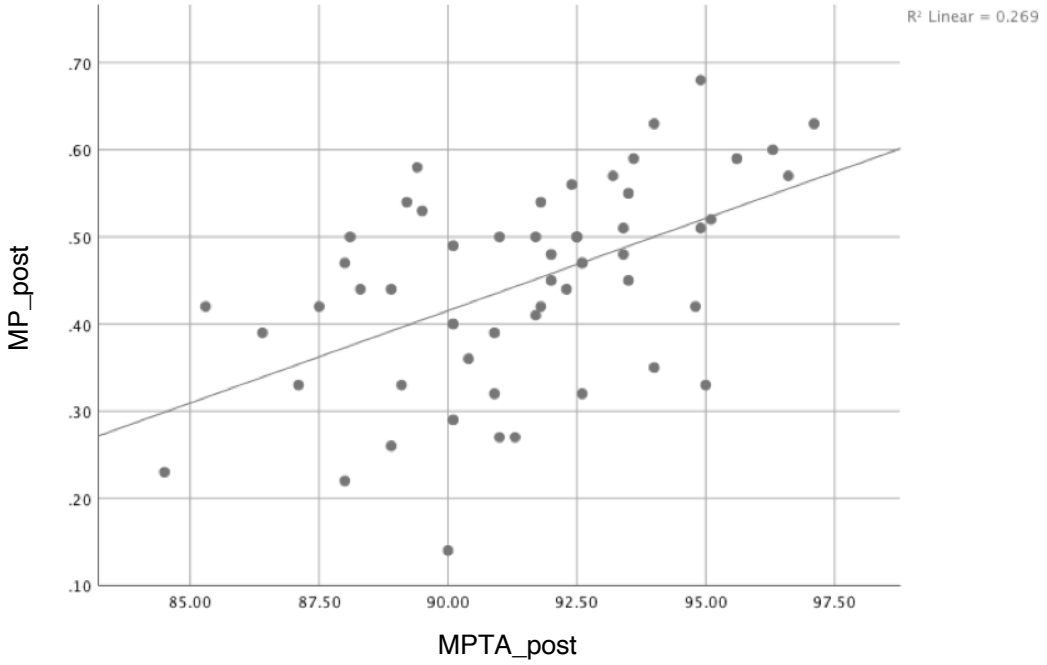

Figure 65: Scatter plot of post-operative MP versus MPTA

Finally, the correlations between the values of the pre- to post-operative differences were assessed (n=55), calculated by subtracting the pre-operative results from the post-operative results (Table 24). A similar picture is seen with the expected significant correlations ($p<0.05$) between the three variables.

	MPTA_diff (°)	**mTFA_diff (°)**	**MP_diff(%)**
MPTA_diff (°)		-0.788 (p<0.001)	0.777 (p<0.001)
mTFA_diff (°)	-0.788 (p<0.001)		-0.951 (p<0.001)
MP_diff (%)	0.777 (p<0.001)	-0.951 (p<0.001)	

Table 24: Pearson's correlation coefficient for pre- to post-operative differences in MPTA, mTFA and MP.

Here again there is the same very strong correlation between mTFA and MP (-0.951). Again, the correlations between MPTA and mTFA (-0.788) or MP (0.777) are less strongly correlated than mTFA and MP to each other but

show a higher strength of correlation than for the pre- or post-operative measures alone.

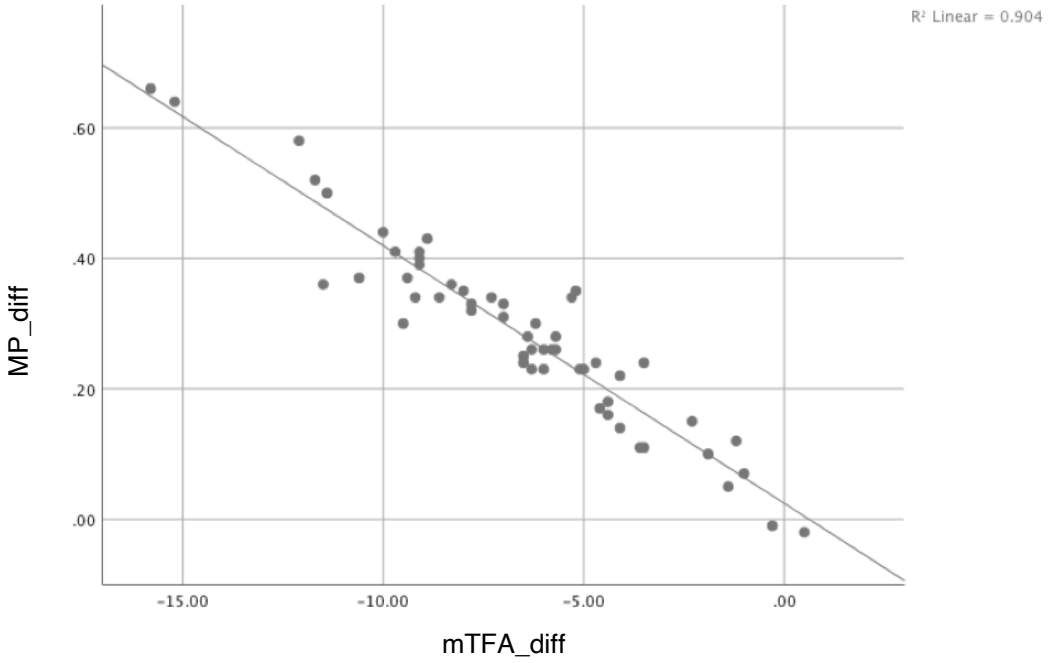

Figure 65: Scatter plot of pre- to post-operative difference MP versus mTFA

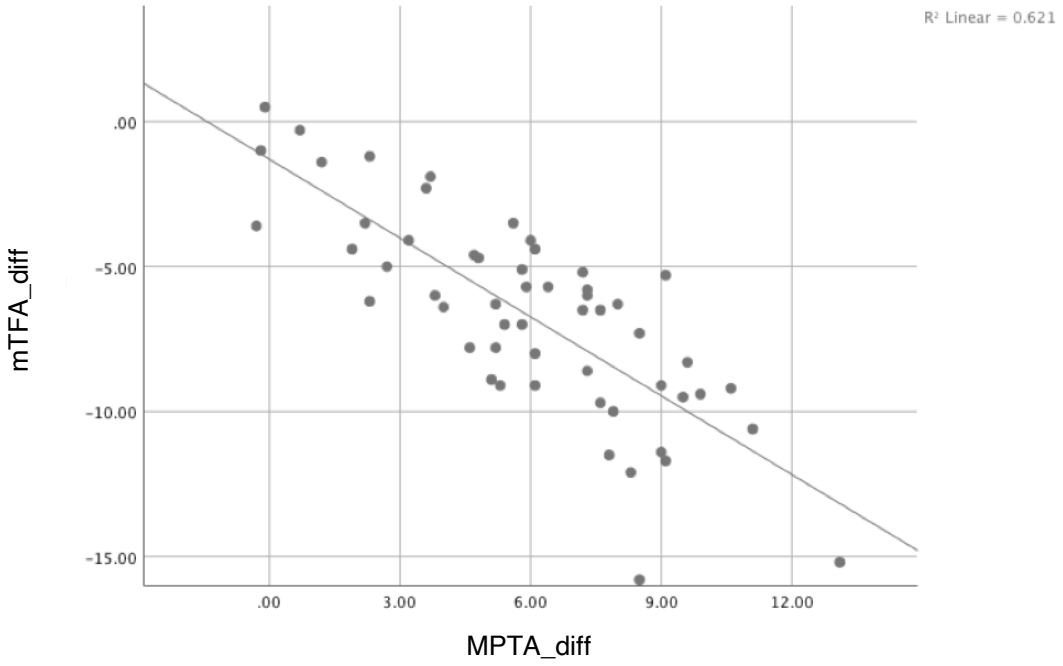

Figure 66: Scatter plot of pre- to post-operative difference mTFA versus MPTA

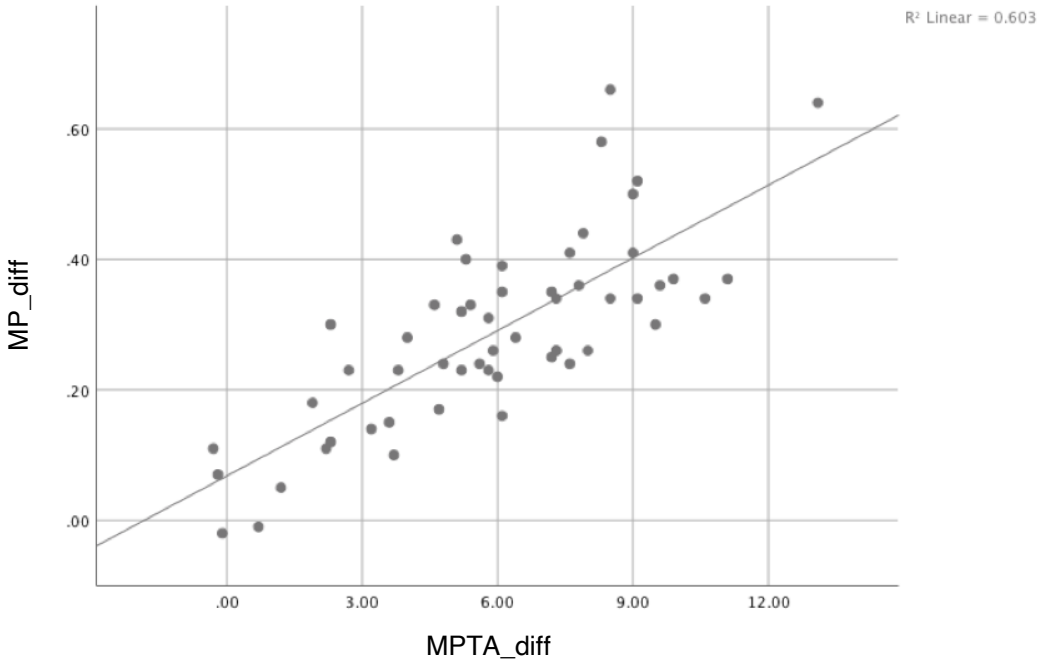

Figure 67: Scatter plot of pre- to post-operative difference MP versus MPTA

4.4.2 Effects on the limb and stance

There were 35 operated limbs in this cohort who had a complete set of radiographs. 33 were male (94%), aged 33 to 62 with a mean age of 51. There was an even split of left and right knees (54% left).

As shown in Table 25, The paired T-test showed significant differences pre- to postoperatively in MPTA, mTFA and MP as expected. Neither JLCA knee or either of the hip measures (GFN, FNS) showed significant change and neither did LDTA or JLCA ankle. However, a significant decrease in ground talus angle (GT), was observed (-5.43°, -157%) as was a significant increase in stance width (1.31 talar widths, 31%).

	Pre-HTO	Post-HTO	Difference	*p* value
mTFA (°)	7.96 ± 4.02	1.52 ± 2.77	-6.45 ± 3.79	<0.001*
Mikulicz point (%)	15.1 ± 16.1	42.1 ± 11.6	27.0 ± 15.6	<0.001*
MPTA (°)	85.7 ± 2.87	91.1 ± 2.84	5.40 ± 3.28	<0.001*
JLCA knee (°)	3.97 ± 1.74	3.57 ± 1.89	-0.40 ± 1.68	0.169
GFN (°)	42.4 ± 6.94	44.3 ± 6.40	1.91 ± 9.03	0.218
FNS (°)	128 ± 6.85	128 ± 6.62	-0.23 ± 3.71	0.718
LDTA (°)	88.3 ± 3.61	87.7 ± 4.33	-0.64 ± 3.62	0.303
GT (°)	3.46 ± 4.46	-1.97 ± 5.30	-5.43 ± 4.50	<0.001*
JLCA ankle (°)	0.03 ± 2.96	0.09 ± 2.91	0.06 ± 2.67	0.900
Stance width (talar-widths)	4.22 ± 1.70	5.53 ± 1.72	1.31 ± 2.24	0.001*

All values are presented as the mean ± standard deviation
Abbreviations: mTFA, mechanical tibio-femoral angle; MPTA, medial proximal tibial angle; LDTA, lateral distal tibial angle; GT, ground-talus angle; JLCA, joint line convergence angle; GFN, ground-femoral neck angle; FNS, femoral neck-shaft angle.
*Significant difference compared to preoperatively

Table 25: Comparison of radiographic measures before and after HTO

When the preoperative correlations were analysed (Table 26), there were moderate, significant correlations between the measures of limb alignment (mTFA, MP) and the level of proximal tibial deformity (MPTA) as seen in the larger data set earlier in the chapter (-0.615 and 0.601 for mTFA and MP respectively). The mTFA and MP were also moderately correlated with JLCA knee, GFN and FNS, all of which were significant.

Looking at the ankle, mTFA was weakly correlated with GT, suggesting that some of the patients who had a higher degree of limb varus may have a more laterally tilted talus. MPTA was moderately positively correlated with JLCA. LDTA was moderately correlated with GT and showed a weak positive correlation with SW preoperatively. Again, all correlations were significant.

	mTFA	Mikulicz point	MPTA	LDTA	JLCA knee	GFN	FNS	GT	JLCA ankle	Stance width
mTFA		-0.971*	-0.615*	-0.050	0.570*	-0.650*	-0.453*	0.365*	-0.211	-0.084
Mikulicz point	-0.971*		0.601*	0.156	-0.621*	0.625*	0.438*	-0.316	0.245	0.101
MPTA	-0.615*	0.601*		0.006	-0.215	0.261	0.058	-0.071	0.492*	0.086
LDTA	-0.050	0.156	0.006		-0.256	-0.133	-0.078	0.528*	0.267	0.391*
JLCA knee	0.570*	-0.621*	-0.215	-0.256		-0.454*	-0.322	0.187	0.120	-0.049
GFN	-0.650*	0.625*	0.261	-0.133	-0.454*		0.911*	-0.403*	-0.193	-0.162
FNS	-0.453*	0.438*	0.058	-0.078	-0.322	0.911*		-0.325	-0.252	-0.102
GT	0.365*	-0.316	-0.071	0.528*	0.187	-0.403*	-0.325		0.264	0.144
JLCA ankle	-0.211	0.245	0.492*	0.267	0.120	-0.193	-0.252	0.264		0.247
Stance width	-0.084	0.101	0.086	0.391*	-0.049	-0.162	-0.102	0.144	0.247	

All values are presented as Pearson's correlation coefficient (*r*).
Abbreviations: mTFA, mechanical tibio-femoral angle; MPTA, medial proximal tibial angle; LDTA, lateral distal tibial angle; GT, ground-talus angle; JLCA, joint line convergence angle; GFN, ground-femoral neck angle; FNS, femoral neck-shaft angle.
*Correlation is significant at the 0.05 level (2-tailed)

Table 26: Correlation of radiographic measures before HTO

Postoperatively (Table 27) there was a similar pattern of moderate correlations between the measures of limb alignment (mTFA, MP) and MPTA. Interestingly, there were no significant correlations of mTFA, MP or MPTA with any of the measures regarding the hip or ankle. However, GT correlated significantly with LDTA (strong) and JLCA ankle (weak). Stance width showed a weak negative correlation with FNS.

	mTFA	Mikulicz point	MPTA	LDTA	JLCA knee	GFN	FNS	GT	JLCA ankle	Stance width
mTFA		-0.980*	-0.524*	-0.029	0.123	0.262	-0.130	0.246	0.228	0.057
Mikulicz point	-0.980*		0.519*	0.064	-0.156	-0.261	0.085	-0.236	-0.194	-0.063
MPTA	-0.524*	0.519*		-0.029	0.278	-0.160	-0.175	-0.076	0.104	-0.008
LDTA	-0.029	0.064	-0.029		-0.131	-0.251	-0.091	0.726*	-0.030	0.139
JLCA knee	0.123	-0.156	0.278	-0.131		0.221	-0.117	0.180	0.326	0.205
GFN	0.262	-0.261	-0.160	-0.251	0.221		0.190	-0.113	-0.096	-0.076
FNS	-0.130	0.085	-0.175	-0.091	-0.117	0.190		-0.226	-0.317	-0.381*
GT	0.246	-0.236	-0.076	0.726*	0.180	-0.113	-0.226		0.352*	0.187
JLCA ankle	0.228	-0.194	0.104	-0.030	0.326	-0.096	-0.317	0.352*		0.288
Stance width	0.057	-0.063	-0.008	0.139	0.205	-0.076	-0.381*	0.187	0.288	

All values are presented as Pearson's correlation coefficient (*r*).
Abbreviations: mTFA, mechanical tibio-femoral angle; MPTA, medial proximal tibial angle; LDTA, lateral distal tibial angle; GT, ground-talus angle; JLCA, joint line convergence angle; GFN, ground-femoral neck angle; FNS, femoral neck-shaft angle.
*Correlation is significant at the 0.05 level (2-tailed)

Table 27: Correlation of radiographic measures following HTO

When the differences pre- to postoperatively were analysed (Table 28), similar to preoperatively, there was strong correlation between the markers of limb alignment and proximal tibial deformity (mTFA, MP, MPTA). However, there was also moderate correlation between all three of these angles and GT. In addition, GT was weakly negatively correlated with SW.

At the hip, GFN was moderately correlated with mTFA, MP and MPTA as well as JLCA ankle but FNS only correlated (moderately) with LDTA. There were no correlations with JLCA knee.

	mTFA	Mikulicz point	MPTA	LDTA	JLCA knee	GFN	FNS	GT	JLCA ankle	Stance width
mTFA		-0.963*	-0.848*	-0.079	0.231	-0.478*	0.092	0.416*	-0.227	-0.089
Mikulicz point	-0.963*		0.833*	0.179	-0.298	0.465*	-0.149	-0.404*	0.273	0.133
MPTA	-0.848*	0.833*		0.033	-0.241	0.459*	-0.069	-0.445*	0.151	0.182
LDTA	-0.079	0.179	0.033		-0.111	-0.084	-0.505*	0.332	0.128	0.028
JLCA knee	0.231	-0.298	-0.241	-0.111		-0.106	-0.023	0.240	-0.294	-0.155
GFN	-0.478*	0.465*	0.459*	-0.084	-0.106		0.203	-0.180	0.457*	0.144
FNS	0.092	-0.149	-0.069	-0.505*	-0.023	0.203		-0.192	0.067	-0.149
GT	0.416*	-0.404*	-0.445*	0.332	0.240	-0.180	-0.192		0.017	-0.377*
JLCA ankle	-0.227	0.273	0.151	0.128	-0.294	0.457*	0.067	0.017		0.021
Stance width	-0.089	0.133	0.182	0.028	-0.155	0.144	-0.149	-0.377*	0.021	

All values are presented as Pearson's correlation coefficient (*r*).
Abbreviations: mTFA, mechanical tibio-femoral angle; MPTA, medial proximal tibial angle; LDTA, lateral distal tibial angle; GT, ground-talus angle; JLCA, joint line convergence angle; GFN, ground-femoral neck angle; FNS, femoral neck-shaft angle.
*Correlation is significant at the 0.05 level (2-tailed)

Table 28: Correlation of radiographic measures pre- to post-HTO

4.5 DISCUSSION

Looking first at the relationship between MPTA and the mechanical axis, it is clear these measures are not as closely related as one might expect given it is the basis of the entire HTO planning procedure. In each analysis (pre-, post- and pre- to post-operative difference), the very strong, significant correlation between mTFA and MP is to be expected as these quantify the same feature (whole limb alignment). However, as these two variables are measured independently, this correlation provides some confirmation of the accuracy of the measurement process.

The correlations between MPTA and these two measures (mTFA and MP) pre- and post-operatively are significant but only moderate in strength suggesting there are other factors for these patients which are contributing to the limb malalignment in addition to the abnormal MPTA. Interestingly, when these are combined to examine the pre- to post-difference, MPTA correlates more strongly with alignment (coefficients in the region of +/- 0.8 rather than +/- 0.5). However, this still suggests that if the MPTA were to increase (via an HTO procedure) by the same amount in two separate patients with the same starting mTFA/MP, this would not guarantee the same post-operative alignment. This is crucial when it comes to the reproducibility of the procedure and ability to counsel the patient on the likely outcome.

This finding also opens the question as to why these two hypothetical patients might respond differently to the same change in MPTA. Is the change in alignment during surgery being in some way modulated by compensatory changes at other joints? The initial T-test suggested that HTO in addition to changing the alignment at the knee was also causing changes to subtalar joint alignment (GT) and stance width. Following HTO, there was a change from mean lateral to mean medial talar tilt, representing

compensation at the subtalar joint in order to keep a plantargrade sole. The magnitude of this change (5.4°) was in line with the two other published studies (6.8° in Lee et al 2015 and 4.3° in Choi et al 2017) (Lee *et al.*, 2015; Choi *et al.*, 2017).

The clinical significance of this is unclear as there is little published work looking into the effects of HTO on the ankle and foot. Normal values for "talar tilt" are approximately 3° when weightbearing (Dowling, Giakoumis and Ryan, 2014; Lau *et al.*, 2022), however this is when the measure is being used to assess ligamentous laxity in the ankle rather than position of the subtalar joint. In this study, there was no significant difference in the JLCA of the ankle, supporting the hypothesis that it is the subtalar joints that are adapting in the coronal plane, rather than the ligaments of the ankle joint itself. Lee et al. do comment that their HTO cohort had more laterally tilted taluses pre-operatively which improved post-operatively but did not normalise relative to their control group (figures for the control group are not quoted)(Lee *et al.*, 2015). Choi et al. presumed that 0° of "talar inclination" (their analogous term to Ground Talus angle) was normal and as such noted that one cohort of their HTO patients went from a laterally tilted talus to a more normal alignment, whereas a second cohort went from a normally aligned talus preoperatively to a medially tilted abnormal position post-operatively. Interestingly they found that those in the first cohort reported improved ankle symptoms whereas those in the second cohort had worsened ankle symptoms (Choi *et al.*, 2017). This aligns with the isolated case reports of HTO being used successfully to treat symptomatic malalignment at the ankle (Takeuchi, Saito and Koshino, 2008; Elson *et al.*, 2013) and the findings that HTO can significantly reduce ankle contact pressures (Suero *et al.*, 2015).

Although it is not possible to be conclusive from the data of this study, it does support the clinical theory that normal subtalar alignment is a GT of 0° and

that HTO may affect the ankle and subtalar joints either positively or negatively by increasing the medial tilt of the talus. This study clearly shows there is a high degree of variability in the starting subtalar alignment in these patients and this is not routinely clinically assessed preoperatively (as witnessed by the paucity of literature in this area). However, without thorough assessment of the ankle and subtalar joints preoperatively (looking for alignment and pre-existing OA) it is impossible to counsel the patient on how HTO may affect these joints.

Further distal compensatory changes were seen in stance width. Post-HTO, when asked to stand comfortably with their knees facing forward for their radiographs, patients were standing with their feet significantly (31%) further apart. This is a novel finding not previously reported in the literature but is of interest given there is evidence that a wide stance gait can mitigate medial compartment loading (Bowd *et al.*, 2019).

Again, the lack of change in LDTA speaks to the accuracy of the data as no change should occur in this measure (as the morphology of the distal tibia is not changed in the surgery) and so any difference in measurements represents variation in radiograph angle or measurement error.

No significant change following HTO was seen within the knee joint (JLCA) which is at odds with much of the published literature (Lee *et al.*, 2016; Ogawa *et al.*, 2016; Oh *et al.*, 2016; Shin *et al.*, 2016; Na *et al.*, 2017). As can be seen in Table 29, all these studies reported larger changes in JLCA of the knee following HTO, albeit with very large standard deviations relative to their measured means. Several also reported correlation between change in mTFA (i.e. degree of correction) and change in JLCA and therefore posited knee joint laxity as a key factor that could potentially reduce the accuracy of correction.

Author (year)	N	mTFA (°of varus)			JLCA (°)		
		Pre	Post	Change	Pre	Post	Change
Oh (2016)	69	6.0 ± 4.0	-3.3 ±3.3	9.4 ± 4.7	1.8 ± 1.8	0.5 ± 1.7	-1.2 ± 1.6
Lee (2016)	86	8.0 ± 3.9	-3.4 ± 2.3	9.78	3.4 ± 2.3	2.1 ± 2.3	-1.3
Shin (2016)	50	7.5 ± 3.1	-2.8 ± 1.8	10.3 ± 3.5	3.6 ± 2.4	1.8 ± 2.0	-1.8 ± 1.6
Ogawa (2016)	50	9.6 ± 4.0	-3.2 ± 2.3	12.8 ± 4.3	4.6 ± 2.2	2.7 ± 1.6	-2.0 ± 1.5
Na (2021)	80	5.6 ± 3.4	-3.3 ± 2.7	8.9 ± 3.5	2.7 ± 1.6	1.8 ± 1.1	-0.9 ± 1.2
This study	35	8.0 ± 4.0	1.5 ± 2.8	6.5 ± 3.8	4.0 ± 1.7	3.6 ± 1.9	-0.04 ± 1.7

All values are presented as the mean ± standard deviation
Abbreviations: mTFA, mechanical tibio-femoral angle; JLCA, joint line convergence angle

Table 29: Recent studies reporting change in joint line convergence angle (JLCA) of the knee after HTO

The current study did not reveal a significant change in JLCA of the knee, however, as seen in Table 29, the correction levels in this study were smaller when compared to the other studies.

The other studies all mention a planned correction target of approximately 62.5%, otherwise known as the Fujisawa point. Although this has been traditional teaching for HTO surgery, there has been increasing evidence concerning the risk of potential overcorrection to the lateral compartment (Hernigou *et al.*, 1987; Marti *et al.*, 2004; Agneskirchner *et al.*, 2007; Amis, 2013). For the current project, the lead surgeon has chosen a default correction target of 50% (neutral alignment) for HTO patients. This would account for the smaller mTFA changes when compared to the other studies and may account for the smaller degree of change in JLCA. No significant correlation between JLCA of the knee and mTFA was found in this study which may be due to a smaller mean correction and a smaller sample size. When the results of this study are plotted along with the studies listed above Figure 68, the graph does suggest that the results from this data set may be in keeping with the published literature, just representing patients with more subtle corrections and therefore more subtle compensatory changes.

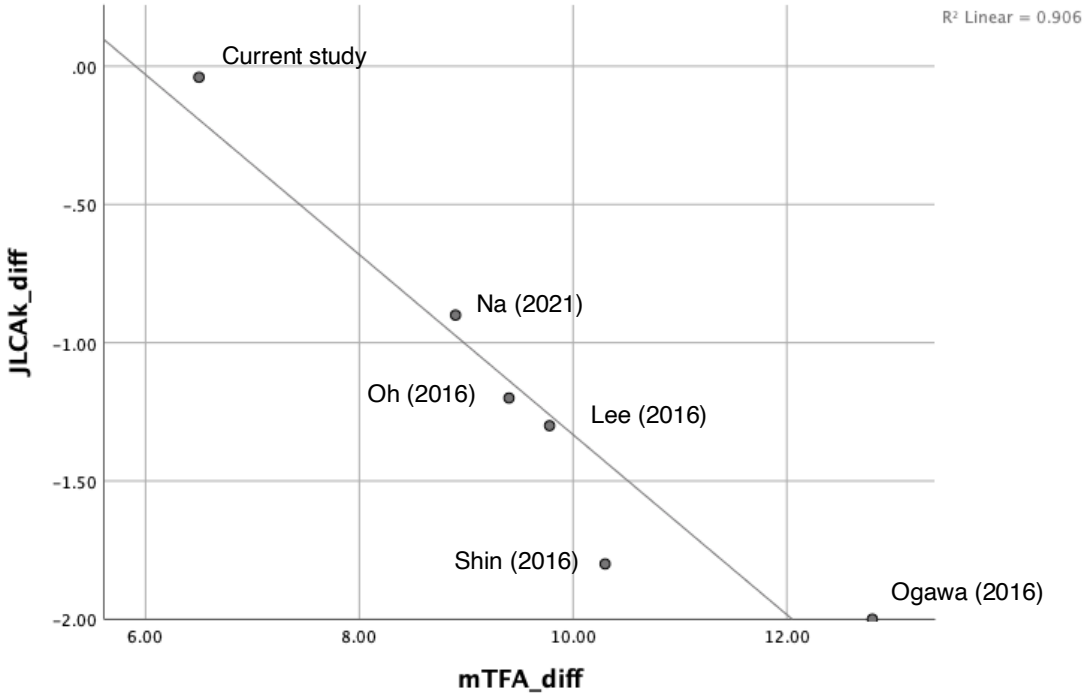

Figure 68: Scatter plot of mean change in JLCA (knee) versus mean change in mTFA following HTO comparing this study's data set against the published literature.

No significant differences were seen in the hip measurements following HTO surgery. Again, FNS angle is a control measure so no difference would be expected (barring radiograph rotation/measurement error). However, there was also no significant change observed for the GFN angle. This suggests either that the adaptation to HTO is taking place solely distal to the knee and as such the hip joint is unaffected, or alternatively that the change in coronal plane rotation at the hip (abduction/adduction) is too subtle to be detected by these measures. This is possible given that the large femoral lever arm means that any rotational adjustment at the hip would likely be very small.

The analysis of the correlations between radiographic measures revealed several important relationships. Focussing first on the correlation across the measures of limb alignment; pre-operatively, in addition to the previously discussed relationship with MPTA; mTFA and MP were significantly

correlated with the JLCA of the knee, GFN and FNS. The directions of these relationships suggests that the patients who have a more varus mechanical axis pre-operatively, in addition to having a lower MPTA, have a higher JLCA of the knee (representing lateral ligamentous laxity and/or medial cartilage loss) and hips that are both more intrinsically varus (lower FNS) and positioned in greater abduction (lower GFN). They may also have pre-operative adaptations in the subtalar joint as mTFA was positively correlated with GT but this was a weaker correlation and was not replicated with MP.

Interestingly, there was no correlation with LDTA suggesting that the distal tibial morphology is not intrinsically different in this group of patients with varus knee alignment. There is little published work looking at the links between distal and proximal tibial morphology although Choi et al. did find differences a lower LDTA in the HTO patients with the most abnormal MPTA (Choi *et al.*, 2017). In addition, work from the Cardiff Versus Arthritis Centre has suggested that LDTA is one of the factors that, in conjunction with MP, best explains variance in peak external knee adduction moment (EKAM), thought to be a key indicator of medial compartment loading during gait (Whatling *et al.*, 2019).

In addition, there were further correlations between the various adaptive measures. Patients with greater JLCA knee showed lower GFN (more hip abduction) and lower GFN was also associated with lower GT angle. JLCA ankle was positively correlated with MPTA and LDTA was correlated with both GT and stance width. This suggests a complex interplay between the various components of alignment in the pre-operative state, i.e., these patients do not have an isolated knee deformity. The alignment of the tibial plafond (LDTA) appears to have a role in determining what distal adaptations are made to malalignment at the knee, correlating with subtalar joint position (GT) and SW. This is logical when considering the LDTA which can either

contribute to or mitigate the resultant limb angle required for the ankle and subtalar joints to square with the floor; possible only by either repositioning the subtalar joint or repositioning the feet. The correlation of GT with LDTA reflects similar findings from Choi et al. (2017) who also reported GT being associated not just with overall limb alignment but with LDTA (Choi *et al.*, 2017). Of course the question then is which came first? Are these patients who have pre-existing malalignment and therefore are predisposed to abnormal loading and medial compartment OA? Or are they patients who have developed malalignment as a result of osteoarthritic joint damage? The sparse existing literature on this suggests the answer may be complex. There is a suggestion that OA may be resultant from malalignment but that some of this malalignment develops as the patient ages (Matsumoto *et al.*, 2015).

Post-operatively there were no significant correlations between the limb alignment measures and any of the adaptive measures at the hip, knee or ankle. This could indicate that once all the limbs have a normalised weightbearing axis, any variation in these measures is very small. There remained weak correlations between some of the ankle/subtalar measurements but the clinical significance of these is hard to assess.

It is in the change in the measured parameters from pre- to post-operative that the effect of HTO can be seen. Here there is a clear picture of the alignment change (MPTA, mTFA and MP) resulting in significant changes at the hip (reduced GFN meaning greater hip abduction) and subtalar joints (increased GT, meaning a more laterally tilted talus). It is also of interest that there is a negative correlation between GT and stance width meaning that these may be independent adaptive mechanisms (i.e. patients either adapt by tilting their subtalar joint or by standing with their feet wider apart). As

these measures are novel, there is unfortunately no existing literature to either support or refute this theory.

There are several limitations to this analysis. As already discussed, it may not be possible to pick up very subtle alignment changes using this method and this may be of particularly relevance in this group of HTO patients where the target correction is smaller than in some previous studies. This analysis is also focussed only on the coronal plane as this is how the patients are clinically radiologically assessed. To fully described limb alignment and joint position would require analysis in all three clinical planes, perhaps requiring cross sectional imaging (CT/MRI) to provide 3D analysis.

Another limiting factor is the fact that the analysis considers only the lower limbs and their response to HTO. The entire musculoskeletal system interacts in response to loading and particular aspects such as lumbar spine mobility, trunk lean and the contralateral limb should be considered (Whatling *et al.*, 2019).

Finally, this analysis is focused only on the clinically defined static situation. How these changes whilst standing translate into dynamic changes within gait is yet to be seen (Whatling *et al.*, 2019, 2020).

Despite these limitations, these finding are of great clinical importance to several cohorts of patients. Firstly, patients undergoing HTO who have no ankle/hip symptoms. In these patients, understanding more about how the other limb joints interact may help to better predict the outcome from their HTO. For example, extra care could be needed in those with hip dysplasia, a cam type hip morphology or abnormal rotational profile. The multiple correlations across, but broad standard deviations within, the measures in

this study lend weight to the theory that there are different subgroups of HTO patients who adapt pre- and postoperatively in different ways.

The second cohort for whom these findings are important are patients undergoing HTO with concomitant ankle/subtalar or hip pathology (e.g. OA or ankle malalignment) (Takeuchi, Saito and Koshino, 2008; Elson *et al.*, 2013; Suero *et al.*, 2015). Here, this study highlights the importance of ankle/foot/hip examination before consideration of HTO as a pre-existing condition may alter or restrict how the patient can adapt following realignment at the knee and it is yet to be seen how this in turn may affect patient outcomes.

Thirdly, the evidence linking limb and knee malalignment with ankle OA (Tallroth *et al.*, 2008; Hubbard, Hicks-Little and Cordova, 2010; Onodera *et al.*, 2012; Suero *et al.*, 2015; Xie *et al.*, 2018) suggests that HTO could have a broader range of indications than just the treatment of unicompartmental knee OA. It is possible that in this cohort of patients presenting with ankle symptoms, a contributory factor may lie in more proximal malalignment. If so, HTO could be an option to address their ankle symptoms. If surgery to the ankle is required, it remains vital to establish any proximal malalignment first, so as to avoid abnormal stresses on the distal fusion/arthroplasty and unanticipated alignment challenges of subsequent surgery at the knee.

4.6 CONCLUSION

The work described in this chapter has confirmed Hypothesis 2, demonstrating that MOW-HTO does cause measurable changes in the radiological alignment of the lower limb beyond those anticipated as a result of the surgical correction. In addition to revealing how a change in proximal tibial morphology does not produce an accurately predictable response in limb alignment, this study has also revealed several of the adaptive mechanisms within other joints of the lower limb which may modulate this response. It is clear that HTO results in measurable, statistically significant effects on the hip and subtalar joints and that there is a complex interplay between compensatory strategies, likely individual to each patient. Through a greater understanding of the mechanisms by which patients adapt following realignment surgery, based on accurate, repeatable and objective measures, clinicians will be able to predict individual patient outcomes and plan surgery more appropriately.

Chapter 5:

Analysis of linked biological and mechanical factors

5.1 INTRODUCTION

There is clear evidence from a number of sources to suggest there are measurable biological changes within the knee joint following HTO. As outlined in the introduction, this comes both from observation of cartilage regeneration (Odenbring *et al.*, 1992; Schultz and Gobel, 1999; Takeshi Kanamiya *et al.*, 2002; Koshino *et al.*, 2003; Parker *et al.*, 2011; Rutgers *et al.*, 2012; Spahn *et al.*, 2012; Jung *et al.*, 2014; D'Entremont *et al.*, 2015; C.-W. Kim *et al.*, 2017; Huizinga *et al.*, 2017; Tsukada and Wakui, 2017) and from changes observed in subchondral bone or synovial fluid (Akamatsu *et al.*, 1997; Nakashima, Koshino and Saito, 1998; Takahashi, Tomihisa and Saito, 2003; Kroner *et al.*, 2007; Bai *et al.*, 2017; Gersing *et al.*, 2018; Kim *et al.*, 2019).

Added to this are the findings from Chapter 3 which suggested possible changes in expression of several biological markers (GRIK4, NR2D, SOST) by subchondral bone in the posteromedial quadrant following HTO.

The fact that these changes are seen following realignment surgery suggests that they may likely be mechanically mediated, as the effect of HTO on the knee joint tissues is to change their mechanical environment. This is particularly true of the subchondral bone marker changes described in Chapter 3 where the changes were observed in a specific quadrant (posteromedial) following offloading. In the case of SOST, reciprocal changes were seen in the PL compartment where load is being increased post HTO.

The exact nature of the mechanical changes seen following HTO is perhaps more complex than previously thought and understanding remains incomplete. As stated in the introduction, several existing studies have described decreased medial compartment load post HTO (Agneskirchner *et*

al., 2007; Ogden *et al.*, 2009; Suero *et al.*, 2015; Trad *et al.*, 2018) and there is a strong body of evidence that a valgus high tibial osteotomy reduces knee adduction moment (KAM) (Wang *et al.*, 1990; Wada *et al.*, 1998; Ramsey *et al.*, 2007; Bhatnagar and Jenkyn, 2010; Lind *et al.*, 2013; Leitch *et al.*, 2015; Marriott *et al.*, 2015, 2019; Birmingham *et al.*, 2017; Whelton *et al.*, 2017; Badie, Katouzian and Rostami, 2018; da Silva *et al.*, 2018; Whatling *et al.*, 2019). However, in addition several other changes have been observed which may complicate the picture, including adaptive mechanisms such as trunk lean, stride length, sagittal plane changes or alteration in foot progression angle (Wang *et al.*, 1990; Takemae *et al.*, 2006; Lind *et al.*, 2013; Lee *et al.*, 2017; da Silva *et al.*, 2018; G. M. Whatling *et al.*, 2019).

Again, the earlier results in this MD study add to this picture of a complex combination of interdependent mechanical factors. As described in Chapter 4, not only is the relationship between correction at the proximal tibia and change in mechanical axis to an extent unclear, there are also further (and hitherto undescribed) compensatory changes seen distally (such as stance width and subtalar joint positioning) which have an impact in the resultant mechanical environment of the knee following HTO.

What remains unknown, with little previous investigation, is the existence of any relationships between these observed biological and mechanical changes and it is this missing link which this chapter will investigate.

5.2 AIMS

To address Hypothesis 3, that the biological and biomechanical changes observed following medial opening wedge HTO are related. In addition, to test the hypothesis generated by the earlier results, that there are phenotypic groups of patients who differ in their response to HTO.

This overall hypothesis will be broken down in the following series of questions:

1) Are there phenotypic groups of patients who can be identified preoperatively?
2) Are there any preoperative markers which are predictive of outcome?
3) Are there any postoperative markers which could be used as surrogate markers for outcome?
4) What biological factors are associated with a change in alignment/HTO?
5) Are there any mechanical variables which show association with the subchondral bone changes identified in Chapter 3.

5.3 METHODOLOGY

Patient recruitment, surgical procedure, sample acquisition, bone core/radiological/gait analysis were performed as described in previous Chapters 2-4.

This analysis will incorporate data from a number of sources. An explanation of the variables used is below (Table 30). All HTO data is attributable to the Biomechanics and Bioengineering Research Centre Versus Arthritis (BBRCVA). Ethics, recruitment, sample collection/transport/processing/storage/records provided by Ms Cheryl Cleary, Mr Paul White and the CAVOC clinical and research teams. Bone sample analysis performed by Ms Carole Elford, Dr Karen Brakspear, Dr Cleo Bonnet, Dr Sophie Gilbert and, as detailed in Chapter 3, a small number by the author. Blood and synovial fluid analysis performed by Dr Nidal Khatib. Biological analysis led by Prof Deborah Mason but data analysis (as described in Chapter 3) performed by the author. Biomechanical/gait analysis and patient outcome scores performed by Dr Gemma Whatling, Dr Jake Bowd, Dr Paul Biggs and staff of the BBRCVA Clinical Laboratory led by Prof Cathy Holt. Radiological analysis performed largely by the author (as detailed in Chapter 4) with a small number of early radiographs analysed by Mr David Elson.

The cross disciplinary analysis of this data to be detailed in this chapter was performed exclusively by the author having learnt the processes and software involved from online tutorials and discussions with Prof. Mason and Dr Nidal Khatib.

Table 30: Explanation of variables.

Data type	Variable	Explanation
Gait analysis	Speed	Gait speed. Increased speed = higher ground reaction forces = increased forces generated at the knee. Slow gait may point toward pain avoidance
	Cycle time	Time taken to complete one gait cycle. Related to gait speed (see above).
	DLST	Double limb support time. Time taken with both feet on the ground. Longer time may suggest pain avoidance.
	EKAM1	External knee adduction moment, first peak. Surrogate of peak medial knee loading during early stance phase
	EKAM2	External knee adduction moment, second peak. Surrogate of peak medial knee loading during late stance phase
	ROM	Range of movement. Maximum arc of flexion/extension movement measured by goniometer.
Patient demographics	Age	In years at time of surgery.
	Height	In metres at time of surgery.
	Weight	In kilograms at time of surgery.
Patient reported outcome scores	OKS	Oxford Knee Score. Knee function score (out of maximum 48, higher score = better function)
	PACS	Pain Audit Collection System. Pain score (out of maximum 100, higher score = greater pain)
Radiological	KL	Kellgren and Lawrence grade. Severity of OA (1-4)
	mTFA	Mechanical Tibio Femoral Axis Measured in degrees of varus, this tells you the alignment of the lower limb as a whole.
	Mik (or MP)	Mikulicz Point. Another marker of lower limb alignment. It is expressed as a percentage compared to the tibial plateau width. 50% is normal, <50% is varus (weightbearing axis of the limb and therefore load is falling on the medial compartment).
	LDTA	Lateral Distal Tibial Angle. Angle of distal tibial joint line relative to tibial shaft (mechanical axis). This measure is inherent to the tibia and may contribute to overall alignment but should not be altered by HTO.
	LDFA	Lateral Distal Femoral Angle. Angle of the distal femoral joint line relative to the femoral mechanical axis. This measure is inherent to the femur and may contribute to overall alignment but should not be altered by HTO.
	GT	Ground Talus angle. Measure of the top of the talus relative to the horizontal on an AP radiograph– therefore representing the alignment of the subtalar joints (see Chapter 4 for further explanation).
	SW	Stance Width. A measure of how far apart the feet are positioned in a weightbearing X-ray. Standardised by talar width (see Chapter 4 for further explanation)

Subchondral bone markers	EAAT1	Excitatory Amino Acid Transporter 1 (AKA Glutamate Aspartate Transporter 1 or GLAST-1). Transporter within the glutamate pathway, found in subchondral bone – potentially in higher levels in OA. Glutamate pathway appears load mediated in bone but which components involved as yet unknown.
	EAAT3	Excitatory Amino Acid Transporter 3. Transporter within the glutamate pathway, found in subchondral bone – potentially in higher levels in OA. Glutamate pathway appears load mediated in bone but which components involved as yet unknown.
	NR2D	Subunit of the NMDA (N-methyl-D-aspartate) type glutamate receptor (glutamate gated ion channel). Glutamate pathway appears load mediated in bone but which components involved as yet unknown. Earlier work by author (Chapter 3) suggests this may be down regulated in the posteromedial compartment following unloading via HTO.
	GRIK4	Glutamate Receptor, Ionotropic, Kainate 4. Kainate type glutamate receptor (glutamate gated ion channel). Glutamate pathway appears load mediated in bone but which components involved as yet unknown. Earlier work by author (Chapter 3) suggests this may be down regulated in the posteromedial compartment following unloading via HTO.
	SOST	Sclerostin. Produced by osteocytes and has an inhibitory effect on osteoblast (bone forming) activity. Some evidence it is downregulated by load (i.e. part of the mechanism for increased load to cause increased bone formation). Earlier work by author (Chapter 3) suggests this may be down regulated in the posteromedial compartment and upregulated in the posterolateral compartment following unloading via HTO.
	IL-6	Interleukin-6. Pro-inflammatory cytokine
Synovial fluid markers	SF_Glu	Glutamate. Signalling molecule and suggested mediator of load mediated pathway.
	SF_RANKL	Receptor activator of nuclear factor kappa-B ligand. Stimulator of osteoclast formation and activity (therefore bone resorption).
	SF_OPG	Osteoprotegerin. Competitive inhibitor of RANKL.
	SF_ALP	Alkaline Phosphatase. A marker of bone turnover.
	SF_SOST	Sclerostin. (See above)
	SF_IL-6	Interleukin-6. Pro-inflammatory cytokine
	SF_IL-8	Interleukin-8. Pro-inflammatory cytokine
	SF_TNFa	Tumour necrosis factor alpha. Pro-inflammatory cytokine

The data analysis followed a two staged approach.

In the first stage, a Principal Component Analysis (PCA) technique was employed using SIMCA (SIMCA® Multivariate Data Analytics Solution, Version 14.1, Umetrics, Sartorius Stedim, Sweden). This was a hypothesis driven investigation (based on the questions listed above) where models were generated to look for potential correlations between either groups of variables or groups of patients. PCA is an exploratory form of data analysis which plots each data point in multidimensional space and then generates axes that best explain the variance (Wang *et al.*, 1990; Takemae *et al.*, 2006; Lind *et al.*, 2013; C.-W. Kim *et al.*, 2017; da Silva *et al.*, 2018; G. M. Whatling *et al.*, 2019). SIMCA allows this data to be displayed as intuitive plots to show groupings of variables or patients. The analysis of these plots can assist in identifying potential correlations, although it does not necessarily imply either significance or causation.

The models generated can be assessed in terms of the proportion of data variance explained (R2X) and how predictive the model is (Q2). The selection of data included in each model is therefore a necessary compromise between including maximum possible variables without drowning any correlations in noise.

Gender was not included in these models as the inclusion of a categorial variable disrupted the analysis. However, given there are very few women in the data set, it is unlikely that gender would be a significant contributor within our patient cohort. The models were checked manually and the female patients did not appear to be grouped together, suggesting that within this cohort, gender was not a confounding variable.

As explained in Chapter 3, the data from the subchondral bone analysis can be expressed in two ways; either absolute quantity of RNA or percentage change from pre- to post-HTO for each quadrant. Where the models are

being constructed to look for a pre- to post-HTO change, the percentage data has been used as the error level in RT-PCR is likely too high to show a pre- to post-difference within an individual. However, where the models are assessing the pre- or post-HTO situation alone, the absolute biological figures were used as this allowed a significantly higher number of participants.

The first four questions listed above were investigated by PCA as follows:

1) **Are there phenotypic groups of patients who can be identified preoperatively?**
   Models were analysed for clustering of variables suggesting associations and then whether groups of patients mapped to these groups. The mechanical variables were tested alone to begin with as this produced the best model and following analysis of this, biological markers were introduced.
2) **Are there any preoperative markers which are predictive of outcome?**
   Change in OKS and PACS scores pre-operatively to post-operatively (OKS_diff, PACS_diff) were used as measures of outcome and analysis examined which preoperative markers were associated with these scores.
3) **Are there any postoperative markers which could be used as surrogate markers for outcome?**
   Again outcome was judged using OKS and PACS and analysis looked for what postoperative markers were associated.
4) **What biological factors are associated with a change in alignment/HTO?**
   Here the percentage change data from the subchondral bone analysis was compared to the change in mechanical variables.

In the second stage of analysis (to answer question 5), the biological variables identified in the subchondral bone analysis in Chapter 3 and any additional biological markers identified in the PCA models were compared to the mechanical variables using Spearman's rank correlation coefficient (chosen in order to take account of the mixture of continuous and ordinal data and the likelihood of non-linear relationships). Data pairings were excluded if $n<10$ so therefore only static radiographic measures able to be tested. Correlations were judged as significant at the 0.05 level. However, those less than 0.1 were also highlighted as, though not meeting the threshold of proven significance, they may represent areas of potential interest in subsequent investigation. This analysis was undertaken using Excel (Microsoft® Excel for Mac, Version 16.45; Redmond, WA, USA) and SPSS (IBM SPSS Statistics for Macintosh, Version 26.0; Armonk, NY, USA).

5.4 RESULTS

5.4.1 Principal Component Analysis

5.4.1.1 Are there phenotypic groups of patients who can be identified preoperatively?

The following selection of mechanical (dynamic and static) markers (Figure 69) produced a model over two axes with R2X 0.476, Q2 0.0643. This model therefore explained just under half of the variability but was poorly predictive.

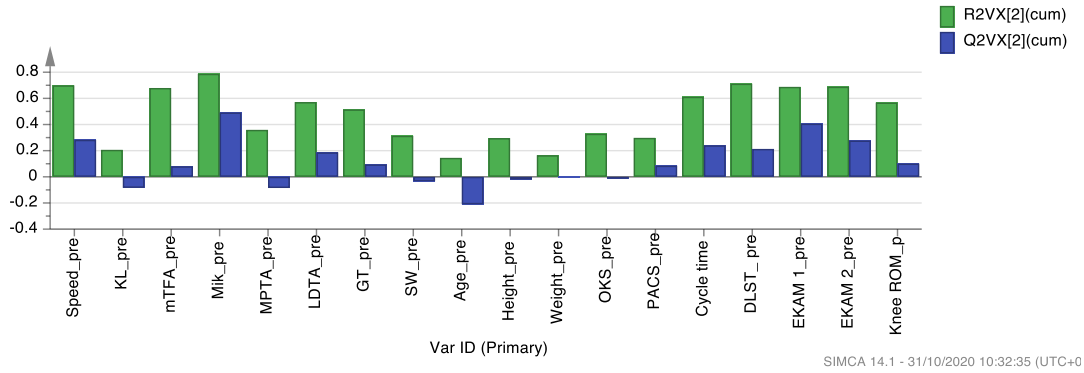

Figure 69: Contribution plot of variables included in model looking at pre-operative mechanical markers only.

The loading plot of these variables is shown in Figure 70:

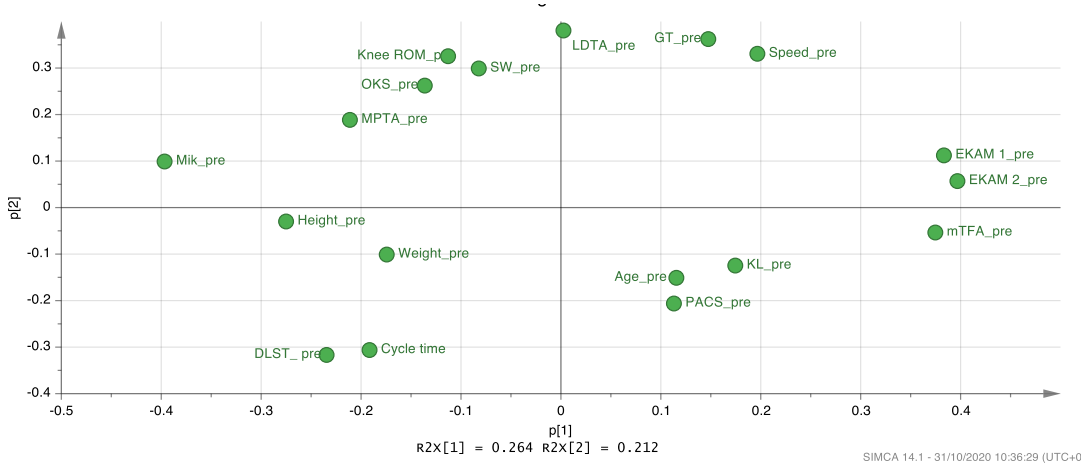

Figure 70: Loading plot of variables included in model looking at pre-operative mechanical markers only.

This model shows MPTA, knee ROM, OKS and stance width all grouping together. This group is all negatively correlated with age, KL and PACS (pain). These groups are highlighted in Figure 71.

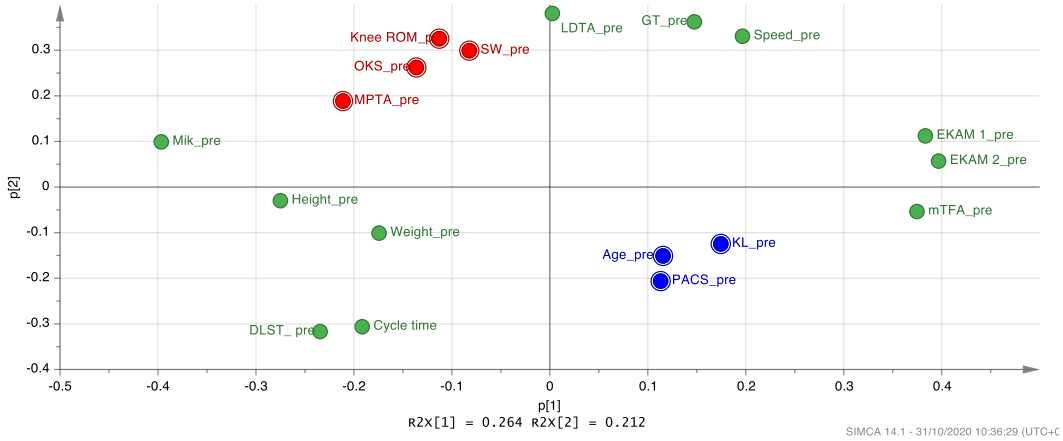

Figure 71: Loading plot of variables included in model (looking at pre-operative mechanical markers only) highlighting first set of variables.

These groups of variables are associated with markers of static and dynamic varus (mTFA, Mik, EKAM) but these associations are not as strong as with MPTA.

The loading plot (Figure 72) does show Mik and varus (mTFA) are strongly negatively correlated as expected given a lower Mikulicz point (i.e. lower than 50%) means the weightbearing axis is travelling medially in the knee, i.e. greater varus). These markers of static limb varus are also grouped with the dynamic varus variables (EKAM). The previously mentioned group of MPTA, ROM, OKS and SW show positive association with Mik and negative with mTFA (and to lesser extent EKAM).

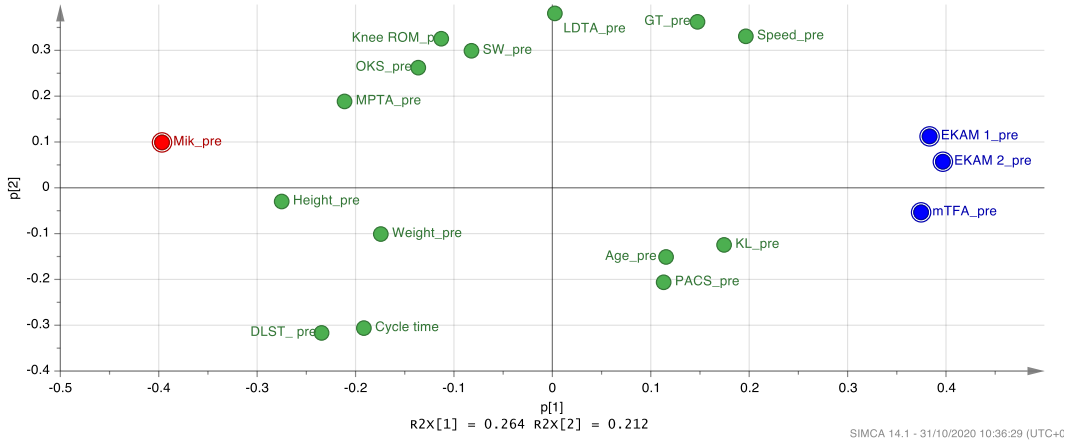

Figure 72: Loading plot of variables included in model (looking at pre-operative mechanical markers only) highlighting second set of variables.

The other variable groupings that come out of this model are as follows (Figure 73)

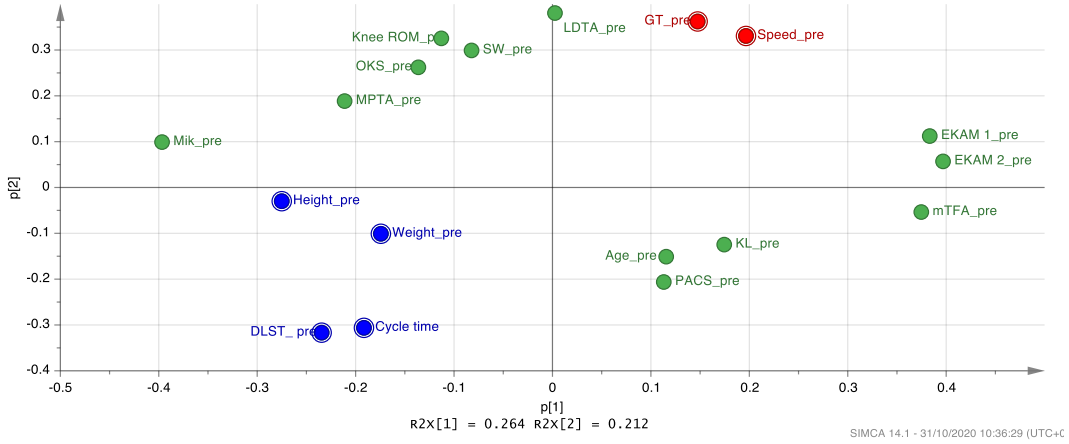

Figure 73: Loading plot of variables included in model (looking at pre-operative mechanical markers only) highlighting third set of variables.

Speed is negatively correlated with cycle time and double limb support time (DLST) but is also correlated with ground-talus angle (GT). The markers of reduced speed are also associated (less strongly) with taller and heavier patients.

LDTA appears related to both the “low symptom group” of variables (ROM/OKS/MPTA), higher speed and markers of distal adaptation (SW, GT).

This model did not provide any obvious distinct groupings of patients (Figure 74) but potential phenotypic groups were investigated.

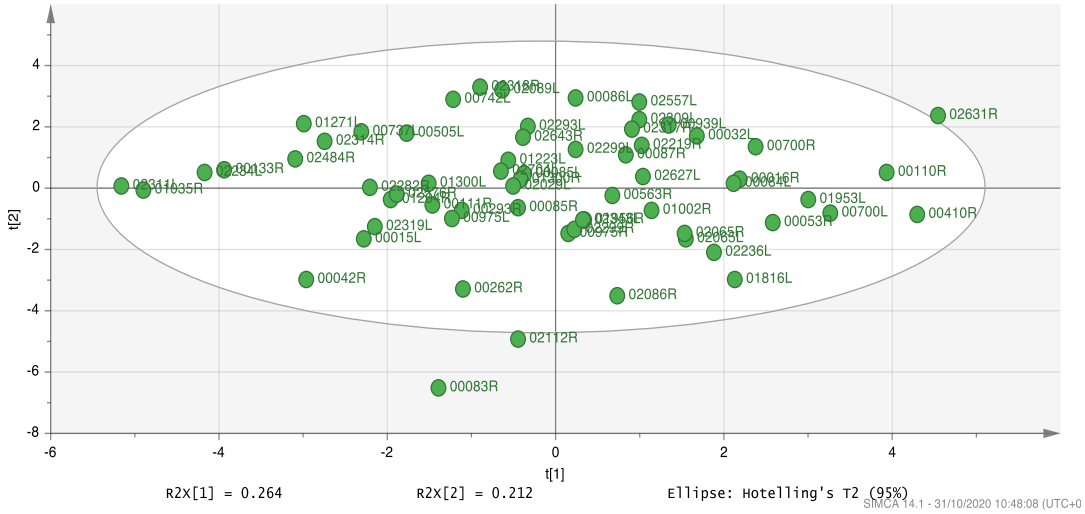

Figure 74: Scores scatter plot showing distribution of patients across the two principal components in model looking at pre-operative mechanical markers only.

The outliers were examined. Several represented groups or individuals with an incomplete data set, however, removing these patients did not improve the model and therefore they were retained.

The group of 00742L, 02089L, 02318R had wide stance, good range of movement, high LDTA and good patient scores compared to the average (Figure 75-6).

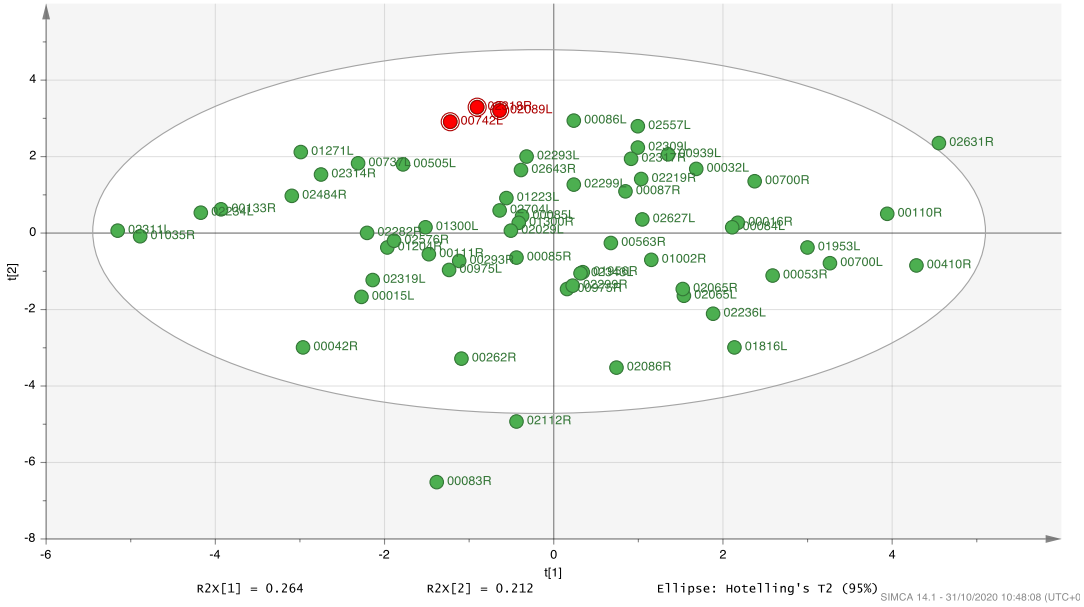

Figure 75: Scores scatter plot showing distribution of patients across the two principal components in model looking at pre-operative mechanical markers only (highlighting first group of patients).

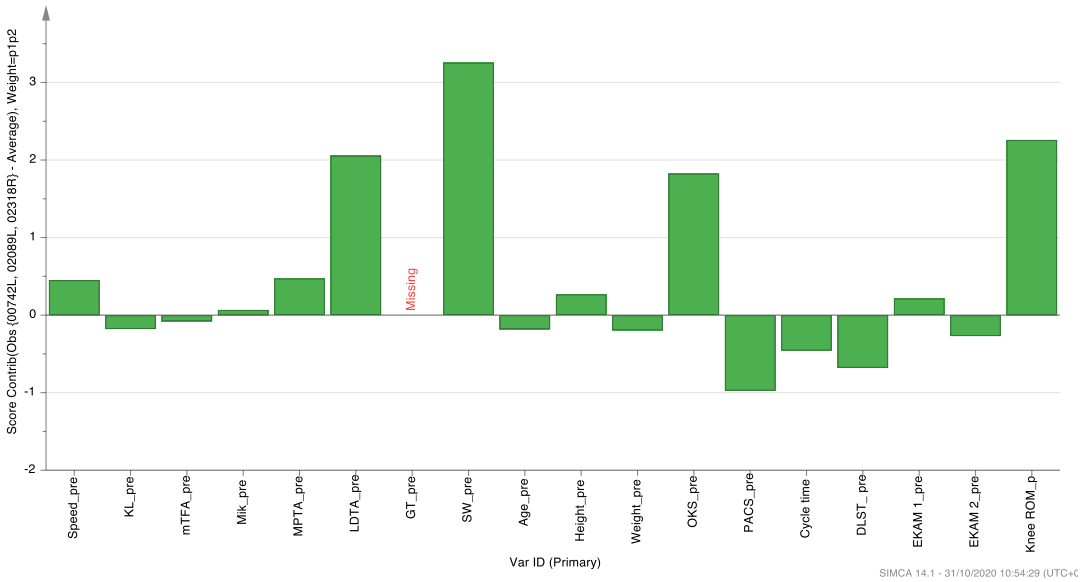

Figure 76: Score contribution compared to average for group highlighted in Figure 75 (00742L, 02089L, 02318R)

This group (02484R, 02314R, 01271L, 00737L, 00505L) showed high MPTA and Mik (i.e. low varus). Associated with good range of movement, low EKAM, good OKS scores and faster speed. Compared to the average they are also taller, heavier, younger and have a lower KL score (suggesting radiologically less severe arthritis).

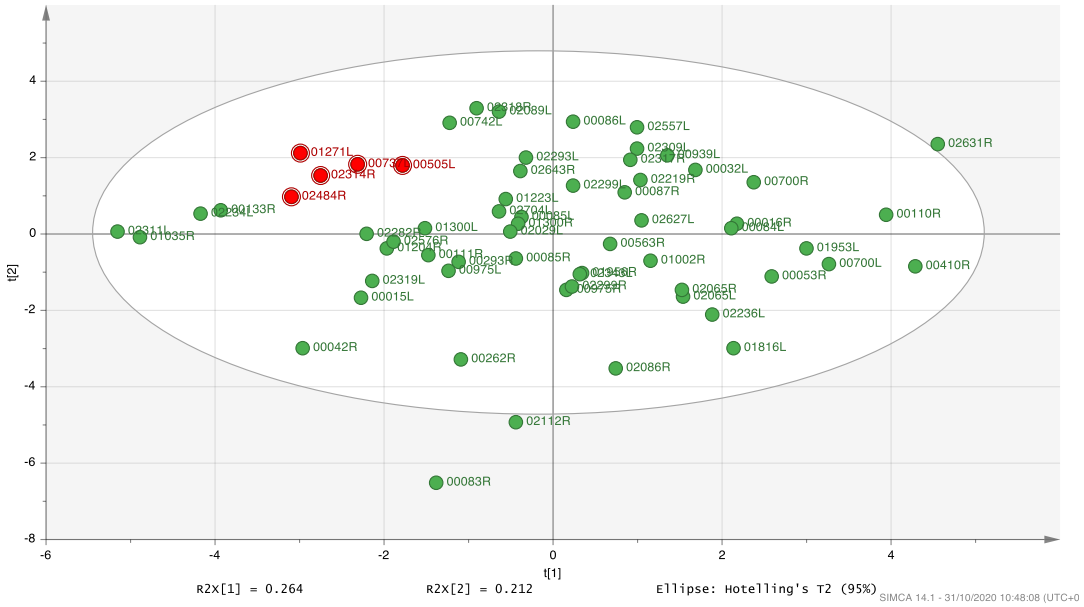

Figure 77: Scores scatter plot showing distribution of patients across the two principal components in model looking at pre-operative mechanical markers only (highlighting second group of patients)

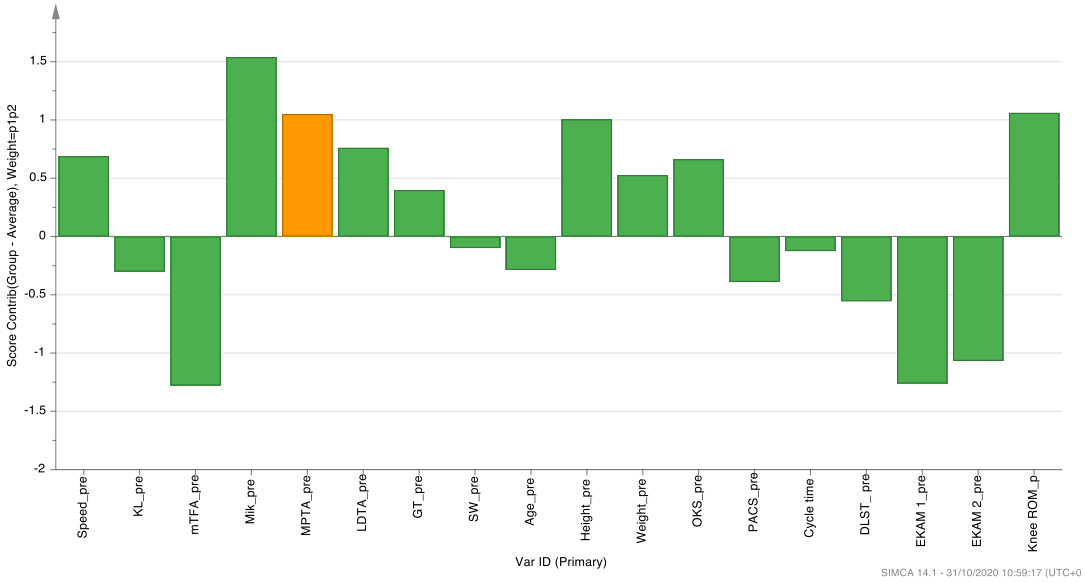

Figure 78: Score contribution compared to average for group highlighted in figure 77 (02484R, 02314R, 01271L, 00737L, 00505L). (Yellow variables outside their 3 standard deviation range).

In contrast, group 00975R, 01956R, 02299R, 02343L had relatively poor speed, range of motion and narrow stance. However, OKS scores slightly better than average and varus level only slightly worse than average (figure 79).

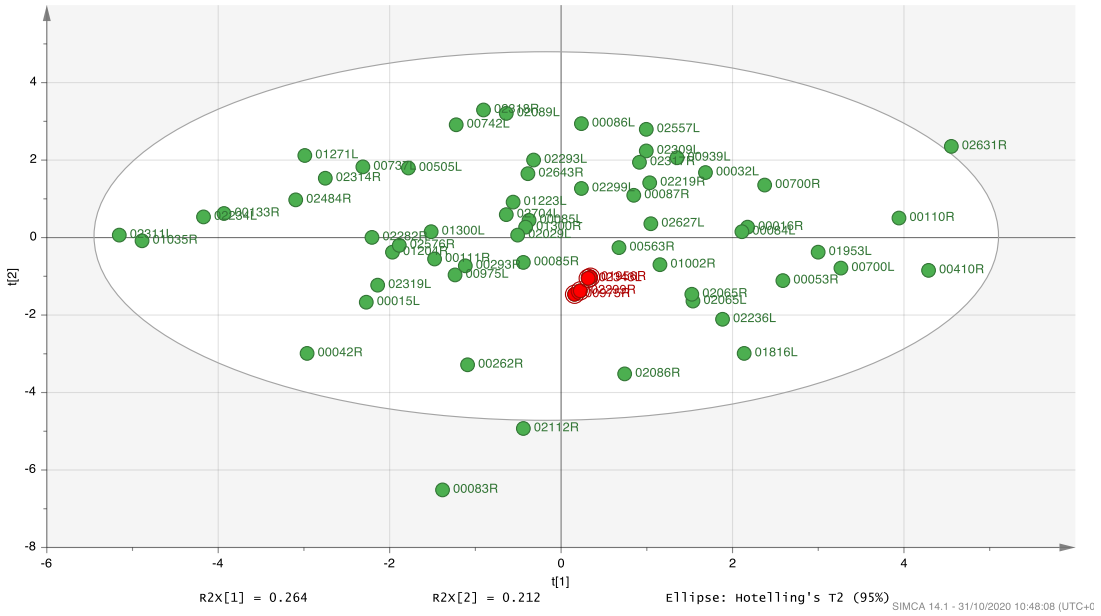

Figure 79: Scores scatter plot showing distribution of patients across the two principal components in model looking at pre-operative mechanical markers only. Highlighting third group of patients.

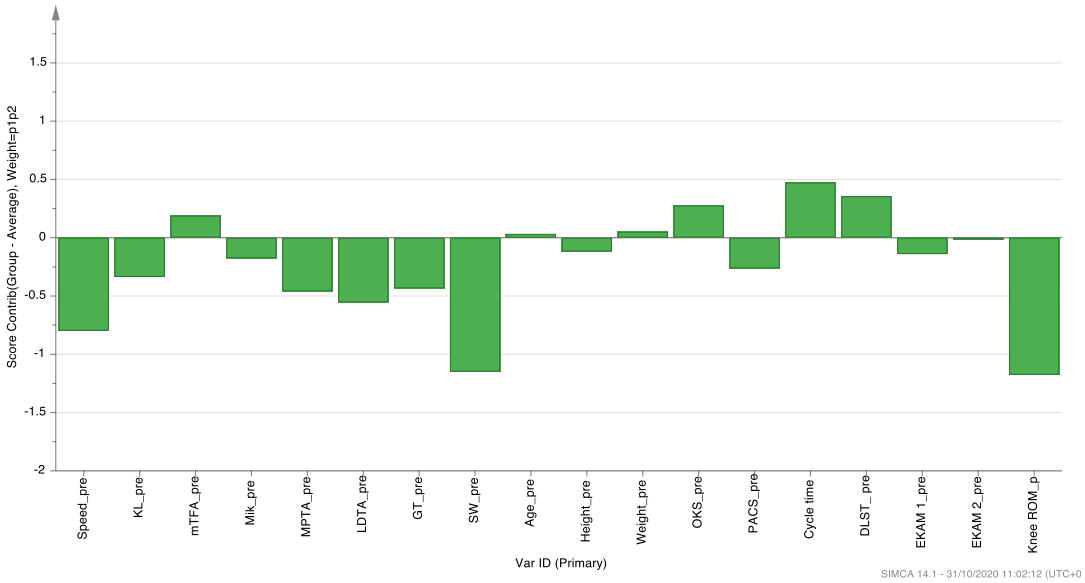

Figure 80: Score contribution compared to average for group highlighted in figure 79 (00975R, 01956R, 02299R, 02343L).

The following larger group (shown in blue, figure 81) had below average levels of malalignment (both static and dynamic) but are slower, older, taller and a little heavier than average (figure 82).

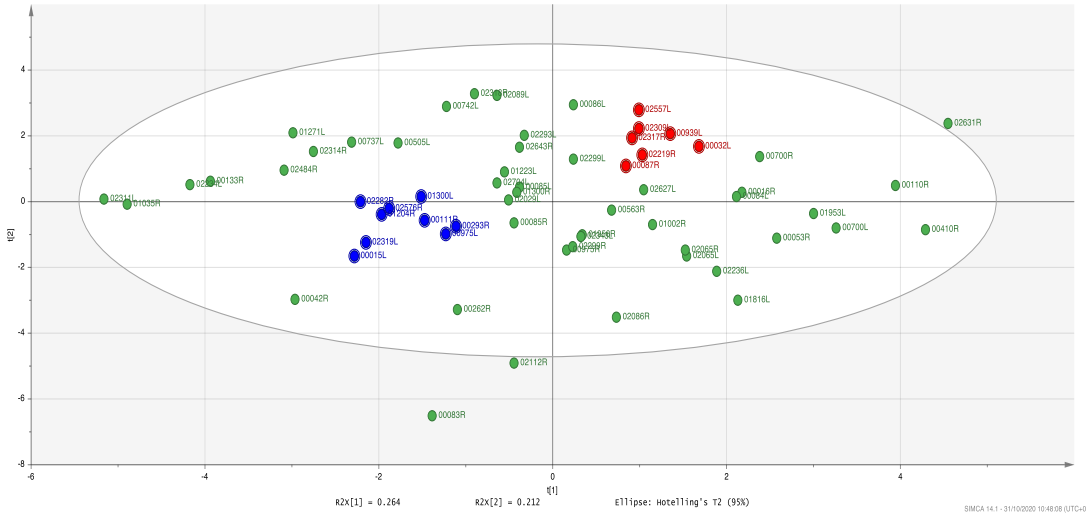

Figure 81: Scores scatter plot showing distribution of patients across the two principal components in model looking at pre-operative mechanical markers only. Highlighting third and fourth group of patients.

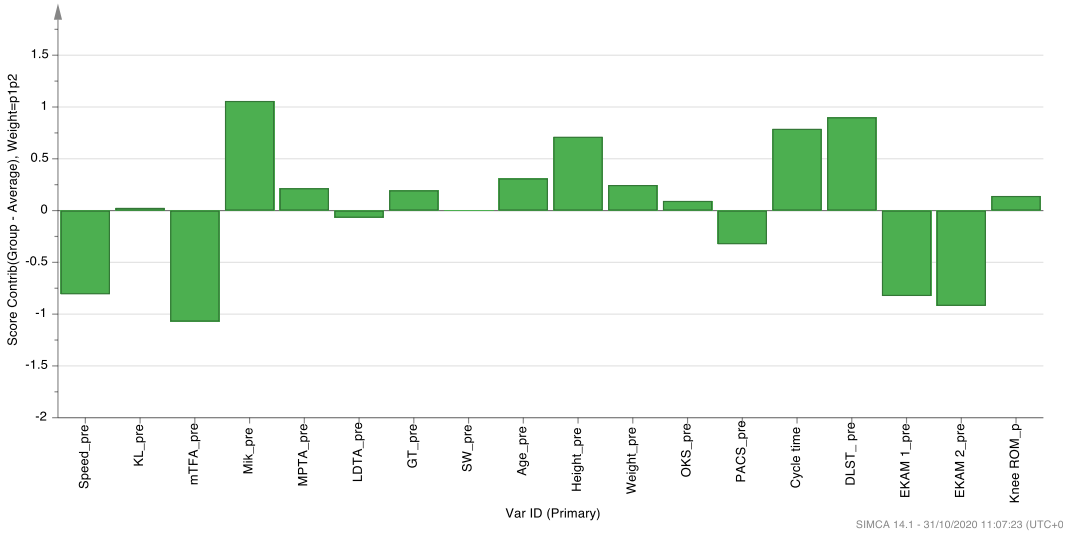

Figure 82: Score contribution compared to average for group highlighted in blue in figure 81.

Whereas the contrasting group here (shown in red, figure 81) compared to the average are faster with good range of motion, significant GT adaptation and good scores despite worse than average alignment.

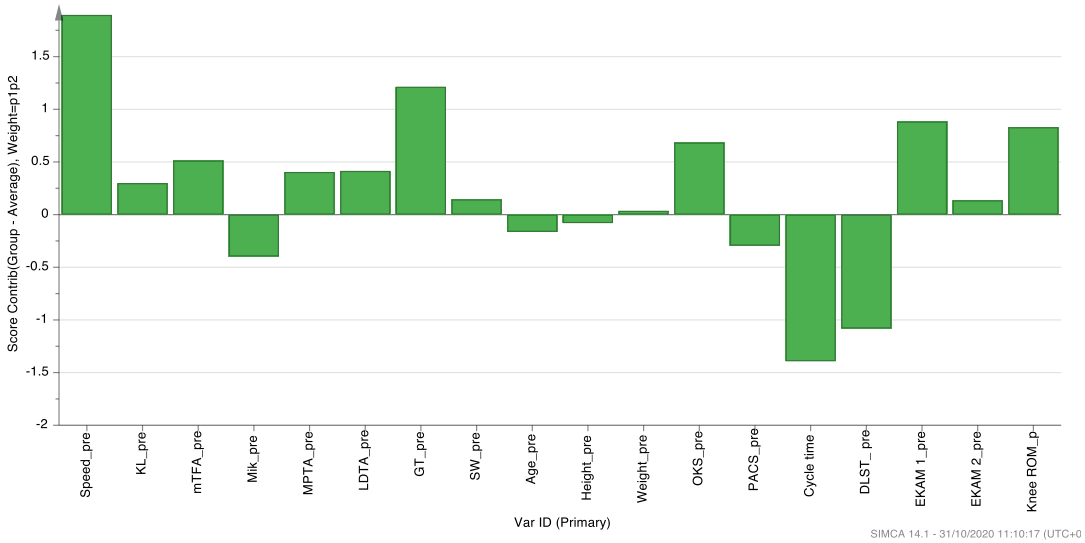

Figure 83: Score contribution compared to average for group highlighted in red in figure 81.

The model was then adapted to include biological markers. Due to the large number of potential biological markers (six subchondral bone markers, each with four quadrants plus multiple synovial fluid markers), including all of them caused increased levels of noise within the model. Therefore, over two axes had scores of R2X 0.276 and Q2 -0.0825.

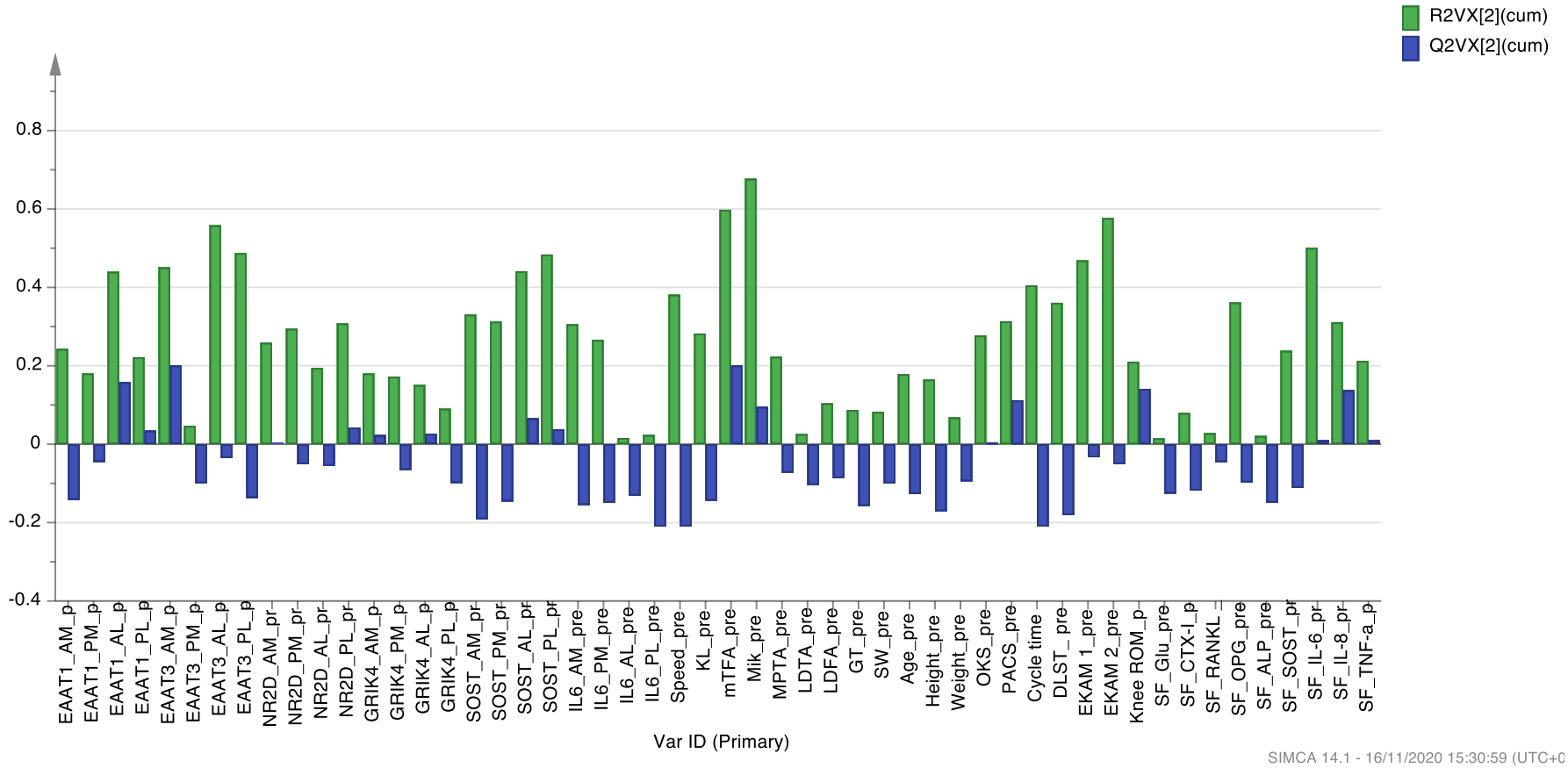

Figure 84: Contribution plot of variables included in model looking at pre-operative mechanical and biological markers.

Variables in this model were arranged as shown in the loading plot:

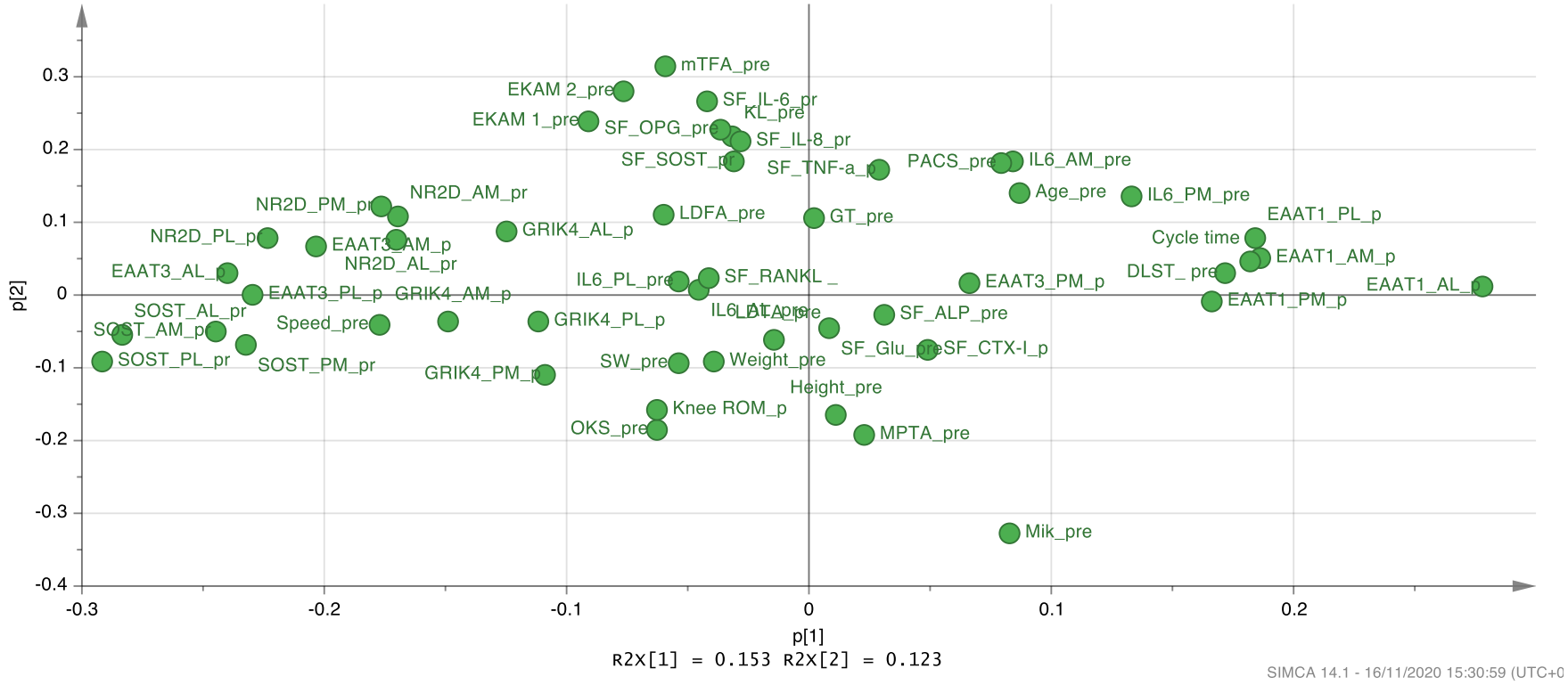

Figure 85: Loading plot of variables included in model looking at pre-operative mechanical and biological markers.

The same crossed pattern of mechanical variables is observed, divided largely by alignment indicators in one direction and speed indicators in the other. This relationship has been adjusted by the biological inclusion and there are some biological indicators now associated with these variable groups (Figure 86).

High Mikulicz is associated with high MPTA and negatively associated with high degrees of varus and EKAM. However, following addition of the biological variables, these alignment measures no longer seem associated with measures of outcome (OKS, PACS).

Higher varus is associated with higher EKAM but is also associated with the group highlighted in blue, including LDFA, KL grade and synovial fluid levels of IL-6, OPG, IL-8 and SOST. This group is negatively associated with the group highlighted in red, which in addition to Mikulicz and MPTA, includes height and synovial fluid levels of glutamate, ALP, and CTX-1 (Figure 86).

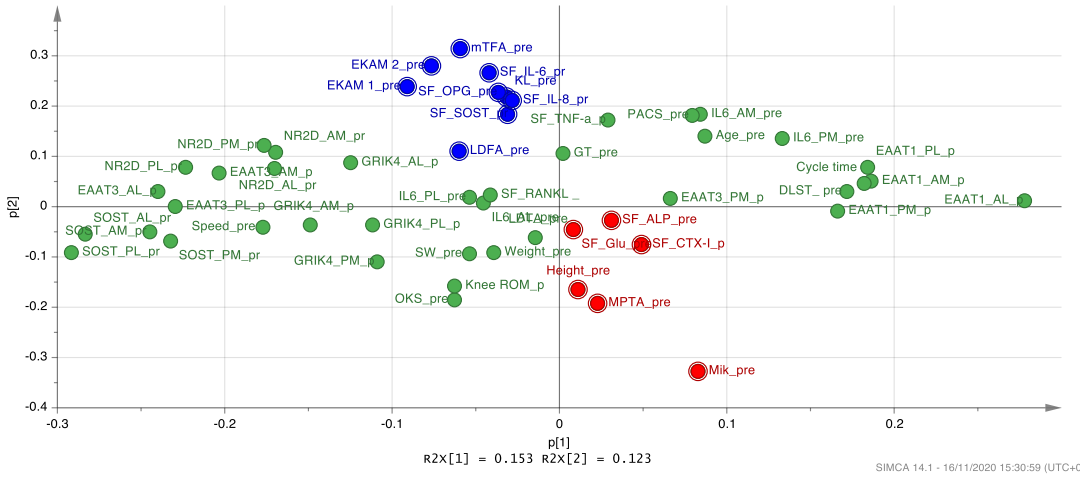

Figure 86: Loading plot of variables included in model looking at pre-operative mechanical and biological markers. First and second group of variables highlighted.

There are groups of variables that are associated with preoperative patient scores (Figure 87). OKS is grouped with ROM, weight, stance width, LDTA and GRIK4 in the posterolateral and posteromedial quadrants (shown in red). This group is negatively associated with PACS and the associated variables of age, IL-6 AM and PM, EAAT3 PM and synovial fluid TNFa.

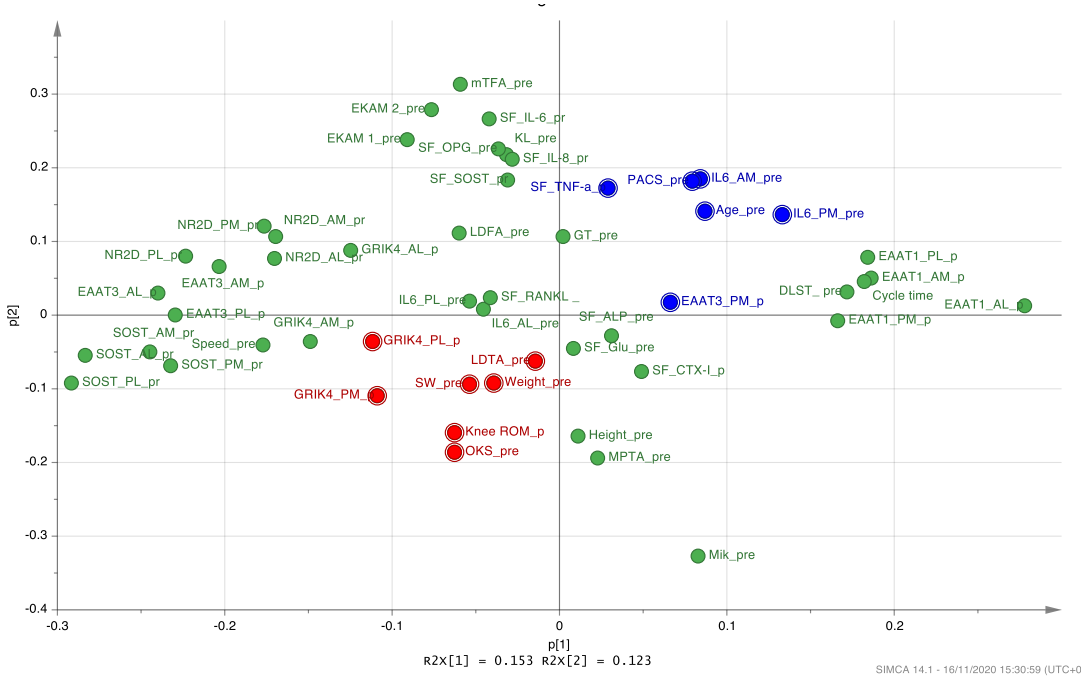

Figure 87: Loading plot of variables included in model looking at pre-operative mechanical and biological markers. Third and fourth group of variables highlighted.

The final variable groupings are those associated with gait speed. All quadrants of EAAT1 are associated with markers of slower gait (DLST and cycle time) and this group are negatively associated with speed and production of SOST, NR2D, GRIK4 and EAAT3 in most quadrants.

Having included the biological variables, the scoring plot for participants was as follows:

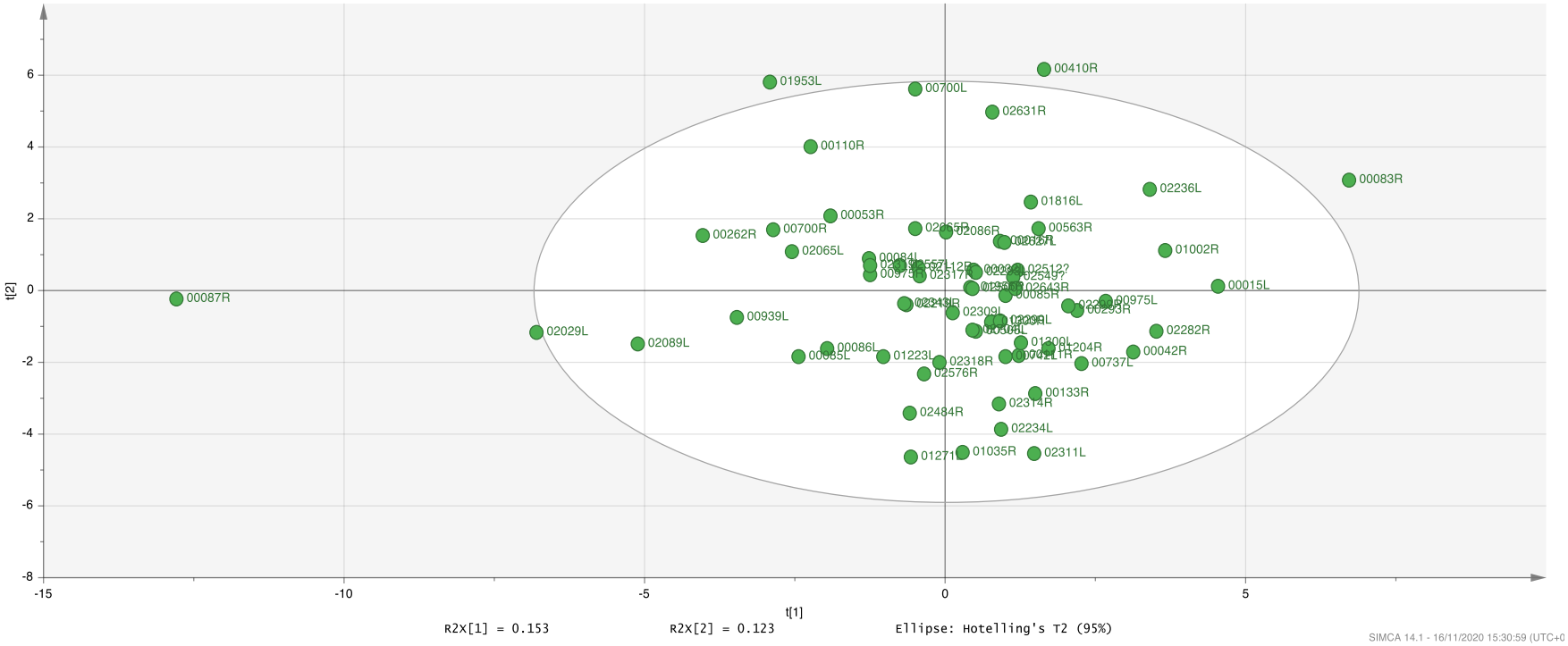

Figure 88: Scores scatter plot showing distribution of patients across the two principal components in model looking at pre-operative mechanical and biological markers.

The outliers were primarily distinguished by high levels of missing data points. However, removing them worsened the overall model scores so they were left in situ. The spread of patients was primarily explained by Mikulicz and mTFA as shown in the colour spectrum plots below (Figure 89):

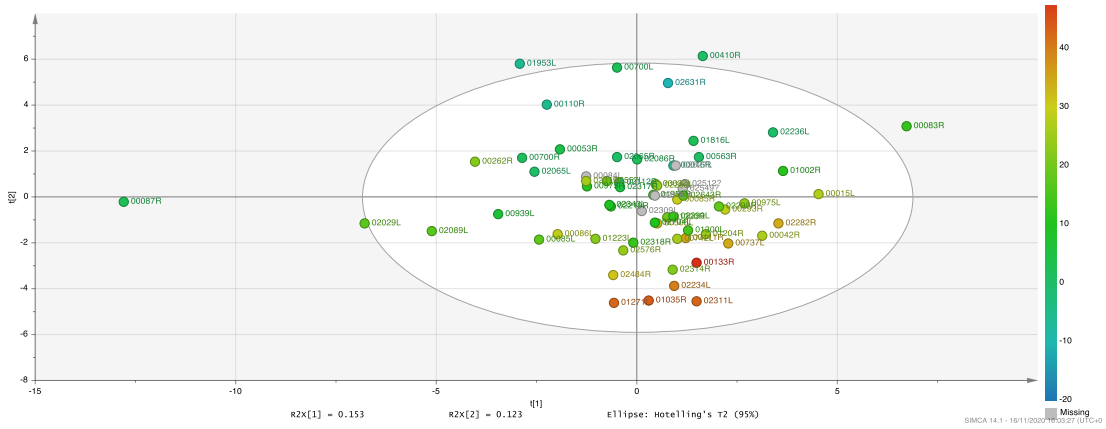

Figure 89: Scores scatter plot showing distribution of patients across the two principal components in model looking at pre-operative mechanical and biological markers. Coloured according to preoperative Mikulicz point.

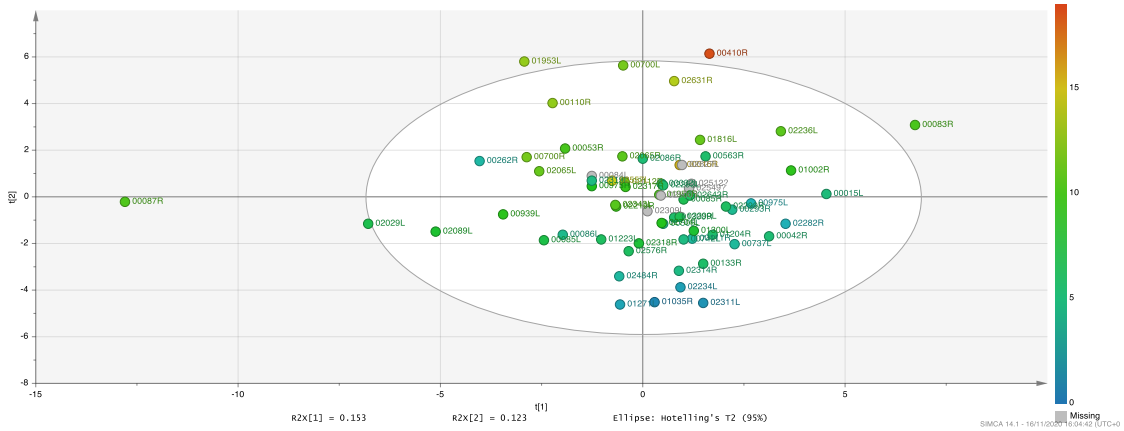

Figure 90: Scores scatter plot showing distribution of patients across the two principal components in model looking at pre-operative mechanical and biological markers. Coloured according to preoperative mTFA.

The only other variable that explained some grouping of patients was KL grade (Figure 91):

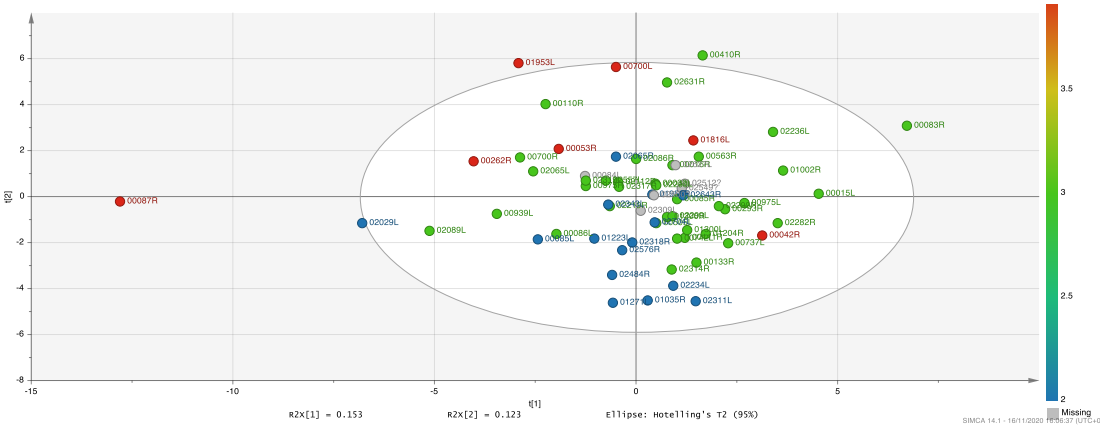

Figure 91: Scores scatter plot showing distribution of patients across the two principal components in model looking at pre-operative mechanical and biological markers. Coloured according to preoperative KL grade.

There did appear to be some clustering of patients into groups (Figure 92). Those shown below in blue were distinguished by high levels of subchondral bone activity, most particularly production of EAAT3 in the AM quadrant, NR2D in all but the PM quadrant, GRIK4 in the lateral quadrants, and SOST in the posterior quadrants. In addition, this group had scores higher than average by over three standard deviations in IL6 AM, weight and SF RANKL (Figure 93).

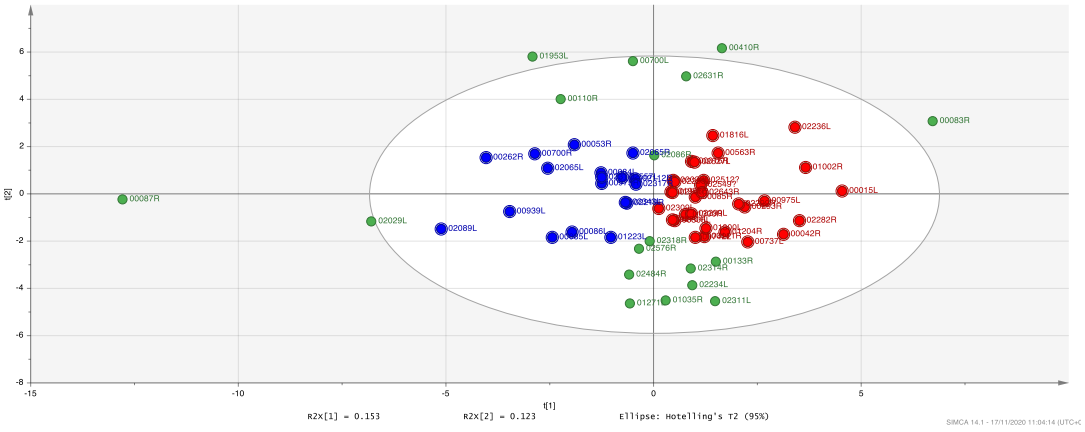

Figure 92: Scores scatter plot showing distribution of patients across the two principal components in model looking at pre-operative mechanical and biological markers. Highlighting first and second group of patients.

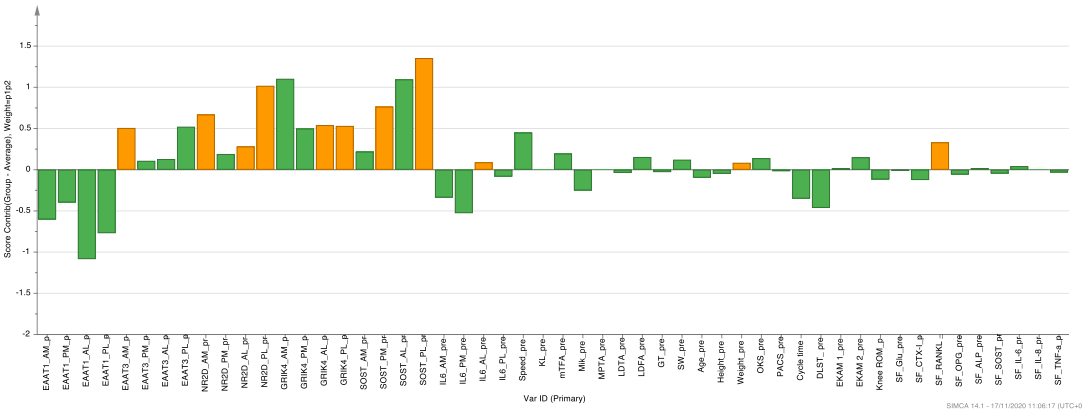

Figure 93: Score contribution compared to average for group highlighted in blue in figure 92. (Yellow variables outside their 3 standard deviation range).

By contrast, the group shown in red showed much lower subchondral bone activity, with the exception of EAAT1 (Figure 94).

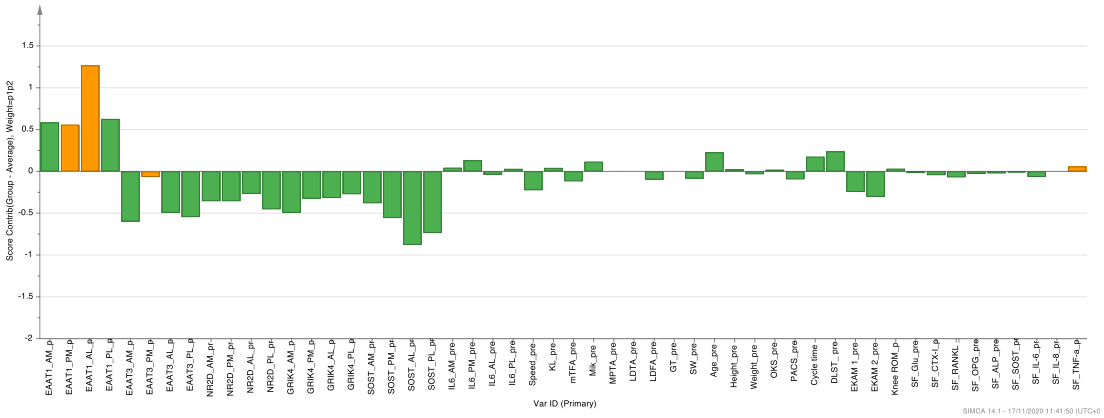

Figure 94: Score contribution compared to average for group highlighted in red in figure 92. (Yellow variables outside their 3 standard deviation range).

Both these groups showed close to average results for most of the mechanical markers.

The other, smaller groupings seen show a less uniform picture (Figure 95). The group shown in blue are characterised by high synovial high levels of OPG and IL6 along with higher subchondral bone levels of EAAT1 PL, NR2D PM and IL6 AM (Figure 96). This groups also show poor alignment and higher KL grades.

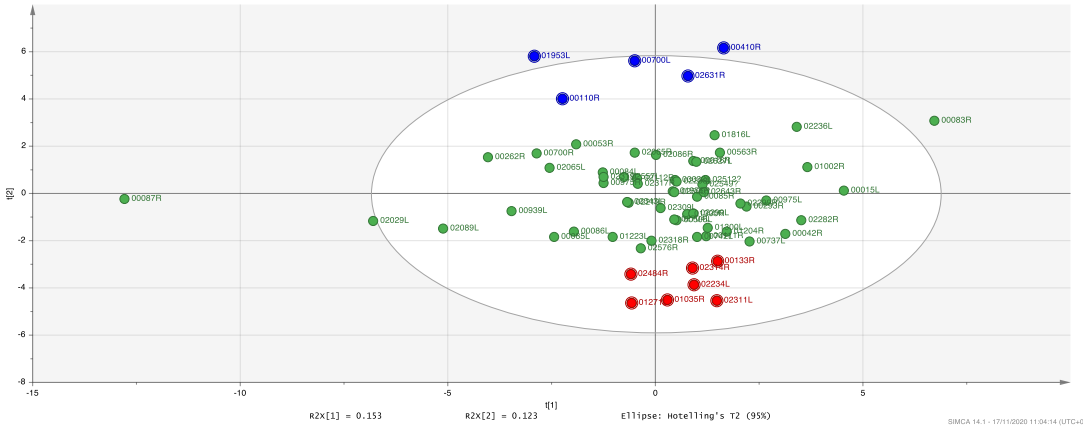

Figure 95: Scores scatter plot showing distribution of patients across the two principal components in model looking at pre-operative mechanical and biological markers. Highlighting third and fourth group of patients.

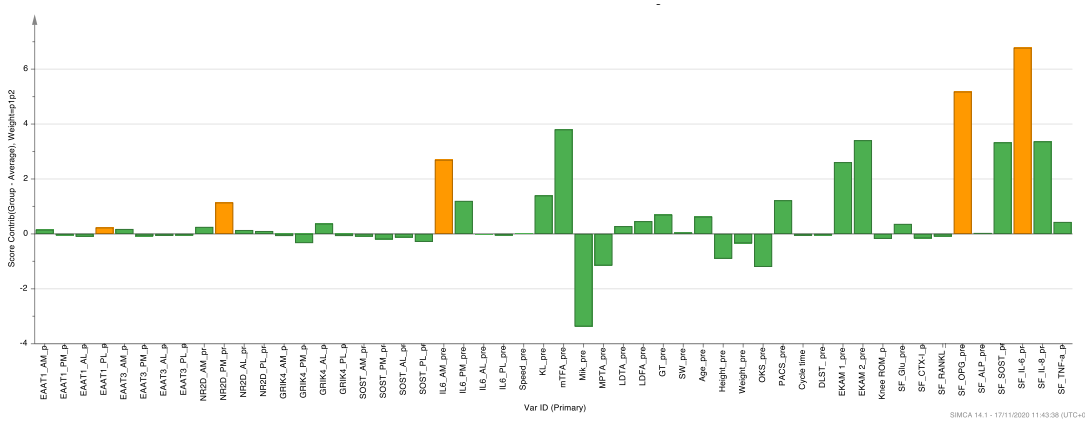

Figure 96: Score contribution compared to average for group highlighted in blue in figure 95. (Yellow variables outside their 3 standard deviation range).

The red grouping by contrast show better than average alignment (particularly MPTA) and LDFA is also lower than average but this group have few distinguishing biological factors (with the possible exception of SF CTX-1) (Figure 97).

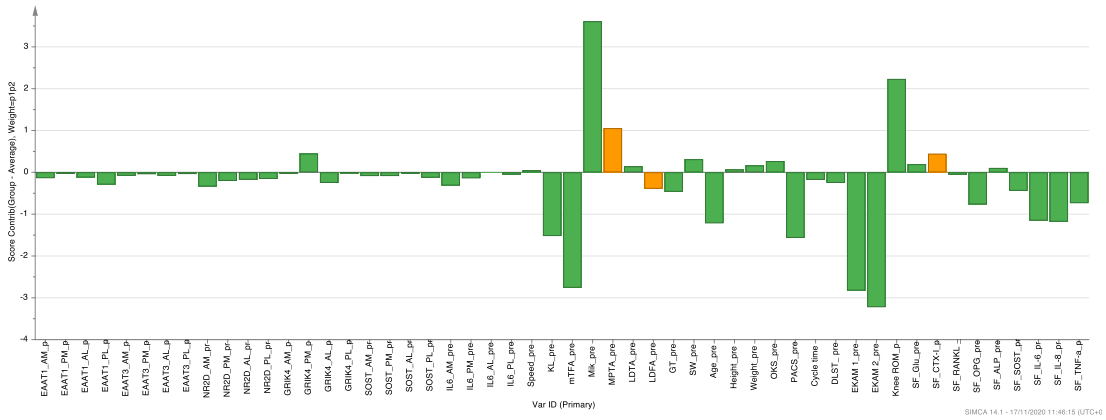

Figure 97: Score contribution compared to average for group highlighted in red in figure 95. (Yellow variables outside their 3 standard deviation range).

5.4.1.2 Are there any preoperative markers which are predictive of outcome?

Modelling the mechanical (static and dynamic) markers gave scores of R2X 0.566, Q2 0.062 over three axes, suggesting the majority of variability (57%) was explained but the model was poorly predictive.

Of note, OKS is a functional score in which a higher result means better function therefore the higher OKS_diff, the greater the functional improvement. Conversely, with PACS, a higher score means greater pain. Therefore a clinical improvement following HTO would mean a negative PACS_diff. This is important to bear in mind when assessing the following associations.

The loading plot (Figure 98) showed positive association between change in OKS (OKS_diff) and preoperative KL grading, age and PACS (shown below in red). This group are negatively associated with preoperative OKS, ROM, stance width, MPTA and change in PACS (shown in blue). There is a possible weak negative association with preoperative Mikulicz and LDTA.

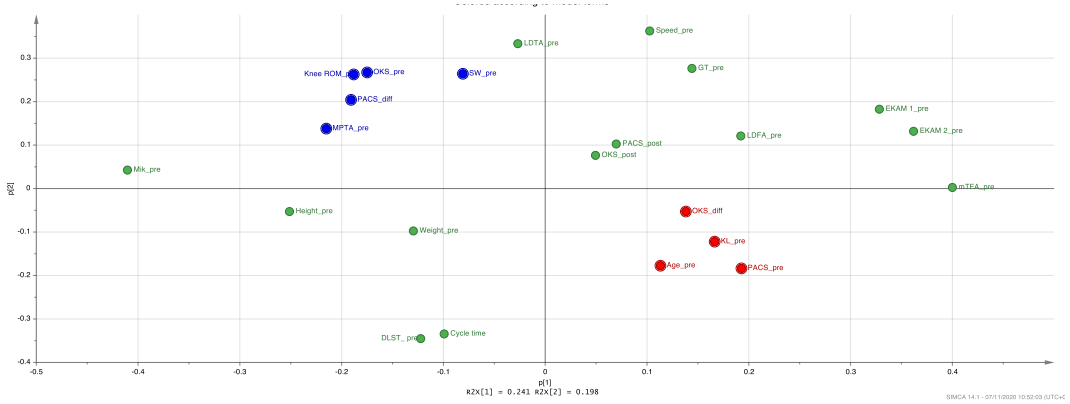

Figure 98: Loading plot of variables included in model looking at pre-operative mechanical markers and patient outcome scores (OKS, PACS). First and second group of variables highlighted.

Postoperative OKS seemed strongly positively associated with postoperative PACS. This pair were more weakly positively associated with preoperative speed, GT, LDFA, EKAM and mTFA. They were negatively associated with height, weight and preoperative DLST and cycle time (Figure 99).

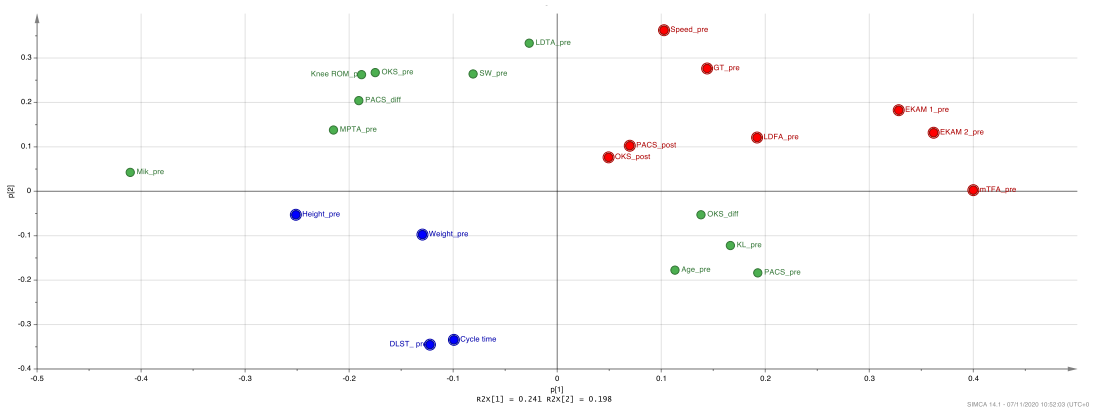

Figure 99: Loading plot of variables included in model looking at pre-operative mechanical markers and patient outcome scores (OKS, PACS). Third and fourth group of variables highlighted

After inclusion of the biological markers to the model, scores across three axes were R2X 0.391, Q2 -0.0295 suggesting moderately poor explanation of variability (39%) and very poor (negative) predictive ability.

Markers associated with improved function (greater OKS_diff) and reduced pain (included preoperative MPTA, SW, Mikulicz, PACS and IL6 PL (shown below in blue in Figure 100). Subchondral SOST production also appears more weakly correlated.

Markers associated with poorer function and less pain reduction (shown in red in Figure 100) include preoperative OKS, mTFA, LDFA, SF RANKL, SF SOST, SF IL8, SF OPG, KL, GRIK 4 in AM and PL, IL6 AL and EAAT 3 in both lateral quadrants.

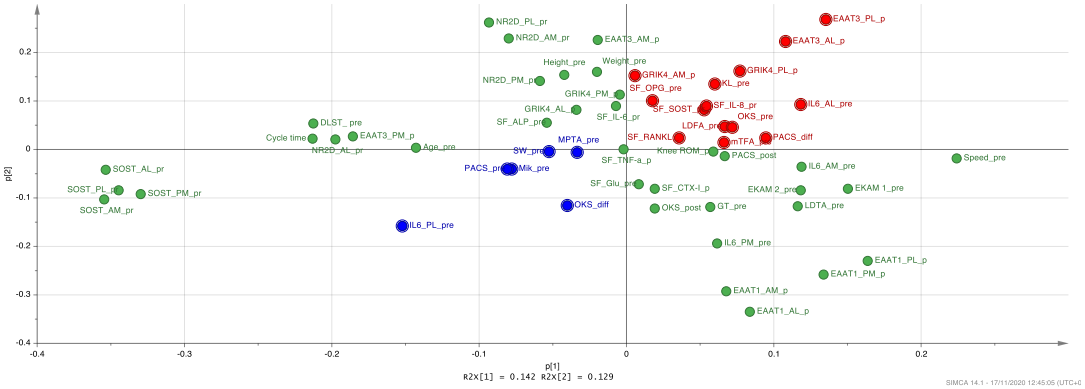

Figure 100: Loading plot of variables included in model looking at pre-operative mechanical and biological markers with patient outcome scores (OKS, PACS). First and second group of variables highlighted.

5.4.1.3 Are there any postoperative markers which could be used as surrogate markers of outcome?

Modelling was performed using change in OKS and PACS (OKS_diff, PACS_diff) along with postoperative bone core and mechanical data. There were very few postoperative synovial fluid results available therefore these had to be excluded. The initial model across two axes had scores of R2X 0.346, Q2 -0.0806, suggesting relatively poor explanation of variability (35%) and very poor (negative) predictive ability.

Postoperative factors showing some association with improved function (shown in blue in Figure 102) include Mikulicz, MPTA, global EAAT1 production in subchondral bone along with most quadrants of IL6 (except AL), GRIK4 PL, NR2D PL, SOST PL and LDFA post.

The group shown in red in Figure 102 are negatively associated with improved function and include mTFA, EKAM, ROM, poorer pain reduction and greater subchondral bone production of EAAT3 (AM, PM, PL), SOST (AM, PM), NR2D (AM, PM), GRIK4 (AM, PM).

Again, speed measures do not appear to be associated with alignment (Figure 103) but may show association with subchondral bone activity in the AL quadrant (EAAT3, NR2D, GRIK4, IL6, SOST).

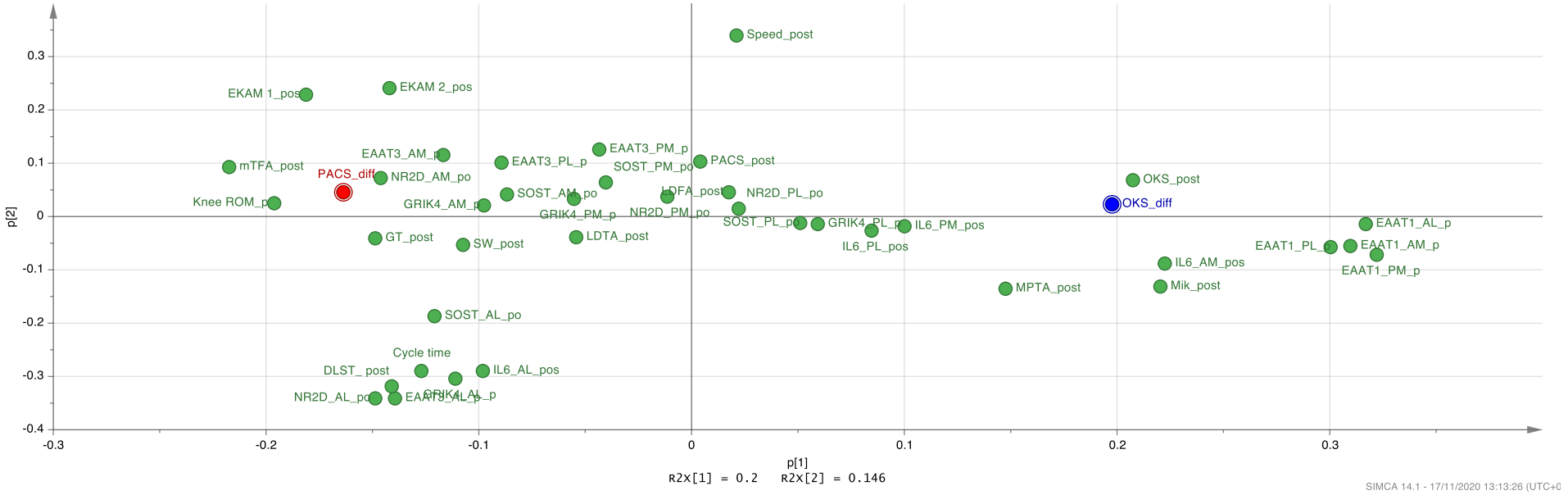

Figure 101: Loading plot of variables included in model looking at post-operative mechanical markers and patient outcome scores (OKS, PACS).

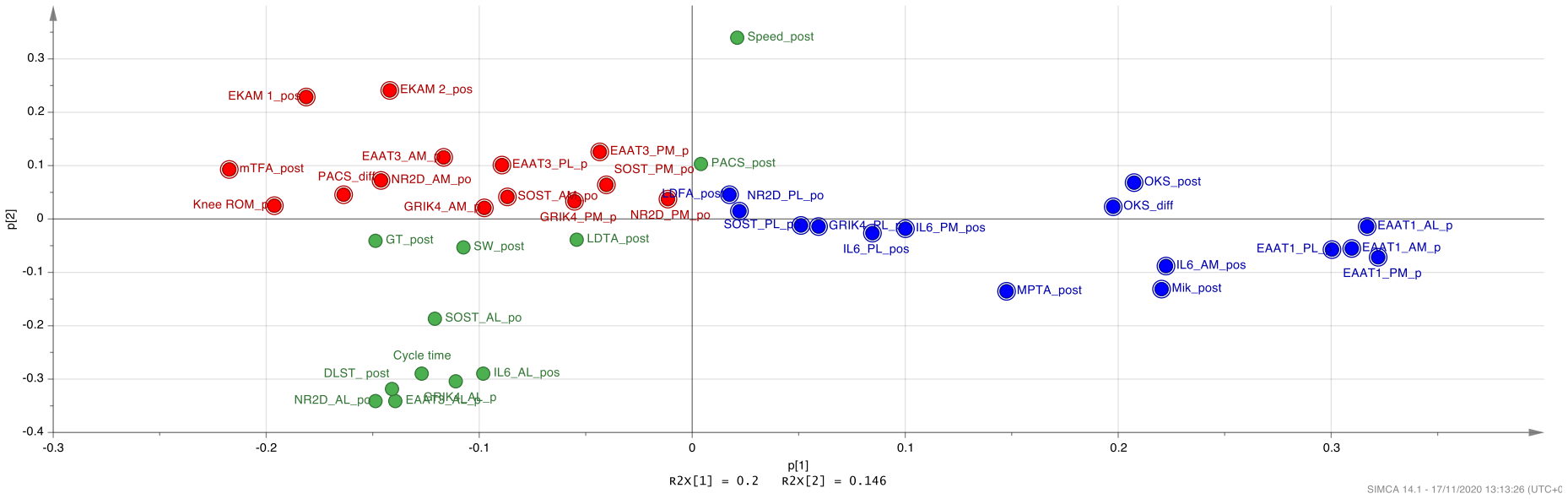

Figure 102: Loading plot of variables included in model looking at post-operative mechanical markers and patient outcome scores (OKS, PACS). First and second group of variables highlighted.

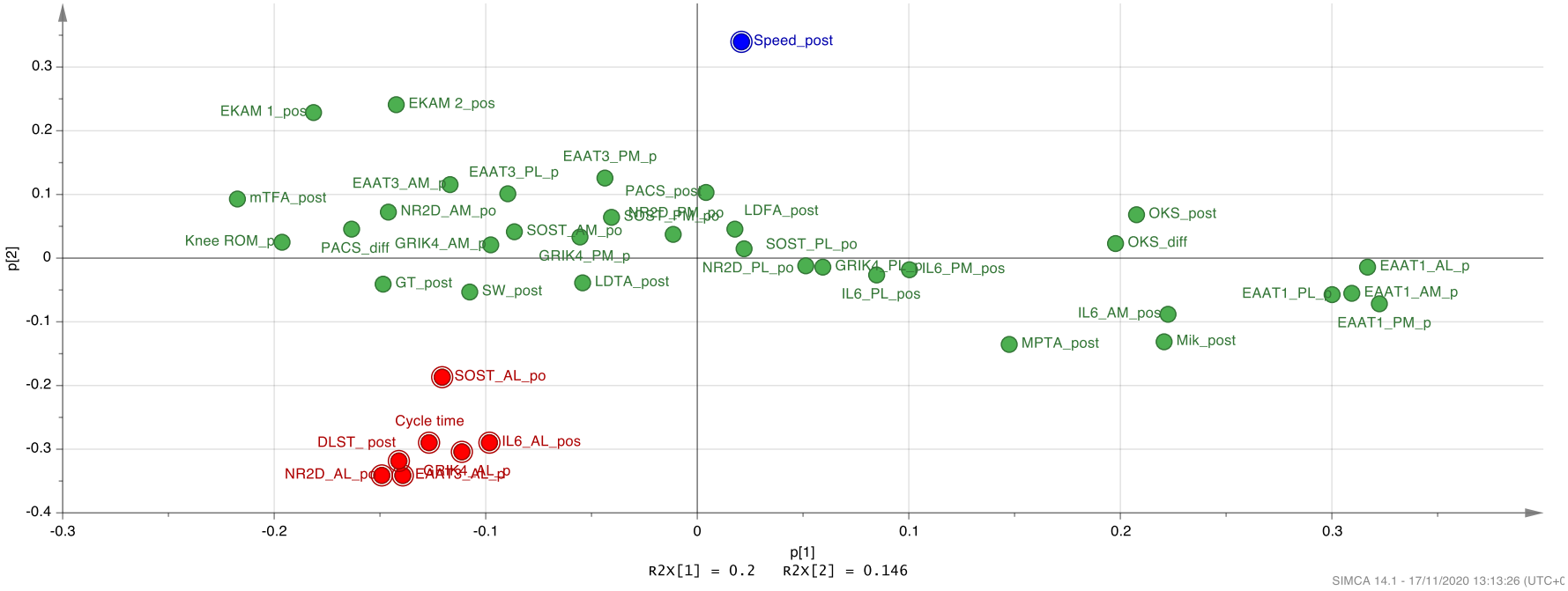

Figure 103: Loading plot of variables included in model looking at post-operative mechanical markers and patient outcome scores (OKS, PACS). Third and fourth group of variables highlighted.

5.4.1.4 What biological factors are associated with a change in alignment/HTO?

Due to the very small number of postoperative synovial fluid results, the biological factors were restricted to the subchondral bone RNA data. In order to look at change in production, the RNA percentage change data for each quadrant was used. As explained in Chapter 3 this method was devised in order to reduce the impact of methodological variability when looking at a loading related change in production across the tibial plateau quadrants. This method reduces the number of patients in the data set down to 25 as need complete data from all eight quadrants (four pre-operative, four post-operative) for the calculation.

This reduction in patient number combined with a large number of variables resulted in poor initial model scores (R2X 0.328, Q2 -0.194 across two axes if all variables used). Analysis of the model and further trials to identify the most significant variables revealed the best model scores if only the PM quadrant data from EAAT3, NR2D, GRIK4 and SOST were included alongside the selected mechanical variables. These were selected both due to their positive effect on creating the best model but also due to the potential significance identified in Chapter 3.

Including just the PM quadrants for these four bone markers (EAAT3, NR2D, GRIK4 and SOST) gave model scores R2X 0.504, Q2 -0.2, i.e. it remained a poorly (negatively) predictive model but did explain 50% of the variability.

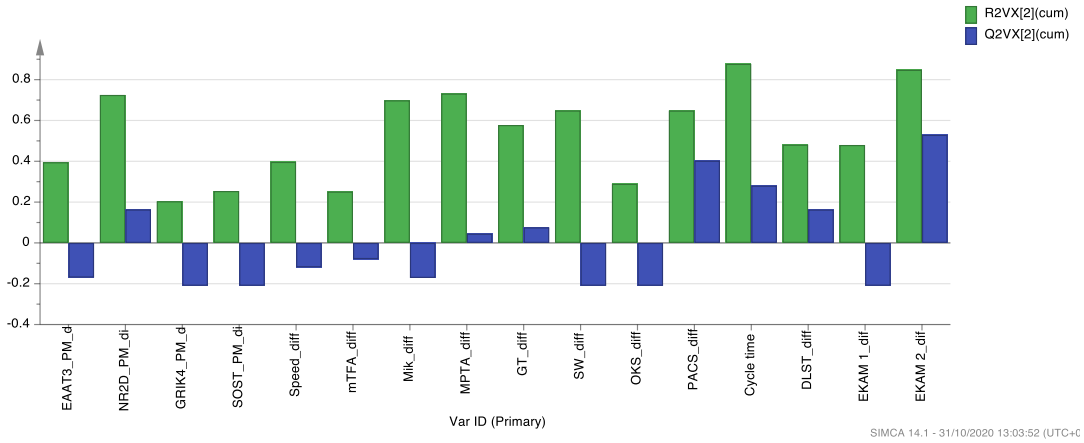

Figure 104: Contribution plot of variables included in model looking at biological factors associated with alignment change.

The variable loading plot shows correlation between percentage change in GRIK4 in PM quadrant and change in EKAM, mTFA, Mik, MPTA and subtalar alignment (GT) (Figure 105).

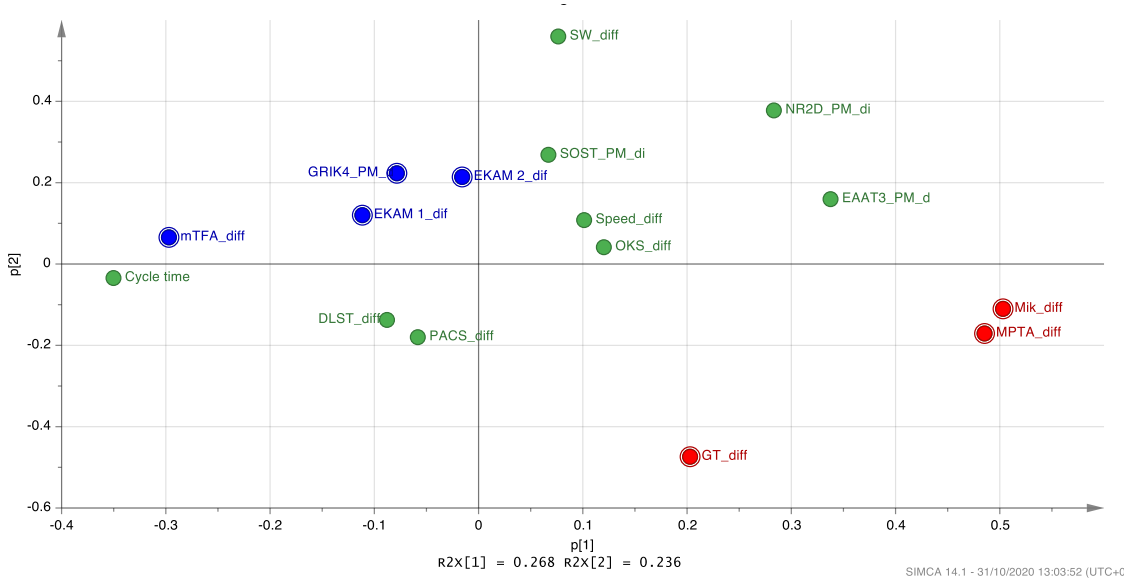

Figure 105: Loading plot of variables included in model looking at biological factors associated with alignment change.

PM quadrant change for NR2D, EAAT3 and SOST appear to be not associated with alignment but potentially associated with speed, function, pain and stance width (Figure 106).

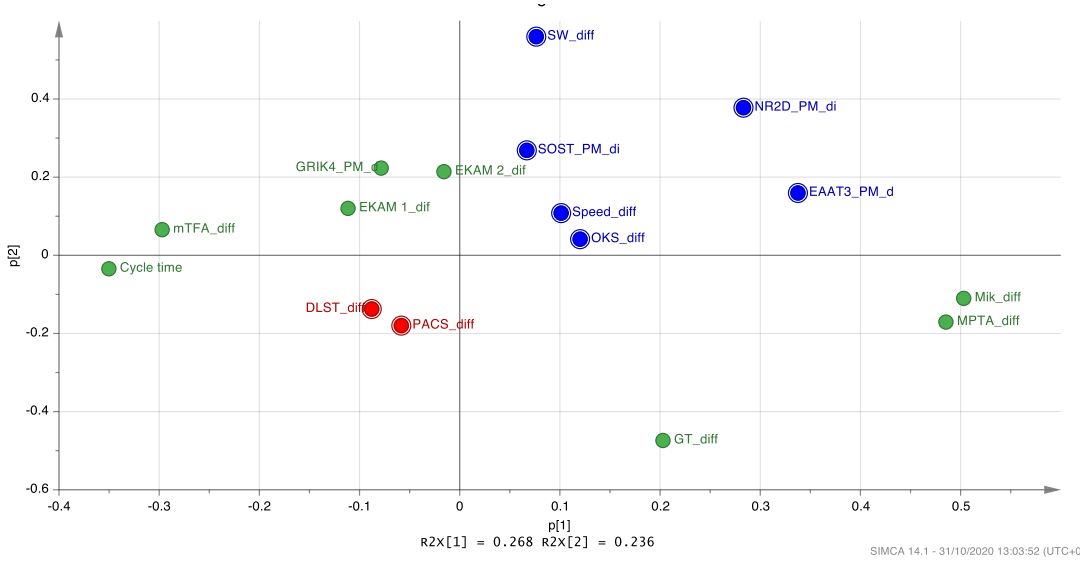

Figure 106: Loading plot of variables included in model looking at biological factors associated with alignment change.

Taking each biological marker individually (i.e. looking at a single bone marker in PM quadrant versus mechanical changes) EAAT3 (R2X 0.633, Q2 -0.12) appears associated with talar position (GT) and negatively correlated with EKAM Peak 2 (Figure 107). There is a weak positive correlation with pain and weak negative correlations with stance width, mTFA and cycle time.

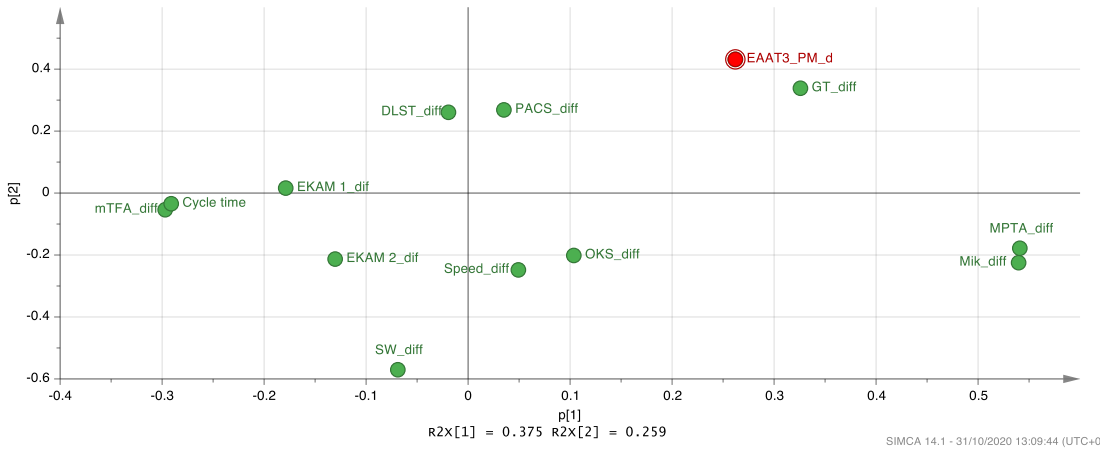

Figure 107: Loading plot of variables included in model looking at mechanical factors associated with change in EAAT3 in PM quadrant.

NR2D (R2X 0.659, Q2 -0.0223) appears associated with stance, speed and outcome but not at all with limb alignment or subtalar alignment (GT) (Figure 108).

.

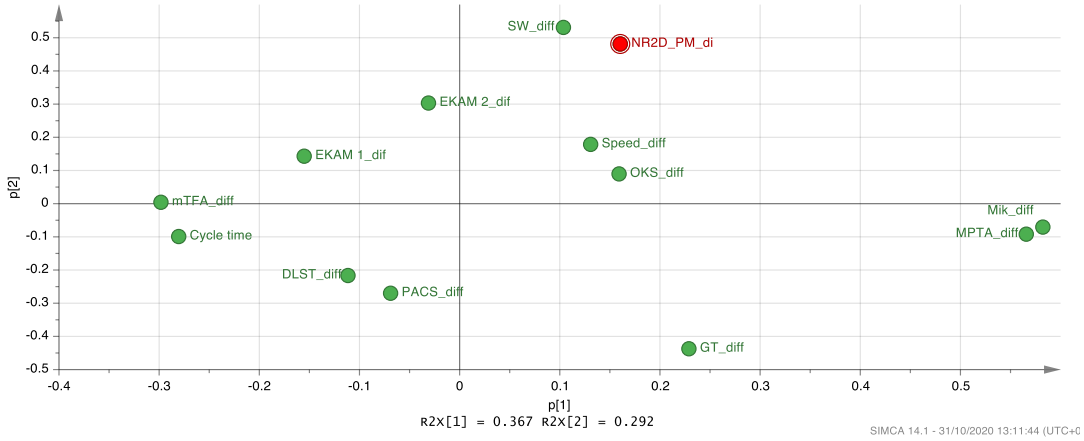

Figure 108: Loading plot of variables included in model looking at mechanical factors associated with change in NR2D in PM quadrant.

GRIK4 (R2X 0.63, Q2 -0.147) appears positively correlated with EKAM, stance width, mTFA and cycle time and negatively correlated with pain and subtalar alignment (GT) (Figure 109).

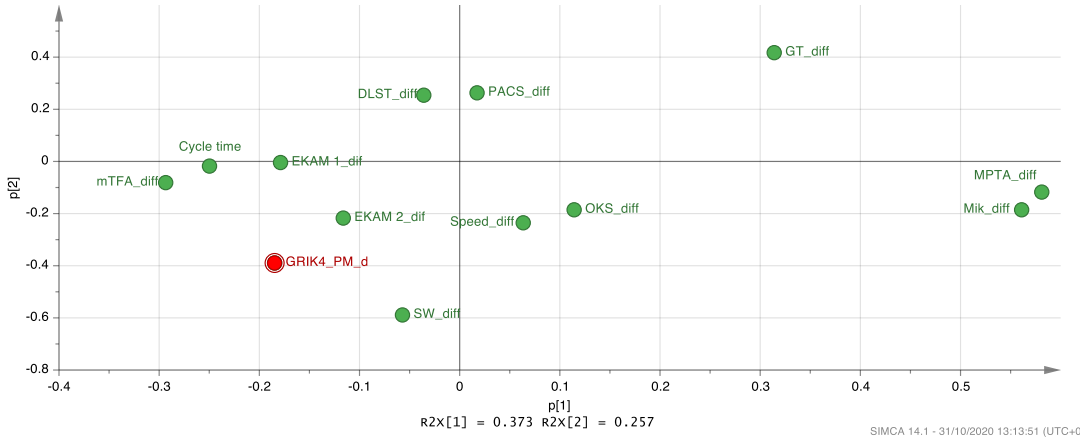

Figure 109: Loading plot of variables included in model looking at mechanical factors associated with change in GRIK4 in PM quadrant.

SOST (R2X 0.617, Q2 -0.0969) shows weak positive correlations with stance width and EKAM. There is a negative correlation with PACS and possible weak negative correlation with GT (Figure 110).

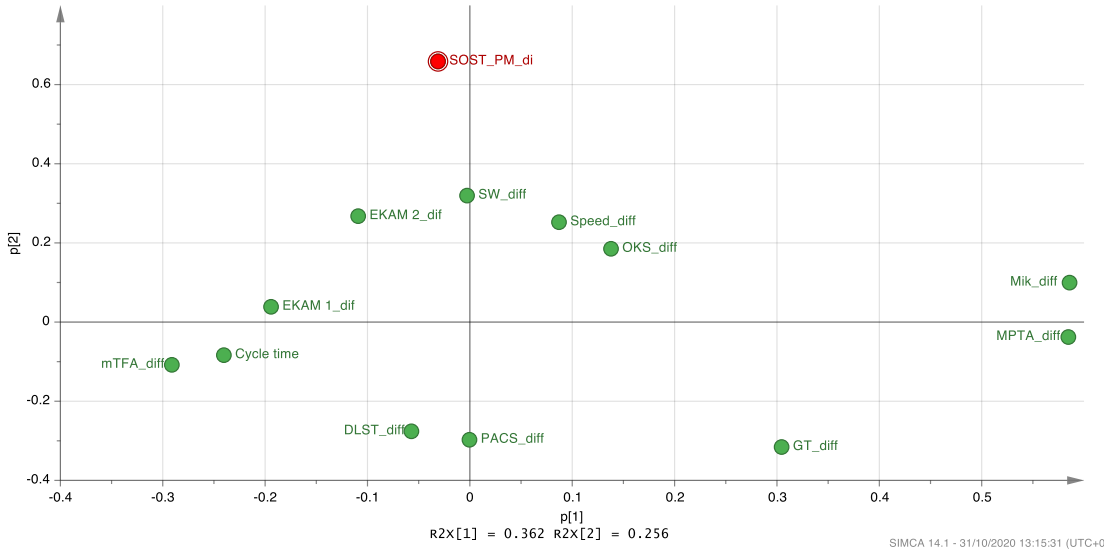

Figure 110: Loading plot of variables included in model looking at mechanical factors associated with change in SOST in PM quadrant.

To investigate the possible link between alignment change and production of these markers by the subchondral bone, GRIK4 was investigated in more detail as it had shown the strongest potential association with change in alignment (both dynamic and static). When the patient scores plot is examined, the patients do appear to fall into two groups (Figure 111):

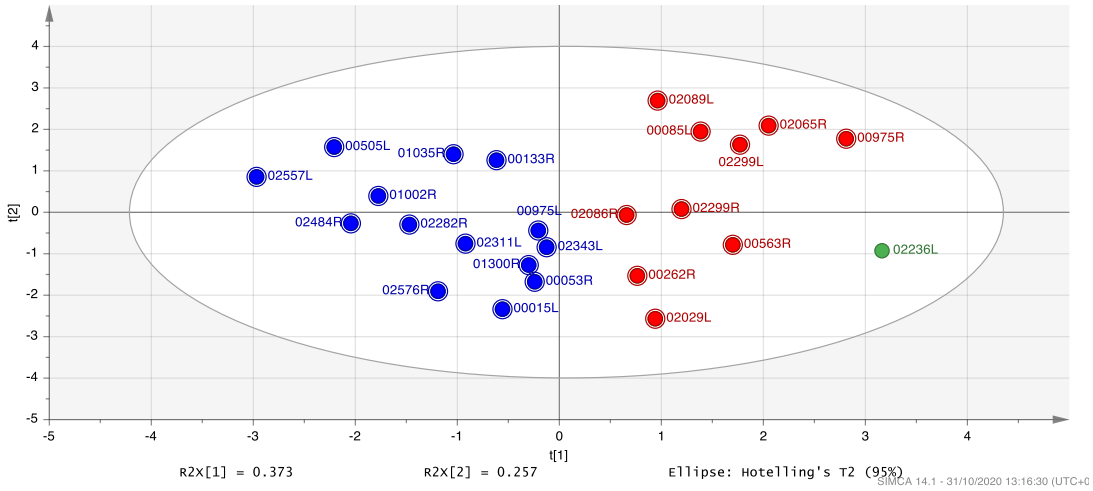

Figure 111: Scores scatter plot showing distribution of patients across the two principal components in model looking at GRIK4 and alignment measures. Highlighting groups of patients.

The blue group appear to show lower than average changes in alignment measures along with greater change in talar tilt and slightly greater than average change in GRIK4 PM (Figure 112).

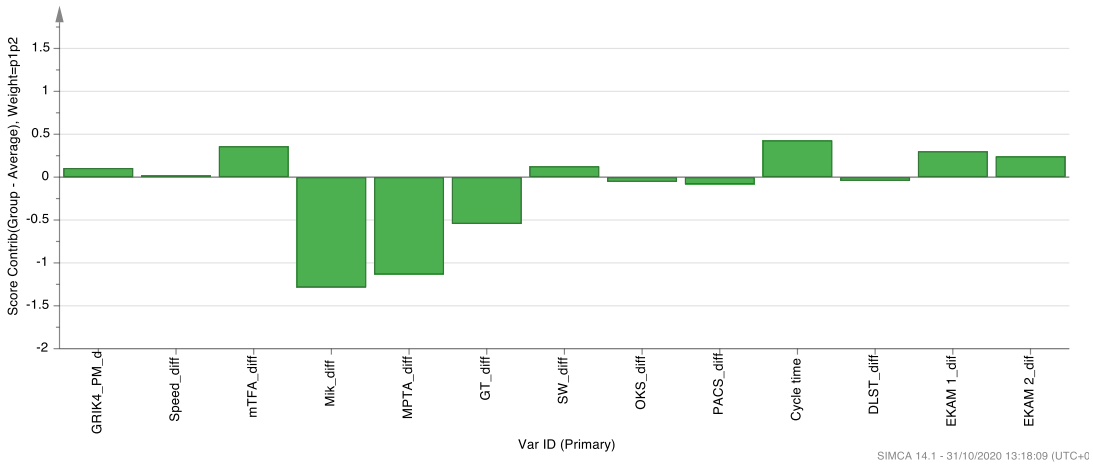

Figure 112: Score contribution compared to average for group highlighted in blue in Figure 111.

The red group have the converse pattern:

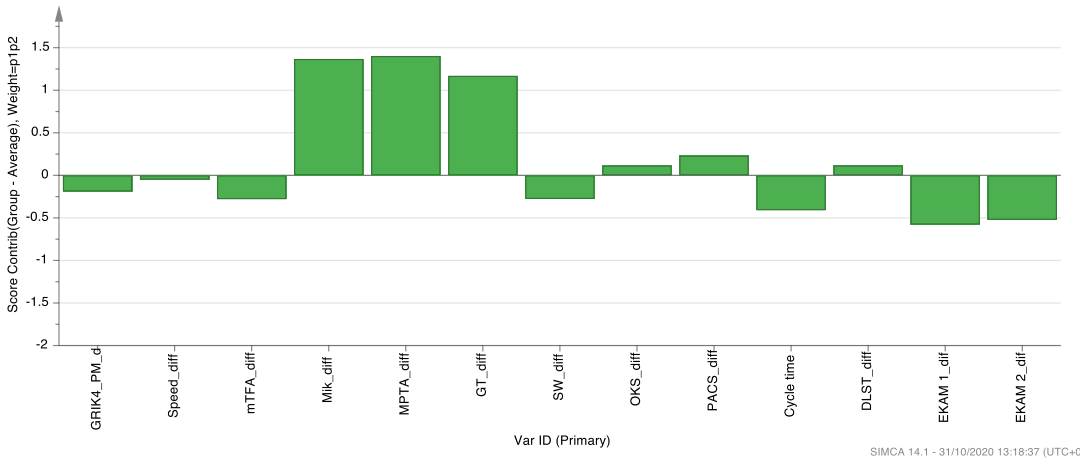

Figure 113: Score contribution compared to average for group highlighted in red in figure 111.

The groups appear to be split to a degree by the change in Mikulicz point, with those on the left (blue group) having higher (i.e. more normal) Mikulicz point (Figure 114).

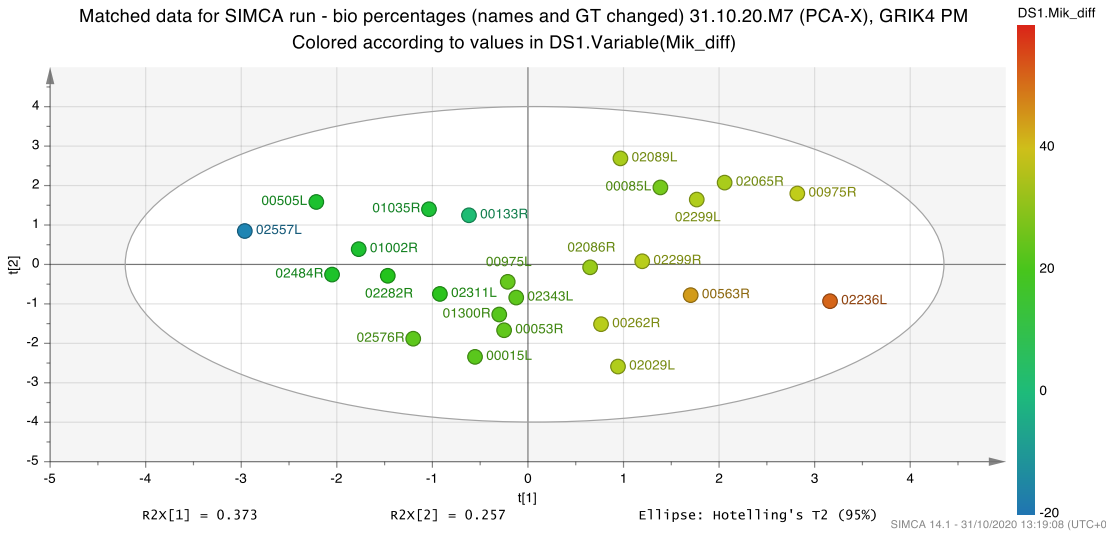

Figure 114: Scores scatter plot showing distribution of patients across the two principal components in model looking at GRIK4 and alignment measures. Coloured according to change in Mikulicz point (Mik_diff).

The equivalent plots for models focussing on EAAT3, NR2D and SOST also show a similar patient grouping (Figures 115-7).

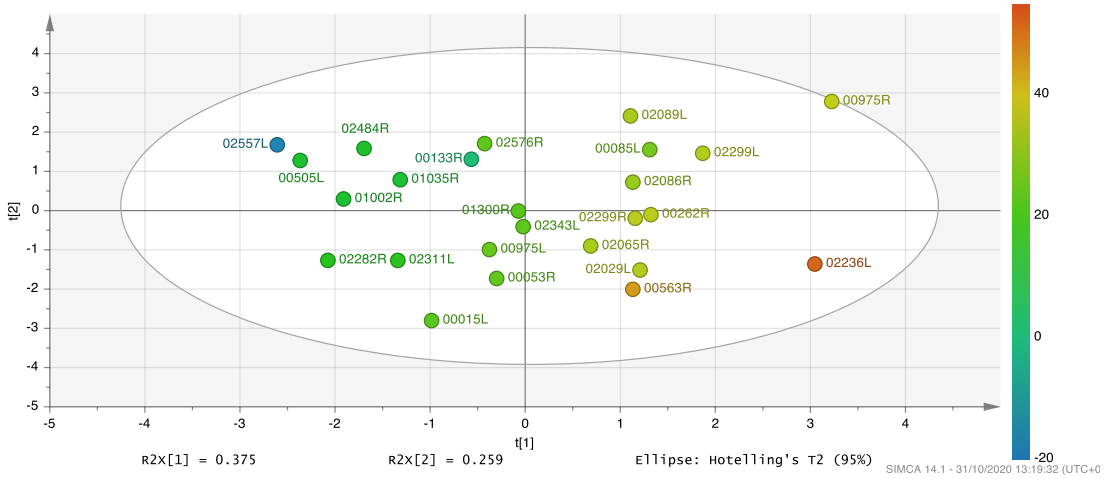

Figure 115: Scores scatter plot showing distribution of patients across the two principal components in model looking at EAAT3 and alignment measures. Coloured according to change in Mikulicz point (Mik_diff).

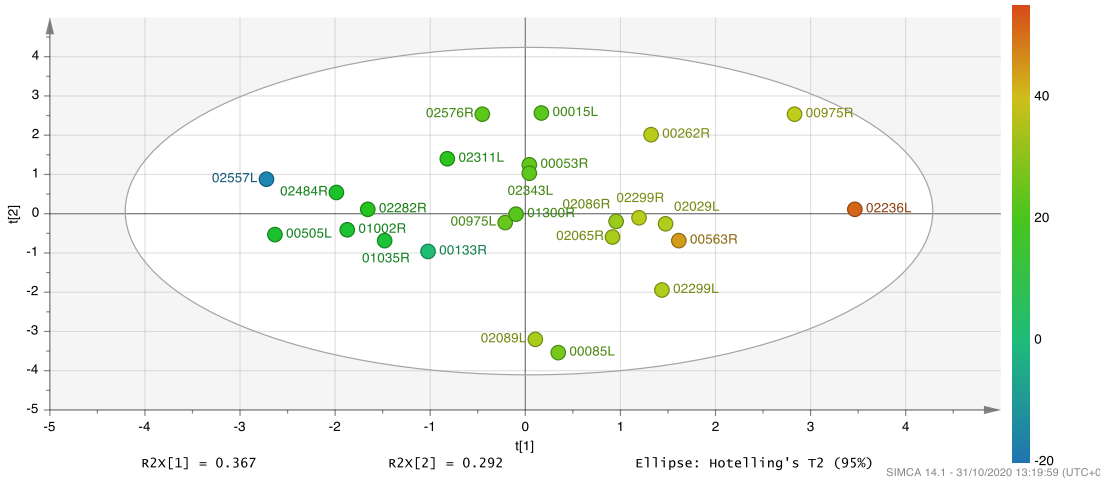

Figure 116: Scores scatter plot showing distribution of patients across the two principal components in model looking at NR2D and alignment measures. Coloured according to change in Mikulicz point (Mik_diff).

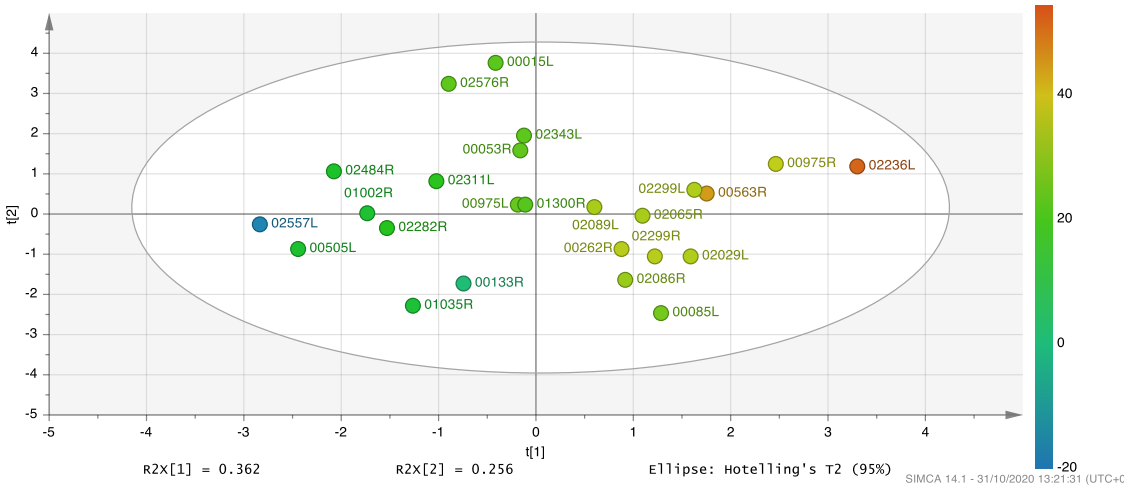

Figure 117: Scores scatter plot showing distribution of patients across the two principal components in model looking at SOST and alignment measures. Coloured according to change in Mikulicz point (Mik_diff).

5.4.2 Correlations between individual variables

The analysis of subchondral bone in Chapter 3 suggested expression of NR2D, GRIK4 and SOST in the PM quadrant could be responsive to the change in load caused by HTO. The PCA analysis described also identified potential correlations with these markers and EAAT3 also showed prominence in some of the models.

These biological variables were therefore compared individually with the mechanical, radiological and clinical variables using Spearman's rank correlation coefficient. Data pairings were excluded if n<10 so therefore only static radiographic measures were able to be tested. Correlations were judged as significant at the 0.05 level. However, those less than 0.1 were also highlighted as, though not meeting the threshold of proven significance, they may represent areas of potential interest in subsequent investigation.

Table 31: Correlations between pre- to post-operative differences in EAAT3, NR2D, GRIK4 and SOST in the PM quadrant and mechanical/clinical/radiological variables

** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).
†Correlation is significant at the 0.1 level (2-tailed).

		EAAT3_PM_diff	NR2D_PM_diff	GRIK4_PM_diff	SOST_PM_diff
EAAT3_PM_diff	Correlation Coefficient		0.495*	0.126	0.22
	Sig. (2-tailed)		0.019	0.586	0.312
	N		22	21	23
NR2D_PM_diff	Correlation Coefficient	0.495*		0.29	0.2
	Sig. (2-tailed)	0.019		0.203	0.385
	N	22		21	21
GRIK4_PM_diff	Correlation Coefficient	0.126	0.29		0.136
	Sig. (2-tailed)	0.586	0.203		0.556
	N	21	21		21

SOST_PM_diff	Correlation Coefficient	0.22	0.2	0.136	
	Sig. (2-tailed)	0.312	0.385	0.556	
	N	23	21	21	
KL_pre	Correlation Coefficient	0.015	0.149	0.008	-0.071
	Sig. (2-tailed)	0.943	0.499	0.973	0.743
	N	24	23	22	24
mTFA_pre	Correlation Coefficient	0.185	0.027	0.183	0.352
	Sig. (2-tailed)	0.386	0.904	0.415	0.092
	N	24	23	22	24
mTFA_post	Correlation Coefficient	0.133	-0.117	0.573**	0.373†
	Sig. (2-tailed)	0.545	0.604	0.007	0.079
	N	23	22	21	23
mTFA_diff	Correlation Coefficient	-0.231	-0.21	0.031	-0.173
	Sig. (2-tailed)	0.289	0.347	0.893	0.431
	N	23	22	21	23
Mik_pre	Correlation Coefficient	-0.096	-0.112	-0.153	-0.325
	Sig. (2-tailed)	0.655	0.61	0.496	0.121
	N	24	23	22	24
Mik_post	Correlation Coefficient	-0.078	0.046	-0.3	-0.39†
	Sig. (2-tailed)	0.715	0.835	0.174	0.059
	N	24	23	22	24
Mik_diff	Correlation Coefficient	0.173	0.173	-0.032	0.054
	Sig. (2-tailed)	0.42	0.43	0.887	0.801
	N	24	23	22	24
MPTA_pre	Correlation Coefficient	-0.202	-0.052	0.056	-0.052
	Sig. (2-tailed)	0.344	0.814	0.805	0.81
	N	24	23	22	24
MPTA_post	Correlation Coefficient	0.043	0.104	-0.134	-0.132
	Sig. (2-tailed)	0.847	0.645	0.562	0.549
	N	23	22	21	23
MPTA_diff	Correlation Coefficient	0.137	0.098	-0.092	-0.082
	Sig. (2-tailed)	0.532	0.665	0.691	0.711
	N	23	22	21	23
LDTA_pre	Correlation Coefficient	-0.227	-0.304	-0.143	0.182
	Sig. (2-tailed)	0.336	0.207	0.57	0.443
	N	20	19	18	20
LDFA_pre	Correlation Coefficient	0.327	0.273	-0.058	0.272

	Sig. (2-tailed)	0.119	0.208	0.798	0.198
	N	24	23	22	24
GT_pre	Correlation Coefficient	-0.533*	-0.121	0.213	0.475†
	Sig. (2-tailed)	0.034	0.656	0.446	0.063
	N	16	16	15	16
GT_post	Correlation Coefficient	-0.394	-0.225	0.265	0.284
	Sig. (2-tailed)	0.163	0.439	0.382	0.324
	N	14	14	13	14
GT_diff	Correlation Coefficient	0.325	-0.126	0.27	-0.231
	Sig. (2-tailed)	0.257	0.667	0.372	0.426
	N	14	14	13	14
SW_pre	Correlation Coefficient	-0.018	-0.407	-0.394	0.026
	Sig. (2-tailed)	0.945	0.105	0.131	0.919
	N	18	17	16	18
SW_post	Correlation Coefficient	-0.119	0.24	0.05	-0.044
	Sig. (2-tailed)	0.639	0.353	0.854	0.861
	N	18	17	16	18
SW_diff	Correlation Coefficient	-0.179	0.478†	0.306	0.059
	Sig. (2-tailed)	0.478	0.052	0.249	0.817
	N	18	17	16	18
Age_pre	Correlation Coefficient	0.098	0.282	0.227	0.005
	Sig. (2-tailed)	0.672	0.242	0.351	0.982
	N	21	19	19	21

Table 31: Correlations between pre- to post-operative differences in EAAT3, NR2D, GRIK4 and SOST in the PM quadrant and mechanical/clinical/radiological variables

5.5 DISCUSSION

5.5.1 Principal Component Analysis

5.5.1.1 Are there phenotypic groups of patients who can be identified preoperatively?

This model revealed some interesting grouping of variables. MPTA, knee ROM, OKS and stance width all grouped together, suggesting that a good clinical score preoperatively (i.e. a less symptomatic knee) is associated with a good ROM, a wide stance and a higher MPTA (i.e. closer to 90 which is normal). This group correlated negatively with age, KL grade (OA severity) and PACS (pain) as may be expected. There may however be some contamination of the data caused by a lack of independence of some variables; for example, outcome scores such as OKS overlapping with measures of pain and stiffness.

Interestingly the associations between these groups of variables and markers of static (mTFA, Mik) and dynamic (EKAM) varus was not very strong. It has been shown in Chapter 4 that the correlation between MPTA and mTFA/Mik is not as strong as may be expected and these findings in the PCA analysis suggest the deformity at the proximal tibial level (indicated by MPTA) is more closely related to symptoms that the overall varus of the limb.

However, reassuringly in terms of model validity, the loading plot does show Mik and mTFA are strongly negatively correlated as should be expected (given a higher mTFA corresponds to a lower Mik) and these measures of static varus are grouped with the dynamic varus variables (EKAM). Severity of pre-operative malalignment also appears to be negatively correlated with higher ROM and function (as might be expected) but also with stance width,

suggesting that a wider stance may be beneficial or protective. This is of interest given there is some evidence that a wider stance gait may reduce medial compartment loading (Bowd *et al.*, 2019) and fits with the evidence put forward in Chapter 4 that stance width changes are associated with HTO.

There also appears to be a separate axis of associations on these plots related to gait speed. Speed was negatively correlated with cycle time and double limb support time (i.e. stance phase) as would be expected but interestingly speed is also correlated with ground talus angle (GT). Adaptation at the subtalar joint (as measured by GT) in response to proximal tibial malalignment was discussed in Chapter 4 where although there was only a weak correlation between GT and mTFA pre-operatively, there was a significant change in GT pre- to post-HTO, as demonstrated in other studies (Lee *et al.*, 2015; Choi *et al.*, 2017). The findings of Chapter 4 however, did posit that change in subtalar alignment and stance width may be separate adaptations occurring in different sets of patients and the PCA models do appear to support this theory; with stance width being associated more with limb alignment and GT associated more with markers of gait speed.

LDTA appeared to be related to both of the “positive” pre-operative groups (both the low symptom/low varus group and the high speed group). This suggests LDTA may have some form of mitigating effect on proximal tibial varus which would support findings from Biomechanics and Bioengineering Research Centre Versus Arthritis (BBRCVA) studies which suggested that LDTA is one of the factors that, in conjunction with MP, best explains variance in peak external knee adduction moment (EKAM), thought to be a key indicator of medial compartment loading during gait (Whatling *et al.*, 2019).

The variable clustering suggests that preoperatively there may be different phenotypic subgroups of patients dependent on which of these profiles they

conform to. When the scores plots were examined, there appeared to be some potential phenotypic groups of patients who showed similar variable associations. However, the groups were far from clearly distinct. Patients selected for HTO are a very heterogenous group and this is apparent clinically (different body habitus, clinical history, severity of symptoms/OA). However, these models suggest that there may be different mechanical phenotypes in addition. For example, there appears to be one group with preserved gait speed and adaptable subtalar joints who are in general taller and heavier. The influence of this phenotype on either their pre-operative state (i.e. to what degree proximal tibial malalignment translates into symptomatic OA) or outcome post-surgery is as yet unknown.

Following addition of the biological markers, the increase in variables caused significantly increased noise and a deterioration in the degree of variability explained by and predictive ability of the model. Similar groupings of mechanical variables were maintained but the relationships were adjusted by the biological inclusion and there were some biological indicators now associated with these mechanical variable groups.

Taking the alignment groups first, markers of limb varus (Mik and mTFA) were associated (in their respective directions) with MPTA, height and LDFA. The biological markers of glutamate, ALP and CTX-1 (components of pain, inflammation and bone remodelling pathways in OA) in the pre-operative synovial fluid positively associated with good alignment/outcomes but by contrast, poorer outcomes and worse malalignment were associated not just with OA severity (KL grade) but with higher levels of SOST, IL-8, OPG and IL6 within the synovial fluid, suggesting a possible link to higher inflammation and bone turnover. However, with few data entries for synovial fluid, true significance is difficult to judge, especially given it is the ratio of several of these mediators which is important (e.g. OPG in its role as an inhibitor of resorption).

Running cross-wise to this alignment-based axis was again the axis based on speed. Here, higher gait speed was positively associated with subchondral bone levels of EAAT3, NR2D, GRIK4 and SOST but negatively associated with EAAT1 levels. With the exception of EAAT3, there did not appear to be particular quadrant distinctions in the preoperative data. This speed-based axis ran at almost 90^{o} to the alignment-based axis, suggesting that these groups of variables are largely independent of each other. However, between these two axes, ran a third, based around patient scoring systems (OKS and PACS). Here, good function is positively associated with ROM, stance width, weight and LDTA but negatively associated with higher pain, age, some subchondral bone markers in the medial compartment (IL6, EAAT3) and synovial fluid levels of TNFa. What is interesting about this patient experience-based axis is that it runs between the other two axes described. This suggests that improved patient function and pain are contributed to both by better alignment and by higher gait speed (along the other mechanical and biological markers grouped with these variables as previously described).

Again, although patient groupings were not very distinct, there do appear to be some clusters of patients displaying particular phenotypic patterns, with alignment and OA severity being the most important distinguishing features across the whole patient group. Four distinct patient groups are potentially detectable; the first two characterised by high subchondral bone activity (and so potentially inflammation) and the second two by alignment.

One clear limitation is the very small numbers of patients with synovial fluid data (as this was contributed from a related study with an overlapping patient cohort). The model was trialled without these markers included to check whether they were causing a disproportionate effect but the scores were only slightly improved and the underlying patterns largely unchanged. Therefore

they were retained as they provide useful indicators for the direction of future research. This is only possible when examining the preoperative data as there are very scant data points for postoperative synovial fluid markers.

Although far from conclusive, this model does add weight to the theory that variation in patient expression of subchondral bone and synovial fluid markers may represent the “missing link” in explaining how mechanical changes result in patient symptoms. It also supports the theory that the variation in this pathway is likely due to different patient phenotypes, both mechanical or biological.

5.5.1.2 Are there any preoperative markers which are predictive of outcome?

The model of mechanical (static and dynamic) markers gave scores of R2X 0.566, Q2 0.062 over three axes, suggesting the majority of variability (57%) was explained but the model was poorly predictive.

The first groups of associations within the loading plot suggested the greatest improvement in function (OKS) was related to older patients, higher levels of preoperative pain, more severe OA, poorer preoperative function, poorer ROM, narrower stance width and more severe proximal tibial deformity (MPTA). There was no strong association with levels of limb malalignment (mTFA or Mikulicz). This suggests that patients with a milder symptomatic burden or minimal tibial malalignment to begin with, may need to be counselled that they may not experience such dramatic improvement. It may also suggest that even those with more severe disease could still experience symptomatic relief. However this data is not sufficient to determine the maximum or minimum thresholds for clinical effectiveness and does not shed any light on longer term outcome measures (for example whether minimal alignment correction has a protective effect on future OA).

The second set of associations were more surprising. There was a strong association between high function post operatively (high OKS) and high levels of pain post operatively (high PACS), which seems counterintuitive. This pairing was positively associated with higher preoperative speed, talar malignment and LDFA, plus, to a lesser extent, EKAM and mTFA. This variable groups was negatively associated with height weight and markers of slower gait. It is difficult to explain the grouping of these factors but it is possible that absolute post-operative OKS or PACS scores do not have sufficient validity in between-patient comparisons.

Following addition of the preoperative biological markers to the model, the model quality scores decreased and the addition of a large number of additional variable makes interpretation challenging. Although no definite conclusions can be drawn, it did suggest that improved function and pain relief was associated with a lower level of pre-operative deformity (higher MPTA, Mik and lower mTFA), a lower starting grade of OA and that a wider stance width was beneficial. Higher levels of IL-6 (an inflammatory mediator) along with SOST, RANKL and OPG (markers of bone turnover) in the synovial fluid were associated with poorer pain relief and functional improvement as measure by the PACS and OKS score changes.

Interestingly however, tibial plateau subchondral bone production of SOST across all quadrants was negatively associated with its level within the synovial fluid, i.e. was a marker of positive outcome (improved function and decreased pain). Higher expression of SOST would suggest greater inhibition of bone formation and therefore reduced sclerosis. The established literature suggests that sclerostin expression is decreased in osteoarthritis (Power *et al.*, 2010; Chan *et al.*, 2011; Weivoda, Youssef and Oursler, 2017) so the correlation of higher pre-operative sclerostin with the “positive

outcome" markers may be explained by these patients having milder OA (as demonstrated by the negative correlation with KL grade). Perhaps this represents patients with early-stage OA who still have reparative potential within the tibial plateau. This is in line with studies suggesting higher levels of sclerostin are associated with later (more sclerotic) stages of OA (Mabey *et al.*, 2014). In addition, the PCA model suggests SOST expression by the subchondral bone is associated with a lesser degree of starting deformity (i.e. less abnormal loading) which is potentially in keeping with the scientific consensus that SOST expression by osteocytes is down-regulated by load (Lin *et al.*, 2009; Delgado-Calle, Sato and Bellido, 2017; Weivoda, Youssef and Oursler, 2017).

However, it is notable in this model made no differentiation of SOST production across the quadrants and appears to run contrary to the findings of Chapter 3, which suggested that the quadrant of the knee most off-loaded by MOW-HTO (the posteromedial quadrant) saw proportional downregulation of SOST post-HTO whereas the quadrant that the load to was transferred to (posterolateral) saw upregulation. There is full discussion of these findings in Chapter 3 but the difference between those results and the suggestion of this PCA model may be explained by different input data. In Chapter 3, it was the change in proportional expression pre- to post-operatively by each quadrant that was being explored whereas in this model it was the raw pre-operative levels being used.

These results provide interesting starting points for further research by identifying potentially involved markers. However, in a poorly predictive model, the inferences drawn cannot be considered conclusive and as this is novel research, there is little existing literature with which to compare. Of the scant available studies in this area, most look at factors associated with cartilage degeneration (of native or following cartilage regeneration techniques) (C.-W. Kim *et al.*, 2017; Kumagai *et al.*, 2017) although some

also looked at clinical outcome scores (Catani *et al.*, 1998; C.-W. Kim *et al.*, 2017; Otsuki *et al.*, 2021). Various pre-operative factors that may assess outcome were suggested including age, BMI, pelvic retroversion, severity of OA and limb external rotation. However the studies are contradictory, several commented on the lack of association between cartilage health and clinical outcome and none included any analysis of biological factors.

5.5.1.3 Are there any postoperative markers which could be used as surrogate markers of outcome?

The model explained only 35% of the variability and was weakly negatively predictive. It suggested that improved patient reported functional outcome (higher OKS_diff) is associated with better postoperative coronal alignment both static (mTFA, Mik, MPTA) and dynamic (EKAM). However, there were also interesting associations seen within the subchondral bone activity. It seems that higher postoperative EAAT1 production by the tibial plateau is associated with better outcome although this did not appear to be quadrant specific (therefore potentially not directly related to change in load). IL-6 showed a similar but weaker pattern although the AL quadrant associated differently. Meanwhile, higher production of SOST, NR2D and GRIK 4 postoperatively within the medial compartment appeared associated with poorer pain reduction and poorer function (in keeping with NR2D and GRIK4 role in glutamate mediated OA pathways of inflammation and pain). This is particularly interesting as it reflects the findings in chapter 3 that posited these markers as the most likely responders to change in load. Again, this model adds weight to the theory that there are a subgroup of patients, potentially with very early OA, who have biologically responsive subchondral tibial bone and who seem to be associated with better improvement in pain and function post-HTO. These pathways are difficult to interpret however as bone remodelling and inflammation can be a good or bad prognostic

indication dependent on the degree. Although the evidence remains tenuous, this does point to the possibility these patients are undergoing modulation of OA-linked pathways and as such that HTO could be affecting the progression of OA.

The picture for EAAT3 appeared more mixed with most quadrants (AM, PM, PL) negatively associated with function.

Also of interest is the clustering of AL quadrant activity for all but one subchondral bone marker (EAAT3, NR2D, GRIK4, IL6 and SOST) with markers of reduced gait speed. This may suggest that gait speed has an effect on differential loading through the tibial plateau, perhaps due to increased rotational or shear forces.

5.5.1.4 What biological factors are associated with a change in alignment/HTO?

It is of great interest that selection of variables for this model again highlighted EAAT3, NR2D, GRIK4 and SOST particularly in the posteromedial quadrant. Not only have these markers and this quadrant also been identified as potentially significant in some of the previous PCA investigation earlier in this chapter but also the previous analysis described in Chapter 3 picked up GRIK4, NR2D and SOST as showing potential down regulation in the PM quadrant following HTO.

This reflects the fact that the PM quadrant is likely the site of greatest load change following HTO. This is because pre-HTO, the varus malalignment will cause load to be disproportionately medial and the combination of knee joint morphology and the roll back effect will cause greater load posteriorly. As previously described, during gait, the contact points of the tibiofemoral joint

are located progressively posteriorly as the knee flexes and engages the "roll-back" mechanism (Masouros, Bull and Amis, 2010).

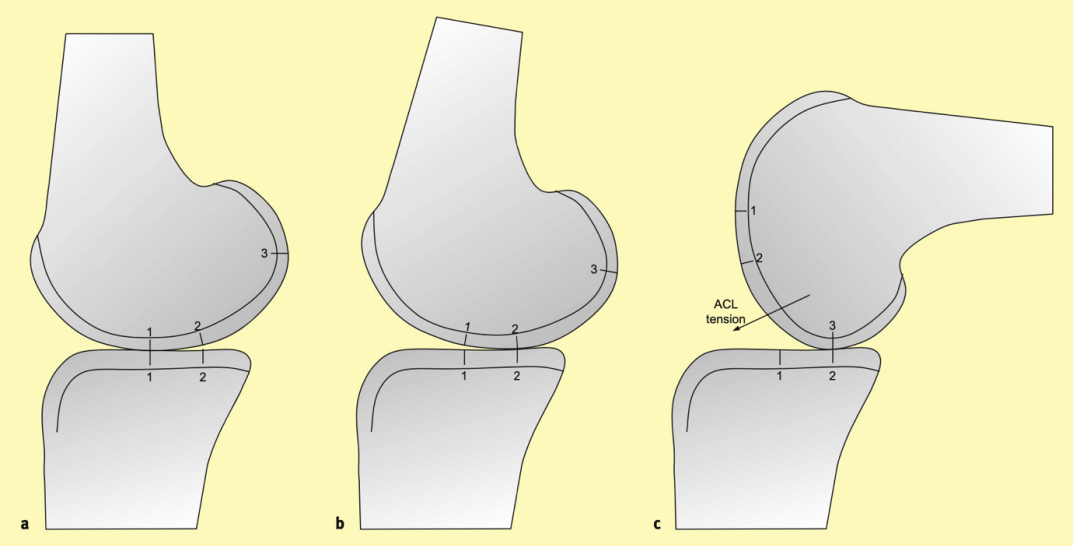

Figure 119: Knee joint kinematics in the sagittal plane during gait (reproduced from Masouros, Bull and Amis 2010). **a** Extension: contact is located centrally. **b** Early flexion: posterior rolling; contact continuously moves posteriorly. **c** Deep flexion: femoral sliding; contact is located posteriorly; the unlocking of the ACL prevents further femoral roll back.

In addition, the more the knee is flexed, the further posterior the body weight is situated relative the knee and therefore the longer the moment arm and the greater the force transmitted through the joint. Previous studies have suggested walking produces forces of 3.4 body weight (BW), increasing to 4.3 BW when climbing stairs and 8.5 when walking downhill or getting up from a chair (Masouros, Bull and Amis, 2010). These increased forces are occurring during flexion and therefore as shown above are being disproportionately transmitted into the PM quadrant of the tibial plateau.

In addition, there is some early evidence that the site of knee contact force is also more posterior in patients with OA than controls (Meireles *et al.*, 2017).

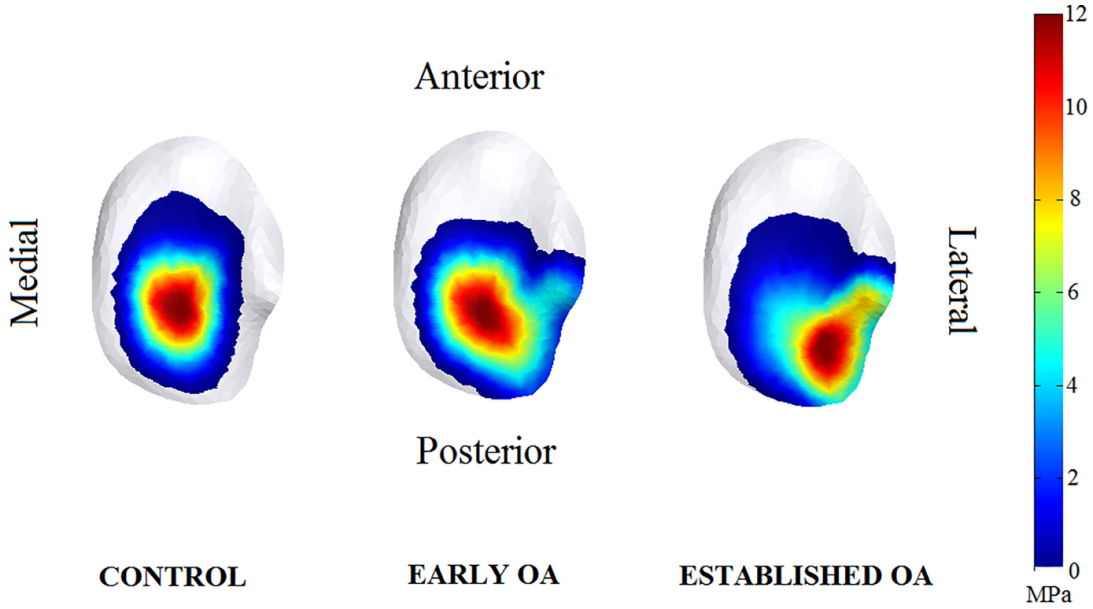

Figure 120: Group-averaged contact pressure distributions on the articular surfaces of medial tibial plateau at the time intant of the first peak medial knee contact force. Reproduced from Meireles et al. 2017.

Including just the PM quadrants for these four bone markers (EAAT3, NR2D, GRIK4 and SOST) produced a model that explained approximately 50% of the variability but remained negatively predictive. In this model, again the interplay between the effect of change in alignment combined with the effect of HTO on gait speed and the rest of the limb (talus alignment, stance width) was seen. It seems that each of these mechanical factors is associated to different degrees with different biological response. The factor with the closest association to alignment change appeared to be GRIK4, supporting the findings in Chapter 3 that it constitutes part of a load modulated, glutamate mediated pathway within osteoarthritis.

Again, different groups of patients appear to have differing biological responses to the change in alignment, reinforcing the earlier discussion about the heterogeneity of patients both clinically, mechanically and biologically.

5.5.2 Correlations between individual variables

The pre- to post-operative change in subchondral bone production of NR2D, GRIK4 and SOST in the posteromedial quadrant were selected due to their identification as potential significant markers of HTO induced loading change, both in Chapter 3 but also within the PCA. EAAT3 PM was identified as potentially significant or load dependent in the PCA so was also included.

Changes in EAAT3 and NR2D were moderately associated but this was the only association between the biological markers. This suggests that, with the exception of EAAT3 and NR2D, each marker represent different responses or mechanisms to load change. This is in keeping with the earlier work in Chapter 3 which suggested they were not associated.

The most significant correlation was seen between change in GRIK4 and post-operative mTFA. The moderately positive correlation is further evidence that the correction of varus malalignment by HTO causes a downregulation in GRIK4 in the posteromedial (off-loaded) quadrant and is in line with the findings in Chapter 3. The fact that it is the post-operative value of mTFA which is significant suggests what is important is how “normal” the knee alignment is post-operatively rather than the degree of change. However, it is interesting that no significant correlation is seen with Mikulicz point given this measures a similar feature of limb alignment and these markers have proved to be closely associated in earlier analysis.

SOST did not show any correlations that met the significance threshold but did show correlations at the 0.1 level to both mTFA and Mikulicz point post-operatively. Although not judged significant in this data set, given the repeated identification of SOST as a potential marker of loading change (in Chapter 3 and in the PCA) and the mirroring of this result with that described for GRIK4, it would represent an interesting marker for further study.

Neither EAAT3 or NR2D showed any correlation with knee or limb alignment change but did show possible correlations of interest in the measures of distal compensatory change. EAAT3 showed significant, moderately negative association with ground talus angle pre-operatively. Interestingly, SOST showed a non-significant ($p=0.064$), moderate positive correlation with the same measure. NR2D showed a non-significant ($p=0.052$) moderately positive correlation with change in stance width. Although most of these correlations did not attain the required significance level ($p<0.05$) in this data set, it is of interest that again, the measures identified as having borderline significance ($p<0.1$) via this analysis were the ones identified as showing potentially significant difference following HTO in Chapter 3.

These findings, although not conclusive, provide further evidence to support these subchondral bone and radiological markers as significant factors in the mechanism of HTO.

5.6 CONCLUSION

This chapter represents an entirely novel, exploratory approach to investigating the biological and mechanical effects of HTO and reveals further evidence to support a linked biological and mechanical hypothesis for the action of HTO on OA. The two separate analysis methods have both highlighted potentially linked variables, with some of the same candidates repeatedly appearing as significant and mirroring findings from previous analysis in Chapters 3 and 4. Again, the picture painted is one of multiple interacting responses and compensatory mechanisms, both biological and mechanical, which may explain some of the heterogeneity seen in patient outcome following HTO.

Chapter 6:

Discussion

6.1 OVERVIEW

Knee osteoarthritis is a painful, debilitating and extremely common condition, estimated to affect 17% of adults aged over 45 in England and Scotland (no Wales data available) (Arthritis Research UK, 2018). Despite being one of the commonest conditions presenting to orthopaedic services, there remain many unknowns regarding the pathogenesis of knee OA and significant gaps in its diagnosis and management. In addition to the personal cost to patients, the economic cost is significant and rising, secondary to increasing age and obesity within the population and more recently, the extensive surgical delays following the COVID pandemic.

For patients with isolated medial compartment OA, high tibial osteotomy offers a joint preserving option with good clinical evidence that it can provide improvement in pain and function (Brouwer *et al.*, 2014). However, despite this procedure being first reported in 1958, there is still little known about how it achieves its clinical effects. It is possible that it functions purely as a temporising symptomatic measure; off-loading the painful arthritic area but without affecting the underlying osteoarthritic process at all. However, some studies have reported evidence of cartilage regeneration following HTO, suggesting that the resultant changes from altering the limb alignment are more profound (Kanamiya *et al.*, 2002; Koshino *et al.*, 2003; Parker *et al.*, 2011).

The challenges of investigating this are manifold. Firstly, there are still so many gaps in our understanding of the underlying mechanisms within OA, especially its early stages. There are as yet no definitive tests or biomarkers although this remains an active area of research (Felson and Lohmander, 2009; Hunter *et al.*, 2014; Kraus *et al.*, 2015; A. Mobasheri *et al.*, 2017). Secondly, there is much that is still under debate regarding HTO; including indications, optimal correction angle, method of fixation etc. Putting just

these two issues together, this thesis was in some respects setting out to compare one unknown against another unknown. But on top of this is added the great heterogeneity of the patient cohort and lastly the multidisciplinary challenges of this topic. This last challenge is perhaps also the greatest opportunity. At present, much of the research into this area is siloed. Biologists work on the pathogenesis of OA, engineers on the mechanics of gait and alignment and clinicians on the “macroscopic” picture of patient outcome. Osteoarthritis however (and by extension all surgeries aimed at influencing it) is a biological and mechanical entity and without a fuller understanding of if and how surgery affects the biological and mechanical environment of the knee joint, it is impossible to accurately counsel patients on the appropriate therapy.

Bearing this in mind, this cross-disciplinary project aimed to link the expertise of the bioscientific, mechanical and clinical teams in order to investigate if and how HTO influences knee OA.

The first strand of this was to investigate the biological environment. The second, to analyse the changes in the mechanical environment resultant from HTO surgery which may be influencing the biological reaction and the third, to look at the potential links between the two.

6.2 BIOLOGICAL CHANGES IN THE SUBCHONDRAL BONE

Hypothesis 1: (Chapter 3) Medial opening wedge HTO causes measurable changes in molecular markers of osteoarthritis in subchondral bone.

There are a myriad of targets that could be investigated when looking for molecular markers of osteoarthritis. The target tissue for this research was the subchondral bone as it is known to be biologically active and responsive to changes in load (Skerry, 2008; McBride and Silva, 2012) and remodels in early stages of OA (Loeser *et al.*, 2012). In addition, it is possible to sample this tissue at the time of surgery with very minimal risk or morbidity to the patient.

On a macroscopic scale, there was already evidence that HTO may alter features of OA seen in the subchondral bone (such as sclerosis and bone marrow lesions) (Akamatsu *et al.*, 1997; Takahashi, Tomihisa and Saito, 2003; Kroner *et al.*, 2007) but on a molecular level, the mechanisms by which a change in load may influence the progression of OA are largely unknown. There is good evidence however that glutamate mediated pathways represent at least one of these mechanisms given that glutamate is increased in OA and has been shown to drive mechanoresponsive pathways involved in pain, inflammation and cartilage degredation (Mason *et al.*, 1997; Mason, 2004a, 2004b; Parada-Turska *et al.*, 2006; Flood *et al.*, 2007; Brakspear and Mason, 2012; Lindblad *et al.*, 2012; Bonnet *et al.*, 2015) . Therefore four components of the glutamate pathway were selected as the molecular markers for analysis (EAAT1, EAAT3, NR2D, GRIK4). In addition, two further markers were chosen to look for evidence of mechanoresponsive change in bone turnover (SOST) or inflammation (with IL-6 having been previously identified as a good predictor of post-traumatic arthritis (Garriga *et al.*, 2021)).

Results from the initial analysis were very encouraging, showing a statistically significant decrease in expression of NR2D, GRIK4 and SOST in the posteromedial (PM) quadrant following HTO. In addition to the decrease in the PM quadrant there was a corresponding statistically significant increase in SOST expression in the posterolateral (PL) quadrant. However, after the addition of five further patients, although the trends remained, the statistical significance was lost.

This is exploratory work and not a powered study. At present, not enough evidence exists on these markers to be able to select a sample size that will give a clear result. The aim was to identify markers which may represent worthwhile targets for further research and so even with the loss of statistical significance, the fact that three of the six markers showed changes all within the same quadrant suggests these results remain worthy of note. When you add to this the fact that the quadrant identified was the region of the tibial plateau with the greatest change in load post-HTO and that evidence of reciprocal changes were seen laterally for both SOST and NR2D, these results do lend weight to the hypothesis that MOW-HTO is causing measurable changes in the biology of osteoarthritis within the subchondral bone.

This then brings up two further questions; what effect are these measurable biological changes having and why are the results so variable?

In answer to the first question, the involvement of the glutamate pathway is perhaps easier to understand. Both NR2D and GRIK4 are known to be expressed in bone and there is good evidence that glutamate pathways are a key component of OA pathogenesis, causing cause pain, inflammation and cartilage degradation (Mason, 2004b; Flood *et al.*, 2007; Brakspear and Mason, 2012; Bonnet *et al.*, 2015, 2020). There is also evidence to suggest this pathway is mechanically regulated (Mason *et al.*, 1997) and has an

anabolic effect on bone mass suggesting a downregulation of bone formation/subchondral sclerosis (Lin *et al.*, 2008; Brakspear and Mason, 2012).

The downregulation of SOST in the offloaded quadrant (and reciprocal upregulation in the loaded quadrant) could be seen as at odds with the established literature about sclerostin and OA. However, as discussed in Chapter 3, the evidence on SOST production in osteoarthritis is in places contradictory and the author has put forward a hypothesis, informed by the literature, that could explain these results by considering the chronicity and severity of OA in different circumstances.(Chan *et al.*, 2011; Mabey *et al.*, 2014; Rauch and Adachi, 2016; Weivoda, Youssef and Oursler, 2017). This hypothesis and the supporting evidence is explained in further detail in Chapter 3 but is summarised in Figure 120.

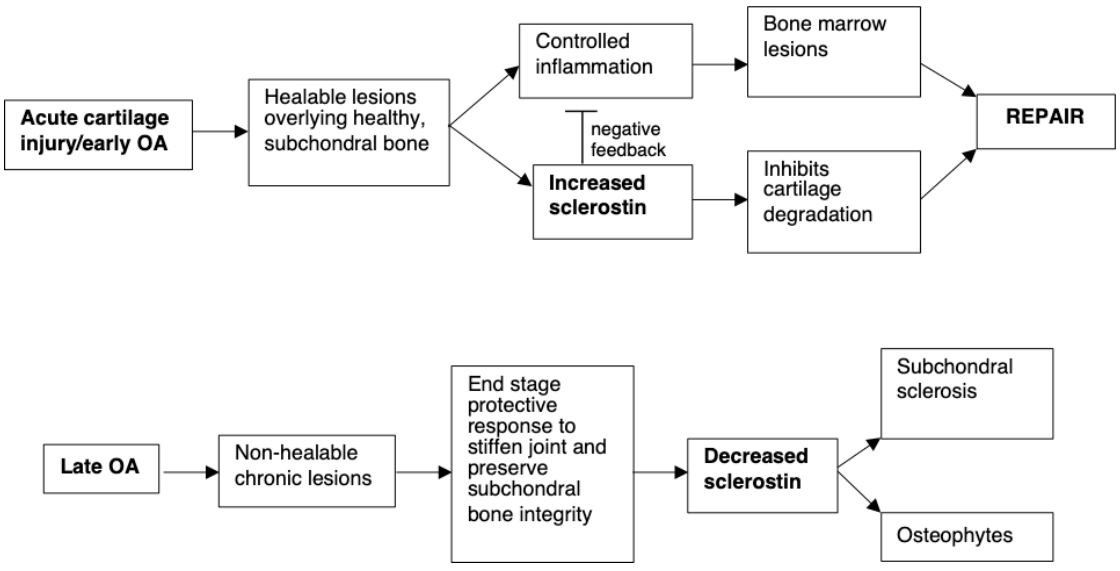

Figure 120: Author's theory on role of sclerostin in early versus late osteoarthritis.

If valid, this hypothesis would question the rationale for the common use of therapies aimed at reducing inflammation in early stage OA or the studies suggesting use of bisphosphonates to reduce symptoms from BMLs

(Conaghan, 2013). Although inflammation causes pain and has the potential to be very destructive to the joint in rheumatoid arthritis for example, it is also the first stage of recovery from injury for most biological tissues including bone (Ramachandran, 2006; Bulstrode *et al.*, 2011). Perhaps BMLs are not always pathological signs of inevitable joint degeneration but evidence of physiological efforts at repair, as evidenced by their temporary presence following long distance running for example (Krampla *et al.*, 2008; Loeser *et al.*, 2012).

The second question thrown up by the results was one of variability. Despite evidence of some mean changes, when considering the group as whole, the patterns of changes in expression within each patient were very variable. These could represent individual biological phenotypes and there is also the undeniable fact that, as with any group of patients, there is a large degree of clinical heterogeneity. For example, patients are of different ages, sizes and backgrounds, have different severities and patterns of OA and have had varied clinical courses leading up to their HTO (e.g. some have had previous surgery or injuries to the knee). They also have different mechanical phenotypes. At its most basic this means they have differing levels of varus but there are a multitude of possible morphologies and adaptations within the lower limb skeleton that could influence this.

The next stage of analysis therefore was to take a closer look at the radiographs of these patients to see what exactly is happening to their leg following HTO.

6.3 RADIOLOGICAL CHANGES TO LIMB ALIGNMENT

Hypothesis 2: (Chapter 4) Medial opening wedge HTO causes measurable changes in the radiological alignment of the lower limb beyond those anticipated as a result of the surgical correction.

The high tibial osteotomy procedure is entirely based on the idea that by changing the proximal tibial morphology, you can change the weightbearing axis of the limb. Therefore the first stage was to investigate this relationship between proximal tibial morphology (as measured by MPTA) and whole limb alignment (as measured by mTFA and Mikulicz point) to see how reliable this correlation is. The analysis of the weightbearing radiographs for the patient cohort suggested that although MPTA is correlated to mTFA and MP, these measures are not as closely related as one might expect. This is the case both pre-operatively (suggestive that other factors are contributing to overall alignment) but also when looking at the association between change of MPTA and change in alignment post HTO.

The clinical importance of this is clear and is a feature that is seemingly overlooked in the literature. The difficulty in achieving a target post-operative alignment following HTO is well reported (Marti *et al.*, 2004; Miller, Maddox and El-Daccache, 2020) and there are multiple clinical studies looking at ways to increase the accuracy of HTO; comparing different planning methods, computer navigation or open versus closing wedge techniques (Marti *et al.*, 2004; Hankemeier *et al.*, 2010; Kyung *et al.*, 2013; Schröter *et al.*, 2016; Han, Kim and Lee, 2017; Jones *et al.*, 2017; Cerciello *et al.*, 2020; He *et al.*, 2020). However, most of these studies seem to overlook the fact, revealed in this thesis, that there is variability inherent in the method as the correlation between change in MPTA and change in mTFA is not perfect.

Given the clinical importance of being able to accurately predict alignment change based on change of MPTA, it is therefore vital to understand the causes of this variability and it was with that in mind that the radiographs were examined to look for compensatory changes at other joints which may be modulating the effect.

The most striking findings were two seemingly independent mechanisms occurring distally. Some patients appeared to be adapting by altering the position of their subtalar joint (as measured by the ground talus angle). There is minimal existing literature in this area and terminology is very variable, however this change has been reported in two previous studies, both giving a similar magnitude of change (Lee *et al.*, 2015; Choi *et al.*, 2017). However, the second adaptive mechanism has not previously been described and is based on a novel measurement devised by the author (pending inter/intraobserver reliability analysis). The radiographs revealed that post-HTO, when asked to stand comfortably, patients were standing with their feet significantly (31%) further apart. This is a novel finding not previously reported in the literature but is of interest given there is evidence that a wide stance gait can mitigate medial compartment loading (Bowd *et al.*, 2019).

With so little previous research in this area, it is difficult to be conclusive about whether these changes are beneficial or detrimental to the patient and are likely part of a more complex mechanical phenotype specific to the individual. It is obvious that when the proximal tibial morphology is changed by HTO, there must be changes elsewhere in the limb to allow the foot to be planted on the ground. It would seem reasonable to suspect that these changes are not uniform and vary dependent on each patient's skeletal morphology (in coronal, sagittal and axial planes), joint flexibility and gait patterns. Certainly the multiple correlations across, but broad standard deviations within, the measures in this study lend weight to the theory that

there are different subgroups of HTO patients who adapt pre- and postoperatively in different ways.

However the findings of Chapter 4 do raise two immediate clinical implications. The first is that it gives weight to the case reports that HTO may have indications in the treatment of ankle/subtalar pathology (Takeuchi, Saito and Koshino, 2008; Elson *et al.*, 2013; Suero *et al.*, 2015). The second is to underline the importance of thorough examination and analysis of the whole limb prior to embarking on HTO and there is the possibility that distal pathology could either be affected by or itself affect the results of the procedure.

Having revealed novel findings concerning both the biological and mechanical changes seen in the limb following HTO, the next stage therefore was to see how these may be linked and what links there may be to clinical outcome.

6.3 CORRELATIONS

Hypothesis 3: (Chapter 5) Biological and biomechanical changes seen following medial opening wedge HTO are related.

As stated earlier, OA is a disease of interlocking biological and mechanical factors and the results from Chapters 3 and 4 show evidence that HTO surgery is also having both biological and mechanical effects. What is unclear however is how these two domains may be linked and subsequently how they link with the third domain of clinical outcomes. It was with this in mind therefore that the data from the previous chapters was combined with further data from studies within the Biomechanics and Bioengineering Research Centre Versus Arthritis (biomechanics and synovial fluid analysis) to explore potential associations using both principal component analysis and paired comparisons.

The principal component analysis models suggested some preoperative phenotypic groups separated by either pain/function or gait speed and the suggestion that the previously identified compensatory mechanism of stance width and subtalar adaptation may be working within each group respectively. There was also the suggestion that distal tibial morphology (LDTA) may have some form of mitigating effect on the functional impact of proximal tibial varus and this would support findings from the Biomechanics and Bioengineering Research Centre Versus Arthritis which suggested that LDTA is one of the factors that, in conjunction with MP, best explains variance in peak external knee adduction moment (EKAM), thought to be a key indicator of medial compartment loading during gait (Whatling *et al.*, 2019). Those in the higher gait speed group also appeared to be those with the highest expression of glutamate pathway factors (EAAT3, NR2D, GRIK4) and sclerostin. Of course PCA merely groups variables together in models that best explain the variance, it does not claim to produce causal

relationships but if this association is valid, it would still leave the question as to whether the subchondral bone activity is allowing increased gait speed or whether these patients have increased cyclical loading due to higher activity levels which is upregulating pain and inflammation pathways. This group was not however associated with increased pain scores but the link between pain pathway activity and a patients subjective pain experience is of course very variable.

When looking for which preoperative markers may be predictive of outcome, the model appeared to suggest that the patients who experienced the greatest improvement were those who had been in the worst state preoperatively (more severe OA, older, poorer ROM, poor function, worse pain, more severe proximal tibial deformity). However interestingly this did not correlate with the overall severity of their malalignment pre-operatively. This highlights a, sometimes awkward, clinical truism that the worse a patient is when you start, the easier it is achieve a satisfactory outcome following surgery. Their expectations and functional demands are low and even a marginal improvement in symptoms causes great relief. In contrast, patients with milder symptoms and higher functional demands require a much greater level of operative success to notice a significant improvement. A clinical version of the “law of diminishing returns”.

With regards to HTO, this may muddy the waters. It is possible that there are two groups of people who may experience clinical benefit from HTO but for different reasons. Those with severe medial compartment OA, with or without significant varus, may experience symptomatic relief from offloading the affected compartment without any possibility of change to the end stage OA. However, those with early OA and malalignment may experience less symptomatic relief post operatively but experience long term benefit from the preservation and potential improvement of their medial compartment cartilage. There is some evidence that although all severities of KL grade

may gain early benefit with HTO, there is a higher rate of TKR in those with higher KL grades, which supports this hypothesis (Kuwashima *et al.*, 2021). Currently, with no clear way to distinguish between these groups (the cut-off for OA stage/severity remains unclear), both could appropriately undergo a HTO procedure but show a very different pattern of results. It is possible that in the future, a better understanding of osteoarthritis biomarkers may allow distinction of these two groups and the model did suggest SOST production as a possible candidate (in line with results in Chapter 3) although the evidence is weak.

Post-operatively it seemed that achieving a better post-operative alignment (mTFA, Mikulicz point and MPTA) and reduced external knee adduction moment (EKAM, a measure previously associated with medial compartment load (Whatling *et al.*, 2020)) is associated with the greatest improvement in patient reported outcome scores. This is an expected and reassuring output in this model. Interestingly this model also suggested that higher production of SOST, NR2D and GRIK4 in the posteromedial compartment post-operatively was associated with poorer pain reduction and poorer function improvement following HTO. This supports the findings in Chapter 3 that these markers are responsive to the change in load produced by HTO and represent a mechanism by which the operation is having a measurable effect on the progression of OA within the medial compartment.

Further support for this hypothesis was provided when these posited correlations were examined individually. The change in GRIK4 in the posteromedial compartment showed a moderate, statistically significant correlation with post-operative mTFA. This provides further evidence that the correction of varus malalignment by HTO causes a downregulation in GRIK4 in the posteromedial (off-loaded) quadrant and is in line with the findings in Chapter 3. The fact that is the post-operative value of mTFA which is significant suggests what is important is how “normal” the knee alignment is

post-operatively rather than the degree of change. The correlations for SOST did not meet the statistical significance threshold although did come close with correlations to measures of post-operative varus. In conjunction with other results from this thesis, this does suggests SOST as a marker and pathway in OA certainly worthy of further investigation.

Again, as suggested in earlier results, each of these markers seemed to be working independently of each other and the correlations also suggested they may be associated with different mechanical phenotypes/adaptive mechanisms, as shown by the potential correlations with ground talus angle and stance width.

Certainly, both the modes of analysis employed in this chapter supported the hypothesis that the measurable changes seen in the biological and mechanical environment of the knee following HTO are linked. However, it also reinforces the previous discussion about the likely complexity of these links and the resultant variability this produces between patients. This represents the first research of this type and as such there is no established literature with which to compare findings. It does however provide multiple avenues for further research.

6.5 BIOMECHANICAL MODEL OF HTO EFFICACY

When the results of all three chapters are considered, they support the theory of HTO having a measurable effect on the underlying OA pathology, rather than purely symptomatic relief from unloading. The findings reveal fragments of a biomechanical model of HTO efficacy as illustrated in Figure 121.

This simplified diagram shows some of the linked mechanisms revealed by this thesis within a possible encompassing theory. The suggestion is that proximal tibial varus (as demonstrated by a reduced MPTA) leads to varus malalignment of the limb but this relationship is modified by other aspects of the mechanical phenotype (e.g. subtalar joint changes, distal tibial morphology, stance width etc). The varus malalignment of the limb has both direct static effects on medial compartment load (particularly PM quadrant as previously explained) as well as indirect effects via the dynamic changes in gait (most notably EKAM). This change in load is suggested to cause upregulation of biological pathways in the subchondral bone related to pain, inflammation, cartilage degradation and bone turnover resultant in early OA.

Medial opening wedge HTO (MOW-HTO), by increasing the MPTA reduces the varus malalignment but, as shown in Chapter 4, with modulation of the effect by the mechanical phenotype. The resultant changes in load of the PM quadrant then result in the biological changes within the subchondral bone illustrated in Chapter 3 and as such, a potential slowing, halting or potential reversal (if possible) of OA.

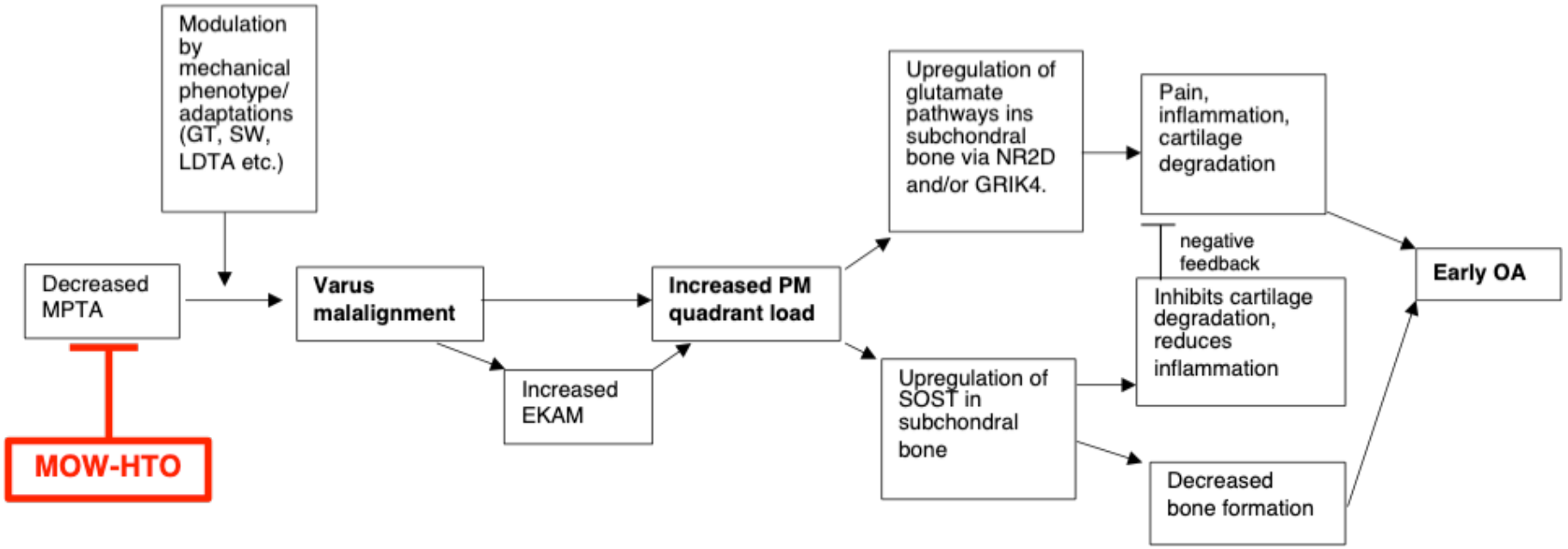

Figure 121: Suggested biomechanical model of early OA and the efficacy of HTO

6.6 CLINICAL IMPLICATIONS

As with all healthcare research, the key question is what this means to the patients. Although this research is only preliminary, it does have both immediate and longer term clinical implications.

The findings of Chapter 4 regarding changes within the ankle/subtalar joint support some immediate changes to clinical practice regarding the screening of these joints prior to considering HTO. As mentioned earlier, it also supports the possible extended indication of HTO to treat ankle OA (Takeuchi, Saito and Koshino, 2008; Elson *et al*., 2013).

As more is discovered about the compensatory mechanisms and the effect of different mechanical phenotypes on the effect of HTO, it is hoped that this will allow better patient selection and more accurate counselling of patients on what to expect from HTO. This is also true regarding the possible different biological responses and phenotypes noted in Chapter 3. What is most striking from all chapters of this study is the wide variability of all these aspects across our patient cohort. This mirrors the wide variability in outcomes reported from HTO (Webb, Dewan and Elson, 2018). During a Public and Patient Involvement (PPI) event hosted by Cardiff Biomechanics and Bioengineering Research Centre Versus Arthritis, one of the recurring themes during the author's discussions with patients was the importance of accurate predications of post-operative function. Some patients were unhappy after being painted a more negative picture of what they would be able to do compared with their results. Others conversely felt disappointed having been told they would be able to return to activities which they had not been able to achieve. This mirrors the author's experience within clinical practice and is particularly the case with HTO as the patients are often high functioning individuals, keen to return to sport for example. The consenting process for any surgery is based on a patient being informed of the likely

risks and benefits before making their decision. However, currently this is largely based on generalised probabilities for these risks and benefits at a population level. With greater understanding of each patient's biological and mechanical phenotype, it is hoped that a more individualised and accurate approach could be employed, leading to patients being able to take a more informed decision.

6.7 INSIGHTS INTO THE PATHOGENESIS OF OA

In addition to providing insights about the efficacy of HTO, this work could also shed some light on the pathogenesis of OA. In addition to supporting the evidence for the involvement of glutamate mediated pathways and the potentially complex role of sclerostin, the findings of thesis regarding the effect of HTO suggest not just that OA is a linked biological and mechanical process but also that it has distinct stages.

It has always been held as orthopaedic doctrine that hyaline cartilage is incapable of repair (Ramachandran, 2006; Bulstrode *et al.*, 2011). However, when this idea is scrutinised, it seems unlikely to be entirely true. Considering the insults that, particularly weightbearing, joints are put through on a day-to-day basis it would seem likely that the cartilage must undergo regular minor injuries; the equivalent of bruises and scuffs. However, when a joint surface in a non-arthritic individual is examined, we do not find a life-time's history of every run, jump and twist written across its surface. A more likely scenario is the "Envelope of Function" hypothesis (Figure 123) proposed by Dye (Dye, 1996). This suggests that the knee's ability to cope with load depends both on the magnitude and frequency of the load applied. There is a level of physiological load that can be repeatedly applied indefinitely without joint injury (e.g. day-to-day walking), a level of load that

can be coped with as long as the frequency is controlled (e.g. high impact sport) and a level of load which would be damaging even if applied only once (e.g. trauma).

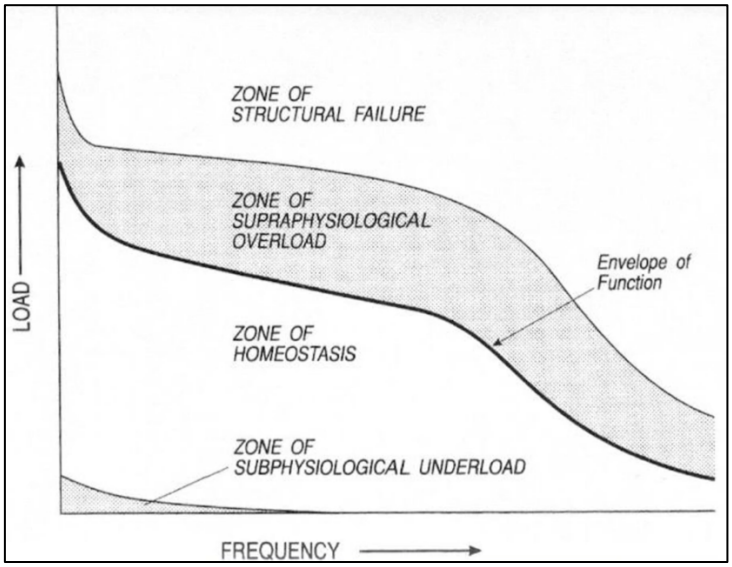

Figure 122: Envelope of Function (Dye, 1996)

The thresholds for these zones however vary between individuals and are modifiable, for example by training.

Drawing from the findings laid out in the previous chapters, the Dye "Envelope of Function" hypothesis and further existing literature on the pathogenesis of OA (Dye, 1996; Burr and Gallant, 2012; Pan *et al.*, 2012), the author proposes the following potential model (Figure 123).

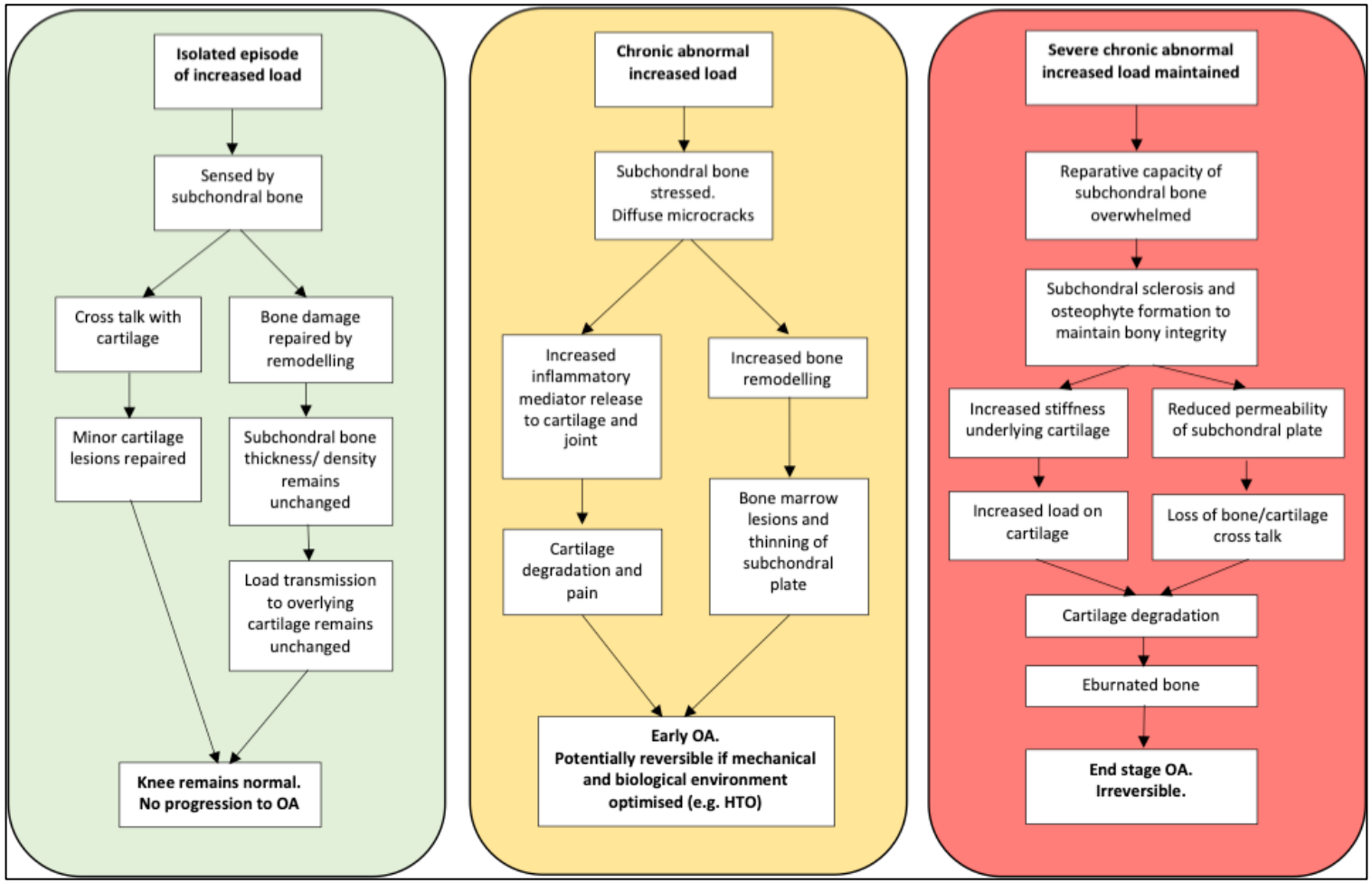

Figure 123: Suggested model of osteoarthritis stages and pathogenesis

The green zone represents physiological normalcy. The knee is constantly subjected to isolated episodes of increased load creating a temporarily abnormal strain environment for both the subchondral bone and cartilage (e.g. if someone were to jump down from a wall or go on a run with no training). The day or so of aching from the knee commonly experienced after this suggests there is some level of injury and repair ongoing. A nociceptive response has been activated along with inflammatory and degradative activity, as part of mechanically regulated pathways. The subchondral bone is known to be both biologically active and responsive to load, plus as with all bone tissue, undergoes a constant cycle of remodelling to repair microfractures. However, there is evidence that there is also some controlled permeability of mediators across the subchondral bone plate allowing cross talk with the more biologically inactive cartilage (Pan *et al.*, 2012). In the normal physiological state, both these tissues are able to return to normal without long term sequelae, explaining why not everyone develops OA.

Those in the yellow zone represent the group that would potentially see the greatest benefit from HTO. Here the subchondral bone is at the limit of its reparative capacity and changes are starting to be seen in the bone and cartilage in keeping with early OA (Burr and Gallant, 2012; Pan *et al.*, 2012). It is the author's suggestion, based on the findings of this thesis and the evidence seen previously regarding the effect of HTO on cartilage (Kanamiya *et al.*, 2002; Koshino *et al.*, 2003; Parker *et al.*, 2011) that for patients in this yellow area, the knee can go one of two ways. If the mechanical environment remains unfavourable, the OA will continue to progress into the end stage changes illustrated in the red zone (joint failure). If however, the mechanical environment is optimised (for example by HTO), there is a chance that inflammation is appropriately regulated and the knee could be either stalled in the yellow zone or returned to the green zone of physiological normalcy. Where the boundary of the zones lie however (and therefore how reversable

the changes are) would be patient-specific; likely influenced by a range of genetic and acquired predispositions.

This would fit with what is known about the injury state of other tissues including bone following fracture. The ability of bone to heal is determined by the mechanical environment and its interaction with the biological stages of repair (Perren, 1979). Without the appropriate mechanical environment, the stages of repair cannot progress and non-union results. By optimising the mechanical and biological environment however, union can be restored (Andrzejowski and Giannoudis, 2019). Similar concepts exist for tendon and ligament injuries also (Killian *et al.*, 2012; Wang, Guo and Li, 2012; Galloway, Lalley and Shearn, 2013; Mahapatra, Horriat and Anand, 2018) so why should cartilage be any different? Perhaps the reason hyaline cartilage is thought to be irreparable is because it has only been studied in relatively advanced OA and rarely has the underlying biomechanical environment been considered.

The model in Figure 123 may explain some of the variability seen in patients undergoing HTO. Currently our clinical diagnosis of OA and the staging systems used for research such as the Kellgren and Lawrence system, are based on plain radiograph findings (subchondral sclerosis, loss of joint space, osteophytes and subchondral cysts). However, these all represent late findings of OA and there are as yet no clear tools for identifying and differentiating early stages of the disease. Without this ability, both our data set and those of other HTO studies will potentially include both “yellow zone” and “red zone” patients who are likely to have significantly different responses to the same procedure. With a better understanding of the underlying biology however, it is hoped that different biomarker profiles (either biological or imaging based) may be a route to better differentiation of these stages and therefore better evaluation of therapies.

6.8 SOURCES OF VARIABILITY

As previously alluded to, a high level of variability is apparent, both in the patient cohort and perhaps unsurprisingly in the results. The majority of clinical research is designed to look at patient reported outcome measures as the focus of all orthopaedic therapies is to improve the quality of life for patients (Gagnier, 2017). However, as demonstrated by the findings of this thesis, there are so many interacting variables between the patient undergoing HTO and the outcome that this approach may not be best suited to evaluating its effectiveness. As discussed above, it is likely that stages of OA are being grouped together inappropriately but as also illustrated, there are a range of clinical, mechanical and biological phenotypes at work.

Figure 124 provides a, likely non-exhaustive, illustration of the cloud of variables surrounding each stage of the proposed pathway between patient and outcome. This is before even considering the variability inherent in the sampling and measurement of each criterion.

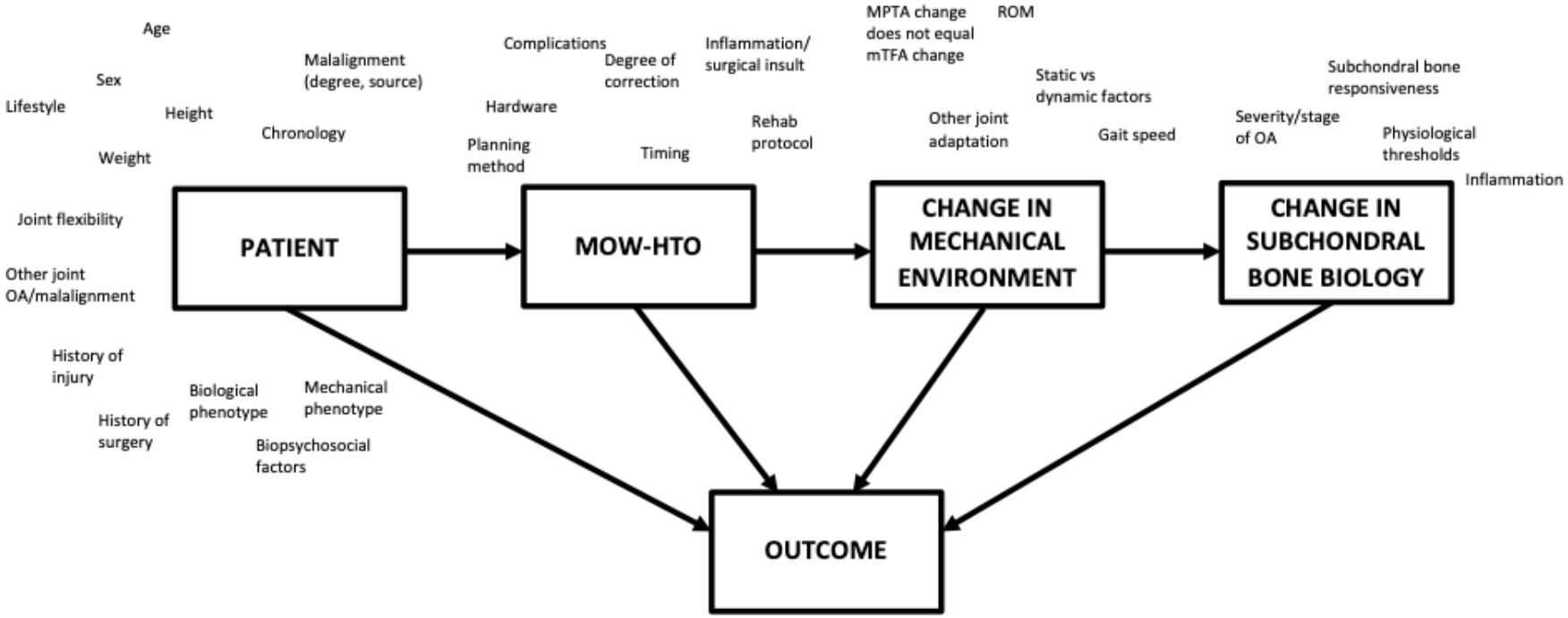

Figure 124: Sources of variability

6.9 LIMITATIONS

There are clearly several limitations to this work. The issue of variability has been discussed but this is particularly apparent when dealing with a relatively small sample size. This work was always intended to be exploratory and with so little known about the various markers (both biological and mechanical) under investigation, it would not have been possible to do a power analysis to set a sample size at this stage. This does however make it difficult to rule on the significance of any findings and as shown most notably in Chapter 3, a small change in sample cohort can have a significant effect on the results.

The other limitation discussed in Chapter 3 was the lack of repeatability of absolute copy number from the RTqPCR method. This was worked around within this project by using the proportionality method and, as there does not appear to be any accepted error for this method within the literature, it is an area that the wider study group are going to investigate further.

The fact that so much of this project is novel research is both its greatest strength and greatest limitation. On the one hand it means that the work has provided some original findings and exciting areas for further research. On the other however, it means that the opportunities to compare findings against existing published literature are scant.

6.10 FURTHER RESEARCH

Regarding planned further research, as previously mentioned, this included an analysis of the variability within the absolute RTqPCR method and there is also work on going within the wider group looking at whether the biological changes observed in the subchondral bone post-HTO are matched by changes in the synovial fluid, blood or urine of these patients.

A powered study looking at the biological markers highlighted by this work would be of great benefit in confirming or contradicting the findings but would be likely to require a cross site collaboration and work is undergoing (supported by the author) into linking with other centres performing high volumes of HTOs.

Collaboration continues with the engineering team looking into whether the static adaptations identified on the weightbearing radiographs in Chapter 4 are carried through into related dynamic changes seen in gait analysis.

Regarding the theories put forward about the differing effect of HTO dependent on stage of HTO, it would be of benefit to subgroup a larger data set of HTO patients based on pre-operative MRI findings. This could allow the effect of HTO on those with early OA (e.g. BMLs but no sclerosis) versus those with later OA (e.g. plain radiograph OA appearances) to be differentiated.

6.11 CONCLUDING REMARKS

In conclusion, the data presented in this thesis has identified novel biological and mechanical changes resultant from HTO surgery in addition to revealing potential linking mechanisms between them. It lends weight to the theory that HTO surgery, beyond causing symptomatic relief, can cause measurable change to the pathology underpinning osteoarthritis and as such has the potential to halt, slow or even reverse its progression. The clinical significance of this work is clear. By enhancing understanding of both HTO surgery and osteoarthritis, and by providing promising avenues for further research, it will enable clinicians to better counsel their patients and as such have a direct positive impact on patient care.

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Appendices

Appendix 1: Patient information leaflet and consent forms

CARDIFF UNIVERSITY
PRIFYSGOL CAERDYDD

GIG CYMRU NHS WALES
Bwrdd Iechyd Prifysgol Caerdydd a'r Fro
Cardiff and Vale University Health Board

PATIENT INFORMATION SHEET

The collection, storage and analysis of Clinical Waste, Blood & Urine Samples

We would like you to take part in a research study

- Before you decide if you would like to take part it is important for you to understand why the research is being done and what it will involve.
- Please take some time to read the following information sheet carefully and discuss it with friends or relatives if needed.
- It is your decision whether or not to take part.
- Ask a member of the study team if you have any questions about the research.
- If you decide to take part in this research but later change your mind you are free to withdraw at any time. This will not affect any of your NHS care.

Important Information about this Research

- This research is part of a series of studies being conducted by the Biomechanics & Bioengineering Research Centre (BBRCVersusArthritis) at Cardiff University.
- You may also be asked to complete some questionnaires relating to your health and daily living.
- We would also like to collect information about your diagnosis and treatment from you and from your medical records
- We do not expect there to be any direct benefit for people who take part in this research

BIOMECHANICS & BIOENGINEERING RESEARCH CENTRE
VERSUS ARTHRITIS

What is the purpose of this research?
This research is part of a series of studies being carried out by the Centre Researchers, Orthopaedic Surgeons and Physiotherapists.

We are interested in analysing samples obtained following your clinical procedure (where appropriate), to help us research into the causes, diagnosis and treatment and/or monitoring of joint problems, (such as following injury or due to disease). Research with such samples can help us to find out more about what causes joint diseases, how to prevent and treat them. The samples you donate might be used for a number of related research studies associated with the Centre.

Why am I being asked to take part?
You have been asked to take part because you fall into one, or more, of the following categories:

- Are currently on a waiting list for orthopaedic, physiotherapy or rheumatology treatment
- Have received treatment for a joint or back problem
- Have previously taken part in Centre research.
- Have a joint problem we are interested in looking at with this technique

If you are on a waiting list for surgery, your surgeon has agreed that you may be suitable to take part in this research.

What does taking part involve?
The Centre would like to collect and store any tissue or bone from surgery which is normally destroyed as clinical waste, samples of Blood and samples of Urine. These samples will be stored in secure laboratories at Cardiff University. The human sample storage facilities at Cardiff University are used to collect tissues for research purposes only.

It is up to you to decide to join the study and you are free to withdraw at any time without giving a reason. This would not affect the standard of care you receive. For each of these studies you will be provided with a further information sheet and have the opportunity to ask questions. For each additional study you will be asked to sign a consent form before and research activity is performed.

With your permission, we will ask a member of your clinical team or researchers who have been suitably trained to collect blood and/or urine samples during your routine clinic visits. In some circumstances, we may ask you to provide blood and/or urine samples at other times. The collection of blood and urine would involve up to half an hour of your time. We will pay any reasonable travel expenses you incur if you are asked to provide samples that do not coincide with your routine clinical visits

We may approach you up to a maximum of 10 times over the course of 5 years to provide additional samples. If you are happy for us to do this please ensure you have initialled the appropriate box on the consent form. This consent will last for 2 years, after this we will provide you with another consent form if we would like to collect further samples. Even if you have signed the form to say you are happy to give additional samples there is no obligation to provide these samples and you can withdraw your consent at any time.

For clinical waste that is routinely discarded, we would ask your consultant to save the samples removed during your clinical procedure for us.

If you are happy to take part in the study we will ask you to sign a consent form
What other types of information do we want?
We would like to collect information from your medical records, such as your weight, and record details of your condition, such as diagnosis and the results of various tests. We would also like to follow your progress after your procedure by looking at information that your doctor has collected from you during your routine follow-up visits. Your doctor may record such information such as the medications you are taking and whether your level of pain has changed. We would also like to ask your permission to send you ethically approved surveys or questionnaires to complete. This enables us to collect standardised information about large numbers of tissue donors. They ask questions about lifestyle choices such as "Do you smoke?" or "Do you exercise?" No personal information will be collected without your permission.

What will happen to my information / Samples?

After you have signed a consent form you will be assigned a unique number. from then on, this number will be used to identify you throughout the study. All electronic data will be held securely on NHS or University computers. Access to this information will be restricted to members of the research team.

As well samples we may also collect some routine data from your medical records. This may include information about your operation, diagnosis and treatment, where it is relevant to your participation in this study. The information that may be given to the researcher includes, but is not restricted to: your age, sex, race, medical history, diagnosis, treatments and possibly some medical history. This information will be collected from your health record by the study staff. They may also look at your medical record in the future in order to update your personal health information.

You may also be asked to complete some questionnaires and be asked to answer some questions on your joint problem and how it affects daily life.

Cardiff University is the sponsor for this study based in the UK. We will be using information from you and your medical records in order to undertake this study and will act as the data controller for this study. This means that we are responsible for looking after your information and using it properly. Cardiff University will keep identifiable information about you for up to 15 years after the study has finished.

Your rights to access, change or move information are limited, as we need to manage your information in specific ways in order for the research to be reliable and accurate. If you withdraw from the study, we will keep the information about what we have already obtained. To safeguard your rights, we will use the minimum personally-identifiable information possible. You can find out about how we use your information by contacting the project lead detailed on the next page.

You can find out more about how we use your information at:
https://www.cardiff.ac.uk/public-information/policies-and-procedures/data-protection
or by contacting the University's Data Protection Officer: inforequest@cardiff.ac.uk

The NHS will use your name, NHS number and contact details to contact you about the research study, and make sure that relevant information about the study is recorded for your care, and to oversee the quality of the study. Individuals from Cardiff

University and regulatory organisations may look at your medical and research records to check the accuracy of the research study. The NHS will pass these details to the Biomechanics and Bioengineering Research Centre (Cardiff University) along with the information collected from you and/or your medical records. The only people in Cardiff University who will have access to information that identifies you will be people who are conducting the research, those who need to contact you about the study or audit the data collection process.

The NHS will keep identifiable information about you from this study for at least 10 years after the study has finished.

With your consent, anonymous data collected in the study may be shared with other institutions, including Universities and commercial organisations.

You will not be identified in any reports, presentations or publications relating to this research.

Sample storage / Use
Samples collected are usually kept at very cold temperatures, and can be stored this way for a very long time. Your samples will be stored indefinitely or until it is used up.

Many different types of research rely on the use of human samples. They can be used to develop new tests or help diagnose diseases, or can be used to help develop new ways to treat or even cure joint problems. Some of the research may lead to new medical products, such as diagnostic tests and drugs, or new procedures.

We may want to carry out genetic research (for example the identification of genes or diseases that run in families) on your tissue. On the consent form you will be given the option to exclude your tissue from this area of research. Genetic testing will be for research not diagnostic purposes and it may be many years before the results of any such tests could have clinical implications.

Access to your samples and any personal data that may be associated with your samples is strictly controlled. The sample you donate may be given to other groups within Cardiff University as well as external research collaborators, for example, other Universities and Companies, in the UK and abroad, for approved medical research but the samples will not be sold for profit to you or the researchers. Such researchers will only receive your donated samples and when appropriate, information about you (such as your sex, age and the reason for your clinical procedure) from your hospital record. The researcher will not receive your name, address, phone number or any other personal identifying information. This is done to protect your confidential information.

Future Research
Your samples may be retained at the end of this study for use in future research within the UK and abroad. At this stage we do not know what the research will involve but some of it could include genetic research (for example the identification of genes or diseases that run in families), or use in the commercial sector. On the consent form you will be given the option to exclude your tissue from these areas of research.

What are the potential risks and benefits of taking part?
There is no intended clinical benefit for people taking part in the study. The information we collect from patients may help us to provide future patients who have joint disease or injury with improved treatment options.

If you agree to give up to a 40ml (8 teaspoons) sample of blood, we will perform this at the same time as one or more of your routine clinical samples if possible. If this is not possible, you may experience some minor discomfort from having the extra blood sample(s) taken.

If you agree to provide a urine sample, there would be the minor inconvenience of you collecting this.

If you agree to donate Clinical Waste Samples they will be collected during your routine clinical procedure and therefore there are no obvious disadvantages to taking part in the study.

Risks associated with the COVID-19 pandemic
Due to the COVID-19 pandemic, new safety measures have been put in place at our Cardiff University research facilities to avoid the spread of COVID-19. Research visits are usually arranged during a phone call. Due to the COVID-19 pandemic, we will phone you twice prior to the research visit. First call will be for arranging a visit date and check if you or anyone you met has had COVID-19 symptoms before, or if you are in a high-risk group to develop COVID-19 complication. Second call will happen 24 hours prior to the visit to check if you have developed COVID-19 symptoms or have met anyone who has had COVID-19 since the first phone call. If you or anyone you met have COVID-19 symptoms, the research visit will be cancelled and you will be advised to follow Welsh Government guidelines on Test, Trace and Protect. Research visits will only go on if it is safe for you and the research team.

During the research visit our research team will be wearing full personal protective equipment (PPE) to keep you and ourselves safe. This equipment includes eye protection, face masks, disposable aprons, and gloves. New cleaning and disinfection procedures will be performed after every volunteer visit. Government guidance on safe working will be followed.

You might be asked to wear a facemask and disinfect your hands at arrival to our research facility. Social distancing measures will be applied where possible. However, during the collection of blood samples, social distancing will not be possible. You might also be asked to pull your mask off, so the person taking blood can monitor if you feel unwell during the procedure.

What will happen to the results of the research using samples I have donated?
The results from these studies will be submitted for presentation at scientific conferences and publication in scientific journals. You will not be identified in any presentation or publication. The findings of this research will also be linked to the results of any of the other interlinking research studies you may undertake.

Other useful Information
Occasionally, during a research project, new information may become available. If this happens you will be contacted by a member of the research team to explain how this may affect you and your participation in the research.

We do not routinely send a letter to the GP to inform them of your participation in this research. However, with your permission we may contact your GP before getting in touch with you in the future to ensure it is suitable for us to do so. For this reason we ask you to provide details (name, address and telephone number) of the GP with whom you are registered.

This study has been reviewed and approved by the Wales Research Ethics Committee 3 (REC 3) and is managed by Cardiff University.
If something goes wrong and you are harmed due to negligence, you may have grounds for legal action. If you wish to make a complaint about the way you were approached or the treatment you have received within the study please contact Cheryl Cleary: Centre Manager 029 2251 0265. If you feel your complaint is not adequately addressed, you may escalate your complaint by writing to: The School Manager, School of Bioscience, Cardiff University, Museum Avenue, Cardiff, CF10 3AX

As well as being asked to take part in this research you may also be asked if you are interested in taking part in some of the other Centre studies.

For each of these studies you will be provided with a further information sheet and have the opportunity to ask questions. For each additional study you will be asked to sign a consent form before and research activity is performed.

What happens next?
This information sheet covers research into a wide range of joint and back problems. The study requirements vary depending on the joint under investigation and the planned treatment.

If you still have questions after reading this information, please contact a member of the research team.

Contact Details:

Centre Manager
Cheryl Cleary
Biomechanics and Bioengineering Research Centre Versus Arthritis
Cardiff School of Biosciences
Cardiff University
Cardiff
CF10 3AX
Tel: 029 2087 5419 or 029 2087 4986
email: ArthritisCentre@Cardiff.ac.uk

NHS Site
Jessica Falatoori/Matthew Williams
CAVOC Research Office
Cavoc.research@wales.nhs.uk (029) 2182 6511

Project Lead / Contact
Mr Tim Matthews (Consultant Surgeon – Principal Investigator)
tim.matthews@wales.nhs.uk

Thank you for taking time to read this information sheet

More information about the Biomechanics and Bioengineering Research Centre Versus Arthritis can be found by visiting:
http://www.cardiff.ac.uk/arthritis-biomechanics-bioengineering-centre

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PATIENT CONSENT FORM

The collection, storage and analysis of Clinical Waste, Blood & Urine Samples

Page 1 of 2

Centre ID: ☐☐☐☐☐ **Project Name:** _____

You DO NOT have to sign this document. Please DO NOT sign this document unless you fully understand it. If there is ANYTHING which you do not understand please do not hesitate to ask for a full explanation.

To confirm agreement with each of the statements below, please initial each box and delete where applicable:

1. I confirm that I have read and understand the information sheet dated 03 August 2020 (Version 12.2) for the above study and have had the opportunity to ask questions ☐

2. I understand that my participation in the study is voluntary and that I am free to withdraw at any time, without giving any reason, and without my medical care or legal rights being affected. ☐

3. I understand that my details will be linked to a unique identifier to allow you to follow me through course of the study ☐

4. I do / do not (please delete as appropriate) give permission for up to a 40 ml (8 teaspoons) sample of my blood to be collected. ☐

5. I do / do not (please delete as appropriate) give permission for one or more samples of my urine to be collected ☐

6. I do / do not (please delete as appropriate) give permission for my clinical waste collected during surgery to be collected ☐

7. I understand that researchers from other organisations in the UK and abroad, including commercial companies, may access my samples, that research may take many years and the information gained will not benefit me or my family directly. ☐

CARDIFF UNIVERSITY
PRIFYSGOL CAERDYDD

GIG CYMRU NHS WALES
Bwrdd Iechyd Prifysgol Caerdydd a'r Fro
Cardiff and Vale University Health Board

PATIENT CONSENT FORM

The collection, storage and analysis of Clinical Waste, Blood & Urine Samples

Page 2 of 2

8. I understand I can withdraw my consent for the storage and future use of my samples at any point and that any unused samples will be destroyed immediately. I understand that any samples used in research prior to the withdrawal of consent may not be destroyed until the end of the study. ☐

9. I give permission for my consent to cover the collection of any additional samples over the next 2 years for this study and I understand that by signing this form I am not obliged to give these additional samples. ☐

10. I agree to my GP being contacted ☐

11. I understand that you may access my Medical notes. ☐

12. I agree to take part in the above study. ☐

Optional – please delete / circle as appropriate

13. You may / may not (please delete as appropriate) contact me in the future to ask if I would be interested in participating in a follow up study. ☐

14. I do / do not (please delete as appropriate) give permission for genetic analysis to be carried out using my samples ☐

15. I would / would not (please delete as appropriate) like to be contacted if genetic information is found that may have implications for me or my family. ☐

Name of Participant	Date (dd/mmm/yyyy)	Signature
Name of person obtaining consent	Date (dd/mmm/yyyy)	Signature

BIOMECHANICS & BIOENGINEERING RESEARCH CENTRE
VERSUS ARTHRITIS

Original Investigator Site File / Trial Master File,
1 copy for the participant;
1 copy for the patient notes (where applicable),
1 copy researcher

PATIENT INFORMATION SHEET

The collection of synovial fluid from joints

We would like you to take part in a research study

- Before you decide if you would like to take part it is important for you to understand why the research is being done and what it will involve.
- Please take some time to read the following information sheet carefully and discuss it with friends or relatives if needed.
- It is your decision whether or not to take part.
- Ask a member of the study team if you have any questions about the research.
- If you decide to take part in this research but later change your mind you are free to withdraw at any time. This will not affect any of your NHS care.

Important Information about this Research

- This research is part of a series of studies being conducted by the Biomechanics & Bioengineering Research Centre (BBRCVersusArthritis) at Cardiff University.
- You may also be asked to complete some questionnaires relating to your health and daily living.
- We would also like to collect information about your diagnosis and treatment from you and from your medical records
- We do not expect there to be any direct benefit for people who take part in this research

What is the purpose of this research?
This research is part of a series of studies being carried out by the Centre Researchers, Orthopaedic Surgeons and Physiotherapists.

We are interested in analysing samples obtained following your clinical procedure (where appropriate), to help us research into the causes, diagnosis and treatment and/or monitoring of joint problems, (such as following injury or due to disease). Research with such samples can help us to find out more about what causes joint diseases, how to prevent and treat them. The samples you donate might be used for a number of related research studies associated with the Centre.

Why am I being asked to take part?
You have been asked to take part because you fall into one, or more, of the following categories:

- Are currently on a waiting list for orthopaedic, physiotherapy or rheumatology treatment
- Have received treatment for a joint problem
- Have previously taken part in Centre research.
- Have a joint problem we are interested in looking at with this technique

If you are on a waiting list for surgery, your surgeon has agreed that you may be suitable to take part in this research.

What does taking part involve?
We would like to invite you to take part in our research studies by donating your fluid (synovial fluid) around your joint to the Centre. Before you decide, we would like you to understand the purpose of you donating samples, and what being part of our research would mean for you. Someone from our team will go through the information sheet with you and answer any questions you may have. If English is not your preferred language and you would like this information in another language, please ask and it will be provided, or an interpreter called. You are encouraged to take this document home and discuss your decision to donate your sample for research with friends and family.

It is up to you to decide to join the study and you are free to withdraw at any time without giving a reason. This would not affect the standard of care you receive. For each of these studies you will be provided with a further information sheet and have the opportunity to ask questions. For each additional study you will be asked sign a consent form before a research activity is performed.

In most cases we will ask for one single sample collection, but we may approach you up to a maximum of 10 times over the course of 5 years to provide additional samples – we will ensure that there is a minimum of 6 months between each collection. If you are happy for us to do this please ensure you have initialled the appropriate box on the consent form. This consent will last for 2 years, after this we will provide you with another consent form if we would like to collect further samples. Even if you have signed the form to say you are happy to give additional samples there is no obligation to provide these samples and you can withdraw your consent at any time. We will pay any reasonable travel expenses you incur if you are asked to provide samples that do not coincide with your routine clinical visits.

If you are happy to take part, we will ask you to sign a consent form

What other types of information do we want?
We would like to collect information from your medical records, such as your weight, and record details of your condition, such as diagnosis and the results of various tests. We would also like to follow your progress after your procedure by looking at information that your doctor has collected from you during your routine follow-up visits. Your doctor may record such information such as the medications you are taking and whether your level of pain has changed. We would also like to ask your permission to send you ethically approved surveys or questionnaires to complete. This enables us to collect standardised information about large numbers of tissue donors. They ask questions about lifestyle choices such as "Do you smoke?" or "Do you exercise?" No personal information will be collected without your permission.

What will happen to my information / Samples?

After you have signed a consent form you will be assigned a unique number. from then on, this number will be used to identify you throughout the study. All electronic data will be held securely on NHS or University computers. Access to this information will be restricted to members of the research team.

As well samples we may also collect some routine data from your medical records. This may include information about your operation, diagnosis and treatment, where it is relevant to your participation in this study. The information that may be given to the researcher includes, but is not restricted to: your age, sex, race, medical history, diagnosis, treatments and possibly some medical history. This information will be collected from your health record by the study staff. They may also look at your medical record in the future in order to update your personal health information.

You may also be asked to complete some questionnaires and be asked to answer some questions on your joint problem and how it affects daily life.

Cardiff University is the sponsor for this study based in the UK. We will be using information from you and your medical records in order to undertake this study and will act as the data controller for this study. This means that we are responsible for looking after your information and using it properly. Cardiff University will keep identifiable information about you for up to 15 years after the study has finished.

Your rights to access, change or move information are limited, as we need to manage your information in specific ways in order for the research to be reliable and accurate. If you withdraw from the study, we will keep the information about what we have already obtained. To safeguard your rights, we will use the minimum personally-identifiable information possible. You can find out about how we use your information by contacting the project lead detailed on the next page.

You can find out more about how we use your information at:
https://www.cardiff.ac.uk/public-information/policies-and-procedures/data-protection
or by contacting the University's Data Protection Officer:
inforequest@cardiff.ac.uk

The NHS will use your name, NHS number and contact details to contact you about the research study, and make sure that relevant information about the study is recorded for your care, and to oversee the quality of the study. Individuals from Cardiff University and regulatory organisations may look at your medical and research records to check the accuracy of the research study. The NHS will pass these details to the Biomechanics and Bioengineering Research Centre (Cardiff University) along with the information collected from you and/or your medical records. The only people in Cardiff University who will have access to information that identifies you will be people who are conducting the research, those who need to contact you about the study or audit the data collection process.

The NHS will keep identifiable information about you from this study for at least 10 years after the study has finished.

With your consent, anonymous data collected in the study may be shared with other institutions, including Universities and commercial organisations.

You will not be identified in any reports, presentations or publications relating to this research.

Sample storage / Use
Samples collected are usually kept at very cold temperatures, and can be stored this way for a very long time. Your samples will be stored indefinitely or until it is used up.

Many different types of research rely on the use of human samples. They can be used to develop new tests or help diagnose diseases, or can be used to help develop new ways to treat or even cure joint problems. Some of the research may lead to new medical products, such as diagnostic tests and drugs, or new procedures.

We may want to carry out genetic research (for example the identification of genes or diseases that run in families) on your tissue. On the consent form you will be given the option to exclude your tissue from this area of research. Genetic testing will be for research not diagnostic purposes and it may be many years before the results of any such tests could have clinical implications.

Access to your samples and any personal data that may be associated with your samples is strictly controlled. The sample you donate may be given to other groups within Cardiff University as well as external research collaborators, for example, other Universities and Companies, in the UK and abroad, for approved medical research but the samples will not be sold for profit to you or the researchers. Such researchers will only receive your donated samples and when appropriate, information about you (such as your sex, age and the reason for your clinical procedure) from your hospital record. The researcher will not receive your name, address, phone number or any other personal identifying information. This is done to protect your confidential information.

Future Research

Your samples may be retained at the end of this study for use in future research within the UK and abroad. At this stage we do not know what the research will involve but some of it could include genetic research (for example the identification of genes or diseases that run in families), or use in the commercial sector. On the consent form you will be given the option to exclude your tissue from these areas of research. You may withdraw your consent for the storage and future use of your samples at any point. If you do withdraw your consent your samples will not be used in any subsequent studies and will be destroyed according to local practices. Any samples already distributed for use in research prior to the withdrawal of consent will continue to be used in that study and any samples remaining at the end of the study will be destroyed.

What are the potential risks and benefits of taking part?
There is no intended clinical benefit for people taking part in the study. The information we collect from patients may help us to provide future patients who have joint disease or injury with improved treatment options.

If you agree to give up to a 40ml (8 teaspoons) sample of blood, we will perform this at the same time as one or more of your routine clinical samples if possible. If this is not possible, you may experience some minor discomfort from having the extra blood sample(s) taken.

If you agree to provide a urine sample, there would be the minor inconvenience of you collecting this.

If you agree to donate Clinical Waste Samples they will be collected during your routine clinical procedure and therefore there are no obvious disadvantages to taking part in the study.

What will happen to the results of the research using samples I have donated?
The results from these studies will be submitted for presentation at scientific conferences and publication in scientific journals. You will not be identified in any presentation or publication. The findings of this research will also be linked to the results of any of the other interlinking research studies you may undertake.

Other useful Information
Occasionally, during a research project, new information may become available. If this happens you will be contacted by a member of the research team to explain how this may affect you and your participation in the research.

We do not routinely send a letter to the GP to inform them of your participation in this research. However, with your permission we may contact your GP before getting in touch with you in the future to ensure it is suitable for us to do so. For this reason we ask you to provide details (name, address and telephone number) of the GP with whom you are registered.

This study has been reviewed and approved by the Wales Research Ethics Committee 3 (REC 3) and is managed by Cardiff University.

If something goes wrong and you are harmed due to negligence, you may have grounds for legal action. If you wish to make a complaint about the way you were approached or the treatment you have received within the study please contact Cheryl Cleary: Centre Manager 029 2251 0265. If you feel your complaint is not adequately addressed, you may escalate your complaint by writing to: The School Manager, School of Bioscience, Cardiff University, Museum Avenue, Cardiff, CF10 3AX

As well as being asked to take part in this research you may also be asked if you are interested in taking part in some of the other Centre studies.

For each of these studies you will be provided with a further information sheet and have the opportunity to ask questions. For each additional study you will be asked to sign a consent form before and research activity is performed.

What happens next?
This information sheet covers research into a wide range of joint and back problems. The study requirements vary depending on the joint under investigation and the planned treatment.

If you still have questions after reading this information, please contact a member of the research team.

Contact Details:

Centre Manager
Cheryl Cleary
Biomechanics and Bioengineering Research Centre Versus Arthritis
Cardiff School of Biosciences
Cardiff University
Cardiff
CF10 3AX
Tel: 029 2087 5419 or 029 2087 4986
email: ArthritisCentre@Cardiff.ac.uk

NHS Site
INSERT LOCAL NHS CONTACT DETAILS HERE

Project Lead / Contact
INSERT CARDIFF UNIVERSITY PROJECT LEAD DETAILS HERE

Thank you for taking time to read this information sheet

More information about the Biomechanics and Bioengineering Research Centre Versus Arthritis can be found by visiting:
http://www.cardiff.ac.uk/arthritis-biomechanics-bioengineering-centre

PATIENT CONSENT FORM

The collection of synovial fluid from joints

Page 1 of 2

You DO NOT have to sign this document. Please DO NOT sign this document unless you fully understand it. If there is ANYTHING which you do not understand please do not hesitate to ask for a full explanation.

To confirm agreement with each of the statements below, please initial each box and delete where applicable:

1. I confirm that I have read and understand the information sheet dated 06 September 2019 (Version 12.1) for the above study and have had the opportunity to ask questions ☐

2. I understand that my participation in the study is voluntary and that I am free to withdraw at any time, without giving any reason, and without my medical care or legal rights being affected. ☐

3. I understand that my details will be linked to a unique identifier to allow you to follow me through course of the study ☐

4. I agree to donate synovial fluid from my the procedure as a "gift" to the Biomechanics and Bioengineering Research Centre at Cardiff University according to the conditions in the information sheet.. ☐

5. I understand that the procedure to obtain my joint fluid is being carried out for research purposes only and is not part of my routine clinical care. ☐

6. I understand that researchers from other institutions may access my samples, that research may take many years and the information gained will not benefit me or my family directly. ☐

7. I agree to my GP being contacted ☐

8. I understand that you may access my Medical notes. ☐

9. I agree to take part in the above study. ☐

PATIENT CONSENT FORM

The collection of synovial fluid from joints

Page 2 of 2

Optional – please delete / circle as appropriate

10. You may / may not (please delete as appropriate) contact me in the future to take part in a follow up study if you require further fluid samples. ☐

11. I do / do not (please delete as appropriate) give permission for genetic analysis to be carried out using my samples ☐

12. I would / would not (please delete as appropriate) like to be contacted if genetic information is found that may have implications for me or my family. ☐

Name of Participant	Date (dd/mmm/yyyy)	Signature
Name of person obtaining consent	Date (dd/mmm/yyyy)	Signature

Original Centre File, 1 copy for the patient; 1 copy for patient notes

PATIENT INFORMATION SHEET

The collection, storage and analysis of Bone Samples during Osteotomy Surgery

We would like you to take part in a research study

- Before you decide if you would like to take part it is important for you to understand why the research is being done and what it will involve.
- Please take some time to read the following information sheet carefully and discuss it with friends or relatives if needed.
- It is your decision whether or not to take part.
- Ask a member of the study team if you have any questions about the research.
- If you decide to take part in this research but later change your mind you are free to withdraw at any time. This will not affect any of your NHS care.

Important Information about this Research

- This research is part of a series of studies being conducted by the Biomechanics & Bioengineering Research Centre (BBRCVersusArthritis) at Cardiff University.
- You may also be asked to complete some questionnaires relating to your health and daily living.
- We would also like to collect information about your diagnosis and treatment from you and from your medical records
- We do not expect there to be any direct benefit for people who take part in this research

What is the purpose of this research?

This research is part of a series of studies being carried out by the Centre Researchers, Orthopaedic Surgeons and Physiotherapists.

We are interested in analysing samples obtained following your clinical procedure (where appropriate), to help us research into the causes, diagnosis and treatment and/or monitoring of joint problems, (such as following injury or due to disease). Research with such samples can help us to find out more about what causes joint diseases, how to prevent and treat them. The samples you donate might be used for a number of related research studies associated with the Centre.

Why am I being asked to take part?
You have been asked to take part because you fall into one, or more, of the following categories:

- Are currently on a waiting list for osteotomy surgery
- Have previously had a osteotemy surgery and are having your plate removed and or revision surgery
- Have previously taken part in Centre research.

If you are on a waiting list for surgery, your surgeon has agreed that you may be suitable to take part in this research.

What does taking part involve?
With your permission, we will ask your surgeon to collect four small samples of bone, called bone cores, from the site where your Osteotomy is being performed. These samples are collected using a bone biopsy needle from the operation site, and are no more than 20mm in length. Collection of these samples cause no additional pain or morbidity, nor does the collection affect your recovery from this surgery. Collection of these samples takes no more than 5 minutes and is carried out during your osteotomy or plate removal should you have this procedure done at a later date.

On the consent form we will ask you if we can collect bone samples during your Osteotomy surgery and again during plate removal if this is within 2 years. This consent will last for 2 years, after this we will provide you with another consent form if we would like to collect further samples. Even if you have signed the form to say you are happy to give additional samples there is no obligation to provide these samples and you can withdraw your consent at any time.

If you are happy to take part in the study we will ask you to sign a consent form

What other types of information do we want?
We would like to collect information from your medical records, such as your weight, and record details of your condition, such as diagnosis and the results of various tests. We would also like to follow your progress after your procedure by looking at information that your doctor has collected from you during your routine follow-up visits. Your doctor may record such information such as the medications you are taking and whether your level of pain has changed. We would also like to ask your permission to send you ethically approved surveys or questionnaires to complete. This enables us to collect standardised information about large numbers of tissue donors. They ask questions about lifestyle choices such as "Do you smoke?" or "Do you exercise?" No personal information will be collected without your permission.

What will happen to my information / Samples?
After you have signed a consent form you will be assigned a unique number. from then on, this number will be used to identify you throughout the study. All electronic data will be held securely on NHS or University computers. Access to this information will be restricted to members of the research team.

As well samples we may also collect some routine data from your medical records. This may include information about your operation, diagnosis and treatment, where it is relevant to your participation in this study. The information that may be given to the researcher includes, but is not restricted to: your age, sex, race, medical history, diagnosis, treatments and possibly some medical history. This information will be collected from your health record by the study staff. They may also look at your medical record in the future in order to update your personal health information.

You may also be asked to complete some questionnaires and be asked to answer some questions on your joint problem and how it affects daily life.

Cardiff University is the sponsor for this study based in the UK. We will be using information from you and your medical records in order to undertake this study and will act as the data controller for this study. This means that we are responsible for looking after your information and using it properly. Cardiff University will keep identifiable information about you for up to 15 years after the study has finished.

Your rights to access, change or move information are limited, as we need to manage your information in specific ways in order for the research to be reliable and accurate. If you withdraw from the study, we will keep the information about what we have already obtained. To safeguard your rights, we will use the minimum personally-identifiable information possible. You can find out about how we use your information by contacting the project lead detailed on the next page.

You can find out more about how we use your information at:
https://www.cardiff.ac.uk/public-information/policies-and-procedures/data-protection
or by contacting the University's Data Protection Officer: inforequest@cardiff.ac.uk

The NHS will use your name, NHS number and contact details to contact you about the research study, and make sure that relevant information about the study is recorded for your care, and to oversee the quality of the study. Individuals from Cardiff University and regulatory organisations may look at your medical and research records to check the accuracy of the research study. The NHS will pass these details to the

Biomechanics and Bioengineering Research Centre (Cardiff University) along with the information collected from you and/or your medical records. The only people in Cardiff University who will have access to information that identifies you will be people who are conducting the research, those who need to contact you about the study or audit the data collection process.

The NHS will keep identifiable information about you from this study for at least 10 years after the study has finished..

With your consent, anonymous data collected in the study may be shared with other institutions, including Universities and commercial organisations.

You will not be identified in any reports, presentations or publications relating to this research.

Sample storage / Use
Samples collected are usually kept at very cold temperatures and can be stored this way for a very long time. Your samples will be stored indefinitely or until it is used up.

Many different types of research rely on the use of human samples. They can be used to develop new tests or help diagnose diseases or can be used to help develop new ways to treat or even cure joint problems. Some of the research may lead to new medical products, such as diagnostic tests and drugs, or new procedures.

We may want to carry out genetic research (for example the identification of genes or diseases that run in families) on your tissue. On the consent form you will be given the option to exclude your tissue from this area of research. Genetic testing will be for research not diagnostic purposes and it may be many years before the results of any such tests could have clinical implications.

Access to your samples and any personal data that may be associated with your samples is strictly controlled. The sample you donate may be given to other groups within Cardiff University as well as external research collaborators, for example, other Universities and Companies, in the UK and abroad, for approved medical research but the samples will not be sold for profit to you or the researchers. Such researchers will only receive your donated samples and when appropriate, information about you (such as your sex, age and the reason for your clinical procedure) from your hospital record. The researcher will not receive your name, address, phone number or any other personal identifying information. This is done to protect your confidential information.

What are the potential risks and benefits of taking part?
There is no intended clinical benefit for people taking part in the study. The information we collect from patients may help us to provide future patients who have joint disease or injury with improved treatment options.

If you agree to donate these bone samples, they will be collected during your sheduled surgery and therefore there are no obvious disadvantages to taking part in the study. As explained previously, collection of these samples cause no additional pain or morbidity, nor does the collection affect your recovery from this surgery.

What will happen to the results of the research using samples I have donated?
The results from these studies will be submitted for presentation at scientific conferences and publication in scientific journals. You will not be identified in any presentation or publication. The findings of this research will also be linked to the results of any of the other interlinking research studies you may undertake.

Future Research
Your samples may be retained at the end of this study for use in future research within the UK and abroad. At this stage we do not know what the research will involve but some of it could include genetic research (for example the identification of genes or diseases that run in families), or use in the commercial sector. On the consent form you will be given the option to exclude your tissue from these areas of research.

All tissue will be supplied anonymously; recipients of the tissue will not be able to identify you from your tissue. Your tissue will not be sold.

You may withdraw your consent for the storage and future use of your samples at any point. If you do withdraw your consent your bone samples will not be used in any subsequent studies and will be destroyed according to local practices. Any bone samples already distributed for use in research prior to the withdrawal of consent will continue to be used in that study and any tissue remaining at the end of the study will be destroyed.

Other useful Information
Occasionally, during a research project, new information may become available. If this happens you will be contacted by a member of the research team to explain how this may affect you and your participation in the research.

We do not routinely send a letter to the GP to inform them of your participation in this research. However, with your permission we may contact your GP before getting in touch with you in the future to ensure it is suitable for us to do so. For this reason we ask you to provide details (name, address and telephone number) of the GP with whom you are registered.

This study has been reviewed and approved by the Wales Research Ethics Committee 3 (REC 3) and is managed by Cardiff University.
If something goes wrong and you are harmed due to negligence, you may have grounds for legal action. If you wish to make a complaint about the way you were approached or the treatment you have received within the study please contact Cheryl Cleary: Centre Manager 029 2251 0265. If you feel your complaint is not adequately addressed, you may escalate your complaint by writing to: The School Manager, School of Bioscience, Cardiff University, Museum Avenue, Cardiff, CF10 3AX

As well as being asked to take part in this research you may also be asked if you are interested in taking part in some of the other Centre studies.

For each of these studies you will be provided with a further information sheet and have the opportunity to ask questions. For each additional study you will be asked to sign a consent form before and research activity is performed.

CARDIFF UNIVERSITY
PRIFYSGOL CAERDYDD

GIG CYMRU NHS WALES
Bwrdd Iechyd Prifysgol Caerdydd a'r Fro
Cardiff and Vale University Health Board

PATIENT CONSENT FORM

The collection, storage and analysis of Bone Samples during Osteotomy Surgery

Page 1 of 2

Centre ID: ☐☐☐☐☐ **Project Name:** _____

You DO NOT have to sign this document. Please DO NOT sign this document unless you fully understand it. If there is ANYTHING which you do not understand please do not hesitate to ask for a full explanation.

To confirm agreement with each of the statements below, please initial each box and delete where applicable:

1. I confirm that I have read and understand the information sheet dated 15 August 2019 (Version 3) for the above study and have had the opportunity to ask questions ☐

2. I understand that my participation in the study is voluntary and that I am free to withdraw at any time, without giving any reason, and without my medical care or legal rights being affected. ☐

3. I understand that my details will be linked to a unique identifier to allow you to follow me through course of the study ☐

4. I do / do not (please delete as appropriate) give permission for four samples of bone to be collected during my osteotomy surgery. ☐

5. I do / do not (please delete as appropriate) give permission for my consent to cover the collection of any additional bone samples over the next 2 years for this study, during a plate removal and I understand that by signing this form I am not obliged to give these additional samples. ☐

6. I understand that researchers from other organisations in the UK and abroad, including commercial companies, may access my samples, that research may take many years and the information gained will not benefit me or my family directly. ☐

BIOMECHANICS & BIOENGINEERING RESEARCH CENTRE
VERSUS ARTHRITIS

CARDIFF UNIVERSITY
PRIFYSGOL CAERDYDD

PATIENT CONSENT FORM

The collection, storage and analysis of Bone Samples during Osteotomy Surgery

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7. I understand I can withdraw my consent for the storage and future use of my samples at any point and that any unused samples will be destroyed immediately. I understand that any samples used in research prior to the withdrawal of consent may not be destroyed until the end of the study. ☐

8. I agree to my GP being contacted ☐

9. I understand that you may access my Medical notes. ☐

10. I agree to take part in the above study. ☐

Optional – please delete / circle as appropriate

11. You may / may not (please delete as appropriate) contact me in the future to ask if I would be interested in participating in a follow up study. ☐

12. I do / do not (please delete as appropriate) give permission for genetic analysis to be carried out using my samples ☐

13. I would / would not (please delete as appropriate) like to be contacted if genetic information is found that may have implications for me or my family. ☐

Name of Participant	Date (dd/mmm/yyyy)	Signature
Name of person obtaining consent	Date (dd/mmm/yyyy)	Signature

BIOMECHANICS & BIOENGINEERING RESEARCH CENTRE
VERSUS ARTHRITIS

Original for Centre File,
1 copy for the patient;
1 copy for the patient notes

Appendix 2: Oxford Knee Score

PROBLEMS WITH YOUR KNEE

During the past 4 weeks..

✓tick one box for every question

1 ***During the past 4 weeks*****.....**

How would you describe the pain you usually have from your knee?

None	Very mild	Mild	Moderate	Severe
❑	❑	❑	❑	❑

2 ***During the past 4 weeks*****.....**

Have you had any trouble with washing and drying yourself (all over) because of your knee?

No trouble at all	Very little trouble	Moderate trouble	Extreme difficulty	Impossible to do
❑	❑	❑	❑	❑

3 ***During the past 4 weeks*****.....**

Have you had any trouble getting in and out of a car or using public transport because of your knee? (whichever you would tend to use)

No trouble at all	Very little trouble	Moderate trouble	Extreme difficulty	Impossible to do
❑	❑	❑	❑	❑

4 ***During the past 4 weeks*****.....**

For how long have you been able to walk before pain from your knee becomes **severe**? (*with or without a stick)*

No pain/ More than 30 minutes	16 to 30 minutes	5 to 15 minutes	Around the house only	Not at all - pain severe when walking
❑	❑	❑	❑	❑

5 ***During the past 4 weeks*****.....**

After a meal (sat at a table), how painful has it been for you to stand up from a chair because of your knee?

Not at all painful	Slightly painful	Moderately painful	Very painful	Unbearable
❑	❑	❑	❑	❑

6 ***During the past 4 weeks*****.....**

Have you been limping when walking, because of your knee?

Rarely/ never	Sometimes, or just at first	Often, not just at first	Most of the time	All of the time
❑	❑	❑	❑	❑

 /P.T.O

During the past 4 weeks...

✓tick one box for every question

7 *During the past 4 weeks.....*

Could you kneel down and get up again afterwards?

Yes, Easily	With little difficulty	With moderate difficulty	With extreme difficulty	No, Impossible
❑	❑	❑	❑	❑

8 *During the past 4 weeks.....*

Have you been troubled by pain from your knee in bed at night?

No nights	Only 1 or 2 nights	Some nights	Most nights	Every night
❑	❑	❑	❑	❑

9 *During the past 4 weeks.....*

How much has pain from your knee interfered with your usual work *(including housework)*?

Not at all	A little bit	Moderately	Greatly	Totally
❑	❑	❑	❑	❑

10 *During the past 4 weeks.....*

Have you felt that your knee might suddenly 'give way' or let you down?

Rarely/ never	Sometimes, or just at first	Often, not just at first	Most of the time	All of the time
❑	❑	❑	❑	❑

11 *During the past 4 weeks.....*

Could you do the household shopping on your own?

Yes, Easily	With little difficulty	With moderate difficulty	With extreme difficulty	No, Impossible
❑	❑	❑	❑	❑

12 *During the past 4 weeks.....*

Could you walk down one flight of stairs?

Yes, Easily	With little difficulty	With moderate difficulty	With extreme difficulty	No, Impossible
❑	❑	❑	❑	❑

Appendix 3: PACS Pain Scoring System

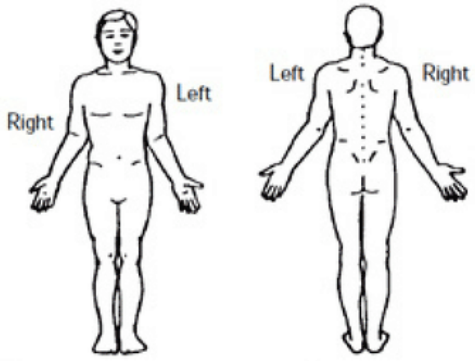
Date: _____ /_____ /_____ Time: _____

Name: _____ _____ _____
Last First Middle intial

1) Throughout our lives, most of us have had pain from time to time (such as minor headaches, sprains, and toothaches). Have you had pain other than these everyday kinds of pain today?

1. Yes 2. No

2) On the diagram, shade in the areas where you feel pain. Put an X on the area that hurts the most.



3) Please rate your pain by circling the one number that best describes your pain at its **worst** in the past 24 hours.

0	1	2	3	4	5	6	7	8	9	10
No pain										Pain as bad as you can imagine

4) Please rate your pain by circling the one number that best describes your pain at its **least** in the past 24 hours.

0	1	2	3	4	5	6	7	8	9	10
No pain										Pain as bad as you can imagine

5) Please rate your pain by circling the one number that best describes your pain on **average**.

0	1	2	3	4	5	6	7	8	9	10
No pain										Pain as bad as you can imagine

6) Please rate your pain by circling the one number that tells how much pain you have **right now**.

0	1	2	3	4	5	6	7	8	9	10
No pain										Pain as bad as you can imagine

7) What treatments or medications are you receiving for your pain?

_____

_____

8) In the past 24 hours, how much **relief** have pain treatments or medications provided? Please circle the one percentage that most shows how much relief you have received.

0%	10	20	30	40	50	60	70	80	90	100%
No relief										Complete relief

9) Circle the one number that describes how, during the past 24 hours, pain has **interfered** with your:

A. General activity

0	1	2	3	4	5	6	7	8	9	10
Does not interfere										Completely interferes

B. Mood

0	1	2	3	4	5	6	7	8	9	10
Does not interfere										Completely interferes

C. Walking ability

0	1	2	3	4	5	6	7	8	9	10
Does not interfere										Completely interferes

D. Normal work (includes both work outside the home and housework)

0	1	2	3	4	5	6	7	8	9	10
Does not interfere										Completely interferes

E. Relations with other people

0	1	2	3	4	5	6	7	8	9	10
Does not interfere										Completely interferes

F. Sleep

0	1	2	3	4	5	6	7	8	9	10
Does not interfere										Completely interferes

G. Enjoyment of life

0	1	2	3	4	5	6	7	8	9	10
Does not interfere										Completely interferes