

# KNEE OSTEOARTHRITIS IS A BILATERAL DISEASE

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## Abstract

Knee osteoarthritis (OA) is a common cause of pain and disability. Patients often complain that they overload the other limb when they walk, resulting in disease in the unaffected knee. However, it is unknown whether this happens or the mechanism by which it occurs.

Data was analysed from an established longitudinal cohort study to examine the development of bilateral knee OA. One hundred and forty-three subjects were examined over a 12 year period with bilateral radiographs. Bilateral knee osteoarthritis was found to be very common over time, and the majority of individuals with unilateral knee OA eventually developed bilateral disease.

A gait analysis study was performed on 20 subjects with unilateral knee OA awaiting arthroplasty surgery and 20 healthy age equivalent controls. Abnormal moments and muscle co-contractions were observed in the other knee and hips when they walked due a characteristic slow, cautious, stiff-legged gait pattern. Fifteen subjects re-attended 12 months following their surgery. Whilst moments returned to normal in most of the replaced knees, they remained elevated at the contra-lateral side and co-contraction failed to recover in either knee.

A novel study design is presented to examine the effect of gait-derived loading waveforms on fresh human osteochondral plugs. By applying mechano-biology techniques and Finite Element Modelling to fresh human tissue, new observations

can be made about the relationship between in-vivo loading and cartilage mechano-biology.

A characteristic gait pattern was observed in knee OA which is not simply antalgic but tends towards symmetry, with an increase in joint loading bilaterally. The observed gait behaviour does not resolve, despite arthroplasty of the affected joint. This would be expected to contribute to the development of disease in an inherently vulnerable joint. Additional training may have a role to play in restoring normal biomechanics and protecting the other knee from disease.

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## List of Abbreviations

ACL	Anterior cruciate ligament
ADAMTS	A disintegrin and metalloproteinase with thrombospondin motifs
AJM	Ankle joint marker
ANCOVA	Analysis of covariance
AR(UK)	Arthritis Research UK
ASIS	Anterior superior iliac spine
B[NL]	Belief in normal
B[OA]	Belief in osteoarthritis
B[Θ]	Belief in uncertainty
BMC	BioMed Central
BMI	Body mass index
BMP-2	Bone morphogenic protein 2
BW	Body weight
C7	Seventh cervical vertebra
CCI	Co-contraction index
cDNA	Complimentary deoxyribonucleic acid

cf(v)	Confidence factor for a variable
CI	Confidence interval
COM	Centre of Mass
COP	Centre of pressure
COX-2	Cyclooxygenase 2
CT	Computed tomography
DNA	Deoxyribonucleic acid
EMG	Electromyography
EULAR	The European League Against Rheumatism
$F_{[x,y,z]}$	Components of the ground reaction vector
FE/FEM	Finite element/finite element modelling
GC	Gait cycle
GRF	Ground reaction force
GRF X, Y, Z	Components of the ground reaction force
HAM	Hip adduction moment
HES	Hospital Episode Statistics
Ht	Height
ICC	Intra-class correlation

IL-6	Interleukin 6
JRF	Joint reaction force
KAD	Knee alignment device
KAM	Knee adduction moment
KAMI	Knee adduction moment impulse
KC	Knee centre
KJC	Knee joint centre
KL or K-L	Kellgren-Lawrence classification system
KOOS	Knee injury and osteoarthritis outcome score
KR	Knee replacement
LED	Light emitting diode
LREC	Local Research ethics committee
MASAC	Mid-anterior superior iliac spine-sacrum virtual marker
MMP	Matrix metalloproteinase
MRI	Magnetic resonance imaging
mRNA	Messenger ribonucleic acid
mSv	Millisieverts

N·m/BW·Ht	Newton·metres/(Body weight·height)
N·m·s/BW·Ht	Newton·metre·seconds/(Body weight·height),
NHS	National Health Service
NICE	National Institute of Health and Clinical Excellence
NL	Normal (when used in reference to the Cardiff classifier)
NSAIDs	Non-steroidal anti-inflammatory drugs
OA	Osteoarthritis
OARSI	Osteoarthritis Research Society International
ONS	Office for National Statistics
OR	Odds ratio
ORLAU	Orthotic Research and Locomotor Assessment Unit
ORUK	Orthopaedic Research UK
P/Phys	Physiological (with reference to loading waveform)
PC's	Principle components
PCA	Principle component analysis
PCR	Polymerase chain reaction
PEDW	Patient Episode Database for Wales

qPCR	Quantitative real-time polymerase chain reaction
R&D	Research and development department
RCT	Randomised controlled trial
REC	Research ethics committee
RJAH	The Robert Jones and Agnes Hunt Orthopaedic Hospital, Oswestry
RMS	Root mean square
RMSE	Root mean square error
RNA	Ribonucleic acid
ROM	Range of movement
SD	Standard deviation
TGF- $\beta$	Transforming growth factor beta
TKR	Total knee replacement
TNF $\alpha$	Tumour necrosis factor alpha
WOMAC	Western Ontario and McMaster osteoarthritis index
$\Theta$	Uncertainty (when used in reference to the Cardiff classifier)

## **Chapter 1. Introduction**

This thesis did not originate as an academic exercise but arose from a clinical question, based on a common statement made by patients in clinic every week: “Does a bad knee cause you to overload onto the other leg and wear out the other knee?”. This question is difficult to clearly answer, and would require a long and costly project to do so. The purpose of this thesis is start to pick apart that overriding question into its component parts, and develop some of the background work needed to start a more definitive study in the future.

Whilst on an orthopaedic placement in North Wales in 2008, I attended a meeting on research techniques in the Oswestry Institute of Orthopaedics, at the Robert Jones and Agnes Hunt (RJA) Orthopaedic Hospital. Dr C Stewart gave a talk on the research potential of gait analysis, and a later talk on potential research plans in the trust by Dr J-H Kuiper introduced the fact that multiple joint osteoarthritis (OA) was an area that was deficient in the literature. This led me to question whether gait analysis would be a useful technique for studying multiple joint OA, initially with an exploratory study and preliminary research over the proceeding year.

After a literature review, it became clear that the topic of multiple joint OA was an area in which there was only a small number of studies, with very different techniques and findings (see sections 2.1.8, 2.3.6 and 2.3.7 for a discussion of those papers). The kind of large longitudinal cohort study required to truly understand multiple joint disease was not then feasible, as the background work required to pilot and develop such a piece of research had not been undertaken. Therefore a number of smaller linked hypothesis were developed, to develop into a body of

work investigating the influence of disease in one joint on the others, and to see if changes in treatment might be required to protect these secondary joints.

The background and literature review contained in Chapter 2 was written between 2010 and 2011, at a time when the initial studies contained in the thesis were running and the later studies were being planned. Further work has subsequently been published from other centres, particularly during 2013. This knowledge supports and adds to the data presented in this thesis, some of which has also been published over the past 12 to 18 months (Appendix 4). These more recent studies are therefore reported later in the thesis in Chapter 7, helping to establish a broader base for the further research proposed in that chapter.

The first two issues to address when examining multiple joint disease were whether there was a clinical problem at all, and whether loading is abnormal in the other joints during gait. The first question had been partially answered in the literature with cross-sectional surveys, but a longitudinal approach was lacking. Researchers responsible for an established longitudinal cohort study in Sweden were approached and the results of that analysis are reported in Chapter 3.

The question of whether loading was abnormal was investigated using gait analysis. The gait laboratory at Oswestry was approached and a study was developed to examine patients with severe knee OA as described in Chapter 4. The results of that study led to a desire to assess the response to treatment (in the form of knee arthroplasty) and so a further post-operative set of gait analyses were performed, as described in Chapter 5.



The pre-and post-operative analysis produced a large quantity of data, some of which was important in the context of the original question and some of which could be used to develop new hypotheses or potential therapies. Therefore, once joint loading patterns had been extracted and assessed, a more in depth statistical analysis was used to reduce the data to a manageable form and to describe the pattern of gait mathematically. A statistical approach to the classification and analysis of gait data in knee OA has previously been described in the School of Engineering at Cardiff University and that technique was employed for the current dataset. That work is also described in Chapters 5.

The link between joint loading and cartilage biology was studied with a view to increasing our understanding of the relevance of the joint loading patterns that we have seen. One way of dealing with the link between joint loading and disease progression would have been to perform a longitudinal cohort study, with serial imaging over a prolonged period of time. This would be expensive and time-consuming, beyond the scope of the current thesis. However an understanding of the link between joint loading and cartilage biology would increase knowledge about the link between the biomechanical findings in the gait laboratory and the impact on the patient. It would also create a model that could be used to assess the potential for treatment to change the biological and subsequently the clinical outcome. The work into this is described in Chapter 6.

Therefore, a series of studies is presented, each attempting to address the wider clinical questions of: 'Is multiple joint arthritis a real clinical problem?', 'Is loading

abnormal in patients with knee arthritis, and does it change with treatment?', 'What is abnormal about the gait pattern of these patients?' and 'What impact does abnormal loading have on cartilage?' It is intended that this thesis will help to answer these questions, or contribute to the understanding of where treatment can be improved. An improved understanding of OA as a disease of the whole musculoskeletal system, rather than just a disease of a single joint, has the potential to change the way we view the patients we treat, and may lead to new and innovative approaches to the management of OA in the future.

## **Chapter 2. Background and Literature Review**

## 2.1 Osteoarthritis the disease

### 2.1.1 History of osteoarthritis

Osteoarthritis (OA) is one of the oldest recorded diseases, and has been traced back many millennia. Fossil remains of dinosaurs as far back as 100 million years ago have shown evidence of degenerative joint disease (Karsh 1960). OA was uncommon in many dinosaurs but evidence of OA has been found in the *Iguanodon* species (figure 2.1), and a recent paper described the discovery of ankle OA in up to 30% of samples of a land-based bird, *Caudipteryx*, which existed 20 million years before the earliest dinosaur discovery. This has been linked to its size and lack of flight, as it was thought to have poorly adapted joints for the load that they encountered (Rothschild and Tanke 1992; Rothschild, Xiaoting et al 2012).

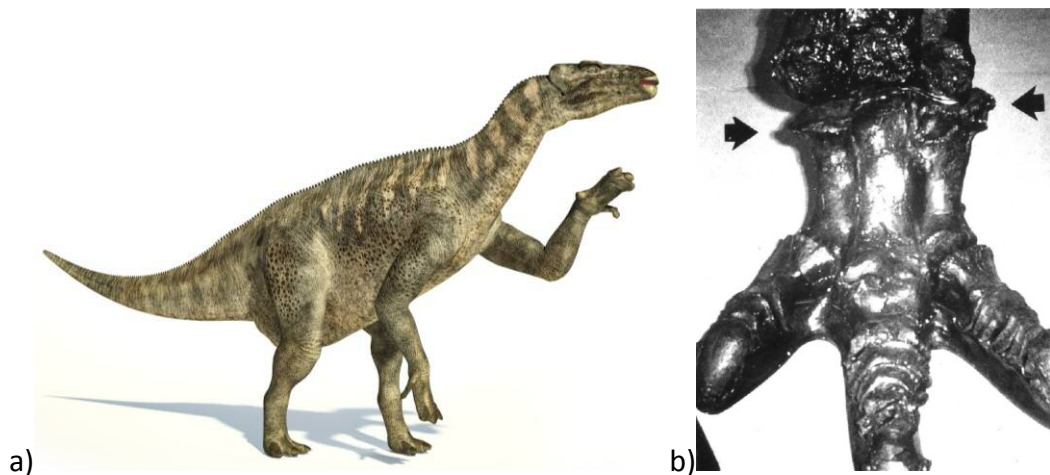


Figure 2.1. a) Artists impression of an *Iguanodon* and b) evidence of ankle OA in the fossilised remains of the same species (Rothschild and Tanke 1992).

Evidence of OA has been found in Neanderthal man and it has been described as a specific disease in Palaeolithic man (Strauss 1957; Dequeker and Luyten 2008). The iced remains of 'Otzi', a traveller from 3000BC found on the Austria-Italian Border,

had evidence of attempts to treat joint pain in the forms of tattoos over affected joints (Dickson 2003). Numerous cases of OA have been found amongst Egyptian mummies of the same era (Ruffer 1912).

Examination of Saxon remains in England from the 9<sup>th</sup> to the 15<sup>th</sup> century shows that OA was a common condition, although more likely to affect the shoulders, hips and patello-femoral joints than the tibio-femoral joints (Rogers, Watt et al. 1981; Rogers and Dieppe 1994). A diagram of knee compartments is given in figure 2.7, on page 23. Typical patterns of joint involvement changed over time with particular differences noted between hunter-gatherer and farming communities, as well as between Caucasian and Asian populations (Inoue, Hukuda et al. 2001).

Although the concept of OA of the hip as a disease entity was described in the early 19<sup>th</sup> century (*'malum coxae senilis'*), it was only by the turn of the 20<sup>th</sup> century and the advent of x-rays that inflammatory (*'atrophic'*) and degenerative (*'hypertrophic'*) joint disease were widely regarded as separate entities (Goldthwaite 1897; Nichols and Richardson 1909). The term *'osteoarthritis'* superseded *'hypertrophic'* arthritis rapidly and despite its pathological inaccuracy, persists in common use (Dequeker and Luyten 2008).

### 2.1.2 Definition of Osteoarthritis

Despite the 100 years that has passed since OA was recognised as a distinct entity, the condition has remained difficult to define clearly. The European League Against Rheumatism (EULAR) recently published a series of guidelines on the definition and the diagnosis of knee OA. Their definition of knee OA is (Zhang, Doherty et al. 2009):

“Knee osteoarthritis is characterised clinically by usage-related pain and/or functional limitation. It is a common complex joint disorder showing focal cartilage loss, new bone formation and involvement of all joint tissues. Structural tissue changes are mirrored in classical radiographic features”

Previously accepted definitions were from the American Rheumatism Association and the American Academy of Orthopaedic Surgeons (Altman, Asch et al. 1986; Kuettner 1995). The EULAR definition has the advantage that its primary focus is on the patient’s subjective experience – the ‘bottom line’, rather than the pathological entity or radiological findings. This is because plain radiography, MRI and arthroscopic findings do not universally correlate with pain, symptoms or physical function (Kornaat, Bloem et al. 2006; Bedson and Croft 2008; Hofmann, Marticke et al. 2009; Link 2009; Neogi, Felson et al. 2009). However, the use of pain as a primary definition of a physical disease also has its limitations, as the perception of pain changes both between individuals and is dependant not only on the condition of the joint itself but also on psychosocial and environmental factors (McAlindon, Cooper et al. 1992; Creamer, Lethbridge-Cejku et al. 1999; Dieppe and Lohmander

2005). For that reason, the definition and classification of OA for a particular piece of research needs to be clearly defined, and appropriate to the study question.

### 2.1.3 Epidemiology of OA: Prevalence and Economic Impact

Joint pain is a common problem in society. With regard to the knee, a systematic review from 2001 estimated that 25% of adults over 55 have knee pain (Peat, McCarney et al. 2001). This is supported by two more recent community surveys which have found similar results amongst communities in different part of the UK (Dawson, Linsell et al. 2004; Keenan, Tennant et al. 2006).

As OA becomes more common with age, epidemiologists consider joint pains in anyone in their 6<sup>th</sup> decade and beyond to be primarily be caused by OA (Lawrence, Bremner et al. 1966; Petersson 1996; Peat, McCarney et al. 2001).

OA and joint pain are significant causes of disability, pain and reduced quality of life in the community. As reported above, approximately 25% of adults in the UK over 55 report knee pain. Ten percent have some reported disability due to knee pain and 1.5% are severely disabled, according to Lesquane knee scores, SF-36 and the Health Assessment Questionnaire (McAlindon, Cooper et al. 1992; O'Reilly, Muir et al. 1996; Peat, McCarney et al. 2001). Disability and pain are increased with radiographic severity of disease, number of affected compartments, mechanical loading, obesity and multiple joint disease, but psycho-social factors also play a significant role (McAlindon, Cooper et al. 1992; Creamer, Lethbridge-Cejku et al.

1999; Dawson, Linsell et al. 2004; Dieppe and Lohmander 2005; Szebenyi, Hollander et al. 2006; Maly, Costigan et al. 2008; Neogi, Felson et al. 2009; White, Zhang et al. 2010; White, Zhang et al. 2010) .

Wales had a population of approximately 2.9 million in the 2001 census, compared to 49.1 million in England, and those numbers have not thought to have significantly changed over the past decade (ONS 2002). In the 2008-2009 financial year, there were 5,211 knee replacements in Wales, and 3,903 hip replacements, taking up a total of 71,598 hospital bed days (PEDW 2010). In England in the same time period, there were 65,871 hip replacements and 74,606 knee replacements taking up a total of 99,4714 hospital bed days (HESOnline 2010).

OA is also costly to society. In the USA, the medical expenditures cost of 'arthritis and rheumatic conditions' for 2003 was estimated to be as high as \$81 Billion (US Dollars, approximately £54 Billion UK sterling), with earning losses totalling \$47 Billion (approximately £31 Billion) (Yelin, Murphy et al. 2007). Equivalent studies have not been performed in the UK, and treatment costs vary widely between different countries. However, it is clear that OA is common a common and disabling condition which has a major economic cost to society in general.



#### 2.1.4 Pathophysiology of osteoarthritis

As described in the definition of osteoarthritis (Section 2.1.2), OA is a disease that involves multiple tissues within a joint, including the synovium, bone and cartilage as well as supporting structures such as the menisci in the knee. Much of the research in OA has focused on the most macroscopically obvious abnormality, which is change in articular cartilage. In early OA, hyaline cartilage softens, fibrillates and fissures, and eventually breaks off to expose subchondral bone in established OA. One hypothesis is that the initiation of OA appears to relate to a change in the composition and properties of the hyaline cartilage in the joint, which is a multi-factorial event (figure 2.2).

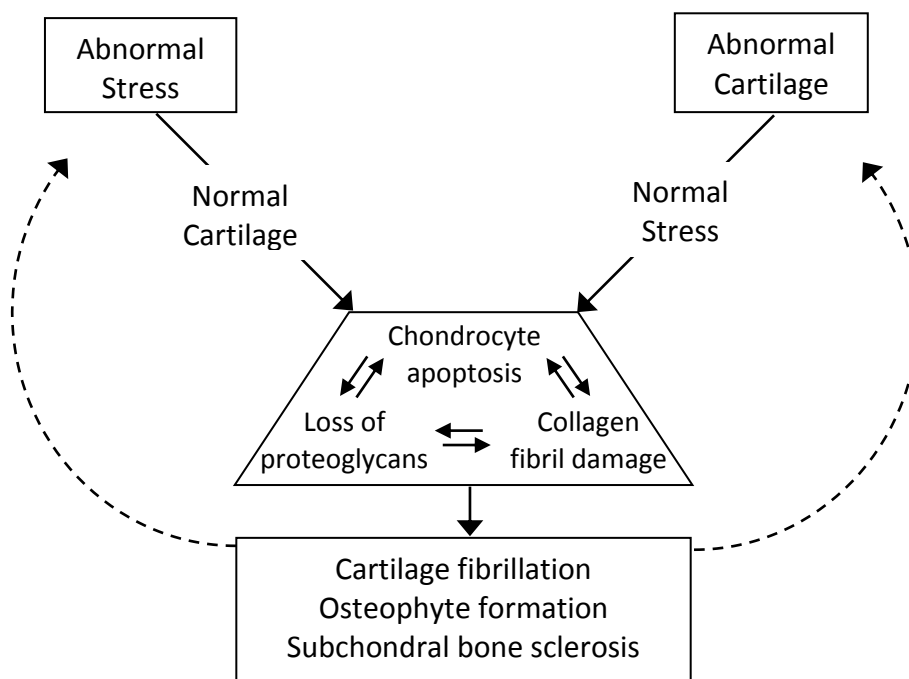


Figure 2.2 Description of the initiation of OA. Although this diagram describes two separate pathways, in many cases the problem is a combination of some abnormal stress on the background of relatively abnormal cartilage, for example due to ageing and obesity. Adapted from (Nuki 2005).

The importance of changes in subchondral bone in early and advanced OA are becoming increasingly recognised. As OA becomes more advanced, sub-chondral bone turnover increases and the bone becomes more sclerotic and stiffer (Ayrar, Dougados et al. 1996; Krasnokutsky, Attur et al. 2008). It is thought that this change in mechanical property may increase the stress on chondrocytes and increase (or initiate) the loss of cartilage in the joint. Osteophytes also develop, and like subchondral sclerosis it is thought that this may be due to the local production of growth factors (such as TGF- $\beta$  and BMP-2) that occur as part of the disease cascade. Glutamate signalling has been highlighted in Cardiff as an important link between mechanical loading, pain and inflammation in OA subchondral bone (Brakspear and Mason 2012).

To properly understand the relationship between mechanical loading and OA, the biology of both bone and articular cartilage needs to be considered (Goldring and Goldring 2010). It is clear, however that cartilage degeneration and loss is a key component of the disease process and the relationship between mechanical loading and cartilage degeneration is important in both disease initiation and progression.

Healthy articular cartilage is a complex structure which is able to resist constantly varying stresses due to its interwoven structure of collagen and non-collagenous proteins. Tensile stresses are resisted by a collagen network which forms arcades, with fibrils longitudinally arranged in the deep zone and transversely arranged in the superficial zone. Compressive stresses are resisted by non-collagenous proteins

such as proteoglycans. Proteoglycans are composite structures, made up of a protein core with hyaluronic acid sugar chains with numerous branches of highly hydrophilic glycosaminoglycans, such as keratan and chondroitin sulphate. The most common proteoglycan in cartilage is aggrecan. These strongly hydrophilic molecules attract water into the matrix, giving a powerful resistance to compression.

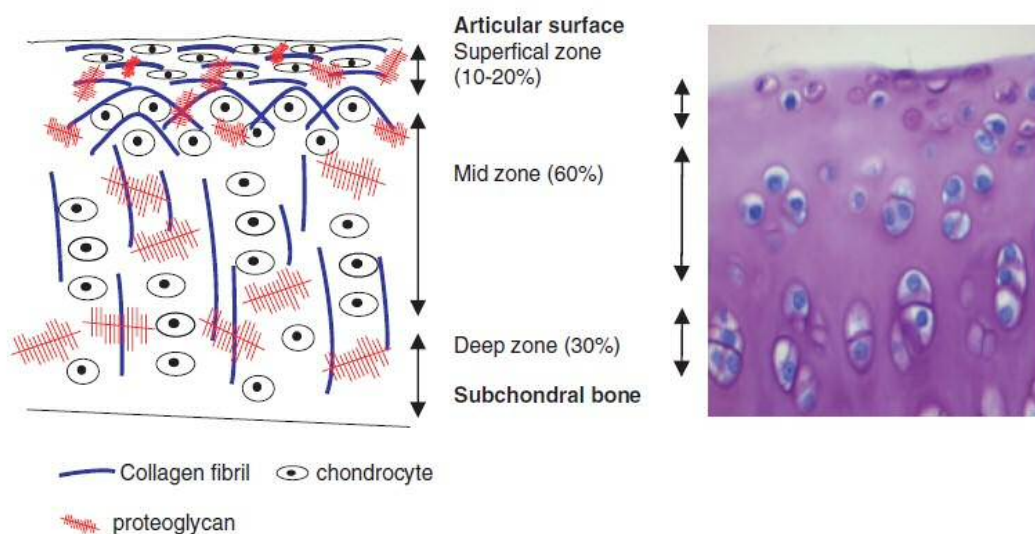


Figure 2.3 Schematic diagram and histology section showing general structure of articular cartilage. From (Ramage, Nuki et al. 2009).

The mechanical function of the collagenous and non-collagenous parts are closely dependent on each other, in a manner that is often considered analogous to a collection of balloons in a string bag. If either the balloons or the string breaks, the structure fails. However if both remain intact the structure is strong and can resist a range of forces applied to it.

The production and maintenance of collagenous and non-collagenous proteins is performed by chondrocytes embedded into the cartilage matrix. They produce matrix proteins such as the collagens, especially type 2 collagen, (the dominant type

in articular cartilage) and aggrecan. This is their main anabolic function, however they are also able to produce a range of catabolic proteins, the most potent of which are the matrix-metalloproteinase's (MMP's). The MMP's break down various parts of the extracellular matrix, such as collagenases (e.g. MMP 1, 8 and 13), gelatinases (MMP 2 and 9), and stromolysins which work on a range of proteins including proteoglycans and fibronectin (e.g. MMP 3, 7, 10 and 11) (Blain 2007). Aggrecans are broken down by proteins such as ADAMTS-4 and ADAMTS-5.

The balance between the production of anabolic or catabolic proteins is, in part, regulated by the stresses that the cartilage is exposed to. This balance is particularly altered in OA with increased expression of catabolic proteins and reduced anabolic function leading to ongoing cartilage damage and loss (Brew, Clegg et al. 2010).

Histologically, the hallmarks of early cartilage degeneration are collagen fibril damage in the superficial zone of cartilage, changes to proteoglycan structure such as fragmentation of proteoglycans, and chondrocyte death or apoptosis. The response to cartilage injury and ongoing mechanical stimulation is mediated by IL-1 $\beta$ , which results in the activation of an inflammatory cascade involving the powerfully pro-inflammatory cytokines IL-6 and TNF $\alpha$  and prostaglandin E2, alongside further matrix-metalloproteinase (MMP) production, such as the collagenases MMP-1, MMP-8 and MMP-13 and the aggrecanase ADAMTS-5 (Goldring and Goldring 2004; Stevens, Wishnok et al. 2009).

It has to be remembered that much of the work on cartilage biology in OA has, by necessity, been done using animal models of disease (Ameye and Young 2006).

These have been confirmed where possible using human cartilage although that is very difficult to obtain in significant quantities, whilst the search for the ideal animal model goes on (Kerin, Patwari et al. 2002; Ameye and Young 2006).

Physiological loads on cartilage produce changes in the production of matrix proteins and glycosamino-glycan concentrations are increased following the introduction of moderate exercise (Roos and Dahlberg 2005). However, excessive mechanical loading of in-vitro bovine and human cartilage has been shown to lead to cartilage matrix abnormalities in the superficial zone of hyaline cartilage, changes in aggrecan structure, up-regulation of genes which code for catabolic proteins such as the MMP's and pro-inflammatory cytokines, and an increased vulnerability to the damage caused by pro-inflammatory cytokines (Lee, Fitzgerald et al. 2005; Stevens, Wishnok et al. 2009; Sui, Lee et al. 2009; Rolauffs, Muehleman et al. 2010).

Cartilage is subjected to a range of forces during normal ambulation, and chondrocytes are sensitive to direct mechanical stresses as well as changes in their local environment created by loading. Chondrocytes can be subjected to hydrostatic compression or tensile stresses, which can be generated by both compressive and shearing forces applied to tissues as tissue is deformed under load (figure 2.4). These stresses cause changes in the cytoskeleton of the cell (actin and vimentin in particular), which lead to a cascade of responses resulting in changes in gene expression (Wong, Siegrist et al. 2003; De Croos, Dhaliwal et al. 2006; Blain 2007).

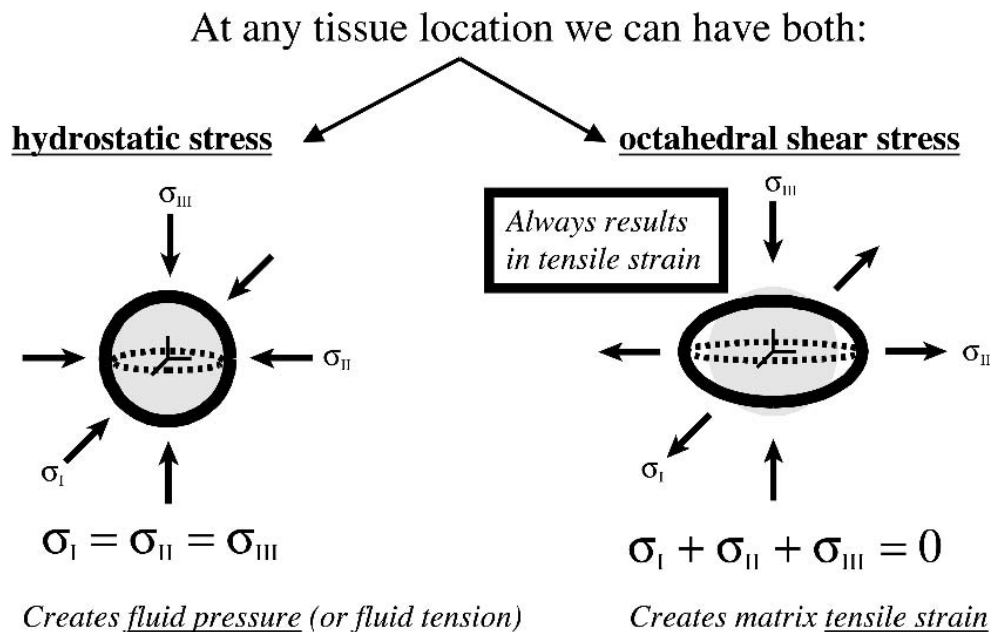


Figure 2.4. Forces applied to chondrocytes can be described in terms of hydrostatic stress or shear stress. From (Carter, Beaupre et al. 2004).

However, changes in the extracellular matrix also stimulate changes, in particular the movement of fluid around the cells. As cartilage is compressed, the local interstitial pressures overcome the osmotic and chemical pressures keeping water in the matrix, and water is displaced, both within the matrix and into the joint. These water flows create fluid shearing around the cells, which are detected by cell surface receptors either directly or via changes in the local ionic gradient, also leading to a change in gene expression (Jin, Sah et al. 2000; Yeh, Chang et al. 2009; Zhu, Wang et al. 2010; Degala, Williams et al. 2012).

Therefore cartilage breakdown in OA is a biologically active process that is stimulated by the mechanical environment (Guilak, Meyer et al. 1994; Kerin, Patwari et al. 2002; Lee, Fitzgerald et al. 2005). The importance of mechanical loading on the initiation and progression of OA was summed up in a recent review by Andriacchi, who concluded that whilst healthy cartilage appears to respond

positively to normal load, abnormal cartilage responds negatively to increasing load, as a combination of mechanical and biological processes lead to cartilage loss and the typical features of OA of a joint (figure 2.5) (Roos and Dahlberg 2005; Andriacchi, Koo et al. 2009; Stevens, Wishnok et al. 2009).

The change to from normal 'homeostatic' cartilage to abnormal cartilage may also be mediated by abnormal load or biological change, as previously suggested by Dye with his theory on the 'envelope of function' of cartilage (Dye 2005).

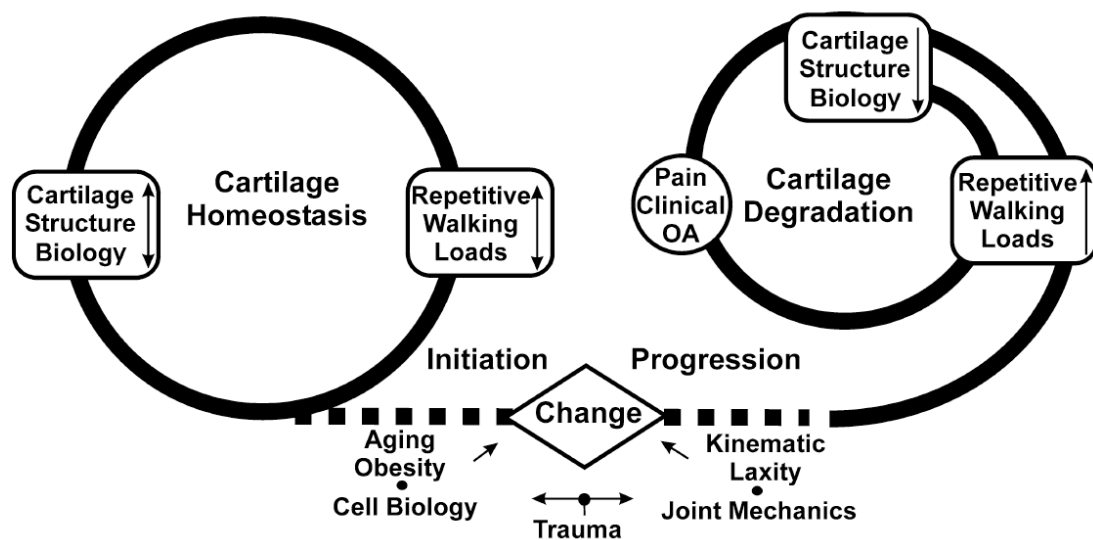


Figure 2.5. Schematic of the relationship between mechanical loading and cartilage homeostasis, as proposed by Andriacchi. Whereas normal cartilage responds positively to moderate load, abnormal cartilage responds increasing poorly to load. Disease initiation could be related to trauma, abnormal biology or abnormal loading, or a combination of factors. From (Andriacchi, Koo et al. 2009)

### 2.1.5 Risk factors for OA development and progression

Whilst basic scientists continue to work on the patho-mechanics and patho-physiology of OA, epidemiologists and clinicians have attempted to elucidate the factors that lead to the initiation and progression of OA. Initiation and progression are thought of as separate processes with separate risk factors, although this is undoubtedly complicated by challenges in defining both the presence and progression of disease.

The factors that have been found to lead to an increased risk of OA initiation were summarised in a recent meta-analysis by Blagojevic et al from the AR(UK) National Primary Care Centre at Keele (Blagojevic, Jinks et al. 2010). The factors they identified were: BMI>25 (Odds Ratio=2.96); Previous knee injury (not defined further, OR=3.86); Smoking (OR 0.87 however this was not present in cohort studies only where OR was 0.97); Female sex (OR 1.84); Heberden's nodes/hand OA (OR 1.49); Age (OR not available, but the oldest of three age groups in the Chingford study had an OR for developing OA of 2.49); Physical occupation (involving lifting or knee bending but not walking alone); Exercise & sports; Increased bone mineral density, previous menisectomy, anxiety and depression, and a shorter index finger than ring finger (Hart, Doyle et al. 1999; Blagojevic, Jinks et al. 2010).

The influence of BMI is clearly important, with numerous studies identifying a clear link between a high BMI and OA (Liu, Balkwill et al. 2007; Reijman, Pols et al. 2007; Niu, Zhang et al. 2009; Toivanen, Heliovaara et al. 2010). Age is also a key factor, and as cartilage changes in mechanical properties and composition with age it is



thought that this leads to an increased susceptibility to load (Kempson 1982; Verzijl, DeGroot et al. 2000).

Genetics certainly play a part in determining susceptibility to disease, although the relationship is complex and multi-factorial. Significant family clustering in longitudinal and twin studies has been established, although it is likely that numerous genetic loci are involved with complex interactions (Spector, Cicuttini et al. 1996; Felson, Couropmitree et al. 1998; Spector and MacGregor 2004; Valdes, McWilliams et al. 2010).

The relationship between joint alignment and the development of OA is important but controversial at present. The presence of a varus knee was not associated with incident disease in the Framingham study based on a case-control study design, but was associated with incident OA in the only cohort study in the literature to date (Brouwer, van Tol et al. 2007; Hunter, Niu et al. 2007; Tanamas, Hanna et al. 2009).

Joint alignment certainly has a major influence on the progression of established disease. In the 'MAK' study, Sharma et al followed up 237 patients over an 18 month period and found that varus mal-alignment increased the risk of medial compartment disease progression (radiographic and clinical) 4-fold and valgus alignment had the same effect in the lateral compartment, with an OR of 4.9 (Sharma, Song et al. 2001). Their findings have since been corroborated by a systemic review comprising 14 studies (8 rated as 'high quality')(Tanamas, Hanna et al. 2009). Varus or valgus alignment also affects the chance of medial or lateral patella-femoral degeneration (Cahue, Dunlop et al. 2004).

The influence of alignment is a mechanical effect, as varus mal-alignment increases medial compartment load and valgus alignment increases lateral compartment load (Hurwitz, Ryals et al. 2002; Foroughi, Smith et al. 2010; Moyer, Birmingham et al. 2010).

The presence of a varus thrust (a dynamic increase in varus angle during gait) was also found to relate to a significantly increased risk of progression by Chang et al, over and above the effect of mal-alignment (Chang, Hayes et al. 2004). Miyazaki, Wada et al found that medial compartment loading as measured by gait analysis was strongly associated with tibio-femoral disease progression in their longitudinal study, and was substantially more predictive of disease progression than static alignment, pain or radiographic status (Miyazaki, Wada et al. 2002). This paper will be discussed further in section 2.3.

Based on the studies described above, a modification of figures 2.2 and 2.5 could be proposed, in which the initiation of OA is described as an interaction between biological and mechanical factors in all cases, as described in figure 2.6. Although similar factors are likely to relate to disease progression, the joint is already damaged and is susceptible by definition. Therefore mechanical loading appears to be the major factor in determining the rate of disease progression.

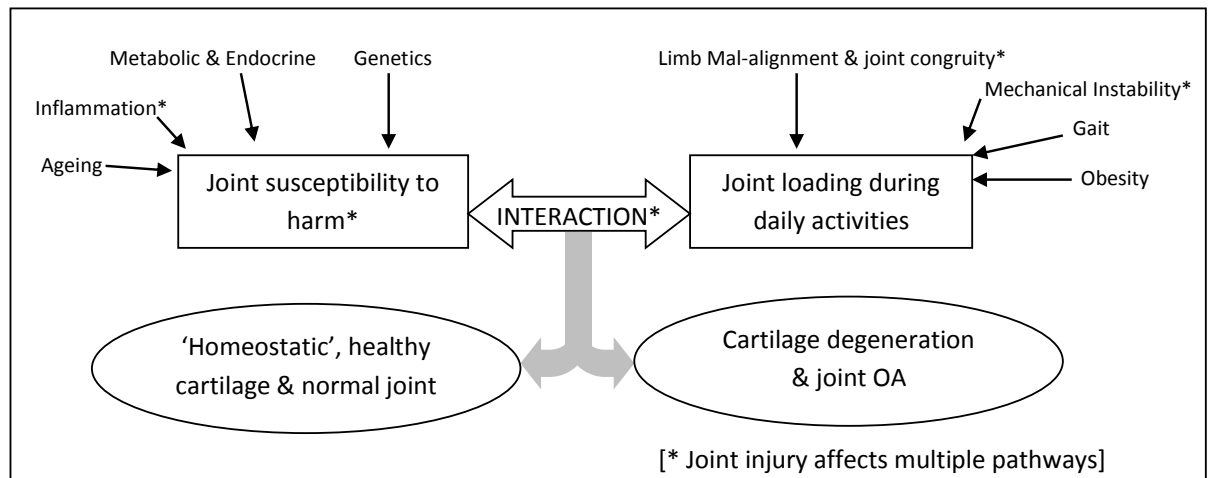


Figure 2.6. Synthesis of figures 2.2 and 2.5 plus above text to emphasise the theory that OA development and progression relates to the interaction between the condition of the cartilage, its susceptibility to load, and the loading applied to it.

#### 2.1.6 Classification of OA

OA has been as difficult a condition to classify as it has to define. The American Rheumatism Association classified OA into 'primary or 'idiopathic' and 'secondary' disease, although the accuracy and clinical utility of this has been questioned (Altman, Asch et al. 1986; McAlindon and Dieppe 1989; Dieppe and Lohmander 2005). Causes for secondary OA included post-trauma, congenital or developmental disease, calcium deposition disease, other joint disease and other diseases such and hypothyroidism (Altman, Asch et al. 1986).

OA of the knee can also be classified according to the presence of disease in the medial, lateral or patella-femoral articulations (figure 2.7). These can occur either separately or in combination and the distinction is important in planning both conservative and surgical treatment (Ledingham, Regan et al. 1993; Lafeber, Intema

et al. 2006; Griffin, Rowden et al. 2007; Duncan, Peat et al. 2008; Zhang, Moskowitz et al. 2008). Patello-femoral disease is more commonly seen on x-rays than medial or lateral compartment OA but may not always be as symptomatic (Ledingham, Regan et al. 1993; Duncan, Peat et al. 2008).

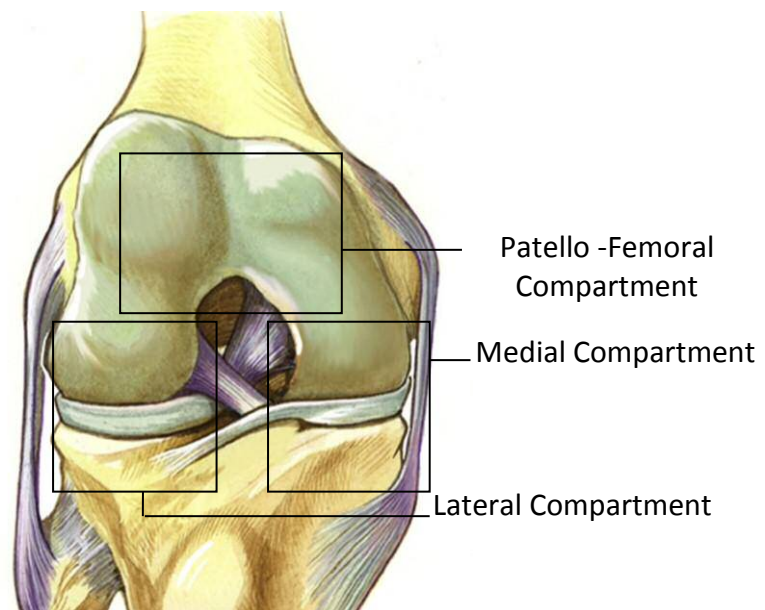


Figure 2.7: Compartments of the knee (the patella has been removed).

Finally, many authors stratify the disease into grades of severity based on the appearance on x-ray. The system devised by Kellgren and Lawrence in the 1950's (figure 2.8) remains the most popular and is used widely in the literature (Kellgren and Lawrence 1957; Kellgren, Jeffrey et al. 1963). The classification is useful as an objective marker of disease severity, uses relatively little radiation, is cheap and quick to perform for research studies and has reasonable intra-observer reliability (Kessler, Guenther et al. 1998; Szebenyi, Hollander et al. 2006). Different authors do not always apply the same criteria and the x-ray findings do not consistently correlate with pain, function, MRI or operative findings (Lawrence, Bremner et al.

1966; Williams, Sharma et al. 2005; Kijowski, Blankenbaker et al. 2006; Szebenyi, Hollander et al. 2006; Schiphof, Boers et al. 2008; Neogi, Felson et al. 2009). However, it is the most widely used tool at present, is well understood by researchers worldwide, and good reliability has been quoted in series in which the authors were experienced in its use. (Boegard, Rudling et al. 1998; Szebenyi, Hollander et al. 2006; Neogi, Felson et al. 2009).

Most other radiographic classifications have failed to improve on the Kellgren-Lawrence Classification in terms of both reliability and relationship to symptoms, although a joint space width classification has been shown to perform well recently (Gossec, Jordan et al. 2008).

For the knee, the Kellgren-Lawrence classification as quoted verbatim from 'The Atlas of Standard Radiographs' (Kellgren, Jeffrey et al. 1963) is: **Grade 0** no disease. **Grade 1**: Doubtful narrowing of joint space and possible osteophytic lipping. **Grade 2**: Definite osteophytes and possible narrowing of joint space. **Grade 3**: Moderate multiple osteophytes, definite narrowing of joint space and some sclerosis and possible deformity of bone ends. **Grade 4**: Large osteophytes, marked narrowing of joint space, severe sclerosis and definite deformity of bone ends.



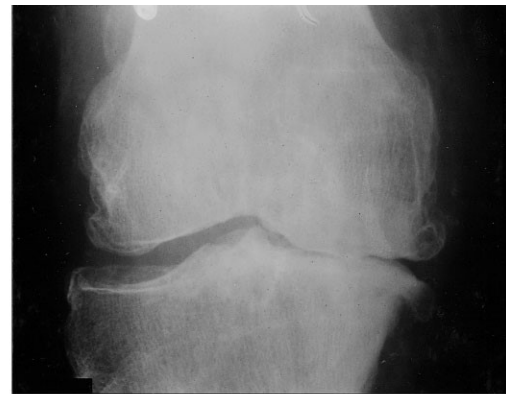
Grade 1



Grade 2



Grade 3



Grade 4

Figure 2.8: The Kellgren- Lawrence Classification. From: (Kellgren, Jeffrey et al. 1963).

### 2.1.7 Measuring Pain and Function in OA

Attempts to define OA by the pain it causes and the effect it has on physical function and disability has thrown up many questions and challenges. As the definition in section 2.1.2 makes clear, OA does not necessarily cause a constant pain but is characterised by pain with use and a restriction in function. There are a number of approaches to measure the clinical severity of the disease and its impact on the patient, most of which have the aim of converting a set of feelings,

experiences and emotions into a figure that can be examined quantitatively for research.

These approaches can be divided into generic health questionnaires, or disease specific instruments, which may be objective (i.e. measured or assessed by an observer, researcher or clinician) or subjective (i.e. reported by the patient). Generic (or global) health questionnaires are designed to examine the overall health or wellbeing of a person. Their benefits are that they measure the influence of a condition against the overall well-being of a person, giving a more global assessment and some scores have also been designed to allow health economic evaluations as well. Examples of commonly used global scores are Short Form 36 (SF36), the Health Assessment Questionnaire (HAQ) and the EuroQol 5 Dimension (EQ5D). Some of the most commonly used scores have been studied extensively, meaning that their characteristics are well understood, including in patients with knee OA (Fransen and Edmonds 1999; Lingard, Katz et al. 2001; Salaffi, Carotti et al. 2005; Escobar, Quintana et al. 2007; Xie, Pullenayegum et al. 2010). The difficulty with generic scores is that they may easily be confounded by other health problems and because of their broad nature, they may provide only a limited assessment of the problems that a specific disease causes (for example, the limitation of certain functions in knee OA) (Hawker, Melfi et al. 1995; Fransen and Edmonds 1999; Lingard, Katz et al. 2001).

The most widely used objective measure of knee function is the American Knee Society Score (Insall, Dorr et al. 1989). Its use is dependent upon the person

measuring the score and the findings are have been reported to be less reliable than scores such as WOMAC or the Oxford Knee scores, although it has performed well in some validation studies (Liow, Walker et al. 2000; Lingard, Katz et al. 2001). The main difference between objective and subjective outcome scores is the aspect of health that they measure, as objective scores tend to measure specific clinical findings (such as range of motion and stability) and focus less on the effect of a problem on a patients function or quality of life (Miner, Lingard et al. 2003; Pollard and Johnston 2006; Johnson, Archibald et al. 2007; Bream, Charman et al. 2010).

The most commonly used subjective score for knee OA in the literature is the WOMAC score (Bellamy, Buchanan et al. 1988). It was developed specifically for knee or hip OA, has been extensively studied and the relationship between the WOMAC score and overall health status is well understood (Hawker, Melfi et al. 1995; Escobar, Quintana et al. 2007; Xie, Pullenayegum et al. 2010). It remains one of the highest performing scores in use for validity, responsiveness and reliability (Bellamy, Buchanan et al. 1988; Ryser, Wright et al. 1999; Jinks, Jordan et al. 2002; Salaffi, Carotti et al. 2005; Chesworth, Mahomed et al. 2008). There is some redundancy in the questions (some questions test the same thing), although the numerous modified versions have not been accepted widely (Ryser, Wright et al. 1999; Davis, Badley et al. 2003; Whitehouse, Lingard et al. 2003).

The score is has 23 questions, each of which is rated on a 5 point Likert scale. It is divided into 3 components, pain (5 questions), stiffness (2 questions) and function (17 questions). Although the pain component has been criticised as an individual



item, it remains one of the few widely used pain scores for OA at present (Creamer, Lethbridge-Cejku et al. 1999; Jinks, Jordan et al. 2002; Stratford, Kennedy et al. 2007).

The Oxford knee score was devised in the early 1990's as a simple to administer score for use before and after knee replacement. It is also widely used in the literature, particularly in the orthopaedic community, and has been used as a monitoring tool for total knee replacements in the New Zealand, Australian and UK National Joint Registries (Dawson, Fitzpatrick et al. 1998). Most of the 12 questions relate to physical function rather than pain and this makes it a simpler tool for patients, however the score cannot be broken down into domains to distinguish between pain and physical function. The score has again performed well in validation studies by both the originators as well as independent groups and although some redundancies have been detected it is a widely used tool which is relatively simple to administer (Dawson, Fitzpatrick et al. 1998; Garratt, Brealey et al. 2004; Whitehouse, Blom et al. 2005; Conaghan, Emerton et al. 2007).

#### 2.1.8 Multiple Joint OA

Joint pain and OA frequently affects more than one joint. There have been three UK studies looking at the prevalence of joint pain in community populations aged over 50, 55 or 65 (Dawson, Linsell et al. 2004; Keenan, Tennant et al. 2006; Peat, Thomas et al. 2006). The three populations studied were Oxfordshire, North Yorkshire and Staffordshire. Their findings are described in table 2.1. All three studies found that

overall disability increased with increasing numbers of affected joint sites, Dawson et al and Peat et al reported increasing overall pain with increasing numbers of affected joint sites, and Peat et al reported greater levels of joint pain in each individual joint in those with multiple joint pain. The latter finding could be due to patients with multiple joint pain having more severe disease, or it could be related to changes with central pain perception in those with more than one source of extremity pain (Dieppe and Lohmander 2005).

All of these surveys found that the most likely 'second' joint to be affected was the opposite side. In other words, bilateral disease is common. A recent study used a longitudinal cohort of patients defined as 'at increased risk' of OA (obesity, female gender, age, previous joint injury) to demonstrate that the development of bilateral OA, as opposed to unilateral disease, was an independent risk factor for poor physical function (as measured with the WOMAC physical function sub-scale) (White, Zhang et al. 2010). Bilateral disease is especially common in patients with severe disease, as a radiological study in the 1980's demonstrated that 87% of those awaiting knee replacements had radiographic disease in the contra-lateral joint (Gunther, Sturmer et al. 1998).

However, there are very few studies that have attempted to document the development of bilateral disease over time. Only one study has reported this clearly, finding that 34% of those with unilateral knee OA developed bilateral disease over a 2 year period, raising the question as to whether bilateral disease is a universal problem in patients with knee OA (Spector, Hart et al. 1994). There is

therefore significant value in examining the development of unilateral and bilateral knee OA individually over a prolonged period of time.

Study	Survey Region & age	Number surveyed	Prevalence of joint pain	Proportion with single joint/multi-joint pain	Joints covered in survey
Dawson, Linsell et al 2004	Oxfordshire Age >65	5039	40.7%	48/52%	Hip & knee only
Keenan, Tennant et al 2006	North Yorkshire Age>55	18227	39.1%	12.5/87.5%	Neck, shoulder, elbow, hands, back, hip knee, ankle, foot
Peat, Thomas et al 2006	Staffordshire Age>50	3883	100%*	26/74%	Hip, Knee Foot & Ankle

Table 2.1. Overview of UK community-based surveys of multiple joint pain. \*Peat et al sent surveys to patients who had previously reported joint pain in a preceding community health survey, the proportion of joint pain in that survey was 35%.

Data on the relationship between hip and knee OA is also limited. A longitudinal study on females in London (the 'Chingford study') demonstrated that a patient was more likely to have progression of pre-existing knee OA if they also had progression of their disease in the hip or hand during the same time period. Proportions of patients with unilateral and bilateral disease were not reported (Hassett, Hart et al. 2006). The implication was that this study demonstrated evidence for a genetic basis of the disease, although other factors could also have been implicated such as variation in BMI, mass distribution, anatomical differences or changes in gait.

Shakoor et al reported on a cohort of patients over a 20 year period who had undergone a replacement of a single joint (hip or knee) and examined the need for

joint replacement in subsequent joints (Shakoor, Block et al. 2002). The most common site for a second joint was the opposite 'cognate' joint (i.e. the other knee after a previous knee replacement and the other hip after a previous hip replacement). However, they also found an association between an affected hip and the opposite knee, as described in figure 2.9.

The findings of this study correlated with the findings of a gait analysis study by the same team, which found increased loading in the contra-lateral (as compared to the ipsi-lateral) knee in patients with unilateral hip OA – implying that altered mechanics were the reason for the distribution of late-stage OA that they had observed (Shakoor, Hurwitz et al. 2003) .

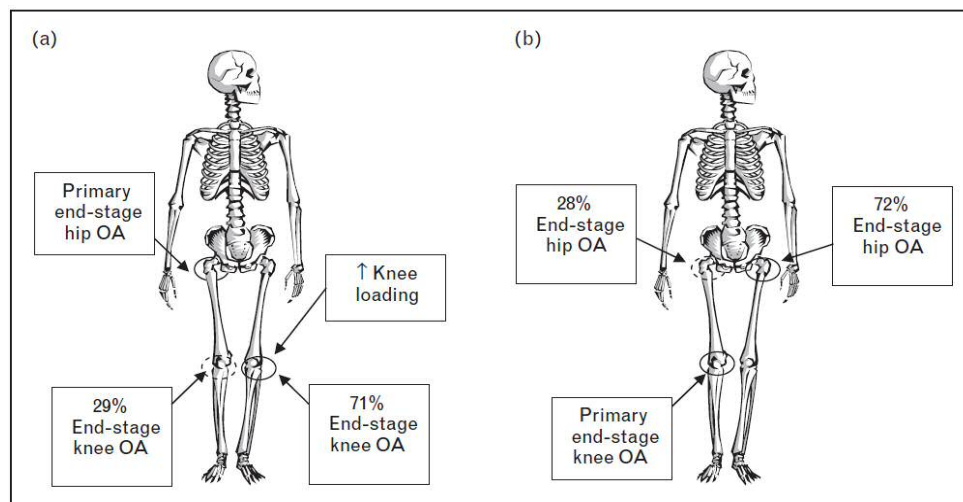


Figure 2.9 Summary of results from Shakoor et al 2002. The definition of 'end-stage OA' was a joint replacement. Image taken from (Block and Shakoor 2010).

It is clear that there is a shortage of longitudinal data on the development of multi-joint OA. In the second chapter of this thesis, data is presented to begin to address that need by examining the development of unilateral and bilateral knee OA over a 12 year period. If the other joints are 'at risk' of disease, it raises the question of

whether treatments to protect those joints should be considered. This again is a question that will be addressed throughout the thesis.

Given the prevalence of multi-joint disease, it is unsurprising that after a knee replacement, there is a risk that the other knee will come to need replacing in time. This was described by Ritter in 1994, who found that in those with OA in the other knee at the time of surgery (87% of the total in their study of 113 patients), 24% would end up having a contralateral knee replacement over 7 years and 37% over 10years (Ritter, Carr et al. 1994). This finding echoed that of McMahon and Block who identified a 37% risk of contralateral knee replacement over a 10year time period (McMahon and Block 2003).

A study of 508 patients over a 2 year period, all with the same implant in Bristol found an improvement in pain in the contralateral knee over the 2 year study period (Smith, Wylde et al. 2013). Of the 1198 patients starting the study, 56 (5%) has simultaneous bilateral TKR, 199 (17%) had had previous surgery on the other knee, 100 had missing data, and 142 (13%) had contra-lateral side surgery within 1 year. 224 were lost to follow up by 2 years and 51 (9% of the available cohort) underwent a subsequent joint replacement between 12 and 24 months. Recovery of function in terms of patient reported outcome scores peaks at 2 years, with a gradual deterioration in scores after that, so it may be that further degeneration and deterioration of the other knee occurs as a later phenomenon, although this remains poorly understood (Williams, Blakey et al. 2013).

### 2.1.8 Treatments for OA

Throughout history, numerous treatments have been attempted for OA. Yet the only treatments to date that have succeeded in altering the natural course of the disease have been surgical - joint replacement and osteotomy.

In order to describe treatments, the most logical approach is to discuss conservative and surgical treatments in turn, using two major current guidelines as a structure. These are the National Institute of Health and Clinical Excellence (NICE) OA Guidelines and the OA Research Society International (OARSI) Recommendations (Conaghan, Dickson et al. 2008; Zhang, Moskowitz et al. 2008). Both were based on formal structured reviews of the literature supplemented with a consensus of expert opinion where evidence was poor, although the guidance development group for the NICE document lacked an orthopaedic member of the panel, which may have weighted the strength of their recommendations.

### 2.1.9 Conservative Treatments

The simplest form of treatment is the provision of lifestyle advice, such as activity modification, appropriate exercises, weight loss and walking aids such as canes (if tolerated) (Conaghan, Dickson et al. 2008; Zhang, Doherty et al. 2009).

Pain relief in OA can be achieved with regular use of paracetamol, intermittent use of Non-Steroidal Anti-inflammatory drugs (NSAIDs) and possibly weak opioids (Bjordal, Ljunggren et al. 2004; Pincus, Koch et al. 2004; Zhang, Jones et al. 2004;

Conaghan, Dickson et al. 2008; Zhang, Moskowitz et al. 2008). Although they are an effective analgesic, NSAIDs have a significant risk profile and the use of gastro-protective agents or selective use of new COX-2 inhibitors remains controversial (Ofman, MacLean et al. 2002; Scheiman, Yeomans et al. 2006).

Corticosteroid injections may be used to reduce the inflammatory component of the pain and although their use can be effective, the effects often do not last beyond a few weeks (Bellamy, Campbell et al. 2006). Hyaluronic acid may be considered for injection although its benefit remains under debate (Bannuru, Natov et al. 2009; Chevalier, Jerosch et al. 2010; Jorgensen, Stengaard-Pedersen et al. 2010).

Two relatively new conservative treatments, valgus knee braces and wedged footwear, have received considerable attention in the recent literature. Shoes or insoles, which alter the weight distribution to the knee by moving the centre of pressure of the foot, have been shown to reduce the 'adduction moment' (an indicator of medial compartment load) in patients with and without OA (Shimada, Kobayashi et al. 2006; Hinman, Payne et al. 2008). This effect is not present in severe OA (KL 3-4), possibly due to the presence of significant mal-alignment in these groups (Shimada, Kobayashi et al. 2006). Numerous small RCTs or non-randomised trials have shown clinical benefit with wedged shoes or insole wedges (Toda and Segal 2002; Hinman, Payne et al. 2008; Barrios, Crenshaw et al. 2009). They were not successful at improving function or reducing pain in the two largest RCTs in the literature although there are methodological reasons to explain the

failure of both studies to identify an effect (use of heel wedges only in Pham et al, which are known to be mechanically less effective, and the inclusion of large numbers with severe OA in Baker et al)(Pham, Maillefert et al. 2004; Baker, Goggins et al. 2007).

Valgus knee braces have a smaller effect on the adduction moment, may be less acceptable to patients and a small clinical benefit has been reported. Their principle effect may be due to a reduction in co-contraction of muscles around the knee, reducing joint contact forces, which will be discussed later in this chapter (Brouwer, van Raaij et al. 2006; Ramsey, Briem et al. 2007).

#### 2.1.10 Surgical Treatments for OA

The simplest surgical treatment for OA is an arthroscopy and debridement. Although theoretically beneficial, in well controlled randomised studies debridement has not been shown to improve pain or function in an osteoarthritic population (Moseley, O'Malley et al. 2002; Kirkley, Birmingham et al. 2008; Reichenbach, Rutjes et al. 2010). The exception to this is those patients who have mechanical symptoms such as locking, where a meniscal tear or chondral fragment could be debrided to improve function (Dearing and Nutton 2008)

The major change to surgical treatment of OA in the last century has been joint replacement. Following Charnley's successes with total hip replacement in the 50's and 60's, numerous attempts were made to replicate that success with the more



complex knee articulation. The first commercially successful knee replacement with acceptably long lifespan were developed in the 70's, most notably by Ranawat and Insall, who designed the Total Condylar Replacement (figure 2.10) (Insall, Ranawat et al. 1976). An evolved version of this design (the 'PFC Sigma', DePuy, Leeds UK) remains the best selling implant in use today (NJR 2010).

Modern knee replacements have received relatively minor design changes since and can broadly be divided into those that retain the posterior cruciate ligament, and those that allow it to be sacrificed, in favour of a mechanism in the implant design which replaces its function (Sierra and Berry 2008). Despite theoretical and biomechanical differences between the two, there remains little evidence of a difference in terms of patient preference or functional recovery whichever design is used (Nelissen and Hogendoorn 2001; Cates, Komistek et al. 2008; Chaudhary, Beaupre et al. 2008; Kim, Choi et al. 2009).

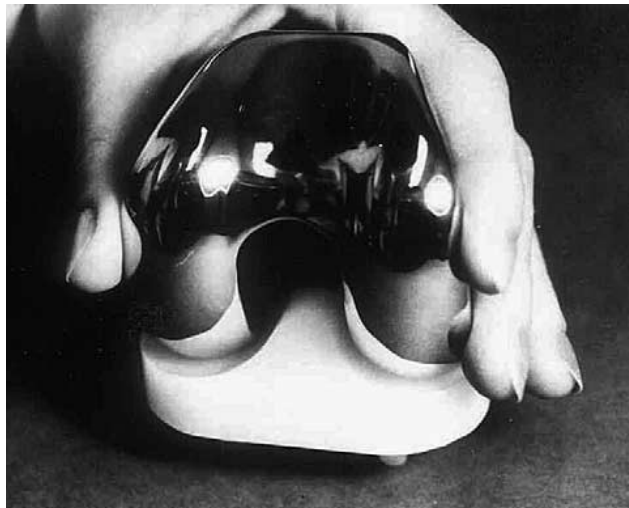


Figure 2.10. The total condylar knee replacement. This was one of the fore-runners of contemporary knee replacements (Robinson 2005).

Eighty to ninety percent of patients who undergo knee replacement are very pleased or pleased with the outcome, and although relief of pain and recovery of function are usually not complete, they are often profoundly improved (Dieppe, Basler et al. 1999; Robertsson, Dunbar et al. 2000; Wylde, Dieppe et al. 2007). The best performing implants in the Swedish and Australian national joint registries would be expected to have a survivorship of at least 95% at 8-10years (AOA 2009; SKAR 2009).

Functional recovery is variable, and often not as good following revision total knee replacement as it is following primary replacement, meaning that optimising function throughout life requires preservation of the primary joint as well as delaying the time until joint replacement is required (Saleh, Dykes et al. 2002; Ghomrawi, Kane et al. 2009).

Unicompartmental knee replacements are becoming increasingly popular. Only the affected compartment is replaced, sparing the cruciate mechanism. This improves functional outcome and in theory makes the first revision significantly more conservative (Saldanha, Keys et al. 2007; Newman, Pydisetty et al. 2009). Their limitation is that many patients have multi-compartment disease and there has been some controversy over their survival compared to total replacement, as well as the survival of the subsequent revision (Johnson, Jones et al. 2007; Pearse, Hooper et al. 2010; Price and Svard 2010).

High tibial osteotomy is a technique which involves realigning the tibia to restore the mechanical axis in patients with medial compartment OA. The effect of this is to

reduce the moment around the knee, to offload the medial compartment (Prodromos, Andriacchi et al. 1985; Birmingham, Giffin et al. 2009). This is an effective, bone sparing technique which reduces pain and improves function. However, its longevity is a problem, as further medial compartment degeneration with time often occurs, although results vary and are dependent on the patient population (Akizuki, Shibakawa et al. 2008; van Raaij, Reijman et al. 2008). Most patients can be counselled to expect 10 to 15 years of benefit before needing a total knee replacement, which should perform as well as a standard 'primary' total knee (Akizuki, Shibakawa et al. 2008; van Raaij, Reijman et al. 2008; van Raaij, Reijman et al. 2009). It is therefore typically used as a technique in young patients, to delay the need for arthroplasty and hence the need for future multiple revision.

Knee OA is a common condition that causes significant functional impairment. Increasing use of both conservative and surgical techniques have benefited patients, although there have been few papers that have looked beyond a single affected joint to examine the effects of treatments on the other joints. The few that have will be discussed in section 2.4, but given the functional impact of multiple joint disease on this population, there is clearly more work to be done in this field. This thesis aims to build on the present knowledge base about multiple joint disease, the mechanical factors that influence its development and the influence of surgical treatment on the biomechanics of the other weight-bearing joints.

## 2.2 Overview of Gait Analysis

### 2.2.1 Principles of modern gait analysis

Modern gait analysis is a technique capable of giving detailed and useful information about one of the most basic functions of human activity – walking. Forms of gait analysis have been in clinical use for many centuries. The Greek philosopher Aristotle wrote a book on animal gait in the year 350 B.C. and clinicians have been taught for many years the importance of an examination of gait in assessing pathology (Aristotle 350BC).

Human locomotion is a complex movement that takes a growing child almost a full decade to develop fully (Chester, Tingley et al. 2006). As such, it is impossible to register and record its nuances with just the naked eye. Numerous techniques have been developed to understand it in more detail, ranging from the pioneering stop motion photography of Eadweard Muybridge (1830-1904), to the modern capture techniques in use today (figure 2.11).



Figure 2.11, part of: 'Male walking, taking off hat'. From the National Archives ([www.nationalarchives.gov.uk](http://www.nationalarchives.gov.uk)).

There are various ways of collecting data during motion. Possibly the most common is the use of simple video recording, either for clinical measures, athletic training or even in sports shops to determine footwear choice. However, the utility of video recording is limited to descriptive information as images are by their nature two dimensional and clear definition of landmarks and their movement is difficult.

Dynamic MRI scanners and video fluoroscopy can both collect detailed measurements during motion in a confined space, limiting the studies to certain movements such as stepping or lunging, rather than walking (Williams and Logan 2004). Mobile fluoroscopy scanners may allow assessments during gait, but remain limited by radiation exposure, time factors and a narrow field of view, meaning that only certain information is obtained (albeit in considerable detail) (Kozanek, Hosseini et al. 2009). Neither technique is able to calculate kinetics (the forces, moments and power involved in locomotion). In order to do that a measure of motion is required that assesses the skeleton on a larger scale.

Motion can also be measured with much greater freedom using accelerometers. These are electrical sensors which record acceleration which can therefore be related to changes in position. As such, they are relatively cheap to use, provide useful data about motion and don't require a patient to be limited to a laboratory (Kavanagh and Menz 2008). However, the data cannot be related to fixed co-ordinates outside of the subject (i.e. in a room) and as such cannot be used to assess forces or loads.

The technique used by most motion analysis laboratories for recording natural gait involves the use of reflective markers (retro-reflective markers) attached to various parts of the body. Subjects walk either on a treadmill or a flat surface and data is collected about the position of the markers. This technique is sometimes referred to as optico-electronic gait analysis or stereophotogrammetry, and it has many advantages in studying gait (Cappozzo, Della Croce et al. 2005).

Natural movements are recorded without significantly impeding the subject and can be used to assess the whole skeleton if required. There is no radiation and the procedure is very low risk. Because of this there is no time limit, so repeated measures can be taken, both during the analysis session and at separate visits. Using force plates built in to the floor, the ground reaction force can be measured and resultant joint forces can be calculated, making the technique a powerful method for assessing kinetics as well as skeletal kinematics (movements) during natural movements.

Optico-electronic gait analysis (referred to as 'gait analysis' for the remainder of the thesis) does have drawbacks. It is very time consuming and requires a lot of staff time for every assessment. Patients are inconvenienced by having to travel to specialist laboratories for assessments. Errors are present, especially from skin movement artefact (discussed in section 2.2.5) – making it useful for analysis of major joints but less so for small joints of the feet or hands.

However, despite these drawbacks it remains the current gold standard method for measuring in-vivo motion and loading of major joints during normal gait, and has

hugely advanced our understanding of the normal and pathological processes that affect or skeleton throughout our daily lives.

In order to interpret gait data, it is important to understand the method by which it is collected and the potential sources of error. By understanding these aspects, useful data can be analysed whilst the more error-prone measurements can be discarded. The remainder of this section aims to explain the technique, alongside its strengths and weaknesses in more detail.

### 2.2.2 Data collection in gait analysis

To understand the gait analysis literature, it is helpful to describe the basic procedures and definitions involved at this stage. More detail will be given on the specific study protocols in the methodology chapters of the thesis.

Prior to any gait analysis, the patient is asked to change into appropriate clothing. Footwear is usually removed, and basic anthropological data is measured, for example height, weight, limb length, epicondylar and malleolar width.

The kinematic data in gait analysis is collected using retro-reflective skin markers. These are usually cork or plastic spheres covered with highly reflective material (figure 2.12). The markers are placed on pre-described landmarks around the sections of the body (the marker sets used for this study are described in appendix 2). In most parts of the body they are attached using sticky tape or an adhesive pad,

although straps or thermoplastic moulds are used by some centres to attach clusters to the thigh and lower leg (Garling, Kaptein et al. 2007).

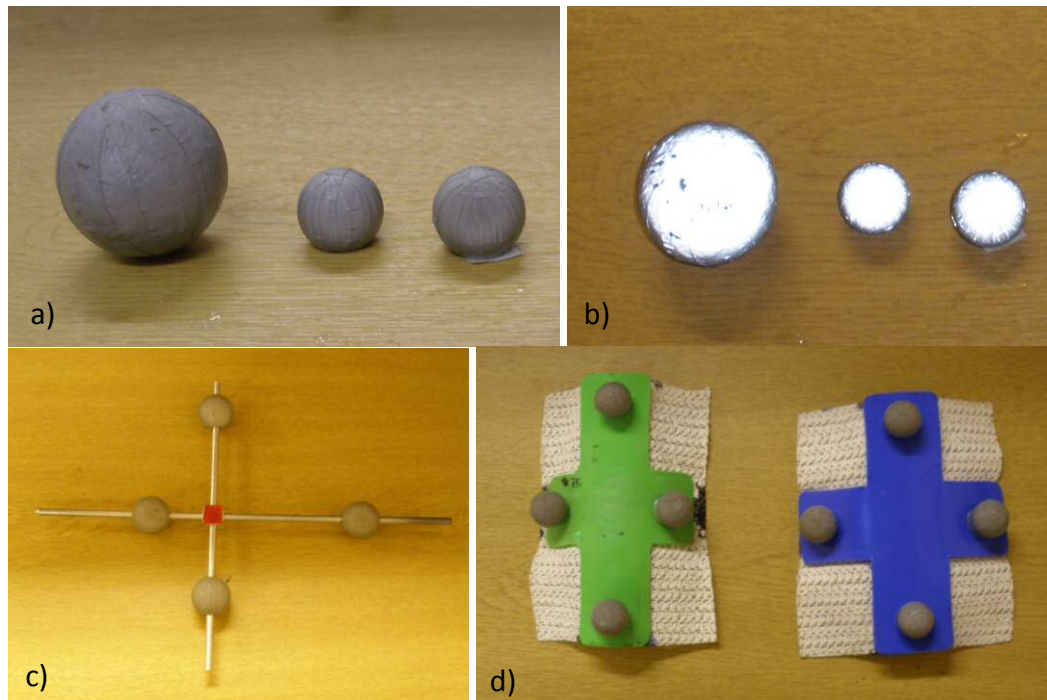


Figure 2.12. Retro-reflective markers. a) The majority of markers are cork spheres of varying diameter. b) The same markers pictured with a flash, demonstrating their highly reflective nature. c) A pointer, which can be used to mark anatomical points whilst being identified by the camera system. d) Marker clusters with non-slip backing, designed to improve tracking and reduce error in tracking the thigh and shank during motion.

Once the equipment has been calibrated, the study starts with an assessment of the patient standing. The positions of the markers are recorded by a number of infra-red cameras around a room (typically 5-10 cameras) which send out, and receive back an infra-red signal (figure 2.13). Each camera records a 2 dimensional image of the markers and as the position of each camera is known, the 3 dimensional position of each marker in space can be calculated.





Figure 2.13 a) A typical motion capture camera, with a ring of infra-red LED's around an infra-red camera. b) A patient fitted with retro-reflective markers (consent has been obtained to use these images). c) The motion analysis laboratory used in one of the studies (ORLAU, Oswestry). The patient walks along the walkway whilst the 12 cameras simultaneously record motion of the reflective markers.

Participants are asked to perform an activity. Most commonly this involves either walking on a flat surface along a path marked on the floor, or walking on a treadmill whilst the cameras track the positions of the markers.

In many laboratories, force plates are built into the floor (or treadmill) to capture the ground reaction force (figure 2.14). This is the force that the floor exerts on the point of contact of the foot. This (kinetic) information can be combined with the kinematic data to calculate the moments applied to joints ('external' forces, i.e. forces and moments that result from the action of the ground reaction force).



Figure 2.14. Two force plates built into the floor. The surrounding removable boxes and vinyl covering are also pictured, so that when in place the surface of the force plate is continuous with the floor.

The movements are repeated a number of times until an adequate quantity of data has been collected. The raw data is analysed using specially designed software based on well described mathematical algorithms (Kadaba, Ramakrishnan et al. 1990; Davis, Gage et al. 1991). To understand the principles of the analysis, a

description of the principles of documenting position in space and relative motion is required.

### 2.2.3 Conventions in describing position, motion and forces

In order to understand the position of any object in space, it has to be described relative to another object according to a pre-defined system for describing position. For example, if we say 'the hospital is 1 mile north of the city centre' we are describing one place relative to another according to an axis system defined by the Earth's magnetic field.

In gait analysis, the position of an object is determined relative to the room, and the co-ordinate system of the room is called the global co-ordinate system. This is defined by three orthogonal axes, usually referred to as x, y and z. The object itself (such as a limb segment) can also be associated with a co-ordinate axis system – this is called the local co-ordinate system.

The position of an object in a room can be described in terms of translation in each of the axes of the global co-ordinate system and rotation relative to these axes (figure 2.15.a). By convention, positive is usually defined by a 'right hand' convention (figure 2.15.b) and a 'right hand rule' is also typically used to define positive rotation (figure 2.15.c).

Motion is described first in translation and this can be expressed in any order by defining three measures, one for each axis. For example point  $[x=1,y=-1,z=3]$  is the same as  $[y=-1,z=3,x=1]$ .

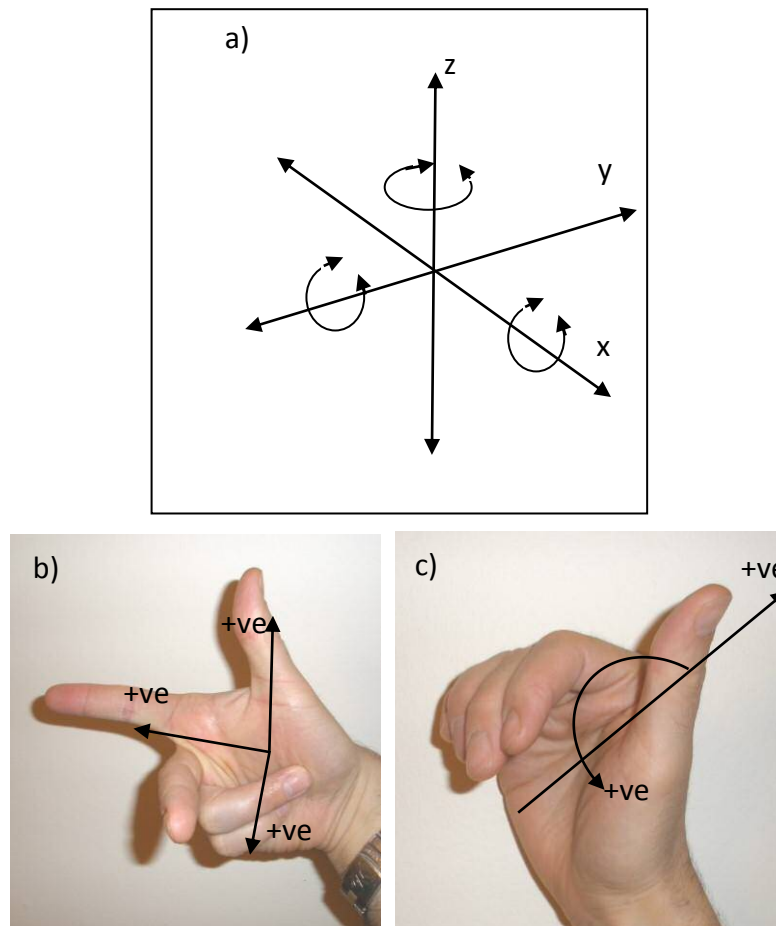


Figure 2.15. a) The position of an object can be described in relation to 3 orthogonal axes (x,y,z in this example). Motion can be described in terms of translations or rotations relative to those axes. Therefore there are 6 degrees of freedom – 3 rotations and 3 translations. b) and c) The 'right hand rule' is typically used to define +ve and –ve by convention – i.e. in b) the direction of the thumb & fingers determines positive directions. In c) the position of the fingers determines +ve rotation with respect to an axis (+ve direction along the thumb).

Rotational alignment and motion of an object in space can be described in many ways, but using Euler or Cardan angles are the commonest ways of doing this. Euler

and Cardan angles describe the three rotations that need to occur for an object to move from one position to another. However, the order in which they are expressed and the axes around which the rotations occur needs to be defined clearly. Euler angles describe the use of 3 rotations to define a change in overall rotation of an object which are typically performed using 3 rotations about 2 axes (such as  $z,x,z'$ ), whereas Cardan angles refer to a sequence of rotations around 3 orthogonal axes (such as  $x,y,z$ ) (Nigg, Cole et al. 2007).

Euler and Cardan angles are dependent on the order in which they are described. This is because the final position of the object that is being moved varies depending upon the order in which the rotations are performed around each axis.

In gait analysis software, a Cardan sequence is typically used, meaning that rotations are described in order around 3 axes. The local orientation of those axes in the limb segments is based around the anatomical position although the method of defining those axes can result in errors (Della Croce, Leardini et al. 2005).

For joint movements, motions can be described around a co-ordinate system known as the 'joint co-ordinate axis system' (figure 2.16) (Grood and Suntay 1983). This system has the benefit of being independent of order – the rotations can be performed in any order as the axis system does not change with each rotation.

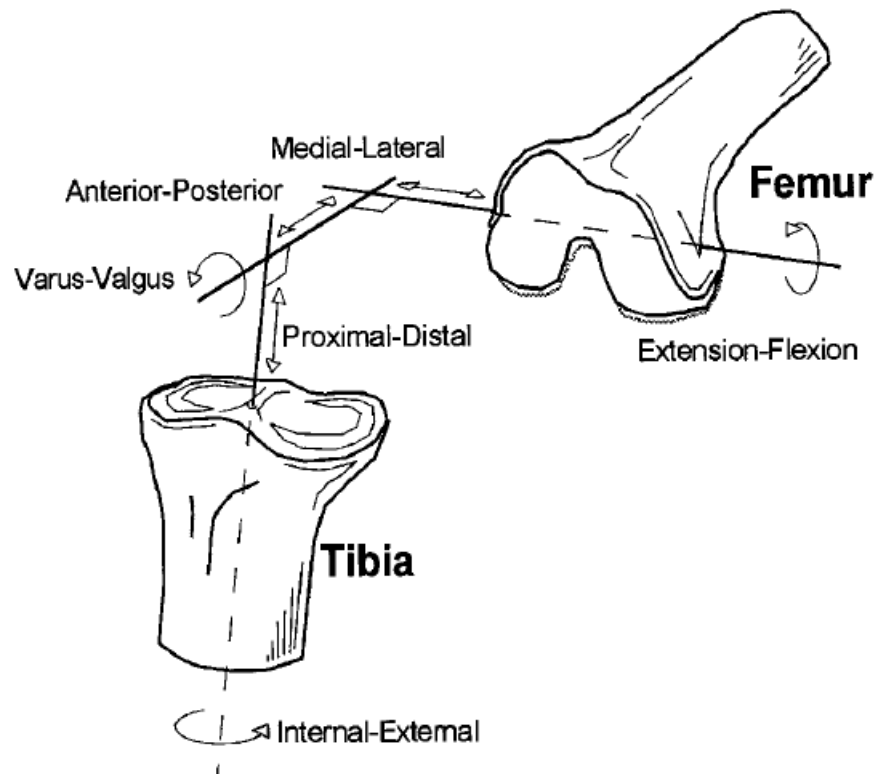


Figure 2.16. The joint co-ordinate system proposed by Grood and Suntay. The varus-valgus axis is a floating axis, and is the cross product of the other two axes (Woo, Debski et al. 1999).

For the knee, the joint co-ordinate axis system is constructed first from the medio-lateral axis of the thigh co-ordinate system based at the distal femur (flexion-extension occurs around this axis) and the long axis of the tibia (rotation occurs around this axis). The cross-product of these two axes (a line that is perpendicular to a plane defined by the two vectors) is the third, 'floating' axis, around which flexion-extension occurs (Grood and Suntay 1983; Lafortune, Cavanagh et al. 1992). A similar result is gained using a Cardan sequence is described in the order: flexion-extension; ab-adduction; internal-external rotation (Grood and Suntay 1983).

#### 2.2.4 Principles of data processing for gait analysis

The reflective markers themselves are spheres and although their size is considered in the calculations, the centre of each marker is identified as the centre of a circle on each individual 2 dimensional camera image. The position of each marker in 3 dimensional space is calculated as a single point corresponding to the centre of the marker. Markers can translate, but not rotate and therefore they have 3 degrees of freedom.

The skeleton is idealised as a series of non-deformable segments, called a 'kinematic chain'. By assuming that bones are non-deformable, classical mechanics can be used to calculate the relative movements of segments, such as the thigh segment (anatomically equivalent to the femur) and the lower leg (equivalent to the tibia and fibula) (figure 2.17) (Cappozzo, Della Croce et al. 2005). The relative movement between 2 segments can be represented by rotations and translations at the joints. The motion of segments can be described in 6 degrees of freedom.

Each segment has a mass of its own, and consequently has inertia when moving which needs to be considered in the calculations. The majority of gait analysis systems use a literature definition of typical segment properties and masses which are then scaled to the subjects body mass (Dempster, Gabel et al. 1959).

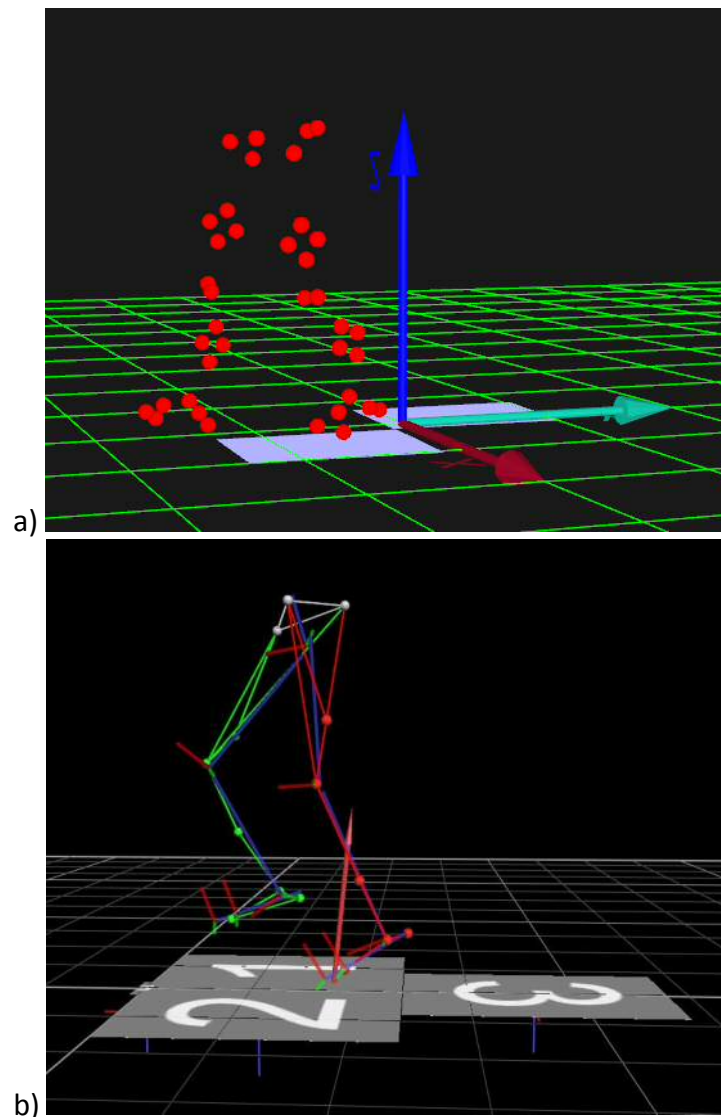


Figure 2.17 Visual outputs from 2 commonly used motion tracking programmes at different stages in processing (Qualisys Track Manager, Qualisys, Sweden and Vicon Nexus, Vicon, Oxford). a) shows the global co-ordinate system and a set of markers. b) shows a further set of markers, this time with the anatomy identified, segments identified from the markers, and a ground reaction vector represented visually.

The first part of the analysis involves associating the known position of the markers to skeletal landmarks and joint centres with the subject standing. Markers are placed over palpable anatomical landmarks and corrections are made to calculate the position of joint centres and segments based on these markers. Joint centres and segments can also be defined using functional, defined movements.



The local co-ordinate system of each segment and the relationship between the markers on the thigh and lower leg is then established. This means that the motion of segments can be tracked using the markers, and the relative motion of segments can be computed – these motions are the kinematic output from gait analysis.

The ground reaction vector is related to the global co-ordinate system, as the force plates are fixed to a consistent position in the room (figure 2.17). Data from the ground reaction force (henceforth known as the GRF) can be related to the kinematic data to calculate moments and powers at joints. This can be done by calculating the distance of the GRF from the joint centre, although this measurement ignores the influence of the inertia of the segments.

A more widely used method of calculating joint moments is done using a technique called ‘inverse dynamics’, which involves resolving the kinematics first, and then calculating the kinetics of each segment & joint in turn, starting at the foot and ankle and working proximally (Davis, Gage et al. 1991). It is important to know which method of calculating moments is used, and the axis definitions used, as the moments that are calculated differ according to the method used and the axis system that was utilised (Newell, Hubley-Kozey et al. 2008; Whatling, Evans et al. 2009).

More sophisticated musculoskeletal models can be constructed, with and without calculation of individual muscle forces or estimation of joint contact forces. The outputs from these models show good agreement with in-vivo measurements of loading using instrumented total knee replacements (Kim, Fernandez et al. 2009).

However, the models are time consuming to construct and analyse and the individual mechanics of joints are often idealised to very simplistic models (such as a hinge at the knee) (Shelburne, Torry et al. 2006; Fregly 2008; Reinbolt, Haftka et al. 2008; Lin, Walter et al. 2010). As such their use is limited to specific research questions on small numbers of patients at present.

Finally, the conventions for +ve and -ve are adjusted from the mathematical conventions to anatomical conventions when the data is reported, to prevent confusion. Positive movement is defined according to the direction that the patient is walking, and positive rotation is described according to a set definition for each joint and plane (e.g. at the knee, a +ve angle in the sagittal plane is defined as flexion).

#### 2.2.5 Sources of Error

In order to understand which components of a gait report are useful, and which are prone to error, a detailed description of the sources of error in gait analysis is worthwhile. The theoretical background to gait analysis and sources of error were reviewed comprehensively in 2005 in a series of 4 papers (Cappozzo, Della Croce et al. 2005; Chiari, Della Croce et al. 2005; Della Croce, Leardini et al. 2005; Leardini, Chiari et al. 2005). The titles of three of those papers form a reasonable framework to use when discussing sources of error in gait analyses. These are instrumental errors, skin movement artefact errors and errors related to anatomical landmark identification.

Instrumental errors refer to errors due to the cameras, their identification of markers and the force plates. Camera errors can be systematic or random (Chiari, Della Croce et al. 2005). Systematic effects include lens distortion of the cameras (which should be considered in the calibration) and errors associated with calibration. Prior to every analysis, calibration is now performed throughout the area visualised by the cameras using a rod of fixed distance with markers on each end, allowing a calculation of the area captured by the cameras but also accounting for changes in image distortion across different parts of each camera's field of view (Cerveri, Borghese et al. 1998).

Random effects include electronic noise, marker flickering and mis-interpretation or identification of markers. These effects are reduced using filtering and smoothing algorithms built in to modern tracking software, the use of appropriately spaced markers and the avoidance of reflective surfaces in the laboratory (Gazzani 1994; Chiari, Della Croce et al. 2005).

Skin movement artefact is probably the major source of error in gait analysis. The phrase is misleading – it is due to deformation of all of the soft tissues between the markers and the bone and occurs with any marker that is not rigidly attached to the bone of interest. Studies have been performed to compare the bony motion to those measured using skin markers, using either bone pins or dynamic fluoroscopy as the gold standard (Reinschmidt, van den Bogert et al. 1997; Stagni, Fantozzi et al. 2005; Garling, Kaptein et al. 2007; Akbarshahi, Schache et al. 2010).

Thigh marker error is generally greater than error at the shank (i.e. tibial). Marker error is highly dependent on which markers are used and how they are attached, as a lateral femoral epicondyle marker may vary by up to 20mm, whereas markers on the thigh have much lower errors (<10mm) and even less so on the lateral malleolus (<5mm) (Garling, Kaptein et al. 2007; Gao and Zheng 2008; Akbarshahi, Schache et al. 2010; Peters, Galna et al. 2010). The positioning of markers and their role in the analysis therefore needs to be well defined when reporting gait analysis studies.

Knee joint kinematics measured using reflective markers have relatively low levels of error with regard to flexion-extension but errors in ab/adduction and internal/external rotation can be equal to, or sometimes greater than the measured motion (Garling, Kaptein et al. 2007; Akbarshahi, Schache et al. 2010; Peters, Galna et al. 2010).

Due to variation in methodology, a systematic review this year was unable to pool data from the various studies of skin movement artefact, so a summary of angular errors from one recent study is given in table 2.2. It must be appreciated that this data was collected from a small number of healthy, young patients who may differ in terms of both gait parameters and tissue behaviour from older osteoarthritic patients (Akbarshahi, Schache et al. 2010; Peters, Galna et al. 2010). Studies which have compared skin markers to bone pins have found lower errors in rotation than the results presented here, although the magnitude of errors are similar (Reinschmidt, van den Bogert et al. 1997; Benoit, Ramsey et al. 2006).

	Flexion- Extension	Abduction/Adduction	Internal/External Rotation
Average RMS (degrees)	4.5	4.45	5.91
RMS/ROM (%)	8.9	93.3	82.6

Table 2.2 Estimated errors in measuring knee kinematics comparing an optical markers to fluoroscopic techniques. From (Akbarshahi, Schache et al. 2010). Average RMS = error in knee joint angulation expressed as the average RMS error. RMS/ROM = percentage of the error in relation to the full range of movement of the joint in that plane.

Whilst motion of the markers relative to each other or to bone gives direct information about error from soft tissue motion, errors are not solely due to skin movement artefact. The identification of bony anatomy and the application of local axis systems to the bony segments are also of importance (Della Croce, Leardini et al. 2005). The errors described in table 2.2 are due to a combination of the 2 effects.

Marker positions are identified by palpation, although bony landmarks are often surfaces or curves, rather than specific points. This leads to both inter- and intra-observer variation in their position, although errors can be significantly reduced by training and with specific instructions on identifying landmarks (Della Croce, Leardini et al. 2005).

Another factor which should also be considered is the estimation of joint centres – a step in the analysis process. As the joint centres are not directly measured they have to be estimated from the position of the markers (such as the pelvic markers

for the hip). This is based on anthropology studies, often from relatively small cohorts (Bell, Pedersen et al. 1990; Kadaba, Ramakrishnan et al. 1990). This is a lesser issue at the knee (which is relatively superficial) whereas the hip is a deeper joint, and so the potential for error is greater (figure 2.18).

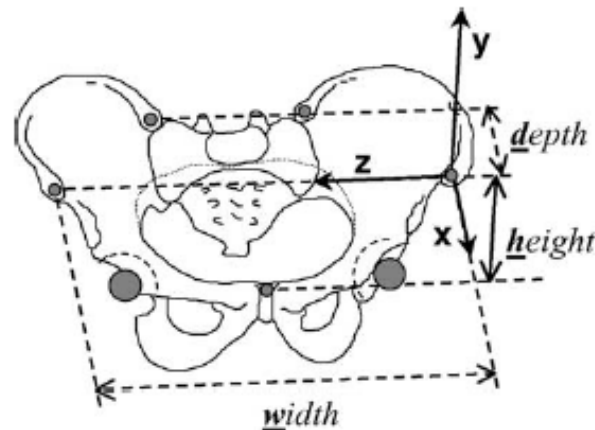


Figure 2.18 Estimation of the hip centre is performed using regression equations based on the position of the markers. As the external pelvic dimensions are known, the position of the hip centre can be estimated as a standard translation relative to those markers. Diagram from (Della Croce, Leardini et al. 2005)

The identification of anatomical landmarks is important, not only for consistency of measurement but also to ensure the co-ordinate axes are consistently aligned. If there is an error in the registration of an axis (for example if it was internally rotated - around  $z$  - by  $30^\circ$ ) then a movement that should be along a single plane will be expressed by the system as having occurred in 2 planes (Della Croce, Leardini et al. 2005; Nigg, Cole et al. 2007). Although not technically an error (the motion is still recorded correctly, just expressed in a different axis system), it can lead to problems inconsistency of data interpretation.

This is a particular issue at the knee, which has a high excursion in one plane and low levels of motion in orthogonal planes. Registration of the co-ordinate axes are dependent upon the correct identification of anatomical landmarks, which can be mistaken. Therefore much of the recorded axial rotation and ab/adduction at the knee may simply be the result of knee flexion occurring out of alignment with the axis system.

Finally, the choice of axis convention is important. A recent study chose to compare normal subjects and those with OA using 3 different axis conventions for describing knee moments (Newell, Hubley-Kozey et al. 2008). They found significant differences in moments and waveforms produced depending on which model was used. A study from Cardiff looking at stair gait also found differences in moments depending on the software used for data processing and analysis (Whatling, Evans et al. 2009).

Given that the primary outcome measure used in much of this thesis is the adduction moment, these studies have particular relevance. It is clear that any study using gait analysis needs to clearly define the model definitions that were used in the study methodology, as well as the techniques for collection of the raw data.

### 2.2.6 Overall Reliability of Gait Analysis

Given the various potential sources of error, it is valuable to know whether variables measured by gait analysis adequately represent motion and loading in real life. In terms of kinematics, we have seen above that sagittal plane rotations are well represented by gait analyses but those occurring in the axial and frontal planes have high levels of error and should be interpreted with caution.

We also need to consider the repeatability of gait analysis. The repeatability of kinematic measurements in normal patients was assessed in a recent systematic review (McGinley, Baker et al. 2009). Twenty three studies were analysed, study protocols varied considerably, and it was difficult to separate the effect of repeated marker placement from the results. The conclusion was that test-retest error in the sagittal plane could be expected to be less than 4° and error in the coronal plane approximated 2°.

Loading of the medial compartment of the knee is represented in gait analysis indirectly by the adduction moment, which will be described in more detail in chapter 2.3.8 and will be the major outcome measure in the majority of the studies in this thesis. The most direct study into the validity of the adduction moment was performed using 15 gait trials in a single patient with an instrumented knee replacement capable of measuring loads directly (Zhao, Banks et al. 2007). This study found that the adduction moment correlated with the medial compartment loads well (overall  $R^2=0.77$ ).



The adduction moment is also a reliable measure, as a study on the test-retest reliability of the peak knee adduction moment found a high mean intra-class correlation (ICC=0.86) and a low magnitude of error (mean error 0.1 on a mean value of 2.54 [% body weight x height])(Birmingham, Hunt et al. 2007). The same group subsequently found similarly high levels of test-retest reliability for the adduction impulse (ICC=0.89), a measure calculated from the moment curve (Robbins, Birmingham et al. 2009). Additionally, there is a substantial body of evidence that the adduction moment is a relevant and important measure of loading in knee OA and these studies will be discussed in section 2.3.2.

We can conclude that despite its potential sources of error, gait analysis is a repeatable and useful measure of the biomechanics of human locomotion. Its advantages are that it is an *in-vivo*, direct measure of the biomechanics of locomotion. In order to interpret results accurately it is important to understand which measures are reliable and which are prone to error. When the errors and limitations are considered in the analysis it can be a powerful research tool.

#### 2.2.7 Electromyography (EMG)

Gait analysis is able to give information on resultant motion and loading, meaning that if two muscles or forces act against each other with equal and opposite forces, no movement will be measured or recorded. Individual muscle strength or action may be inferred when only single muscles or muscle groups act to produce a movement. However, most joints are crossed by multiple muscle groups in each

plane and contractions of these muscle groups often occur together, cancelling their action out but having important actions in terms of joint loading. It is therefore useful to have another measure of muscle action during gait. The most common way of doing this is with electromyography (EMG).

Electromyography measures the electrical signal that occurs when motor units (a group of muscle cells and their associated motor neuron) depolarise. A higher signal corresponds to a greater number of depolarising motor units, greater size of the units, or a greater firing rate of each unit, all of which result in an increased muscle force. The output is therefore a single variable that represents a combination of all of those factors (Staudenmann, Roeleveld et al. 2010).

There are numerous potential sources of error. One of the greatest issues is that the EMG signal itself is usually measured using an electrode placed on the skin surface, and is therefore subject to the quality of the contact between the electrode and the skin, as well as the resistance across the skin, subcutaneous tissues and fat. It can also be affected by the signal from adjacent muscle groups (Rau, Schulte et al. 2004; Staudenmann, Roeleveld et al. 2010). These problems can be overcome with the use of fine needles but this invasive technique may interrupt gait, only represents the part of the muscle that the needle is in, and adds significantly to the study complexity (Rau, Schulte et al. 2004).

Each activated motor unit produces a very small amount of electrical activity as it depolarises and the 'spikes' of activity recorded by EMG are a summation of numerous motor units acting at the same time. This produces an interference

pattern, which is characterised by the appearance of a series of spikes in the reading in close proximity to each other. The apparently random nature of these spikes can be filtered out, using a technique called low pass filtering which essentially averages out the electrical signals over a 'moving window' of time, to produce a smooth 'enveloped' signal.

Changes in this signal relate to changes in the force generated by the muscle during the period of measurement (Staudenmann, Roeleveld et al. 2010). Because of the variability in skin contact and subcutaneous tissues between individuals, it can be difficult to compare strength between individuals using EMG signals (Rau, Schulte et al. 2004). Therefore, the relationship between some sort of maximum is usually recorded, either a maximum contraction achievable by the patient or a maximum during gait (Murley, Menz et al. 2010).

EMG data therefore provides useful data which needs to be interpreted within the confines of its potential for error. As with gait analysis techniques, powerful information about human locomotion can be gained but needs to be interpreted carefully if valuable conclusions are to be drawn.

## 2.3 Gait Analysis in OA

### 2.3.1 Gait changes in knee OA

There is a large body of literature on gait analysis in patients with knee OA. Patients with OA have been shown to walk at reduced speed and cadence, with a reduced loading response and reduced knee flexion in both stance and swing phase, and a greater trunk swing (Mundermann, Dyrby et al. 2004; Astephen and Deluzio 2005; Hunt, Birmingham et al. 2008; Briem and Snyder-Mackler 2009; Hatfield, Hubley-Kozey et al. 2010).

The difficulty with analysing data in knee OA is that there is a wealth of information produced by a gait analysis, some of which may be relevant and some of which is not and much of it gives conflicting answers.

In Cardiff, a classification system has been developed which aims to address this (Jones, Beynon et al. 2006; Jones, Holt et al. 2008). Gait data was collected on samples of healthy and osteoarthritic patients. Multiple waveforms and individual pieces of data were analysed using principle component analysis to determine the ten pieces of information that best discriminate between healthy and osteoarthritic subjects. A classification was subsequently developed using the Dempster-Shafer theory of evidence to convert these complex pieces of information into a format that is easy to interpret. One benefit of this system is that it can be 'reverse

engineered' in the sense that the factors that determine the score are retained and can be drawn out to explain the findings.

Two numerical factors (belief values) between 0 and 1 are produced – giving a scale that runs from 'definitely normal' to 'definitely OA'. Another scale between 0 and 1 describes the uncertainty. These are described in figure 2.19. The classification has subsequently been used to describe functional recovery following total knee replacement. Its utility and functional relevance has also been improved with the addition of functionally challenging tasks, such as stair climbing. This work is continuing, as well as work to understand the factors that affect functional recovery following knee replacement, and part of this thesis will be exploring function using the classifier with regard to the opposite limb.

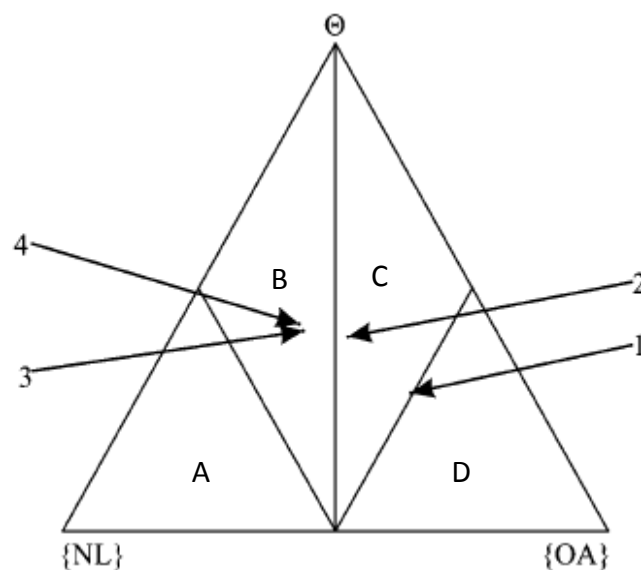


Figure 2.19 The Cardiff functional classifier (from Whatling 2009, adapted from Jones and Holt 2008). The horizontal axis represents a belief that the subject is either normal or has OA based on their gait data, whereas the vertical axis represents uncertainty. Triangle A represents normal (high certainty), triangle B represents normal but with uncertainty, triangle C represents an OA finding with uncertainty, and triangle D represents OA with a high level of certainty. Points 1 to 4 represent the path of a patient recovering from a knee replacement (1 = pre-op, 2 = 3months post op, 3 = 6months post op, 4 = 12 months post op).

### 2.3.2 Gait changes in Total Knee Replacement

Improvements in subjective outcome and pain scores have been well described following TKR but there have been relatively few studies using gait analysis to describe objective function and biomechanics after knee arthroplasty (Baker, van der Meulen et al. 2007; McClelland, Webster et al. 2007; Bourne, Chesworth et al. 2010).

A systematic review of gait studies in TKR was published in 2007 by McClelland, Webster and Feller. They found wide variation in methodology between different studies but despite this there were a few consistent findings, such as a reduction in total knee range of motion during gait, a reduction in knee flexion during swing and a reduction in the range of knee flexion during the loading response.

McClelland et al also found that after knee replacement a loss of the normal 'biphasic' pattern of the sagittal knee moment was common, although the reasons for this were poorly understood in the literature and subsequent studies by the same authors and others have found that a biphasic pattern was present in the majority of post-TKR patients (McClelland, Webster et al. 2007; Catani, Ensini et al. 2009; McClelland, Webster et al. 2010). The presence or absence of a biphasic pattern is a coarse generalisation of gait, however and closer inspection of both these papers and others suggests that gait often improves following knee arthroplasty but that it rarely returns to normal (Saari, Tranberg et al. 2005; Jones, Beynon et al. 2006; Catani, Ensini et al. 2009; Hatfield, Hubley-Kozey et al. 2010; McClelland, Webster et al. 2010; Alnahdi, Zeni et al. 2011).

This has been shown previously in the PCA and classification papers by Jones et al (2005), and more recently by Hatfield et al (2010) who used a similar PCA technique (although without ranking or classification) and demonstrated significant persisting abnormalities in patients 1 year following knee replacement (Jones, Beynon et al. 2006; Hatfield, Hubley-Kozey et al. 2010). That study failed to document the condition of the other knee, despite two published studies which found that the primary determinant of functional performance after TKR was the strength in the opposite limb (Mizner and Snyder-Mackler 2005; Farquhar and Snyder-Mackler 2010). The interpretation of gait analysis in TKR patients is challenging and it is becoming increasingly clear that the condition of the other leg should be accounted for, either in the study design or the subsequent analysis of the complex data that can be produced with these analyses.

### 2.3.3 The Knee Adduction Moment

There has been a large amount of literature over the past two decades focusing on one key measure – the knee adduction moment. The knee adduction moment is the rotational force that is applied to the knee as a result of the ground reaction vector in the coronal (frontal) plane. As such, it is an ‘external’ load – it does not consider the effect of balanced muscle activation either side of the joint (‘unbalanced’ muscle activation results in movements which are measureable by gait analysis). The factors that influence the adduction moment at any point in time are described in figure 2.20.

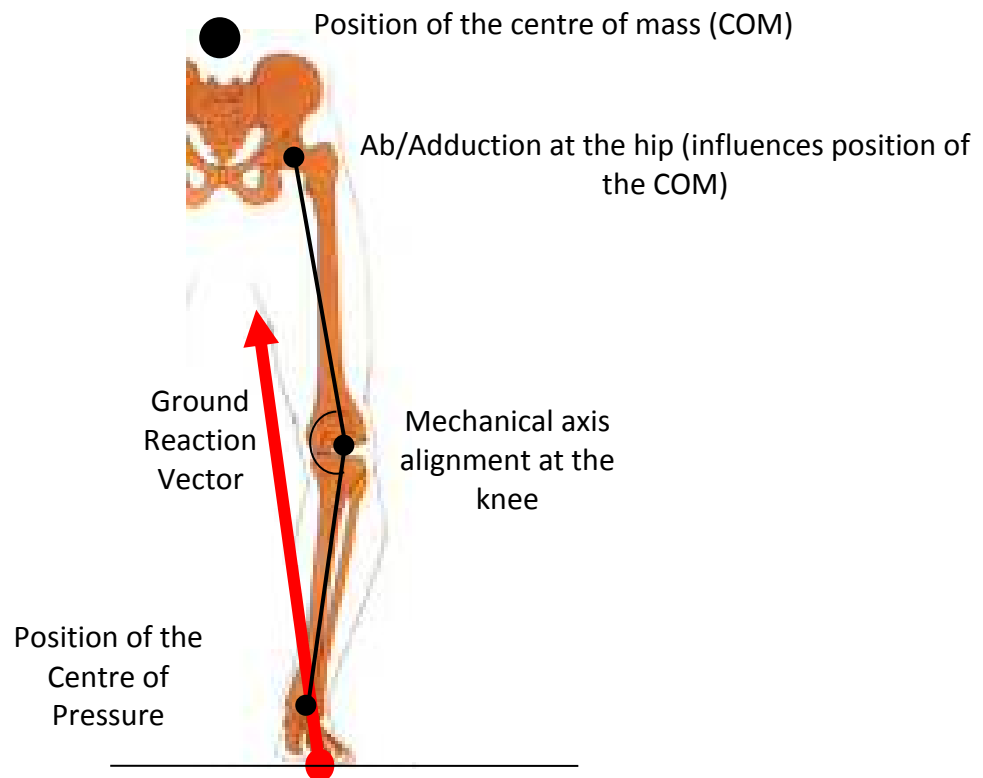


Figure 2.20. Factors affecting the knee adduction moment at any given time. Axial rotation of the leg - often described in terms of foot progression angle - is the one other factor missing from the list and influences the moment by effectively converting an adduction moment to a flexion/extension moment at the joint itself.

The majority of the literature on the knee adduction moment has focused on the peak knee adduction moment. This is the maximum that the adduction moment reaches during the gait cycle. A higher knee adduction moment is associated with a greater medial-to-lateral bone density ratio, which is often taken as evidence that it is representative of increased joint loading (although increased disease severity causing a higher moment is another possible explanation) (Hurwitz, Sumner et al. 1998). The peak adduction moment is higher in patients with more severe disease and is influenced by limb alignment, which becomes more varus as medial



compartment disease progresses (Sharma, Hurwitz et al. 1998; Hurwitz, Ryals et al. 2002; Mündermann, Dyrby et al. 2004; Mündermann, Dyrby et al. 2005).

Knee pain often results in efforts by the patient that reduce their knee adduction moment (including the peak) through reduced gait speed or adaptive strategies such as toe-out gait or trunk swing (Hurwitz, Ryals et al. 2000; Chang, Hurwitz et al. 2007; Hunt, Birmingham et al. 2008; Lynn and Costigan 2008; Kito, Shinkoda et al. 2010). It may be argued that the excessive use of analgesia results in increased joint loads and disease progression – an ‘analgesic arthropathy’ (Hurwitz, Sharma et al. 1999).

In the late 1980’s, Prodromos and Andriacchi showed that the peak knee adduction moment was predictive of the recurrence of deformity 3 years after high tibial osteotomy (Prodromos, Andriacchi et al. 1985). This has been confirmed recently in a large study using modern osteotomy techniques (Birmingham, Giffin et al. 2009).

Although there have been numerous subsequent studies of knee moments, there have only been two longitudinal studies of reasonable quality which have examined its relationship to disease incidence and progression independent of an operative intervention.

Amin et al studied 118 patients (75 with no history of knee pain previously) over the age of 60 with gait analysis during a range of functional activities at baseline, and telephone follow up after 4 years (Amin, Luepongsak et al. 2004). Only 7 people developed new chronic knee pain over that time period, however those patients had greater adduction moments at baseline in all functional activities compared

with those that didn't develop knee pain. Although the study was severely limited by numbers, it remains the only study to link gait analysis findings with disease initiation.

Miyazaki et al examined the progression of OA over a 6 year period in 74 patients with radiographs and gait analysis at baseline and radiographs at follow up (Miyazaki, Wada et al. 2002). 32 patients developed progression of their disease over the course of the study. The peak adduction moment was significantly better at predicting future outcome than static radiographic alignment, joint space width or pain at baseline. An increase of the moment of 1%Body Weight x Height (a 20% change) corresponded to an increase in the odds ratio of progression of the disease of 6.46. Therefore, mechanical loading was the major influence on disease progression in their population of OA sufferers in Japan. Unfortunately, the only measure of the moment waveform that was studied in that paper was the peak value – whereas other measures of loading are now gaining popularity.

Alongside reporting single values in the gait cycle as a measure of joint loading, many papers now report the adduction impulse as well. This is the integral of the adduction waveform, although reports of how it is calculated vary (Robbins and Maly 2009; Bennell, Creaby et al. 2010). The majority of authors integrate the part of the curve that corresponds to single limb stance only, using standard definitions of single limb stance (18-50% of the gait cycle) or patient derived definitions – although the latter skew the results according to individual variation in the length of stance phase.

Theoretically the impulse is a better representation of the energy absorbed by the cartilage and bone during gait. Studies have shown that the impulse is more closely correlated with bone marrow oedema on MRI and more closely correlated with pain than the peak moment in osteoarthritic patients, although both of these findings could be explained by patients with more severe or active disease walking slower (Robbins and Maly 2009; Bennell, Creaby et al. 2010; Kito, Shinkoda et al. 2010). The measure is gait speed dependant for the simple reason that the standing leg spends longer on the ground when gait is slower.

Bennell et al recently reported a study in which they looked at the relationship between knee adduction moment impulse, peak adduction moment and cartilage loss at 1 year in a cohort of 144 patients with early knee osteoarthritis (Bennell, Bowles et al. 2011). They found no relationship between cartilage loss and the peak adduction moment over 12 months but a strong relationship between the knee adduction moment impulse and cartilage loss. The impulse remained an independent factor in predicting cartilage loss, even when alignment was accounted for. In this study the impulse was calculated as the total area under the positive section of the moment curve, which is the definition that has subsequently been used in this thesis.

It was noted by McClelland et al that few studies had examined coronal plane moments in knee replacements, with those papers mostly finding similar or lower peak values in the affected knee to controls. Hatfield et al (2010) and McClelland et al (2010) also noted an increase in mid-stance moment and although the reasons

for this were not explored statistically, a failure to recover normal gait speed may explain the findings in both of these papers (Saari, Tranberg et al. 2005; McClelland, Webster et al. 2007; McClelland, Webster et al. 2010).

McClelland noted in 2007 that no studies had examined the contra-lateral knee in patients undergoing TKR, and the two that have since then have limitations (see section 2.3.7) (McClelland, Webster et al. 2007). The challenges of recruitment of patients with unilateral knee OA were discussed but it was believed by the authors to be a valuable study that was lacking in the literature (McClelland, Webster et al. 2007).

The direct relationship between the knee moment and joint loading has not yet been fully established, and although it is an indirect measure of joint loading the adduction moment is a useful measure that has important consequences for the patient both at the time of assessment and with regard to future progression.

Abnormal moments are a potent cause of disease progression. Although the evidence is weak, abnormal moments may also represent a potent cause of disease initiation, although further research is needed to define this clearly.

#### 2.3.4 The Hip Adduction Moment

The hip adduction moment has received much less interest in the literature than the knee adduction moment. The hip adduction moment can be easily represented on a free body diagram (figure 2.21). The moment is resisted by the hip abductors

in the frontal plane and the increased action of the hip abductors would be expected to increase resultant joint loads.

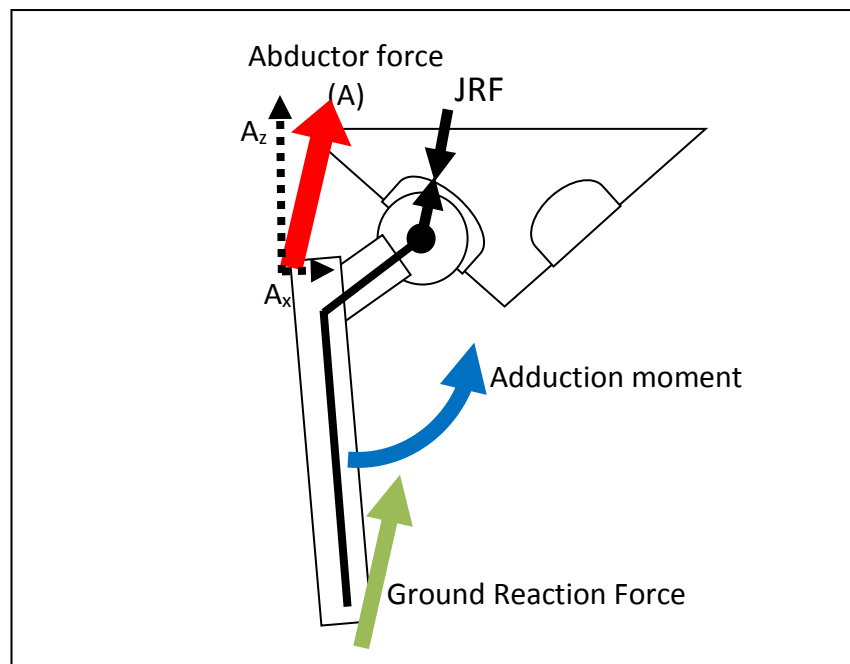


Figure 2.21. Free body diagram to describe the importance of the hip adduction moment during single limb stance. The adduction moment at the hip (blue arrow) is resisted by the abductors (red arrow), whose action can be described in 2 components (dashed arrows). The vertical ( $A_z$ ) and horizontal ( $A_x$ ) components of the abductor force, and the vertical ground reaction force contribute to the joint contact force at the acetabulum (JRF), which has an equal and opposite reaction from the femoral head. The action of the hip adductors has been excluded for simplicity.

The relationship between the hip adduction moment and OA of the hip has received surprisingly little attention, despite the relationship between the moment and resultant joint contact forces. One paper by has shown that patients with OA of one hip were more likely to develop disease on the contra-lateral hip if the moment was elevated (Hurwitz, Sumner et al. 2001).

Some authors have made the assumption that the hip adduction moment is therefore a direct representative of hip strength, which might be modified with training (Chang, Hayes et al. 2005). However, subsequent studies have shown that weakening the hip abductors actually increases the moment during gait (presumably by stopping the patient from lateralising the centre of mass over the hip) (Henriksen, Aaboe et al. 2009; Rutherford and Hubley-Kozey 2009). To further complicate the issue, it has also been observed in a cadaveric study that increased abductor forces change the morphology of the acetabulum and alter the contact area between the femoral head and acetabulum (Bay, Hamel et al. 1997).

Given the small number of studies on the subject, the relationship between the hip adduction moment and hip OA is not clear. It might be expected from classical mechanics that when the adduction moment is high, there would be increased joint contact loads and subsequent cartilage damage, although this remains largely a supposition.

#### 2.3.5 Muscular Co-Contraction

One feature of gait in OA that has received some interest recently is the combined contraction of the quadriceps and hamstring muscles across the knee during the gait cycle. This is important as the two muscles acting together would be expected to increase resultant joint loads but would not be detected using standard gait analysis.

Co-contraction is recognised as a strategy used to stabilise joints to protect them from injury – it is considered a protective mechanism in young men playing contact sports or landing from a height (Ford, van den Bogert et al. 2008). Co-contraction occurs more readily in the elderly in response to trips and falls as well as stepping down, although some co-contraction occurs in both age groups (Hortobagyi and DeVita 2000; Hortobagyi and Devita 2006).

It is presumed throughout the literature that co-contraction is used to stiffen a joint and possibly improve control. Pronounced co-contraction is often seen in both young and old individuals performing novel tasks or those that require stability (Patten and Kamen 2000).

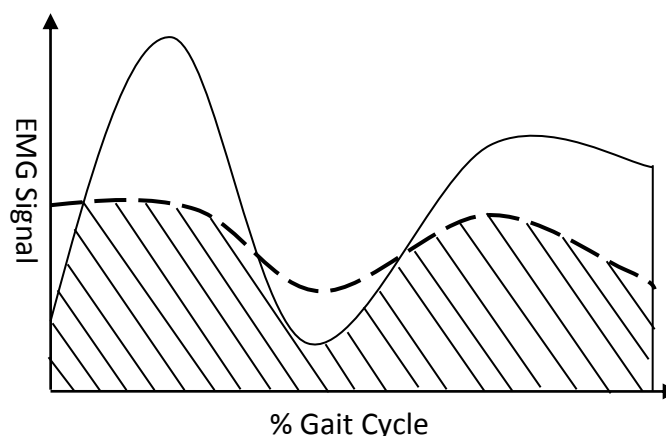


Figure 2.22 Sample chart of two imaginary enveloped EMG waveforms. Co-contraction is the area in which they are both active, and can be defined as an area under a curve, or as an average over a section of the gait cycle. Adapted from (Winter 2009).

Co-contraction is calculated in many ways throughout the literature. All of the described methods aim to represent the period of time and magnitude that the two

muscles are active together, usually either as an average or as an area under a curve (figure 2.22).

In 2004 Lewek et al used surface electrode measurements of quadriceps and hamstrings to examine co-contraction in 12 osteoarthritic knees and 12 normal subjects. They produced a co-contraction index (documented in chapter 3) and found that it was elevated in individuals with OA. They related co-contraction to joint laxity measured using stress radiographs, demonstrating that co-contraction of those muscles was related to both joint laxity and the knee adduction moment (Lewek, Rudolph et al. 2004).

A subsequent study by the same group found that co-contraction could be reduced with the use of a valgus off-loading brace, which may explain the clinical benefit associated with their use (Ramsey, Snyder-Mackler et al. 2007). Similar reductions in co-contraction were also seen following high tibial osteotomy surgery (Ramsey, Snyder-Mackler et al. 2007). A recent paper has demonstrated that co-contraction in the affected knee is sensitive to knee OA severity (Hubley-Kozey, Hill et al. 2009).

At present, there is very little literature about co-contraction in knee OA, although it is clearly relevant to joint loading. In OA, co-contraction might stabilise the joint but it would also increase joint loading. The one paper that looked at bilateral co-contraction is described below, although it is clear that there is significantly more work to be done with regard to co-contraction and OA.



### 2.3.6 Loading of Unaffected joints in Patients with Knee OA – the Same Side

There have been very few studies on the loading of other joints in patients with knee OA and no study has determined whether loads in the opposite leg are abnormal.

Two papers have described elevated moments in the ipsilateral hip and ankle in patients with OA of the knee compared to normal subjects, although neither study examined the opposite limb (Mündermann, Dyrby et al. 2005; Astephan, Deluzio et al. 2008). Both studies had study groups with 'mild or moderate' disease and 'severe' disease (Kellgren-Lawrence 3-4, or awaiting TKR). The study by Mündermann et al recruited patients with bilateral disease, whereas the status of the other limb was not defined in Astephan et al.

Mündermann et al described elevated peak moments at the knee and hip in the 'moderate' group but decreased peaks at the hip in the severe group, with similar walking speeds in all groups (Mündermann, Dyrby et al. 2005). Astephan et al described progressively increasing mid-stance moments at the hip, knee and ankle as severity increased, with a reduced peak at the hip in the severe group. Walking speeds were lower in the OA patients than the controls, and lower again in the severe OA group (Astephan, Deluzio et al. 2008).

### 2.3.7 Loading of Unaffected joints in Patients with Knee OA – the Other Side

There have been four studies which have reported loading in the contralateral leg, two in patients with knee OA and two in patients after total knee replacement (Hunt, Birmingham et al. 2006; Milner 2008; Briem and Snyder-Mackler 2009; Catani, Ensini et al. 2009). Only one of these studies utilised control patients without disease (Catani, Ensini et al. 2009) and the recruitment strategy and condition of the other knee was not always reported, or even considered in recruitment. Given the high frequency of bilateral disease and the subsequent difficulty in recruiting patients with unilateral disease, this raises questions about the presence of disease or deformity in the other leg – a subject that will be discussed in detail later in this thesis.

The studies by Hunt et al and Briem et al both set out to study gait adaptations to OA, focusing particularly on the hips and the trunk (Hunt, Birmingham et al. 2006; Briem and Snyder-Mackler 2009). In both cases, the loading of the opposite limb was a secondary outcome measure. Briem et al described a clear recruitment strategy and entry criteria, in which bilateral limb alignment radiographs were taken although unilateral disease was defined by symptoms. The paper by Hunt et al had greater numbers but less details on recruitment. Bilateral cases were included and the less affected side was defined as 'unaffected'.

Hunt et al found a difference in moments between knees which they described as being due to the 'frontal plane lever arm' – essentially the distance between the knee centre and the GRF vector (measured perpendicular to the GRF vector). This

was presumably due to either a lateralisation of the knee centre relative to the centre of pressure or a medial shift in the ground reaction vector.

Briem et al described a related phenomenon, in which patients used their trunk to lateralise the GRF vector as a compensation mechanism, effectively reducing the lever arm on that side – although whether that resulted in an increase on the other side relative to normal was not discussed (figure 2.23). Briem et al found similar adduction moments in both knees but differences existed between the hips – with a lower peak moment on the affected side.

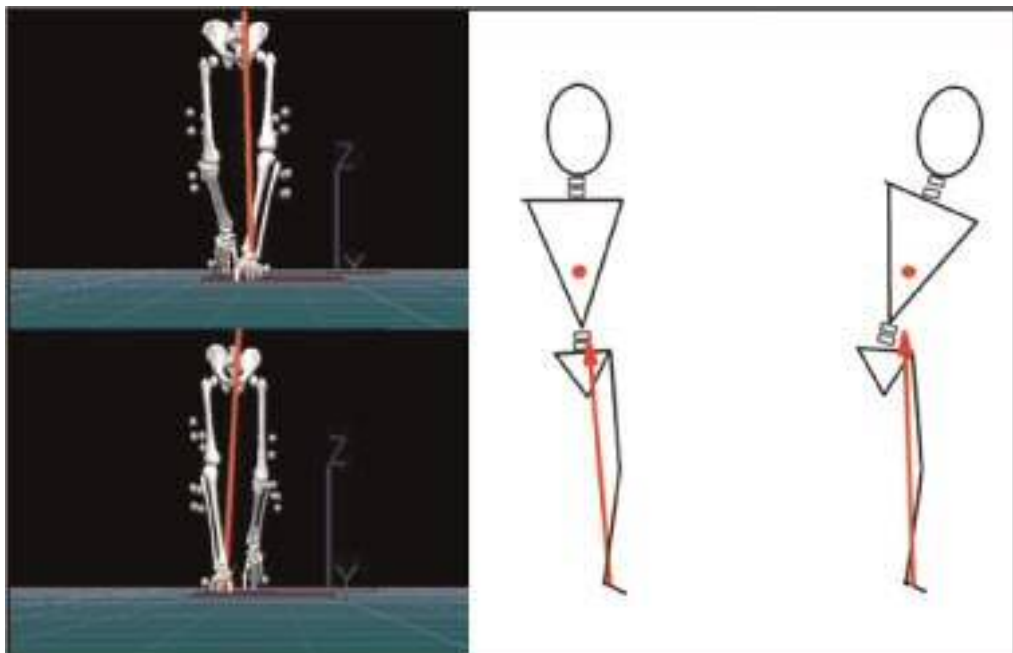


Figure 2.23. Taken from (Briem and Snyder-Mackler 2009). The authors reported the use of trunk lean to lateralise the centre of mass and reduce the knee moment as a compensation strategy. The effect of this on the other limb was not clarified.

Compensation strategies change as disease severity increases and this may explain the differences in findings between the two studies (Mündermann, Dyrby et al. 2005; Astephen, Deluzio et al. 2008). The study group in the paper by Hunt et al

mostly had Kellgren-Lawrence 4 disease, as opposed to 2-3 in the study by Briem et al. As neither study had a control group it was difficult to say whether the other joints were loaded abnormally, although given that loads in the opposite knee were equal to the OA knee in the latter paper, it is likely that they were (Briem and Snyder-Mackler 2009).

Two recent studies reported inter-limb differences in patients after knee replacement however neither study recruited patients with unilateral disease, or reported the condition of the other knee or hips in their paper (Milner 2008; Catani, Ensini et al. 2009). Neither study included a pre-operative assessment. Both studies reported similar moments in the replaced and the contra-lateral knees and although a control population was described in Catani et al, the moments in this population were not reported. These studies are therefore difficult to interpret, and are not able to determine whether the joint replacement influenced kinetics of the other joints.

One study reported on co-contraction in both legs in a cohort of 15 patients with unilateral OA defined by symptoms and x-rays and 15 normal control subjects (Lewek, Scholz et al. 2006). Knee moments were not reported, and co-contraction was only examined up to the peak adduction moment, but not beyond. Differences in co-contraction between groups were small relative to large confidence intervals. Unfortunately, there was no data on moments, or co-contraction through the rest of stance phase which may have given interesting data especially in such a well controlled study group.

Clearly there are large gaps in knowledge with regard to the other joints in knee OA. This is particularly important when we realise the large number of people affected by multiple joint disease.

Although the loading of other joints has been reported, these have often been either as secondary outcomes or used as a control. There have been no studies which have determined whether the joints of the lower limb are abnormally loaded or whether those loads change with treatment. Also, the effect of varying patterns of loading on the mechano-biology of articular cartilage is also poorly understood. The majority of this thesis is dedicated to answering those questions.

## 2.4 Aims and Objectives

Multiple joint OA is a common and disabling problem. However, there are a number of gaps in the literature on this subject and the natural history of this aspect of OA is poorly understood. No study has identified if loading is abnormal in the other joints in patients with knee OA, or clearly studied whether the other joints are influenced by treatment of the osteoarthritic knee.

The aims of this thesis are therefore:

To explore the development of multiple joint OA using a longitudinal cohort.

To test the hypothesis that abnormal forces occur during gait in the contra-lateral knee and both hips in patients with knee OA.

To test the hypothesis that joint replacement alters the joint forces in the other weight bearing joints and to gain an understanding of the sources of variation between individuals.

To test the effect of the observed changes in loading on the biomechanics and biology of human articular cartilage.

## **Chapter 3. Is Knee OA a Symmetrical Disease?**

**Analysis of a 12 year Prospective Cohort.**

### 3.1 Introduction

This chapter of the thesis came out of a realisation that we needed to understand the natural history of the condition that we were interested in. Knee OA was historically considered an 'asymmetric' disease and most research continues to focus on each joint without consideration of the other side. The little research that has been done looking at multiple joint disease has been either cross-sectional, or has involved relatively short term follow up (i.e. 2 years).

As we have seen from the previous chapter, cross sectional studies have shown that bilateral knee pain is a frequent problem in the community (Dawson, Linsell et al. 2004; Keenan, Tennant et al. 2006; Peat, Thomas et al. 2006). Each additional joint affected by OA results in a decrease in physical function and an increase in overall pain (Dawson, Linsell et al. 2004; Keenan, Tennant et al. 2006; Peat, Thomas et al. 2006). A recent study demonstrated that bilateral knee pain was an independent risk factor for poor physical function over a two year period (White, Zhang et al. 2010). However there is a lack of longitudinal data on to inform us about the natural history of bilateral disease. Therefore, the aim of this chapter is:

To explore the development of bilateral knee OA using a longitudinal cohort.

The null hypothesis to be tested is:

**Knee osteoarthritis develops and progresses in one knee only.**



## 3.2 Methods

### 3.2.1 Development of the study

A thorough literature search was performed for longitudinal studies of multiple joint disease. This revealed very few longitudinal studies which had examined more than one joint over time. One possible source of information was an analysis of the 'Bristol OA500' series, by Prof P.Dieppe, in which the development of hip and knee OA were reviewed over an 8 year period (Dieppe, Cushnaghan et al. 2000). Unfortunately, the affected side was not recorded in the paper.

To see if this question could be answered in retrospect, Prof Dieppe was contacted by e-mail. His response was that the data from this study was no longer available, but that there were a few potential studies which may have adequate data to use in understanding multiple joint OA in more detail. He contacted a number of researchers in the field to see if a collaboration to answer this question might be possible. One of the people that was contacted was Prof Ingemar Petersson from Sweden, and he made contact with his colleagues Dr Carina Thorstensson and Dr M Andersson to ask for their assistance.

Dr Thorstensson and Dr Andersson worked in Spenshult in Sweden and had recently reported on the 12 year results of the Spenshult cohort (Thorstensson, Andersson et al. 2009). With the help of Dr Thorstensson and Dr Andersson, a plan was developed to re-analyse the data with regard to the development of bilateral knee

OA. Over a series of conversations and an assessment of the available data, the aim for this study was refined to become:

To document the development of bilateral knee OA over a 12 year period using a middle-aged population-based cohort with knee pain at inclusion.

### 3.2.2 Description of the cohort study

Data was extracted from an ongoing prospective longitudinal population based cohort study which has been reported previously (Petersson, Boegard et al. 1997; Thorstensson, Andersson et al. 2009). This section relates to the work that was done in Spenshult to recruit the cohort of patients, collect and process the data, and is therefore not work done for this thesis. The subsequent section (analysis of data) is work that was done by the author in collaboration with researchers at Spenshult as a novel piece of work for this thesis.

The study population was recruited from a cohort of 279 patients aged 35-54 who had reported knee pain on most days during the past three months in a community survey. People reporting previous knee trauma, inflammatory joint disease or any other known causes of knee pain were excluded. Height and weight was measured at the study start and body mass index (BMI) was calculated.

A flowchart of participant numbers and loss to follow up over the 12 year period has been published previously (figure 3.1) (Thorstensson, Andersson et al. 2009). Two hundred and four patients were included at baseline, with 143 (63 female and

80 male) having had radiographs of both knees taken at baseline and 12 year follow up, giving a follow up rate of 78%. Eighteen patients out of the 143 did not have radiographic examination at the 5-year follow up time point.

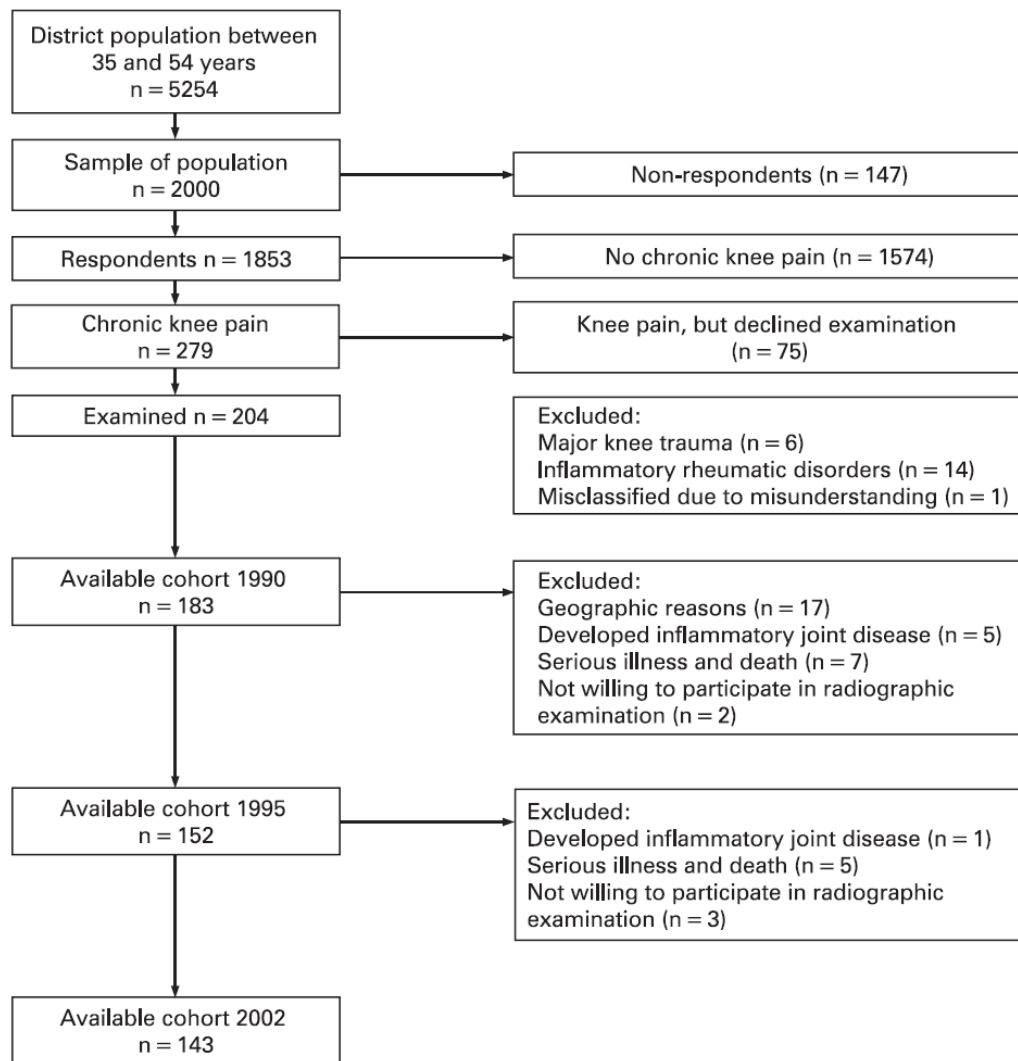


Figure 3.1. Flowchart of the sample population through the study period. Taken directly and without modification from (Thorstensson, Andersson et al. 2009).

The condition of the patella-femoral joint was not assessed at baseline and so was not included in the analysis. Therefore, only the tibio-femoral data was examined for the purposes of this paper.

The Kellgren-Lawrence (KL) classification system was used to assess the severity of OA (Kellgren, Jeffrey et al. 1963). All radiographs were assessed by an experienced radiologist blind to the patient details, and inter- and intra- observer agreement has been found to be high ( $\kappa=0.72-0.98$ ) (Boegard, Rudling et al. 1997; Petersson, Boegard et al. 1997; Boegard, Rudling et al. 1998).

Joint space width was also recorded from the medial and lateral compartment at the 5 and 12 year assessment point. The Ahlback classification was recorded at baseline, but a direct measurement of joint space width was not (Petersson, Boegard et al. 1997). The relative insensitivity of the Ahlback classification for mild to moderate OA has been discussed previously in relationship to this cohort, and so was not considered in the analysis of the data for this thesis (Petersson, Boegard et al. 1997).

The following data was provided to the author to perform the analysis:

Unique identifying number for the study

Age (at baseline) & Sex

BMI (baseline & 12 years)

Pain in each knee over past 3 months (12 months for baseline) at each time point

Pain in each knee on a daily basis at each time point

K-L grade of each knee at each time point

Joint space width in medial and lateral compartment at 5 and 12 year follow up

### 3.2.3 Analysis of the data

#### *3.2.3.1 Definitions*

The definition of radiographic tibio-femoral OA was defined as KL grade 1 and above (Boegard, Rudling et al. 1998; Hart and Spector 2003). Although some authors consider KL1 to be 'doubtful' and KL2 to define disease, studies from both Spenshult and elsewhere have demonstrated that the presence of an osteophyte alone (the definition of KL1) was predictive of further disease development and was likely to be an adequate definition of the presence of disease (Boegard, Rudling et al. 1998; Hart and Spector 2003; Thorstensson, Andersson et al. 2009). This was therefore used as the primary determinant of the presence of OA on x-ray. A secondary definition of KL grade 2 was also recorded so that other centres could compare their own data or other sources in the literature to the current study.

In order to determine which compartment was most affected, measures of joint space width from the medial and lateral compartments were reviewed. Minimum joint space widths at the 5 and 12 year time points were re-expressed as a categorical joint space width score between 0 and 3, according to the OARSI-OMERACT taskforce report from 2008 (Gossec, Jordan et al. 2008). Each joint was classified as having predominantly medial disease (a higher score in the medial compartment), predominantly lateral disease (a higher score in the lateral compartment), equal disease in both compartments, or no joint space narrowing.

A cut off of BMI > 30 kg/m<sup>2</sup> was used to determine obesity, whereas age was analysed as a continuous variable.

### *3.2.3.2 Statistical analysis*

The principle outcomes of this study were descriptive, and were expressed as proportions. Ninety-five percent binomial confidence intervals (CI) were calculated for proportions. Excessive statistical testing was avoided, but where this was relevant, effect sizes for between-group comparisons were expressed as relative risks.

In order to examine the effect of age, gender and BMI on the development of bilateral disease, a subgroup was formed that allowed for a direct comparison between those who developed bilateral disease versus those who progressed in one knee only. Therefore, patients with either bilateral disease at baseline or no disease at final follow up were both excluded from this part of the analysis. Therefore the group used to examine the effect of age, gender and BMI had no disease or unilateral disease at baseline and subsequently had unilateral or bilateral disease in 2002 (n=95).

Probability testing was performed using Fishers exact tests for 2x2 tables, except for age, which was analysed using students t test. All significance testing was done using SPSS version 16 (SPSS Inc., Chicago, Illanois, USA).

### 3.3 Results

The age, gender, BMI, unilateral/bilateral pain and radiographic severity of the group at baseline is given in Table 3.1. The age distribution is described by the histogram in figure 3.2

Mean Age	45.0 years (SD 5.9)
Gender	63 Female; 80 Male
Mean Body Mass Index	25.6 kg/m <sup>2</sup> (SD 3.9)
Pain at baseline (number of subjects)	
Unilateral	64
Bilateral	79
KL grade at baseline (all knees, n=286)	
0	182
1	73
2	14
3	17
4	0

Table 3.1 Demographics of the study group at baseline.

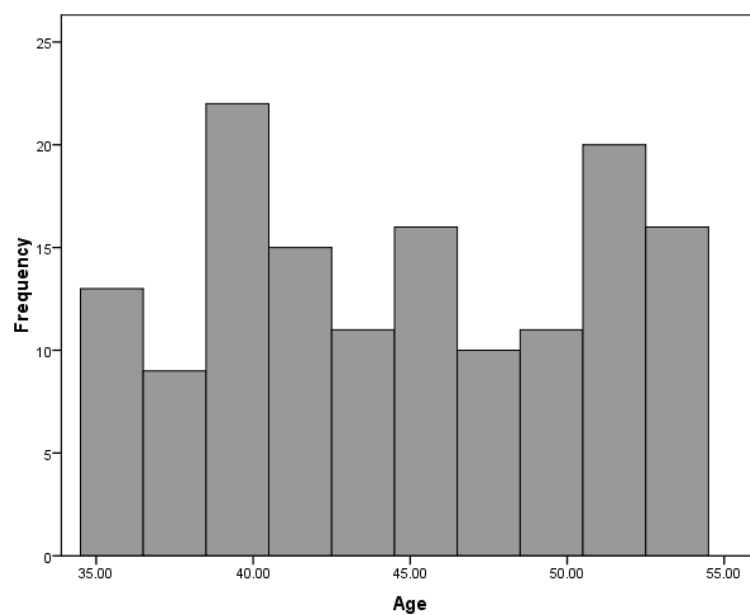


Figure 3.2 Histogram demonstrating the age distribution of the cohort

At baseline, 76 out of 143 (53%) participants had no changes on x-ray, and 37 (26%) had changes in both knees (Figure 2.3). At 5 and 12 years the number with bilateral disease had increased to 65 (52%) and 100 (70%) respectively.

If a definition of OA as  $KL \geq 2$  was preferred, then at baseline 118 patients had no OA, 19 had unilateral OA and 6 had bilateral OA. Using the same definition at 12 years, there were 41 with unilateral OA and 40 with bilateral OA. Of those who had unilateral disease at baseline, 12/19 (63%) subsequently developed bilateral OA (defined as  $KL \geq 2$ ).

Of those who started the study with knee pain but no radiological changes, 23/76 (30% (95%CI 20.3 to 41.9%)) developed unilateral changes after 12 years, whereas 41/76 (54% (95%CI 42.1 to 65.4%)) developed bilateral changes. 24 of the 30 (80% (95%CI 61.4 to 92.3%)) patients with unilateral disease at baseline developed bilateral disease after 12 years.

Using the joint space width scoring, there were 79 patients (63%) with bilateral disease at five year follow up, and 106 patients (74%) with bilateral disease at 12 year follow up.



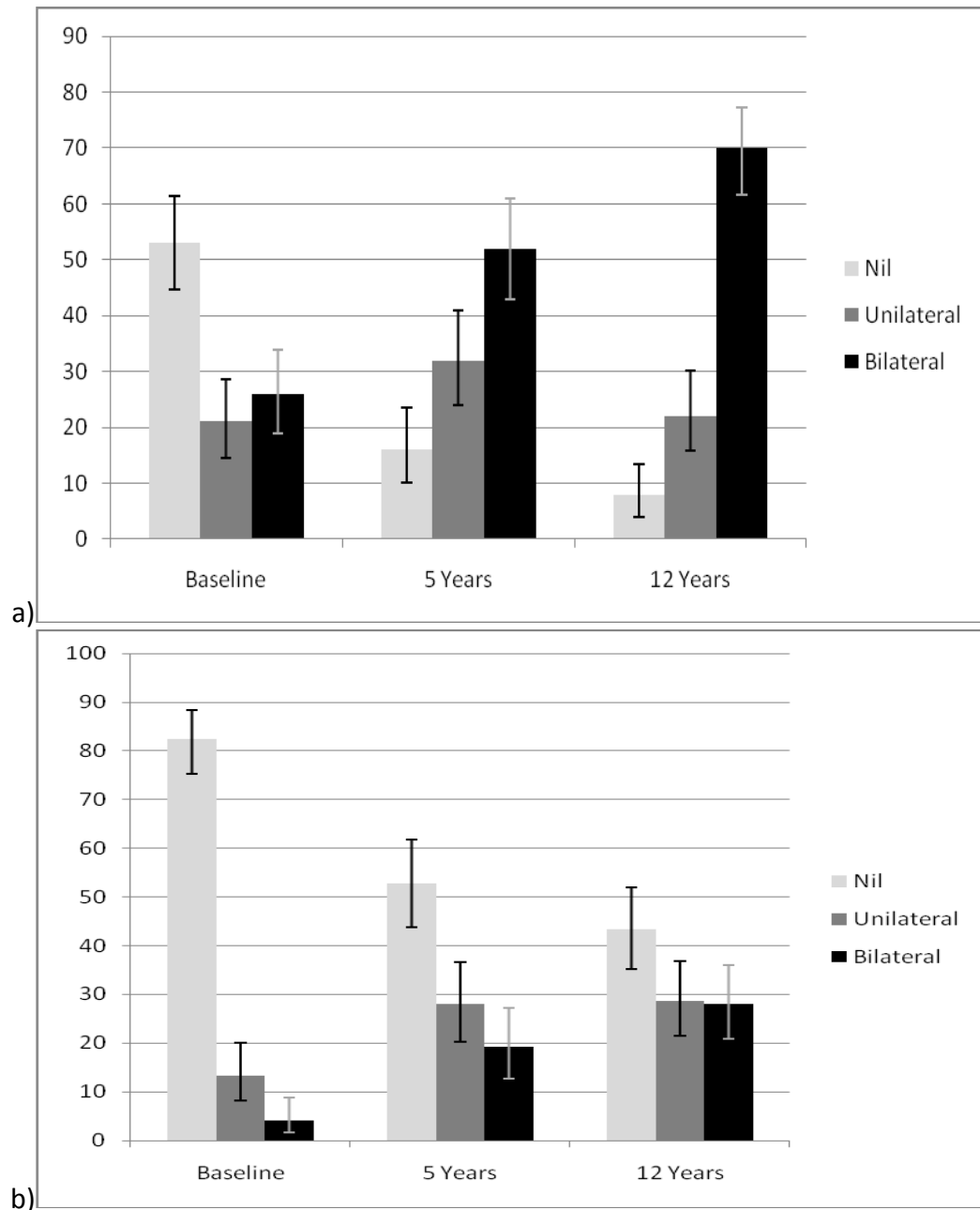


Figure 3.3. Percentage of subjects with no radiographic change, unilateral and bilateral changes at each time point. Error bars represent 95% confidence intervals a) using the primary definition of KL $\geq$ 1 b) using the definition of KL $\geq$ 2.

There was an increasing association between radiological severity in the most affected knee and bilateral changes (table 3.2 & figure 3.4). At the final follow up, of the patients whose most severe knee was KL grade 1, 27/51 had bilateral disease,

whereas 39/44 of those with KL grade 2 and 34/37 of those with KL grade 3 and above had bilateral involvement.

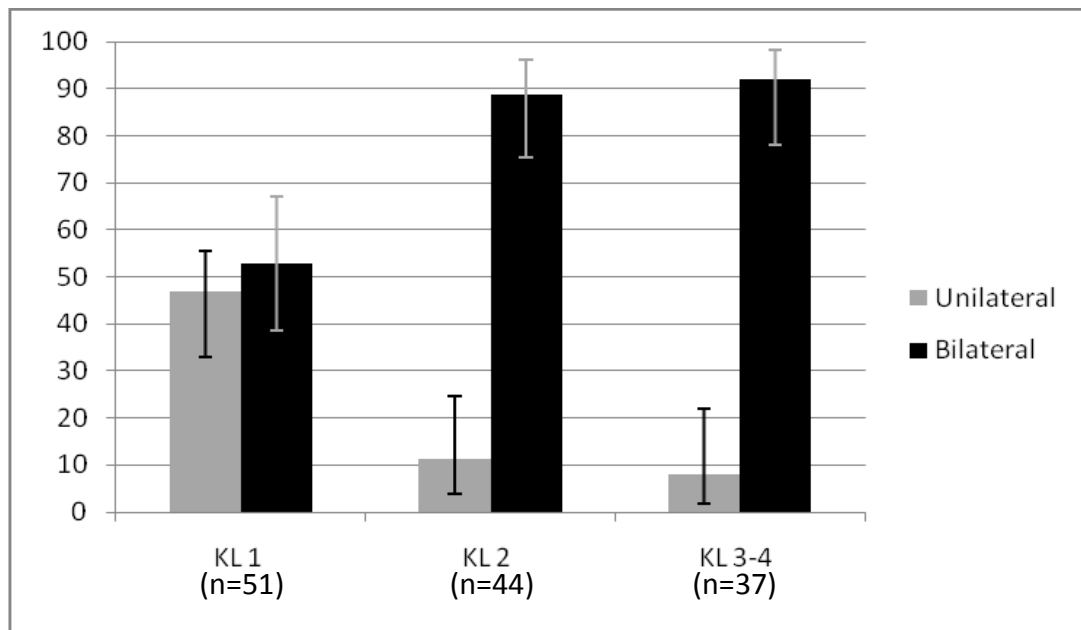


Figure 3.4. Percentage of subjects with unilateral and bilateral OA at 12 year follow up, according to the Kellgren Lawrence grade of the most severely affected knee. Error bars represent 95% confidence intervals. (n=number of subjects for each comparison).

a) Baseline:

	Less severe knee KL				
Most severe knee KL (down)	0	1	2	3	4
0	76				
1	22	20			
2	3	6	1		
3	5	5	3	2	
4	0	0	0	0	0
Total	106	31	4	2	

b) 5 years\*:

	Less severe knee KL				
Most severe knee KL (down)	0	1	2	3	4
0	20				
1	33	13			
2	4	21	13		
3	1	4	4	2	
4	2	3	0	3	2
Total	60	41	17	5	2

c) 12 years:

	Less severe knee KL				
Most severe knee KL (down)	0	1	2	3	4
0	11				
1	24	27			
2	5	25	14		
3	3	4	10	2	
4	0	3	2	6	4
KR	0	1	0	0	2
Total	43	60	26	8	6

Table 3.2 a-c. Cross-sectional findings of disease severity at each time point. The severity is determined by Kellgren and Lawrence (KL) grade, reported as number of patients in each group. 'Most severe knee' represents the knee with the highest radiographic score of the two joints (vertical column). Note increasing severity and an increased tendency toward bilateral disease over the course of time. KR = Knee replacement (3 patients had unilateral knee replacements, there were no bilateral replacements). \*18 patients not seen at 5 year follow-up.

The data can also be expressed using arrow charts (figures 3.5-3.7). These describe the flow of data, with each individual arrow representing a single patient. Movement of data downwards indicates increasing severity of the most affected knee, whereas movement to the right indicates an increasing tendency towards bilateral involvement. The charts are divided into the timepoints (0-5 years, 5-12 years, and 0-12 years representing the 18 patients who were not assessed in the middle time point). Vertical arrows suggest that the worst knee progressed with no change in the other side. Horizontal arrows would suggest that the worst knee was unchanged and better knee was progressing, tending towards symmetry. Oblique arrows would suggest that the both knees were progressing with time.

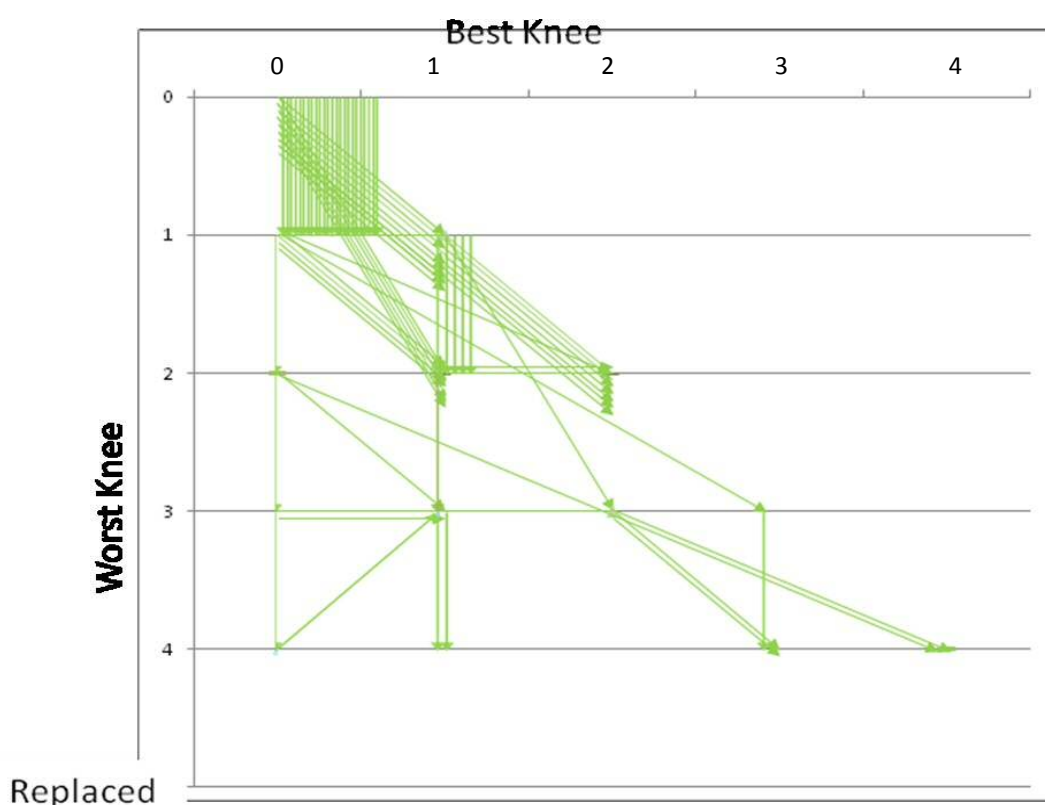


Figure 3.5. Arrow chart representing flow of radiographic data between baseline and 5 years

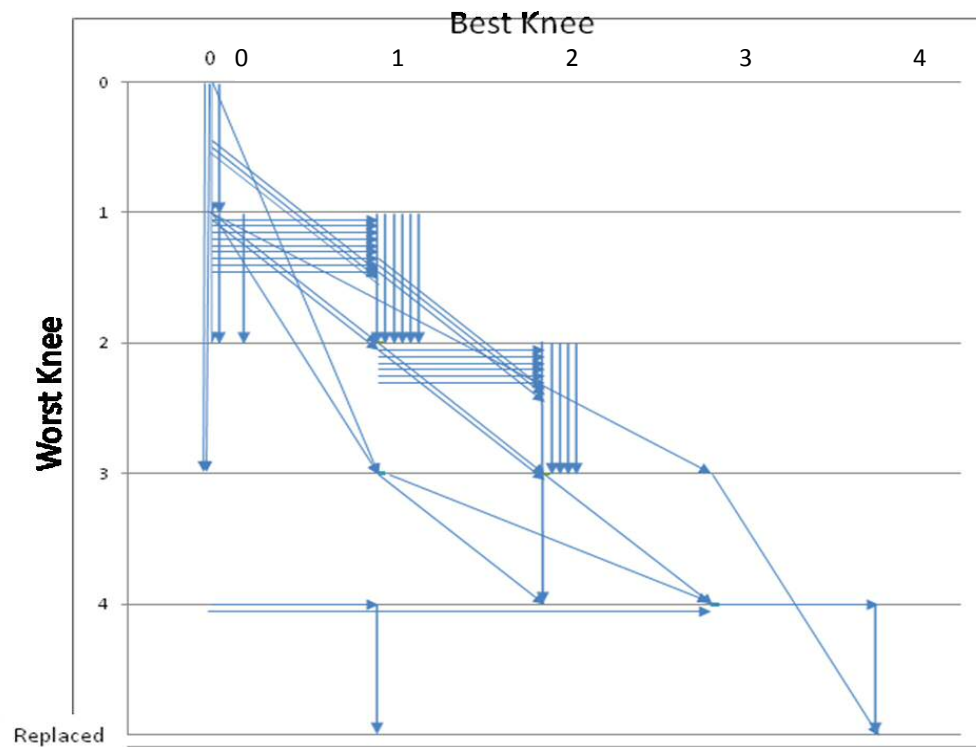


Figure 3.6 Flow of data from 5 to 12 year follow up.

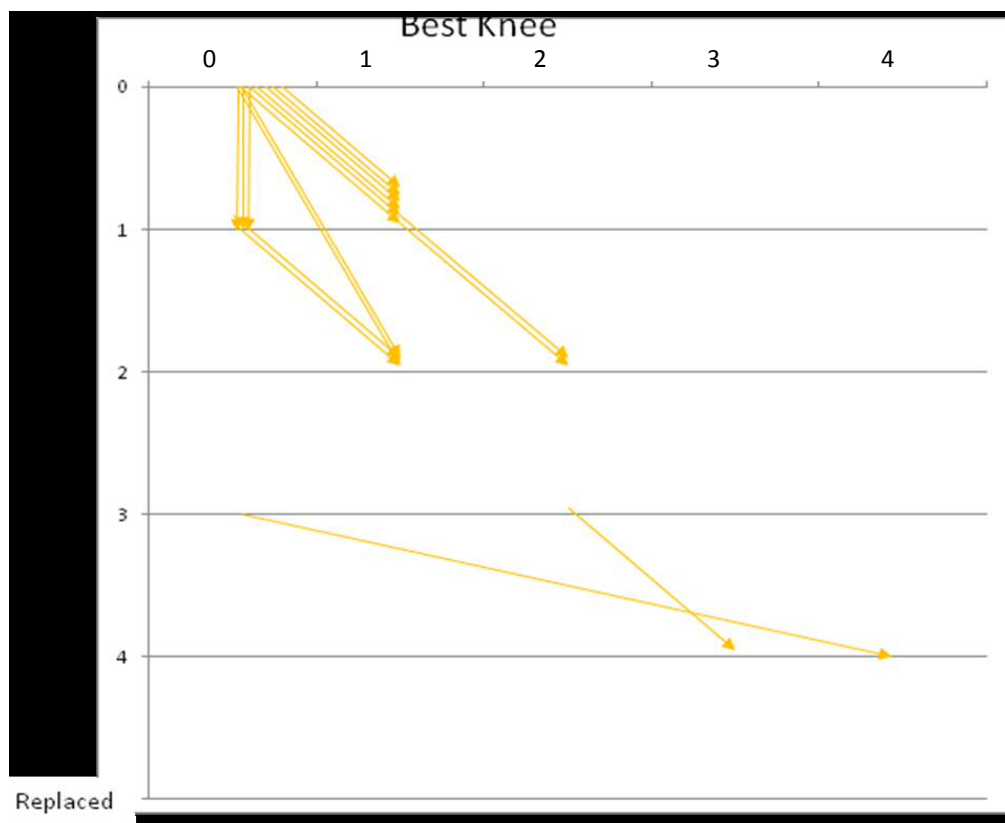


Figure 3.7. Arrow Chart representing the flow from baseline to 12 years in the 18 patients who were not seen at the 5 year follow up time point.

Age, gender, or BMI>30 had no influence on the development of bilateral as opposed to unilateral disease (p-value for age=0.48, relative risk for a female=1.2, p=0.27, Relative risk for BMI>30=0.97, p=0.87). The presence of bilateral pain at baseline was not predictive of the development of bilateral, as opposed to unilateral disease (relative risk=0.92, p=0.66).

Of those patients with bilateral disease at 12 year follow up, 73 patients had predominantly medial compartment disease in both knees, 6 patients had medial compartment disease in one knee and lateral compartment disease in the other knee whereas only 2 had predominantly lateral disease in both knees. Twenty-five patients had equal scores in the medial and lateral compartment of at least one knee.

### 3.3 Discussion

#### 3.3.1 Overview of findings

In this population based middle-aged cohort, a majority had early disease (i.e. joint pain with no or minor radiographic changes) at baseline. Despite this, 70% had bilateral radiographic changes 12 years later. Of those with unilateral knee OA at baseline, 80% developed bilateral disease over 12 years. OA may have an asymmetrical onset but it has a tendency to affect both joints with time.

Previous work on the Spenshult cohort has shown that knee pain is the first sign of knee OA (Thorstensson, Andersson et al. 2009), and the results from the present study add to this message that early knee OA, without any known previous injuries to the anterior cruciate ligament or the meniscus, in most cases is a bilateral disease. However, it is important to note that cause cannot be established on the basis of these findings.

Medial compartment OA was the most common finding at 12 years, and this is very much expected. However, it was interesting to find that even amongst patients with lateral compartment changes in one knee, medial compartment disease was more common in the other knee. Numbers were too low to examine statistically and would need to be confirmed in larger cohorts, but this may be an interesting sub-group to study in the future.

### 3.3.2 Limitations of the study

It may be argued that the contra-lateral knee is not necessarily free of disease in patients with unilateral radiographic disease using standard radiographic protocols (Mazzuca, Brandt et al. 2003). However, the development of incident radiographic OA in this joint does demonstrate significant progression of the disease process and therefore remains a relevant end point.

The change in pain over the 12 year period was not considered during this study. This is because relatively little information was collected about pain at each time point, as the main focus of the cohort was on radiographic disease.

It may also have been difficult for subjects to clearly remember whether pain was unilateral, or bilateral, as the way pain is recalled and described is known to be very variable amongst individuals (Litcher-Kelly, Martino et al. 2007). It was therefore concluded that including the measure of pain into the analysis could only serve to confuse the study and over-complicate the analysis.

The variable relationship between pain and radiographic disease is well documented (Kellgren 1961; Neogi, Felson et al. 2009; Thorstensson, Andersson et al. 2009). However, more severe radiographic OA is associated with increased frequency and intensity of pain, as well as reduced physical function (Neogi, Felson et al. 2009; White, Zhang et al. 2010). The major focus on radiographic disease in



this study is therefore clinically relevant, as bilateral structural disease progression would be expected to result in increased pain and functional decline over time.

The lack of association between gender, age or obesity and bilateral OA needs to be interpreted with caution, since the current study did not have sufficient numbers to separate out the effects of these factors properly. As this was a population based cohort that would traditionally be considered at low risk for OA, with a high proportion of males, low levels of obesity and a low age range compared to most OA population studies, the high incidence of bilateral radiographic disease after 12 years was striking.

A recent analysis of a large longitudinal cohort of patients at high risk of OA found that bilateral knee pain was an independent risk factor for poor physical function, even when pain severity was accounted for (White, Zhang et al. 2010). The authors of this paper speculated that this may be due to the loss of a 'good limb' to compensate during functional activities. Given the high frequency of bilateral disease after 12 years in the current study, it is likely that the development of disease in a second joint is a significant cause of additional disability in the population.

#### 3.3.4 Implications and Conclusion

These findings have implications for future research in OA but also for clinicians. Researchers need to remember to account for both knees when assessing the

relationship between pain, function and structural disease. The non-affected knee should not be used for comparison as a control.

Clinicians should be aware that the presence of OA in one knee is likely to herald a process that also involves the other side in the future. The opposite knee should be considered a 'joint at risk'.

Future interventions aiming at slowing the progression to bilateral disease may well be of benefit to the patient with OA. In order to do this, it is important to understand the modifiable and non-modifiable factors that contribute to the process.

Whereas joint injury (bony or soft tissue) usually affects one joint alone, there are many reasons why knee OA would tend to progress to bilateral disease. Genetic influences and inherent mal-alignment would both be expected to lead to bilateral disease (Sharma, Lou et al. 2000; Valdes, McWilliams et al. 2010).

However, patients frequently tell us in clinic that they feel that changes in the way they walked lead to them 'overloading' the opposite joint to protect the first. At present there is no basis to either accept or reject that theory – it has to be tested.

The mechanical contribution to multiple joint disease is poorly understood but it is a factor which may be amenable to treatment. This thesis represents one of the first attempts to address this question.

As such, the global aim of this thesis is to give an initial insight into loading of the other joints during walking, to identify areas which may be amenable to treatment,

and to plan further study into this important but under-represented part of the disease process.

## **Chapter 4. Abnormal Loading of the Major Weight Bearing Joints in Knee OA**

## 4.1 Introduction

Multi-joint OA is a common and disabling problem. We have seen from both the literature review and the previous chapter that bilateral knee OA is a very common problem which develops over time in the majority of people with knee pain (chapter 3). Bilateral knee OA (as opposed to unilateral disease) is an independent risk factor for poor physical function and disability (White, Zhang et al. 2010). Hip and knee OA often co-exist and there may be a mechanical explanation for the association in many patients (Shakoor, Block et al. 2002; Shakoor, Hurwitz et al. 2003).

There are many reasons why OA would tend towards bilateral involvement. Genetic factors clearly play a significant role, particularly in determining the vulnerability of a joint to mechanical injury (Felson 2004; Spector and MacGregor 2004). Mechanical factors are known to play a significant role in the initiation and development of knee OA (Miyazaki, Wada et al. 2002; Amin, Luepongsak et al. 2004; Andriacchi, Koo et al. 2009).

Patients frequently complain that gait changes due to OA cause them to subject the unaffected joints of the other limb to abnormal loads, leading to new disease in previously healthy joints. However, there is little objective information in the literature to determine whether this actually happens, or to understand the mechanism by which it occurs.

Knee OA is associated with a number of changes in gait pattern. These include changes in the ground reaction force, reduced gait speed, an atypical loading response, and proximal adaptations such as increased trunk lean or swing (Hurwitz, Ryals et al. 2000; Jones 2004; Mündermann, Dyrby et al. 2005; Hunt, Birmingham et al. 2006; Astephen, Deluzio et al. 2008; Hunt, Birmingham et al. 2008; Andriacchi, Koo et al. 2009; Briem and Snyder-Mackler 2009). It is reasonable to assume that these changes might also impact upon the other limb.

Adduction moments are generally abnormal in patients with knee OA and high moments have been linked to increased rates of both development and progression of OA, as well as pain and structural changes (Hurwitz, Sumner et al. 2001; Miyazaki, Wada et al. 2002; Amin, Luepongsak et al. 2004; Bennell, Creaby et al. 2010; Kito, Shinkoda et al. 2010). Muscular co-contraction of the quadriceps and hamstrings has also been identified as a cause for excessive joint loading and pain in patients with knee OA, although there have been no studies examining the levels of co-contraction bilaterally in this patient group (Lewek, Rudolph et al. 2004; Ramsey, Briem et al. 2007).

Previous gait studies have identified abnormal loading of the ipsilateral hip and ankle in patients with knee OA, presumably due to changes in gait (Mündermann, Dyrby et al. 2005; Astephen, Deluzio et al. 2008). Whilst some gait analysis studies have provided data on the other leg, healthy control groups were not used and the other leg was often used as an internal control, which may be a source of error

when interpreting those findings (Hunt, Birmingham et al. 2006; Briem and Snyder-Mackler 2009).

The aim of this study was to determine whether the contralateral knee and both hips are abnormally loaded in patients with single joint knee OA during normal level gait and to explore the mechanism by which this occurs.

The null hypothesis to be tested was:

**There is no difference in the adduction moment impulse and quadriceps-hamstring co-contraction between normal individuals and subjects with unilateral knee osteoarthritis.**

## 4.2 Materials and Methods

### 4.2.1 Study development and management

An initial literature review was performed to explore previous studies into loads in other joints in individuals with knee OA. This search found very little that specifically dealt with the hypothesis, but confirmed that gait analysis as a methodology could be used to study joint loads in an accurate and relevant manner. A preliminary protocol was prepared with advice from experts in gait analysis research at the Orthotic Research and Locomotion Assessment Unit (ORLAU) at the Robert Jones and Agnes Hunt Orthopaedic Hospital in Oswestry (RJAH).

Given that the study was addressing an aspect of knee OA on which there was very little literature, it was decided that the investigation should begin with patients with severe disease, on the presumption that they would have more severe gait changes. This would be increase the chances of detecting an abnormality given that this first study would be in a relatively small number of subjects.

Whilst there are many ways of defining severity in knee OA, the most pragmatic definition of the severe group is to select patients that require knee arthroplasty. To be placed onto the knee arthroplasty waiting list requires both a patient and a clinician to accept that a disease is 'end stage' and it was therefore decided that this would be a clinically relevant and logical cohort to start the research with.



Following a detailed literature review a full study protocol was written. This is contained within Appendix 1. The only two deviations from the initial protocol has been the use of the adduction moment impulse as the primary outcome measure, a result of progress in the literature since the time the protocol was initially written (specifically the work of Bennell et al 2011), and a change in the statistical analysis away from controlling for gait speed using ANCOVA, as discussed in sections 4.4 and 5.4 and recognised more recently in a review of the topic (Bennell, Bowles et al. 2011; Astephen Wilson 2012).

An application was made to the North Wales NHS Trust (Now the Betsi Cadwaladr University Health Board) for research funds of £7400, which was awarded. This covered the costs of the gait analysis for all subjects and travel expenses were offered to all participants. This was a significant cost as the patients travelled from across North Wales, sometimes as far away as Colwyn Bay (a 3 hour round trip to Oswestry). The funds were held and managed by the Trusts Finance Department although this was monitored to prevent any overspend and to forward travel claim forms. The North Wales NHS trust also agreed to act as Sponsors and a Good Clinical Practice online course in research governance was undertaken.

A Local Research Ethics Committee (LREC) application was made with the author as the Chief Investigator for the study and Principle Investigator for the North Wales NHS Trust site. This involved developing the detailed study plan, writing a full set of documents for patient recruitment and generating information sheets covering the procedure of the study as well as storage and use of the data. At the first meeting,

the committee requested a number of small amendments, and subsequently approval was granted by the sub-committee following minor amendments.

Approval was received from both North Wales and RJAH Research Departments and Site-Specific Approval was granted by the relevant LREC's at the first sitting (this process has since changed to merge R&D approval and Site-Specific Approval into one, but the two were still distinct processes at the time of starting the study). All consultants who performed knee arthroplasty surgery at the two sites were approached for verbal permission and the Clinical Lead from each department approved the study formally as part of the LREC and Site-Specific Approval.

#### 4.2.2 Recruitment and patient selection

The recruitment process was performed with the assistance of Mr Alex Dodds, a Registrar based in Wrexham at the time of the study, at the two sites of the North Wales NHS Trust (now the Central and Eastern Sites of the Betsi Cadwaladr University Health Board). The central site incorporates Abergele and Glan Clwyd Hospital and the Eastern site is Wrexham Maelor Hospital.

The knee arthroplasty waiting lists (total and unicompartmental) were obtained from consultant secretaries and booking clerks on a monthly basis. The radiographs of 610 consecutive patients on these waiting lists (over a 9 month period) were screened, and clinical notes were requested and examined if radiographs were not clear. Patients with recorded joint disease, surgery, trauma or joint replacement in

any lower limb joint other than the single affected knee were excluded from the study.

Medial compartment OA is a common indication for replacement and is known to have a significant effect on overall gait pattern (Mündermann, Dyrby et al. 2005; Hunt, Birmingham et al. 2006; Briem and Snyder-Mackler 2009). The inclusion criteria that patients should have predominantly medial compartment disease was also applied (mild patello-femoral or lateral compartment changes were not exclusion criteria, as long as they were less severe radiologically than the disease in the medial compartment). As different surgeons have varying indications for unicompartmental knee replacement (Beard, Holt et al. 2012), patients listed for both total and unicompartmental knee arthroplasty were included in the study, as both groups could be defined as having end-stage medial compartment OA.

Where no contra-indications could be found from the radiograph review, telephone numbers were accessed from the hospital patient information system with the assistance of the secretarial staff. Access to this information had been discussed in the LREC application and had been formally approved by the North Wales NHS Trusts information manager.

One hundred and fifty seven patients were subsequently contacted by telephone and those reporting no history of joint pain in any other joint, no thoraco-lumbar or cervical spine pain, and no concurrent neurological disorder were invited to attend for a screening interview.

Telephone calls were typically made between the hours of 5pm and 7pm in order to maximise the chance of successfully making contact with an individual. If there was no answer, further calls were made weekly and if there had been more than three failures to get through then telephone numbers were re-checked with the information system. No number was called more than four times to prevent complaints that patients were being telephoned excessively.

The telephone conversation always followed the same pattern. Patients were told that the call was from an Orthopaedic doctor, that it was about research and a few minutes of their time was politely requested. They were then asked if they had pain in any other joint other than the one due for operation, or if they had any other problems with their legs or back, or previous operations or injuries. Even slight knee pain or ache ("I don't have any pain, just a little niggle" was a common reply) in the other knee was considered a contra-indication to the study. If they reported no pain at all in any other joint than the affected one, then the details of the full study were explained to them, permission was gained to write to them with more details, and they were invited to a screening interview. The telephone calls typically took 5 minutes per call (15 minutes for those invited for screening) but could be significantly longer depending on the patient.

Twenty three patients attended the screening interview at either Abergele hospital or Wrexham Maelor Hospital. Prior to the interview, the address of the patient was collected from the hospital patient information system and a cover letter and two information sheets (one for the study and one for data storage) were posted to the

patient, although fresh copies of these were also provided at the interview in case they had been lost.

The interviews were organised on a weekly basis (if there were patients were to be seen that week) in an afternoon on days that the outpatient department had spare available rooms, with full permission from the senior sister in charge of each outpatient suite. As these were research interviews in which the patient was helping the doctor, and in which a full examination needed to be accompanied by a full explanation of the study and formal consent, significantly more time was required for each appointment than was usually needed for a clinic appointment. The maximum number of patients seen in an afternoon was therefore three, although most clinics were with one or two patients at a time due to the rate of recruitment.

Travel expenses were paid for the clinic appointments as well as for the gait analysis and were also paid to those who attended the clinic but were subsequently excluded. These were organised by calculating the mileage formally and submitting this information to the trust who paid expenses on a per-mile format.

At interview, inclusion and exclusion criteria were checked and an examination of both knees was performed, as well as both hips, feet and ankles and a screening examination of the lumbar spine. If the study criteria were satisfied, informed consent was obtained and WOMAC and Oxford scores were collected (Bellamy, Buchanan et al. 1988; Dawson, Fitzpatrick et al. 1998). These scores were used because they had both been used and validated in patients with severe knee OA.

They have both been widely reported in the literature and have been used in related studies, making the results easier to interpret in the context of the wider literature (Astrophen, Deluzio et al. 2008; Hatfield, Hubley-Kozey et al. 2010; NJR 2010).

Radiographs of the affected knee were reviewed, the tibio-femoral angle was measured and disease severity was classified by the Kellgren-Lawrence scale (Kellgren, Jeffrey et al. 1963).

The inclusion criteria were patients with predominantly medial compartment disease (determined on radiographs) who were on the knee arthroplasty waiting list for unilateral total or unicompartmental knee replacement. The exclusion criteria were: Any history (past or present) of pain or discomfort in any other lower limb joint than the one due for replacement; current lower back pain; previous surgery or trauma to the lower limbs, pelvis or spine (except diagnostic arthroscopy of the affected knee); medical co-morbidities limiting walking distance or affecting gait; previous stroke or neurological disease (including diabetic neuropathy); unable to travel to the gait laboratory; age over 85; and BMI over 40.

Twenty subjects conformed to the inclusion and exclusion criteria and were included in the study. Three others were interviewed of which one had evidence of sciatica on examination, one had pain in the contra-lateral knee on examination and one patient had decided that his affected knee was not severe enough to warrant replacement. The recruitment process was performed over a 9 month period in total.

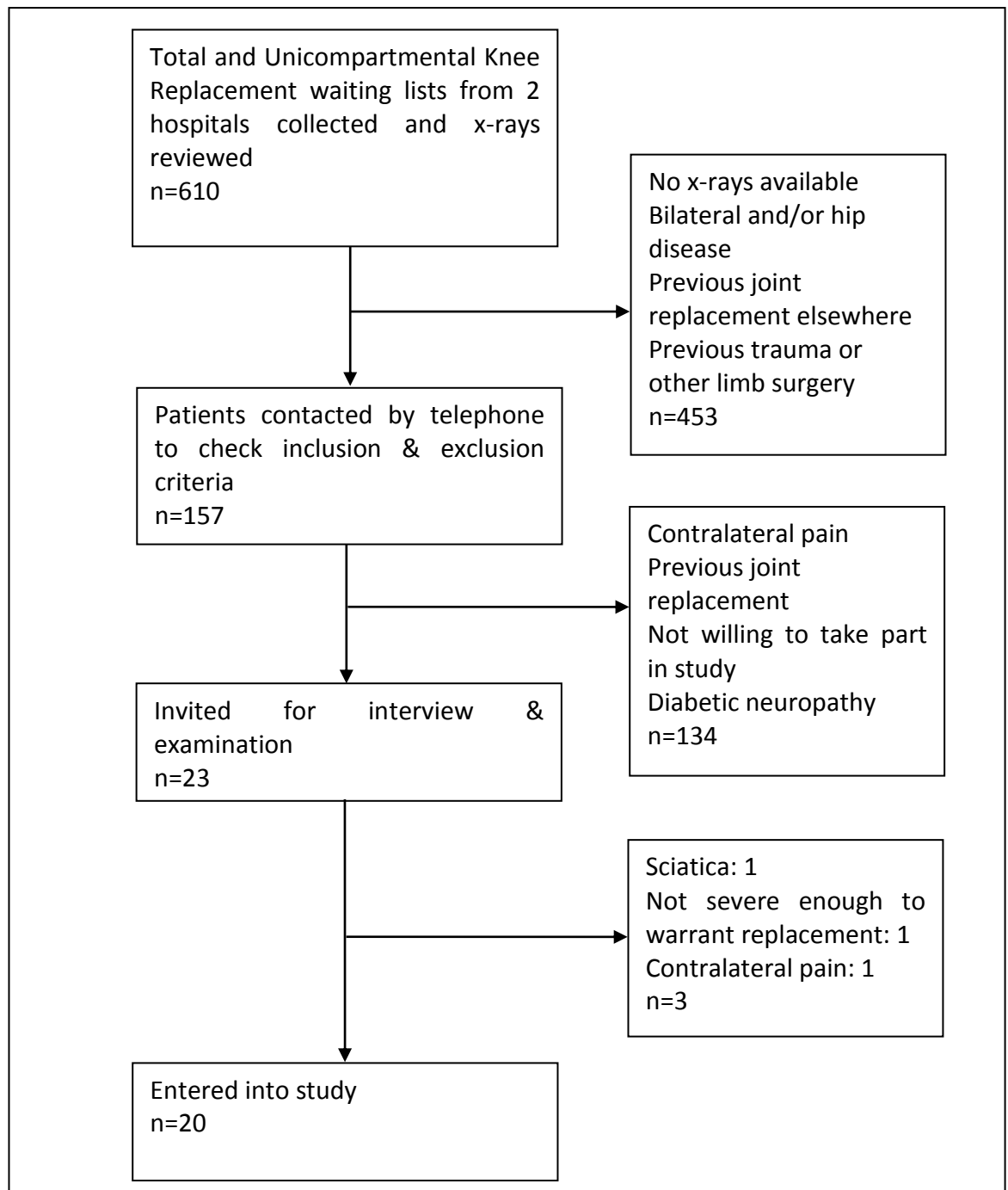


Figure 4.1. Flow-chart of recruitment to the study.

For a control group, twenty healthy subjects between the ages of 60 and 85 were recruited from community advertisements. They were assessed by the Physiotherapy team in ORLAU to confirm that they were free of disease, pain or other abnormality that may have affected their gait, and were consented at the

time of the assessment, both for the analysis and for data storage and ongoing use of the data in ORLAU. This was funded through ORLAU as part of the unit's process for collecting normal healthy data which can be used to interpret clinical gait analyses. The control subjects all had no history of lower limb pains or disorders, no history of stroke or neurological disease and all had BMI's of less than 40.

#### 4.2.3 Gait Analysis Protocol

The gait analysis was performed at the Orthotic Research and Locomotor Assessment Unit (ORLAU) at the Robert Jones and Agnes Hunt Orthopaedic and District Hospital, Oswestry (RJAH). The marker placement and collection of gait data was performed by trained technicians and specialist physiotherapists working in teams of two for each analysis. The technicians and physiotherapists are regularly assessed in terms of their ability to re-produce marker placements and to perform consistent and reliable gait analysis. This is part of the quality assurance process in ORLAU and meant that the study was performed in as consistent and reliable way as possible. ORLAU as a department is run to comply with ISO 9001, and to achieve this standard regular internal and external audits of the quality and reliability of data collection are performed.

In order to achieve this level of precision, the author would have had to undergo a prolonged training period on 'dummy' subjects who could not have been used for the study, which has a substantial cost given that the study was performed in a busy clinical laboratory. The effect of this would have been that the recruitment



process would have been curtailed early. It was therefore decided that the physical gait analysis should be performed by the technicians and engineers in ORLAU.

Training was undertaken to understand the process from the start of the study since a thorough knowledge of the study protocol was required for the LREC application and consenting process. Gait analysis sessions were attended at ORLAU and teaching was received from Dr Stewart and Dr Postans. The marker set and technicalities of EMG skin surface electrode placement was reviewed in detail, which was also important to understand for the study process.

Gait sessions were attended in the Motion Analysis Laboratory in the School of Engineering at Cardiff University. Proficiency was gained to enable the author to act as part of the data collection team. A gait analysis session always requires at least two individuals – one to instruct the patient and place markers, and one to run the computer and check that the data has been collected correctly with an adequate number of force plate contacts. Markers from the Cardiff data set were tracked in 3D using Qualisys QTM software (Qualisys, Sweden) and the full analysis process was performed. Although this data has not contributed to the thesis it formed part of the training process.

The ORLAU laboratory has 12 Vicon Mx2 Cameras sampled at 100Hz. Three AMTI force plates are built in to the floor, sampled at 1040Hz, in the centre of a 12 metre walkway. All assessments were supervised by a physiotherapist who recorded basic anthropometric measurements at the start of the study, such as height, weight and leg length.



Figure 4.2. Photograph (front and side views) of the marker set used in this study as well as EMG lead positioning (protocol given in Appendix 2). Formal written consent has been taken for the use of these images.

The Plug-in-Gait (Vicon, Oxford, UK) marker set was used for the lower limbs (Davis, Gage et al. 1991). The marker set is outlined in figure 4.2 and the formal protocol is given in Appendix 2. Trunk markers were added as a result of recent studies which showed that trunk sway was an important compensatory mechanism in knee OA patients (Mündermann, Asay et al. 2008; Briem and Snyder-Mackler 2009). The

trunk was defined by a marker on C7 and two markers on the sternum (manubrium sternum and xiphisternum).

A standing trial was performed initially with the subject standing on the force plate facing in the direction of motion. A knee alignment device (a large clamp embedded with markers) was positioned at the epicondyles to define the epicondylar axis and knee centre. The alignment device was removed and a lateral epicondyle marker was placed in its place. Subjects were then requested to walk at self-selected speed along a level walkway. This was done barefoot. Walking trials were continued in both directions in the laboratory and full contact with the force plate was discreetly noted by the technician so as not to disturb the pattern of gait.

In order to achieve a force plate strike by a particular foot, the start point of the walk is adjusted by asking the subject to start the walk with their toes touching a particular coloured line (which are spaced 10cm apart). Assuming a subject has a consistent stride length (not always true, but relatively consistent) then if they start at a certain point then a force plate strike during natural gait can usually be achieved.

Six trials were recorded for each limb with an adequate foot strike on a force plate (this typically took between 10 and 20 walks to achieve). Markers were then removed and the EMG assessment was performed.

Skin preparation was performed with alcohol wipes, although if a leg was particularly hairy a small patch of skin was shaved to achieve a good contact (this was included in the consent for the study). The electrodes had a small space for

conductive gel which was placed in the centre of the electrode before application. The skin surface electrodes were placed over the palpable muscle bulk of the vastus medialis, vastus lateralis, semitendinosus and biceps femoris muscles of both legs using the laboratory standard anatomical reference points (ORLAU Quality Assurance Manual MAS OP 112, Oct 2002 Appendix 2). Leads from each electrode were attached to a small (<1Kg) backpack attached to a belt worn by the patient. Six walking trials were performed on a level walkway at self-selected speed. Electromyographic data was collected at 1000Hz for a minimum of three strides per walking trial and two of those strides were selected (the first two with complete cycles, unless there was an obvious error in the data) to give 12 gait cycles for each EMG assessment.

#### 4.2.4 Data Processing

Markers were tracked and processed using Vicon Nexus software (Vicon, Oxford, UK) with Plug-in-Gait by Dr Stewart, and extracted using Vicon Polygon (Vicon, Oxford, UK).

Forces were normalised to weight and expressed as percentage of body weight (%Bw). External moments and impulses were calculated and normalised to height and weight. Moments were expressed as Newton·metres/(Body weight·height) percentage and impulses as Newton·metre·seconds/(Body weight·height) percentage as used widely elsewhere in the literature (Mündermann, Dyrby et al. 2005; Bennell, Bowles et al. 2011).

Whilst the software automatically normalises for weight, normalisation for height was performed in the secondary processing stage using Excel (Microsoft, USA). It was decided that moments should be normalised to both height and weight as the moment is a product of the ground reaction vector acting on a lever arm formed by the thigh and shank segments. The magnitude of the ground reaction vector is proportional to the weight of the individual and the length of the thigh and shank are proportional to height. Therefore, for the purpose of comparison between two groups, both factors were accounted for when normalising moments.

Mid-stance was defined as 50% of the stance time (between heel strike and toe-off) and calculated for each limb individually. Adduction moment impulses were calculated in Excel by integrating the whole of positive section of the curve (exported in 2% points by Polygon) between heel strike and toe-off and multiplying it by 2% of the stance time. Whilst there are numerous definitions of the moment impulse in the literature, the calculation used was based on the definition used by the group with the strongest longitudinal data that was current in the literature (Bennell, Creaby et al. 2010; Bennell, Bowles et al. 2011).

Knee extension during gait was considered as a possible explanatory factor, and was calculated as the minimum value on the knee flexion-extension curve.

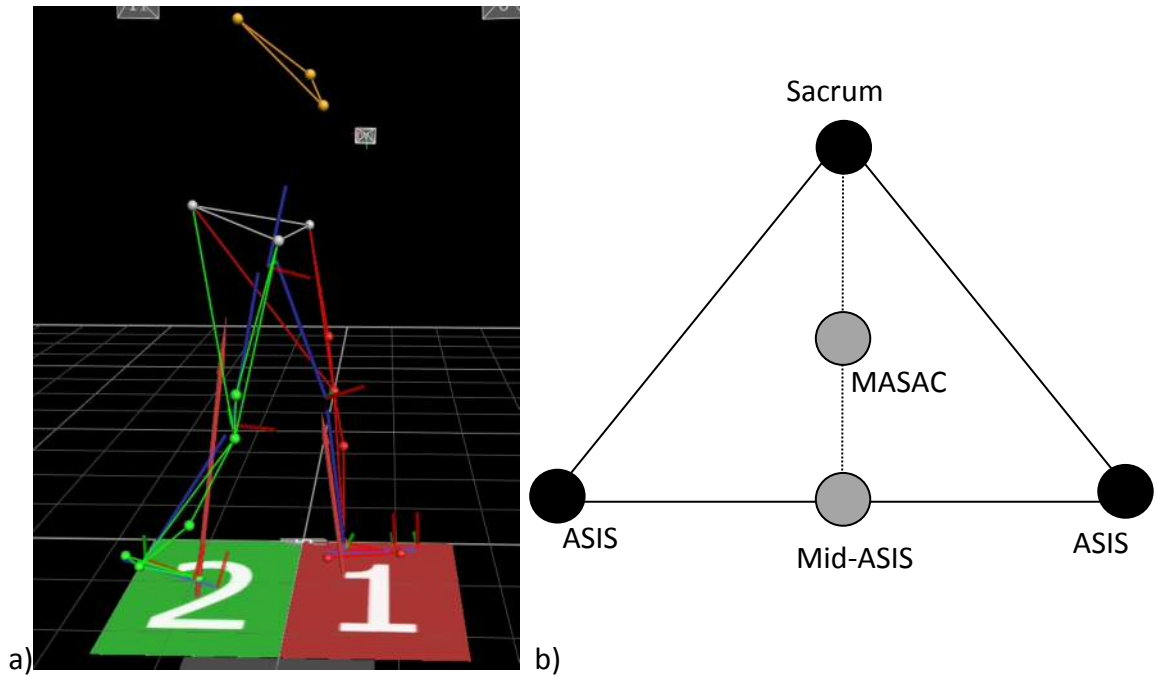


Figure 4.3. a) Image from Vicon Nexus of the marker set including the trunk markers, b) Schematic of virtual markers created at the pelvis, used to calculate trunk sway. The black markers are true markers and the grey markers are virtual markers defined in the software.

The trunk was defined by a virtual marker at the midpoint between the C7 marker and the marker at the manubrium sternum (figure 4.3). An additional virtual marker was created in the pelvis, by taking the mid-point of the two ASIS (Mid-ASIS) markers and then creating a virtual marker between the Mid-ASIS point and the sacral marker, which was labelled MASAC. A vector was created between the virtual trunk marker and the MASAC marker. The Trunk angle was defined as the angle between this angle and the z-axis of the global co-ordinate system in the frontal plane. This was performed using specially designed software written in Vicon Bodybuilder by Dr Stewart and the angle was exported into Microsoft Excel. Trunk lean was defined as the mean angle throughout the gait cycle and trunk swing as the range in angle for each gait cycle.

Dynamic limb alignment in the coronal plane was calculated using a technique developed for this study using the marker data. This will be described in detail in the next section (4.2.5).

Electromyography signals were enveloped by Dr Postans using a 2<sup>nd</sup> order low-pass Butterworth-Chebyshev filter at 6Hz using institutionally designed software written in Delphi (Embarcadero, San-Francisco, USA). The maximum signal in each trial was used as a sub-maximal peak value for normalization (Murley, Menz et al. 2010). It was decided that co-contraction would be the primary measure taken with EMG as it represents a form of additional loading to the knee which would not be detected by gait analysis derived moments alone. This is especially important as muscle forces contribute at least as much, if not more, to the joint reaction force as external moments (Shelburne, Torry et al. 2006; Gardinier, Manal et al. 2013).

Co-contraction was calculated for the medial quadriceps and hamstrings and the lateral quadriceps and hamstrings separately. The signal from the least active muscle (either quadriceps or hamstrings) was divided by the signal from the most active muscle, and then multiplied by the sum of the two signals at each percentage point through stance phase [i.e.  $(\text{lower EMG signal} / \text{higher EMG signal}) \times (\text{lower EMG signal} + \text{higher EMG signal})$ ].

A mean was taken of all 100 points throughout stance phase, and that value was halved to give a value between 0 and 1, to make the value easier to interpret. This gives the following equation (Equation 1):

$$Co - contraction\ index\ (I) = \frac{\sum_{100\%}^{0\%} \left( \frac{lower}{higher} \right) (lower + higher)}{200} \quad [Equation\ 1]$$

Where the term ‘lower’ represents the lower of the two signals at each time point and the term ‘higher’ represents the higher of the two signals at each time point.

The formula that was used was similar to the methods described previously by Lewek et al and Ramsey et al, although a value was produced which represented co-contraction throughout stance rather than focusing on early stance only (Lewek, Rudolph et al. 2004; Lewek, Ramsey et al. 2005; Ramsey, Briem et al. 2007). Good test-retest reliability has recently been reported for co-contraction in patients with medial compartment OA (ICC 0.89 medially and 0.76 laterally) (Hubley-Kozey, Robbins et al. 2013).

EMG data on the control group was incomplete and the EMG equipment in the laboratory was changed before the control group were recruited. Therefore the subjects from the standard laboratory control group (who had been examined using the same EMG system as the patient group in the current study) were used as a control group for the EMG analysis. This was a cohort of twenty volunteers between the ages of 20 and 60 who were also recruited from the community with no history of musculoskeletal disease or joint pain, BMI less than 40 and no neurological disease. No kinematic or kinetic differences were noted between the gait analysis and EMG control groups and previous studies have shown either very small differences, or no differences in co-contraction between young and old adults



for level walking at preferred speed (Schmitz, Silder et al. 2009; Monaco, Ghionzoli et al. 2010).

#### 4.2.5 Development of a measurement for dynamic limb alignment

One of the concerns in the analysis of the data was the technique for measuring alignment. In order to avoid the need for unnecessary radiographs with their associated cost and radiation dose, long leg alignment films were not taken in this study and neither were views taken of the other knee. It was therefore important to correctly assess the alignment of both limbs using the gait data. This has been done before with good correlation to radiographic measurements, although both of the described techniques are cumbersome to perform practically using the available data (Mundermann, Dyrby et al. 2008; Kornaropoulos, Taylor et al. 2010).

The measurement of alignment using gait data has two major potential sources of error – estimation of joint centres and the choice of axis system used. These are described in detail in the literature review on sources of error in gait analyses, section 2.2.5.

Functional estimation of joint centres and axis definitions can be useful to reduce errors in gait analyses and a functional approach has been shown to significantly improve the ability of gait analysis to measure limb alignment (Kornaropoulos, Taylor et al. 2010). However, it requires the patient to put their hip and knee through a considerable range of motion which may be difficult or painful to perform

in pre-operative individuals (Piazza, Okita et al. 2001; Kornaropoulos, Taylor et al. 2010).

Current functional techniques include methods both for identifying joint centres and then for defining axis systems (Schwartz and Rozumalski 2005; Ehrig, Taylor et al. 2007). Both cadaver and clinical studies have shown that regression techniques can estimate the medio-lateral position of the hip joint centre with reasonable accuracy and similar errors are seen when comparing functional joint centres to standard regression based techniques (Seidel, Marchinda et al. 1995; Schwartz and Rozumalski 2005; Andersen, Mellon et al. 2013).

In order to prevent inconvenience and discomfort for the patient, the standard regression approach to estimate joint centres was used. However, it was still desirable to correct the other potential source of error, the axis definition. This has been shown to vary considerably between the standard Plug-in-Gait regression method for defining axes and functionally derived axis definitions (Schwartz and Rozumalski 2005). It was decided that a functional approach to the description of varus/valgus would allow for this potential error to be reduced.

As motion is described in Plug in Gait using a form of Euler/Cardan sequence, the same motion may be described differently depending on the location of the embedded axis system. If a rotation occurs around only one of the axes of the co-ordinate system (for example if the knee flexes around the epicondylar axis, parallel to the zx plane), then only one rotation chart will show any motion (rotation around y in this example) whereas the other two charts will show no motion. However, if

the motion does not occur around the epicondylar axis, but around an axis that is oblique to that, then exactly the same motion will be recorded in two or three charts (figure 4.4). This is called 'crosstalk', and although the same motion may be perfectly described by both systems in a Euler/Cardan sequence, it introduces an inaccuracy if data is extracted from only one of those components (Piazza and Cavanagh 2000; Della Croce, Leardini et al. 2005).

The principle underlying this was demonstrated in a technical note by Piazza and Cavanagh published in 2000 (Piazza and Cavanagh 2000). They demonstrated that a knee model composed of a single uniplanar hinge could be shown incorrectly to have a 'screw home mechanism' (i.e. coupled internal rotation with flexion) using a marker set rigidly attached to the bones and motion described in a Cardan sequence. This was because the femoral co-ordinate system was not perfectly aligned with the flexion-extension axis of the hinge, and so a coupled ab-adduction and internal-external rotation motion was registered as the hinge was flexed and extended. A mean rotation of  $6.4^{\circ}$  was required to bring the axis system in line with the correct axis of the hinge, such that a correct uniplanar representation of motion could be described.

One approach to this problem is to use a non-orthogonal axis system. The system recommended by the International Society of Biomechanics was described by Grood and Suntay in 1983 and describes three rotations based around different axes, with ab/adduction occurring around a 'floating' axis which is orthogonal to the epicondylar axis of the femur and the long axis of the tibia (see section 2.2.3)

(Grood and Suntay 1983) (Wu and Cavanagh 1995; Wu, Siegler et al. 2002). Whilst a floating axis might conceptually resolve the problem, the calculation of abduction angles in both Plug-in-Gait and the Grood and Suntay method is performed with the same equation (Grood and Suntay 1983; Kadaba, Ramakrishnan et al. 1990).

Whilst Kadaba et al and Grood and Suntay used conceptually different approaches to solving the mathematical problem of describing motion between the femur and tibia (Kadaba used a Cardan sequence, whereas Grood and Suntay linked the two axis systems by presenting a secondary non-orthogonal axis system), it can be shown that the two are mathematically equivalent for all three angles (Grood and Suntay 1983; Kadaba, Ramakrishnan et al. 1990). Whilst small differences exist between the two in the technique for establishing axis systems based on the marker sets, these are likely to be relatively minor in practical terms. Sensitivity studies have reported that the Grood and Suntay method is still prone to crosstalk error if the epicondylar axis is incorrectly defined due to poor marker placement (Della Croce, Leardini et al. 2005).

In the case of knee motion, a small amount of frontal plane motion occurs relative to the large range of flexion/extension. If the axis system is not aligned it would be easy to report a value for ab/adduction which actually is an out-of plane representation of some degree of flexion. Whilst this is technically correct when a full Euler/Cardan sequence is reported, it becomes an error when it is misinterpreted by expressing only one value (i.e. “the abduction of the knee is....”).

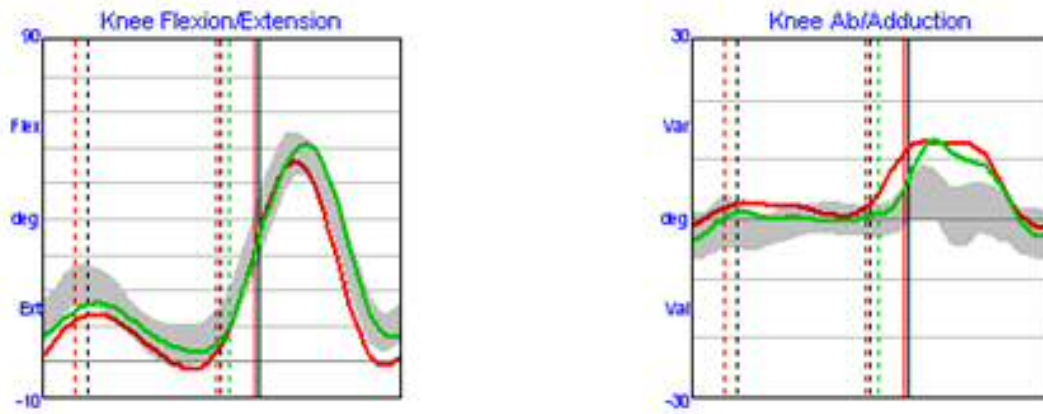


Figure 4.4. One example of flexion/extension crosstalk taken directly from the Vicon Polygon report of a subject in this study. Where the axis system does not perfectly align with the flexion/extension axis of the limb, some of the flexion motion is represented in the ab/adduction graph.

One approach to this is to assess the knee in full extension, generally performed during the standing trials. However, many knee OA patient have significant fixed flexion deformities and stand with some degree of flexion, resulting in the same problem.

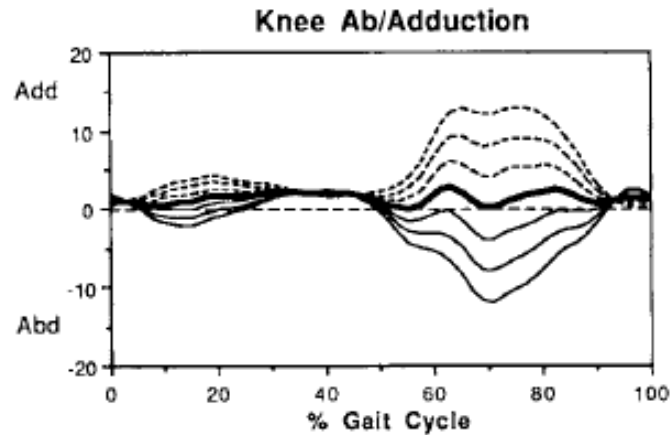
In Plug-in-Gait, the ab/adduction angle at the knee is given by equation 2, which is taken directly from Kadaba, Ramakrishnan et al (1990), where  $\theta_2$  represents the ab/adduction angle,  $-K_3$  represents the z axis of the shank segment and  $J$  represents the y axis (the epicondylar axis) of the thigh segment. As the y axis of the thigh is  $90^\circ$  to the long axis of the thigh, and arcsin has been used in preference to arccos (effectively achieving a 90 degree offset), the equation gives the angle between the long axes of the shank and the thigh, with 0 representing 'neutral' alignment.

$$\theta_2 = \arcsin(-K_3 \cdot J)$$

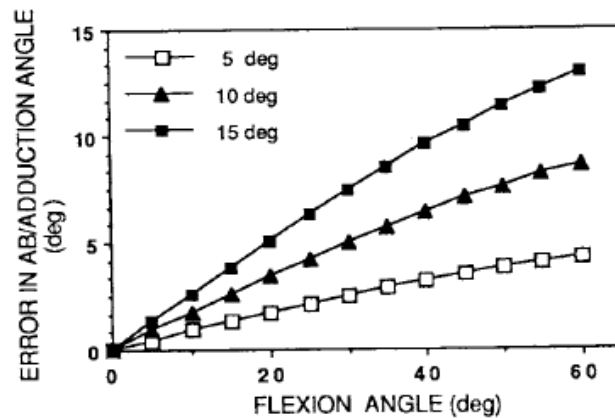
(Equation 2)

This measurement makes the important assumption that the axis has been rotationally aligned correctly. Whilst the knee flexion-extension axis should approximate the anatomical epicondylar axis it does not perfectly correspond to this and an error in the definition of that axis would result in flexion-extension occurring around an axis oblique to the computed epicondylar axis (Eckhoff, Bach et al. 2005). Accurate recording of the epicondylar axis is dependent upon the knee alignment guide being placed accurately on the centre of the epicondyles, which can be difficult points to clearly define.

The registration of the frontal plane of the knee (that is, the rotational alignment of the epicondylar axis) was one of the main concerns raised in the early papers about Plug-in-Gait written by its developers and is a well-recognised source of error in the analysis of ab-adduction angles (figure 4.5) (Kadaba, Ramakrishnan et al. 1990; Della Croce, Leardini et al. 2005). Patients with knee OA frequently walk with fixed flexion deformity and therefore this could be a significant source of bias in our study.



a)



b)

Figure 4.5. Summary of the sensitivity analysis of Kadaba, Ramakrishnan et al. 1990 (images taken from the paper) examining the risk of errors in abduction/adduction angles when using Plug-in-Gait a) With changing internal/external rotation of the epicondylar axis by 5 degrees over a range -15 to +15degrees, differences in the ab/adduction chart can be seen. This assumes a normal knee with full range of motion. b) With increasing internal/external rotation of the epicondylar axis, the relationship between error in ab/adduction angle and flexion angle of the knee is plotted.

This potential error has been dealt with differently by different authors. Mundermann, Dyrby and colleagues (2008) used in-house software to identify the frontal plane by first rotating the limb until the axis of the foot was pointing anteriorly in the laboratory co-ordinate axis system (giving an agreement of  $R=0.74$ ), although this technique is difficult to achieve with Vicon software and also is dependant upon ankle and foot anatomy which may be variable between

individuals. Kornaropoulos, Taylor et al. (2010) used a fully functional approach to derive joint centres and the rotational axis, which required the individual to pass the limbs through a series of complex manouvers before starting the analysis. Whilst this technique reported very good agreement with long leg alignment measured on CT ( $R=0.91$ ), it requires additional effort and potentially discomfort for the patient.

Rather than rely on the palpated epicondylar axis, a new technique was developed for this study to process the gait data in such a way that the measurement of ab/adduction was taken orthogonal to the functional flexion-extension of the knee. This has the potential advantage of being more anatomically relevant to the knee, and should provide a more consistent and reliable result than palpation of the epicondyles.

The aim was therefore to develop a measurement of limb alignment based on a functionally defined axis system that could be derived from gait data collected using the standard Plug-in-Gait data collection protocol.

The candidate and Dr Stewart worked together to write the following code and algorithms. First, software was written for Vicon Bodybuilder (a part of Nexus software) which could express the ankle joint centre in the thigh local co-ordinate axis system, as opposed to the laboratory axis system. The ankle joint centre was defined as the mid-point between the malleoli. The thigh local co-ordinate system has an origin at the knee centre. The primary axis of the co-ordinate system is defined by the vector between the hip centre and knee centre and the epicondylar



axis is used to define the frontal plane. This new virtual marker was labelled AJM (short for Ankle Joint Motion). Therefore, a vector between the origin (the knee centre) and the AJM marker (the ankle centre) represented the shank segment, expressed relative to the thigh.

The new Bodybuilder code was run for each individual and the AJM marker for each walk which was saved with the rest of the gait data. A single step was chosen from each individual by examining the coronal plane kinematic and kinetic variability charts (the non-averaged data taken from each individual step) in polygon to pick a trace which was fully representative of that gait session. The timing and detail of that step was recorded manually and the co-ordinates of the AJM marker for the step were exported from Polygon to Excel.

The equations used to analyse the AJM data were derived as follows. Two vectors at different time points were selected (one in extension, one in flexion) and defined as  $a$  and  $b$ . The vectors  $a$  and  $b$  were both defined by the knee centre and the AJM marker. As the knee centre was the origin of both vectors (i.e. with co-ordinates [0,0,0]), each vector can be defined by the co-ordinates of AJM, as follows:

$$a = [x_a, y_a, z_a]$$

(Equation 3)

$$b = [x_b, y_b, z_b]$$

(Equation 4)

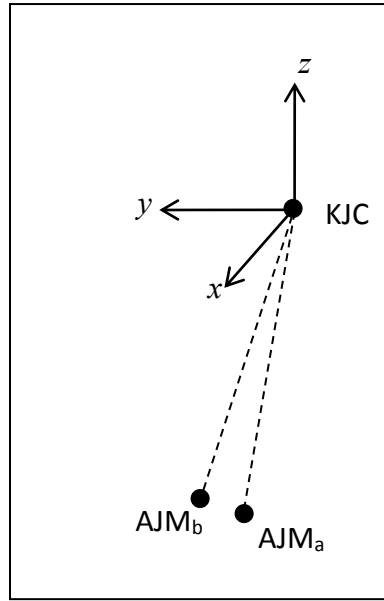


Figure 4.6. Pictorial representation of the steps taken to derive the alignment equation. The bold lines are the axis system for the thigh (z points to the hip centre), the vectors  $a$  ( $AJM_a$ ) and  $b$  ( $AJM_b$ ) are represented by dashed lines. The knee joint centre (KJC) is the origin of the axis system.

These two vectors can be combined to give a cross-product ( $a \times b$ ) (Stroud and Booth 2013). The cross-product can be thought of as being perpendicular to a plane formed by the two vectors. Its co-ordinates can be found by solving a matrix made up of the vectors  $a$  and  $b$ :

$$a \times b = \begin{bmatrix} \mathbf{i} & \mathbf{j} & \mathbf{k} \\ x_a & y_a & z_a \\ x_b & y_b & z_b \end{bmatrix} \quad (\text{Equation 5})$$

The cross-product can be defined by resolving the matrix determinant into a 3x1 matrix (based on (Stroud and Booth 2013)):

$$a \times b = \begin{bmatrix} x \\ y \\ z \end{bmatrix} = \begin{pmatrix} y_a z_b - y_b z_a \\ z_a x_b - x_a z_b \\ x_a y_b - y_a x_b \end{pmatrix} \quad (\text{Equation 6})$$

The angle  $\theta_{yz}$ , is the angle of the cross-product  $axb$  relative to the y axis in the frontal (yz) plane, which is given by:

$$\theta = \text{atan} \left( \frac{x_a y_b - y_a x_b}{z_a x_b - x_a z_b} \right)$$

(Equation 7)

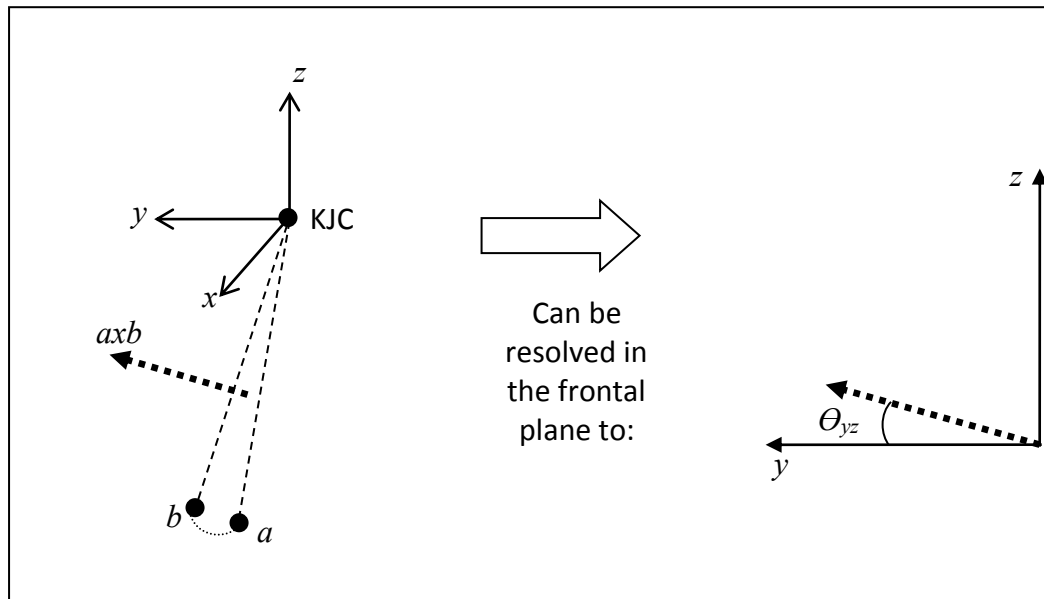


Figure 4.7. A cross product has been taken of the two vectors  $a$  and  $b$  giving a new vector  $axb$  (bold dotted lines, this has been represented as taking origin from the plane but it actually takes its origin at the knee joint centre). This can be resolved in the frontal plane  $yz$  to give an angle  $\theta_{yz}$  which is geometrically equal to the alignment angle in the frontal plane.

However, this gives the angle for in the frontal plane ( $yz$ ) which is still susceptible to mal-alignment of the axes. If the plane of flexion does not coincide with the  $y$  axis, then the value of  $y$  will be reduced and the angle will be mis-represented.

Therefore the alignment angle was formed by taking the angle between the  $xy$  plane (the horizontal plane) and the crossproduct  $axb$ . This avoids the problem of

axis mal-rotation by essentially taking the angle in the plane of the deformity (the plane perpendicular to the cross product). This was achieved by representing the projection of the  $axb$  vector in the xy plane (defined as  $l$ ), given by:

$$l = \sqrt{(x_{ab}^2) + (y_{ab}^2)}$$

(Equation 8)

Therefore:

$$l = \sqrt{(y_a z_b - y_b z_a)^2 + (z_a x_b - x_a z_b)^2}$$

(Equation 9)

Finally, the correct angle for the alignment  $\theta$  can be given by:

$$\theta = \text{atan} \left( \frac{x_a y_b - y_a x_b}{\sqrt{(y_a z_b - y_b z_a)^2 + (z_a x_b - x_a z_b)^2}} \right)$$

(Equation 10)

This was written into a format that could be used by Excel (Microsoft, USA), so that the AJM co-ordinates could be copied from Vicon Polygon (Vicon, Oxford, USA) into Excel and the formula would automatically be applied, giving a value for  $\theta$  over two time points in a single gait cycle.

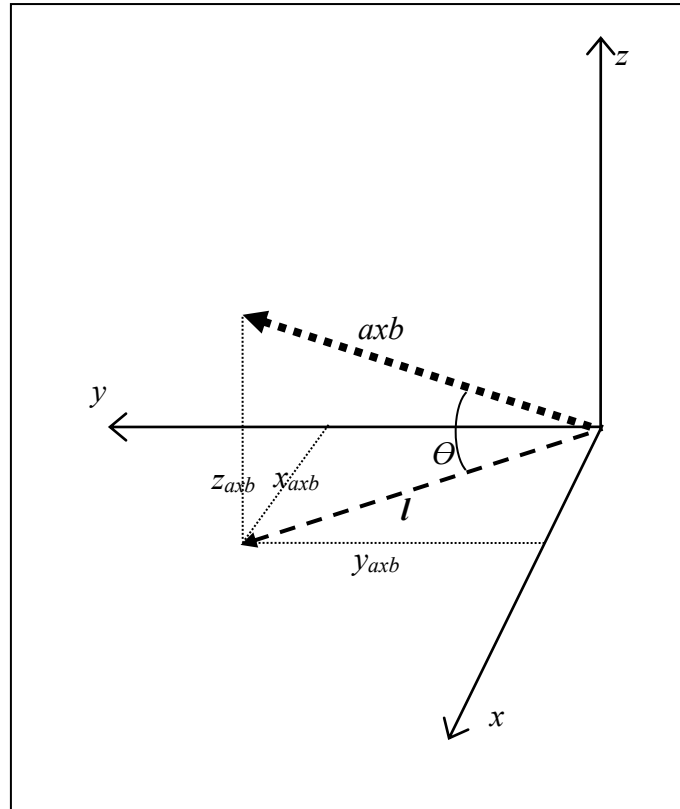


Figure 4.8. The crossproduct  $axb$  (strong black arrow) has been represented in 3 dimensions and its components have been described by the narrow dotted lines. The line  $l$  is the hypotenuse of  $x_{axb}$  and  $y_{axb}$  and is the projection of  $axb$  in the  $xy$  plane. The angle between the  $xy$  plane and  $axb$  gives  $\Theta$ , the alignment angle.

An additional angle can also be described using the same approach, representing the angle of the crossproduct  $axb$  to the  $y$  axis in the  $xy$  plane,  $\sigma$ . This is effectively the angular difference between the epicondylar axis (as defined by the original axis system) and the newly derived flexion-extension axis. Whilst this value is not of use in analysing the main study, it is useful to examine when considering the relevance and use of the new alignment measure described here.

$$\sigma = \text{atan}\left(\frac{y_a z_b - y_b z_a}{\sqrt{(y_a z_b - y_b z_a)^2 + (z_a x_b - x_a z_b)^2}}\right)$$

(Equation 11)

As discussed in the results, the most consistent result was found when the largest plane could be formed by the two shank vectors (that is, when the measurement could be taken over a wide arc of flexion). In order to do that, vectors were taken when the majority of both normal and arthritic subjects were in peak extension (40% of the gait cycle was chosen for this) and when the majority were in peak flexion. Between this range, the motion of the AJM was seen to be reasonably planar, implying that this was a reasonable part of the gait cycle to take (see results section).

As the timing of peak flexion appeared to vary between different individuals vectors were taken at each time point between 64% and 84% of the gait cycle. An angle was calculated for each one of these vectors and a mean was taken of those to give the final result and a standard deviation was also produced to check that the angle was consistent between the different calculations.

The new alignment measure was compared against what would be considered a standard measure of alignment. This could be derived either from the static trial or from the walking data. As many of the static trials were performed with the knee in some degree of flexion (for patient comfort and to ease identification of bony points), it was decided to use the walking data. As the knee is typically in maximum extension at 40% of the gait cycle, the varus/valgus (or ab/adduction) angles were extracted from Vicon Polygon and the value that corresponded to 40% of the gait cycle was used as the standard measure of limb alignment.

#### 4.2.6 Statistical Analysis

The purpose of the statistical analysis was to analyse the differences between the pathological and control groups in the primary outcome and secondary outcome measures and to explore the data using well accepted statistical methods. A more in depth statistical analysis is also presented in chapter 5.

The sample size was 40 in total (20 OA subjects, 20 controls) with data on both legs for all participants. As prior data was not available to plan the sample size for the study, a post hoc calculation was performed at the end of the study based on the experimental data. This was performed using a well accepted equation from the literature (Campbell, Julious et al. 1995; Armitage, Berry et al. 2002; Kirkwood, Sterne et al. 2003).

$$\text{Sample size of each group} = \frac{(u+v)^2(\sigma_1^2 + \sigma_2^2)}{(\mu_1 - \mu_2)^2} \quad (\text{Equation 12})$$

Where  $u$  is the one-sided percentage point of the normal distribution corresponding to (100%-the power), making  $u=1.28$  for 90% power, and  $v$  is the percentage point of the normal distribution corresponding to the two-sided significance level, making  $v=1.96$  for an  $\alpha$  of 0.05.  $(\mu_1 - \mu_2)$  is the difference between the means and  $\sigma_1, \sigma_2$  are the standard deviations for the two groups.

Using the adduction moment impulse of the unaffected knee as the primary measure (two sided  $\alpha=0.05$ ), 19 patients in each group were required to achieve a

power of 90% to detect the observed difference between the controls and the unaffected knee of the subjects.

Comparisons between arthritic and normal subjects were made with independent sample t-tests using a Bonferroni correction for multiple testing. Adjusted p-values are presented throughout the text (p-values for the outcome measures in Table 4.3 have been multiplied by 16), with an overall significance level of 5%. The data was approximately normally distributed and variances were similar, although equal variance was not assumed.

Stepwise multiple regression was used to examine potential explanatory factors for knee moments in the OA and control groups (n=40). The factors examined were: alignment, gait speed, trunk swing and knee extension during gait.

Given that gait speed has a significant effect on moments via changes in the ground reaction vector, the relationship between the gait speed and both the vertical and horizontal components of the ground reaction force was examined using Pearsons correlation coefficient.

The analysis was subsequently reviewed by Professor Peter Jones of Keele University, a biostatistician, who was satisfied that appropriate statistical methods had been used.

Data was processed using Excel 2007 (Microsoft, US) and SPSS v16.01 (SPSS Inc, Illanois, USA) was used for statistical calculations.



#### 4.2.7 Examination of component parts of frontal plane moments

Whilst the planned statistical analysis answered the primary question about whether loading was abnormal, a further question remained regarding what was driving the change in moments, particularly in mid-stance. The moment can be abnormal as a result of either a change in joint position relative to the ground reaction vector, or because of a change in the ground reaction vector itself. The ground reaction vector can also be described in three components (of which 2 have a major influence on the frontal plane moment), and so changes in either its vertical or its medio-lateral component might lead to an abnormal moment.

The purpose of this further analysis was to examine the component parts of the moment in more detail, to provide insight as to the relative influence of knee centre location and the components of the GRF on the results.

As a way of exploring the data, the raw marker and force plate data was extracted from Vicon Nexus in order to calculate the frontal plane moments at the knee from its components manually. This approach does not give a precise 3 dimensional moment as it does not account for limb rotation, the position of the reference segment, or the inertia of the limb segments themselves. Whilst this was not intended to examine a pre-identified hypothesis, it was intended that the analysis would inform further studies by giving additional insight which was not available from the standard parameters.

Gait traces were examined visually for each patient and single gait cycle was selected for each patient which was representative of their mean moment

waveform. The co-ordinates of the knee centre in the global co-ordinate system for that representative cycle were extracted from Nexus. The co-ordinates of the centre of pressure in the global co-ordinate axis system and the two components of the force in the frontal plane were extracted from the force plate data. Estimated moments were calculated according to figure 4.9 at three time points corresponding to 10%, 20% and 30% of the gait cycle (arbitrarily selected to correspond to the stages in the gait cycle around the peak moment and the most likely position of the mid-stance moment).

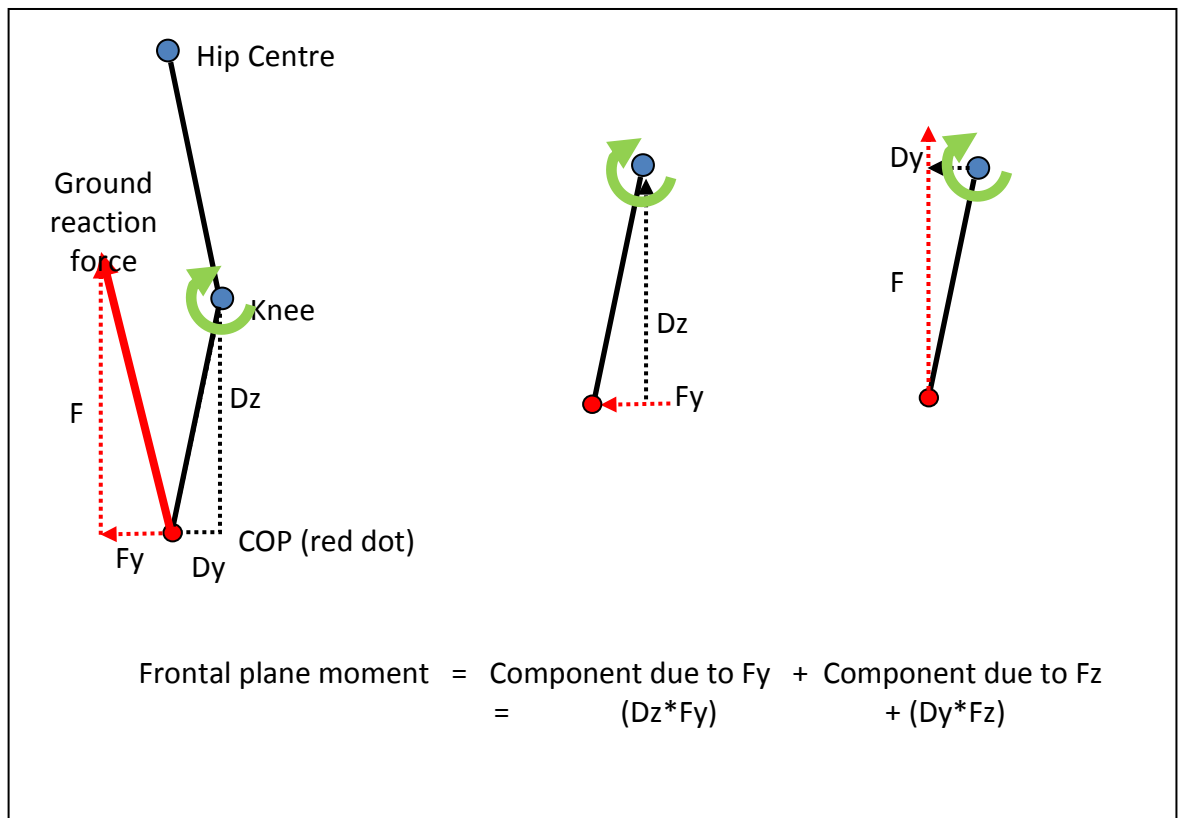


Figure 4.9. Derivation and method for calculating frontal plane moments. Green arrows represent the moments and red arrows the ground reaction vector and its components ( $F_y$  and  $F_z$ ). The position of the knee centre relative to the centre of pressure (COP) is defined by  $D_y$  (the horizontal distance) and  $D_z$  (the vertical distance). By understanding the components separately we hoped to understand more about the factors that influence moments.

Where  $D_{[x,y,z]}$  is the co-ordinates of the knee centre ( $KC_{[x,y,z]}$ ) relative to the centre of pressure ( $COP_{[x,y,z]}$ ), the formula for estimating frontal plane moments can be derived by:

The base of the centre of pressure is set at ground level, so:

$$COP_z \equiv 0. \text{ Therefore: } D_z = KC_z$$

The horizontal distance between the knee centre and the centre of pressure is:

$$D_y = KC_y - COP_y$$

Therefore:

$$\text{Estimated moment} = (KC_z \times F_y) + ((KC_y - COP_y) \times F_z)$$

Finally, the sign conventions were altered where needed to match the Vicon output (for example, to adjust for the direction of walking in the laboratory) and divided by 10 to correspond to the same units. The data was presented graphically in order to explore the relationship between the calculated moment and the true moment, as well as the relative influence of the two components of the calculated moment.

#### 4.2.8 Exploration of the effect of gait speed in normal individuals

As discussed in the results section, one of the significant factors in determining loading at the knee was gait speed. Changes in gait speed result in changes in the profile of the centre of mass in both its medio-lateral and vertical components, resulting in changes in peak and mid-stance moments at the knee (Orendurff, Segal

et al. 2004; Tesio, Rota et al. 2010). As stance time is directly proportional to gait speed, the adduction moment impulse also changes with regard to gait speed. However, kinematics are also dependant on gait speed and moments are dependent on both the GRF and the kinematics, so changes in GRF do not necessarily directly correspond directly to changes in moments (Roislien, Skare et al. 2009).

Correlations between gait speed and moments were only moderate in the current study, and there is little data in the literature to clearly tell us what happens to the moment waveform in normal individuals with changing gait speed, both in terms of its overall shape as well as its summary measures. In an attempt to understand this in more detail, a normal subject was assessed in the ORLAU gait laboratory as described in the methods above (section 4.2.3). A series of walks were performed, at normal speed, slow speed and very slow speed.

Visual assessment of the moment charts showed that the individual tested had very low knee moments overall, but that both the peak and mid-stance moments fell during slow speed gait and it was theorised that the findings in OA subjects may not have been typical of 'normal' slow speed gait but of other compensations. Therefore a further 9 normal individuals were recruited by staff at ORLAU during research sessions to undergo gait analysis at varying speed as part of a process to expand the ongoing normal data collection in ORLAU.

Each individual was screened by a physiotherapist prior to undergoing analysis to conform the absence of any lower limb pathology, deformity, pain or any

neurological problems which might affect the analysis. All subjects had BMI's of less than 35.

Markers were placed as described above with the same standing trial. Subjects were then requested to walk at their normal pace and 6 force plate strikes for each foot were recorded. Subjects were then requested to walk slowly, and a further 6 force plate strikes with each foot were recorded. Finally, subjects were requested to walk quickly and again 6 force plate strikes for each foot were recorded. Slow, normal and fast speed were all self-selected. The gait speed was not formally controlled to hit a pre-set target and no metronome was used, despite this being used in one other prominent centre, as it was believed that this create a less natural pattern of gait that would not be representative of normal gait (Mündermann, Dyrby et al. 2005).

The data was processed as described in section 4.2.3 and 4.2.4. Means were recorded for gait speed, peak and mid-stance knee adduction moment, knee adduction moment impulse, as well as peak and mid-stance values for the vertical and medio-lateral components of the GRF. These were analysed using paired t-tests primarily, which were performed in SPSS version 20.0. The data was subsequently analysed in comparison with the experimental data for the OA cohort that is presented in section 4.3.2.

## 4.3 Results

### 4.3.1 Demographics

The demographics for the OA subjects and healthy controls are given in Table 4.1. The age range was 53 to 82 (mean 69.0) for the OA group and 60 to 83 (mean 68.3) for the control group. The mean Oxford score for the study group was 25.2 out of a possible 48 (range 12-33) and the mean WOMAC score was 46.2 out of a possible score of 96 (range 31-80). Seven patients had a Kellgren-Lawrence grade of 3 in the affected knee and the remaining 13 had grade 4 changes. Sixteen patients were waiting for total knee replacements and four patients were awaiting medial uni-compartmental replacements.

	OA Subjects	Healthy Controls
Sex	9 Female: 11 Male	10 Female: 10Male
Age (years)	69.0 (7.2)	68.3 (5.9)
Height (metres)	1.66 (0.97)	1.69 (0.98)
Weight (Kg)	85.8 (10.8)	75.0 (14.8)
BMI	31.1 (3.5)	26.3 (3.6)

Table 4.1. Demographics of the OA subjects and healthy control groups, expressed as means (standard deviation).

#### 4.3.2 Gait Analysis Findings

Table 4.2 reports the differences in temporal parameters. Overall, gait speed was significantly slower in the OA group due to a combination of reduced step length and reduced cadence. There was only a small difference in stance times between the two legs (mean difference 1.8%) and stance time was prolonged in both legs. The unaffected leg was significantly different to the affected leg using an independent sample t-test ( $p=0.009$ ). The lack of a clinically significant difference and the prolonged stance time in both legs implies that the gait pattern was not typical of the traditionally described 'antalgic' gait. Trunk swing (the range of trunk motion) was increased in the OA subjects, and foot progression angle was greater on the affected limb, but the same as healthy controls at the unaffected limb of the OA subjects.

	OA Subjects	Healthy Controls	Significance
Gait Speed ( $\text{ms}^{-1}$ )	0.97 (0.23)	1.33 (0.21)	$p < 0.001$
Cadence (steps/min)	105 (11)	118 (9)	$p < 0.001$
Stance percentage Affected limb Unaffected Limb	62.6% (2.0%) 64.4% (2.6%)	60.5% (1.6%)	$p = 0.003$ $p < 0.001$
Step Width (cm)	18.9 (4.2)	15.5 (3.4)	$p = 0.004$
Trunk Swing (degrees)	2.71 (1.52)	1.41 (0.68)	$p = 0.003$
Foot progression angle (degrees) Affected limb Unaffected Limb	10.82 (5.43) 7.59 (7.77)	7.87 (4.75)	$p = 0.006$ $p = 0.53$

Table 4.2. Temporal and kinematic variables in the two study groups, expressed as means (standard deviation).

The moments, impulses and co-contraction indices are reported in Table 4.3. The mean values for both legs are recorded for the control group as there were no side-to-side significant differences seen in the healthy controls (mean knee adduction impulse for left leg 0.84; mean knee adduction impulse for right leg 0.81; paired students t-test:  $p = 0.62$ ).

Adduction moment impulses were significantly elevated for both knees and the contra-lateral hip, mid-stance moments were significantly different between groups for both knees and hips, whilst peak values were comparable between groups. These findings are best explained by the differences in moment waveform seen in the two groups (Figure 4.10 a-h).



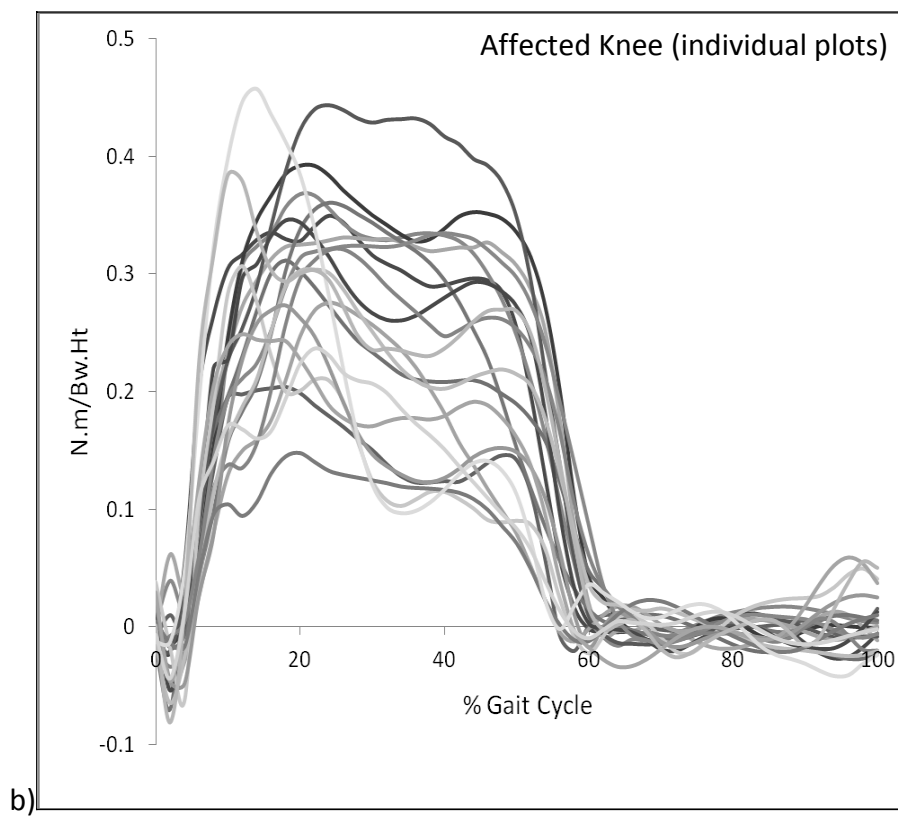
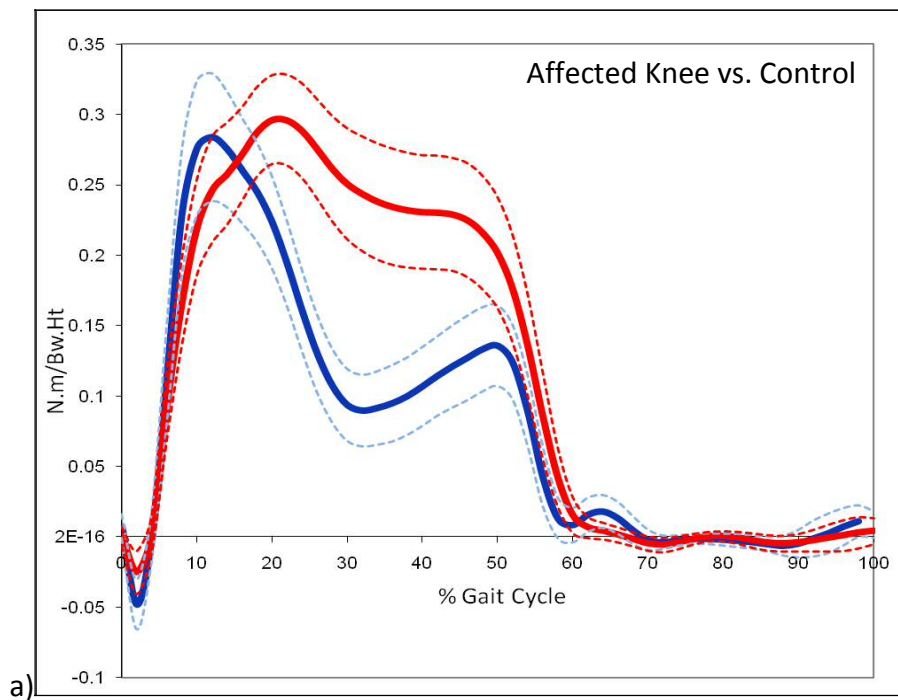
	OA Subjects	Healthy Controls	Significance (adjusted p-values)
Adduction Moment Impulse (N·m·s/BW·Ht)			
Hip			
Affected side	2.00 (0.26)	1.76 (0.13)	p=1.0
Unaffected side	2.23 (0.24)		p=0.030*
Knee			
Affected side	1.45 (0.20)	0.84 (0.12)	p<0.001**
Unaffected side	1.16 (0.15)		p=0.048*
Hip Adduction Moments (N·m/BW·Ht)			
Peak			
Affected side	4.25 (0.56)	4.71 (0.33)	p=1.0
Unaffected side	4.49 (0.41)		p=1.0
Mid-stance			
Affected side	3.74 (0.41)	2.70 (0.29)	p<0.001**
Unaffected side	3.58 (0.40)		p<0.001*
Knee Adduction Moments (N·m/BW·Ht)			
Peak			
Affected side	3.23 (0.33)	3.09 (0.42)	p=1.0
Unaffected side	2.80 (0.30)		p=1.0
Mid-stance			
Affected side	2.46 (0.40)	0.94 (0.25)	p<0.001**
Unaffected side	1.85 (0.30)		p<0.001**

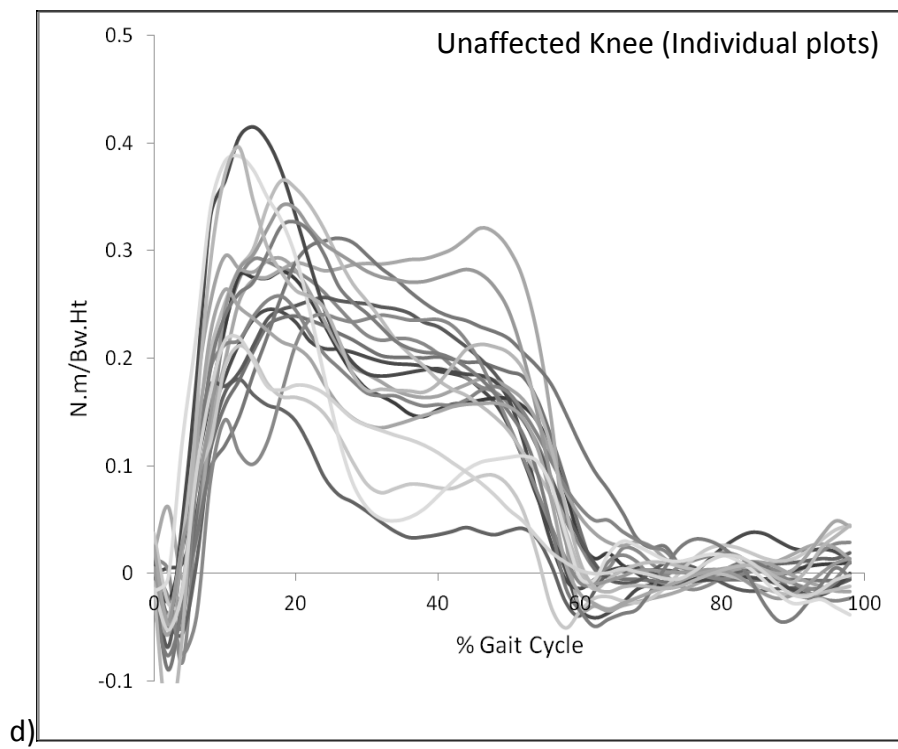
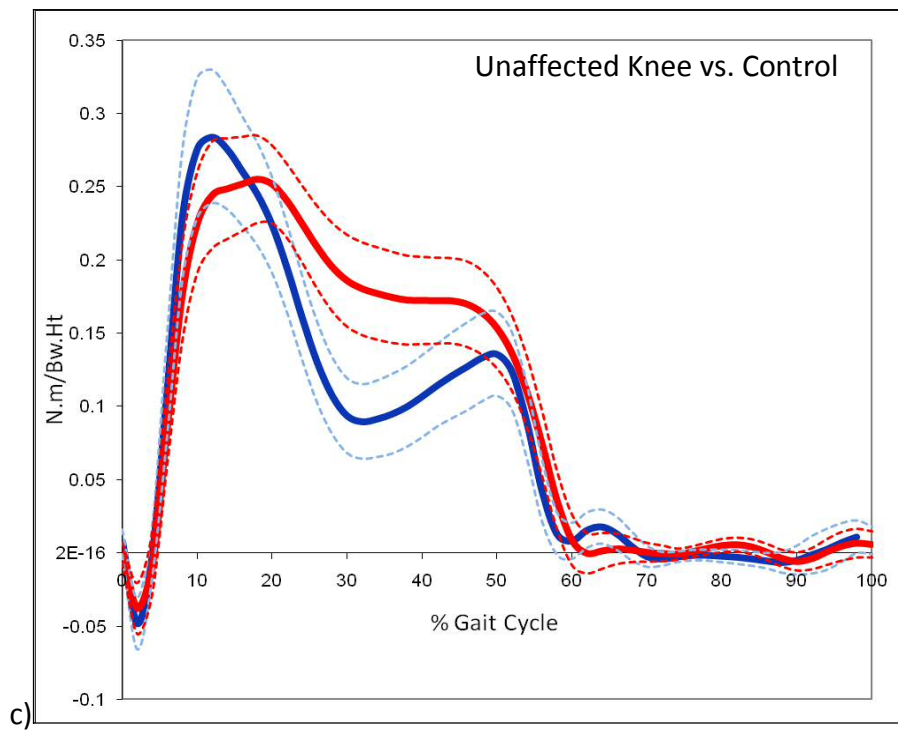
Table 4.3. Moments and moment impulses expressed as mean (+/- 95% confidence interval). Significance testing was performed using t-tests with Bonferroni correction (p-values multiplied by 16). \*=significant (p<0.05) \*\*=highly significant (p<0.01).

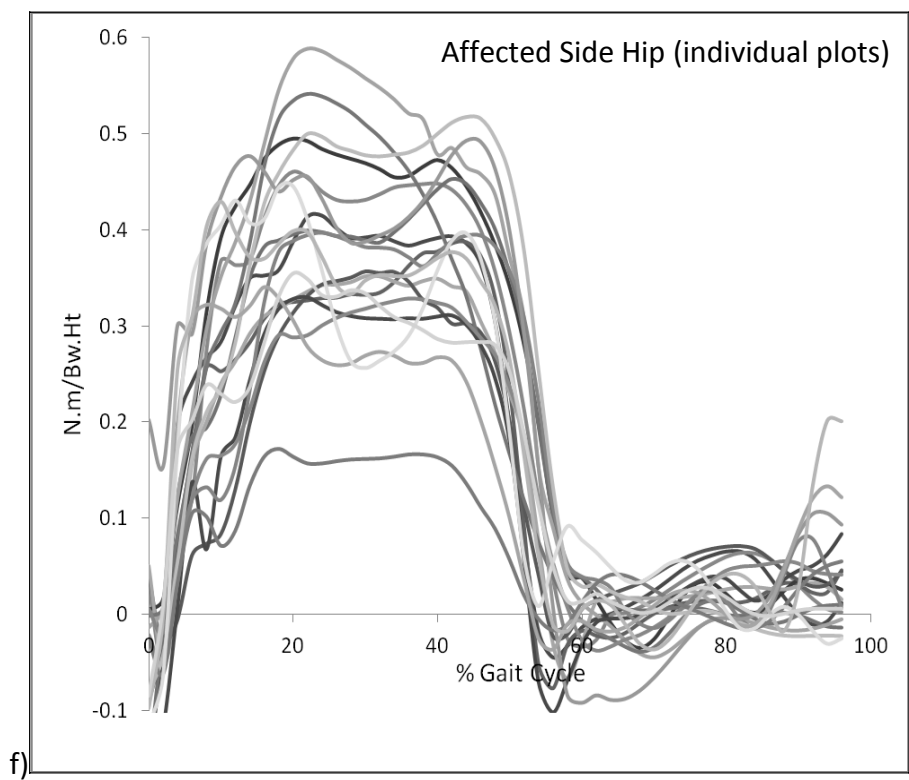
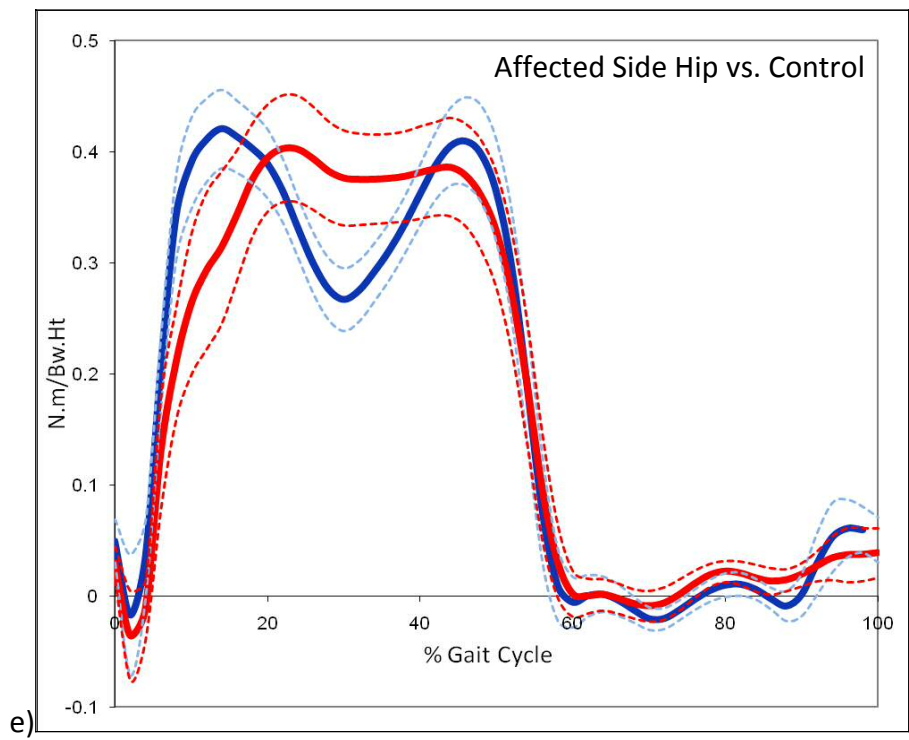
Whilst the averaged traces give an impression of the shape of the waveforms, it is also clear that the exact patterns presented in the individual plots are the results of an average of different patterns, some relatively normal and some which are quite

different. The most abnormal traces could be likened to either an inverted U, or a 'square wave' pattern, with no definable peak in early stance. As discussed later, this pattern was reflected in the observed patterns of the GRF.

When examined using stepwise multiple regression, peak knee moments were associated with limb alignment alone ( $p=0.024$ ). Mid-stance moments were associated with (in order of strength of association): Gait speed; knee extension during gait; and limb alignment, with trunk swing just failing to reach significance (gait speed  $p<0.001$ , knee extension  $p=0.006$ , limb alignment  $p=0.042$ , trunk swing  $p=0.054$ ).







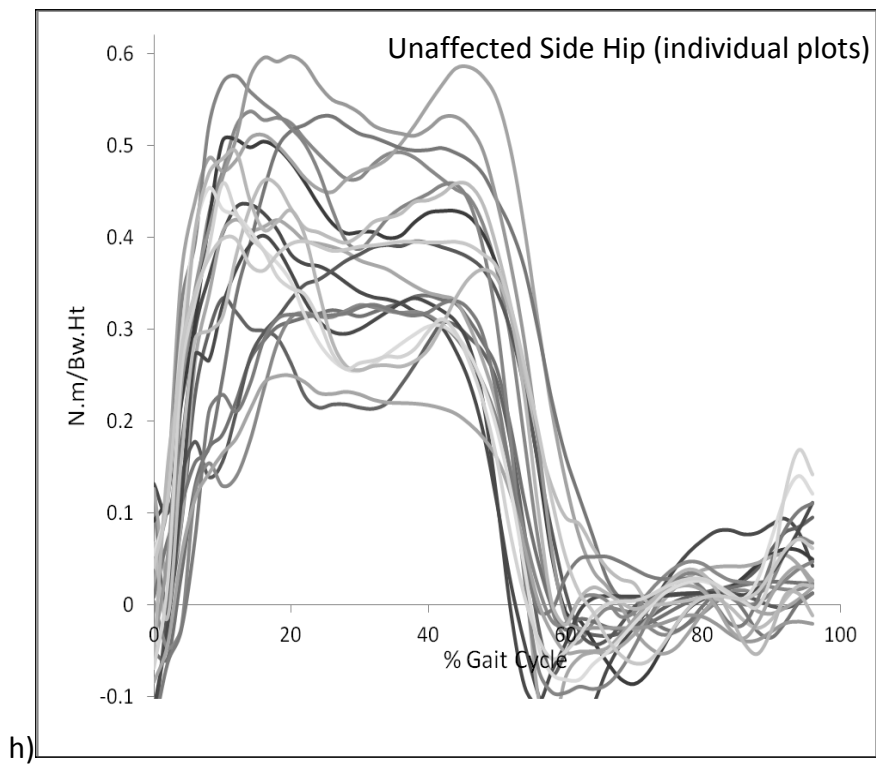
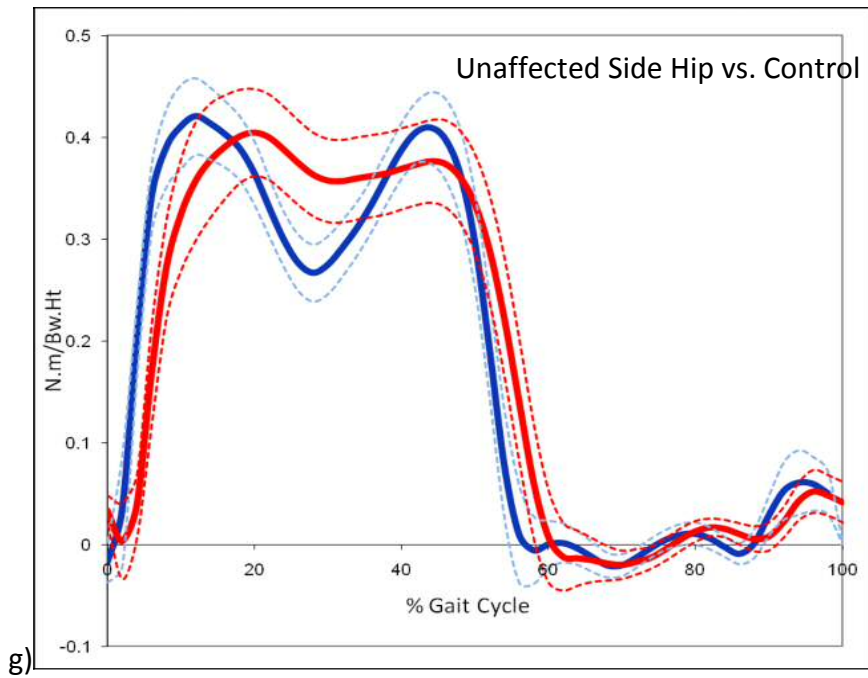


Figure 4.10 Mean external moment charts for the healthy controls (blue line) and OA subjects (red line) and individual plots for the subjects for: a) and b) the affected knee; c) and d) the unaffected knee; e) and f) the ipsilateral hip; g) and h) the contralateral hip. Dotted lines represent 95% confidence intervals for healthy controls (blue) and OA subjects (red).

The vertical and medio-lateral components of the ground reaction force ( $F_z$  and  $F_y$ , respectively) were also examined to understand the changes in gait between the two groups. OA subjects had lower peak values of the vertical ground reaction force ( $F_z$ ) than healthy controls and higher mid-stance values. There was also a difference in the medio-lateral ground reaction force ( $F_y$ ) between groups at mid-stance, although there was no significant difference in  $F_y$  at peak. This is expressed in table 4.4.

	OA Subjects	Healthy Controls	Significance
Ground reaction force vertical component (%BW)			
Peak	101.1 (6.5)	112.4 (8.4)	$p=0.003$
Mid-stance	86.0 (6.3)	72.1 (7.5)	$p<0.001$
Ground reaction force horizontal component (%BW)			
Peak	5.89 (1.43)	5.93 (1.85)	$p=0.81$
Mid-stance	4.58 (1.30)	3.46 (1.01)	$p=0.003$

Table 4.4. Differences in the ground reaction force between subjects with OA and controls, normalised to body weight and expressed as mean (standard deviation). The mean of both sides is reported as there were no side-to-side differences in the GRF in either control or affected subjects.

Although the medio-lateral component is smaller than the vertical component in overall value, it has the potential to greatly increase loads on the medial compartment owing to its moment arm (see exploration of moments in 4.3.3). As such, both components of the ground reaction force make a significant contribution to the moment at both the knee and the hip despite their difference in magnitude.

Gait speed was the major determinant of Fz (Gait speed and peak Fz  $r=0.747$ ; Gait speed and mid-stance Fz  $r=-0.934$ ) but was only weakly correlated to Fy (gait speed and peak  $r=0.10$ , gait speed and midstance  $r=0.38$ ).

#### 4.3.3 Co-contraction findings

Co-contraction indices were abnormally high on both sides of the knee, bilaterally (Table 4.5, Figure 4.11 and 4.12). As can be seen from figure 4.12, this was related to changes in activation of both hamstrings and quadriceps, especially at mid-stance, where the muscles remained active. Mid-stance is normally a relatively passive phase in the gait cycle, as demonstrated by the normal gait traces, whereas the persistent activation of the two muscle groups throughout stance was a consistent finding in the OA group.

	OA Subjects	Healthy Controls	Significance
Medial			
Affected side	0.24 (0.03)	0.14 (0.02)	$p<0.001^{**}$
Unaffected side	0.21 (0.03)		$p=0.005^{**}$
Lateral			
Affected side	0.30 (0.04)	0.15 (0.03)	$p<0.001^{**}$
Unaffected side	0.24 (0.04)		$p<0.001^{**}$

Table 4.5. Co-contraction indices ( $0<x<1$ ), expressed as mean ( $\pm$  95% confidence interval). Significance testing was performed using t-tests with Bonferroni correction (p-values multiplied by 16). \*=significant ( $p<0.05$ ) \*\*=highly significant ( $p<0.01$ ).



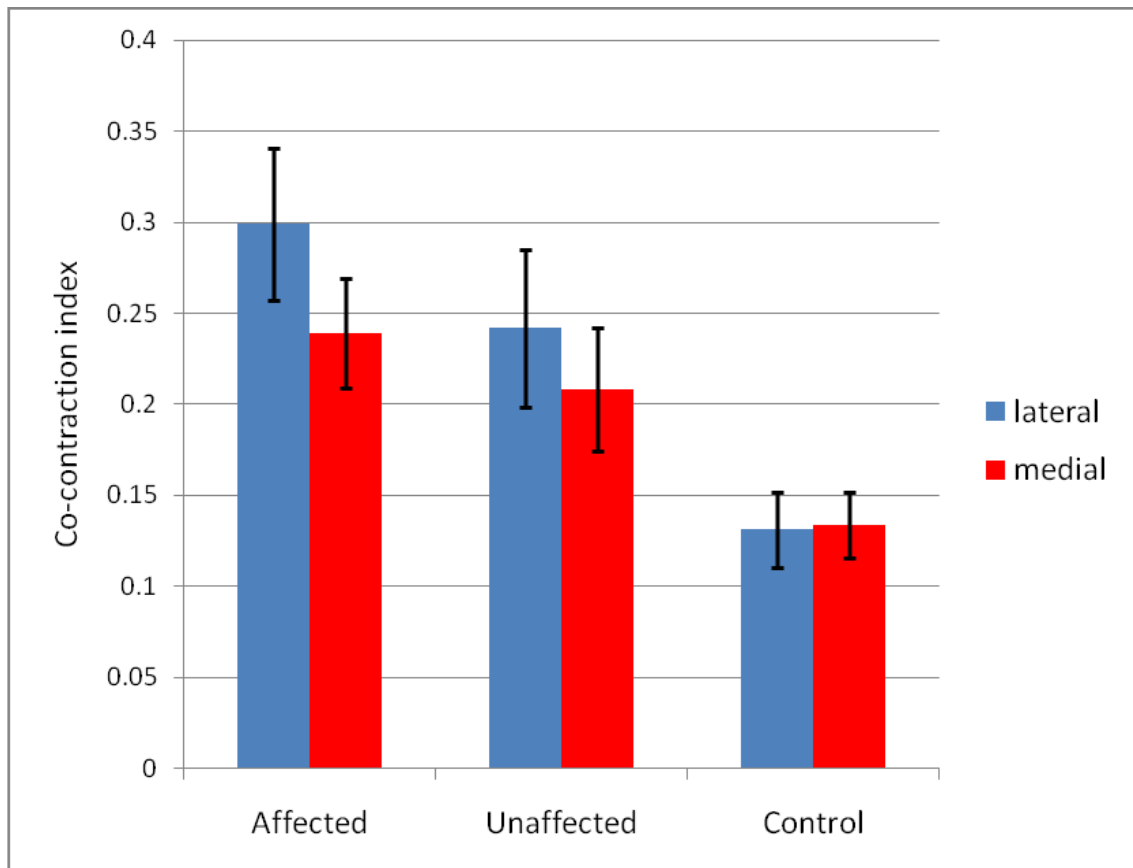


Figure 4.11. Summary of co-contraction indexes for affected and unaffected knees of OA subjects and healthy control knees (no side to side difference noted amongst control subjects).

In the regression analysis, the adduction moment impulse correlated with lateral co-contraction (strength of association 34%;  $p < 0.001$ ,  $\beta = 0.60$ ) but had no relationship to medial co-contraction. There was no relationship between gait speed and co-contraction on either side of the joint.

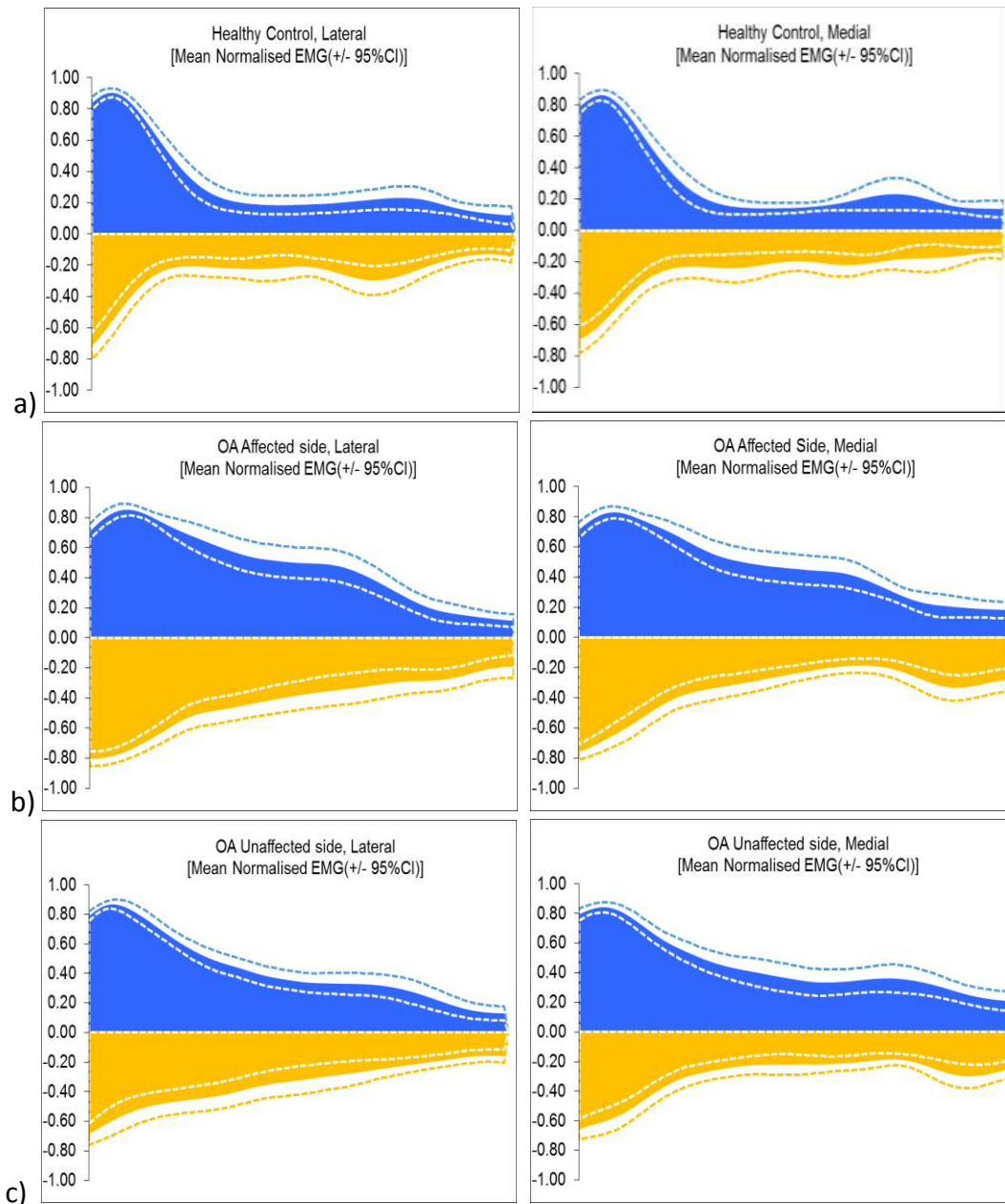


Figure 4.12. EMG traces (Mean  $\pm$  95% CI) for quadriceps (blue) and hamstrings (yellow) for: a) the healthy control population; b) affected knees of OA subjects; c) unaffected knees of OA subjects.

#### 4.3.4 Findings from the dynamic alignment measure

The purposes of examining data from the alignment measure were:

- 1) To examine the assumption that the knee can be modelled as a hinge joint. To do this, the assumption that flexion-extension can be defined as a linear motion during the chosen range of flexion was examined.
- 2) To determine if the correct points in the gait cycle had been chosen.
- 3) To examine the relationship between the (standard) epicondylar axis and the (newly calculated) flexion-extension axis in both healthy controls and OA subjects.
- 4) To extract the relevant data for the current study.

In order to examine the first assumption, the traces of the AJM marker were visually examined in Vicon Nexus. However, this is both subjective and hard to represent clearly in a thesis. In order to give a more accurate view, plots were made of the AJM marker in the x-y plane, essentially describing the ankle joint centre in the horizontal plane of the knee. Plots of a typical healthy control subject and a typical OA subject are found in figure 4.13.

The primary observation from these are that whilst overall the ankle moves in a relatively linear direction, there is some change in the path of motion throughout the gait cycle, and taking two vectors close together to form the cross-product may introduce significant error. However, there is a more linear section of the chart, which occurs between peak extension (approx 40% of the GC) and peak flexion

(approx 70% of the GC), corresponding to the regions of the gait cycle that were chosen to be used for the alignment measure.

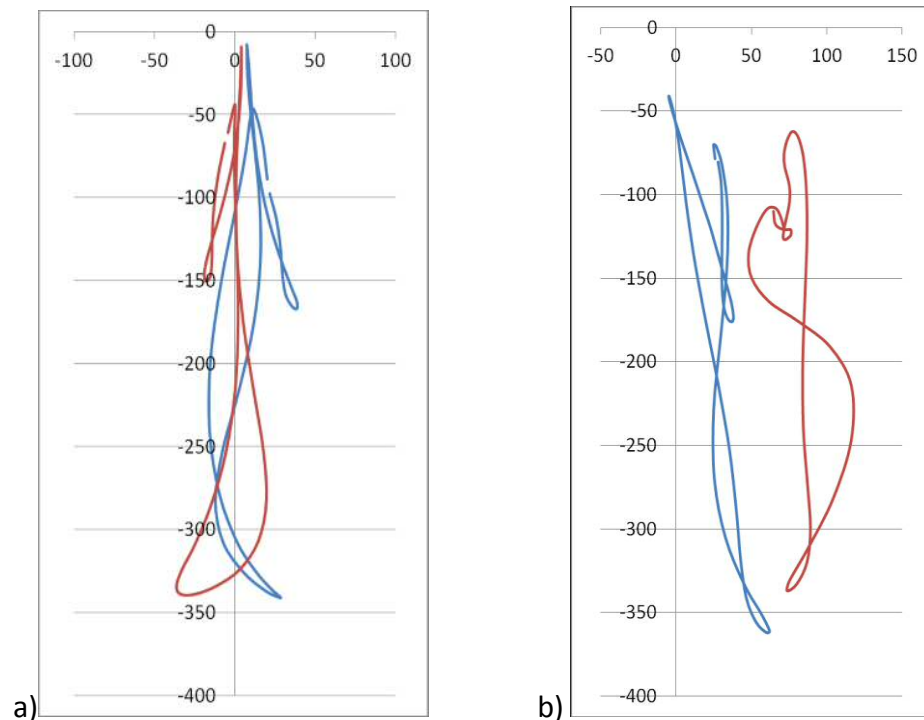


Figure 4.13 x-y plots (in mm) of a) a typical healthy control subject and b) a typical OA subject throughout the whole gait cycle. Red is the right leg and blue is the left.

These charts are difficult to interpret well in view of the method for the alignment measure, as the points in the gait cycle are not marked. A more relevant way of plotting the AJM marker would be to just represent the region of the gait cycle that was examined for the alignment measure. Therefore, figures 4.14 and 4.15 represent x-y plots from 40% of GC to 84% for both OA subjects and healthy controls.

The majority of these plots can be seen to be relatively linear, although a ‘tail’ is noted on most where the path of motion deviates as the knee goes back into extension. This would imply that the range of flexion values that the ‘flexion vector’

was taken over was too broad. Closer inspection of the data would suggest that this only happens for the last two to three data points and in future it is recommended that the calculations are not taken so late in the gait cycle, with the 'flexion' vector being taken between 64% and 78% (rather than 64% to 84%).

The internal consistency of the calculations across a range of flexion for each individual was assessed by taking the standard deviation of the 10 calculations made for each gait cycle. The mean of these SD's was  $0.57^{\circ}$  and was under  $1^{\circ}$  in 30 of the 40 cases, suggesting that it was a stable result across the ranges of flexion that were assessed, despite the concern about the curve seen at the end of flexion in the xy plots.

The patients had a mean varus at the affected knee of  $4.7^{\circ}$ , consistent with their medial compartment disease. However the unaffected side was on average normally aligned, with a mean valgus of  $0.1^{\circ}$ . This was consistent with the derived alignment for the normal individuals, who had a mean valgus of  $0.6^{\circ}$  (table 4.6).

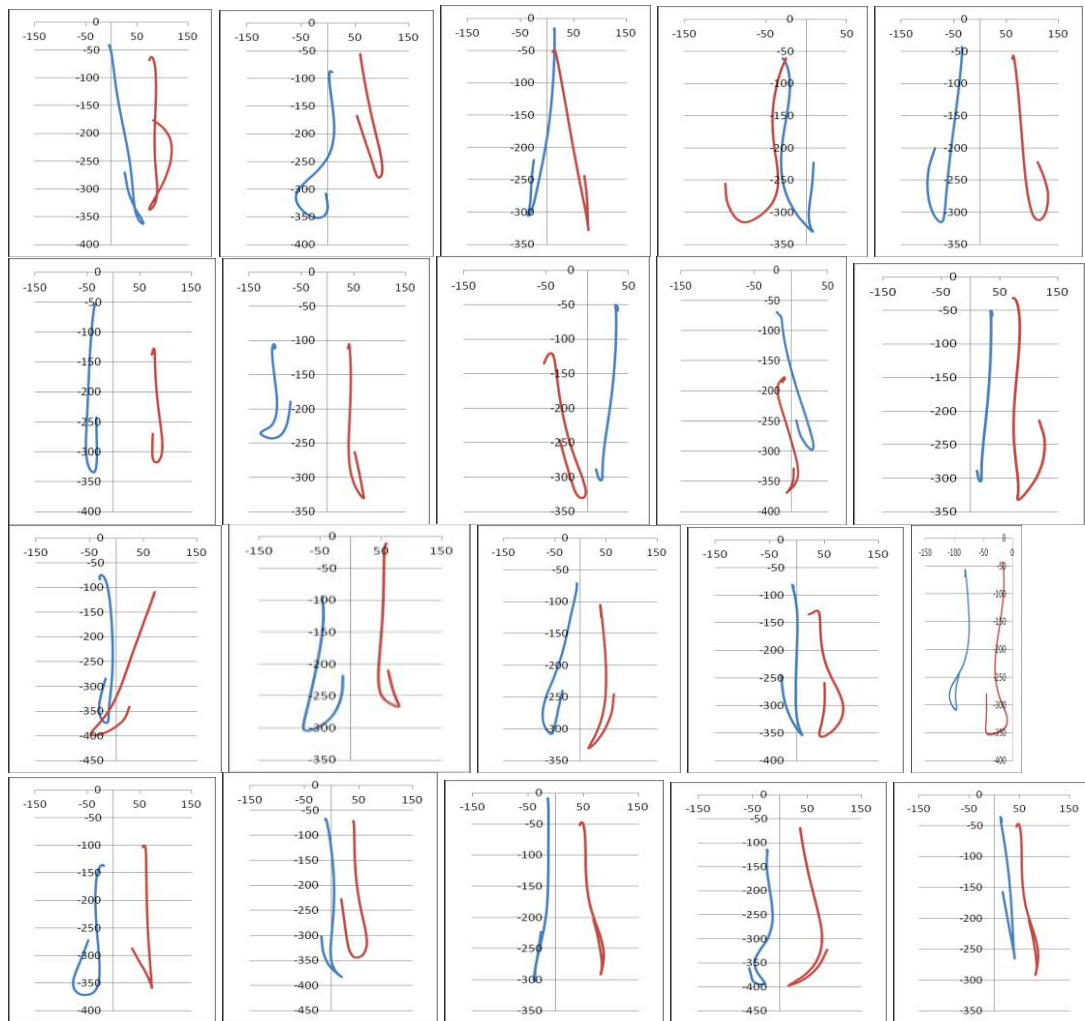


Figure 4.14 x-y plots of the AJM marker in the x-y plane of the thigh axis system for OA subjects

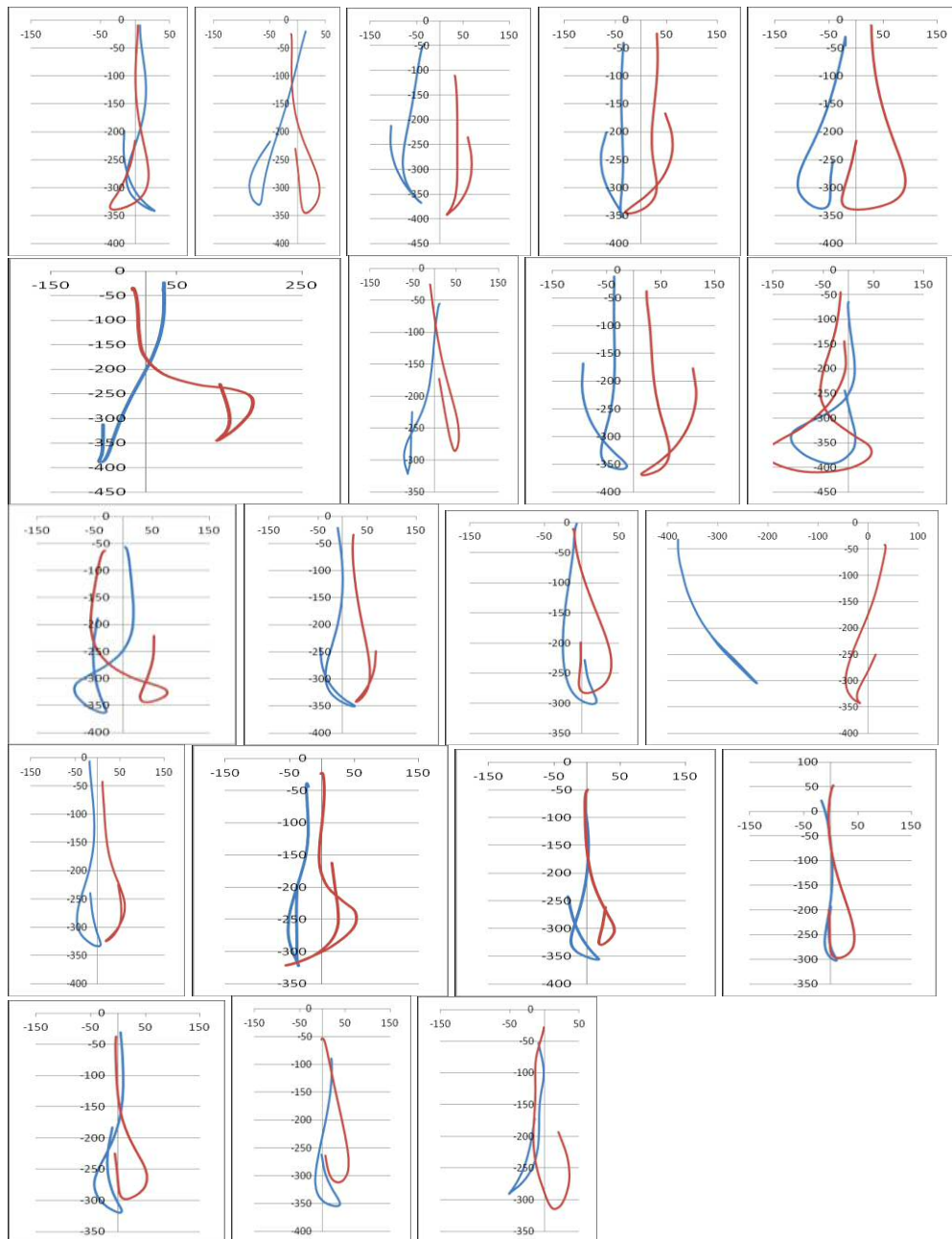


Figure 4.15 x-y plots of the AJM marker in the x-y plane of the thigh axis system for healthy control subjects

	Standard Alignment	New Alignment Measure	RMS Difference	Difference in Axis
Healthy control: Left	-0.65°	0.10°	1.11°	6.70°
Healthy control : Right	-0.49°	0.01°	0.88°	7.16°
OA subject: Ipsilateral	-5.59°	-4.69°	1.99°	8.53°
OA subject: Contralateral	-0.331°	0.08°	1.73°	6.68°

Table 4.6 Mean alignment, comparing the standard and new approaches to this calculation. A negative result represents varus alignment and a positive result represents valgus alignment.

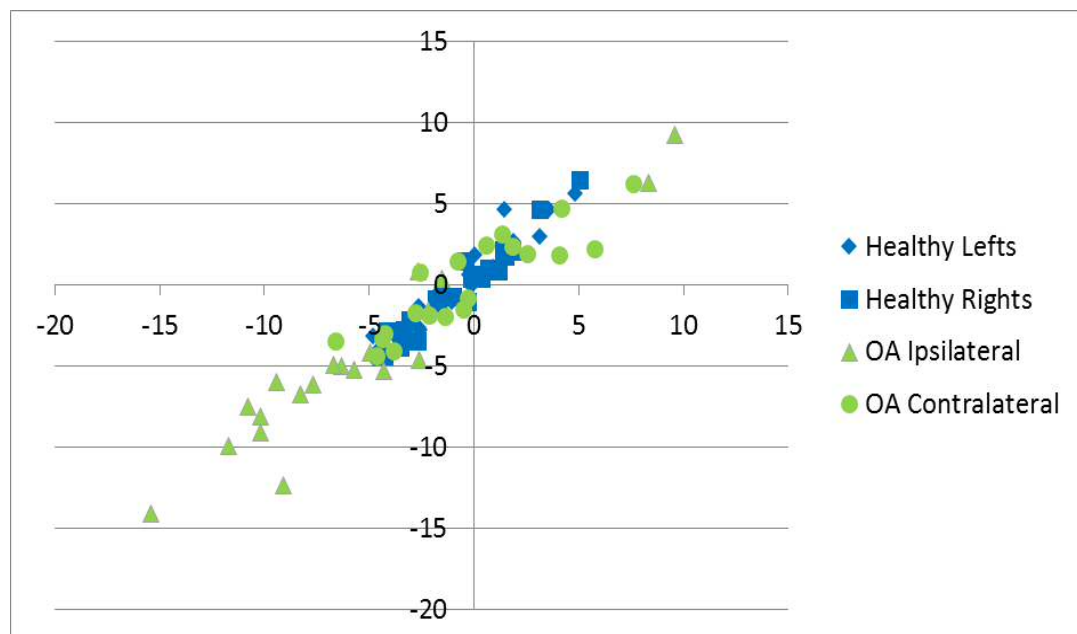


Figure 4.16 Scatter plot comparing new and standard techniques for measuring alignment

The means from the standard and new methods of measuring alignment were relatively similar, and the individual values for the healthy control population were very similar regardless of which method of measuring alignment was used (table 4.6, figure 4.16). Root-mean-square (RMS) values for the difference approximated 1° for the control group, and good agreement would be expected as the control



population would be expected to come into full extension during gait and therefore not have problems with flexion/extension crosstalk. The RMS values were higher for the OA subjects, implying that agreement was less good in the arthritic group.

The calculated axis difference (the difference between the functionally derived axis and the original axis defined by the epicondyles) varied from 0.42° and 28.1°, with a mean across the study group of 7.94° and a standard deviation of 4.95°.

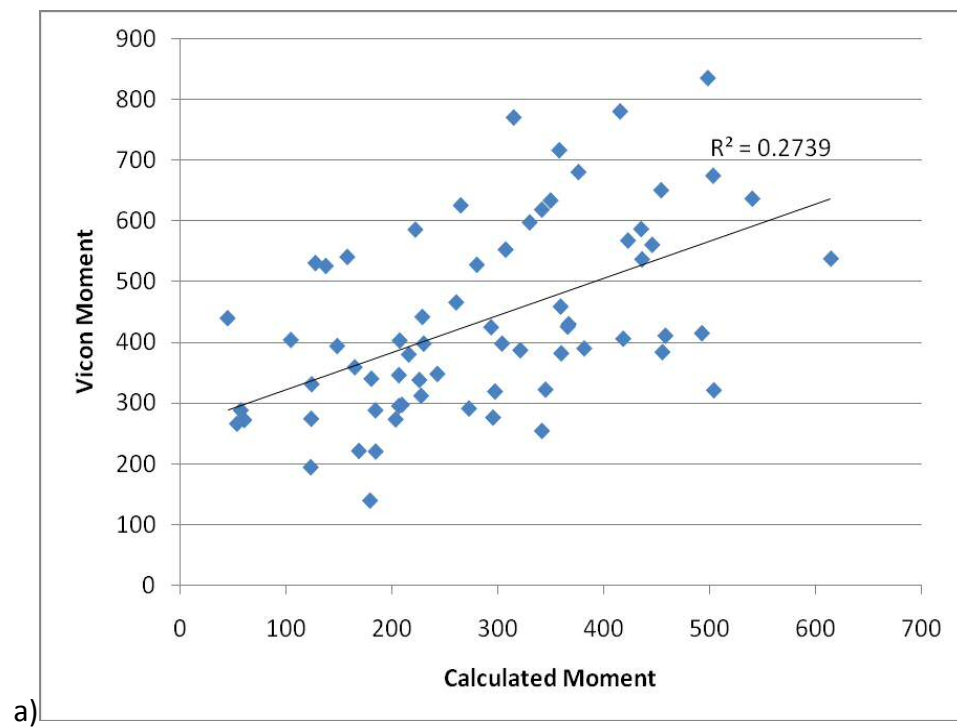
However, this study has not defined which method was the best representation of the subjects true anatomy. Whilst many laboratories do run repeatability studies as part of their quality control, these are typically on healthy individuals, whilst we are most concerned about preventing errors due to flexion deformities in the arthritic group in this study. To determine the best technique for measuring alignment, a further study would be required using a gold standard, and this will be examined further in the discussion.

#### 4.3.4 Exploration of Moments

The value of the approach for estimating moments using the formula described in 4.2.7 was assessed by examining the correlation between our estimated moments and the moments calculated using Vicon Nexus.

As the result would be influenced to some degree by a number of factors, including limb rotation, the relationship between 'estimated' and 'measured' moments was analysed using stepwise multiple linear regression which included the foot

progression angle as a co-variate. However, for each time point (10, 20 and 30%) the foot progression angle was a non-significant factor and was excluded from the final model (which thus became a simple regression analysis between 2 continuous variables). The scatter plots and  $R^2$  values for the relationship between calculated moments and estimated moments for all 80 knees in the study are included in figure 4.17.



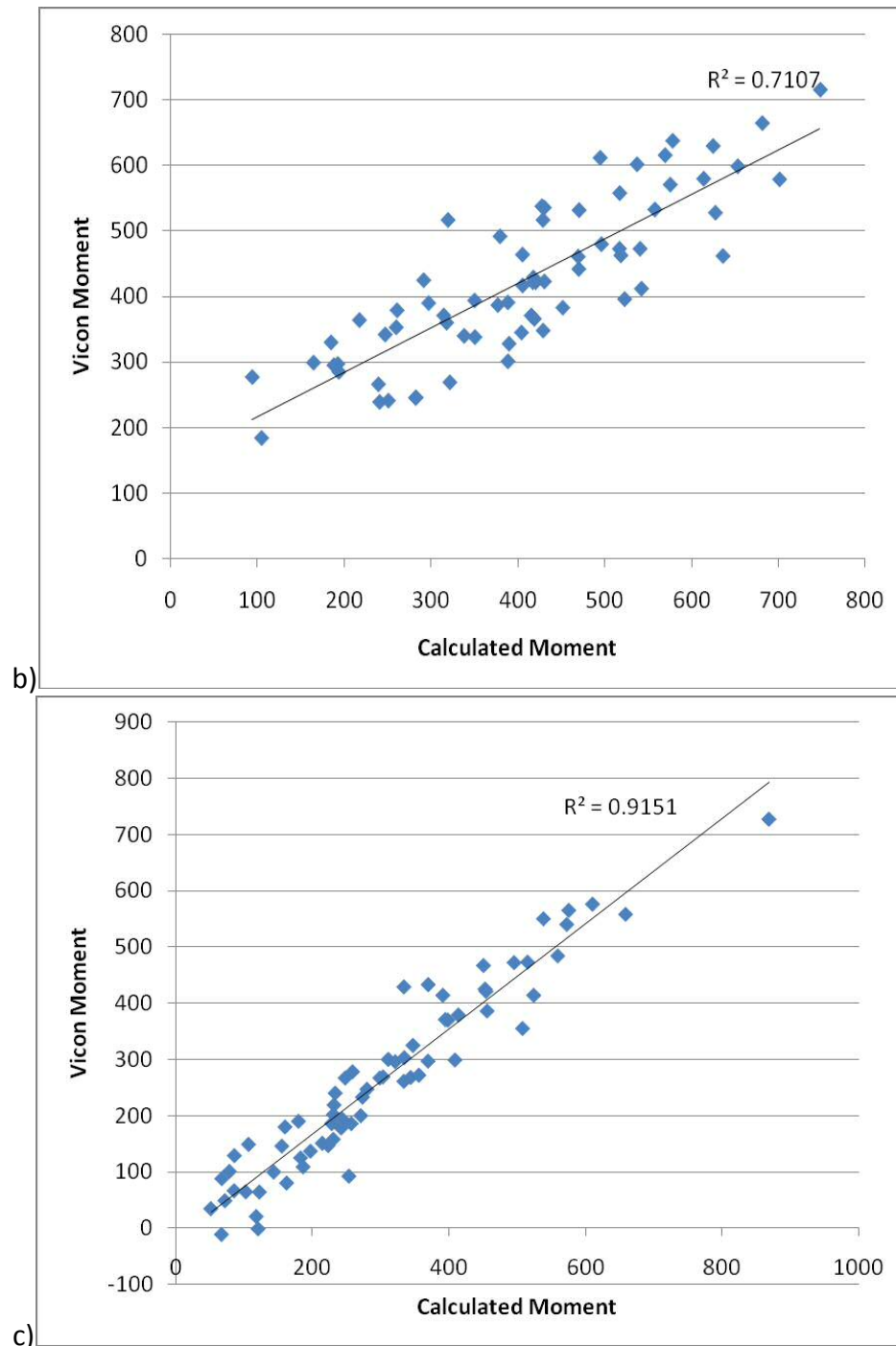


Figure 4.17. Scatter plots demonstrating the relationship between the calculated moments for a single representative gait cycle, and the overall moment for the time point as calculated from all the walking trials using Vicon Nexus a) 10% of the gait cycle b) 20% of the gait cycle c) 30% of the gait cycle.

These results showed that mid-stance moments could be represented using this technique with enough accuracy for the approach to give some insight into the

effect of the different components of the GRV on the moment and could therefore be used as part of the exploratory analysis.

The poor relationship between the two calculations at 10% is not unexpected as the moments change rapidly during this phase (see figure 4.10 a-h) and so analysing single gait cycles is likely to introduce a lot of variability due to subtle changes in timing of the gait cycle, therefore the time points at 10% and 20% were not studied further.

The findings from this exercise are described in figure 4.18. They are presented as the full estimated moment for each subject, broken down into the two components:  $D_z.F_y$  in green and  $D_y.F_z$  in blue.

The findings are simple but relevant in terms of understanding the effect of the ground reaction force on the moment at mid-stance. Both  $F_y$  and  $F_z$  influence the overall moment in healthy control subjects, with  $F_y$  having the strongest effect due to its longer lever arm (i.e. the green bars are larger in most normal individuals, compared to the blue bars).

At the affected joint of OA subjects,  $F_z.D_y$  takes over as the 'dominant' contributor to the moment, as the value of  $D_y$  increases significantly due to the varus alignment of the joint (i.e. the centre of rotation moves laterally relative to the centre of pressure) which is amplified by the high mid-stance moment. However, at the unaffected knee of OA subjects, both values increase. This is not due to a change in  $D_y$  or  $D_z$  – these are affected by alignment only – but due to an increase in  $F_y$  and  $F_z$ .

In other words, the changes at the unaffected knee are driven by changes in both components of the the ground reaction vector, whereas changes in the affected knee are due to a combination of knee varus and the abnormal ground reaction force. Therefore, to understand the changes in joint moments observed in this study, both components of the ground reaction vector need to be examined closely.

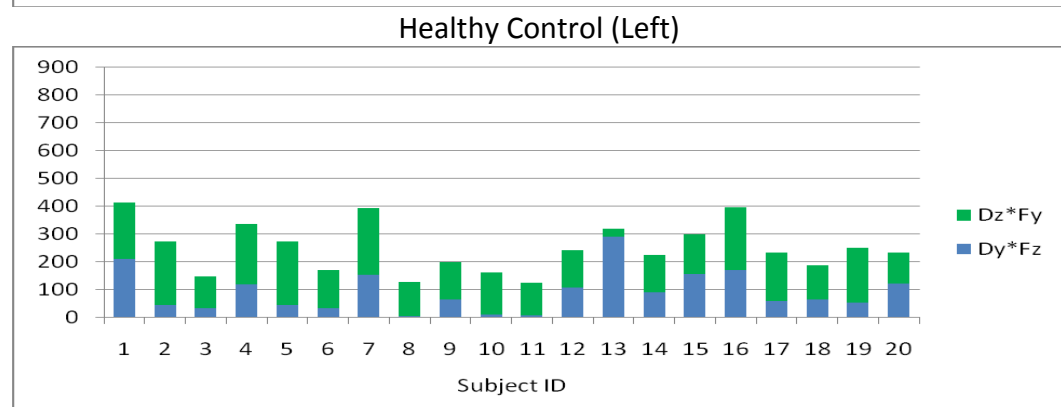
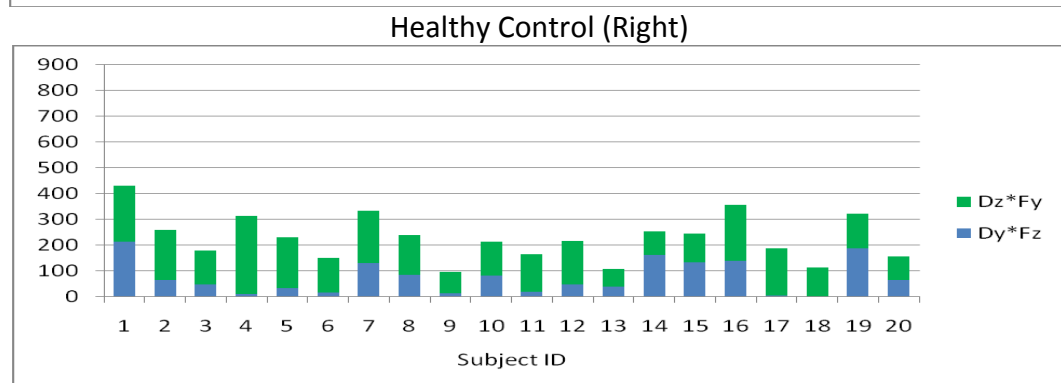
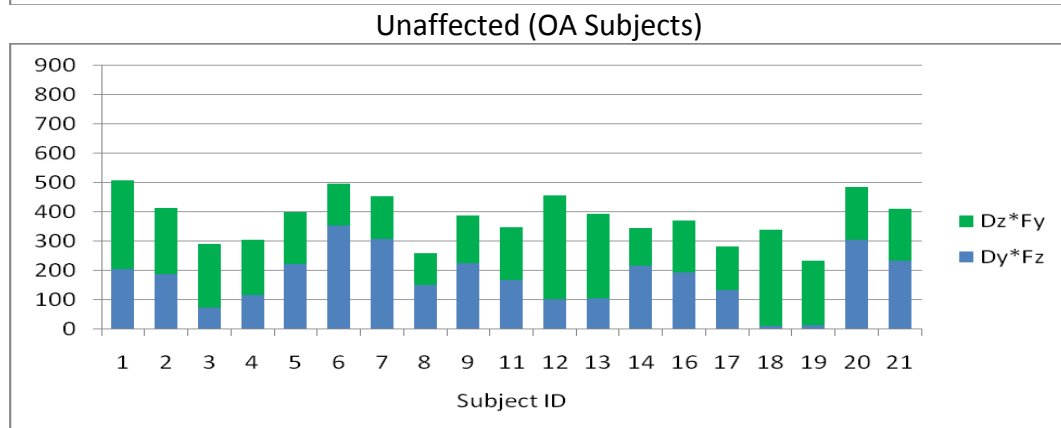
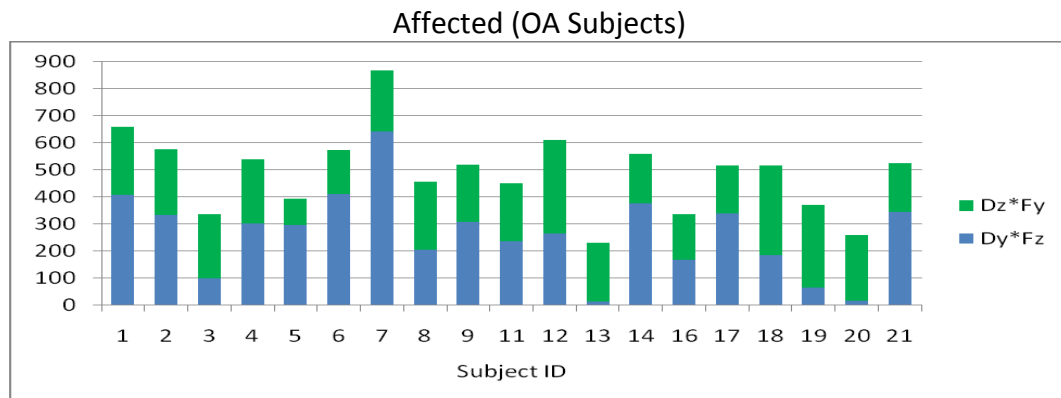


Figure 4.18. Frontal plane moments divided into separate components. Units are Nmm/Kg

#### 4.3.5 The relationship between gait speed and moments

The initial analysis was performed with a single subject walking at progressively slower speeds. Two self-selected speeds (normal speed, which was at an average of 1.25 m/s and very slow, at an average of 0.78 m/s) were selected to produce reports and the moment graphs from these are reported in figure 4.19. Subjectively, it was noted that walking at or below 1 m/s was surprisingly difficult and inefficient, reflecting the fact that gait speed is closely related to physical function and performance.

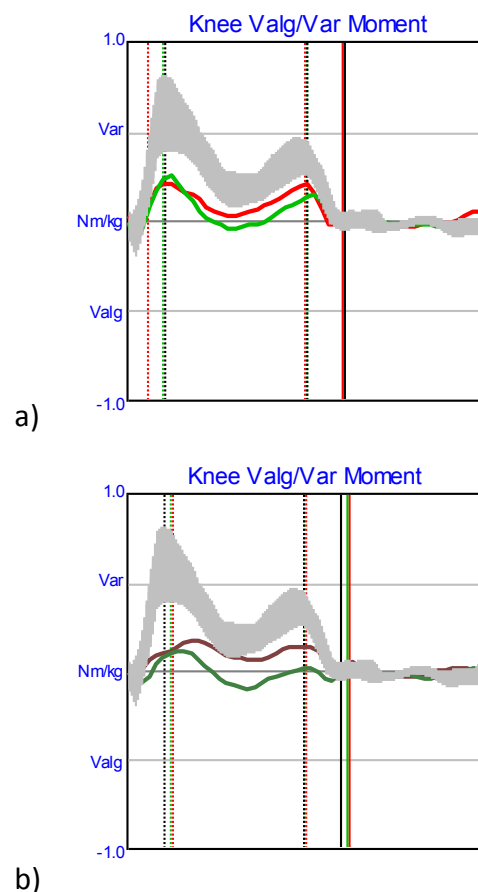


Figure 4.19. Knee adduction moments for normal and very slow speed for the initial healthy subject. The grey band represents a normal young adult (aged 20-60) population.

The peaks were lower in the slow speed group, although the effect of speed was less strong than had been expected, and the waveform shape did not change as expected, as a mid-stance dip in the moment was retained even at slow speed. However, the moment waveform was significantly different to the young adult healthy database (2SD's of normal are represented by the grey band in the charts), and so the speed changes observed in this single sample may not have been representative of a healthy population. It was therefore decided to extend the investigation to examine more healthy individuals walking at different speeds, to see if this pattern was a consistent finding or if the initial data simply represented a misleading outlier.

The results from the additional 9 individuals recruited to this investigation are reported in figure 4.20 and table 4.7. Mean alignment was in relative varus of  $2.52^{\circ}$  (SD 2.43). The mean gait speed in the slow speed group corresponded well to the OA subjects in the primary study (1.03 in slow speed group vs 0.97m/s in the OA subjects) and between the normal speed in this population and the age-equivalent population used in the primary study (1.41m/s in this population and 1.33 m/s in the main study reported in 4.3.2).



	Slow	Normal	Fast
Gait Speed (m/s)	1.06 (0.11)	1.41 (0.10)	1.72 (0.13)
Adduction Moment Impulse (N·m·s/BW·Ht%)	1.14 (0.22)	0.96 (0.16)	0.91 (0.17)
Peak Adduction Moment (N·m/BW·Ht%)	2.71 (0.47)	3.45 (0.84)	4.30 (1.01)
Mid-Stance Adduction Moment (N·m/BW·Ht%)	1.40 (0.50)	0.94 (0.37)	0.65 (0.43)

Table 4.7. Gait speeds and moments for the slow, normal and fast walking. Expressed as mean (SD).

It can be seen that greater speed did result in greater impulses, lower mid-stance moments as well as higher peaks. The impulses of the slow speed group and the contra-lateral legs of the OA subjects are also very similar, although it should also be noted that a slightly higher impulse was seen in this population at normal speed than was seen in the healthy controls presented in 4.3.2. Therefore whilst the absolute values are similar, the change in impulse between normal and slow speed in this cohort was on average 0.18Nm/BwHt, whereas the difference between the impulse at the unaffected knee and the healthy control reported in 4.3.2 was 0.32Nm/BwHt.

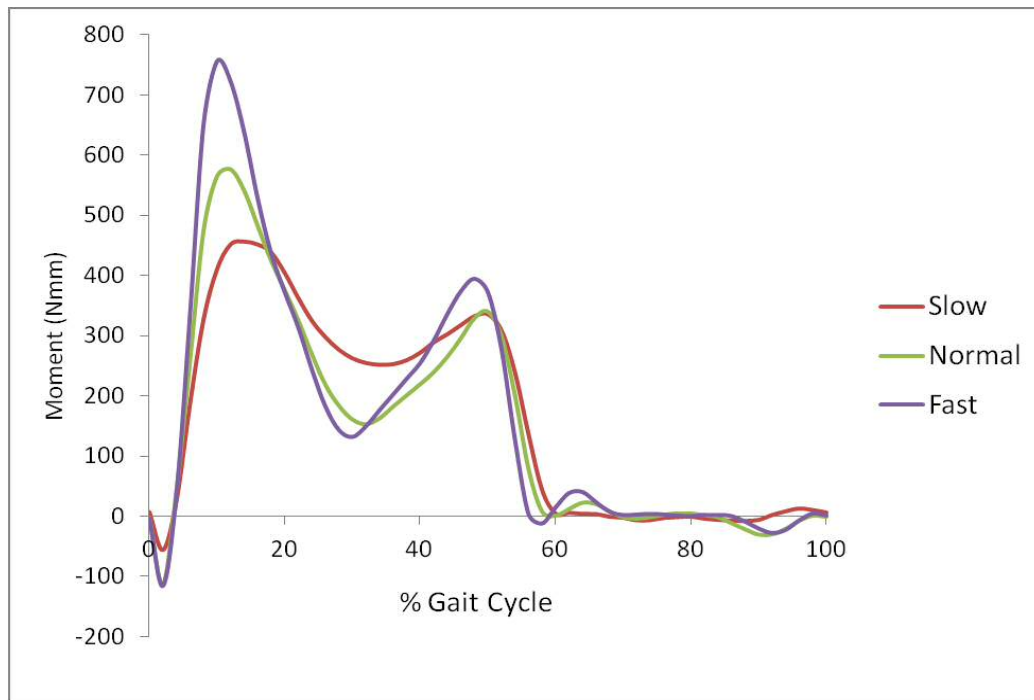


Figure 4.20. Averaged moment waveforms for the three gait speeds.

The impulse is calculated by integrating the moment waveform with time as the x-axis rather than %gait cycle (which is normalised to time). It is therefore the equivalent of the integral of the normalised waveform (the time-normalised impulse), multiplied by stride time (the time taken between heel strikes of the same limb). When these two components are separated, the time-normalised impulse did not change significantly across the three speeds (Expressed as mean [SD], slow speed: 0.96Nm/BwHt [0.18], normal speed: 0.92Nm/BwHt [0.14], fast speed 1.02Nm/BwHt [0.17]). This would suggest that the relationship between gait speed and moment impulse in healthy individuals is entirely due to the change in cycle time, so as the foot is on the ground for longer, the moment impulse increases proportionately.

Comparing the OA subjects to their age-equivalent controls, the time-normalised integral was 1.26Nm/BwHt [0.25] for the affected leg, 0.99Nm/BwHt [0.34] for

the unaffected leg and 0.82NMs/BwHt [0.27] for the controls. Therefore, whilst some of the differences in the impulse presented in table 4.3 may be due to gait speed, there were also some differences seen in waveform shape between the groups.

The effect of gait speed on the ground reaction vector was also examined (Table 4.8). As expected, changes in the peaks and troughs correspond to the peaks and troughs seen in figure 4.20. These can be compared to the results from the main study in table 4.4. The only major difference seen between the this cohort and the OA subjects is in the medio-lateral GRF at mid-stance, which had a very weak relationship with gait speed in this cohort, but was substantially higher in both legs of the OA group.

	Slow	Normal	Fast
Ground reaction force vertical component (%BW)			
Peak	105.9 (4.9)	116.4 (6.3)	125.8 (9.3)
Mid-stance	84.0 (5.3)	69.7 (7.1)	56.5 (7.8)
Ground reaction force horizontal component (%BW)			
Peak	5.28 (1.06)	6.02 (1.81)	6.93 (1.71)
Mid-stance	3.00 (0.98)	2.81 (0.70)	2.71 (0.85)

Table 4.8. Ground reaction forces for the multiple-speed normal cohort, normalised to body weight. Expressed as mean (SD).

## 4.4 Discussion

### 4.4.1 Overview

This is the first study in the literature to demonstrate that subjects with unilateral severe knee OA have abnormal joint loading in both lower limbs compared to a control population. Both joint moments and muscular co-contraction are abnormal in the unaffected limb and both abnormalities would be expected to increase joint reaction forces.

Patients frequently state that they load the opposite limb excessively due to changes in gait caused by knee pain, and this is supported by our findings. Abnormal patterns of loading were seen in both knees and both hips, despite only a single joint being affected.

It is clear that patients with advanced knee OA do not walk with what is traditionally considered an 'antalgic' gait. Stance times were relatively equal between legs, and ground reaction forces were equal between the affected and unaffected joint.

Instead of an antalgic gait, OA subjects walk with a 'severe OA gait', characterised by a slower gait speed using a stable but inefficient pattern of walking. The data from this study will be discussed over the next few sections in terms of the kinetic findings, then the EMG findings with a separate discussion of the the dynamic alignment measure. The weaknesses of this study will then be discussed prior to the conclusion.

#### 4.4.2 Kinetics

The primary measure in this study was the adduction moment impulse, as it represents loading throughout the whole of stance phase. The benefits of this measure have been discussed in section 2.3.2. However, the adduction moment impulse, the peak moment and mid-stance moments are all relevant measures and should all be considered to understand the changes seen in this study (Astephen, Deluzio et al. 2008; Bennell, Creaby et al. 2010).

Whilst peak moments were not elevated in this study, mid-stance moments were abnormal in both hips and both knees of patients with knee OA. Impulses were significantly elevated at the contra-lateral hip only, but were substantially elevated at both knees despite only one joint being involved. These changes can be readily appreciated in inspecting the moment curves in figure 4.10.

The moment around a joint is primarily dependent on the relationship between the ground reaction force (GRF) and the position of the joint centre relative to the GRF vector (Hunt, Birmingham et al. 2006). The relative position of the joint to the GRF vector is mainly determined by the alignment of the limb. Varus alignment is well known to increase loads on the medial compartment of the knee. This is well described in the literature, and is pronounced in early stance because that is when the ground reaction force is at its greatest (Hurwitz, Ryals et al. 2002; Hunt, Birmingham et al. 2006; Foroughi, Smith et al. 2010).

The effect of the ground reaction vector on the joint moment was exacerbated at the affected joint by the presence of mal-alignment, but loads at the opposite knee

were abnormal despite normal measures of alignment. Whilst the alignment may have affected the height of the curve – explaining the difference between the ipsilateral and contralateral leg, the change in waveform shape is due to changes in the ground reaction vector.

The ground reaction force is the force that accelerates the centre of mass. It is therefore the differential of the velocity of the centre of mass, which in itself is the differential of the displacement of the centre of mass. To understand the changes in the ground reaction force that we have seen, we need to consider the motion of the centre of mass during gait.

A healthy individual accelerates their centre of mass both vertically and horizontally during early stance as they transfer weight on to the leading foot. This leads to a peak in the ground reaction vector in both vertical and horizontal components (figure 4.21).

During single limb stance the centre of mass reaches its vertical peak and moves laterally over the leg that is in stance. During this phase, the ground reaction force falls in both vertical and medio-lateral directions (seen as a 'dip' in the waveform), which results in a reduction in joint loading at mid-stance. These changes are discussed in figure 4.21.

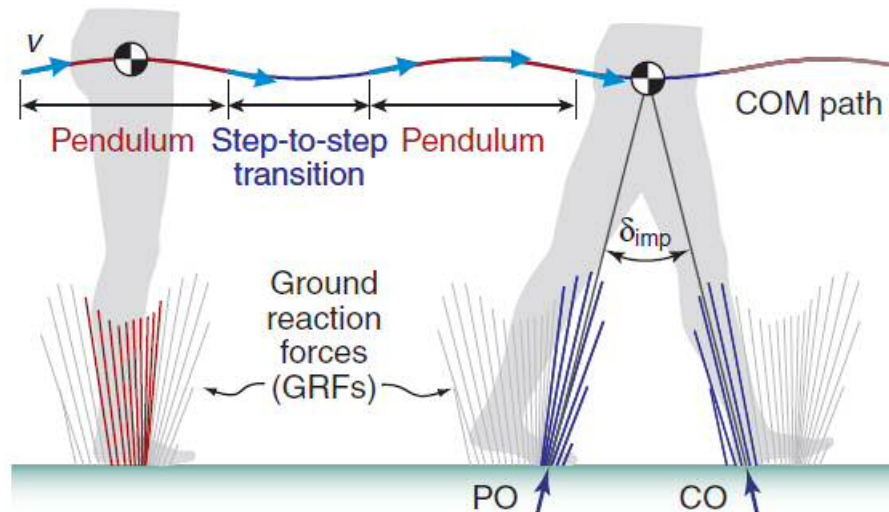


Figure 4.21 A representation of the ground reaction force during normal gait (Adamczyk and Kuo 2009). In this article, the authors were making the point that the centre of mass is accelerated and decelerated during the 'step-to-step transition', but that the movement at mid-stance is more of a pendular movement. This change from an active process (deceleration at weight acceptance and acceleration at push off) to a 'passive' deceleration/acceleration of the centre of mass during mid-stance may explain the shape of the ground reaction force, as discussed above.

In this study, the dip (or biphasic waveform) in the ground reaction vector was seen in the normal population but was diminished or absent in the subjects with knee OA. The loss of the biphasic shape in both components of the ground reaction force in the OA subjects resulted in the abnormal waveforms seen in figure 4.10.

As described by figure 4.21, the shape of the ground reaction force is to be due to an active deceleration and acceleration in early and late stance respectively, affecting both components. This appears to be absent in the OA population, with gait been driven by the pendular movement of the body and trunk over a rigid leg without the active motion seen in healthy individuals. This could be driven either by a change in gait pattern specific to OA, or by the change in gait speed seen in this group.

The amount of knee extension during gait (the minimum value on the flexion-extension curve) was also found to affect the shape of the moment curve. Subjects with a fixed flexion deformity would be unable to bring the centre of mass to its normal peak during stance, and so it is likely that knee extension influenced the moment waveform via an alteration in the trajectory of the centre of mass, as described above.

It is clear that the most important factor in determining waveform shape and moments is gait speed, via its effect on the GRF. The evidence for gait speed being a major explanatory factor in our study is particularly strengthened by the results in section 4.3.5, which show that when gait speed falls in healthy individuals, the mid-stance moment rises and the peak moment falls. However, it can also be concluded that gait speed could not explain all of the changes observed in the primary study, as the difference between slow and normal speed walking in the 'multiple speed' cohort was not as great as the differences seen between the OA and control population reported in table 4.3. This is further supported by the observation the changes in  $F_y$  (the mediolateral component of the GRF) in the OA subjects were not replicated in the slow speed walking group.

The speed of walking has an especially strong relationship to the moment impulse, as has been described previously (Robbins and Maly 2009). As gait speed slows, the cycle time and time in stance inevitably increases, increasing the area under the curve. Whilst a slow gait speed also changes the shape of the curve, the area under the time normalised curve was the same whatever the speed. This was not true of



the OA population, where the increase in impulse was due to both an increase in the area under the curve as well as an increase in the stance time.

It can therefore be concluded that the abnormal impulse in the arthritic population was in part due to slow gait speed, although gait speed was unlikely to be the only reason for the two groups being having a different moment impulse.

A relationship was not found between moments and trunk swing or lean in this study, unlike other studies of knee OA patients (Hunt, Birmingham et al. 2008; Mündermann, Asay et al. 2008). Despite these studies implying that trunk lean reduced knee moments by changing the angle of the GRF, there was no association between trunk lean & trunk swing and the medio-lateral component of the ground reaction vector in this cohort. However, the current study was on an 'end-stage' population, and it is likely that their gait pattern is different to those with earlier disease, where compensation mechanisms may be stronger.

The factors that lead to abnormal loading in the study are described in Figure 4.22, along with their effect on the loading waveform. A number of these factors may be modifiable with education, training and perhaps the use of orthotic devices such as shoe wedges or braces. The role of treatment of the affected knee also needs to be considered, as a functional and painless joint with a normal range of motion would be expected to lead to a restoration of normal joint function and subsequent normalisation of joint forces and muscle activity in the other lower limb joints.

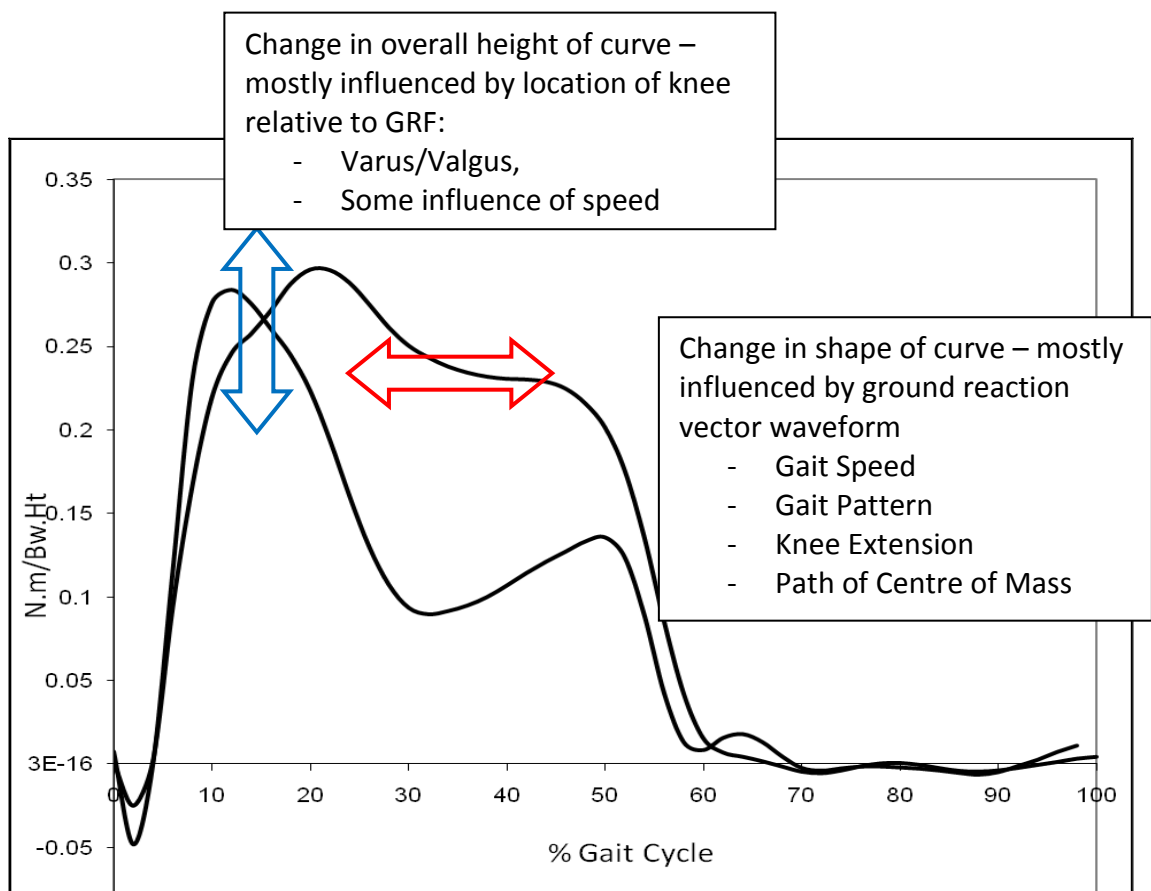


Figure 4.22 Summary of explanatory factors identified in this study, and their apparent effects on the moment profile (waveforms taken from figure 4.10).

The reasons for the difference in gait pattern and gait speed seen in this population has still not been clearly described. Simple step-by-step pain avoidance seems an unlikely explanation, as the gait was relatively symmetrical, despite the OA subjects asymmetrical disease patterns.

It is very unlikely that the gait pattern was a lifelong phenomenon for these individuals, although the possibility remains that such a highly selective study criteria resulted in the selection of an atypical patient cohort. Slow walking is inefficient and tiring. Also, the speeds seen in this study would be noticeably

different from the majority of individuals and would have been identified at a young age as abnormal, which was not a comment that any of the OA subjects made during interviews. It remains more likely that this phenomenon was acquired as part of the disease process.

It may be that by walking more slowly, the OA subjects reduced sharp accelerations and decelerations in load that might otherwise have been painful. Alternatively, the slow speed may be driven by a loss of strength, or by a loss of confidence related to poor strength. Finally, it may be a cautious pattern designed to protect the joint from the effects of slips or accidents. Further studies would be required to define this more clearly. However, it can be concluded that the gait pattern seen in this study is slow and stable but that the sacrifice for this is higher joint loading bilaterally, potentially causing further disease at new joint sites.

#### 4.4.3 Co-contraction

Quadriceps and hamstring co-contraction during stance was found to be abnormal bilaterally in OA subjects, despite only a single joint being affected. Co-contraction would be expected to substantially increase joint contact forces, by compressing the joint from both sides simultaneously. As co-contraction would not be registered in the kinetic data, co-contraction has an additive effect on joint loading above that measured using moments.

Lateral co-contraction may play a part in counteracting the adduction moment at the knee, acting as a 'brace' and the evidence for this was strengthened by the association between lateral co-contraction and the moment impulse. The impulse was chosen for this comparison as, like the measure of co-contraction we have used, it is a summary of the waveform covering the whole of stance phase, rather than a single point in the gait cycle. However, there was no association between any of the gait factors and medial co-contraction, and the reason for these changes remain unclear.

Lewek et al (2004) previously demonstrated that medial co-contraction in an arthritic joint was related to joint pseudo-laxity and instability, and Ramsey et al demonstrated that the primary effect of valgus bracing was a reduction in medial co-contraction at the knee (Lewek, Rudolph et al. 2004; Lewek, Ramsey et al. 2005; Ramsey, Briem et al. 2007). This study has found that co-contraction is a bilateral phenomenon. Although pseudo-laxity might be responsible for co-contraction in a joint which is aligned in varus, there is unlikely to be significant pseudo-laxity on the opposite knee if it is well aligned and not painful.

Co-contraction has been described as a normal physiological response to unfamiliar events or anticipation of a fall (Marigold and Patla 2002; Cappellini, Ivanenko et al. 2010). It presumably acts to stabilise and stiffen a joint, although it may be a 'readiness' strategy, in which high contraction is accepted in order to protect against potential falls. Co-contraction may also be a physiological response to perceived instability or a perceived risk of falling (Lewek, Ramsey et al. 2005).

It is worth noting that similar patterns of muscle co-contraction and prolonged muscle firing during stance are seen when normal subjects walk on a slippery surface (figure 4.23) (Marigold and Patla 2002; Cappellini, Ivanenko et al. 2010). There are also striking similarities in kinematics and kinetics, with slow stable gait speeds, widened base of gait and similar profiles of the ground reaction vector (Marigold and Patla 2002; Cappellini, Ivanenko et al. 2010).

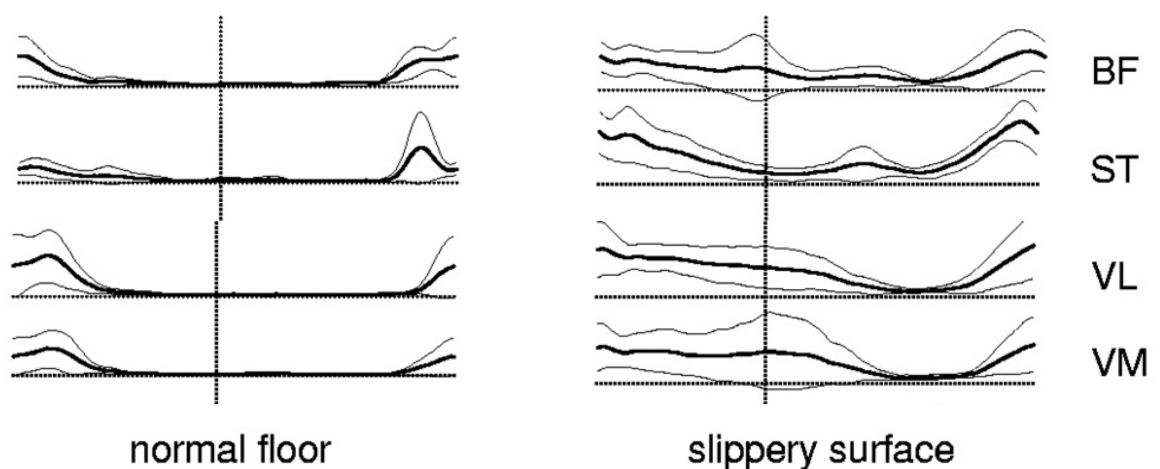


Figure 4.23. EMG signals from healthy individuals on normal floor and a slippery surface. From (Cappellini, Ivanenko et al. 2010).

Studies using instrumented hip replacements have demonstrated that joint loads increase dramatically even during minor stumbles and muscles around the knee contract forcefully during a stumble or trip, increasing joint loading (Marigold and Patla 2002; Bergmann, Graichen et al. 2004; Pijnappels, Bobbert et al. 2005). It is therefore hypothesised that co-contraction is a protective strategy, in order to protect against slips or stumbles that might otherwise cause substantial pain in individuals who have become apprehensive of pain. Thus, patients accept moderate

increases in joint loads during gait to protect against intermittent episodes of more severe pain.

The relationship between EMG signals and strength has not been examined in this study. The force generated by co-contraction can therefore not be directly estimated by the EMG data alone. It may be that a loss of muscle power compounds a loss of confidence in the knee as a part of the pathological process. Future studies into co-contraction should include strength as a covariate or examine strength training as a treatment alternative and this will be considered in the conclusion (Section 7.4).

On the basis of these findings, co-contraction is likely to represent a major potential cause of increased bilateral joint loading in this population. This is an important aspect for investigation in the future.

#### 4.4.4 Dynamic Alignment

The new dynamic alignment measure used in this study was an attempt to reduce one potential source of error in the measuring alignment, whilst avoiding the need for complex calibration protocols to establish functional joint centres. The potential benefits of this approach are that it provides a measure of alignment in a functionally derived axis system, which should be robust to the amount of knee extension that the subject can achieve. Once the code is written it is simple to

perform and can be performed post-analysis on a widely used system for gait analysis both in the UK and worldwide.

Previous studies of dynamic alignment measures have shown good correlation to radiography (plain x-ray and CT) and both of these papers used some form of rotational correction to correct for mis-registration of the axes (Mundermann, Dyrby et al. 2008; Kornaropoulos, Taylor et al. 2010), although neither is identical to the technique described above.

Functional methods have been used by a number of centres to establish joint centres and axis definitions, and the optimum technique remains a matter of debate (Piazza, Okita et al. 2001; Schwartz and Rozumalski 2005; Ehrig, Taylor et al. 2007). The 'SARA approach' (from Charite University, Berlin) was used by Kornaropoulos et al successfully to measure alignment, and this technique has been shown to give very similar results to the 'Schwartz approach' (from Gillette Children's Hospital, USA) despite different mathematical formulae being used in the two studies (Schwartz and Rozumalski 2005; Ehrig, Taylor et al. 2007).

Conceptually, the Schwartz and SARA approaches are similar to the technique described in this thesis with the knee approximated as a hinge and the calculations based on the relative transformation of two segments over two or more time periods. Whilst the assumption that the knee can be modelled as a hinge could be challenged, functional techniques that describe the knee as a helical axis have not shown additional benefit (Ehrig, Taylor et al. 2007). The x-y plots presented in section 4.3.4 (figures 4.13-4.16) demonstrate that in the majority of individuals, a

section of the gait cycle can be identified that is sufficiently linear for that approach to be a reasonable approximation.

The method presented remains susceptible to skin movement artefact (as all the marker-based techniques do) and the estimation of the joint centres, particularly the hip joint centre. Using the standard regression equations of Davis et al and Bell error in the measurement in the medio-lateral direction is likely to be in the order of 0.5-1cm, which would approximately correspond to an error of 0.5-1 degree in alignment (Bell, Pedersen et al. 1990; Davis, Gage et al. 1991; Seidel, Marchinda et al. 1995; Schwartz and Rozumalski 2005; Andersen, Mellon et al. 2013). However a recent paper has described a regression equation which has since been shown independently to more closely approximate the hip centre and the application of this technique might improve the accuracy of our measure (Harrington, Zavatsky et al. 2007; Andersen, Mellon et al. 2013).

The technique described in 4.2.5 and reported in 4.3.4 gave similar results to the 'standard' technique in terms of mean values for the normal and abnormal study groups. The assertion that the unaffected knee from the OA group was normally aligned is strengthened by the fact that the same finding is made even when using different methods for measurement.

Examining the means alone disguises random differences between the two methods and the RMS values painted a different picture, with greater degrees of error in the OA population than the healthy population. This is a predictable finding, as many OA patients have some degree of deformity or more difficult anatomy to



clearly define, greater BMI and a tendency to walk with some degree of flexion deformity.

Whilst the equations should logically provide a stable, reproducible measurement, it is accepted that these have not been proven in comparison to a radiographic measure. The presented study did not have radiographic measures included in its protocols and so a different dataset would need to be used, with appropriate radiation protection assessment and ethical approval.

The radiation dose from a long-leg radiograph is small, but not inconsequential, at 0.36mSv in Cardiff (J.Roberts and A.Rust, Radiation Protection Assessment, May 2011) and measures to avoid this where possible would be worthwhile. A correct measurement of alignment that is user-friendly, radiation free and accessible to users, as well as being acceptable to research volunteers and patients is certainly desirable, and further research is planned into this area.

#### 4.4.5 Weaknesses of the study

The study might be criticised for the lack of radiographic evidence that the contralateral knee was normal, and absence of radiographs in the control populations. However, the data shows that there was no difference in alignment between the control population and the unaffected knees of the subjects. What is more, the study recruitment was very specific in that no patient had any history of pain in the opposite joint. Therefore, if there were subtle 'pre-symptomatic' changes in the

unaffected knee, it is unlikely that they had any influence on gait either through pain or structural deformity.

Whilst the control group in the age matched population could be used for the kinetics component of the study, the control group for co-contraction were adults aged 20-60. This was because of limited time and available laboratory space, as EMG analysis added over an hour to each assessment at the time. This problem has since been resolved as the laboratory has recently purchased a 'wireless' EMG system, so that recording can be done simultaneously, rather than in turn.

No kinematic or kinetic differences were noted between the gait analysis and EMG control groups and previous studies have shown either very small differences, or no differences in co-contraction between young and old adults for level walking at preferred speed (Schmitz, Silder et al. 2009; Monaco, Ghionzoli et al. 2010).

It is accepted that a population of patients with single joint pain may not be considered representative of the general population of patients with severe OA, the majority of whom have pain in multiple joints. It would have been very difficult to isolate the most significant pathology in patients with multi-joint pain, and the restrictive inclusion and exclusion criteria that was used means that the changes identified are highly likely to be due the subjects strategy for coping with the arthritic joint. It may therefore be seen as a relative strength of the study. This may be investigated further in time as new and larger studies are likely to require broader, more representative study populations.

The inclusion of patients undergoing both total and medial uni-compartmental joint replacement was to include a broad range of patient function and disease severity whilst maintaining the emphasis on single joint medial compartment disease. It also ruled out a 'listing bias' between surgeons. By definition, patients in this study were all awaiting arthroplasty of some form and so can be considered to have 'end-stage' disease.

## 4.5 Conclusions and further investigation

Gait changes in knee OA should not be considered as simply 'antalgic'. Rather, characteristic changes in severe OA include a reduction in gait speed in favour of a stable gait pattern with loss of the normal biphasic pattern of joint loading that occurs during healthy gait.

Abnormal moments were observed in both knees and both hips of subjects with single-joint medial compartment knee OA. Co-contraction of the quadriceps and hamstrings of was present in both knees medially and laterally, which could cause substantial increases in joint loading. Based on these findings, patients with severe unilateral knee OA are at risk from abnormal biomechanics in their other major weight-bearing joints, particularly at the opposite knee.

Further investigation was planned on the basis of the findings. Having identified abnormalities in this group of patients, the next step was to see if they would respond to treatment of the affected knee in the form of arthroplasty, and this work is reported in chapter 5.

A longitudinal study with a large population of patients would be an ideal way of further investigating the link between mechanical loading and ongoing degeneration in this population. This kind of study is associated with substantial time and cost, well beyond the scope of this thesis. However, the relationship between mechanical load and biological response in cartilage warranted further investigation. Therefore an in-vitro study was planned to study the relationship

between the pattern of mechanical loading and the response of chondrocytes to that load, and that study is reported in chapter 6.

## **Chapter 5. Biomechanics of the affected and unaffected joints following knee replacement**

## 5.1 Introduction

### 5.1.1 Overview

As described in chapter 4, a population of 20 OA subjects with unilateral knee OA awaiting arthroplasty were found to have abnormal patterns of joint loading in both knees and both hips. This is despite the subjects being carefully selected as having no symptoms or deformity in any joint other than the affected knee. This raised the question as to how they would respond to treatment, and whether treatment in the form of the planned joint replacement would result in restoration of loads in the opposite side to normal.

Whilst the primary outcome measures have already been defined in the previous chapter, an analysis of changes in physical function and the relationship between function and joint loading was also desired. This could be done using very simple metrics, such as gait speed, but given the wealth of data available a further, more in depth analysis of the data was planned. The aim of this analysis was to explore the data to identify potential causes and drivers of the change in gait pattern, and identify which areas, if any, might be targeted with novel or altered treatments.

### 5.1.2 Analysis of complex gait data sets

The previous analysis focused on examination of single factors or summary measures. Whilst that approach is able to answer the primary research question ('is loading abnormal?'), it excludes a large amount of the data that is collected during

a gait analysis. Whilst some of the gait data may be considered prone to error (such as internal-external knee rotation, see section 2.2.5 and 2.2.6), other pieces of data may be considered reliable and may provide insights to the pattern of gait or the functional deficits that are a part of the package of abnormal gait and abnormal loading described in the last chapter.

Gait data can be difficult to analyse using simple statistics. Multiple waveforms with complex physical and causal inter-relationships make it very difficult to interpret using a typical multiple regression approach. Neural network approaches could be considered but their inherent complexity can make it difficult to understand the meaning of a result, how it was reached or where errors could have been introduced inadvertently (Jones 2004).

The use of the Dempster-Schafer theory of evidence to analyse gait data was briefly described in section 2.3.1. This had been used in Cardiff originally to predict future success in business based on multiple and potentially conflicting datasets (Beynon 2005; Beynon, Andrews et al. 2010). It was introduced to medical engineering in the early 2000's by Dr Cathy Holt and Dr Lianne Jones in an attempt to create a classification system that could be used to distinguish differences between fixed and mobile bearing total knee replacement patients (Jones, Beynon et al. 2006; Jones, Holt et al. 2008). The simplex plot was introduced by the same authors to give a visual assessment of gait function that could be readily interpreted. It has since been used to study hip and shoulder function, as well as gait in cerebral palsy



before and after selective rhizotomy (Whatling, Dabke et al. 2008; Whatling, Evans et al. 2009; Stroud 2012; Aranganathan 2013; Whatling, Beynon et al. 2013).

The classifier considers each piece of evidence in a Bayesian-like approach (Jones 2004; Jones, Beynon et al. 2006). A Bayesian approach to decision making assesses forward probability based on known probabilities, and assigns mutually exclusive probabilities. In Bayesian theory, if a Manchester football fan has a probability of 0.6 of supporting United, then there is a probability of 0.4 that the same fan supports City. The Dempster-Shafer approach extends this by considering the possibility that the result could be uncertain (Dempster and Weisberg 1968; Shafer 1976). A 'binary frame of discernment' describes two exhaustive, mutually exclusive hypothesis (United or City in football fans living within Salford and central Manchester) and assigns a belief value to each based on the available evidence.

So for example, we might look at the colour of a football fan's t-shirt and the location of their house and define two belief values: Belief 1 (United), Belief 2 (City), Belief 3 (both) or Belief 4 (neither). As these hypotheses are considered mutually exclusive and exhaustive, 3 and 4 are discarded, giving 2 potential belief values. A fan with a sky blue t-shirt living in Moss side (a traditional City supporting area) would have a high belief value for B(City) and a low value for B(United). However, the evidence may give little credence to either, or may conflict. In our example, a fan might wear a white top and live directly between the two football grounds (little evidence for either), or might live in Moss Side and wear red (a conflict). Therefore a third belief value is also given to the possibility of either or neither

condition, and that value is referred to as uncertainty. Each belief is assigned a value based on the evidence that supports it, so that the three belief values total 1.

Each piece of data ( $v$ ) is assigned a value between 0 and 1 (called a confidence factor) based on a sigmoid function (the equation is defined in Jones, Holt et al 2006), and is dependent on the distance of the true value from a value that would have a 50% chance of being in either group ( $\theta$  in figure 5.1a). This can be used to generate 3 belief values for that variable (figure 5.1b). If there are multiple pieces of data, their belief values are combined mathematically using Dempster's rule of combination, to give a final result with three belief values (Jones, Beynon et al. 2006).

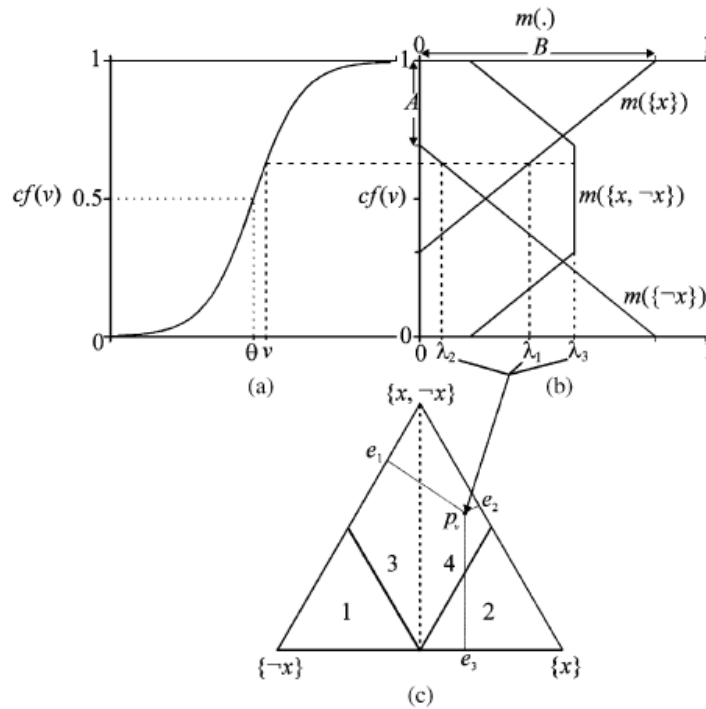


Figure 5.1. From Jones, Holt et al 2006. Conversion of a scalar value  $v$  into three belief values plotted onto a simplex plot. a) demonstrates the sigmoid function which is used to define the confidence factor, b) a single confidence factor is used to define the three belief values c) a simplex plot can be drawn, with a point for each case plotted based on the three values.

To develop a classifier for a specific purpose, it first needs to be trained. This is performed by having two dichotomous groups of inputs (football fans, or gait analyses) whose true identity is known. Waveform data is analysed using principle component analysis, which identifies sections of a waveform that best define variance in the combined population, and assigns a scalar value (the principle component value) to those sections. The shape of the sigmoid curve can be defined according to the mean and spread of the data, allowing a confidence factor to be calculated. The conversion of confidence factors to belief values are also dependant on setting the values A and B. The input variables can be chosen either by a mathematical process or based on their interest to the observer.

The final classification is therefore an analysis of where a subject sits based on a broad range of observations from the training group. The belief factors and confidence factors can be defined and even ranked, therefore allowing the mathematics to be 'reverse engineered', so the effect of single variables can be studied, or the relative importance of variables can be examined. It does depend on well defined and screened training groups, and it may be sensitive to different gait labs, which may produce slightly different waveforms and results for similar patient groups due to variations in laboratory protocols.

In this study, it was decided that the classifier could be used as an exploratory technique to understand and describe the gait changes pre- and post-operatively in as objective a manner as possible.

Therefore, the three aims of this chapter were:

- 1) To assess the change in joint loading before and after knee replacement in subjects with unilateral knee osteoarthritis.
- 2) To analyse the data mathematically to determine the primary factors distinguishing OA subjects from normal.
- 3) To assess the difference in objective physical function between healthy controls and subjects with knee arthritis before and after surgery.

The null hypotheses to be tested were:

**There is no change in the adduction moment impulse in either the affected or unaffected leg following unilateral knee arthroplasty surgery.**

**There is no factor that predicts differences in function between individuals with OA and controls.**

**Physical function measured in each leg of patients with knee OA does not return to normal after surgery.**

## 5.2 Methods

### 5.2.1 Study management

A substantial amendment was made to the North Wales (East) LREC for the post-operative analysis but the panel felt that this represented a new study, so the amendment was rejected. Therefore a new ethical application was made, which was approved in full at the first meeting of the LREC (REC number 10/WNo03/8). A new funding application was again made to the North Wales NHS trust (now Betsi Cadwaladr NHS Trust), who agreed to provide funding for £7000 for this phase of the study, making their total contribution £14,400. The trust again agreed to sponsor the study from a research governance perspective.

It was decided that a post-operative recovery period of 12 months would be appropriate, as studies at the time suggested that restoration of physical function (to the maximum for that patient) start to plateau at 6 months and should have reached a plateau by 12 months (Whatling 2009).

As the study population was already defined, a sample size calculation was technically redundant. However for information purposes a calculation was performed based on the difference between the opposite leg and normal individuals from the data in 4.3.2 and equation 13 in 4.2.6. This gave a sample size of 16 to achieve a power of 80% ( $\alpha=0.05$ ).

Prior to contacting the OA subjects, the relevant consultant's secretaries were contacted and a search was made of the hospital patient information system to

check that there had been no deaths in the intervening time and no changes in the contact details. Dates of surgery were also collected. Subjects were re-invited by means of a letter, with study information sheets enclosed, approximately 11 months following their surgery. If no contact was received after 2 weeks, a telephone call was made to invite them to return. All subjects were contacted using this approach and no further approaches were required. One subject asked to be contacted again after an additional 6 months to allow for changes in employment, at which point he subsequently agreed to take part.

If the individual agreed to attend, an appointment was made and they were consented formally prior to starting the session. WOMAC and Oxford scores were collected as before. Travel expenses were paid through the trust on a mileage basis, the only difference to the previous study being that where taxis were refunded after the event in the first study, an agreement was made with a taxi company that payment for transport would be by invoice only direct to the trust, resolving one of the administrative challenges experienced previously.

#### 5.2.2 Gait and electromyography methods

After the forms had been filled in and anthropometric data re-recorded, a gait analysis session was undertaken by the staff at ORLAU. The gait analysis protocol described in 4.3.2 was used again for the follow-up. There was no change in physiotherapy staff or technicians and the same laboratory was used with the same cameras and force plates. A yearly audit of the gait analysis process is performed

using healthy volunteers analysed repeatedly with different staff to ensure that the laboratory staff and physiotherapists remain consistent with their marker placements and data collection protocols and levels of reliability were found to be high.

The only significant change in protocol was the introduction of a new EMG system. A 16 channel Delsys Trigno wireless EMG system was purchased by ORLAU in June 2011 and used for this cohort. Skin preparation and marker placement were the same as described above but as the electrodes are wireless, the small backpack and trailing wire of the previous system was unnecessary. This allowed the EMG and the gait analysis to be collected during the same walks, and therefore the gait analyses themselves were more efficient, reducing the burden on a primarily clinical laboratory. As the EMG recordings were normalised to the peak value during the trials, any difference in sensitivity between the two systems would not result in significant changes in the registered co-contraction index.

### 5.2.3 Statistical analysis

Raw gait and electromyography data was processed in the same manner as described in 4.2.4. Measurements were normalised to height where appropriate (weight normalisation is performed within the Plug in Gait pipeline in Vicon Nexus) and converted to the units and definitions given in section 4.2.4. Trunk data and limb alignment measures were taken as before.

Changes in gait data were analysed visually, and arrow charts were prepared to show pre- to post- operative changes in the primary measures of interest. Pre- to post-operative change was assessed using paired t-tests which were not corrected for multiple testing as the primary hypotheses had been clearly defined by the previous analysis and would be interpreted as distinct entities. Post-operative findings were compared to normal values using independent t-tests.

#### 5.2.4 Principle Component Analysis

A Cardiff Classifier was trained for the affected leg using the pre-operative data (presented in chapter 4) and a separate classifier was trained using the equivalent dataset for the unaffected leg. The analysis started with the full available kinematic and kinetic data set. A full set of kinematic data (temporal measures, rotations in 3 planes for the pelvis, hip, knee and ankle) and kinetic data (ground reaction forces, moments and joint powers in 3 planes) was exported using Vicon Polygon into Microsoft Excel.

Joint translations were not measured, as a linked model was used in the Plug in Gait pipeline (i.e. it was assumed that there were no joint translations, only rotations). Knee internal-external rotation and coronal plane ankle rotation were excluded as they were known from the literature to be too unreliable (see section 2.2.5 sources of error). The six temporal variables that are displayed as standard in Vicon Polygon reports were all entered as individual variables, namely gait speed, cadence, step



length, step time, double support time and step width. This gave 28 potential variables from the gait data.

A principle component analysis (PCA) was performed on each waveform independently, using the gait data of the affected leg in comparison to the right legs of the control group, and a further principle component analysis was performed on each waveform independently, using the gait data of the unaffected leg in comparison to the left legs of the control group. The sides were assigned in this way as there was a greater number of affected right knees to lefts on the OA group.

This was performed using software written for Matlab 12 (Mathworks, MA, USA) in Cardiff by Dr Lianne Jones and modified by Mr Paul Biggs (a PhD student in the department). The code is included in appendix 3. The temporal data was not analysed using PCA as it was expressed as a single variable and therefore could be inputted directly into the classifier.

Each waveform had been exported in 2% increments between 0 and 100%, and was collated into a single sheet for each waveform. Therefore the PCA was performed on 51 'variables' arranged in columns with 40 subjects arranged in rows.

PCA is an 'orthogonal rotation' technique which follows a least-squares approach to produce linear combinations of the original data which best represents that data. It can be used to reduce complex datasets into more manageable forms whilst retaining the maximum amount of information available in that dataset. For each variable in the original dataset, a new principle component is produced, so if there are 3 original variables then there will be 3 new principle components (PC's). As

these PC's are 'orthogonal' (mathematically unrelated to the other PC's), they can be thought of as axes in a multi-dimensional scatter plot.

The method of selecting principle components were based on the methods described by Dr Jones in her thesis, although with a larger number of variables (Jones 2004). This was developed in Cardiff around the time that other units were using PCA to interpret gait analysis, with similar methods to those chosen in Cardiff (Deluzio, Wyss et al. 1997; Deluzio, Wyss et al. 1999; Deluzio and Astephen 2007).

Principle components were included until the eigenvalues were less than 1 according to Kaiser's rule, as PC's with a value under one explain less variance than the original dataset (which has unit variance). Next, the factored loadings were examined, which give the weighted relationship between the original values and the PC's. Principle components were only selected if they had at least one time point with a factor loading above 0.71. That threshold had been chosen by Dr Jones as above that threshold a variable (the time percentage point) can be primarily represented by only one PC, so each PC can be interpreted separately (Comrey 1973). This method of selection gave between 1 and 4 PC's for each waveform.

Using the same principle, a cut off in the factor loading of 0.71 and above for each time point was used to select regions of the waveform that were defined as being of primary importance for each principle component. These were referred to as principle component regions, an example of which is given in figure 5.2. Their use is in allowing a practical interpretation of the PCA data by relating it back to the original data.

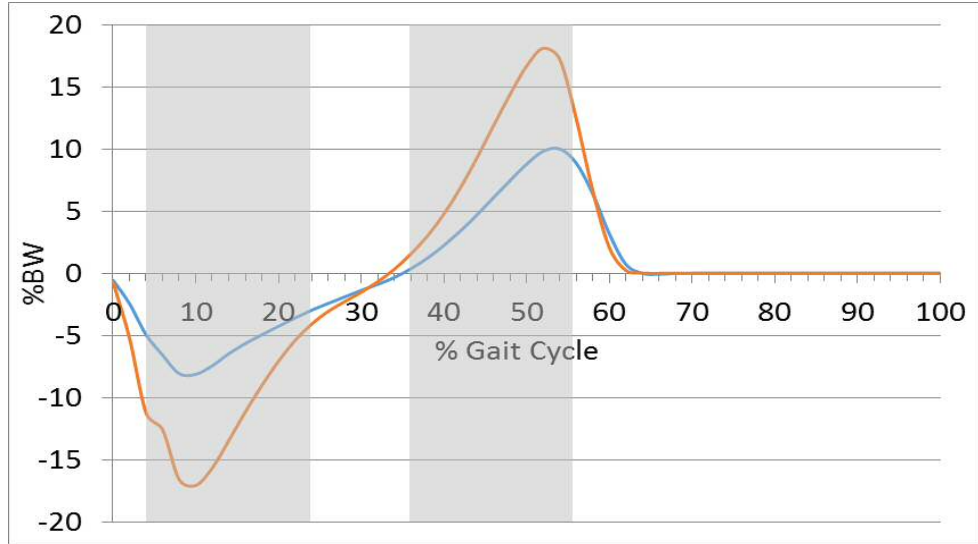


Figure 5.2. An example of the use of factor loading to define principle component regions, using the first PC for GRF-X as an example. The chart is the mean antero-posterior GRF for the normal subjects (red line) and the OA pre-operative subjects (blue line). Using the PCA factor loadings, two regions were identified with factor loadings for all time points greater than 0.71 (shaded in grey).

For each of the principle components selected, PC Values were extracted to be used as the variables entered into the classifier. These were calculated as part of the Matlab function according to equations 1 and 2 for each waveform separately.

$$\Omega = ZE$$

(Equation1)

Equation 1 describes how a 40x51 matrix of PC Values ( $\Omega$ ) are calculated by multiplying the 40x51 matrix of standardised scores ( $Z$ ) by the 51x51 eigenvector matrix ( $E$ ), essentially projecting the standardised scores in the new orthogonal 'axis system' defined by the PCA. The matrix of standardised scores was calculated as described in Equation 2, where the original values ( $x$ ) are normalised to their mean and then divided by their standard deviation.

$$x_{standardised} = \frac{x - \bar{x}}{\delta_x}$$

(Equation 2)

The values for  $\Omega$  that corresponded to the selected PC's were then collated and used to train the classifier.

#### 5.2.5 Training the Dempster-Shafer Classifier

There were an excessive number of PC's selected using the rules above, some of which had poor classification accuracy and little relevance to the condition, and therefore a further process was required which would reduce the number of variables to a manageable number. Dr Jones found (with similar group sizes to this study) that 15 to 20 variables produced a good classification which adequately distinguished individuals between groups, so for the current study a similar number was targeted (Jones 2004).

A process of variable reduction was then performed. A mathematical or logical approach was preferred where possible, to prevent an 'observer bias' where variables might have been selected that supported the theories of the researcher, rather than those which mathematically described the observed gait pattern.

There is no defined limit to the number of variables that may be entered into a classification, although in principle the variables used should each contribute new information to the classifier (although there will inevitably be some crossover between variables), they should be relevant to the biomechanics of the disease

being studied and they should describe a variable that differentiates between diseased and normal states.

In order to select variables further, initial Cardiff classifiers were trained with all of the variables included (55 variables for the affected leg and 54 for the unaffected leg), and those variables were ranked. This was performed using Matlab software again designed originally by Dr Jones and modified by Dr Whatling and Mr Biggs (Appendix 3).

Two classifiers were trained for the pre-operative data, one for the affected and unaffected leg. As a further exploration to see if the variables selected were the most representative variables when examining post-operative gait, an additional two classifiers were trained to compare the post-operative results to the normal population, to see if similar variables separate out the pathological group to the normal group before and after surgery.

Ranking was performed using the percentage classification accuracy achieved by that variable alone. If there was two variables with the same accuracy, the rank was determined based on the lowest score for a calculation referred to as  $OB_{rank}$ . The  $OB_{rank}$  value was defined as the Euclidean distance of the mean data point for a group to the vertex associated with that group. That is, the shortest distance between a perfect classification and the mean of the plotted points for that variable.

Based on this initial classifier training data, the top 20 variables were selected, which was considered from Dr Jones' experience the highest number of variables

that should be included in the final classifier. Only one PC was included for each variable, so if there were 2 PC's from the same variable, then the second was excluded.

As temporal variables were not represented by PC's, a similar selection process was used to exclude redundant or repeated data. As some of the temporal variables were mathematically related, a variable was excluded if it could be completely explained mathematically by a variable or combination of variables that had been ranked higher. Therefore step time and cadence were not included together and only the higher ranked of the two was entered (as the two measures are the inverse of each other). If gait speed, step length and step time (or cadence) were ranked in the top 20, then only the first two were included (as gait speed can be calculated from step time and step length).

Based on this selection process, two definitive classifiers were trained using only the chosen variables from the pre-operative and normal datasets.

Sigmoid curves to calculate the confidence factor for a variable (figure 5.1) were established using Equation 3 (adapted from Jones 2004), and values for A and B (the control variables that determine the relationship between  $cf(v)$  and the belief values) were assigned.

$$cf(v) = \frac{1}{1 - e^{-k(v-\theta)}} \quad \text{(Equation 3)}$$

In this equation,  $cf(v)$  represents the confidence factor (0 to -1) for a value ( $v$ ),  $\theta$  represents the value of  $v$  at which  $cf(v)=0.5$  (i.e. there is even belief that either state

could be true, this is typically estimated by taking the mean of the value) and  $k$  is a constant for that variable.

The four control values ( $\Theta$ ,  $k$ ,  $A$ ,  $B$ ) were determined by Dr Jones using 2 approaches, the first being an optimisation approach, in which serial iterations are performed until the four values are optimal, or a simple mathematical approach. The mathematical approach was found to give the better eventual classification accuracy and therefore this approach was used in the current study.

$\Theta$  was defined as the variable mean,  $k$  was defined by a Pearsons correlation coefficient calculated between the variable and the knee type column of data (0 or 1),  $A$  and  $B$  were determined from equations 4 and 5, based on set values of the upper  $\Theta_U$  and lower  $\Theta_L$  belief values of 0.8 and 1 respectively, which were kept constant for all of the variables. These values were chosen as they achieved optimal spread of the classified data in Dr Jones' thesis without increasing uncertainty excessively (Jones 2004).

$$A = \frac{\Theta_U - \Theta_L}{1 + \Theta_U - 2\Theta_L}$$

(Equation 4)

$$B = 1 - \Theta_L$$

(Equation 5)

Finally, a leave-one-out validation of classification accuracy was performed to assess the discriminatory strength of the classifier. This is performed by re-training the classifier with all but one of the subject's data, and determining whether that

classifier has correctly placed the remaining subject in the right half of the plot. This is repeated for all of the subjects, and then a percentage classification accuracy can be calculated.

#### 5.2.6 Plotting the post-operative results

Once the classifiers were trained, the post-operative analyses were plotted in the new classifier. The first step in this process was to calculate PC values for the post-operative assessment, in the principle components selected during the training process. This was done in Matlab with software written specifically for this analysis by Mr Biggs (Appendix 3). The following two calculations were used:

$$x_{post.stand.} = \frac{x_{postop} - \overline{x_{training}}}{\delta_{xtraining}} \quad (\text{Equation 6})$$

$$\Omega_{postop} = Z_{post.stand.} E_{training} \quad (\text{Equation 7})$$

The purpose of Equation 6 is to standardise the scores relative to the training cohort, so the mean training value is taken from the new post-op value, and then divided by the training standard deviation. In equation 7, the new PC values  $\Omega_{postop}$  are calculated by multiplying the matrix of new standardised scores  $Z_{post.stand.}$  by the eigenvector matrix  $E_{training}$  from the training PCA. This was done for each of the selected variables.



The new PC values and selected temporal values were then entered into a further piece of Matlab software which established belief values for each variable, combined the values according to the Dempster-Shafer principles to give 3 combined belief values for the analysis, and then plotted those variables in the classifier, as described in section 5.1.2. This was performed separately for each individual (to visualise pre-to post op change) in each leg, and together to give an overall classification for the group, again for both affected and unaffected legs.

Change in classifier belief values were correlated against the medial and lateral co-contraction indices. Given that a linear relationship could not be assumed, and the data contained an outlier in terms of functional recovery (patient 15), a non-parametric correlation method (the Spearman rank correlation coefficient) was preferred. Given the small size of the study, correlations can only be considered exploratory and significance testing was not performed.

## 5.3 Results

### 5.3.1 Gait analysis results

Of the initial 20 OA subjects, two subjects (both initially listed for unicompartmental knee replacement) did not have their surgery as planned, one because of medical problems precluding anaesthetic and another was made as a personal decision to wait longer before arthroplasty surgery. One subject had a knee replacement and reported a good result but did not feel able to travel to the gait laboratory due to general health issues and declined the second appointment. Two further subjects indicated that they would like to return but were unable to attend as they were attending to partners with serious illnesses.

Table 5.1 charts the pre-operative mean demographics, impulse and co-contraction index for the 5 who were unable to attend for a second appointment, in comparison to the 15 who were able to attend. It can be seen that whilst the demographics were similar, mean moments and mean co-contractions were greater in the population who were unable to attend.

Therefore, fifteen subjects from the original cohort attended for a second visit. The follow up appointment was performed at a mean of 14.0 months post-operatively (SD 1.3), median 14.3 months (range 11.9 to 24.7 months). The longest follow up was 24.7 months (subject 13), at the request of the individual who was in process of changing employment, and this was an outlier in comparison to the rest of the group, where the next longest follow up was 16.9 months. There were no early

revisions and one re-operation, a manipulation under anaesthetic 3 months post-operatively in a subject who continued to suffer pain in the replaced knee at the follow up appointment.

	Attended 2 <sup>nd</sup> visit (n=15)	Unable to attend (n=5)
Age	67.8	72.8
BMI	31.4	30.3
Womac	48.4	42.2
Oxford	24.5	27.4
HAM impulse (N·m·s/BW·Ht)		
Affected	1.84	1.87
Unaffected	1.98	1.86
KAM Impulse (N·m·s/BW·Ht)		
Affected	1.32	1.84
Unaffected	1.09	1.35
Lateral Co-Contraction		
Affected	0.28	0.40
Unaffected	0.27	0.30
Medial Co-Contraction		
Affected	0.22	0.22
Unaffected	0.18	0.22

Table 5.1 Demographics and primary outcomes for the group who attended in comparison to this who were unable to attend the post-operative visit (KAM=Knee Adduction Moment; HAM= Hip Adduction Moment).

	WOMAC (0-100) mean (SD)	WOMAC Range	Oxford (0-48) mean (SD)	Oxford Range
Pre-operative	48.4 (12.1)	16 to 65	24.5 (5.7)	12 to 33
Post-operative	10.5 (17.5)	0 to 72	40.8 (8.5)	12 to 48
Change	38.0 (24.6)	-40 to 65	16.3 (7.3)	0 to 28

Table 5.2. Patient reported outcome measures

Patient-reported outcome scores are summarised in table 5.2. A number of the scores were close to normal but, for the Oxford score particularly, were only limited by kneeling, which was commonly reported as ‘no, impossible’ simply because

they'd been told not to by clinicians, or occasionally as a result of scar tenderness. The majority of scores post-operatively were normal or close to normal, but the mean was skewed by one subject who reported a poor result, with a post-operative WOMAC of 72 and Oxford of 12. The median post-operative scores were 6 for the WOMAC and 44 for the Oxford. Three subjects reported a recent onset of mild contra-lateral knee pain, whereas the others remained pain free on the other side.

	Pre-operative	Post-operative	Healthy Controls
Gait Speed ( $\text{ms}^{-1}$ )	0.97 (0.20)	1.12 (0.18)	1.33 (0.21)
Cadence (steps/min)	107 (10)	114 (8)	118 (9)
Stance percentage			
Affected limb	62.3% (2.1%)	62.0% (1.8%)	60.5% (1.6%)
Unaffected Limb	64.6% (2.8%)	62.1% (2.4%)	
Step Width (cm)	19.0 (4.2)	18.0 (4.2)	15.5 (3.4)
Limb Alignment			
Affected Limb	3.3° Varus (5.4°)	0.4° Varus (4.2°)	0.1° Valgus (2.9°)
Unaffected Limb	1.2° Valgus (2.6°)	0.4° Valgus (2.5°)	

Table 5.3. Temporal and limb alignment measures for the 15 subjects with pre-and post-operative data.

Change in temporal measures is reported in table 5.3, with the corresponding pre-operative means for the 15 subjects, and the equivalent results from the control group as comparison. Whilst gait speed improved, it did not return to normal. Limb alignment, measured using the new technique reported in section 4.2.5, was restored in the majority of cases at the affected knee and changed very little at the unaffected knee.

	Pre-op	Post-op	Controls	Pre-Post change p-values	Post-op to control p-values
Adduction Moment Impulse (N·m·s/BW·Ht)					
Hip					
Affected side	2.05 (0.33)	1.84 (0.20)	1.76 (0.13)	p=0.164	p=0.646
Unaffected side	2.34 (0.29)	1.98 (0.16)		p=0.003**	p=0.045*
Knee					
Affected side	1.32 (0.19)	0.89 (0.17)	0.84 (0.12)	p<0.001**	p=0.418
Unaffected side	1.09 (0.18)	0.99 (0.16)		p=0.132	p=0.164
Hip Adduction Moments (N·m/BW·Ht)					
Peak					
Affected side	4.38 (0.72)	4.40 (0.51)	4.71 (0.33)	p=0.973	p=0.202
Unaffected side	4.70 (0.49)	4.87 (0.46)		p=0.383	p=0.332
Mid-stance					
Affected side	3.82 (0.54)	3.43 (0.45)	2.70 (0.29)	p=0.155	p=0.010*
Unaffected side	3.66 (0.52)	3.46 (0.35)		p=0.203	p=0.005**
Knee Adduction Moments (N·m/BW·Ht)					
Peak					
Affected side	3.11 (0.40)	2.52 (0.31)	3.09 (0.42)	p=0.021*	p=0.037*
Unaffected side	2.78 (0.36)	2.88 (0.44)		p=0.654	p=0.464
Mid-stance					
Affected side	2.25 (0.43)	1.58 (0.34)	0.94 (0.25)	p=0.003**	p=0.004**
Unaffected side	1.70 (0.35)	1.55 (0.30)		p=0.352	p=0.008**

Table 5.4. Moments and moment impulses pre-and post-operatively for the 15 with follow-up appointments expressed as mean (+/- 95% confidence interval). Significance testing was performed using paired t-tests (for the pre-to post-operative comparisons) and independant sample t-tests (for the post-operative to control comparison). \*=significant (p<0.05) \*\*=highly significant (p<0.01).

Changes in moments are reported in table 5.4. As before, moments are reported in terms of the peak, the mid-stance moment and the adduction moment impulse,

with the primary outcome being the adduction moment impulse. The knee adduction moment impulse for the 15 subjects with pre- and post- operative follow up is given in figure 5.3, with the individual values as well as means and confidence intervals plotted.

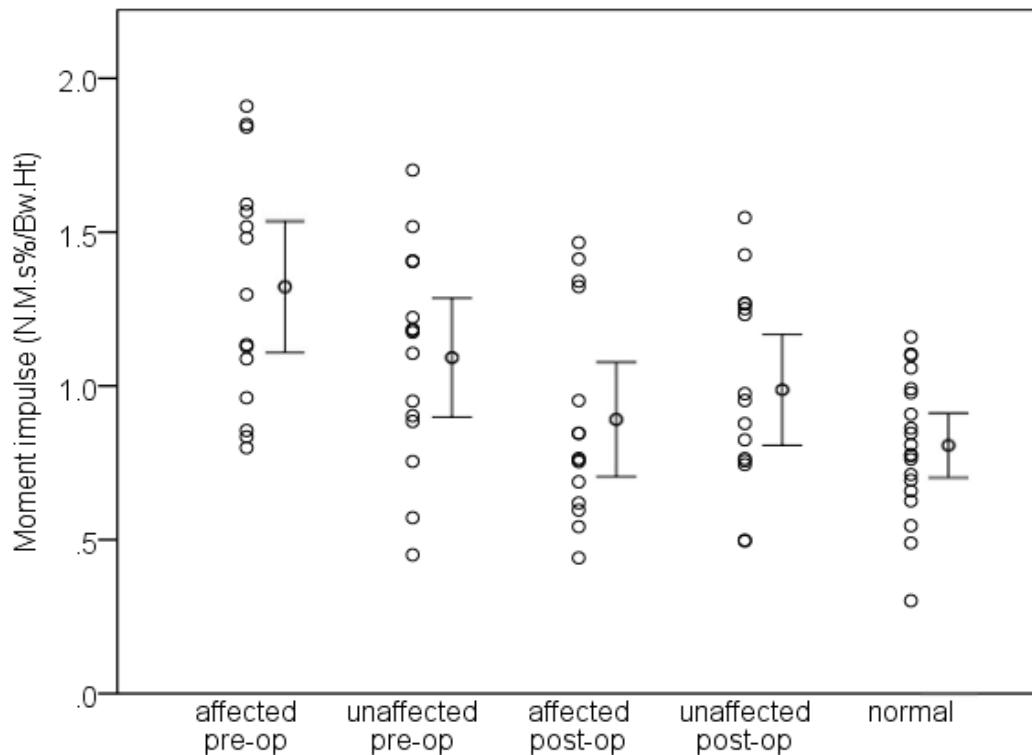


Figure 5.3. Knee adduction moment impulse for the affected and unaffected knees before and after operation, along with values from the healthy control population. For each group, a scatter plot of the actual values is given, along with a mean and the 95% confidence interval error bars.

Whilst moment impulse was the primary outcome for the study, this chart fails to describe the paired nature of the data, and does not show the variability in response seen in the waveforms between different patients. This is better explained by thinking of the peak and mid-stance values individually, as was described in chapter 4. In order to give a visual assessment of the changes and

variability in response, arrow charts were produced which described individual changes in the peak and mid-stance moments separately (figures 5.4 to 5.7).

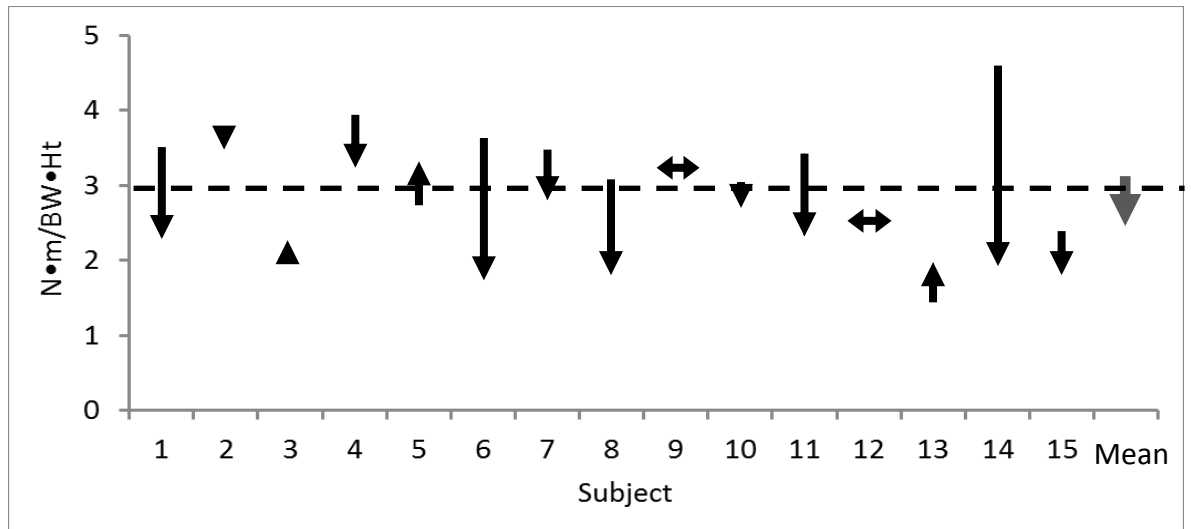


Figure 5.4 Arrow chart for the peak moment at the affected knee (the control group mean is represented by the dashed line)

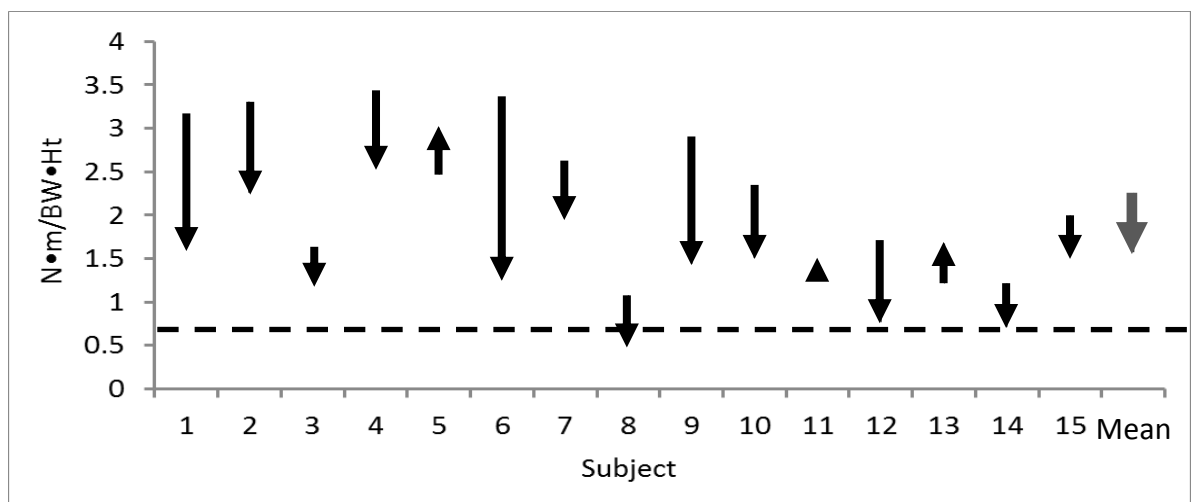


Figure 5.5 Arrow chart for the mid-stance moment at the affected knee

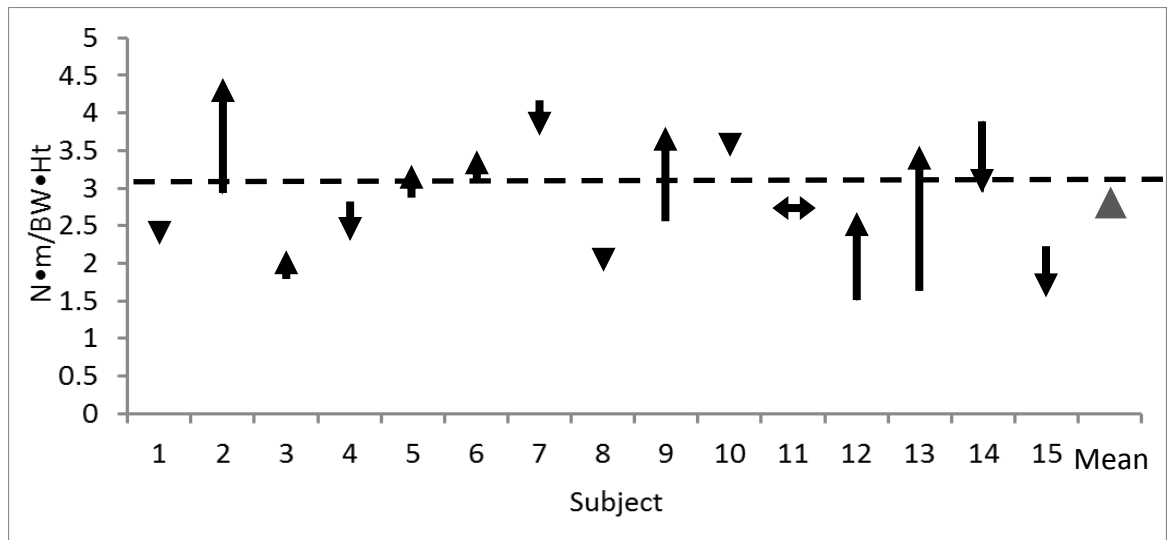


Figure 5.6 Arrow chart for the peak moment at the unaffected knee

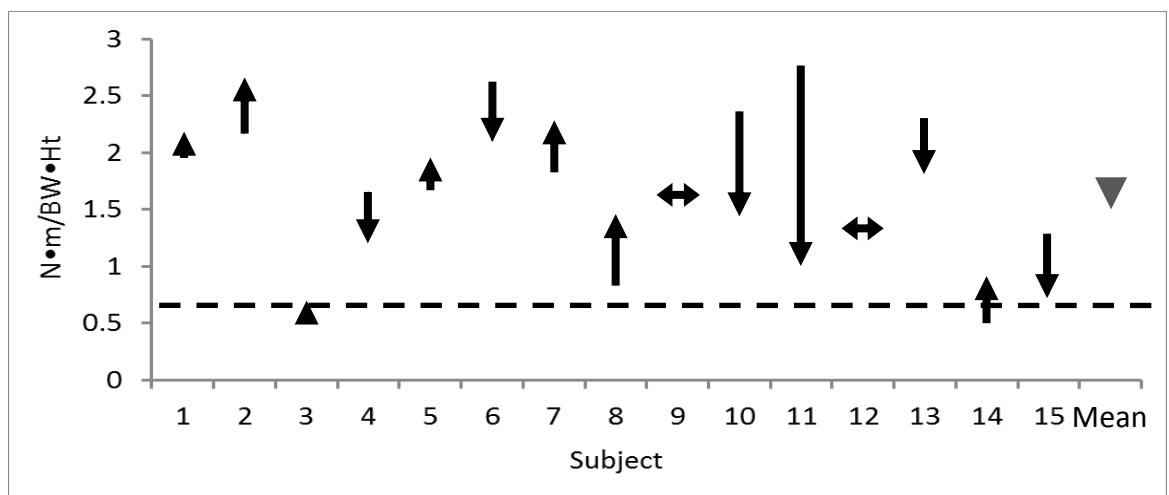


Figure 5.7 Arrow chart for the mid-stance moment at the unaffected knee

### 5.3.2 Co-contraction results

The co-contraction results for the 15 subjects with pre- and post-operative data are given in table 5.5. As before, individual values have been plotted on arrow charts to give an idea of the spread of the data and the magnitude of change for individuals (figure 5.8 and 5.9). The medial and lateral co-contraction results are plotted together, and each subject is separated by a grey line. The charts demonstrate the



variability of response from subject to subject, with a neither side seeing a full recovery and little change seen in the majority of subjects in the unaffected leg.

	Pre-operative	Post-operative	Controls	Pre-Post op significance	Post op to control significance
Medial					
Affected side	0.27 (0.03)	0.22 (0.03)	0.14 (0.02)	p=0.018	p<0.001
Unaffected side	0.18 (0.03)	0.18 (0.04)		p=0.74	p=0.17
Lateral					
Affected side	0.28 (0.04)	0.24 (0.04)	0.15 (0.03)	p=0.071	p=0.003
Unaffected side	0.22 (0.05)	0.21 (0.04)		p=0.74	p=0.016

Table 5.5. Co-contraction indices for the 15 subjects with two visits, and control values, expressed as mean (+/- 95% confidence intervals).

Overall, we can conclude that the change in co-contraction was very variable, with a good response at the affected knee in a few individuals but little response at the unaffected knee.

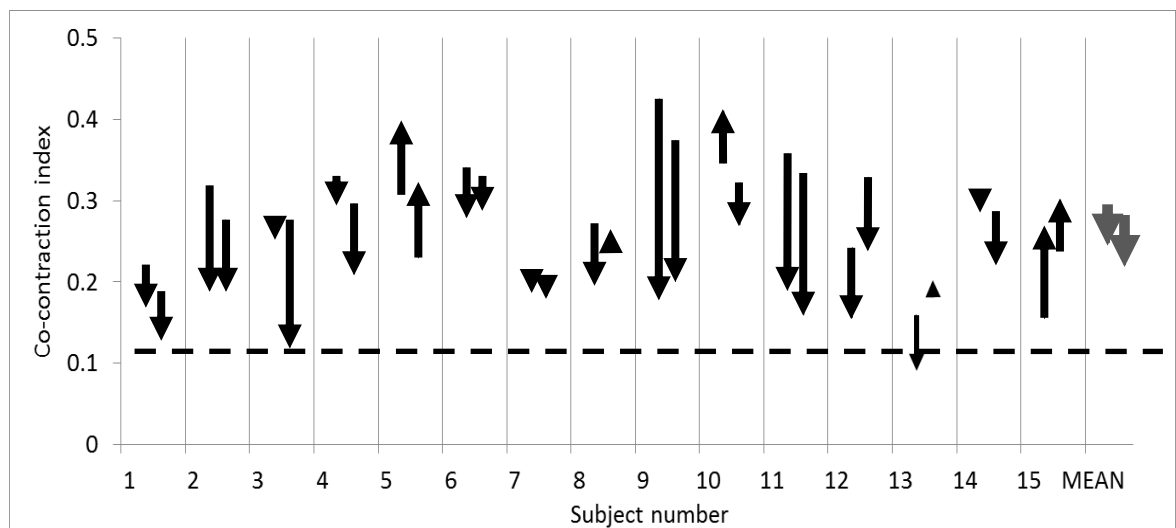


Figure 5.8. Arrow chart for change in co-contraction at the affected knee, with lateral co-contraction plotted on the left and medial on the right. The dashed line represents the control group mean value.

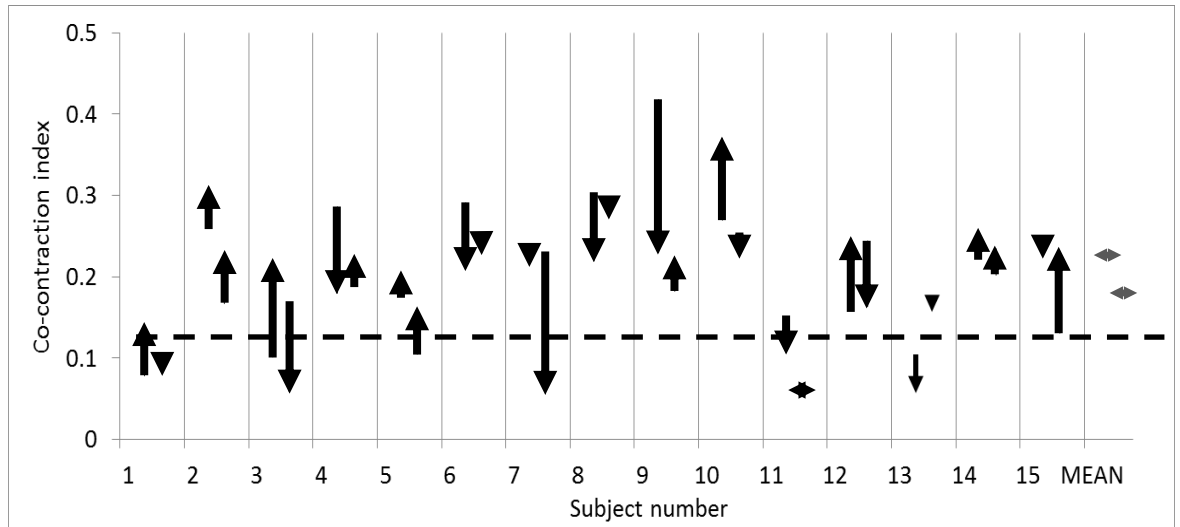


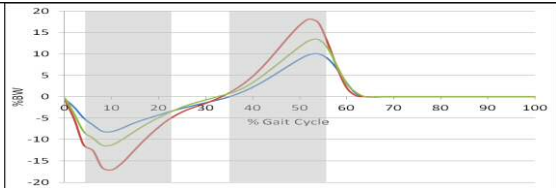
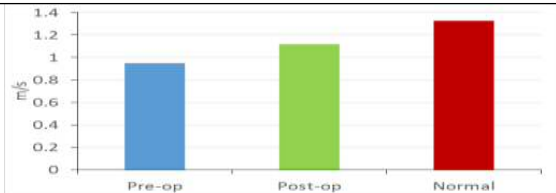
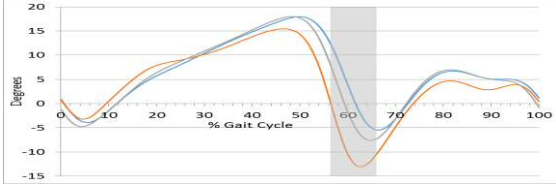
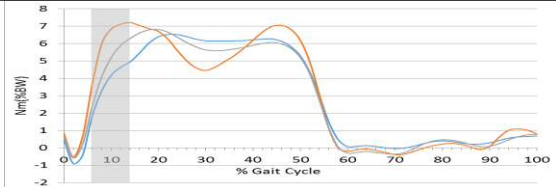
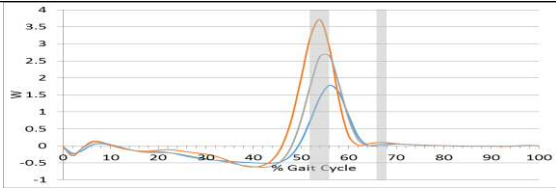
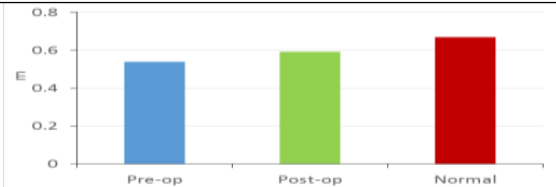
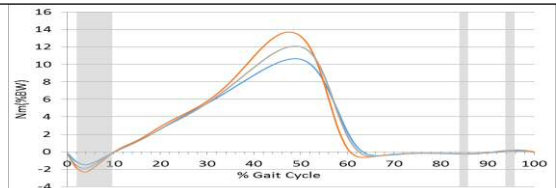
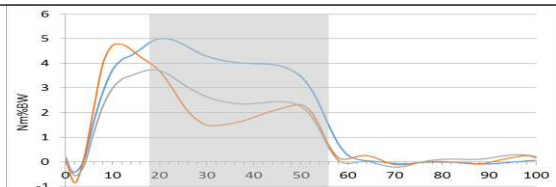
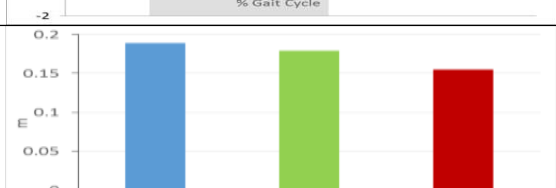
Figure 5.9. Arrow chart for change in co-contraction at the unaffected knee, with lateral co-contraction plotted on the left and medial on the right. The dashed line represents the control group mean value.

### 5.3.3 Principle Component Analysis and Classification Results

Using the process described in 5.2.4 and 5.2.5, 49 principle components were selected for the affected leg and 48 for the unaffected leg. With the six temporal variables also entered, this gave 55 and 54 variables respectively. Following the ranking process, the top 20 variables were selected for the affected and unaffected legs. Three further variables were removed from each classifier as a result of the rules given in 5.2.5. These were ankle power 2, cadence and step time for the affected leg and cadence, step length and hip power 2 for the unaffected leg. Therefore the final classifiers for each leg had 17 variables each.

The top ranked variables, charts plotting the averaged waveforms and the chosen principle components of those variables are represented in table 5.6 (for the affected leg) and table 5.7 (for the unaffected leg).

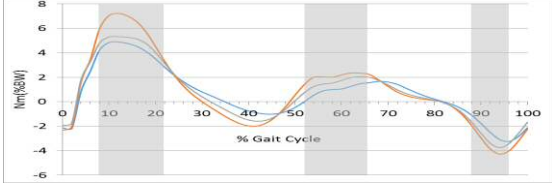
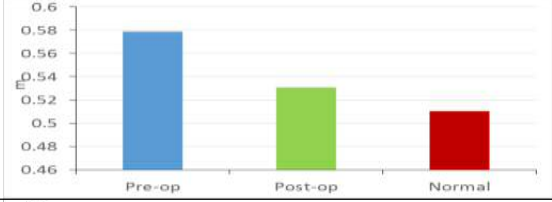
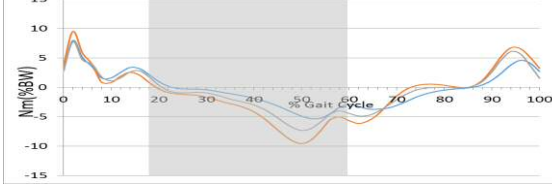
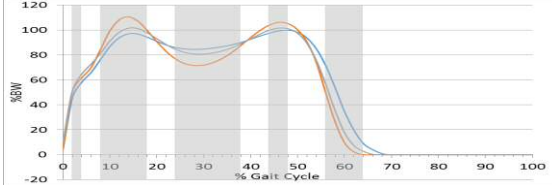
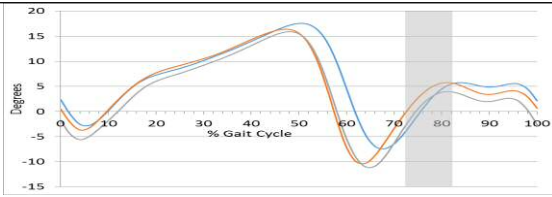
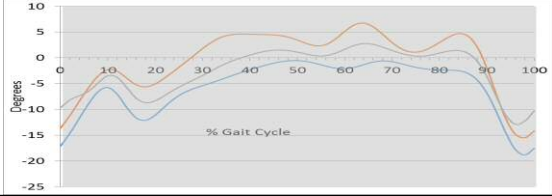
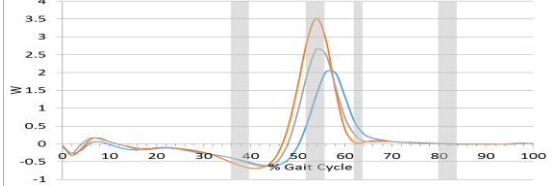
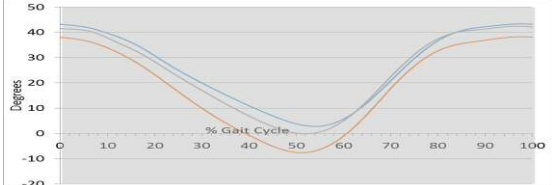
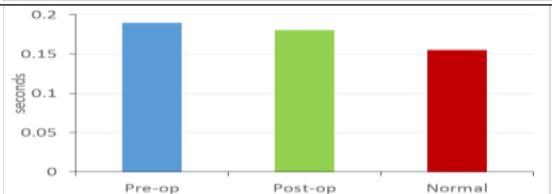
Rank	Variable	<div> <div>Charts</div> <div>Key:</div> <div> <div>Pre-op</div> <div>Post-op</div> <div>Control</div> </div> </div>	Classification accuracy
1	Hip Power 1		92.5%
2	Knee Power 1		87.5%
3	GRF Z 1		85%
4	Sagittal Knee Moment 1		85%
5	Sagittal Hip Moment 1		85%
6	Double Support Time		82.5%

7	GRF X 1		82.5%
8	Gait Speed		80%
9	Saggital Ankle Dorsi/Plantar 2		80%
10	Coronal Hip Moment 2		80%
11	Ankle Power 3		77.5%
12	Step Length		75%
13	Sagittal Ankle Moment 2		75%
14	Coronal Knee Moment 1		72.5%
15	Step Width		70%

16	Coronal Hip Angle (Ab/Ad-duction) 1		70%
17	Sagittal Pelvis 1		62.5%

Table 5.6. PC's for the affected leg

Rank	Variable	Charts Key: <div> <span style="color: blue;">—</span> Pre-op               <span style="color: grey;">—</span> Post-op               <span style="color: orange;">—</span> Control             </div>	Classification Accuracy
1	Hip Power 1		87.5%
2	Sagittal Knee Angle 2		85%
3	Knee Power 1		82.5%
4	Double Support Time		80%
5	Gait Speed		80%

6	Sagittal Knee Moment 1		80%
7	Step Time		77.5%
8	Sagittal Hip Moment 1		77.5%
9	GRF Z 1		77.5%
10	Sagittal ankle 4		75%
11	Axial Hip		72.5%
12	Ankle Power 1		72.5%
13	Sagittal hip 1		70%
14	Step width		65%

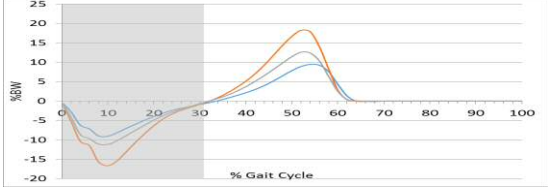
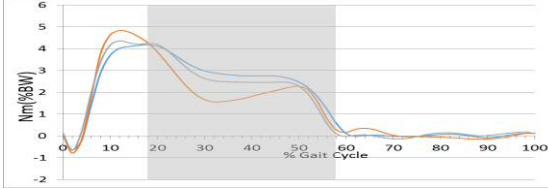
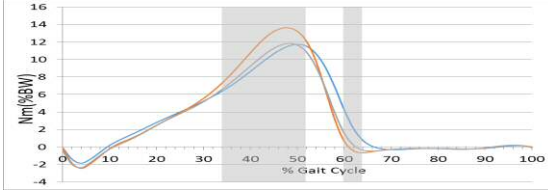
15	GRF X 1		65%
16	Coronal Knee Moment 1		65%
17	Sagittal Ankle Moment 1		65%

Table 5.7. PC's for the unaffected leg

Table 5.8 contains the details of the top ranked PC's for the classification training that compared post-operative to healthy control results. The variables were chosen using the same criteria as the PC's in table 5.6 and 5.7, as described in 5.2.5. This secondary training process was not to be used for the final classifier but was performed to confirm that the variables chosen in tables 5.6 and 5.7 would allow a reasonable assessment of post-operative gait function, and therefore to assess whether post-operative gait function can reasonably be represented in terms of a scale varying between normal and OA, using this technique.

Whilst the order of variables were different in terms of rankings, it can be seen that a similar variables would be selected, regardless of whether the PCA and ranking process is performed for the pre-operative data against control data, or post-operative data against control data. The belief values for each variable are added together to form the formal classifier without weighting according to rank, so the fact that the variables presented in table 5.8 are in a different order to those in 5.6

and 5.7 is not a concern. This was considered adequate evidence that the variables used in the final classifier could also be used to define objective function post-operatively.

Affected Leg			Unaffected Leg	
Ranking	Variable		Ranking	Variable
1	Ankle Power 1		1	Coronal Knee Moment 2
2	Knee Power1		2	Saggital Hip 1
3	Gait Speed		3	Gait Speed
4	Saggital Knee 2		4	Saggital Pelvis 1
5	Saggital Knee Moment 2		5	Double Support Time
6	GRF Z 1		6	Step Length
7	Saggital Pelvis 1		7	Saggital Ankle 1
8	Saggital Ankle Moment 2		8	Step Width
9	Saggital Hip 1		9	Saggital Ankle Moment 3
10	Double Support Time		10	Axial Hip 1
11	Hip Power 2		11	Knee Power 1
12	Saggital Hip Moment 2		12	GRF Z 3
13	Step Time		13	Saggital Hip Moment 1
14	Step Width		14	Coronal Ankle Moment 1
15	Coronal Knee Moment 5		15	Saggital Knee Moment 2

Table 5.8. Ranking of variables when post-operative results are compared to control results.

Based on the selected variables, the two final classifiers were trained, giving the classifications comparing pre-operative results to control results in figures 5.10 and 5.11. Whilst there is some clustering around the extremes in these examples (which could have been adjusted by changing the uncertainty boundaries in the training), this was accepted as it was presumed that the post-operative data would be distributed between these two extremes. Therefore the planned control values were accepted and the post-operative data was plotted in the trained classifier, giving the results in figures 5.12 and 5.13.



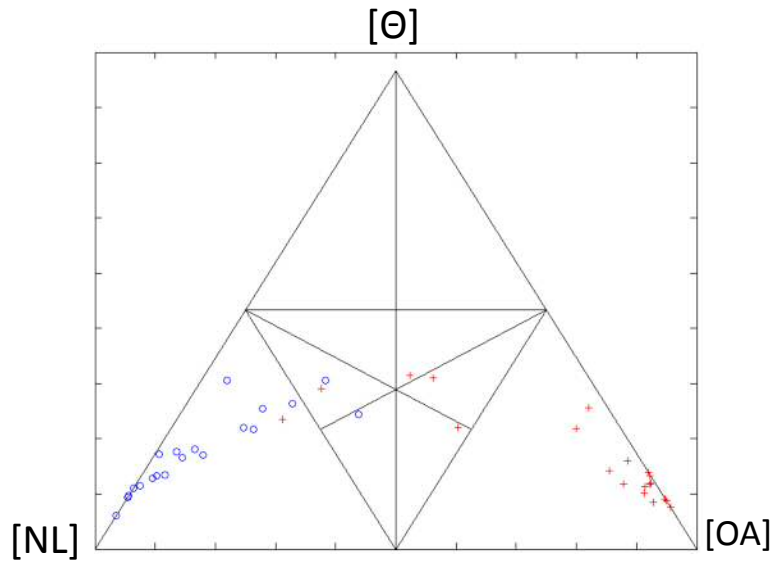


Figure 5.10. Training results for the affected leg, with healthy controls plotted with blue circles and OA subjects plotted with red crosses.

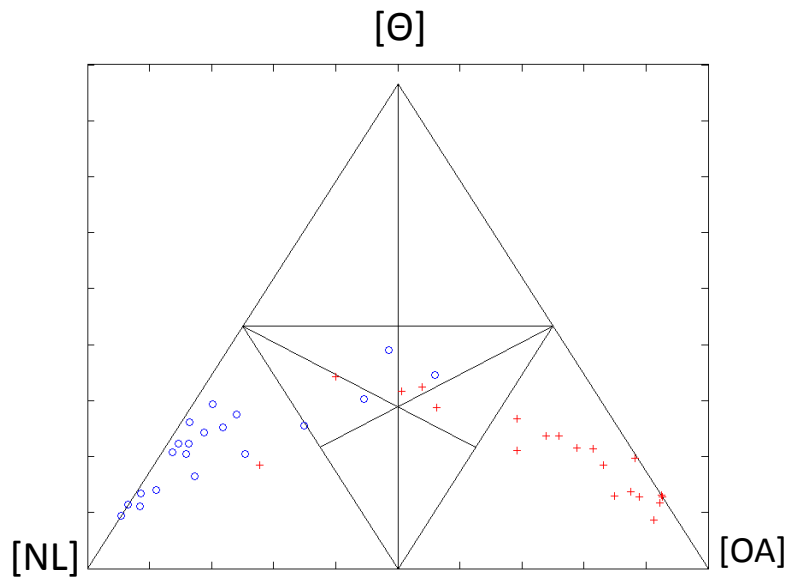


Figure 5.11. Training results for the unaffected leg, with healthy controls plotted with red crosses and OA subjects plotted with blue circles.

The classifiers for the full dataset comparing pre- and post-operative results are given in figures 5.12 and 5.13. It can be seen that the data is well spread across the classifier, although the post-operative results did form into two apparent clusters (figures 5.14 and 5.15).

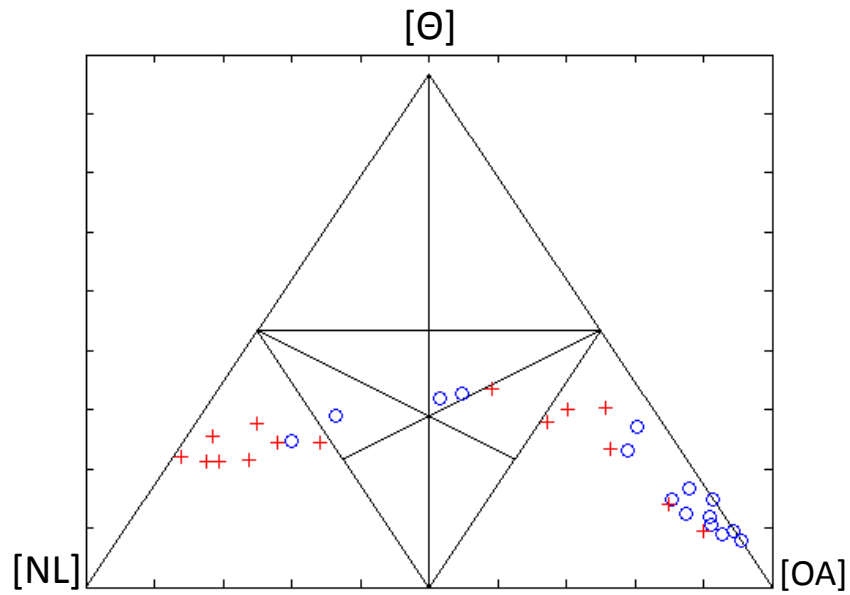


Figure 5.12. Pre- to post-operative results for the affected leg, with pre-operative scores plotted with blue circles and post-operative plotted with red crosses.

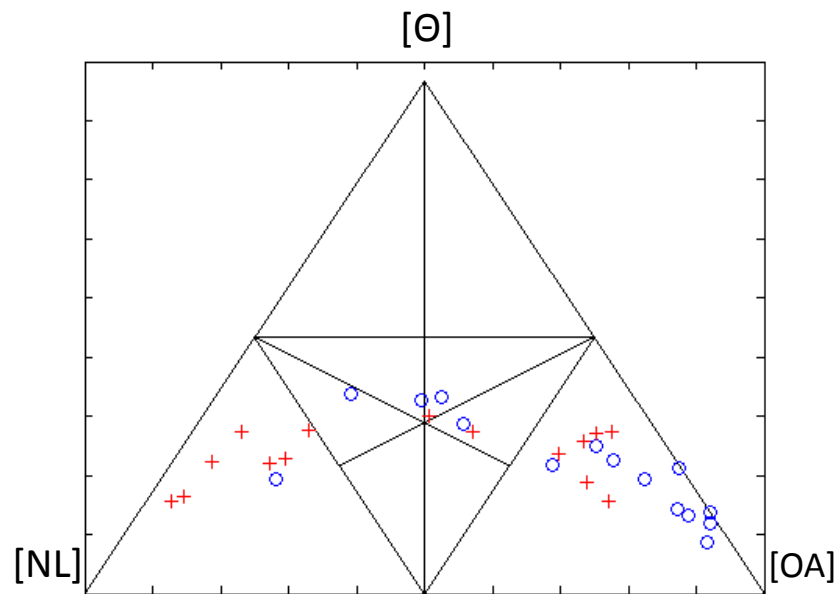


Figure 5.13. Pre- to post-operative results for the unaffected leg, with pre-operative individuals plotted with blue circles and post-operative scores plotted with red crosses.

	Pre-operative	Post-operative	Controls
Belief in OA			
Affected leg	0.66 (0.26)	0.33 (0.28)	0.13 (0.12)
Unaffected leg	0.58 (0.25)	0.34 (0.25)	0.15 (0.17)
Belief in Normal			
Affected leg	0.13 (0.18)	0.39 (0.27)	0.79 (0.16)
Unaffected leg	0.16 (0.18)	0.39 (0.26)	0.75 (0.20)
Belief in Uncertainty			
Affected leg	0.21 (0.10)	0.27 (0.07)	0.08 (0.04)
Unaffected leg	0.24 (0.09)	0.27 (0.05)	0.10 (0.05)

Table 5.9. Belief values summarised for pre and post-operative analyses and for controls (control data taken from the training results figures 5.9 and 5.10). Expressed as means (standard deviations).

Pre- to post-operative belief values are given in table 5.9 and individual plots are given in figures 5.16 and 5.17. The individual subject charts also emphasise the symmetry of the change, with similar pre- and post-operative classifications in the affected and unaffected legs.

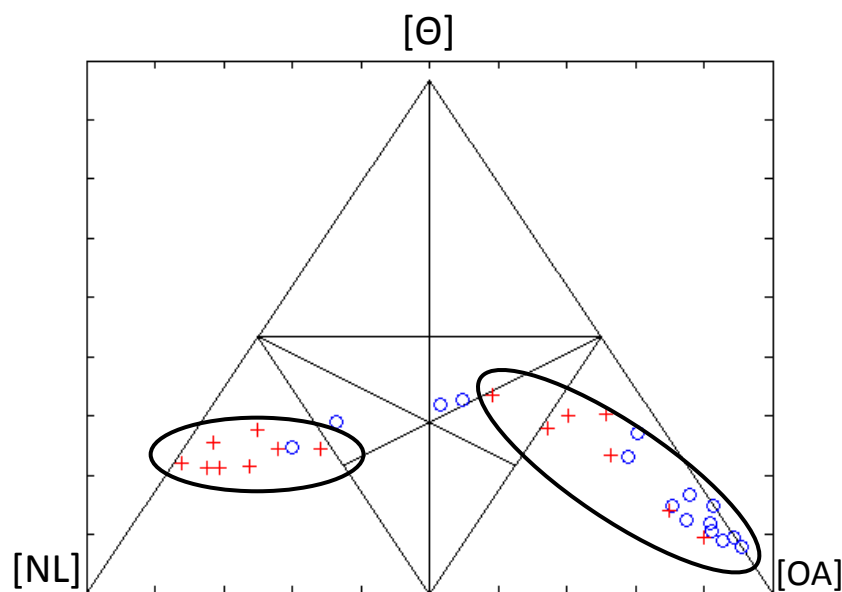


Figure 5.14. Pre- to post-operative results for the affected leg (as in figure 5.11), with the two clusters of good and poor post-operative function (red crosses) marked with ovals.

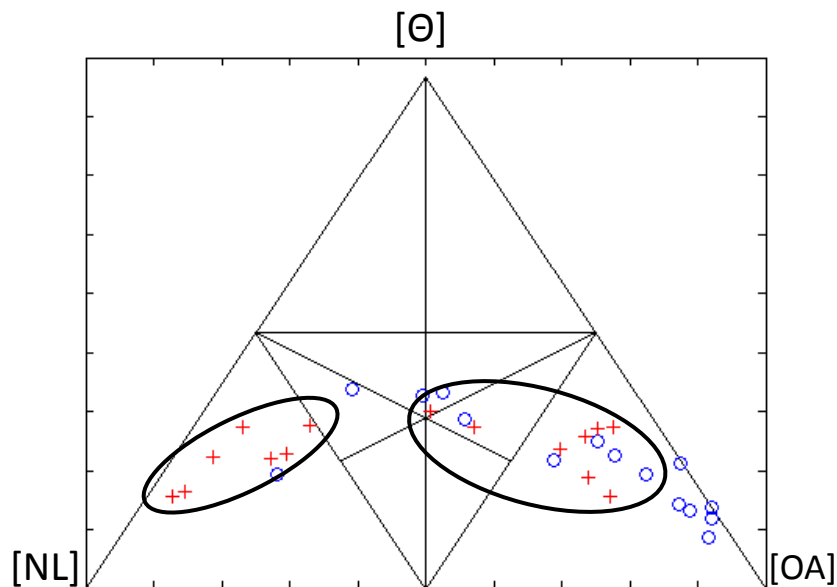


Figure 5.15. Pre- to post-operative results for the unaffected leg, (as in figure 5.12), with the two clusters of good and poor post-operative function (red crosses) marked with ovals.

The two clusters seen in figures 5.14 and 5.15 were examined, with the cluster of 7 subjects that ended up in the normal half of the classifier for both legs compared to the 7 who were classified in the OA half of the classifier for both legs post-operatively. One subject had a different classification in each leg and was not included in this part of the analysis. Given the small sample sizes, significance testing was not performed. Overall, the cluster with good function were younger (mean age 65 compared to 72) and walked faster at the pre-operative assessment (mean gait speed 1.06 compared to 0.94).

Pre-operative belief values were higher in the cluster that ended up with good function (table 5.10). Only one patient in the 'good function' post-operative cluster had a B[OA] in the affected leg greater than 0.8 pre-operatively (subject 9) and only one patient in the 'poor function' post-operative cluster had a B[OA] in the affected leg of less than 0.8 pre-operatively (subject 7).

	Good post-operative function cluster	Poor post-operative function cluster
Belief in OA		
Affected leg	0.55 (0.26)	0.84 (0.04)
Unaffected leg	0.45 (0.25)	0.77 (0.11)
Belief in Normal		
Affected leg	0.17 (0.17)	0.02 (0.02)
Unaffected leg	0.26 (0.20)	0.05 (0.06)
Belief in Uncertainty		
Affected leg	0.28 (0.10)	0.14 (0.04)
Unaffected leg	0.29 (0.09)	0.18 (0.06)

Table 5.10. Baseline belief values for the two post-operative clusters, expressed as mean (SD). Subject 8 was excluded as the affected leg was in the normal half of the classifier and the unaffected leg was in the abnormal half of the classifier.

At the affected leg, change in B[OA] was had a weak correlation with change in lateral CCI ( $\rho=0.35$ ) but a good correlation with change in medial CCI ( $\rho=0.66$ ). Change in B[NL] had similar relationships, although a little weaker (lateral CCI  $\rho=0.30$ , medial CCI  $\rho=0.47$ ). At the unaffected leg, correlation coefficients were very low for change in B[OA] and co-contraction (lateral CCI  $\rho=-0.17$ , medial CCI  $\rho=0.10$ ) and a similar pattern was seen for B[NL] (change in lateral CCI  $\rho=0.28$ , medial CCI  $\rho=0.13$ ).

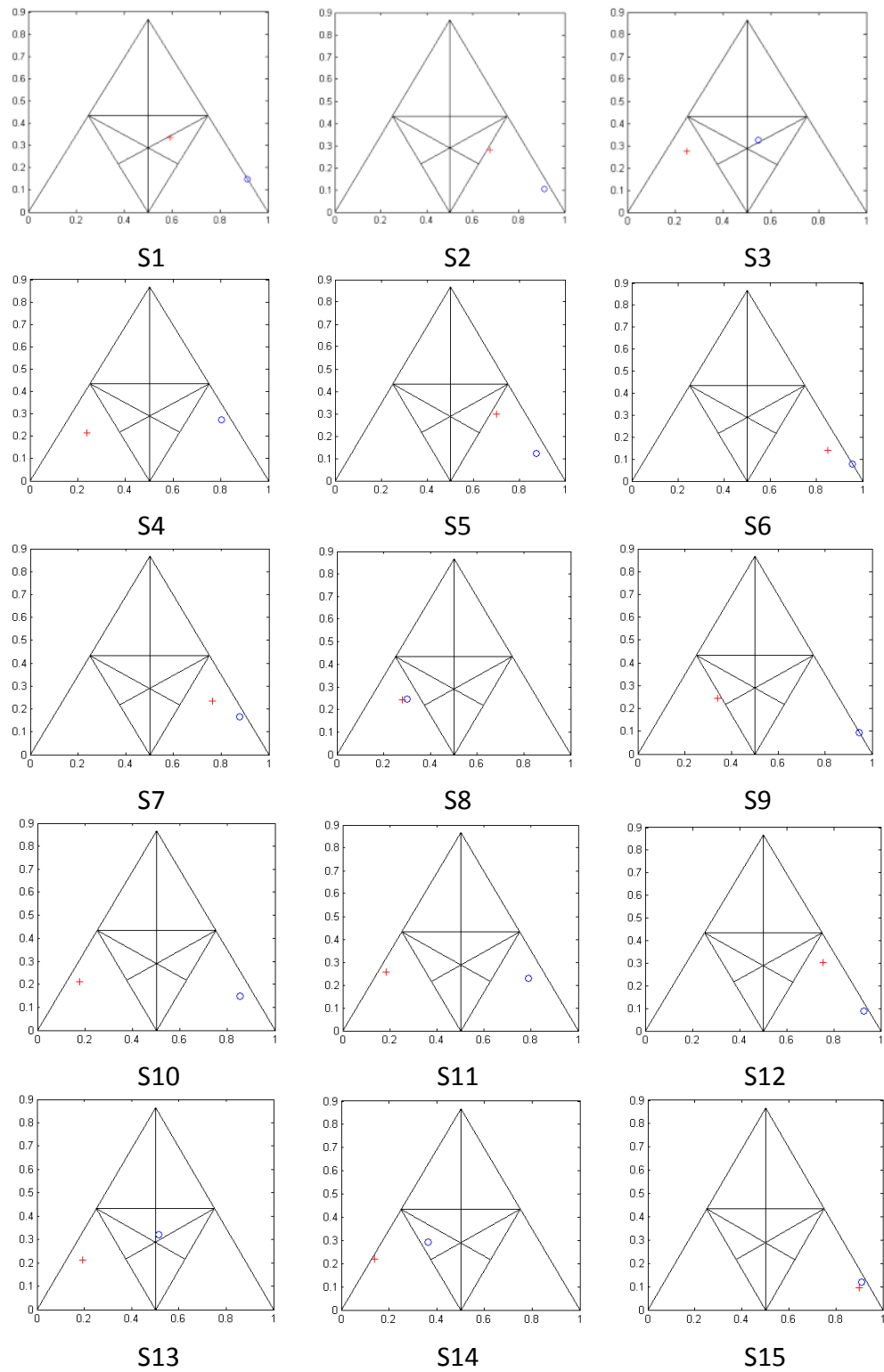


Figure 5.16. Individual classifiers for the affected legs of each subject (S1-15), with blue circles for the pre-operative assessment and red crosses for the post-operative assessment.

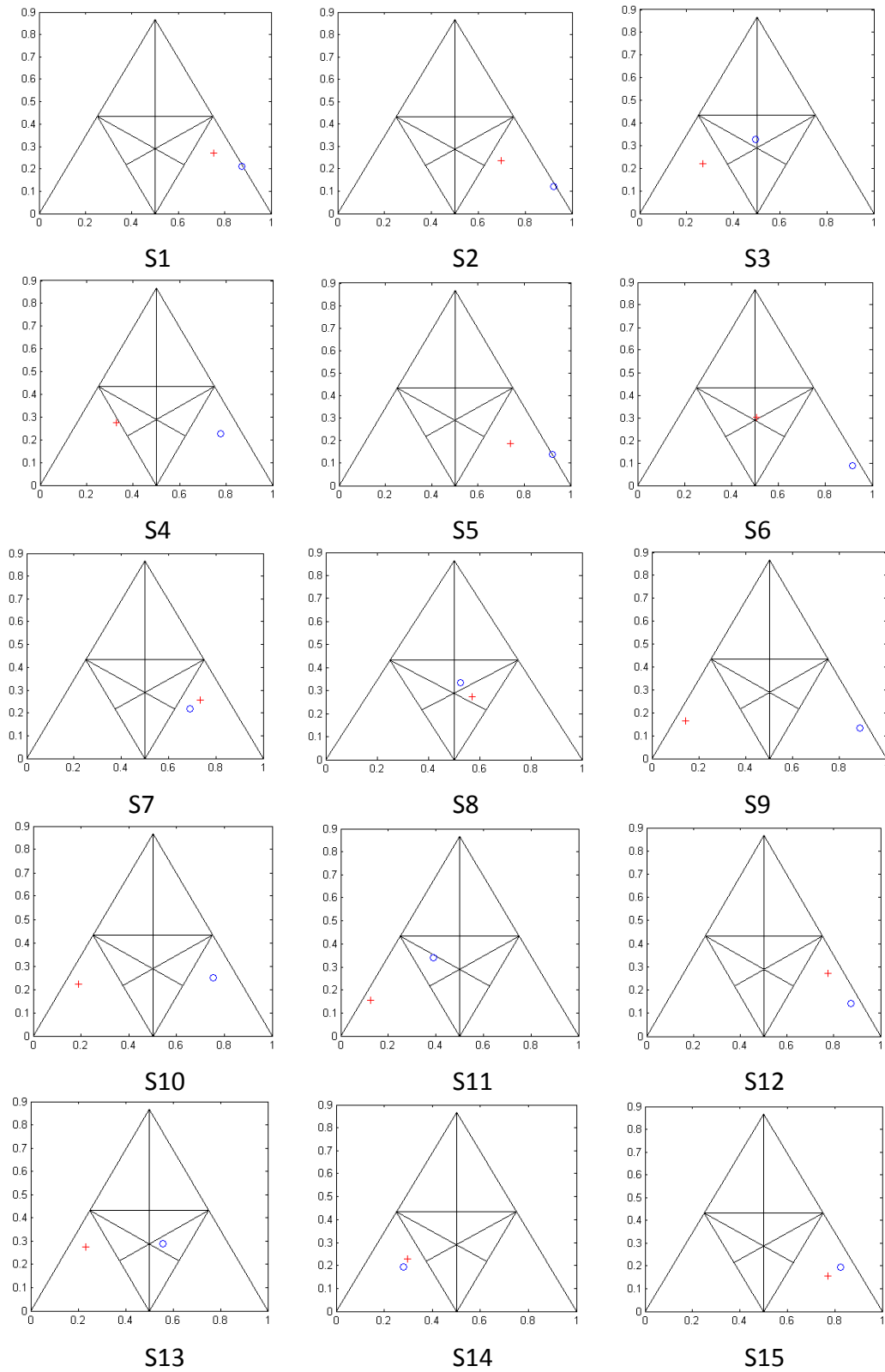


Figure 5.17. Individual classifiers for unaffected legs of each subject (S1-15), with blue circles for the pre-operative assessment and red crosses for the post-operative assessment.

## 5.4 Discussion of Results

### 5.4.1 Post-operative findings

It can be seen from the results that whilst subjective function normalised in almost all subjects, moment waveforms and co-contraction remained abnormal for the majority of subjects after the operation, particularly at the unaffected leg. Clinicians should be aware of the fact that successful treatment of the affected side does not mean that the other side will return to normal, and patients should be warned that 'protecting the other leg' should not be a consideration in the decision to go ahead with surgery.

A drop-out rate of 25% was not unexpected given the context of the study. The participants in this study were spread across a wide geographical area and many had to travel a long distance to visit the laboratory. The age of the subjects meant that the development of new co-morbidities either in themselves or their partners was relatively likely over the study period. However, some factors maintained recruitment to acceptable levels, such as the payment of travel expenses and time taken to talk to patients and their relatives both in recruitment and most importantly by the staff at ORLAU during the visit. Most felt that they enjoyed the visit and this helped maintain recruitment.

It is disappointing that the study was 1 participant short of the number required to achieve 80% power. However this number could only be established after the initial study, beyond which the study numbers were set, and it may therefore be argued



that a power calculation in this setting lacks any value. In a study of this size the risk of both type 1 and type 2 errors are high and should be carefully considered in the interpretation of the results.

The 5 subjects who did not attend (table 5.1) did not vary widely from those who did in most of the metrics, although the mean knee moment pre-operatively was slightly higher in the group who were unable to return. It is therefore unlikely that an assessment of the additional 5 subjects would have changed the conclusion that knee adduction moment fails to normalise post-operatively, although a greater number of subjects might have changed the statistical significance of the results.

Given the small size of the study, the lack of significance when comparing the post-operative knee adduction moment impulse of the unaffected knee to healthy controls should not be interpreted as no difference, as a type 2 error appears to be a more likely explanation (table 5.4). The means were notably different and on the edge of each other's 95% confidence interval. What is more, the mid-stance moment was significantly abnormal compared to healthy controls and the gait speed did not fully normalise. The moment impulse at the unaffected hip was also significantly different from the control population.

These findings point strongly towards a persistently abnormal pattern of loading in the unaffected limb, which does not return to normal over time. This is particularly clearly demonstrated in figure 5.7, which shows that very few of the subjects saw a clinically significant drop in their mid-stance moment.

It cannot be assumed that the findings were purely due to ongoing dysfunction in the affected knee. Greater falls were seen in the adduction moment at the affected knee especially at mid-stance. Although this will partly be due to the change in knee alignment that is associated with the surgery, a greater change at mid-stance (see figures 5.4 and 5.5) would suggest that the main reason for this change was a functional improvement. Overall, the post-operative analyses were relatively symmetrical with similar moment profiles on both legs. The increase in gait speed may also explain a significant proportion of these findings, and will be discussed further in the next section.

Subjective function either normalised or came close to normal in the majority of individuals, and had it not been for the 'kneeling' part of the Oxford score, where subjects were limited either by the advice of hospital staff or some scar tenderness, many of the scores would have been fully normal. This supports the belief that the other knee was normal in these subjects. The poor subjective result experienced by one subject and moderate restoration of scores in another is consistent with the literature, as approximately 20% of patients remain dissatisfied and have minimal change in PROMS following knee arthroplasty surgery (Hamilton, Lane et al. 2013; Kempshall, Hickey et al. 2013).

Co-contraction did not normalise in either leg, and whilst greater pre-to-post operative change was seen at the affected leg, both sides remained significantly different from normal. The lack of change despite a change in gait speed would agree with the analysis in chapter 4 that co-contraction appears to be independent

of gait speed. Increased activation of quadriceps and hamstrings occur relatively early in the disease process, are even seen after ACL reconstruction and may be relatively independent to the gait changes which occur later in the disease process (Lustosa, Ocarino et al. 2011; Mills, Hunt et al. 2013; Rutherford, Hubley-Kozey et al. 2013).

As noted in chapter 4, ongoing co-contraction and abnormal moments would both be expected to increase joint reaction forces, placing the other knee at risk of ongoing degeneration. The difference in timing would also exacerbate the loading waveform changes seen in the OA group, the effect of which will be discussed in chapter 6.

Taking these findings as a whole, it can be concluded that there is an ongoing pattern of abnormal gait which persists after surgery, characterised by reduced gait speeds, abnormal moments and excessive muscular co-contraction. The reasons for this lack of change are not yet understood. The three most logical explanations are that the gait was always abnormal (and was the cause of OA rather than the result of the disease), that ongoing problems in the affected knee results in persisting abnormalities of gait, or that a gait behaviour becomes established as part of the chronic disease process, which persists despite treatment of the affected knee.

The first of these would seem unlikely, as the adopted gait pattern is inefficient and therefore unlikely to be adopted by an individual without pathology. The other two explanations are difficult to separate using the current data, and may occur in combination rather than separately. Further analysis of this was performed using

the classifier, and a plan for future research to address this question is proposed in chapter 7.

#### 5.4.2 Principle Component Analysis and Classification

The principle aim of this additional analysis was to explore the data in a mathematical and consistent manner and to give a more detailed understanding of the observed gait changes. The numerous observed abnormalities described in the last two chapters cannot be considered independently, they are due to a global change in gait pattern and as such the different waveforms are intrinsically inter-related. This makes traditional approaches to statistics difficult to interpret, as multiple co-dependencies in the data and a complex within- and between subject dataset mean that the use of linear regression techniques is limited.

One advantage of the approach that was taken in this study was that these co-dependencies could be analysed in a series of steps that allows the most important components of the dataset to be picked out and analysed without relying on the subjective opinion of an observer who may be biased by the findings that support their personal viewpoints.

The combination of PCA and a classification based ranking of variables meant that the final dataset did not just include PC's with a broad, but potentially irrelevant spread of data, as might occur with random sampling error. The final set of PC's could be considered the most discriminatory parts of the gait dataset, with the

maximum amount of available data used to provide the greatest discrimination between individuals.

Principle component analysis of gait data has recently been shown to have good reliability in a test-retest study for the majority of variables, with only coronal plane knee angle and internal-external knee rotation having poor reliability, both of which were excluded from the final classifier (Robbins, Astephen Wilson et al. 2013).

The dominance of the powers amongst the chosen variables is of particular interest, especially as these were ranked above gait speed. Each joint has only one measure of power, which is calculated by multiplying the moment with the angular velocity in each plane and summing the values for the three planes together. This ignores the effect of co-contractions, as any balanced muscle action across a joint will neither produce an angular velocity nor resist a moment. Therefore the powers are a measure of resultant muscle power (that is, the effect of an agonist muscle group over its opposing antagonists).

The selected PC's for hip moment and knee power correspond to a lack of active propulsion during stance in the OA subjects, in the vertical acceleration and deceleration at early and late stance (as discussed in 4.4.2), as well as in the forward propulsion from the hip that happens at mid-stance. Further down the list of variables, a reduction in peak ankle power implies less of a push-off and less vertical acceleration in late stance. This is consistent with a stiff-leg, pendular pattern of gait, (also discussed in chapter 4.4), resulting in lower peak in the ground reaction vector but greater mid-stance values – changing the loading waveform.

When combined with the EMG analysis, it is likely that the reduced powers are the result of co-contraction, where greater antagonist muscle contraction results in a smaller resultant muscle action and a stiff leg gait, although similar findings could be obtained with a reduction in peak muscle activity in favour of a more prolonged muscle action through stance. Whilst the EMG studies favour the second of those theories, the latter is also a possibility as the EMG was normalised to the peak during the walking trials.

It is also important to recognise the possibility of strength deficits in these individuals, which would not be registered by the EMG traces. The inter-relationship between muscle strength, muscle activity and confidence in knee stability are difficult to separate and would require a more complicated study than is presented in this thesis.

It appears from the data that the changes in vertical and forward propulsion are also reflected in the GRF and the moments. The interplay between the ground reaction vector and the joint moments explain why many of the remaining PC's are at relatively similar phases of the gait cycle. The GRF changes may also be explained by a stiff leg gait, with a more pendular and slower motion in the arthritic population that reduces peak moments in exchange for a more prolonged loading through mid-stance.

In two recently published articles, Resende et al (2012) and Levinger et al (2013) identified significant abnormalities in hip, knee and ankle powers in the affected legs of patients with knee OA (Resende, Fonseca et al. 2012; Levinger, Menz et al.

2013). Levinger et al (2013) also found that these abnormalities persisted following arthroplasty surgery. Neither study reported results from the other leg. Their conclusion agreed with that of this thesis, that despite treatment, gait abnormalities persist and may be related to persisting gait behaviour.

The trained classifier represented a further step forward for the use of the Cardiff Classifier in that it had not been used before to classify the other leg, and it had not been trained using such a large number of variables before. Overall, the classification accuracy between pre-operative subjects and healthy controls was good for both legs, with all but one of the normal individuals being correctly classified in both legs, and all but two of the patients being correctly classified.

The post-operative classification of OA subjects also gave very similar results between affected and unaffected legs, whether plotted using data from the affected leg or the unaffected leg. Slightly better function was seen in the replaced knee rather than the asymptomatic, unaffected knee, although results were similar in the majority. These findings are not consistent with the local pathology at the affected knee being solely responsible for the ongoing functional deficit following arthroplasty. Gait behaviour should also be considered as a factor in future studies and this may be amenable to gait re-education or biofeedback tools.

Two clear clusters of subjects were seen, and an analysis of these clusters demonstrated a clear relationship between pre-operative function and post-operative function, in which very high values of B[OA] and low values of B[NL] pre-operatively were strongly predictive of a failure to return to normal gait. A failure of

many individuals to return to normal subjective function is well defined in the literature, although pre-operative predictors of this are difficult to identify (Hamilton, Lane et al. 2013; Williams, Blakey et al. 2013). Objectively defining the reasons for poor pre- and post-operative function may be of value in larger datasets in the future.

A much higher proportion of subjects returned back to the normal side of the classifier than was observed in previous cohorts (Jones 2004; Whatling 2009). This may be due to OA in the other knee of individuals in those studies as previous classifier studies have included a more general cohort of patients from waiting lists. Therefore, the results of gait studies as a marker of outcome must be taken in context, and the functional success of knee replacement may be understated as a result of the other knee.

Correlations between pre-to-post operative change in the belief values and change in co-contraction were weak at the unaffected leg but moderate to good correlation was observed at the affected leg, particularly for medial co-contraction. The effect of moment or alignment change were not considered as co-variables and this may explain some of the variation in lateral co-contraction at the affected leg. Overall, improvements in the affected leg following knee surgery are related to improvement in co-contraction, although this does not return to normal and no such changes are seen in the unaffected leg.

Co-contraction appears to be a common theme in knee pathology and as with gait performance it may be the result either of a lack of confidence in the knee or a gait



behaviour that occurs in chronic disease and persists after treatment. These two explanations are not mutually exclusive and may be explained by fear of pain or instability.

Co-contraction around the ankle has previously been shown to be related to fear and anxiety in healthy populations and those at risk of falling (Okada, Hirakawa et al. 2001; Nagai, Yamada et al. 2012). Pain related fear and anxiety are known to occur in patients with knee osteoarthritis before and after knee replacement and further research into the link between co-contraction and fear of pain or instability is warranted (Somers, Keefe et al. 2009; Sullivan, Tanzer et al. 2009; Pua, Ong et al. 2013).

Co-contraction occurs symmetrically, and is likely to be dependent on the condition of the worst joint. Given that co-contraction remains abnormal in the majority of subjects after knee surgery, even with good recovery of PROMS, further neuromuscular training and rehabilitation should be developed if patients are to make a full recovery after surgery.

There have been attempts to train individuals to reduce co-contraction, with some results in early series. A reduction in co-contraction was observed in one study using a process of 'stochastic resonance' (a subconscious electrical stimulation) although the physiological basis to this is not clear and no further studies have been reported so far to confirm these findings (Collins, Blackburn et al. 2011).

One study of normal individuals showed that quadriceps and hamstring co-contraction during a balancing task could be reduced following a period of training

involving varying forms of perturbation (Asaka, Wang et al. 2008). An organised programme of perturbation training has also been shown to reduce co-contraction following cruciate ligament disruption and improve outcomes (Chmielewski, Hurd et al. 2005).

The aim of perturbation training is to teach new coping strategies to an individual, so that a broader range of movement patterns are available in any given situation, allowing an individual to use more efficient motor patterns in the majority of instances (Latash and Anson 2006). Whilst perturbations may be too painful for the arthritis patient, a form of training that allows individuals to learn new and varied gait strategies may be one approach to increasing confidence and reducing co-contraction in this patient population, and further research to develop techniques for this are recommended.

## 5.5 Conclusion

Gait does not return to normal after knee arthroplasty, even if the other leg is unaffected by disease and the arthroplasty has been successful by subjective measures. Knee moments on the affected side recover to close to normal values but moments at the other knee fail to recover in the same way. Co-contraction only partially recovers at the affected knee and does not recover at the unaffected knee. Based on the current literature, it is likely that these changes will increase the risk of future degeneration in the other knee.

Gait changes observed in OA include reduced powers, especially at the peaks, with changes typical of a slow, pendular, stiff-leg gait. Given the associated co-contraction and abnormalities in joint loading, it is likely that patients are accepting a chronic increase in joint reaction forces for a safer gait pattern. This may either be to avoid joint movement or to protect from slips or stumbles. A combination of the change in kinetics and the prolonged muscle co-contraction means that the cartilage of the opposite knee is subjected to prolonged loads during stance phase compared to healthy controls.

The observed waveform changes may be considered abnormal but the effect of these waveform changes on cartilage behaviour and biology are not yet understood. This question is addressed in the next chapter, with the final conclusions of the thesis and plans for further study presented in chapter 7.

Objective gait function and its recovery is largely symmetrical and abnormalities in one side are closely reflected in changes on the opposite leg. The observations made in this chapter would suggest that either a neuro-muscular or a behavioural approach to retraining would be required to bring patients gait patterns closer back towards normal.

## **Chapter 6. Analysis of cartilage behaviour under different loading patterns**

## 6.1 Introduction

Throughout the thesis, changes in joint loading have been described due to changes in gait. Specifically, loading of a joint is prolonged through stance phase and the impulse is raised, a measure of the area under the moment curve. This would be exacerbated by excessive co-contraction throughout stance.

Based on the current gait analysis literature, it is reasonable to expect that these changes would be damaging to the joint. A recent study found that the moment impulse was predictive of cartilage loss at 12 month follow up on MRI (Bennell, Bowles et al. 2011). Previous studies have linked the peak moment to progression of OA and one paper demonstrated a relationship between the peak moment and disease initiation in older adults (Prodromos, Andriacchi et al. 1985; Miyazaki, Wada et al. 2002; Amin, Luepongsak et al. 2004).

The response of chondrocytes to excessive mechanical stimuli is an important part of the pathological process in knee OA. This was discussed in chapter 1 (section 1.1.4). Various studies have examined the effects of differing magnitude, frequency and duration of load on gene expression in chondrocytes (Blain, Mason et al. 2003; De Croos, Dhaliwal et al. 2006; Fitzgerald, Jin et al. 2006; Madhavan, Anghelina et al. 2006; Blain 2007; Wolf, Ackermann et al. 2007; Ramage, Nuki et al. 2009). However, studies in this field so far have used sinusoidal loading, which is not typical of joint loading in humans.

Also, studies have typically involved either cells suspended in artificial media or animal tissue (Blain, Mason et al. 2003; Fitzgerald, Jin et al. 2006; Yeh, Chang et al. 2009; Degala, Williams et al. 2012). These approaches make the research easier to perform but cannot necessarily be directly translated to human cartilage in vivo. A search of the literature failed to identify any instances where human cartilage on bone has been used.

As the previous chapters in this thesis make clear, human joint loading is not sinusoidal. During gait, load is applied to a joint over 60% of the gait cycle, with 40% in swing phase. Loading patterns vary depending on pathology, with a relatively short period of maximal loading occurring in early stance phase in normal individuals. However, patients with OA may expose their joints to high loads throughout stance phase.

These types of 'real-life' waveform have never before been applied to cartilage for biological research. However, if the influence of different aspects of the waveform can be clarified, potentially beneficial treatments can be identified and trialled.

It is reasonable to anticipate that changes in loading waveform would lead to changes in cartilage metabolism. Under compression, water is pushed from the extracellular matrix into the joint, leading to compression of the cartilage and fluid flows within the tissue (Lu and Mow 2008). Chondrocytes are sensitive to both fluid flow, hydrostatic and shear stresses and changes in these parameters can lead to catabolic gene expression (Blain, Mason et al. 2003; Fitzgerald, Jin et al. 2006; Ramage, Nuki et al. 2009).

The aim of this part of the thesis was to develop a study to investigate the gene expression profiles derived from human cartilage chondrocytes following the application of two different waveforms, quantified from a recent gait analysis study comparing normal and osteoarthritic patients.

The null hypothesis to be tested was:

**The loading waveform or magnitude has no effect on the mechanical behaviour or gene expression of human articular cartilage.**



## 6.2 Methods

### 6.2.1 Study design and acknowledgment of support

The potential need for investigation into this area was confirmed by an initial literature search. Special expertise in cartilage mechano-biology was sought from Dr Emma Blain, an academic fellow in the school of Biosciences who has a special interest in matrix biology and mechano-biology of articular cartilage. Dr Blain assisted the author in performing all of the work contained in this chapter. Further assistance was received from Professor Sam Evans in the School of Engineering who helped with the study design and provided practical help in setting up and resolving difficulties with the loading apparatus. An initial study plan was prepared and then modified further following the pilot work described in 6.2.2.

It was decided that the study would be performed using human articular cartilage taken from discarded tissue from total knee replacements. Bovine articular cartilage is often used for mechano-biology studies as it is widely available, highly cellular and therefore easier to study biologically, and is easier to use in loading studies as numerous samples can be discarded until a sample with optimal geometry can be identified. However, it is unlikely that healthy young bovine cartilage would respond to relatively subtle changes in load in the same way as cartilage taken from human patients who have already demonstrated their pre-disposition to OA. It was therefore decided that despite the technical difficulties, human articular cartilage should be used to answer the study question, if possible.

One of the challenges of using human cartilage on bone was that a consistent flat surface of sufficient size was not usually available, and the shape of the samples was not consistent between samples. It was therefore decided that this should be recorded and accounted for in the analysis. As the effect of the curve on the loads within cartilage were difficult to predict, Dr Li, a PhD student in the School of Engineering, was approached as she was able to provide expertise in measuring the surface geometry and performing finite element modelling of the samples. Advice and methodological support was also given by Dr Cathy Holt and Dr Gemma Whatling.

A full research plan was written and funding application was made for £1300 to the small grants sub-committee of the Arthritis Research UK Biomechanics and Bioengineering Centre in Cardiff, which was successful. The study was included in the umbrella ethical application for the Arthritis Research UK Centre which was granted in November 2010 (Multi-Centre Research Ethics Committee for Wales: 10/MRE09/28).

#### 6.2.2 Preliminary work

A pilot of the study design and biomechanical testing was performed prior to the start of the study. Two patients undergoing knee replacement for medial compartment OA were consented for the use of their surgical waste following total knee replacement. The explants were examined and the femoral condyles

determined to be macroscopically undamaged by the surgery (the tibial plateau was injured by the surgeon in both cases when removing the meniscus).

The weight-bearing distal femoral condyle removed from macroscopically intact lateral femoral condyles was chosen as the preferred specimen because it was (1) most representative of the weight-bearing cartilage in the medial compartment in patients susceptible to OA, and (2) gave the most undamaged tissue for analysis of a relatively consistent shape compared to the other cuts, such as the tibial plateau.

Testing different areas of a single explant, without dividing it first, was rejected in favour of taking a series of osteochondral plugs. This was to prevent cross-communication of biological responses between explants under different loading regimes.

Various methods for mounting the plugs in the testing machine were attempted until a stable construct of acceptable stiffness was developed. Loading waveforms were trialled successfully and saved for future use.

### 6.2.3 Recruitment and Preparation of Explants

Inclusion criteria were patients with primary medial compartment knee OA with lateral joint space width measurement of >5mm on plain radiographs undergoing knee replacement. Exclusion criteria for recruitment to the study were a history of inflammatory arthropathy (either this joint or others) or related disease (such as

psoriasis), previous injury to the joint in question or refusal or inability to consent independently.

With the agreement of the relevant consultants, theatre lists for the Cardiff and Vale Orthopaedic Centre were examined a week in advance of an experiment and all patients undergoing total knee replacement were identified. Radiographs were reviewed to confirm the inclusion criteria. If these were deemed suitable then the patient was approached, and the inclusion and exclusion criteria were formally confirmed with radiographs and the clinical notes. An information sheet was provided and after a period of time to read the information, consent was taken. A KOOS and Oxford score was also taken to document pain and disease severity.

Ten osteochondral explants were taken from the distal cut of the lateral femoral condyle generated as surgical waste during total knee replacement. Explants were placed in a sterile pot and covered in Hank's Balanced Salt solution (Invitrogen, Paisley, Scotland) to maintain the metabolic activity of the cartilage chondrocytes during transfer. The explants were handled and prepared in a class 2 hood in a Human Tissue Authority approved laboratory. Specimen dimensions were measured using a digital calliper with resolution down to 0.01mm (Moore and Wright, Bradford, UK).

The surface shape and dimensions were recorded using Aquasil (Soft Putty/Regular Set, Dentsply, Addlestone, UK) dental moulding putty. Base (5mls) and Catalyst (5mls) were mixed together, producing a soft putty. This was placed in a Petri dish, covered in cling film and the explants were gently pressed into the putty, with the

application of further pressure to ensure a good “imprint”. The cement hardened without any heat production in approximately 5 minutes. The methods used to measure the resulting moulds is given in section 6.2.9.

Five osteochondral plugs (10mm diameter) were taken from macroscopically normal tissue using a metal corer. Efforts were made to ensure that two pairs of osteochondral plugs were routinely taken next to each other, preferably of similar curvature. The pairs were labelled separately (A1 and A2 and B3 and B4), going from anterior to posterior and the fifth was designated as the unloaded control (figure 6.1).

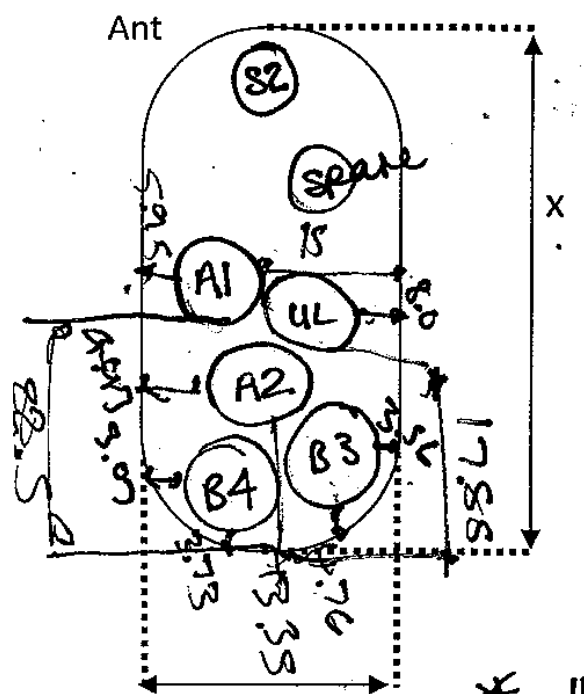


Figure 6.1 Schematic diagram of one of the samples taken from one of the datasheets from the study showing where the cores were taken. The measurements were taken to assist interpretation of the shape data from the moulds. ‘Spare’ and ‘S2’ were additional cores taken to tune the testing rig prior to loading the samples for the experiment.

The cancellous bone underlying the plug was shaped using a bone nibbler, and filed to ensure that the surface was relatively flat and parallel to the chondral surface of the plug, whilst ensuring that there was at least 2mm of bone remaining at the base of the plug. This was done to all samples irrespective of the need to flatten them in order to maintain consistency in the manipulation that had been applied.

Each plug was placed in a separate well of a 12 well plate covered with 2ml of Dulbecco's Modified Eagle's Medium/Hams F12 (DMEM/F12 (1:1); GIBCO, Paisley, UK) supplemented with 100 µg/ml penicillin, 100 U/ml streptomycin and 1x insulin-transferrin-sodium selenite (1x ITS); the presence of ITS maintains the chondrocyte phenotype (Chua et al., 2005). Explants were stabilised overnight in a 37°C incubator (5% CO<sub>2</sub>) following removal from the condyle.

The remaining femoral condyle was re-moulded using the Aquasil putty to record the position of the explanted plugs, and the position of the plugs measured using callipers. The remaining femoral condyle was incubated overnight as described for the osteochondral plugs.

#### 6.2.4 Biomechanical Testing

Following an overnight stabilisation, osteochondral explants were subjected to mechanical loading using a Bose Electroforce 3200 (Bose Corporation, Minnesota, USA) rig (Figure 6.2) in conjunction with Bose Wintest 4.1 software (Bose Corporation, Minnesota, USA).

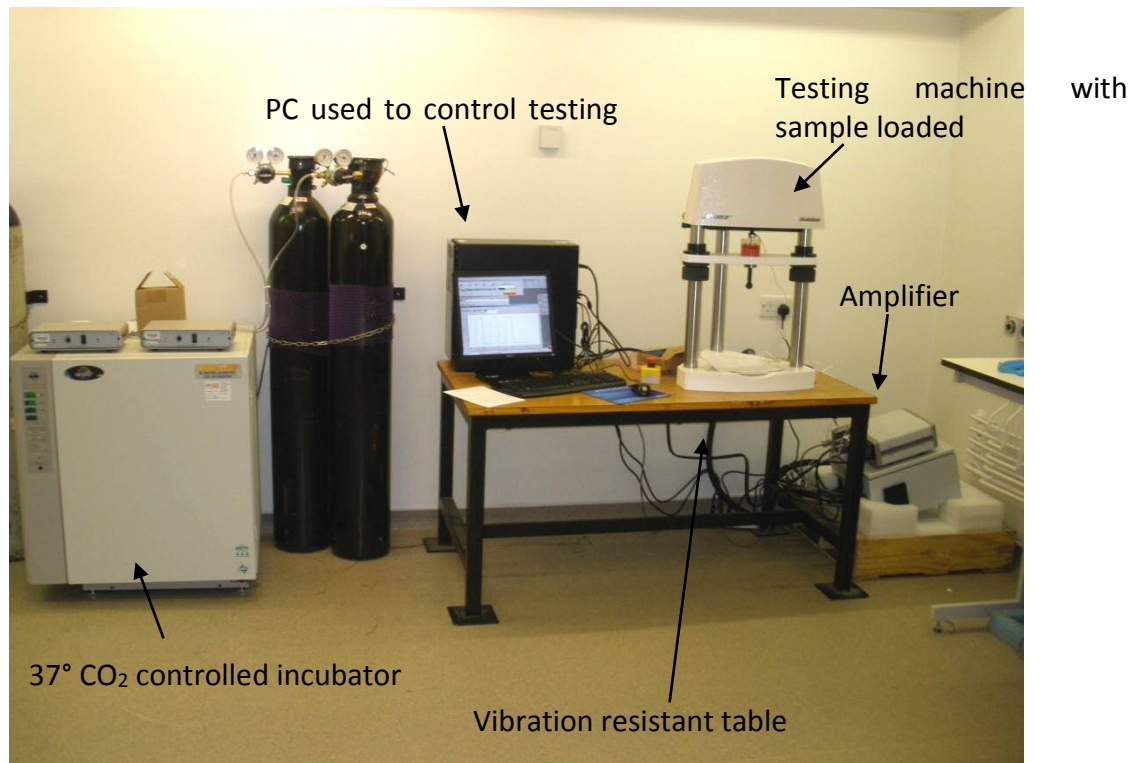


Figure 6.2 Labelled photo of the testing apparatus for the loading component of the study.

The Bose Electroforce 3200 relies on electromagnetic motors to control the testing apparatus and as such can be finely tuned and controlled at high speeds – allowing complex loading patterns to be applied to the tissue. For this study, the Electroforce 3200 allowed more realistic loading waveforms to be applied, compared to previous studies, achieving fine control of load whilst allowing for the visco-elastic properties of the tissue. For this study, a 250N load cell was used (figure 6.3b).

The rig was initially tuned for displacement in air, and for load to 50N using the spare osteochondral plug (the highest expected load for the study based on the pilot samples).

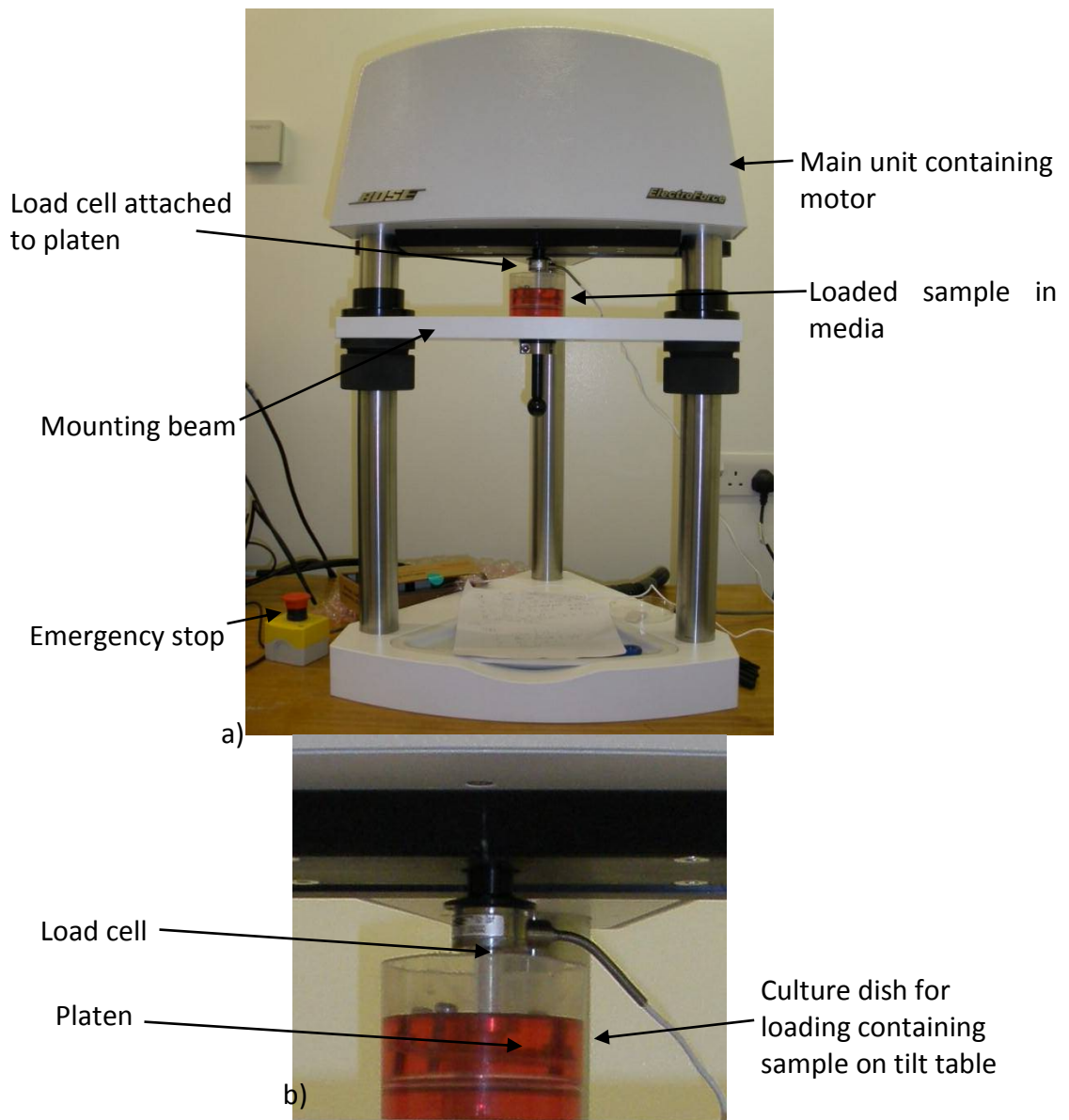


Figure 6.3 The Bose Electroforce 3200 testing machine, with a sample loaded. a) Labelled photo of the machine. b) Close up, demonstrating the load cell (the silver block with attached cable) attached directly to the platen.

The cartilage thickness was measured at the cut edges of the remaining condyle for each osteochondral plug using digital callipers. This was conducted by two independent observers who each measured two points around the edge of the cartilage for each specimen.

Prior to the start of the study, a random number generator had been used to assign each one of the four osteochondral plugs into one of four strain/waveform



categories. The waveforms and their rationale are described in section 6.2.5. For each explant there were 4 potential scenarios (in addition to the unloaded control sample):

- 10% strain, physiological waveform
- 10% strain, osteoarthritic waveform
- 15% strain, physiological waveform
- 15% strain, osteoarthritic waveform

These categories were documented and sealed in ten opaque envelopes. Just prior to loading, the envelope was opened and the plugs assigned to their randomly generated loading regime, preventing selection bias in the coring and preparation of the samples. Osteochondral plugs were mounted on a tilt table, which was adjusted so that the surface of the cartilage was parallel to the loading platen (figure 6.4).

A flat platen with a polished base was designed by Professor Evans and produced from aluminium, reducing the weight and thereby reducing momentum artifacts. This was screwed onto the load cell. A culture dish was prepared by cutting a histology sample pot to a height that would allow the sample to be loaded whilst submerged in medium but to avoid impingement of the lead to the load cell on the edge. An upturned pyrex petri dish was placed in the base to make a stiffer construct than was available with standard plastic containers, which have gently curved bases that were noted to flex under load during the pilot phase of this study.



Figure 6.4. One of the two identical tilt tables used in this study, designed and made by Professor Evans. Both had an aluminium plate with a small spike in the centre (a Kirschner-wire tip) which had been driven through the plate and protruded by 1.5mm, which was used to stabilise the subchondral cancellous bone to prevent horizontal slippage of the core during testing. The angle of the plate could be adjusted in any plane by adjusting the length of the tripod screws individually.

The tilt table containing the specimen was placed into the culture dish and covered in media (as described in Section 6.2.3). The culture dish was then placed on the beam of the machine, the platen was brought down to within 1-2mm of the core, and the position of the core was visually centred on the platen in both planes (Figure 6.5). The load was tared (zero) and the platen brought into proximity with the specimen – with a recorded load of 0.2N indicating contact. The position of the sample was re-checked and the displacement setting was subsequently tared to zero.

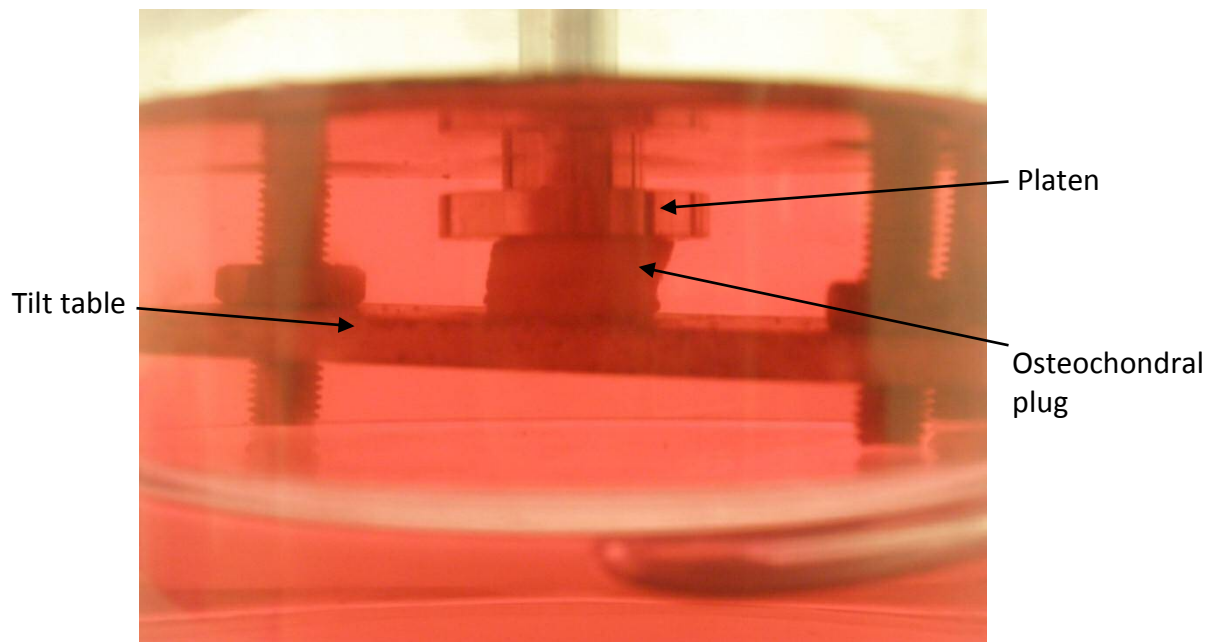


Figure 6.5. Osteochondral plug, immersed in culture media, on the tilt table during loading. The magnifying effect of the liquid is also seen in this photograph although there is also some distortion of the image as a result.

Thirty cycles were initially applied at a frequency of 1Hz using a sinusoidal waveform with a trough of 0.05mm and a peak which corresponded to the intended strain (i.e. for 10% strain of a 3mm thick piece of cartilage, a displacement of 0.3mm was applied). During the last cycle of this pre-conditioning period, the peak load was recorded. The machine was tuned to that load, and the subsequent waveforms were programmed to use in load control. Using load control ensured that the correct load and waveform would continue to be applied to the tissue, even if the tissue became impacted over time. Displacement control was not used as it would cause the platen to fail to contact the tissue correctly once compression had occurred due to creep. The result of this would be that the stresses and strains experienced by the tissue in the later stage of the test would be lower than at the

start. A baseline for load was set at 0.1N (rather than zero) to ensure that the platen always remained in contact with the tissue.

A total of 1800 cycles was applied to each specimen, to represent a 30 minute walk. At the end of the loading protocol, the osteochondral plug was placed in a new well of a 12 well plate containing 2mls of fresh media, and returned to the 37°C incubator for a 4 hour period to allow time for a transcriptional response to occur. Media in the culture dish (Figure 6.5) was replaced in preparation for the next specimen to be loaded.

Following a 4 hour incubation period post-load, the osteochondral plug was removed from the media and a 5mm core was taken from the centre of the sample. This was to exclude the cut edges of the sample from the subsequent biological analyses, as the perimeter of the specimen would have experienced atypical biological and mechanical stresses which were unrepresentative of the scenario being studied. The 5mm core was placed immediately into a 2ml cryo-vial and snap-frozen in liquid nitrogen to inhibit any further metabolic activity. Explants were then transferred to a -80°C freezer for storage until required for processing. The media, in which the explants had been immersed, was also collected and stored at -20°C until required.

#### 6.2.5 Loading Regimes and tissue handling after loading

The two loading waveforms for comparison were designed to be as representative of medial compartment loading as possible. The waveforms needed to be distinct enough to be able to make a comparison at the end of the study, but still applicable to *in vivo* loads.

Zhao et al observed that the shape of the load observed in an instrumented knee replacement was closely related to the adduction moment waveform measured using gait analysis (Zhao, Banks et al. 2007). Therefore, using the collected moment data (Chapter 4) a typical normal moment waveform and a typical osteoarthritic waveform were selected (Figure 6.6). The normal waveform was noted to be similar to the pattern of loading determined recently for a modelling study of normal gait, confirming that this was an appropriate choice. The selection of these two waveforms allowed for analysis of two possible extremes of waveform shape, and also permitted varying the moment impulse for the same peak load in order to test the importance of impulse and waveform shape over peak load.

The moment waveforms were approximated by using ramp settings on the testing machine. A full stride (heel strike to heel strike of the same limb) was observed to last approximately 1 second (section 4.3.2). Once completed and tested, both waveforms lasted 0.98seconds, with the same 60% 'stance' and 40% 'swing' distribution as would occur in normal gait. For the same peak load, the OA waveform had a 30% higher impulse than the 'normal' waveform (Figure 6.6b).

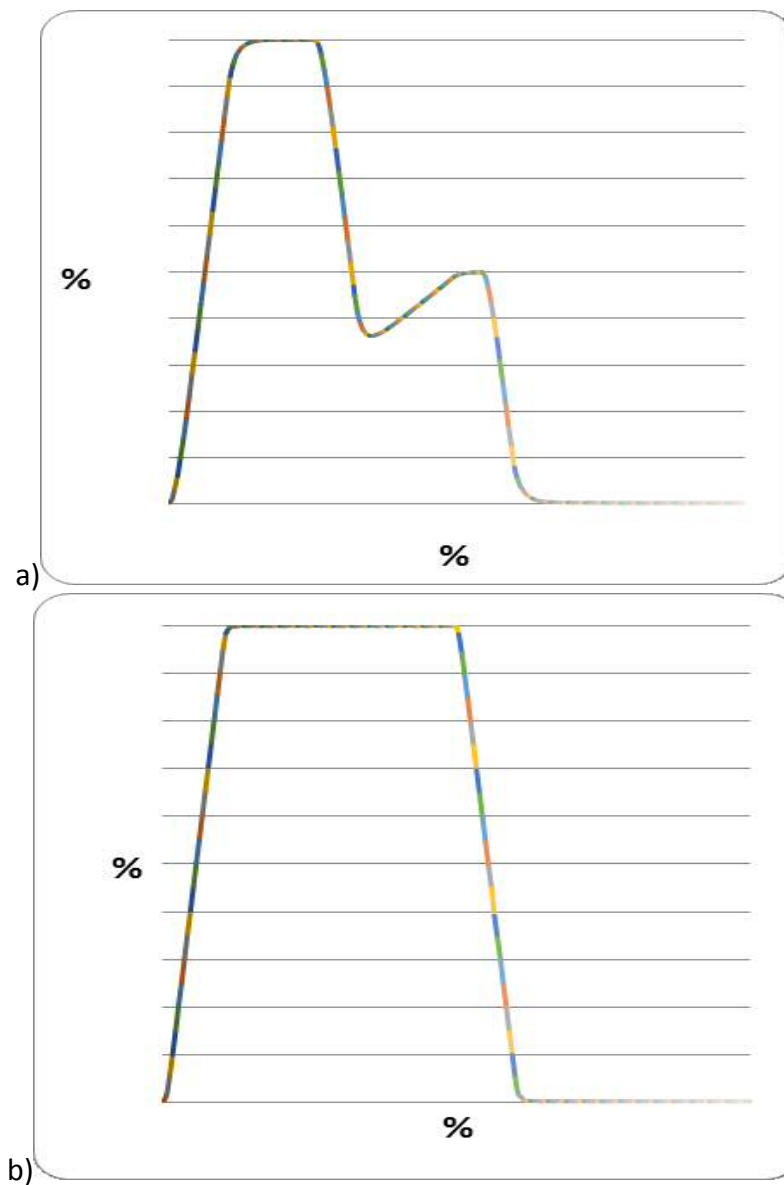


Figure 6.6. The two loading waveforms, normalised to percentage for both time and peak load. a) Physiological and b) Osteoarthritic. A short dwell time was built in to the peak of waveform a) to allow for the visco-elasticity of the tissue. These waveforms can be compared to the moment waveforms from figure 4.9 section 4.3.2.

'Physiological' Loading Waveform	'Osteoarthritic' Loading Waveform
Ramp to F at 10F N/sec	Ramp to F at 10F N/sec
Dwell for 0.15sec	Dwell for 0.39sec
Ramp to F/3 at 10F N/sec	Ramp to 0.1N at 10F N/sec
Ramp to F/2 at 1F N/sec	Dwell for 0.4sec
Ramp to 0.1N at 10F N/Sec	Repeat 1800 cycles
Dwell for 0.4 sec	
Repeat 1800 cycles	

Table 6.1 Ramping regimes programmed in for each waveform. F is the peak force calculated for each sample.

Data was recorded directly from the machine at 200Hz. For the first 4 samples, errors in setting up the software led to the recording of force and displacement data for only small sections of each ramp, whilst force & displacement data was recorded continuously for samples #5 to #10. The reaction force on the platen was recorded directly by the 250N force transducer (also referred to as the load cell) attached directly to the platen (Figure 6.4b).

#### 6.2.6 RNA extraction

The process of RNA extraction can be broken down into 3 parts: Disruption of the tissue; isolation of RNA and purification and production of cDNA. All plasticware, pipette filter tips and reagents used were certified free of DNases and RNases to protect against contamination or degradation. All procedures were performed using gloves, in a laboratory designated for molecular biology.

All labelling for this study was done according to the sample code, without reference to the loading pattern, to blind the analysis and prevent bias being introduced into the processing.

To pilot the assessment process and to ensure adequate tissue availability, the first samples were processed, RNA was extracted and quantitative PCR (qPCR) performed on selected genes of interest. Based on these findings, the rest of the samples were processed in the same manner (see discussion in results, section 6.3).

#### *6.2.6.1 Disruption of cartilage tissue*

Samples were removed from -80°C storage and the cartilage dissected from the bone whilst still frozen using a scalpel. The excised cartilage specimens were placed in 1.5ml eppendorfs and placed on ice. Specimens were powdered using a dismembrator (B.Braun Mikro-dismembrator, Germany). Chambers were cooled in liquid nitrogen and a single cartilage specimen placed inside with 200µl of Trizol™ reagent (Invitrogen, Paisley, Scotland) - a phenol reagent used to stabilise the tissue to prevent RNA degradation. The chamber was further cooled in nitrogen, a ball bearing placed into the cup, the cap fitted and the chamber inserted into the dismembrator. Specimens were powdered by oscillation at 2000rpm for 1.5 minutes, whereby the ball bearing ricochets against the frozen tissue causing its disruption. The powdered contents were transferred to a 1.5ml eppendorf and the volume made up to 1ml with Trizol™, before placing the samples on ice. If any unpowdered tissue was observed, the procedure was repeated. The chamber was



cleaned with ethanol, followed by sterile water, dried and returned to nitrogen in preparation for the next sample.

#### 6.2.6.2 Isolation and purification of RNA

Samples were incubated at room temperature for 5 minutes prior to the addition of 175µl of chloroform (Sigma, Poole, UK). Samples were mixed by inverting four times and the aqueous phase separated by centrifugation at 4°C (Eppendorf Centrifuge 5402) at 14000rpm for 15minutes. The aqueous phase (Figure 6.7) was aspirated carefully, in order to prevent contamination of the sample with any of the solid or organic phase.

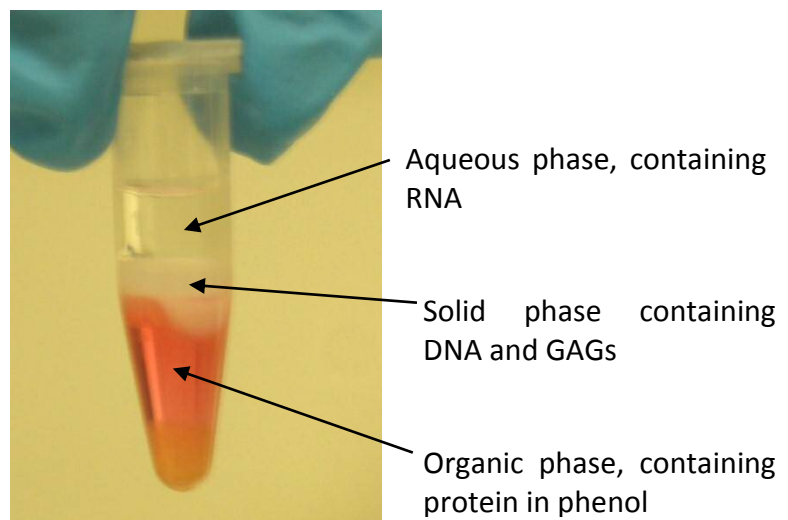


Figure 6.7 Appearance of tube following centrifuge, demonstrating the different phases.

An equivalent volume of isopropanol (Sigma, Poole, UK) was added to the aqueous phase and the RNA precipitated overnight at -20°C. Following RNA precipitation, samples were pelleted by centrifugation at 4°C (14000rpm, 15mins; figure 6.8). The

supernatant was aspirated and discarded, and the sample returned to ice. The pellet was then washed by adding 1ml of 75% ethanol, vortexed and samples pelleted by centrifugation (14000rpm, 5mins, 4°C). The supernatant was again aspirated, and the samples left to air dry.

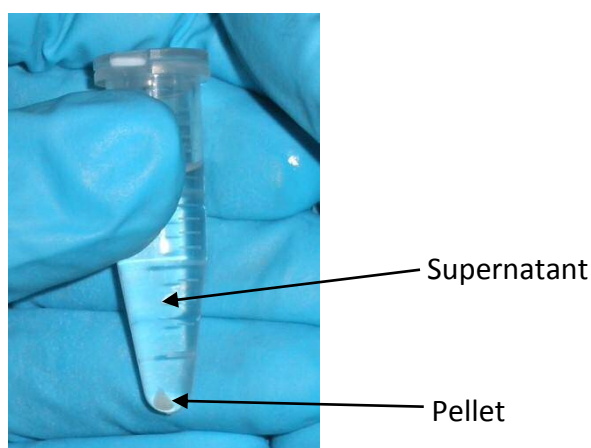


Figure 6.8 Following centrifugation, the pellet of RNA is seen at the base of the tube.

Samples were resuspended in sterile water and DNase treated, following the manufacturer's instructions (Promega, Madison USA), to remove traces of genomic DNA. 0.01 volumes of DNase (Promega, Madison USA) and 0.1 volumes of buffer (40mM Tris-HCl, pH 8.0, 10mM MgSO<sub>4</sub>, 1mM CaCl<sub>2</sub>; Promega, Madison USA) were added to the RNA in a final volume of 22µl, and incubated at 37°C for 30minutes. Samples were then incubated with 0.1 volumes of DNase inactivating agent ((DNA-free™, Ambion, UK), and samples pelleted by centrifugation (10000rpm, 2mins, ambient). The RNA-containing supernatant was transferred to a sterile eppendorf, an aliquot removed for reverse transcription, and the remainder stored at -80°C to avoid degradation.

#### *6.2.6.3 Production of cDNA*

cDNA was synthesised by adding 200ng of RNA to 500µM dNTPs (Invitrogen, Paisley, Scotland) and 0.5µg of random primers (Promega, Madison USA) and samples incubated at 60°C for 5minutes, followed by 4°C for 2minutes (Techne TC312 PCR machine). Samples were then returned to ice prior to the addition of 20U of recombinant RNasin® inhibitor (to remove non-enriched RNA i.e. non-messenger RNA Invitrogen, Paisley, Scotland), 1x 1<sup>st</sup> strand buffer (50mM Tris-HCl, pH 8.3, 75mM KCl, 5mM MgCl<sub>2</sub>: Invitrogen, Paisley, Scotland), 10mM DTT (which breaks disulphide bonds to prepare RNA for copying, Invitrogen, Paisley, Scotland), and 200U of superscript III reverse transcriptase (the enzyme which makes compliment copies of the RNA, Invitrogen, Paisley, Scotland). Samples were then incubated at 25°C for 10 minutes, 42°C for 50minutes (allowing the enzyme to make copies of the RNA, resulting in cDNA), and then up to 70°C for 15minutes, inactivating the enzyme.

#### 6.2.7 Quantitative Polymerase Chain Reaction (qPCR)

Quantitative PCR was conducted on the experimental cDNA samples to determine the expression level of genes of interest; genes of interest were selected on the basis of relevance to the disease process and/or their known mechano-responsiveness in cartilage. The genes chosen were:

- A housekeeping gene (internal control): 18S

- Matrix Genes: Type 2 collagen (Col2A1), Aggrecan
- Cytoskeletal:  $\beta$ -Actin, Vimentin
- Collagenases and Aggrecanases: MMP3, MMP13, ADAMTS-5
- Early response gene: *c-fos*

Primers were designed, acquired and optimised by Dr Blain (Table 6.2). For each gene of interest a master-mix was prepared comprising 12.5 $\mu$ l of SYBR® Green Jumpstart™ Taq Readymix™ (Sigma, Poole, UK), 400nM primers (forward and reverse combined: MWG; Germany) and 10.5 $\mu$ l of water. Master-mix (24 $\mu$ l) and experimental cDNA (1 $\mu$ l) were placed in the well of a 96 well qPCR plate; master mix omitting the addition of cDNA which was replaced with water acted as a negative control for the reaction. A Quantitative PCR was performed using an MX3000P machine (Stratagene) and programmed as indicated: reaction conditions as follows; DNA polymerase activation and cDNA denaturation at 95°C for 10 mins, followed by 40 cycles of 95°C for 30 seconds, primer annealing for 30 seconds (for annealing temperatures see Primer Table) and primer extension at 72°C for 30 seconds. Dissociation curves were generated by an additional cycle of 95°C for 1 min, 55°C for 30 seconds and 95°C for 30 seconds. Relative quantification was calculated using the  $2^{-\Delta\Delta CT}$  method (Livak and Schmittgen, 2001), using the unloaded controls as a reference group to quantify relative changes in target gene expression after normalisation to the housekeeping gene 18s.

Gene of Interest	Forward (F) and Reverse (R) primer sequence	Annealing Temp (°C)	Product size (bp)
$\beta$ -actin	F: 5'-TTCGAGACCTTCAACACCCC-3' R: 5'-GGCCAGAGGCATACAGGGA-3'	60	70
Vimentin	F: 5'-AAGAGGAAATCCAGGAGCTG-3' R: 5'-AGGTCAGGCTTGGAACATC-3'	58	79
Aggrecan	F: 5'-CCTCTGGACAACCAGGTGT -3' R: 5'-AAACCAGGTCAGGGACTCCT -3'	58	174
Col2A1	F: 5'-GCAACGTGGTGAGAGAGGAT-3' R: 5'-ACCACGATCACCTTGACTC-3'	60	216
MMP3	F: 5'-CCCACGGAACCTGTCCCTCCAG-3' R: 5'-GGATTGCGCCAAAAGTGCCTGTC-3'	60	129
MMP13	F: 5'-CCATTACCAGTCTCCGAGGA-3' R: 5'-GGAAGTTCTGGCCAAAATGA-3'	58	118
ADAMTS-5	F: 5'-GAGGCCAAAAATGGCTATCA -3' R: 5'-ATCGGTCACCTTTGGAGAAA-3'	53	159
c-fos	F: 5'-GGCAAGGTGGAACAGTTATCTC-3'	60	192

	R: 5'-CTTCTCCTTCAGCAGGTTGG-3'		
18S	F: 5'-GCAATTATTCCTCCATGAACG-3'  R: 5'-GGCCTCACTAAACCATCCAA-3'	60	122

Table 6.2. Amino acid sequences for the primers used in this study.

#### 6.2.8 Analysis of pooled mRNA samples

As reported in the results (section 6.3.4), a number of the processed samples were failing to amplify for the qPCR. This was presumed to be due to low levels of mRNA in the cartilage samples (further discussion of this is given in 6.3.4). Therefore, a precipitation step was performed to increase the concentration of the RNA yield; the remaining RNAs from all 10 samples were pooled, for each respective loading condition, to increase the likelihood of obtaining adequate RNA concentrations to provide some data.

The remaining 10µl of RNA for each sample was pooled according to the loading condition that was applied, giving 5 samples of 100µl each. RNA was re-precipitated (3 volumes of ethanol and 0.1 volumes 3M NaCl) overnight at -20°C. Following RNA re-precipitation, samples were pelleted by centrifugation and washed as described above (Section 6.2.6.2) with a final re-suspension in 27.5µl sterile water. RNA was analysed using the nanodropLite® ND-1000 Spectrophotometer (with NanoDrop 3.0.1 software) to determine RNA yield and integrity. The RNA concentration was

determined by absorbance at 260nm on a spectrophotometer (1 absorbance unit = 40µg/ml RNA). The A260/A280 ratio should be approximately 2.0 with the acceptable range considered to ideally be 1.8-2.1.

Reverse transcription to produce cDNA was performed as described in section 6.2.6.3 ('Production of cDNA').

After pooling and re-precipitation of the RNA, cDNA was produced and quantitative PCR performed for aggrecan and MMP13, using 5µl cDNA per reaction using the methods described in Section 6.2.7.

#### 6.2.9 Measurement of sample surface shape using digital image correlation

As discussed in section 6.2.3, moulds were taken of the femoral condyle samples prior to coring and then again after coring. This resulted in one mould which could be used to define the surface shape and one which could be used to locate the position of the cores (figure 6.9). This was cross checked by calliper measurements taken of the core locations for confirmation (see figure 6.1, section 6.2.3).



Figure 6.9 Moulds of the condyle samples before and after coring. Pen marks on the surface are approximations to assist in recording of the data and were not used for the actual measurements.

The shape of the moulds was measured using digital image correlation. This is a technique that has been in use in many engineering departments around the world, including Cardiff, for a number of years for measuring shape, strain and deformation in 3-dimensional structures. An object is covered in a random speckle pattern (Figure 6.10). Two cameras record an image of the same object. The picture is divided up into small segments, and the shape of the individual speckles are analysed by the software. Just like the stereo-vision that our eyes use to determine depth, the subtle differences between the two shapes allow the software to infer a 3 dimensional shape based on two-dimensional images.

Using cameras of a similar specification as the ones used in this study, the accuracy of digital image correlation has been estimated as approximately  $1/50,000^{\text{th}}$  of the



image size. Given that the image size in our study was approximately 10cm, this would imply an accuracy of one 500<sup>th</sup> of a millimetre, or 2 $\mu$ m (Orteu 2009).

The moulds were sprayed with repeated thin coats of white matt paint (car paint primer) from a distance of over 6 inches to achieve a thin coat of even thickness. They were then daubed with speckles of black paint using a sponge and sprayed using a toothbrush. This was to achieve a speckled pattern of black paint on the white background. This was random in shape and distribution and dense enough that there was approximately 30-50% coverage of black paint (Figure 6.10).



Figure 6.10 Example of mould covered with speckle pattern

A 2 camera Limesse (Limesse, Pforzheim, Germany) system was used to acquire the data, using Vic-3D 2006 software (Correlated Solutions, South Carolina, USA). This system consists of two black and white digital cameras fixed on to a beam and focused on the area of interest (figure 6.11). The area was backlit using a white

fluorescent light which was specifically used to avoid flickering, which can distort the images. The system was calibrated using a standardised 5mm matrix of dots. The total area covered by the matrix corresponded to the size of the moulds, ensuring calibration of a sufficiently large enough area. A series of 10 photographs were taken of the 5cm matrix with the matrix at different angles and rotations for system calibration (Figure 6.12).

Once the system had been calibrated, the moulds were immediately photographed using this system set-up. The data was fed into Vic-3D, the area of interest was selected for all of the moulds, and the 3-dimensional model was created by the software.



Figure 6.11 Calibration and system set up for digital image correlation.

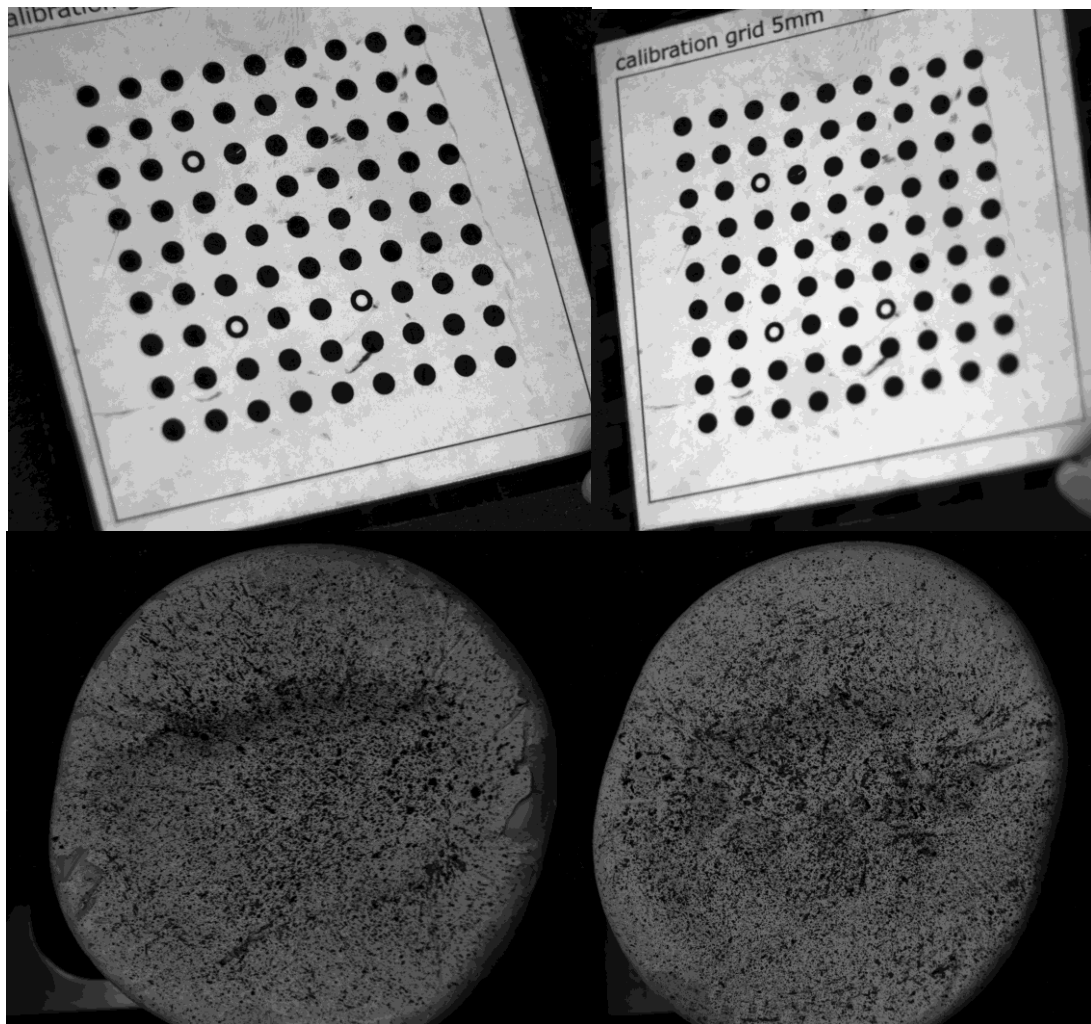


Figure 6.12. Pictures from the Lines cameras of the correlation matrix at 2 different angles, and the spray painted moulds (both of these correspond to the same sample).

Once the models had been created (figure 6.13), the size and shape of each sample was measured, and a centre point in the x-y plane identified for each sample. The post-coring sample was processed first, and the location of each core was identified, marked out and a centre point for each core determined. The central position of each core was then recorded relative to the centre of the sample in the x-y plane.

These core locations were then mapped onto the pre-coring sample, relative to its calculated centre point, again in the x-y plane. A 9.5mm circle was drawn and a series of co-ordinates were taken, one along the y-axis crossing the centre of the core, another in the x-axis crossing the centre of the core. The 3D co-ordinates of each of these points were then extracted into Excel. This allowed calculation of the surface curvature of the cores across a number of points in the both the x and the y axis, allowing an accurate 3D FE model to be built for each core.

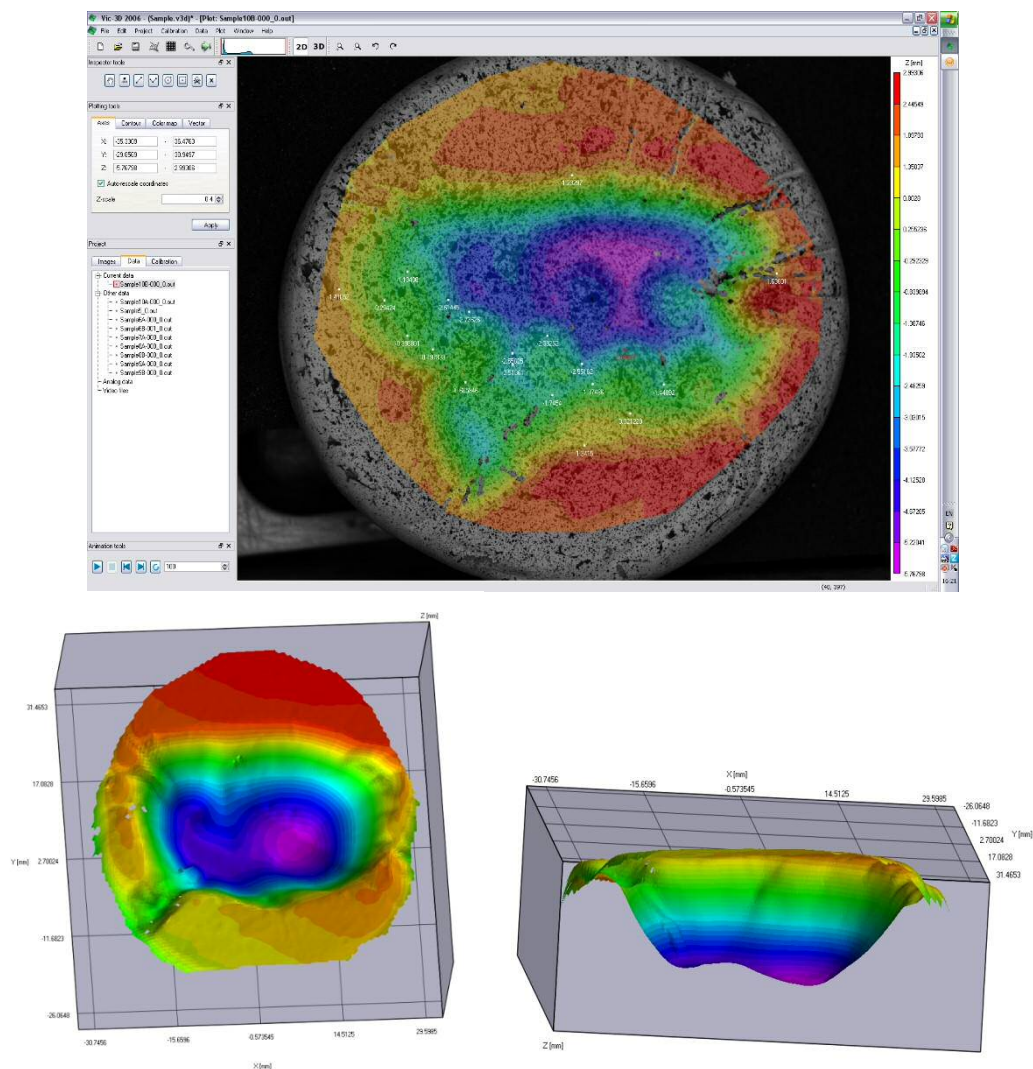


Figure 6.13. Screen shots from the processed data. The first picture shows a temperature map of 'z' overlaid onto a digital photo, with a series of points marked. The second shows an example of a 3D representation of the sample (the z-axis is not in proportion – the sample looks deeper than it is in reality).

#### 6.2.10 Finite element modelling methods

The finite element modelling for this study was performed by Miss J Li as part of her PhD thesis and all of the methods described in 6.2.10 were performed by Miss Li. An outline of this process is given below. The aims of this part of the study were:

- 1) To establish whether there is a benefit in using sample specific FE models, or whether an assumption that the surfaces were flat would give an adequate approximation of tissue stresses and strains.
- 2) To gain insight into the local tissue stresses and strains during testing both to help interpret the results of the current study and to help in planning future work.
- 3) To examine the use of reverse optimisation to determine the tissue properties of the cartilage samples.

It was decided that 3 patients would be used for this section of the study, and the final 3 patients were selected as it was believed that the training effect in the moulding and biomechanical testing would ensure that the samples later in the study would have been handled in the most consistent manner.

The shape data from Vic-3D were exported into Geomagic software (Geomagic, USA) as point clouds and the software was used to identify areas in which the shape deviated significantly (figure 6.14). Using an optimisation algorithm written in Matlab (Mathworks Inc, USA), an ellipse was fitted to the core locations on the post-coring moulds and the centre co-ordinates of each explant were calculated.

The centre co-ordinates were applied to the pre-coring moulds and the surface geometry of each explant was extracted as a point cloud with approximately 33points per mm<sup>2</sup>.

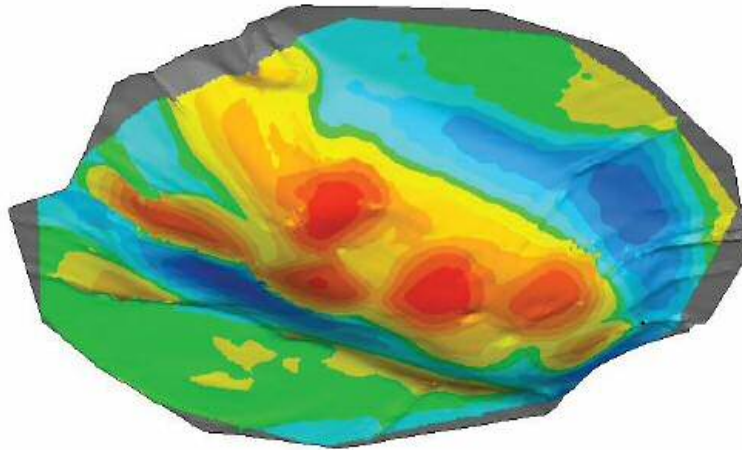


Figure 6.14. Contour plot (from Geomagic software) showing differences between the pre-and post-coring moulds for a sample. The red indicates significant differences.

Simple mathematical measures of curvature were hard to define as the curves were not uniform and varied across the shape of the samples. Therefore, instead of measuring a simple radius of curvature, a measure of surface curvature that accounted for this variation was chosen that measured the overall difference between the sample shape and a tangential flat surface.

The similarity between the point cloud data and a flat surface was determined by establishing a flat plane tangential to the apex of the surface of the cartilage using a shape fitting tool in MATLAB® (MathWorks, Inc.), and then making a plot of the difference between the flat surface and the curve at each point, called the residuals. An example of a layered residual plot is given in figure 6.15b. The root



mean square value for the residuals was calculated to give a measure of surface curvature.

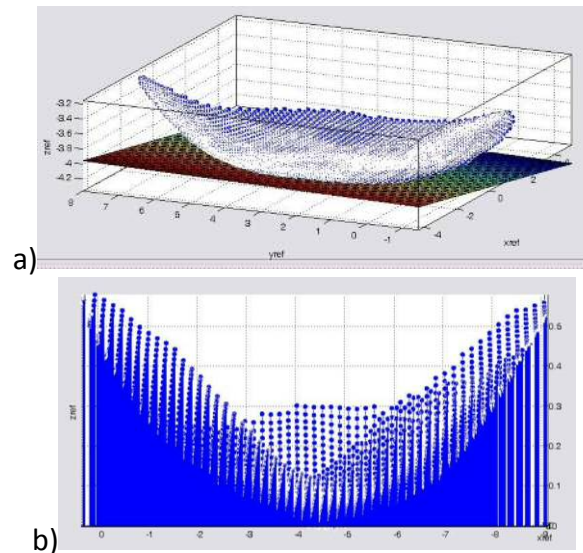


Figure 6.15. Point cloud plot with a flat plane fitted, and the subsequent residual plot used to compare the two surfaces.

The cartilage was assumed to be of even thickness throughout the samples and so the point cloud data was applied to the cartilage surface and the bone surface in creating the models, which were created in Abaqus software (Simulia Corps., USA). An alternative model using flat cartilage and bone surfaces was created as a comparator. The loading platen, the bone and the testing apparatus were all modelled as being non-deformable, and a frictionless contact between the platen and the cartilage surface was assumed. In testing the difference between curved and flat surfaces for modelling tissue stresses and strains, a literature derived Young's modulus was defined as  $E = 4.2\text{MPa}$  and Poisson's ratio,  $\nu = 0.47$  (Shepherd and Seedhom 1999). For the second part of the modelling process, the Poisson's

ratio was kept the same but the Young's modulus was unrestricted, and a reverse optimisation sequence was used to determine the modulus which would give the closest stress-strain relationship to that observed from the experimental data.



## 6.3 Results

### 6.3.1. Description of study cohort

A total of 22 subjects were recruited into the study, from which surgical waste was collected following total knee replacement (TKR). This was to ensure that an adequate number of samples were available on the days that testing commenced (generally one subject sample was tested per day, although occasionally two samples were processed in one day). All 22 subjects underwent TKR for medial compartment (+/- patello-femoral) OA. No subject was known to have any history of inflammatory joint disease. Of those 22, 10 subjects were used for the study and the remaining 12 were discarded because they were either damaged by pre-existing disease or at the time of surgery, or sufficient numbers of samples were obtained. Their demographics and scores are reported in table 6.3.

Patient	Age	Gender	Oxford (0-48)	KOOS (%age) (0-100)
1	84	m	-	64
2	77	m	14	69
3	76	f	13	77
4	68	f	17	60
5	59	f	13	60
6	87	f	21	51
7	67	f	31	63
8	60	m	12	76
9	64	m	21	61
10	56	m	24	45
Mean	69.8	5M:5F	18.4	62.5

Table 6.3 Demographics and subjective scores for the 10 subjects in the study. The Oxford score is scored 0-48 with 48 being normal/full function, whereas KOOS is given as a percentage, with a score of 0 being normal.

### 6.3.2 Mechanical Data

The first sample was loaded as described above, although the dwell time for the loaded plateau of the OA waveform (the second stage in table 6.1) was initially set at 0.35sec. This resulted in a total cycle time of 0.948 seconds for the OA waveform group and 0.982 seconds for the physiological group; the 0.002 seconds time difference resulted from the loaded part of the cycle, as the unloaded rest time was the same for both cycles. The dwell time for the OA group was therefore increased to 0.39 seconds as described (Table 6.1).

The mechanical data that was saved from the loading rig for samples 1 to 4 was incomplete as the settings on the software were incorrect. Therefore, load-time-displacement data was only obtained for the first 0.01 seconds of each ramp. Whilst this is adequate data to confirm that the machine achieved the intended displacements and loads (and therefore confirming that the loading was correctly applied for the biological analysis) this data could not be used to examine the behaviour of the tissue under mechanical load.

Hence, the mechanical findings are based on 6 samples (samples #5 to #10 inclusive). The thickness of the plugs for these samples was recorded (Table 6.4), with a mean cartilage thickness of 2.28mm (SD  $\pm 0.22$ mm). The mean sample size measured by observer 1 was 2.29mm (SD  $\pm 0.24$ mm) and the mean sample size measured by observer 2 was 2.27mm (SD  $\pm 0.23$ mm). The intra-class correlation coefficient for the average was 0.85 and for single values was 0.74, implying that

there was good agreement between observers in cartilage thickness measurement with a benefit in two observers taking measurements.

The mean specimen thickness for each loading parameter were very similar, indicating that the random selection of which loading type corresponded to which plug meant that differences in cartilage thickness averaged out over repeated tests (Table 6.4).

Sample	10% Phys.	10%OA	15%Phys.	15%OA	Mean (SD)
5	2.06	2.35	2.47	2.24	2.28 (0.17)
6	2.22	1.90	2.02	2.05	2.05 (0.16)
7	2.56	2.41	2.54	2.62	2.53 (0.09)
8	2.32	2.41	2.37	2.41	2.38 (0.04)
9	1.84	2.13	2.35	2.25	2.14 (0.22)
10	2.57	2.43	2.15	2.26	2.35 (0.19)
Mean (SD)	2.26 (0.29)	2.27 (0.21)	2.31 (0.20)	2.30 (0.19)	

Table 6.4 cartilage thickness in the osteochondral plugs. Each measurement is a mean of 4 measurements (two for each observer).

Prior to use, the stiffness of the loading construct was tested. A nut was placed on the tilt table instead of an osteochondral plug, with the testing set-up otherwise identical. 25 seconds of sinusoidal loading was applied from 0-50N at 1Hz (Figure 6.15).

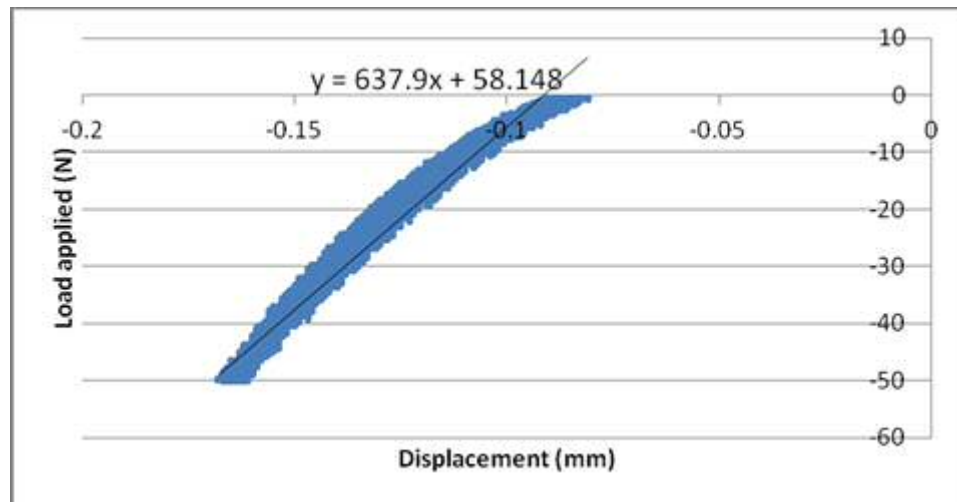


Figure 6.16. Load-displacement trace for the experimental set-up with a nut substituted for a plug

This was felt to be adequately stiff to ensure that the load displacement results would be an accurate representation of the osteochondral plugs rather than the loading construct. Typical load-time and displacement-time curves for one representative sample (sample #8) are presented (Figures 6.17-6.20).

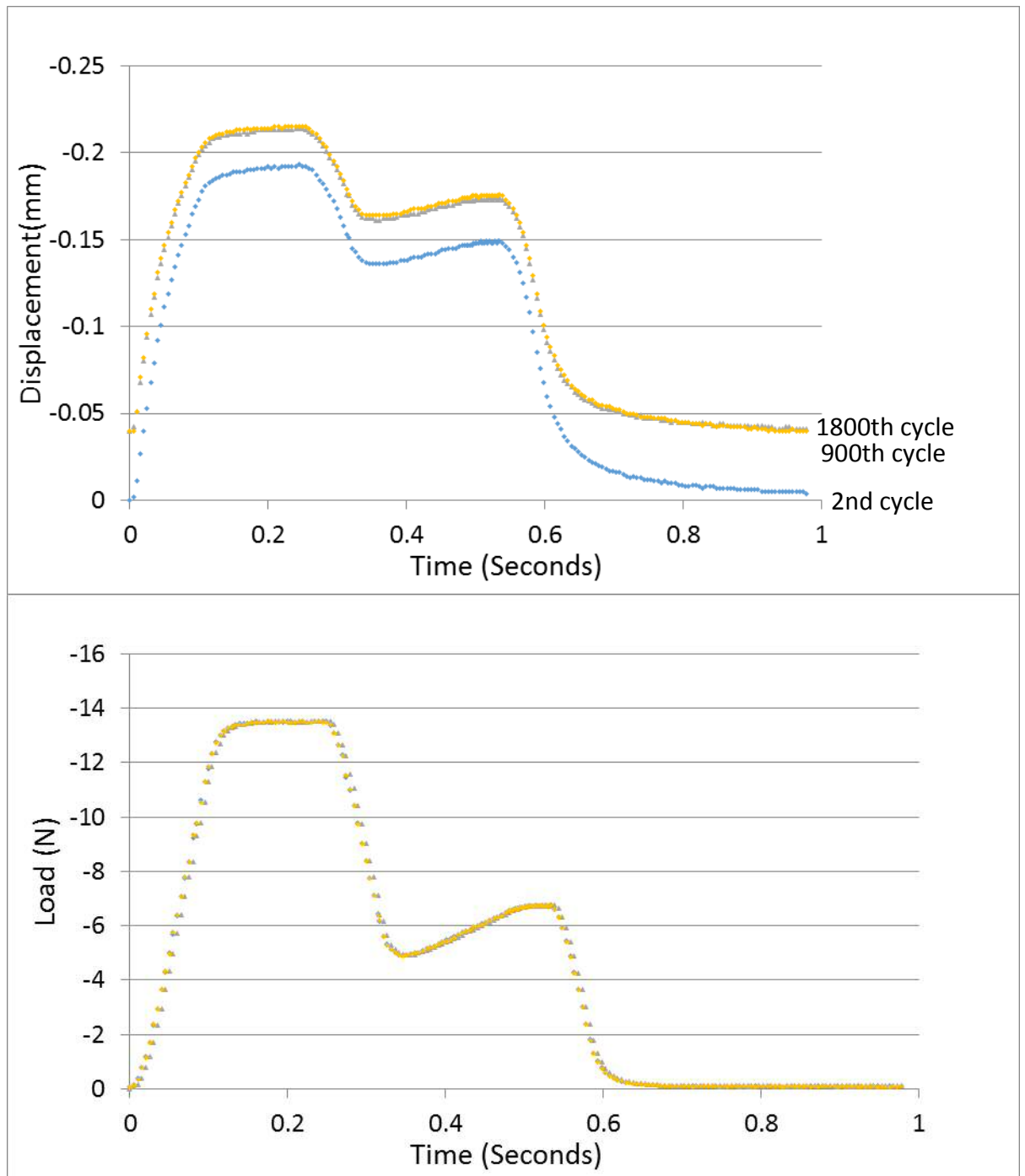


Figure 6.17 (a) Time displacement curve for the start (2<sup>nd</sup> cycle is given, as the start of the first waveform might have been altered by the pre-testing position of the platen), middle and last loading cycles for sample #8, under 10% strain with a 'physiological' loading waveform. (b) Time-force curve for sample #8, 10% strain, 'physiological' loading waveform. These are experimental values sampled from the force transducer and all 3 cycles have been plotted as above. The appearance of all 3 plots as a single line confirms that the loading was applied in a very consistent manner cycle-to-cycle over the full 1800 cycles.

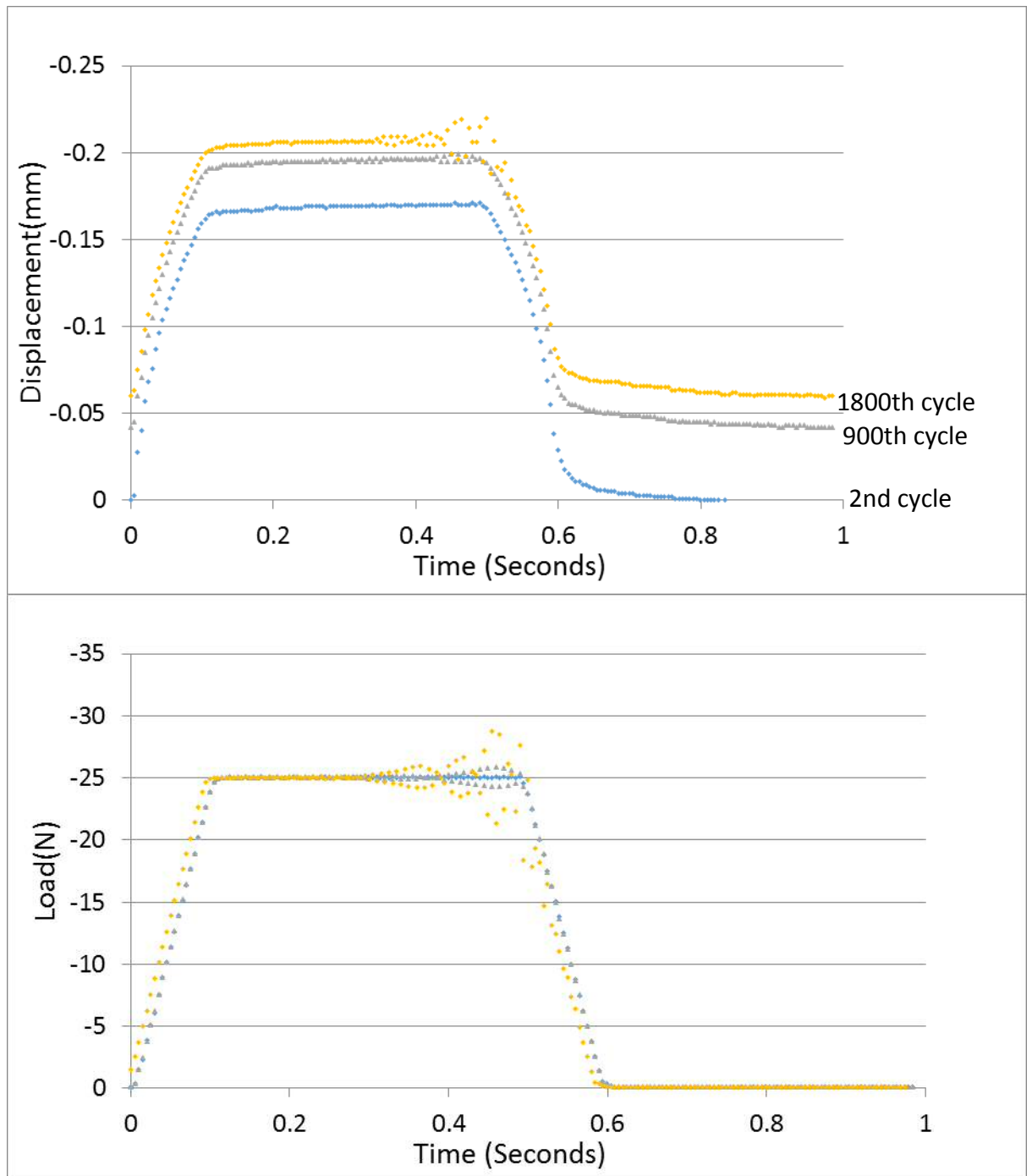


Figure 6.18 a) Time displacement curve for the start (2<sup>nd</sup> cycle), middle and last loading cycles for sample #8, under 10% strain with an 'OA' loading waveform. b) Time-force curve for sample #8, 10% strain, 'OA' loading waveform.

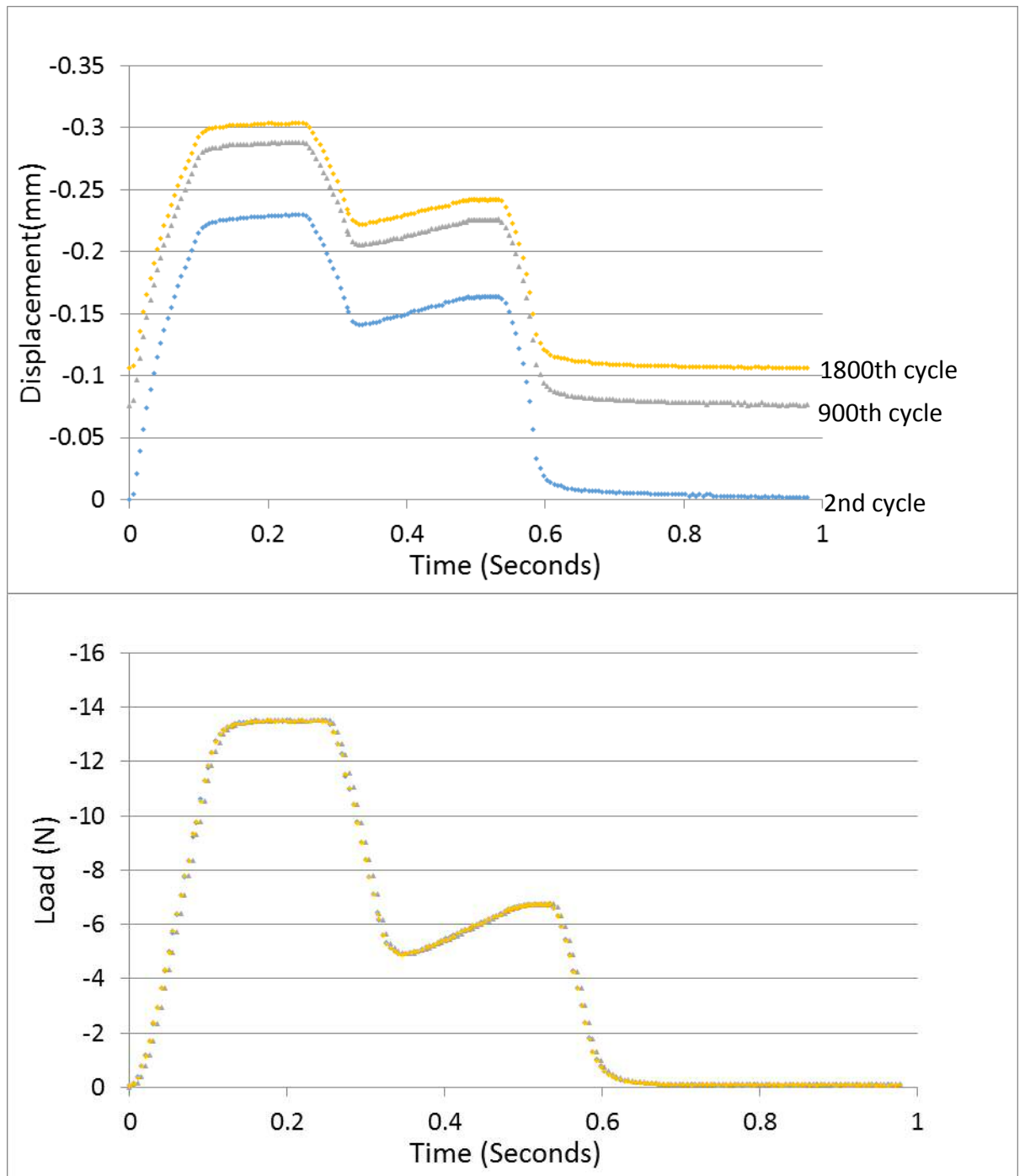


Figure 6.19 a) Time displacement curve for the start (2<sup>nd</sup> cycle), middle and last loading cycles for sample #8, under 15% strain with a 'physiological' loading waveform. b) Time-force curve for sample #8, 15% strain, 'physiological' loading waveform.

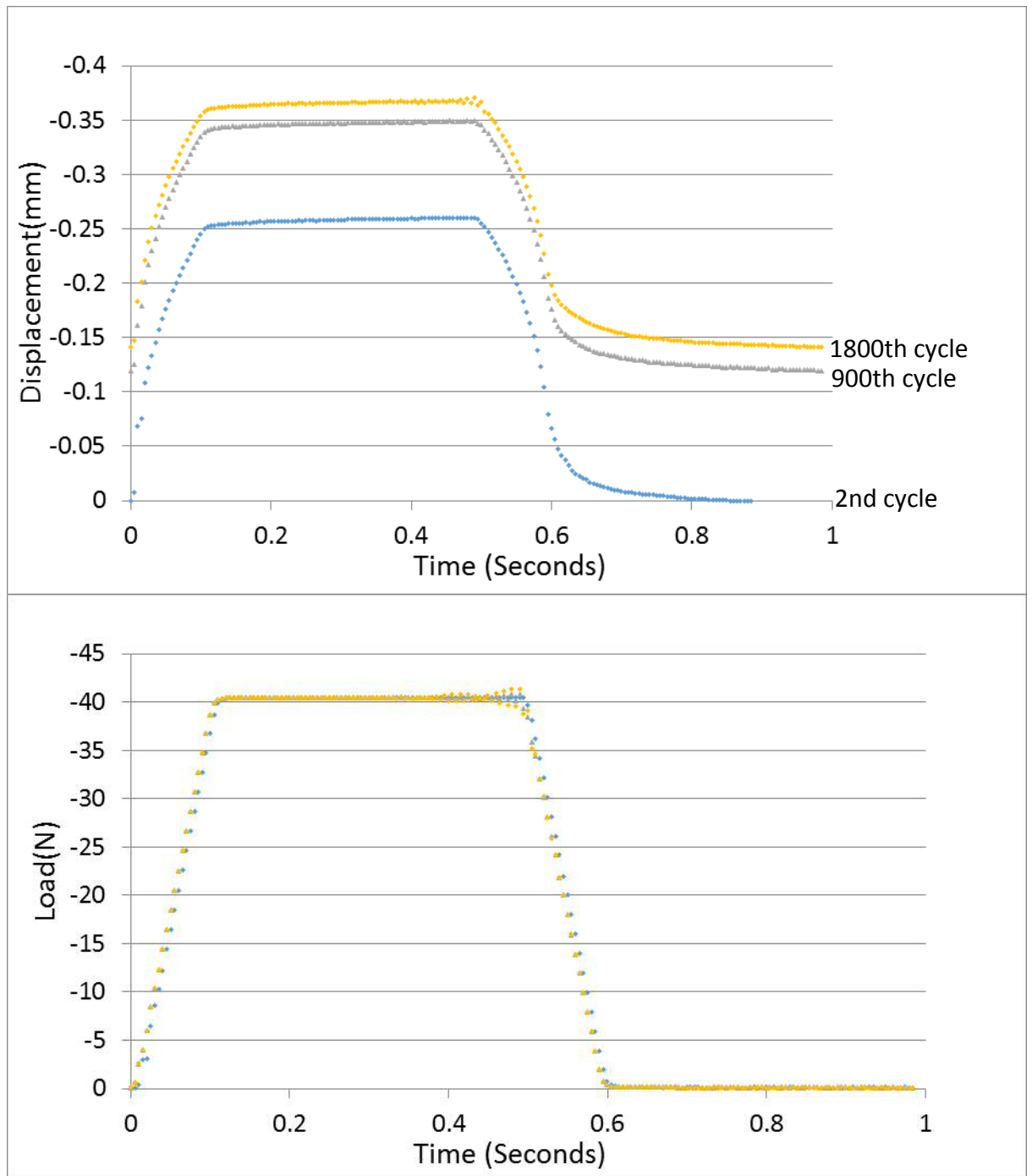


Figure 6.20 a) Time displacement curve for the start (2<sup>nd</sup> cycle), middle and last loading cycles for sample #8, under 15% strain with an 'OA' loading waveform. b) Time-force curve for sample #8, 15% strain, 'OA' loading waveform.

Loading was applied in a consistent manner throughout the 1800 cycles (Figure 6.17-6.20), resulting in the generation of an appropriate strain in the tissues which



reflected the loading waveform applied. After the loaded ('stance' phase), the material demonstrated a period of stress relaxation, which came close to equilibrium at the end of the 0.4second 'rest phase', as demonstrated by the fact that the line almost, but not quite, flattened. However where higher strains and/or the OA waveforms were applied (compare figure 6.19 to 6.20, for example), the end of the curve did not 'flatten' to the same degree, implying that the material was still undergoing a period of stress relaxation by the time the next cycle started.

A small amount of variability was observed in both the loading and displacement traces recorded at the very end of the loaded section of one of the samples (#8, 10%OA), however the waveforms were examined during the recording and this was observed to be very small, representing a amplitude of less than 0.02mm (i.e. well under 1% strain). This had been a problem on some of the early tests – and large amounts of vibration at the end of 'stance' had caused us to discard three tests throughout the process of this study. This was resolved with very careful tuning of the machine prior to every test using a 'spare' osteochondral core taken after the standard 5 cores which was not otherwise used for the formal analysis.

On further discussion with Professor Evans, and in discussion with BOSE engineers at the BOSE study day 2011 (Royal Brompton Hospital, London) it was felt that this was probably due to the high sensitivity of the feedback loop used by the software controlling the testing machine, which caused some resonance as the system had to adjust to subtly changing mechanical properties of the tissue which was behaving in

a viscoelastic manner. Adjustments to the tuning of the machine would be used in further studies using this methodology to solve this problem.

Over the 1800 cycles the plug was therefore slowly compressed. This is demonstrated by the fact that the displacement increased over the 1800 cycles, as seen by comparing the 2nd cycle to the mid-point (900<sup>th</sup> cycle) and final cycle (1800<sup>th</sup>) (Figure 6.21). Over the period of testing, the 'baseline' displacement (i.e. the displacement at the end of the relaxation phase and just prior to a new cycle, where the load remains at -0.1N) increased. This baseline displacement can also be charted (Figure 6.21) indicating the displacement of the platen (the last value at which the force is -0.1N prior to the commencement of the next cycle) for each cycle number. This shows that, under all load conditions, the sample continued to progressively compress over the 1800 cycles and did not reach equilibrium.

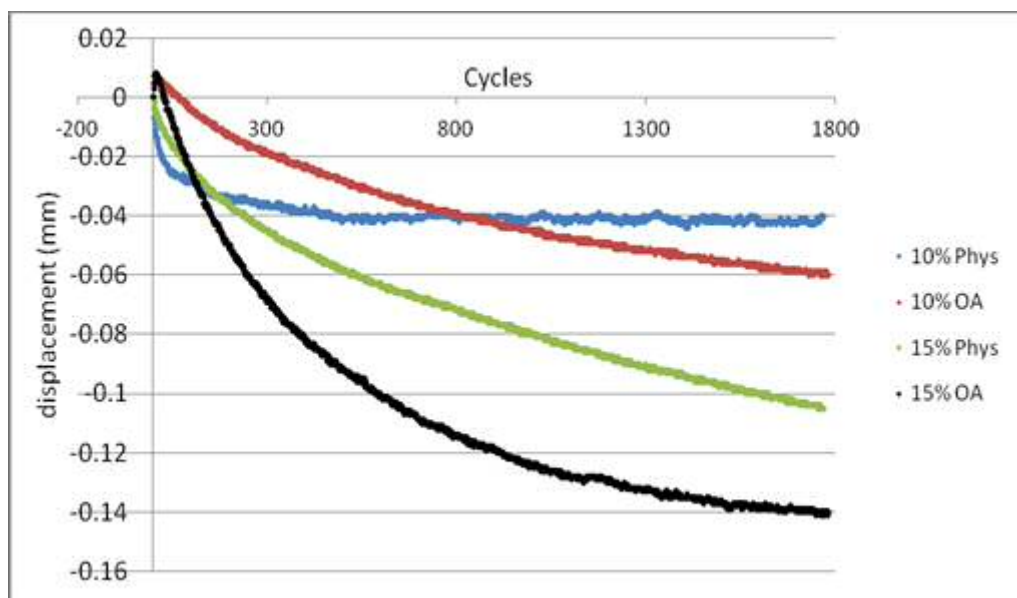


Figure 6.21. Baseline displacement results from #8. The chart represents the 'Resting' position of cartilage over the 1800 cycles, with displacement sampled at the end of each cycle (with the load transducer at -0.1N, just before the start of the loading ramp of the next cycle).

The observation that the eventual displacement was dependant on both strain magnitude and waveform shape was made after examining the time-displacement charts (Figure 6.17-6.20) and the baseline displacement charts (Figure 6.21 and 6.22).

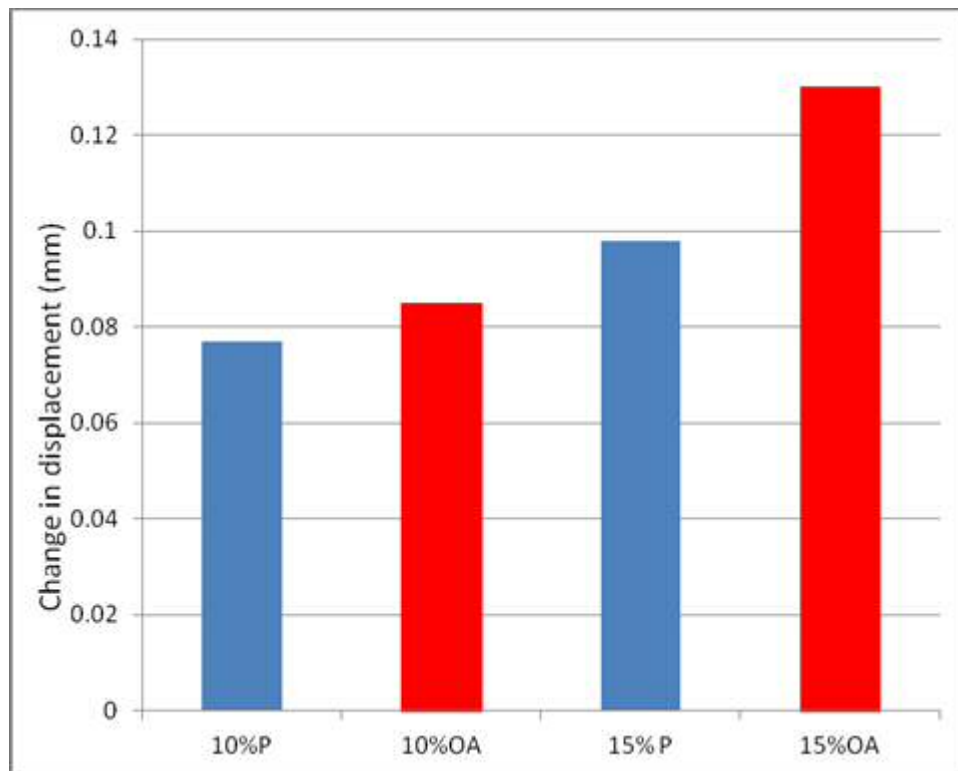


Figure 6.23. Mean change in displacement of samples (all measured at last time point of a full cycle) measured in mm, between cycle 2 and cycle 1800.

Both strain and waveform shape had a notable effect on the mean change in sample displacement (Figure 6.23, Table 6.5). Using Wilcoxon signed rank tests for paired data (processed using SPSS 16.0), the difference in displacement between 10% and 15% strain was not significantly different but showed a trend ( $p=0.0711$ ), whereas for waveform type, the differences narrowly crossed into significance ( $p=0.0498$ ).

	Sample			
subject	10%P	10%OA	15%P	15%OA
#5	0.056	0.071	0.136	0.145
#6	0.162	0.217	0.146	0.16
#7	0.093	0.041	0.046	0.09
#8	0.04	0.06	0.106	0.141
#9	0.069	0.051	0.123	0.193
#10	0.042	0.07	0.03	0.073
Mean (SD)	0.077 (0.046)	0.085 (0.066)	0.097833 (0.049)	0.134 (0.045)

Table 6.5 Numerical values for each subject for change in sample displacement, expressed in mm.

The variation in the load-displacement relationship observed across the samples might partially be explained by the differences in mechanical properties of individual cartilage specimens, as well as differences in cartilage thickness; the load-displacement relationship appeared to most closely correlate to differences in the curvature of the samples. During preliminary testing of the pilot samples it became obvious that flat samples required significantly greater forces to achieve a given deformation than curved samples. This led to the use of strain as the 'fixed' mechanical variable in the study rather than applying a constant force or displacement. Whilst the latter two options would have been easier to programme, they would have resulted in highly variable strains and stresses at the tissue level, which would have impacted on the cellular responses and tissue behaviour observed.

As a consequence of the varying curvatures of the specimens, it was important to ascertain whether the strains and stresses in the tissues were consistent between samples and whether the stresses and strains experienced within the samples could be calculated to use as explanatory factors when examining the biological response. To address this important question necessitated the use of FE modelling (Section 6.3.4).

#### 6.3.3 Gene Expression – Pilot data

In order to confirm that the correct methodology was being used and that the differing waveforms were inducing a response in the cartilage, two genes were processed for sample #1 before any other testing was undertaken. Three genes were studied in the pilot analysis including the housekeeping gene 18S and the mechano-responsive genes Aggrecan (Figure 6.24a) and Vimentin (Figure 6.24b).

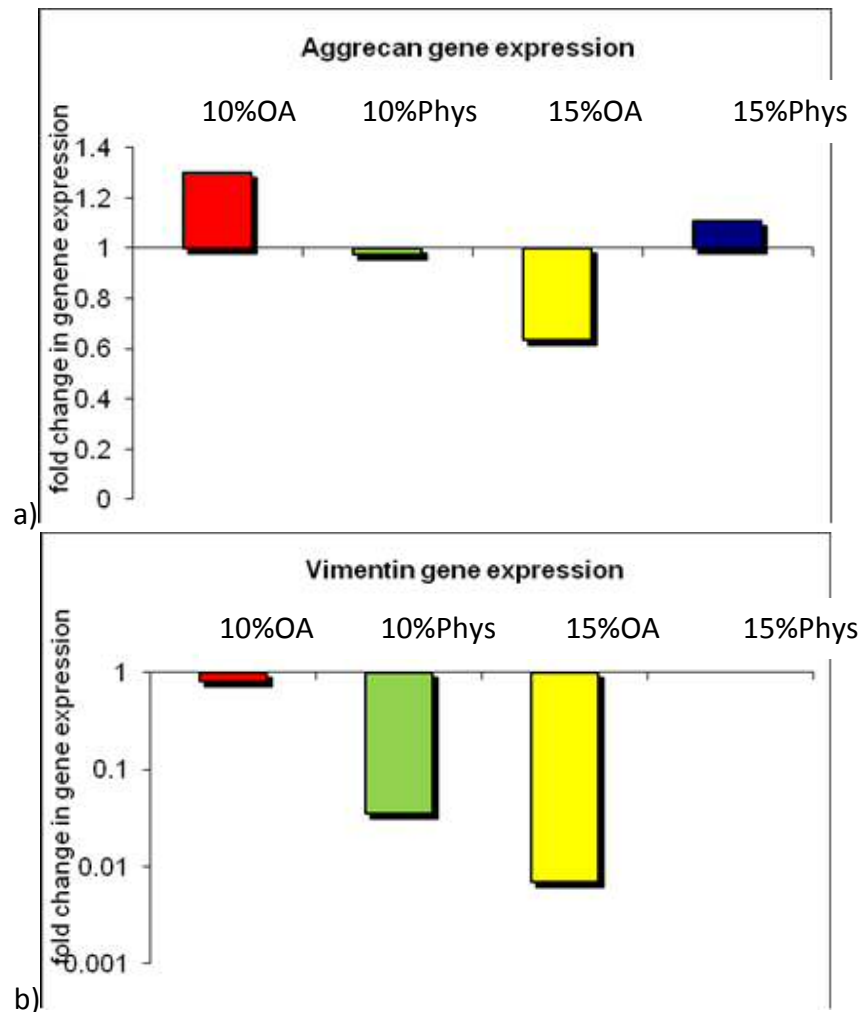


Figure 6.24 a and b. Effect of physiological and OA waveforms with 10% or 15% strain on a) aggrecan, and b) vimentin gene expression in articular cartilage, as assessed using quantitative PCR. Data are normalised to the housekeeping gene 18s and relative to the unloaded sample, which was arbitrarily given a value of 1. Vimentin was not detected in sample B4 (15% physiological waveform).

It was interesting to note that the cartilage sample which had undergone 15% strain with an OA waveform showed the greatest suppression of the selected genes when examined (Figure 6.24), indicating that the loading protocols and analytical methods were suitable.

#### 6.3.4 Gene expression: further analysis and troubleshooting

The remaining 9 samples were processed according to the methods described above (section 6.2.6 to 6.2.8) in order to extract RNA and perform qPCR. Samples were initially processed for Aggrecan and 18S. 18S amplified much later for most of the samples than had previously been observed i.e. at approximately cycle 30 instead of 15-17; aggrecan failed to amplify in any of the samples. This was presumed to be due to low levels of cDNA. The experiment was repeated for aggrecan by increasing the volume of cDNA in the reaction i.e. with 5 $\mu$ l of cDNA instead of 1 $\mu$ l. This had no effect on the amplification of this gene. Additional genes of interest were also tested on these cDNA samples, but they too yielded no amplification. Due to the failure of these amplification reactions, it was interpreted as having insufficient mRNA amounts due to the size of the samples and the age of the patients; cartilage is less cellular than most other tissues, and in aged tissue this is even more evident which could have resulted in low levels of extracted mRNA.

After pooling and re-precipitation of the RNA (mean RNA concentration:  $90.96 \pm 35.71$  ng/ $\mu$ l, mean ratio:  $1.75 \pm 0.14$ ), cDNA was produced and quantitative PCR performed for aggrecan and MMP13 as described in 6.2.8. Amplification occurred for aggrecan (Figure 6.25) but not for MMP13.

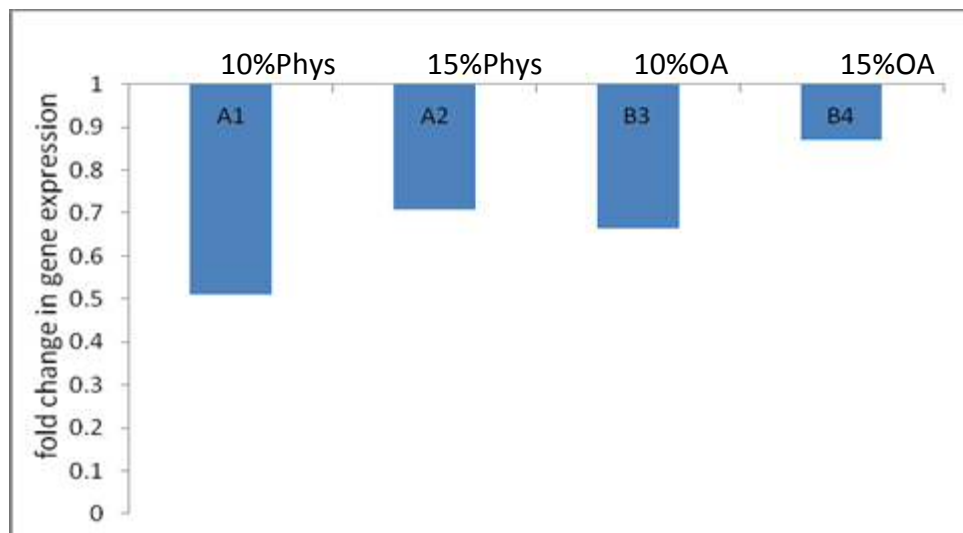


Figure 6.25. Effect of physiological and OA waveforms with 10% or 15% strain on aggrecan gene expression in articular cartilage, as assessed using quantitative PCR. Data are normalised to the housekeeping gene 18s and relative to the unloaded sample, which was arbitrarily given a value of 1.

It was subsequently noted that other researchers in the same laboratory had had recent difficulties with amplification in qPCR studies and an examination was performed of reagents used in the laboratory. qPCR was performed (by Dr E. Blain) using bovine cartilage cDNA and primer sets which had amplified well previously in conjunction with four different batches of SYBR® Green Jumpstart™ Taq Readymix™ (Sigma, Portsmouth UK), including the one which had been used for this study. These tests showed substantial differences, with the batch used in this study performing substantially worse than the other batches. Sigma was contacted and a defective number of SYBR® Green Jumpstart™ Taq Readymix™ vials had been sold even though they had previously been reported as faulty.

Unfortunately whilst this explains the problem that was encountered, it resulted in the loss of all of the independent RNA samples (which were pooled in the mistaken belief that the RNA population required enriching). The pooled samples were



analysed using a reagent which was subsequently determined to be faulty; it is unclear whether the yields could have been affected by repeated precipitation, or whether the pooled results were affected by an outlying sample which skewed the rest. The only sample which was processed successfully was the pilot sample, as this was performed using reagents already present in the laboratory prior to the delivery of new sets of reagents for the study. It is therefore suggested that the pooled results from figure 6.25 may not be representative and a repeat experiment would need to be conducted to confirm the trend observed for specimen #1.

#### 6.3.4 Finite element modelling results

As with section 6.2.10, the results reported in this section were generated by Miss Li and have been reported in detail in her thesis. The primary purpose of the following few paragraphs is to summarise these findings with a particular focus on the results that were relevant to this thesis.

The curvature of the implants, as measured using the RMSE of the residuals, varied from 0.26 to 0.10 (mean 0.19 SD  $\pm 0.09$ ). There was no relationship between implant curvature and the change in displacement reported in 6.3.2.

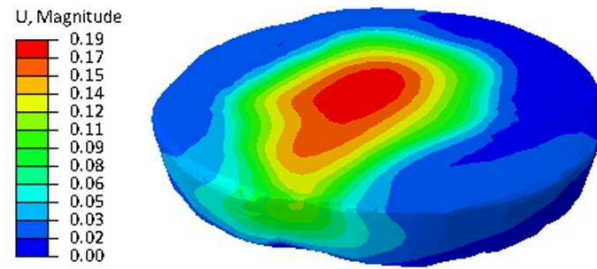


Figure 6.26 Contour plot of nodal displacement for one of the most curved explants. The Blue represents low displacement and the red represents high displacement.

The use of implant specific FE models was found to show significantly different patterns of stress and strain at a tissue level to those estimated using the flat model. Peak displacement under load in the flat-surface model was found to be 0.047mm at the cartilage surface and peak stress was 0.030MPa. In the explant specific models, peak displacements reached 0.185mm and peak stresses reached 0.236MPa, both experienced at the central region of cartilage in the superficial zone, just under the platen (figure 6.26 and 6.27). Shear stresses in the flat surface model were predicted to reach 0.0001MPa, but in the explant specific models these were seen to reach up to 0.15MPa.

Whilst the maximum stresses were compressive stresses experienced in the central region, tensile stresses of a lower magnitude were experienced in the peripheries of the samples (figure 6.27). Shear stresses were seen at the junction between the areas of compressive and tensile stress, and were at their maximum at the bone surface (figure 6.28).

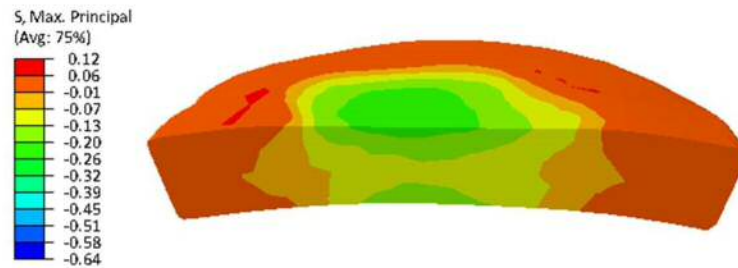


Figure 6.27. Diagram of typical principle stresses, in which the green represents an area of high compressive stress whilst the red represents an area of high tensile stress.

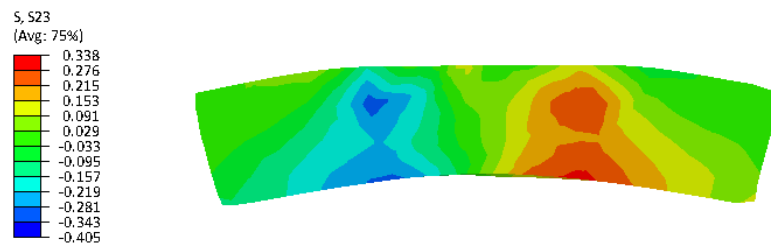


Figure 6.28. Example of shear stress in a relatively curved sample, showing high shear stresses at the margins between areas of compressive and tensile stress.

The Young's modulus of the cartilage samples was calculated to be significantly lower when a flat model was used (mean 3.21MPa, range of 1.41-5.51MPa) compared to when a explant-specific model was used (mean 7.58MPa, range of 2.06 to 12.87), and the values observed in the explant specific models more consistent with previous measurements in the literature (Roberts, Weightman et al. 1986; Shepherd and Seedhom 1999). A ratio was calculated of the experimentally determined stiffness and the FE modelling derived stiffness (termed the stiffness correction factor), and this was found to correlate well ( $R^2=0.55$ ) with the curvature of the sample (the RMSE described above).

Higher values for the modulus were found for samples at the zenith of the condyle than any other region, and those at the medial side of the condyle were higher than those at the lateral side.

## 6.4 Discussion

The aims of this chapter were to develop a method to study the effect of loading waveform on cartilage biomechanics and biology. Unfortunately the complexity of the study design, the learning curve experienced during the loading of the first few samples and a reagent problem in the qPCR reactions meant that a full data set could not be obtained. There is enough data, however to show that cartilage biomechanics are influenced by loading regime, and that changes in chondrocyte gene expression would be expected.

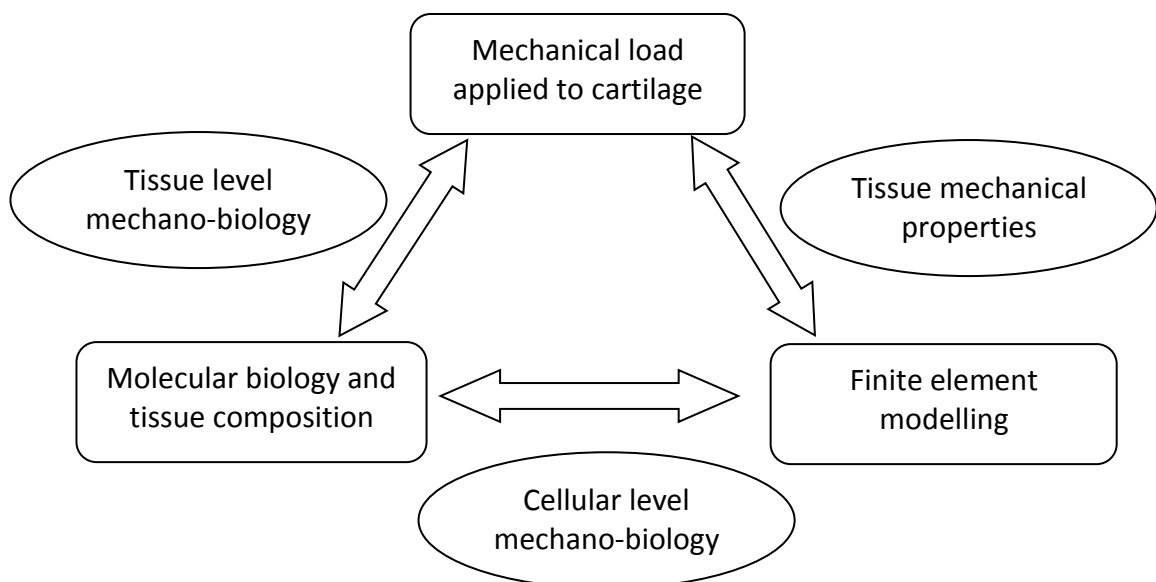


Figure 6.29. Model of approach to the study methodology and results for future work

By combining experimental biomechanics, mechano-biology, and computational modelling, the study method has the potential to produce a large body of important data (figure 6.29). Each step of the study has been performed successfully and a method has been developed which could be refined to produce a

unique and relevant collection of data that could be translated into changes in treatment in the future.

From the available results, it can be concluded that a change in loading waveform towards a square wave pattern causes cartilage to deform to a greater degree over a 30 minute period of simulated gait than is observed with a normal loading waveform. Over a 30 minute period, cartilage continues to deform and does not reach an equilibrium. It may be that more prolonged loading times produce more pronounced changes. The peak load is not the only important factor but the time spent under load is also important. This appears to be due to the visco-elastic properties of cartilage, and is most likely to be due to increased fluid flow under the more prolonged loading pattern.

The testing was designed to simulate true joint loading during gait as much as possible. Whilst the waveform timings are likely to be representative, it is not known exactly how much strain is experienced by the cartilage *in vivo* during normal or pathological situations (such as meniscal or ligament injury). The strains chosen were assumed to be representative of 'normal' and 'heavy' loading, although estimations of cartilage strain vary significantly in the literature, from 5% to 25% maximum principle strains depending on the complexity of the model used and the loading applied to the model (Yang, Nayeb-Hashemi et al. 2010; Sibole and Erdemir 2012; Guo, Maher et al. 2013). A deeper understanding of *in vivo* stress and strain patterns from increasingly sophisticated models which use gait and EMG

derived input variables along with more life-like anatomy and material properties will assist in optimising these studies further.

Chondrocytes are known to be sensitive to changes in direct loading as well as to fluid shifts in the tissue (Jin, Sah et al. 2000; Blain 2007; Zhu, Wang et al. 2010; Degala, Williams et al. 2012). It is reasonable to expect that the observed changes might lead to abnormalities in the biological response of the chondrocytes and this was seen in the pilot sample under the higher loading condition (15% strain). The lower loading condition did not show the same biological changes although at lower load the chondrocytes may be more tolerant of loading pattern. However, this is only a single sample, and the reality for the remainder of the individuals, or the response of other genes, may vary significantly. Whilst a different transcriptional response was observed in the pooled samples, the repeated re-processing to pool RNA samples and the unknown use of defective reagents may have affected the data collected, therefore this experiment would need to be repeated to validate the pilot observations.

At present, it can only be concluded that the method is able to produce a biological response, and that a repeat of the study with new reagents is required to validate the pilot observations for the genes selected i.e. aggrecan and vimentin. It is therefore important to consider what changes would be required in the future to optimise the study and give the greatest possible chance of a full biological and mechanical dataset.

The use of human cartilage is an important step in gaining a relevant comparison between subtleties in loading pattern and magnitude. Whilst the inflammatory milieu within an arthritic joint might have effects that influence the biological behaviour of the chondrocytes, it is believed that this still remains the most representative tissue to use when translating the results of the research to the management of patients at risk of knee arthritis.

For a study such as this, the use of human tissue has 2 particular challenges which need to be addressed: the shape and size of the available sample and the paucity of cellular material to study biologically. It was decided that the sample would be best kept on bone, as severing the link with bone would intrinsically affect the mechanical properties of the tissue. This means that the shape of the surface needs to be considered.

The curvature of the explants was quite variable. The variability was reduced as much as possible by visually selecting regions of the condyle for coring that were relatively homogenous in shape, although that was made more complicated by the need to take so many explants from each condyle. All explants were convex to some degree to ensure that the central region was loaded in preference to the edges.

Only the central region of the samples was taken for biological processing and the finite element models confirmed that for these samples, this approach ensured that the study was focused on the loaded region of the samples. The size of the loaded region varied with the curvature of the samples, with more curved pieces having

smaller areas under significant compressive load (figure 6.30). The finite element modelling was shown to be a valuable step as it allowed the surface curvature and loading pattern to be used as a covariate, allowing for the difference in peak stresses and distribution of stress and strain to be accounted for in the final analysis.

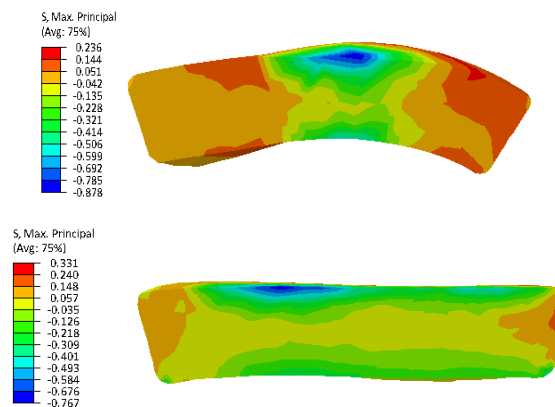


Figure 6.30 Example maximal principle stress curves for a) one of the least curved and b) one of the most curved samples, showing the difference in the area of cartilage that was loaded.

Variation in the shape of the cores made loading particularly challenging, as the load-displacement behaviour of the samples varied and therefore both tuning the machine and determining the correct stress required was difficult. Changes to the settings of the machine have been discussed with Professor Evans and in future advice will be taken from Bose about how to manually adapt the damping settings once tuning is complete, which at present is an automatic process performed by the Bose software.

As a result of the modelling, it is now clear that the location of the cores on the condyle (whether central, medial or lateral) was also important in terms of the stiffness of the explant, a point that was not appreciated visually whilst loading.



The lack of available material for biological processing was the other major challenge. Previous studies have successfully performed qPCR on a range of genes (such as those that we tested for, including the less abundant MMP genes) although using larger quantities of tissue (Brew, Clegg et al. 2010). Brew et al in 2010 reported a study where a number of genes were analysed using qPCR based on 200-300mg of tissue per sample. This was approximately 4 times the volume achieved in this study. Whilst the problems that were encountered due to an unknown defect in one of the reagents could not have been anticipated, there would probably still be a quantity of RNA left over as lower volumes would have been needed in each reaction.

In future studies, it is suggested that only three cores are taken – two for comparison and one as a control and this would ensure that greater consistency could be achieved in the location of the cores and hopefully in their geometry. The use of larger samples would be allowed by this, increasing the genetic material available. Using the current loading regime, the finite element models have demonstrated that there is a size limit beyond which the cartilage being studied is not being loaded adequately, especially if a relatively curved sample is used.

There are a number of possible approaches that could be used to prevent this problem. The first would be to avoid taking samples from the more curved area at the posterior aspect of the samples as the radius of the lateral condyle reduces as you move posteriorly (the 'J' curve) (Howell, Howell et al. 2010). Another option might be to use a subtly concave platen, although this would have to be minimal to

avoid edge contact in the flatter specimens, or a platen with some subtle elasticity to achieve a wider contact during loading. Finally, a mould could be taken of the explant to ensure a more congruent contact, and used as a 'sample specific' platen, although this would add complexity and move away from representing the clinical scenario.

The finite element modelling was found to be a valuable addition to the study protocol. The method of moulding created technical challenges in shape measurement and fitting that would not have been necessary if the explant shapes were measured directly, although this would now be recommended as the author has become more confident with the dental putty. One criticism of this model may be that the tissue properties were not depth dependant, which might have altered the results to some degree although this adds further complexity to the model, making the modelling process more difficult to perform and requiring greater time and computational resources.

Further reverse optimisation studies could also be used to examine the visco-elastic properties of the cartilage, given the wealth of time dependant information collected in these studies. This would allow a greater understanding of the fluid shifts in the tissue and the effects of prolonged periods of walking *in vivo*, and may be considered for future research (Guo, Maher et al. 2013).

Overall, a method is presented for studying the mechano-biology of human cartilage on bone, combining experimental and computational engineering techniques with established molecular biology methods. Financial and time

constraints mean that repeating this study is beyond the scope of this thesis, as modifications to the methods to make the study more robust would mean extending the workload considerably. It is therefore planned that this will be the subject of a new grant application, allowing at least a year and ideally two years to complete the work.

Recommended changes to the study for future would include:

- The use of a larger number of experiments, each one making only a single comparison between two explants for each condyle (i.e. 10% strain vs 15% strain, or one waveform vs. the other).
- Larger explants of 12mm taken from the zenith in the region of contact when the knee is in extension (which has a larger radius of curvature), with a 7.5mm core taken for qPCR, effectively doubling the volume of cartilage for testing.
- If available, a flat platen made of a material of similar modulus to articular cartilage (if available and sufficiently low friction).
- Moulding taken of the explants themselves rather than moulding the condyle.
- Depth dependant finite element modelling.
- Regular qPCR sessions after the first, 5<sup>th</sup> and each subsequent 5 tests to pick up problems at an earlier stage.

## 6.5 Conclusion

A method is presented which combines mechanical testing of human articular cartilage on bone, finite element analysis and molecular biology. This is a novel approach to mechano-biology research which has been designed to be as representative of the clinical situation as possible; furthermore it has the potential to produce valuable data on the relationship between joint loading and biological response. The use of finite element modelling allowed for the variation in sample shape to be accounted for and this was a valuable addition to the study.

A comparison between the two waveforms presented found greater compression of cartilage over a 30minute simulated walking cycle in the abnormal loading pattern compared to a physiological loading waveform. Results are consistent with increased fluid flows in the cartilage samples loaded with the abnormal loading pattern. This might be expected to impact on chondrocyte behaviour, although this remains theoretical at present. Given the pilot data that has been presented, a repeat of the study with minor modifications is likely to provide a clear answer to that question.

## **Chapter 7. Conclusion and Further Study**

## 7.1 Hypotheses tested

A series of studies have been presented in this thesis, each with the aim of filling a knowledge gap in the literature surrounding the development and progression of knee OA in the second knee of individuals who already suffer with OA on one side. The collective aim of the thesis was to build a base of knowledge which could be used to plan future studies into the pathogenesis and treatment of knee osteoarthritis, both at the most affected side as well as the less affected side. In this first section, the primary hypotheses for each chapter will be examined, and the following section will examine the results of further exploratory analyses and give an overview of the thesis. This will be then placed in the context of new developments in the literature and plans for further study.

The following hypotheses were tested in this thesis.

### **Chapter 3: Knee osteoarthritis develops and progresses in one knee only.**

This null hypothesis was rejected. The radiological study was performed over a 12 year period in a relatively young cohort who reported knee pain at baseline and no history of trauma in a community survey. Of those with no x-ray changes at baseline, 41/76 (54% (95%CI 42.1 to 65.4%)) developed bilateral changes. 24 of the 30 (80% (95%CI 61.4 to 92.3%)) patients with unilateral disease at baseline developed bilateral disease after 12 years. Overall, it can be concluded that knee osteoarthritis tends towards bilateral involvement with time.

**Chapter 4. There is no difference in the adduction moment impulse and quadriceps-hamstring co-contraction between normal individuals and subjects with unilateral knee osteoarthritis.**

The null hypothesis was rejected for both the adduction moment impulse and for co-contraction. In this chapter, a cohort of 20 individuals with knee osteoarthritis and no joint pain elsewhere were found to have elevated adduction moment impulses at both knees compared to an age matched control population (table 4.3,  $p < 0.001$  for the affected and  $p = 0.048$  for the unaffected knee respectively). Co-contraction indices were also elevated both medially and laterally (table 4.5,  $p < 0.001$  for affected leg medially and laterally,  $p < 0.001$  for the unaffected leg laterally and  $p = 0.005$  for the unaffected leg medially). It can be concluded from chapter 4 that patients with unilateral knee osteoarthritis place abnormal loads on the other knee during level gait at self-selected speed.

**Chapter 5. There is no change in the adduction moment impulse or co-contraction in either the affected or unaffected leg following unilateral knee arthroplasty surgery.**

The null hypothesis was rejected for the affected knee for both the adduction moment impulse (table 5.4,  $p < 0.001$ ) and for co-contraction (table 5.5,  $p = 0.018$ ). At the unaffected knee, a small fall in the mean adduction moment impulse was observed, with no statistical significance found in pre-to post-operative changes (table 5.4,  $p = 0.132$ ). Co-contraction did not change at the unaffected leg (table 5.5

$p=0.74$ ). Given that gait speed did not return to normal, and that mid-stance moments remained abnormal (table 5.4  $p=0.008$ ), it is likely that a type 2 error due to a small sample size resulted in the lack of significance comparing adduction moment impulse between the unaffected knee of patients and the control population. The same can also be concluded for medial co-contraction at the unaffected leg, although lateral co-contraction at the unaffected leg was significantly different to the control population ( $p=0.016$ ). It can be concluded that the overall pattern of loading at the unaffected leg does not return to normal following knee arthroplasty, although there is significant inter-individual variation in this.

**Chapter 5. There is no factor that predicts differences in function between subjects with OA and controls.**

This null hypothesis was rejected. The variable with the highest classification accuracy in comparing osteoarthritic and normal individuals was hip power, with classification accuracy of 92.5% for the affected leg and 87.5% for the unaffected leg. Ten variables in the affected leg had classification accuracy of above 80% and 6 in the unaffected leg also had classification accuracies of above 80%. In total, 17 variables for each leg were chosen to be entered into the functional classifier. It can be concluded that kinetic and kinematic changes in knee osteoarthritis are relatively consistent, and that pathology in one knee is reflected in kinetic and kinematic changes in both knees.



**Chapter 5. Physical function measured in each leg of patients with knee OA does not return to normal after surgery.**

This null hypothesis was tested using a Bayesian-like approach, based on the Dempster-Shafer theory of evidence. Using this approach, the null hypothesis can be rejected in part, as there was significant inter-individual variation in the results. Seven of the 15 individuals returned to the normal half of the classifier for both legs, one was classified on the normal side for the affected leg and OA side for the unaffected leg, and 7 stayed in the osteoarthritic portion for both legs. Physical function, as measured by gait performance, returns to normal in a proportion of individuals following TKR, even when the other joint is normal.

**Chapter 6. The loading waveform or magnitude has no effect on the mechanical behaviour or gene expression of human articular cartilage.**

The null hypothesis was rejected for mechanical behaviour but could not be answered either way for gene expression. The change in baseline displacement of the samples (at the end of each cycle, with 0.1N force applied only) was greater for the OA-derived waveform than the normal pattern ( $p=0.0498$ ), and a non-significant trend was observed for the difference between 10% and 15% strain ( $p=0.0711$ ). Larger numbers would be required to confirm this finding with more confidence. Due to problems with the reagents used in the study, the biological data was insufficient to be able to draw a reliable conclusion. A repeat of the study design with some modifications (section 6.4) is required to properly address this

hypothesis, although it can be concluded that a repeat study would be both technically feasible and likely to find differences between groups.

## 7.2 Impact

One of the initial aims of the thesis was to determine whether bilateral knee osteoarthritis exists as a clinical problem. If changes in biomechanics are part of the progression to bilateral disease, a pattern of step-wise progression of one knee followed by the other would be expected over time. A significant number of the individuals presented in chapter 3 progressed in a step-wise fashion through a predominantly unilateral stage, tending towards bilateral disease with time and increasing severity, with a smaller proportion of individuals progressing evenly over time. This study does confirm that progression from unilateral to bilateral disease is a relevant clinical entity, and raises the potential for an intervention to protect the other knee in a 'secondary prevention' manner.

Such an approach would be most effective if it was performed early in the disease process (figure 3.4). However, even if radiographic disease is present bilaterally, improved biomechanics in the secondary knee would help the patient both in terms of pain relief and preventing disease progression, and therefore biomechanical studies in this field are warranted.

Gait changes in knee osteoarthritis are a bilateral process. The observed gait changes were not simply 'antalgic' (implying a discrepancy in stance percentage between the two legs) and instead a characteristic 'chronic OA' gait pattern could be described from the data. Slowing of gait, increased double support, a wider base of gait and loss of the typical loading response was seen.

From the kinetics, reduced hip and knee powers and changes in the ground reaction vector imply a tendency in OA subjects to keep the centre of mass stable, rather than accelerating it vertically and horizontally in the normal manner. The change in stance time combined with the change in the shape of the ground reaction vector resulted in changes in knee loading. The accompanying quadriceps and hamstring co-contraction contributed to these observed changes. Co-contraction exacerbates joint reaction forces and may even be the cause rather than the effect of the gait patterns seen here, as a stiff knee gait would give many of the features observed in the kinetic and kinematic data, such as the reduced powers and 'pendular' pattern of gait described in section 4.4.2.

Gait speed made a clear contribution to the change in loading pattern seen in this study. When analysing the results of study 4 the question of using ANCOVA to account for gait speed was raised (note the discrepancy between the protocol in appendix 1 and the methods in 4.2.6), however it was felt that this would deal falsely with a covariate that was not a true source of error but was part of the true physiology of the OA patient. Whilst this was initially criticised in early reviews of one of the published papers, the approach has since been ratified in a recent expert review of dealing with gait speed in OA studies (Astephens Wilson 2012; Metcalfe, Stewart et al. 2013).

Gait speed is clearly a significant part of the patho-mechanics of OA gait. However, normal individuals walking at slow speed do not fully replicate the waveform changes seen in the OA cohort (section 4.3.5), and in the classification ranking

process, gait speed was not the highest ranking variable in either leg. Whilst it must be considered a significant co-factor in causing the changes in loading seen in this study, gait speed is likely to be only part of the process that changes gait pattern in OA.

The biomechanical changes that were observed in chapter 4 would be expected from the literature to result in more rapid progression of disease in both knees (as discussed in section 2.3.2). This assertion is supported by the cartilage loading study presented in chapter 6. One of the aims of the study was to help determine whether peak loading or loading impulse was more important when looking at biomechanical data, as the main difference between the two measures is in the shape and duration of the loading waveform. It must be remembered that cartilage is a strongly visco-elastic material, and prolonged periods of loading will create significant fluid shifts, which would be very likely to influence chondrocyte behaviour (Zhu, Wang et al. 2010; Guo, Maher et al. 2013). Therefore the reporting of peak loads should always be accompanied by time-related data, such as impulse or other summaries of the loading waveform.

Knee replacement was not a perfect solution for the subjects in this study, and whilst subjective function normalised in most, objective function remained abnormal in half of the subjects and abnormal co-contraction persisted in the majority. The close relationship between pre-operative function and post-operative function is an important observation. Predictive models may be developed based on these techniques, allowing patient care to be tailored and stratified to improve

clinical outcomes, and pre-operative training may have some benefit in further improving outcome.

Patients should not be told that a knee replacement will protect the other knee from disease by restoring normal biomechanics. However, there is a potential for training at this stage to improve gait and protect the other joints from disease. As such, an intervention aimed at improving confidence, fluidity and speed may be of benefit for patients in the future.

The underlying reason that patients walk differently has not been defined. Reduction of joint forces is clearly not the aim, and the gait pattern could not be considered efficient. The most likely reason (see discussions in chapter 4 and 5), is that the pattern was a change in gait behaviour. This may have been stimulated by a loss of confidence in the knee, either because of a fear of intermittent pain, a loss of strength or as a result of a loss of proprioception. The observed pattern of co-contraction and ground reaction profiles are subjectively similar to the patterns described for slippery surface gait and it is hypothesised that the gait pattern is a cautious, tentative gait pattern in which stability is prioritised, with a sacrifice of chronic increases in joint loading accepted by sufferers.

In summary, bilateral knee OA commonly follows unilateral disease. Patients with unilateral knee OA place abnormal loads through the other knee and hips when they walk to due a characteristic pattern of gait characterised by a slow, stable, stiff gait pattern. The magnitude of these changes would be expected to lead to mechanical and biological changes in articular cartilage which is by definition

susceptible to disease in this patient group. Recovery of gait function after knee replacement is usually incomplete, even with good recovery of subjective function. Additional training may have a role to play, both pre- and post-operatively, in restoring normal biomechanics and protecting the other knee from disease.

## 7.3 Recent developments in the literature

There have been a number of advances in the knee osteoarthritis literature over the time that this thesis has been prepared. During 2013 in particular there have been a number of studies closely related to the work presented here, with significant overlap in findings, and these will now be discussed.

In 2012, Nishimura et al published a radiographic study which included 65 Japanese patients with unilateral knee OA (KL $\geq$ 2) at baseline (Nishimura, Hasegawa et al. 2012). The mean radiographic follow up was 5.3 (range 2-12 years), and 49% progressed to bilateral knee OA. Using a Kaplan-Meier analysis, the chance of bilateral knee OA at 10-12 years was 80%, agreeing well with the findings from chapter 3.

In a paper reporting two related studies, Dr Jones from Salford University examined the possibility of secondary prevention of the unaffected leg (Jones, Chapman et al. 2013). In the first part of the paper, a cohort study of 152 patients with knee OA from the Framingham OA study was examined, with bilateral serial radiographs over a mean of 8.6 years. Sixty of the 68 (88%) with unilateral knee OA at baseline subsequently developed bilateral disease, also confirming the findings of the study presented in chapter 3. A second study was reported in the same article of a cohort of 51 patients with radiographic knee pain who were treated with laterally wedged insoles, where significant reductions in knee moments were demonstrated. Overall, the study concluded that secondary prevention in knee OA was a valid concept in knee OA and was worthy of further research (Jones, Chapman et al. 2013).



Shao et al (2013) followed a total of 2917 patients for a minimum of 10 years after total knee replacement (mean 17.1 years), and found that 46.0% had a contralateral knee replacement, at a mean of 3.05 years (SD 3.46), with a gradual and ongoing progression over the 20 year study period (Shao, Zhang et al. 2013). It can be concluded from this that further work into the modification of both pre- and post-operative gait mechanics has the potential to improve function in a significant proportion of the knee arthroplasty population.

A gait and strength study performed by Yoshida et al (2012) found persistent strength and function deficits in the affected and unaffected legs of patients up to 3 years following TKR, which tended towards being symmetrical after a time but differed from normal controls (Yoshida, Zeni et al. 2012). The same authors published their findings on co-contraction at 1 year in the same patient cohort, and a further paper on co-contraction in both limbs of TKR patients was also published by the same group, although it is unclear if there was any overlap in the study cohorts (McGinnis, Snyder-Mackler et al. 2013; Yoshida, Mizner et al. 2013). They both used the same technique as Lewek et al to measure co-contraction, which was also the calculation chosen for this thesis, but the calculation was limited to the loading response (Lewek, Rudolph et al. 2004; Yoshida, Mizner et al. 2013).

Co-contraction was found to be correlated to quadriceps strength in the paper by Yoshida et al, but McGinnis et al could not find a correlation between co-contraction and dynamic joint stiffness, although this complicated measure may have been confounded by differences in strength and gait speed. Whilst co-

contraction during loading response was not found to differ from control subjects in these studies, quadriceps recruitment and ankle co-contraction were both different to controls (McGinnis, Snyder-Mackler et al. 2013) (Yoshida, Mizner et al. 2013).

The difference between co-contraction findings in these studies and the results presented in this thesis might be explained by the different parts of the gait cycle that were examined. Co-contraction results from this thesis were significantly affected by the waveform during mid-stance (see figure 4.11) and this has been recently described by other authors, justifying the decision in this thesis to examine co-contraction across the whole of stance phase (Rutherford, Hubley-Kozey et al. 2013).

Fallah-Yakhdani et al (2012) defined co-contraction according to timings of the EMG signals, and noted bilateral abnormalities of co-contraction times in 14 patients following total knee replacement who had not been selected with regard to the unaffected knee (Fallah-Yakhdani, Abbasi-Bafghi et al. 2012). Again this supports the findings in this thesis that co-contraction remains abnormal as the muscles are active for a much longer period through stance than would occur in normal individuals.

A recent review on co-contraction in knee OA referred to a conference presentation by Hodges et al from 2012 which described a relationship between cartilage volume loss and co-contraction, presumably using similar methods to the study from the same unit on knee adduction moment impulse (Bennell, Bowles et al. 2011; Hodges, van der Hooft et al. 2012; Mills, Hunt et al. 2013). The full results of

this study are awaited with interest, as it is reasonable to expect that co-contraction would substantially increase joint loads given the contribution they make to knee joint loads measured using modelling and in-vivo (Shelburne, Torry et al. 2006; Hillstrom, Minacori et al. 2013).

Worsley et al (2013), from the University of Southampton used a musculoskeletal modelling approach driven by gait analysis (without EMG) to determine joint loading in affected and unaffected knees of a cohort of patients before and after TKR (Worsley, Stokes et al. 2013). They found greater loads on the unaffected knee than the affected side pre-operatively, with only small changes 1 year post operatively. Many of their findings were similar to those presented in this thesis, although there was very little detail about the selection of the patient group in terms of symptoms or deformity in the other leg (which was a significant challenge in the studies presented in this thesis).

Overall, there has been a number of papers on joint loading and disease development in the contralateral limb over the past 18 months, including the papers published as a result of work presented in this thesis. The majority of studies have supported the findings within this thesis, increasing confidence in the findings from this work. It is becoming increasingly recognised that future work aimed at protecting the other joints may provide significant benefit for patients in the future.

## 7.4 Further Study

A series of recommendations for further research is presented here. Some of the studies are already in progress and others directly follow on from work done in this thesis, such as a repeat of the work performed in chapter 6. The final proposal is an interconnected group of studies which deal with the overall aim of this thesis, to develop potential strategies for the secondary prevention of disease in the other knee in patients with knee OA. Proposals and recommendations for these studies are listed below.

### **The relationship between in-vivo loading patterns, tissue level stresses and gene expression in human articular cartilage and subchondral bone.**

The first recommendation for further study is a repeat of the work in chapter 6, with modifications as described in the discussion (section 6.4). Biological material could also be taken from subchondral bone, which also contributes to the complex relationship between mechanics and biology in OA. A grant application is proposed to combine the study with ongoing finite element modelling, and a sufficient period of time to compare waveform types and loading magnitude in a sufficient number of samples. With time, this study could be expanded further using modelling derived data, demonstrating (for example) the potential biological effect of a bracing intervention, or a gait retraining programme.

## **The Relationship between Alignment, Function and Loading in Total Knee Replacement**

Whilst working on the study in chapters 4 and 5, research support was also given to colleagues working on the 5-year follow up of a cohort of patients from a regional treatment centre with known high rates of TKR mal-alignment and failure (Kempshall, Metcalfe et al. 2009; Hickey, Kempshall et al. 2012). A study was planned to use the biomechanical techniques learned during this PhD to study the relationship between knee joint alignment and joint loading, objective function and kinematics in this unique patient population. Some of the early data from the study in chapter 5 was used as pilot data, and a grant application to ORUK for £39,516 was successful, with additional financial support also received from the Arthritis UK Biomechanics and Bioengineering Centre of Excellence in Cardiff. A post-doctoral fellow (Dr J Madete) was employed and the data is now in the final stages of analysis. The early results from this study have been presented recently, suggesting a relatively narrow window for 'correct' alignment of a total knee replacement (Metcalfe, Madete et al. 2013).

## **Optimisation and validation of gait analysis techniques for measuring limb alignment.**

One other aspect of the study that warrants further work is a re-examination and validation of methods for using gait analysis to determine limb alignment. A technique is presented in this thesis which can be used to improve on the measure

of limb alignment used by a typical Vicon Plug-in-Gait analysis whilst avoiding the need for cumbersome functional joint assessment, but it still requires testing and further refinement. It may also be that rather than using the presented technique, that it is more accurate to use the described flexion plane to rotate the thigh axis co-ordinate system, correcting for rotational errors in the thigh axis co-ordinate system due to incorrect identification of the epicondyles, before using the standing trial data in which skin movement artefact is less of a problem. The dataset described in the TKR alignment study above may be suitable to assess this and this is being examined with a view to developing a study plan for the future.

### **The biomechanics of the other knee following ACL reconstruction and the link to pain related fear.**

Anterior cruciate ligament injury is well recognised to be a cause of knee osteoarthritis, although there remains little evidence that that risk reduces following ACL reconstruction. On-going biomechanical deficits have been identified following ACL reconstruction, including quadriceps and hamstrings co-contraction. Fear of pain and injury is also well recognised following ACL reconstruction, however biomechanics and pain fear have not been linked previously. A physiotherapist working at ORLAU, Justine Bee, has recently been successful in obtaining a grant for £25,000 to examine the biomechanics of the other limb of patients post-ACL reconstruction using gait analysis and EMG. If abnormalities in

the other knee are confirmed, this patient group may be amenable to training as discussed below.

### **Development and testing of novel advanced rehabilitation techniques following TKR and in early knee OA**

This proposed programme of inter-related studies is also an appropriate conclusion to the thesis, whose aim was to direct further research into protecting the other knee. Two patient cohorts should be examined, an early OA group as examined in chapter 3 and a post-TKR group, as discussed in chapter 5. The aim would be to modify gait behaviour to influence moments and co-contraction, with a view to reducing initiation or progression in the other leg. The findings of chapters 4 and 5 would suggest that a form of neuromuscular training would be worthwhile, with the aims of improving gait speed, improving mobilisation of the centre of mass, and reducing co-contraction.

Given that maintenance of normal gait function appears to be a product of either chronic weakness or abnormal gait behaviour, a three-arm comparative study is proposed comparing no treatment, muscle strengthening and gait re-training. Muscle strengthening could be performed in a controlled way using functional electrical stimulation. There are a number of gait retraining techniques currently in use, from shoe modifications which stimulate a neuromuscular response, to biofeedback training using a treadmill or skin mounted accelerometers. Therefore a short pilot would be required (a gait analysis study of the short term effects of the

most promising techniques), prior to a larger study with gait analysis and EMG but also change in cartilage volume and bone marrow lesions on MRI to establish the effect of treatment over time.

Alongside patient reported outcome measures, kinesiophobia, muscle strength and a measure of proprioception would also be collected as important co-variables and a pre-operative assessment of objective function would be examined to determine if an analysis of pre-operative objective function is able to identify those at risk of poor function (as it was in this study), potentially allowing targeted early treatment to improve post-operative function.

This would give practical benefit to patients but it would also help inform future research, as data from the other knee could be used to understand the effects of biomechanics on degeneration of the vulnerable knee joint. The contra-lateral knee should be considered a 'knee at risk' and studies aimed at developing and refining new treatments for this are recommended.



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## Appendices

## Appendix 1. Ethical Approval

The following pages contain copies of the ethical approval for the studies presented in chapters 4, 5 and 6, with the original study protocols used in those submissions.



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North Wales NHS Trust

### North Wales East Research Ethics Committee

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29 January 2009

Mr Andrew Metcalfe  
Orthopaedic Registrar  
Central Region, North Wales NHS Trust  
Glan Clwyd Hospital, Rhyl,  
Denbighshire, WALES, UK  
LL18 5UJ

Dear Mr Metcalfe

**Full title of study:** The Biomechanics of the Opposite Leg in Knee Osteoarthritis: a pilot study.  
**REC reference number:** 09/WNo03/1

Thank you for your letter of 26 January 2008, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information was considered at the meeting of the Sub-Committee of the REC held on 29 January 2009. A list of the members who were present at the meeting is attached.

### Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

### Ethical review of research sites

The favourable opinion applies to the research sites listed on the attached form. Confirmation of approval for other sites listed in the application will be issued as soon as local assessors have confirmed they have no objection.

### Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission at NHS sites (“R&D approval”) should be obtained from the relevant care organisation(s) in accordance with NHS research governance arrangements. Guidance on applying for NHS permission is available in the Integrated Research Application System or at <http://www.rdforum.nhs.uk>.

## Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

<i>Document</i>	<i>Version</i>	<i>Date</i>
Participant Consent Form: Study consent	2	09 December 2008
GP/Consultant Information Sheets	1	15 December 2008
Letter of invitation to participant	2	15 December 2008
Advertisement	1	09 December 2008
Questionnaire: Oxford Knee Score		
Covering Letter		15 December 2008
Protocol	3	09 December 2008
Investigator CV		21 December 2008
Application	1	21 December 2008
Response to Request for Further Information		
Participant Consent Form: ORLAU Consent	3	22 January 2009
Participant Information Sheet	3	22 January 2009
Letter of invitation to participant		26 January 2009
Questionnaire: WOMAC	2	25 January 2009
Covering Letter		26 January 2009

## Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

## After ethical review

Now that you have completed the application process please visit the National Research Ethics Website > After Review

You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.

The attached document “After ethical review –guidance for researchers” gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Progress and safety reports
- Notifying the end of the study

The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.



We would also like to inform you that we consult regularly with stakeholders to improve our service. If you would like to join our Reference Group please email [referencegroup@nres.npsa.nhs.uk](mailto:referencegroup@nres.npsa.nhs.uk).

<b>09/WNo03/1</b>	<b>Please quote this number on all correspondence</b>
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With the Committee's best wishes for the success of this project

Yours sincerely

**Mr P Richards**  
**Associate Specialist, Surgery**  
**Vice-Chair North Wales East REC**

Email: [tracy.hughes@new-tr.wales.nhs.uk](mailto:tracy.hughes@new-tr.wales.nhs.uk)

Enclosures:                      List of names and professions of members who were present at the meeting and those who submitted written comments  
   "After ethical review – guidance for researchers"  
   Site approval form

Copy to:                              Mrs Lona Tudor-Jones  
   R&D office for North Wales NHS Trust Central

# Study Protocol: Biomechanics of the Opposite Leg in Knee Osteoarthritis

## ***Principle Investigator:***

Mr Andrew Metcalfe MBChB BMedSc MRCS; Registrar in Orthopaedics, North Wales NHS Trust

## ***Other Investigators:***

Dr. Caroline Stewart MSc PhD; Senior Bioengineer, Orthotic Research and Locomotor Assessment Unit, RJA Orthopaedic Hospital, Oswestry

Dr. Heather Smith; Clinical Trials Manager, RJA Orthopaedic Hospital, Oswestry

Mr. Andrew Roberts FRCS(Orth) ;Medical director, Orthotic Research and Locomotor Assessment Unit and Consultant Surgeon, RJA Orthopaedic Hospital, Oswestry

## ***Abstract***

Osteoarthritis commonly affects multiple joints. Patients with knee arthritis often complain that excessive load on the opposite leg led to the development of osteoarthritis in a previously pain free joint. However, there is very little in the literature to explain what happens mechanically to the opposite leg in patients with knee arthritis. Our hypothesis is that abnormal forces occur in the opposite knee and hip joints in patients with osteoarthritis of the knee. We plan to perform 3-dimensional gait analysis with EMG recording in 20 patients with unilateral medial compartment osteoarthritis and 20 matched asymptomatic controls. Based on these findings larger-scale studies would be planned. This study will increase our knowledge of the mechanical factors that cause multiple-joint arthritis and may lead to the development of new treatments to prevent the development and progression of osteoarthritis in the opposite limb.

## ***Background***

Osteoarthritis is a common and disabling condition which frequently occurs in more than one of the major joints of the lower limb. Osteoarthritis of one knee is frequently complicated by the development of osteoarthritis in the other knee<sup>1</sup>. The cause of osteoarthritis in the second joint is often explained by patients as being the result of alterations in the pattern of walking due to pain in the first knee. However, the biomechanical effects of knee osteoarthritis on the other joints of the lower limb has been poorly described in the literature.

Polyarticular osteoarthritis (and bilateral knee arthritis in particular) has been identified by NICE as an area in which current research is deficient<sup>2</sup>. Despite the frequency of bilateral knee arthritis, no attempt has been made to determine whether the forces in the contra-lateral knee are abnormal or how they may be treated.

Gait analysis, (or motion analysis) is a method by which the movement of a limb can be recorded whilst someone walks. When combined with a measurement of force applied to the ground, the mechanical forces applied to bones and joints during walking can be calculated. It is a real time, *in-vivo* analysis of the biomechanics of walking. Oswestry was the first centre in the country to use gait analysis, which was

introduced by Gordon Rose, and now has one of the country's most advanced motion analysis laboratories.

A number of papers have described the use of gait analysis techniques to calculate the forces that occur across osteoarthritic knee and hip joints. The term *knee adduction moment* describes the rotational force (called a *moment*) that tends to increase the 'bow leg' (called an *adduction* or *varus* deformity) of a limb. The knee adduction moment is likely to be representative of loading of the medial compartment of the knee joint in vivo<sup>3,4</sup>. Studies of knee osteoarthritis have demonstrated that high adduction moments occur in arthritic knees and it is thought that they are closely related to both the initiation and progression of knee osteoarthritis<sup>5-9</sup>. It has recently been demonstrated that knee adduction moments can be reduced by changing the point of loading at the foot using orthoses<sup>10-11</sup>. This may represent a future method of treatment for patients with knee osteoarthritis.

A recent study described the use of a combination of EMG recording (recording the electrical activity in skeletal muscle) and 3-dimensional motion analysis to examine patients with osteoarthritis<sup>12</sup>. They recorded high levels of muscular contraction occurring on both sides of the joint (called muscle co-contraction). Co-contraction would be expected to increase loading through the knee joint as muscles around the joint pull together and increase the force applied to the joint. However, that increase in force would not be demonstrated by gait assessment alone as there would not necessarily be any movement around the joint. Motion analysis alone may therefore be missing an important part of the picture in terms of the forces applied to joints in normal and abnormal ambulation.

Two studies in the literature (both of which used 3D motion analysis but not EMG studies) have demonstrated abnormal biomechanics in the hip and ankle of the ipsilateral leg (the same side) in patients with osteoarthritis of the knee<sup>8,9</sup>. A further study demonstrated abnormal contralateral knee mechanics in patients with hip osteoarthritis<sup>12</sup>. One recent study included an analysis of the contra-lateral leg in patients with osteoarthritis of the knee as part of a broader study of gait adaptation in osteoarthritis<sup>14</sup>. The authors found that knee adduction moments in the opposite leg were equal to those experienced in the arthritic knee, however there was no control group for comparison and they did not determine whether the forces were abnormal.

### ***Aims***

To test the hypothesis that abnormal forces occur in the contra-lateral knee and hip in patients with knee osteoarthritis.

### ***Methods***

20 patients with radiological and clinical evidence of medial compartment osteoarthritis will be recruited from the North Wales NHS Trust (Central and Eastern Regions) joint replacement waiting list by the principle investigator. Radiographs will be examined and patients with predominantly medial compartment changes (medial osteophytes and subchondral sclerosis and medial joint space narrowing, with or without varus knee alignment) will be recruited. This study will examine patients who are experiencing pain in one knee and who have an asymptomatic knee on the other side. Exclusions will be: any other painful lower limb condition; any

medical condition that stops the patient from walking uninterrupted for 10 metres; patients with knee arthritis secondary to ACL deficiency or previous fracture; previous knee surgery (except arthroscopy); and patients with lower limb, pelvic or spinal deformity (except in the affected knee).

A control group of 20 asymptomatic patients matched for age, sex and approximate weight and height will be recruited through user groups and adverts placed in the RJAH Orthopaedic Hospital. The spouses of patients in the study may also be invited to participate in the control group. The same exclusion criteria will be applied. These numbers are comparable to other similar studies that have examined simple hypotheses regarding forces in the arthritic knee and should be adequate to allow calculation of population data and spread for the planning of further studies. Formal power studies cannot be calculated from data in the literature although it is expected that the current study will allow formal sample size calculations to be done for further studies.

Each patient (and control) will be given a WOMAC and Oxford Knee Score to complete once consented in an initial visit with Mr Metcalfe, who will also perform a brief knee examination. The most recent clinical X-rays will be examined to assess for limb alignment and a radiographic score will be recorded (Kellgren-Lawrence score). Patients will be requested to take no more than their normal analgesia and to come with their normal flat footwear.

Data collection will be performed at ORLAU, which has the facility to perform 3D motion analysis and EMG recording concurrently. The study does not cause pain or discomfort for the patient. A 3-dimensional movement analysis will be performed using reflective markers placed over bony prominences of the lower limbs and the trunk and the electrical activity of major muscle groups will be monitored with EMG recording. A force plate in the floor will record ground reaction forces. Calculation of forces from this data will be performed by Dr Caroline Stewart.

Comparisons will be made between patients and their matched asymptomatic counterparts. Statistical analysis will be done by Mr. A Metcalfe (with the support of Dr H.Smith) using a mixed two factor Analysis of Covariance to take account of walking speed.

### ***Support Requested***

- The calculated cost of gait analysis for 20 patients allowing for staff time and consumables is £250 per patient (as agreed with the finance department) = £5000
- The 20 control patients will be funded separately to allow the department to build up its database of normal adults (separate consent will be taken for this, as previously agreed with the Shropshire Research Ethics Committee).
- Costs of £1000 for the time of Heather Smith in developing and managing the study (including preparing and checking applications, advertising for control group, managing finance and final analysis).
- Costs of £1000 for the time of Dr Caroline Stewart in study development, analysing the raw data, calculating the forces and final analysis.

- Travel costs for 20 patients from the Rhyl area to attend clinics in Abergele/Glan Clwyd/Wrexham and the ORLAU in Oswestry = £500  
Total cost = £7500(?)

### ***Future Study***

If the study is successful, it is suggested that re-assessment of the patients following their joint replacements would allow us to see whether abnormal mechanics were improved by treatment. This would have implications in the timing of treatment of knee arthritis in clinical practice. Studies would also be planned to attempt to alter the point of contact of the foot to improve the mechanical environment and prevent the initiation or further deterioration of osteoarthritis.

Regarding the biomechanics of multiple-joint arthritis, further studies could include examining patients with arthritis of differing severity or disease stages, and the assessment of patients with bilateral knee pain in comparison to unilaterals. The current study can therefore be considered a pilot study with a simple hypothesis, providing useful data for designing a large-scale clinical trial to develop effective treatments for patients.

It is hoped that further studies would attract either larger charitable grants (such as Joint Action, who have specific grants for registrar led research) or a National Institute for Health Research (NIHR) Research for Patient Benefit grant. It could also be used as evidence to help support the application of a larger NIHR programme grant into polyarthritis if this is required.

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14. Briem K, Snyder-Mackler L. Proximal Gait Adaptations in Medial Knee OA. *Journal of Orthopaedic Research*. 2008 (in press)



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11 March 2010

Mr Andrew Metcalfe  
Registrar in Orthopaedics  
Royal Glamorgan Hospital  
Ynysmaerdy  
Llantrisant  
Rhondda Cynon Taff  
CF72 8XR

Dear Mr Metcalfe

<b>Study Title:</b>	<b>Biomechanics of the other joints in patients with knee osteoarthritis - the influence of knee replacement</b>
<b>REC reference number:</b>	<b>10/WNo03/8</b>
<b>Protocol number:</b>	<b>1</b>

The Research Ethics Committee reviewed the above application at the meeting held on 10 March 2010. Thank you for attending to discuss the study and confirming that you had no objection to the observer being present.

#### **Ethical opinion**

A concern was raised by the Committee that an invitation to participate in the study could be sent out to a now deceased patient and cause distress to the bereaved.

Assurance was provided by you that every attempt would be made to ensure this did not occur as before any correspondence was sent out the Patient Management System would be checked to identify whether the participant was deceased.

The members of the Committee present gave a favourable ethical opinion of the above research on the basis described in the application form, protocol and supporting documentation, subject to the conditions specified below.

#### **Ethical review of research sites**

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

## Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

- Host organisation details to be updated to Betsi Cadwaladr University Health Board from North Wales NHS Trust.

**It is responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).**

## Approved documents

The documents reviewed and approved at the meeting were:

Document	Version	Date
Covering Letter		
REC application 47379/98834/1/730	1	23 February 2010
Protocol	1	15 February 2010
Investigator CV		15 February 2010
Participant Information Sheet	1	15 February 2010
Participant Consent Form	1	15 February 2010
Participant Consent Form: ORLAU	1	15 February 2010
Letter of invitation to participant	1	15 February 2010
GP/Consultant Information Sheets	1	15 February 2010
Questionnaire: WOMAC	1	15 February 2010
Questionnaire: Oxford Knee Score		
Investigator CV		17 February 2010

## Membership of the Committee

The members of the Ethics Committee who were present at the meeting are listed on the attached sheet.

## Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

## After ethical review

Now that you have completed the application process please visit the National Research Ethics Service website > After Review

You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.



The attached document "After ethical review – guidance for researchers" gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Progress and safety reports
- Notifying the end of the study

The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

We would also like to inform you that we consult regularly with stakeholders to improve our service. If you would like to join our Reference Group please email [referencegroup@nres.npsa.nhs.uk](mailto:referencegroup@nres.npsa.nhs.uk).

**10/WNo03/8**

**Please quote this number on all correspondence**

With the Committee's best wishes for the success of this project

Yours sincerely



**Mr Philip Richards**  
**Vice-Chair**

Email: [Tracy.Hughes4@wales.nhs.uk](mailto:Tracy.Hughes4@wales.nhs.uk)

Enclosures: List of names and professions of members who were present at the meeting and those who submitted written comments  
"After ethical review – guidance for researchers"

Copy to: Mrs Lona Tudor-Jones, R&D Manager, Betsi Cadwaladr University  
Health Board Central & East, H. M Stanley Hospital, St Asaph

# Study Protocol: Biomechanics of the Other Joints in Patients with Knee Osteoarthritis - the Influence of Knee Replacement

## ***Principle Investigator:***

Mr Andrew Metcalfe MBChB BMedSc MRCS; Registrar in Orthopaedics, Royal Glamorgan Hospital, Llantrisant, South Wales.

## ***Other Investigators:***

Dr. Caroline Stewart MSc PhD; Senior Bioengineer, Orthotic Research and Locomotor Assessment Unit, RJA Orthopaedic Hospital, Oswestry

Mr David Barlow MBChB MRCS; Registrar in Orthopaedics, Betsi Cadwaladr University Health Board, Wrexham Maelor Hospital, Wrexham

Dr Neil Postans PhD; Bioengineer, Orthotic Research and Locomotor Assessment Unit, RJA Orthopaedic Hospital, Oswestry

Dr Cathy Holt PhD; Senior Lecturer in Biomechanical Engineering, Cardiff School of Engineering, Cardiff University

Mr Andrew Roberts FRCS(Orth) ;Medical director, Orthotic Research and Locomotor Assessment Unit and Consultant Surgeon, RJA Orthopaedic Hospital, Oswestry

## ***Background***

Patients with knee osteoarthritis frequently complain that pain in one knee has led to changes in the way they walk – resulting in abnormal loading and further disease in other joints. Many patients want to know if treatment will not only reduce their current pain but also prevent arthritis developing elsewhere. There is no data in the literature to help answer that question at present.

Multiple joint osteoarthritis is a common clinical problem, especially in those with severe disease, and leads to a significant increase in pain and disability<sup>1-3</sup>. However, the aetiology of multi-joint disease and the effects of treatment on other joints is poorly understood at present<sup>4-6</sup>.

Knee replacement is a common and successful treatment for knee osteoarthritis, and the pattern of walking returns towards normal (although not fully) within 6 months to 1 year post operatively<sup>6-8</sup>. The aim of this study is to see if this change in walking leads to an improvement in the biomechanics of the other (previously unaffected) knee and hips.

Gait analysis, (or motion analysis) is a method by which the movement of a limb can be recorded whilst someone walks. When combined with a measurement of force applied to the ground, the mechanical forces applied to bones and joints during walking can be calculated. It is a real time, *in-vivo* analysis of the biomechanics of walking. Oswestry was the first centre in the country to use gait analysis, which was introduced by Gordon Rose, and now has one of the country's most advanced motion analysis laboratories.

Previous studies have shown that measures of load recorded by gait analysis closely correlate to loading at a joint<sup>9,10</sup>. Gait analysis studies have also shown that abnormal loading at the knee is predictive of both the initiation and progression of osteoarthritis<sup>11,12</sup>.

This is a continuation of a previous study (REC No. 09/WNo03/1; protocol version 3) in which 20 patients with severe medial compartment osteoarthritis underwent a gait analysis at the Orthotic Research and Locomotor Assessment Unit in Oswestry (part of the Robert Jones and Agnes Hunt Orthopaedic and District Hospital).

Early analysis of the data (compared to asymptomatic adults aged 18-60) has identified significant changes in the gait of patients with single joint osteoarthritis.

In particular, patients swing their trunks more than normal to shift weight over the affected leg. This appears to cause abnormal loads at both hips as well as the unaffected knee whilst they walk. Muscles around both knees were seen to contract excessively to stabilise the joint. This would be expected to increase the loads on the cartilage, worsening the problem.

Given these significant abnormalities, we would be interested to see if these potentially pathological patterns resolve after joint replacement. The data from this study will be used to answer the null hypothesis: **Joint replacement has no effect on the kinetics, kinematics or muscle activation patterns in both lower limbs of patients with single joint knee osteoarthritis.**

### ***Proposed Methods***

All of the participants were recruited from the joint replacement waiting list of the North Wales NHS Trust/Betsi Cadwaladr University Health Board (this was initially done to ensure that only patients with advanced disease was included in the study) and so potentially all of the participants from the study would be suitable candidates. There are no specific exclusion criteria.

We propose to review the participants x-rays, hospital episode data and patient access systems again to determine the timing of the replacements, check the current address of the participants and ensure there has been no deaths. They will then be contacted 11 months post-operatively by mail. If they agree to return, post operative radiographs will be reviewed to determine the post-operative joint alignment and no new x-rays will be required.

If they do not initially respond to the postal request, the participants will be contacted once by telephone to check they have received the information and see if they would be happy to take part, emphasising that this is entirely voluntary. We will make it clear that their previous involvement in the study was valuable even if they do not wish to return.

If they agree to take part, only one visit to the gait laboratory will be required. We will pay travel expenses as before. New consent forms for this part of the study will

be filled in (also attached) and WOMAC and Oxford scores will be repeated (no changes to previous documents). A brief examination will be performed of both knees and hips by a trained physiotherapist.

The gait analysis will be identical to the one performed previously. This involves a 3D gait analysis using the same 12 camera Vicon laboratory and EMG recording of the medial and lateral quadriceps and hamstrings bilaterally. There is no risk to the patient and no ionising radiation is required.

The primary outcome measure will be adduction moment at the unaffected knee and both hips. Statistical analysis will be done using ANCOVA to account for variation in gait speed. The non-identifiable gait analysis data files will also be examined at Cardiff University using a previously described statistical technique to differentiate between normal and arthritic gait in pre- and post-operative cohorts<sup>8</sup>. No identifiable data will be required for this part of the analysis.

If the null hypothesis is rejected, it will provide valuable information to a surgeon who is trying to decide when to perform a joint replacement on a patient. Whether the hypothesis is proved or not, the study will also determine the need for ongoing assessment or physiotherapy for patients post operatively to assess or correct abnormalities in gait in order to reduce the impact on their other joints.

The data may also help by identifying factors that lead to greater improvements in gait, leading to further studies to improve surgical decision making and possibly implant design.

#### ***Support Requested for New Amendment***

- The calculated cost of gait analysis for 20 patients allowing for staff time and consumables is £250 per patient (as agreed with the finance department) = £5000
- Costs of £750 for the time of Dr Caroline Stewart PhD in analysing the raw gait analysis data, calculating the forces and final analysis.
- Costs of £750 for the time of Dr Neil Postans PhD in processing and analysing the EMG data and final analysis.
- Travel costs for 20 patients from North Wales to attend the ORLAU in Oswestry = £500

Total cost = £7000 (maximum estimated cost – costs will be reduced by £250 per participant if study participants decline to return for follow up assessment).

#### ***References***

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## Appendix 2. Reflective marker and EMG electrode protocols

The following two protocols are relevant extracts from the ORLAU Quality Assurance Manuals which were written in ORLAU to standardise the gait analysis process. They are combined with regular staff training and audit of accuracy and repeatability to ensure marker and EMG electrode placements are as accurate, repeatable and consistent as possible. The reflective marker protocol is based on the Plug-in-Gait marker set and the EMG protocols are based on the SENIAM guidelines (found at [www.seniam.org](http://www.seniam.org)) (Davis, Gage et al. 1991).

### MAS OP 111 3D Movement Analysis Marker Placement Protocol April 2010

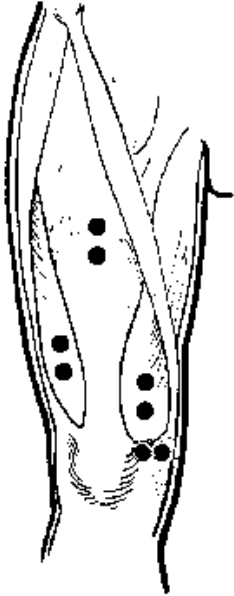
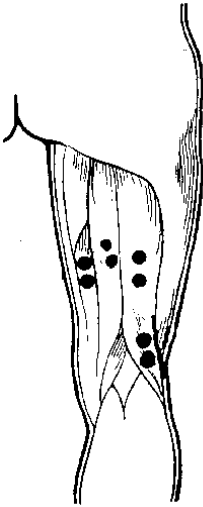
	<b>ANTHROPOMETRIC MEASUREMENTS</b>
1.1	<b>Inter ASIS Distance</b> Ask the subject to lie supine on the plinth For the palpation of each ASIS, stand on the side of the ASIS being palpated. Palpate the iliac crest to identify the general area of the ASIS with the outer hand. Using the inner hand flat, approach the ASIS from below. The first bony prominence should be the ASIS. Identify the tip of the ASIS with the middle finger. Mark the point immediately above the middle finger as the ASIS. Repeat the process on the opposite side, then use the metal callipers to measure the distance in cm between the two points. Record on MAS QF 110
1.2	<b>Leg Length</b> Measure with the patient supine, the knees maximally extended, and the operator stood on the side to be measured. Using a fabric tape measure hold the end on the point marking the ASIS with the proximal hand. Gently pull the tape taught on a direct line to the medial malleolus with the distal hand. Hold the tape here with a finger just distal to the MM. Gently slide this finger up the tape until a bony ledge is

	<p>felt. At this point record the measurement. Repeat on the opposite side. Record measurements on MAS QF 110</p>
1.3	<p><b>Knee Width</b> Identify and Surface Marking Knee Axis <i>Lateral surface marking</i> With the patient supine, stand at the side of the plinth, level with the knee. Flex the knee to 90° and palpate the lateral joint line. Use the other hand to identify the lateral epicondyle of the femur by sliding the hand along the outside of the femur. Now palpate the dip of the popliteal groove between the epicondyle and the joint line. Move along the popliteal groove until between the tendon of biceps femoris and the lateral collateral ligament. The ITB should be above the palpating finger, and the lateral head of gastrocnemius should be below. Move anteriorly and proximally onto a bony nodule - the origin of the lateral collateral. Keep this point under the palpating finger as an assistant slowly extends the knee. Re-palpate (the ITB tends to obscure the point of palpation on extension). In extension mark this point.</p> <p><i>Medial surface marking</i> With the patient supine, stand at the side to be palpated level with the knee. Flex the knee to 90° and from the patella tendon palpate the medial joint line. Identify the broad tibial collateral ligament and grasp this loosely between the thumb and forefinger of the “distal” hand. Maintaining this grasp extend the knee with the other hand. Then run the flattened fingers of the proximal hand down the lower medial side of the thigh to find the adductor tubercle. Mark this with the middle finger and place the index finger on the mid-point of the line that joins the adductor tubercle to the middle of the collateral ligament at the joint line. This is a flat, rather featureless area, but a small depression may be felt. This should be distal and slightly anterior to the adductor tubercle. Remove the finger from this point and mark the same spot with a pen.</p> <p>The distance between the surface markings of the knee joint axis, measured using the red callipers with the patient lying supine (cm). Record measurements on MAS QF 110</p>
1.4	<p><b>Ankle Width</b> Measure the widest part of the ankle malleoli measured using the red callipers with the patient lying supine (cm). Record measurements on form MAS QF 110</p>
1.5	<p>Ensure that the patient’s height, weight and bimalleolar axis are recorded on MAS QF 110</p>

	<b>MARKER PLACEMENT</b>
2.1	<p><b>ASIS Marker Approach from the anterior</b></p> <p>Palpate along the crest to identify the ASIS with the thumb to give an indication of the general location of the ASIS.</p> <p>Stabilise the pelvis posteriorly on the ipsilateral side.</p> <p>Slide the middle finger of the flat medial hand up to hit the inferior edge of the ASIS. Place the marker immediately above.</p> <p>Repeat for other ASIS</p>
2.2	<p><b>Sacrum</b></p> <p>Observe the subject to see if dimples are visible. If not, palpate the PSIS directly by palpating medially over the posterior crest.</p> <p>Use the left hand to support the left ilium. Put a finger of the right hand into the left dimple and move superiorly and laterally until the edge of the bony eminence can be felt. Now holding the pen in the left hand as close as possible to the right finger mark the PSIS.</p> <p>Change hands and repeat for the right PSIS.</p> <p>Now use the ruled edge of the clear plastic goniometer to identify the mid-point between the two marks PSISs. Mark this with the pen, and place a marker over it.</p>
2.3	<p><b>Thigh Markers</b></p> <p>Make sure that the marker is away from the muscle bulk to minimise movement, below the level of the swinging arm, is not obscured by clothing, but is at least 6-10cm away from the knee markers. As KADs are used precise alignment is not necessary.</p>
2.4	<p><b>Shank Markers</b></p> <p>Make sure that the marker is away from muscle bulk to minimise movement. But is at least 6-10cm from the ankle markers. Normally the cameras best see the marker when placed laterally.</p>
2.5	<p><b>Ankle Markers</b></p> <p>Place the marker on the most prominent point of the lateral malleolus</p>
2.6	<p><b>Forefoot Markers</b></p> <p>Place the marker on the dorsum of the foot directly over the head of the second metatarsal.</p>
2.7	<p><b>Calcaneal Markers</b></p> <p>Using the metal gauge, check the height of fore foot marker above the ground. Palpate the calcaneum and place a marker in the centre of the posterior aspect of the calcaneum, at the same height above the ground (or at the same height above the plantar surface of the heel if the heel doesn't touch the ground) as the forefoot marker.</p>
2.8	<p><b>Knee Alignment Devices</b></p> <p>Place the feet of the KAD over the points marked when palpating the knee joint axis, making sure that the marks lie at the centre of the feet. Apply the inside rest first, let the bar lean on the calf, and then apply outside rest. If</p>



	there is excessive anterior movement of the skin over the boney point as the KAD is applied, compensate for this by moving the feet posteriorly. Align the three perpendicular markers approximately so that one points horizontally forwards and one vertically down.
2.9	Following removal of the KAD at the end of the static trial, a knee marker is placed in the same lateral position on the outer aspect of the knee as the lateral foot of the KAD. Marker positions are marked with the soft pencil.

Muscle Group	Diagram
<p><b>Vastus Lateralis</b> With the subject in supine ask the subject to press the knee back into the bed and palpate the lateral aspect of the thigh just above the knee to identify the bulk of vastus lateralis.</p> <p><b>Vastus Medialis</b> With the subject in supine ask the subject to press the knee back into the bed and palpate the medial aspect of the thigh just above the knee to identify the bulk of vastus medialis. Mark electrode position.</p>	
<p><b>Lateral Hamstring</b> With the subject in supine ask the subject to bend the knee. Palpate behind the knee to find the tendon of biceps femoris, move fingers along the tendon to find the start of the muscle belly. Surface mark electrode position</p> <p><b>Medial Hamstring</b> Estimate the mid-point between ischial tuberosity and insertion of semimembranous and semitendinous. Palpate the bulk of the muscle whilst the subject flexes the knee against resistance. Mark this as the electrode position.</p>	

## Appendix 3. Matlab software used for the classification process

The following software is copied direct from the Editor window of Matlab 2013, 8.1.0.604 (The Mathworks Inc, USA) and was prepared by Mr Paul Biggs, a PhD student in the Cardiff School of Engineering based on previous codes written by Dr Lianne Jones and Dr Gemma Whatling. The use of this software in this thesis is described in sections 5.2.4 to 5.2.6 and the results are reported in 5.3.3.

### Matlab function: Principle Component Analysis of training data

```
% PCA_fe_22_08_03.m This performs linear PCA analysis for a set of
normals
% and OAs where the input variables are the 100 points of the gait
cycle (2% increments)
% initially use flexion extension curve
%This code was edited by Paul Biggs for Andy Metcalfe to run PCA
%on his worksheet of data

clear
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% Path where all the data is stored:

%EDIT PAUL
%-----
---
%Specify default file path (saves time)

Defaultpath='E:\PCA\unaffected';

[FileName,PathName] = uigetfile({'*.xls;*.xlsx','Excel Files
(*.xls,*.xlsx)'},'Select the PCA file',Defaultpath);
%-----
---

Datafolder=[PathName '\results'];
mkdir(Datafolder);
```

```

p=path;

%EDIT PAUL
%OLD CODE
%path(p,)

%NEW CODE

path(p,PathName)

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%
% Reading in the file containing the input variables in tabular form
% where the rows are observations (people) and the columns are
variables:
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%

%EDIT PAUL
%OLD CODE
%Filename=['FE']
% eval(['load ' Filename '.txt'])
% eval(['data=' Filename ';' ])

%NEW CODE
%-----
-----
%Seperates filename and extensio
[pathstr, name, ext] = fileparts(FileName);

% eval(['load ' name '.txt'])
% eval(['data=' name ';' ])

[status,sheets] = xlsfinfo(FileName);

for sheetnumber=1:22

% Define new name

name=sheets(sheetnumber);

[numbers,text,row] = xlsread(FileName, sheetnumber);

[height,width]=size(row);
data=cell2mat(row(2:height,2:width));

```

```

[m,n]=size(data);

for i=1:n
Meandata(:,i)=mean(data(:,i));
Stddata(:,i)=std(data(:,i));
zeromeanunitvardata(:,i)=(data(:,i)-Meandata(1,i))/Stddata(1,i);
d=mean(zeromeanunitvardata(:,:));
e=std(zeromeanunitvardata(:,:));
end

data=zeromeanunitvardata(:,:);
% data(find(isnan(data))) = 0;

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% Calculate the correlation matrix, corrmatrix
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
corrmatrix=corrcoef(data)

%[PC1,SCORE,latent1,tsquare] = princomp(zeromeanunitvardata)
%[PC2,latent2,explained] = pcacov(corrmatrix)

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% Find the eigenvalues and the corresponding eigenvectors of this
matrix:
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
[eigenvector,eigenvalues]=eig(corrmatrix);

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% Calculate the eigen energy
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

eigenvals=diag(eigenvalues) '
%eigenvals(2,:)=(eigenvals(1,:)/n)*100;

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% Flip matrix so eigens are in descending order

eigenvals=fliplr(eigenvals)
eigenvector=fliplr(eigenvector)

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% Need to discard the principal components which have an eigenvalue
of less than x, or sum of energy < y%
% where x is some predefined value (e.g.< 1) and y is a
predetermined % (e.g. 80%)
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

sqrteigenvals=(sqrt(eigenvals))

```

```

    for col=1:50
        factorloadings(:,col)=eigenvector(:,col)*sqrt(eigenvals(1,col));
    end

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%
% Calculating the # principal component values for the n individuals
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%

[m3,n3]=size(eigenvector);

for i=1:m          %for every individual in data input file
    for j=1:n3
        PCval(i,j)=sum((data(i,:)).*eigenvector(:,j)');
    end %for j
end %for i

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%Saving eigenvalue and eigenvector matrices in a file:
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

%EDIT - Paul - Saves values to a 4 significant figure precision
%         (without
%         %e+001 etc...)

% OLD CODE
%-----
%
%Filename3=[Filename 'eigenvals'];
%eval(['save ' Filename3 ' eigenvals -ascii -tabs']);

%Filename5=[Filename 'eigenvector'];
%eval(['save ' Filename5 ' eigenvector -ascii -tabs']);

%Filename6=[Filename 'factorloadings'];
%eval(['save ' Filename6 ' factorloadings -ascii -tabs']);

%Filename7=[Filename 'pcvals'];
%eval(['save ' Filename7 ' PCval -ascii -tabs']);
%-----
-%

oldFolder = cd(Datafolder);

% PAUL'S CODE
%supress the "Warning: Added specified worksheet."
warning('off', 'MATLAB:xlswrite:AddSheet');
temp=char(name);
%fOut is just the file name for saving
fOut = [(char(name)) 'PCA_results.xls'];

% Filename=[name 'PCA_results.xls'];

% dlmwrite(fOut, eigenvals, 'delimiter', '\t', 'precision', '%.6f')
xlswrite(fOut,eigenvals, 'eigenvals')

```

```

%dlmwrite(fOut, eigenvector, 'delimiter', '\t', 'precision', '%.6f')
xlswrite(fOut,eigenvector, 'eigenvector')

%dlmwrite(fOut, factorloadings, 'delimiter', '\t', 'precision',
'%.6f')
xlswrite(fOut,factorloadings, 'factorloadings')

%dlmwrite(fOut, PCval, 'delimiter', '\t', 'precision', '%.6f')
xlswrite(fOut,PCval, 'pcvals')

%% Deleting the default sheets of the xls file
% Open Excel file.
excelObj = actxserver('Excel.Application');
excelWorkbook = excelObj.workbooks.Open(fullfile(pwd, fOut)); % Full
path is necessary!

worksheets = excelObj.sheets;
sheetIdx = 1;
sheetIdx2 = 1;
numSheets = worksheets.Count;
while sheetIdx2 <= numSheets
    sheetName = worksheets.Item(sheetIdx).Name(1:end-1);
    if ~isempty(strmatch(sheetName, 'Sheet'))
        worksheets.Item(sheetIdx).Delete;
    else
        % Move to the next sheet
        sheetIdx = sheetIdx + 1;
    end
    sheetIdx2 = sheetIdx2 + 1; % prevent endless loop...
end
excelWorkbook.Save;
excelWorkbook.Close(false);
excelObj.Quit;
excelObj.delete;
%%
A=factorloadings;
A(find(A>0.71))=1;
A(find(A<-0.71))=1;
A(find(A<1))=0;
filteredloadings=A;

xlswrite(fOut,filteredloadings, 'filteredloadings');

cd(oldFolder)

end

```

## Software for training the classifier

```
%Run_DST.m
clear
% Path where all the data is stored:
p=path;
path(p, 'E:\final classifiers\unaffected final classifier training')

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% Reading in the file containing the input variables (characteristic
variables)
% in tabular form where the columns are variables and the rows are
observations :
% and the last row contains the patients classification 0 or 1

Filename=['unaffectedtrainingdata'];
eval(['load ' Filename '.txt'])
eval(['data1=' Filename ';' ])

[m,n]=size(data1);

Optimise=menu('How to calculate control variables?', 'Non-
optimisation', 'Optimisation')
Leaveoneout=menu('LOO or non-LOO?', 'LOO', 'non-LOO');
whichk=menu('Which k definition?', 'corrcoeff', '1/std');
Upperbounduncertainty=1
Lowerbounduncertainty=0.8

whichvars=menu('What variables do you want to use?', 'all', 'subset');

if whichvars==1,
    vars=[1:n-1];

elseif whichvars==2,
    featureselect=menu('Do you want to input the variables yourself
or use feature selection?', 'Input', 'Feature selection')

    if featureselect==1
        vars=input('Please give the variable numbers you want in a
vector: ');

    elseif featureselect==2
        NIND = 4%200; % Number of individuals per
subpopulations i.e. no of subsets of variables
        MAXGEN = 3%20; % Maximum number of generations
        GGAP = 0.8; % Generation gap, how many new
individuals are created
        aim = 0
        crossoverrate = 0.8
```



```

        mutationprob = 0.001
        GA=1; %Need to change this line so that it calls the GA
function/stepwise etc

[vars]=DS_GA(data1,whichk,Upperbounduncertainty,Lowerbounduncertain
y,NIND,MAXGEN,GGAP,aim,crossoverrate,mutationprob,Filename)
    end
end

if Optimise==2
    starttemp=0.5;
    iterationpertemp=100;
    fitnesslevel=0;
    percreducetemp=0.5;
    finaltemp=1e-05;
    maxboundarieskthetaAB=[999999,999999,0.5,0.2];
    minboundariesktheatAB=[-999999,-999999,0,0];
    boundaries(1,:)=maxboundarieskthetaAB;
    boundaries(2,:)=minboundariesktheatAB;
    noofruns=1;
end %if Optimise

if Optimise==1&Leaveoneout==1 %Non-optimisation with leaveoneout

[ouptut]=DS_noop_LOO(data1,vars,whichk,Upperbounduncertainty,Lowerbo
unduncertainty,Filename)
elseif Optimise==1&Leaveoneout==2 %Non-optimisation with no
leaveoneout

[output]=DS_noop_noLOO(data1,vars,whichk,Upperbounduncertainty,Lower
bounduncertainty,Filename)
elseif Optimise==2&Leaveoneout==1 %Optimisation with leaveoneout

[output]=DS_op_LOO(data1,vars,whichk,Upperbounduncertainty,Lowerboun
duncertainty,starttemp,iterationpertemp,fitnesslevel,percreducetemp,
finaltemp,boundaries,noofruns,Filename)
elseif Optimise==2&Leaveoneout==2 %Optimisation with noleaveoneout

[output]=DS_op_noLOO(data1,vars,whichk,Upperbounduncertainty,Lowerbo
unduncertainty,starttemp,iterationpertemp,fitnesslevel,percreducetem
p,finaltemp,boundaries,noofruns,Filename)
end

```

## Software for calculating new PC values based on previous training

```
clear
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% Path where all the data is stored:
p=path;
path(p, 'D:\NEW CHAPTER')
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

% Path where all the data is stored:

%EDIT PAUL
%-----
---
%Specify default file path (saves time)

Defaultpath='J:\lpostop      PCA      for      pre-trained      classifier
26sept\unaffected';

[FileName,PathName] = uigetfile({'*.xls;*.xlsx','Excel      Files
(*.xls,*.xlsx)'},'Select the postop file',Defaultpath);

[FileNameetraining,PathNameetraining] =
uigetfile({'*.xls;*.xlsx','Excel Files (*.xls,*.xlsx)'},'Select the
training(preop) file',Defaultpath);

Datafolder=[PathName '\newresultsPaul'];
mkdir(Datafolder);

p=path;
path(p,PathName)
%Seperates filename and extensio
[pathstr, name, ext] = fileparts(FileName);

% eval(['load ' name '.txt'])
% eval(['data=' name ';''])

[status,sheets] = xlsinfo(FileName);

for sheetnumber=1:22

    % Define new name

name=sheets(sheetnumber);

[numbers,text,row] = xlsread(FileName, sheetnumber);
```

```

[numberstraining, texttraining, rawtraining] =
xlsread(FileNametraining, sheetnumber);

% EDIT
rawtraining=rawtraining(:,1:52);
raw=raw(:,1:52);
% rawtraining(find(isnan(cell2mat(rawtraining)))) = 0.0001;
% raw(find(isnan(raw(cell2mat(raw))))) = 0.00001;
numberstraining(find(isnan(numberstraining)))=0.001;
numbers(find(isnan(numberstraining)))=0.001;

trainingresultsfolder=[PathName '\newresults'];
addpath(trainingresultsfolder)

resultsfilename=[cell2mat(name) 'PCA_results' ];

[numberseigenvector, texteigenvector, raweigenvector] =
xlsread(resultsfilename, 2);

[height,width]=size(numbers);
data=numbers(2:height,1:51);

[height,width]=size(numberstraining);
Trainingdata=numberstraining(2:height,1:51);

[height,width]=size(numberseigenvector);
eigenvector=numberseigenvector;

[m,n]=size(data);

% for loop for going through each patient
for patient=1:m

PatientVariable=data(patient,:);

[mapf,napf]=size(Trainingdata);

for i=1:napf
    Meandata(:,i)=mean(Trainingdata(:,i));
    Stddata(:,i)=std(Trainingdata(:,i));
    newdata(:,i)=(PatientVariable(:,i)-Meandata(1,i))/Stddata(1,i);
end

[mvfe,nvfe]=size(eigenvector);

for j=1:nvfe
    PCval(patient,j)=sum((newdata(:,:)).*eigenvector(:,j)');
end %for j

end

```

```

oldFolder = cd(Datafolder);

% PAUL'S CODE
%supress the "Warning: Added specified worksheet."
warning('off', 'MATLAB:xlswrite:AddSheet');
temp=char(name);
%fOut is just the file name for saving
fOut = [(char(name)) 'PCA_newresults.xls'];

% Filename=[name 'PCA_results.xls'];

% dlmwrite(fOut, eigenvals, 'delimiter', '\t', 'precision', '%.6f')
xlswrite(fOut,PCval, 'NewPCVals')

%% Deleting the default sheets of the xls file
% Open Excel file.
excelObj = actxserver('Excel.Application');
excelWorkbook = excelObj.workbooks.Open(fullfile(pwd, fOut)); % Full
path is necessary!

worksheets = excelObj.sheets;
sheetIdx = 1;
sheetIdx2 = 1;
numSheets = worksheets.Count;
while sheetIdx2 <= numSheets
    sheetName = worksheets.Item(sheetIdx).Name(1:end-1);
    if ~isempty(strmatch(sheetName, 'Sheet'))
        worksheets.Item(sheetIdx).Delete;
    else
        % Move to the next sheet
        sheetIdx = sheetIdx + 1;
    end
    sheetIdx2 = sheetIdx2 + 1; % prevent endless loop...
end
excelWorkbook.Save;
excelWorkbook.Close(false);
excelObj.Quit;
excelObj.delete;
%%

cd(oldFolder)

end

```

## Matlab code for plotting the classifier

```
%TKRstudy.m This program positions new people on a simplex plot
using the kthetaAB calculated
% from the original training set
clear
% Path where all the data is stored:
p=path;

path(p, 'E:\final classifiers\unaffected final classifier training')
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% Reading in the file containing the input variables (characteristic
variables)
% in tabular form where the columns are observations and the rows
are variables:

Filename1=['unaffectedclassificationdata'];
eval(['load ' Filename1 '.txt'])
eval(['data=' Filename1 ';' ])

% data1=data(53,:);
% data2=data(86,:);
%
% data=data1;
% data(2,:)=data2;

[m,n]=size(data);

whichvars=menu('Do you want to use all the variables or a
subset?', 'all', 'subset');
if whichvars==1,
    vars=[1:n-1]
elseif whichvars==2,
    vars=input('Please give the variable numbers you want in a
vector: ')
end
selectvar=length(vars);

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% Reading in the file containing the BestkthetaAB (k 1st col, theta
2nd col, A 3rd col B 4th col)
p=path;
path(p, 'E:\final classifiers\unaffected final classifier training')
Filename2=['kthetaAB'];
eval(['load ' Filename2 ])
eval(['kthetaAB=' Filename2 ';' ])

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
```

```

for j=1:selectvar
    newdata(:,j)=data(:, (vars(1,j)));
    newkthetaAB(j,:)=kthetaAB((vars(1,j)),:);
end %for j

newdata(:,selectvar+1)=data(:,n);

% Calling function to convert charcteristic variables to confidence
values

[newconfidencevalues]=confidencefactor(newdata,newkthetaAB,Filename1
);

    if selectvar==1

[newcboe]=bodyofevidencelvar(newconfidencevalues,newkthetaAB,Filename1);
        newBOE=newcboe;
        newconflict=1;

        [xp,yp]=convertBOEintopv1var(newcboe);

    else

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
        % Calling function to convert each confidence value into a
body of evidence

[newBOE,newcboe,newconflict]=bodyofevidence(newconfidencevalues,newk
thetaAB,Filename1);

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
        % Calling function to convert BOE into simplex coordinates

        [xp,yp]=convertBOEintopv(newBOE,newcboe);

        [a,b]=size(xp)
    end %if selectvar==1

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
    % Calling function to plot points on simplex plot
    newpeoplesp=1;
    if newpeoplesp==1

        Title=['Newpeople simplex plot'];

[newmessage]=simplexplotTKRstudyALL_PB(xp,yp,newdata,newBOE,Filename1,T
itle);

```

```

end % if initialsp

Filename10=['cfv' Filename1];
eval(['save ' Filename10 ' newconfidencevalues -ascii -tabs'])

Filename8=['BOE' Filename1];
eval(['save ' Filename8 ' newBOE -ascii -tabs'])

Filename9=['cboe' Filename1];
eval(['save ' Filename9 ' newcboe -ascii -tabs'])

Filename10=['xp' Filename1];
eval(['save ' Filename10 ' xp -ascii -tabs'])

Filename11=['yp' Filename1];
eval(['save ' Filename11 ' yp -ascii -tabs'])

%Filename6=['xp'Filename1];
%eval(['save ' Filename6 ' xp -ascii -tabs'])

%Filename7=['yp'Filename1];
%eval(['save ' Filename7 ' yp -ascii -tabs'])

```

## Appendix 4. Published Papers

### Papers

The following pages contain copies of the three peer-reviewed papers published so far based on work contained in this thesis. Their references are:

A.J.Metcalf, M.L.E.Andersson, R.Goodfellow, C.A.Thorstensson. Is knee osteoarthritis a symmetrical disease? Analysis of a 12 year prospective cohort study. ***BMC Musculoskeletal Medicine*** (2012)13:153 (*Highly accessed article*)

A.J.Metcalf, C.Stewart, N.Postans, A.L.Dodds, C.A.Holt, A.P.Roberts. The effect of knee osteoarthritis on the biomechanics of the other joints in the lower limb. ***Bone and Joint Journal*** (2013) 95(3): 348-353

A.J.Metcalf, C.Stewart, N.Postans, D.Barlow, A.L.Dodds, G.Whatling, C.A.Holt, A.P.Roberts. Abnormal loading of the Major Joints in Knee Osteoarthritis and the Response to Knee Replacement. ***Gait and Posture*** (2013) 37(1):32-6