Biomechanics and Osteoarthritis:
A Novel Rat Model

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Declaration of Originality

I declare that all material presented in this thesis which is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other University
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Abstract

This thesis has made a number of significant contributions to the evaluation of rodent joint biomechanics, and the relationship between these biomechanics and osteoarthritic pathology. It has presented the first use of static optimization based techniques to evaluate *in vivo* muscle and joint contact forces in the rat, with model outputs comparing well to experimentally collected kinematics and joint kinetics. The sensitivity of the model to errors in marker placement, muscle geometry and segmental properties was evaluated using elementary effects methods. It was found that errors in marker placement had the largest effect on most model outputs, with the exception of muscle forces, which showed far greater sensitivity to changes in muscle geometry. After validating the model outputs and their sensitivity to potential sources of experimental error, the effect of inclination and speed on joint kinetics and kinematics was investigated. This was undertaken both to predict the potential of the techniques to differentiate pathologically different gait and to evaluate whether variations in speed and/or inclination could increase medial joint loading to provide a non-invasive method to accelerate osteoarthritic progression in future disease models. It was found that increasing inclination increased the total knee joint contact forces, whereas increasing speed preferentially increased medial side loading. Finally a novel surgical model of malalignment induced osteoarthritis in rats was developed via a high tibial osteotomy (HTO). Biomarkers of cartilage turnover as well as tibial compressive loading in the medial knee compartment measured via musculoskeletal modelling were shown to be significantly higher in HTO operated animals and correlated well with histological grading. This model demonstrates the fact that increasing medial joint loading alone is enough to induce the development of OA and future work will hope to develop the idea of malalignment induced osteoarthritis as a distinct disease phenotype.
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<td>OA</td>
<td>Osteotomy</td>
</tr>
<tr>
<td>YLD</td>
<td>Years lost to disability</td>
</tr>
<tr>
<td>ACL</td>
<td>Anterior cruciate ligament</td>
</tr>
<tr>
<td>ECM</td>
<td>Extra-cellular matrix</td>
</tr>
<tr>
<td>MMP</td>
<td>Matrix metalloproteinase</td>
</tr>
<tr>
<td>HTO</td>
<td>High tibial osteotomy</td>
</tr>
<tr>
<td>OCT</td>
<td>Optical coherence tomography</td>
</tr>
<tr>
<td>MMT</td>
<td>Medial meniscal tear</td>
</tr>
<tr>
<td>ACLT</td>
<td>Anterior cruciate ligament transection</td>
</tr>
<tr>
<td>GRF</td>
<td>Ground reaction force</td>
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<tr>
<td>JCF</td>
<td>Joint contact force</td>
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Chapter 1

Introduction

1.1 Motivation and Objectives

The objective of this thesis was to investigate the relationship between joint mechanics and osteoarthritis (OA) via the development of a novel model of malalignment induced knee OA in rats. In order to carry this out it was necessary to develop an in vivo model capable of generating osteoarthritic changes through purely mechanical means and to develop the use of a musculoskeletal model for quantifying these mechanical interventions. A description of how this was achieved and the thesis layout is presented below:

1. Chapter 2: Background and Literature Review
   A review of the background to osteoarthritis, animal models of the disease and methods for quantifying biomechanical alterations in rats with a focus on mechanical factors throughout

2. Chapter 3: Gait Analysis and Musculoskeletal Modeling Methods
   A detailed description of the methodologies used in all three experimental chapters to analyze rodent gait and calculate internal joint kinetics via a musculoskeletal model

3. Chapter 4: Evaluation of Errors Present in Musculoskeletal Modeling of the Rat Hind Limb
   Kinematic and kinetic model outputs using in-vivo data were seen to correspond well
to previous studies in the literature. A detailed analysis of the methods described in Chapter 3 was undertaken, including an analysis of the sources and magnitudes of errors as well as sensitivity analysis to quantify the effect of these errors on the output measures of the method. It was found that errors in the placement of markers on the animal had moderate effects on most model outputs and that errors in the placement of the knee marker caused the largest variations in musculoskeletal output variables. As a result of this it was proposed that a single experimenter should be used to place markers on the animals and that sem-permanent markings should be used during longitudinal studies. Errors in muscle geometries were seen to have large effects on muscle forces, with variations of up to 100%. The results of this chapter were used to optimize the in vivo data collection used in the subsequent chapters.

4. Chapter 5: Effect of Speed and Inclination on Biomechanics of Rodent Gait
An evaluation of the effect of both speed and inclination on joint biomechanics was carried out using the techniques developed in Chapter 3 to both provide a prediction of the model’s ability to detect pathologically different gait (as required in Chapter 6) and also as a potential guide as to which combination of inclination/speed may produce conditions most likely to lead to osteoarthritic changes (increases in medial joint contact forces). Future work could use this analysis in combination with the in-vivo model presented in Chapter 6 to accelerate or retard the progression of OA. Among other things, it was found that increasing inclination increased knee joint contact forces overall and increasing speed increased the medial joint contact force preferentially.

5. Chapter 6: In-Vivo Biomechanical Model of Knee OA in Rats
The presentation of a novel malalignment induced model of knee OA in rats validated both histologically and via serum biomarkers. This is presented alongside the result of biomechanical analyses of the hindlimb before and after surgery using an adjusted version of the model evaluated and tested in the previous chapters. Analyses of the correlations between the quantified biomechanical changes at the joint and histological levels of OA was carried out to investigate the relationship between mechanical forces at the knee and the progression of OA. It was found that varus malalignment of the tibia was able to induce histological cartilage degredation 14 weeks post-surgically alongside upregulated CTX-II levels and increased medial joint loading.
6. **Chapter 7: Conclusion** A summary of the achievements of the thesis and the potential directions for future work
Chapter 2

Background and Literature Review

2.1 Chapter Summary

This chapter provides a background to osteoarthritis as a disease, explaining the pathophysiology, aetiology and potential treatments as well as the role that biomechanics are thought to play in the initiation, propagation and treatment of the condition. This will hopefully aid in putting much of the thesis into a more translational context, but will specifically be relevant to Chapter 6. A thorough review of the use of animal models is also presented, focussing on models of osteoarthritis in rats. The use of biomechanical and biochemical biomarkers to validate the presence of OA in these models is also reviewed. Finally, a review of methods relating to the analysis of rodent gait and biomechanics is presented, with a focus on how these methods have been used in models of osteoarthritis. A more concise review of the literature as relevant is presented prior to chapters 4-6.
2.2 Osteoarthritis

2.2.1 Epidemiology

It is known that osteoarthritis is one of the leading causes of disability worldwide, with estimates in 2012 suggesting there may be up to 250 million people suffering from some form of the disease [Vos et al., 2012]. This equates to almost 2.2% of all years lost due to disability (YLD) and 10% of years lost from musculoskeletal disorders, which as a group cause 21% of all YLD [Murray et al., 2012]. When looking at trends over time, the incidence of osteoarthritis as measured by YLD is growing at a faster rate than almost all other major health problems, with an increase of 64% between 1990 and 2010 [Hunter et al., 2014]. This increase is thought to be due to a number of factors, but the main reasons are hypothesised to be the ageing population, especially in the western world, and an increasing obesity epidemic in certain countries. With life expectancy worldwide expected to increase significantly over the coming century, we can expect the burden of osteoarthritis and other musculoskeletal disorders to increase dramatically [Cross et al., 2014].

In epidemiological studies of the disease, a distinction is often made between those patients who are discovered to have radiographic OA with no symptoms as the result of a study [Leyland et al., 2012] and those who present with symptomatic OA [Kim et al., 2014]. Studies looking at the incidence rate of radiographic OA often focus on elderly population groups and attempt to correlate the prevalence of osteoarthritic joint morphology seen using radiographic imaging methodologies with joint pain or other clinical symptoms [Szebenyi et al., 2006]. Most of these studies have shown that there is a strong association between patients reporting with classical symptoms of OA and radiographic evidence of the disease [Lethbridge-ejku et al., 1995], however, they also show that there are a large number of patients who are shown to have radiographic features normally thought to correlate with OA and no symptoms or pain. In one such study, researchers looked at the relationship between hip OA and pain [Kim et al., 2015]. Only 23.8% of hips with radiographic OA were painful and only 15.6% of patients with joint pain were found to have radiographic OA. This discordance between symptoms and radiographic disease markers makes it very difficult for clinicians to provide an accurate diagnosis as to the stage and/or scale of disease based on either of these metrics alone.
For this reason, symptomatic OA is usually used when looking at incidence rates of the disease. Figure 2.1 shows the number of people who have received treatment in the UK for osteoarthritis, split by anatomical disease location. In a report by Arthritis Research UK, 33% of people aged over 45 (8.75m) were found to have received treatment for osteoarthritis between 2006-2013, with the number of women receiving treatment (5.2m) almost double that of the number of men (3.55m). When breaking it down into the locations on the body where osteoarthritic disease is most prevalent it can be seen that for both men and women the knee is the site with the most patients reporting, followed by the hip joint. It is for this reason that many studies choose to focus on osteoarthritis at the knee or lower limbs in general [Arthritis Research UK, 2013].

![Pie charts showing the distribution of osteoarthritic sites for patients reporting to general practitioners in the UK between 2006-2013 split into overall patient data and that for male and females respectively [Arthritis Research UK, 2013] (Adapted).](image)

**2.2.2 Burden of Disease**

When thinking about the burden of disease, consideration needs to be given both to the individual suffering from the condition, as well as the burden to society as a whole. From the individual viewpoint, osteoarthritis often presents with joint pain and lack of mobility, which has significant impacts on the quality of life experienced [Hunter et al., 2014]. Joint pain experienced due to OA has been postulated to come in two forms: a dull consistent pain that becomes more pronounced over time, and short more intense pain that occurs irregularly [Hawker et al., 2008] The unpredictable nature of these intense moments was found to have a more signifi-
cary direct effects on quality of life, leading to avoidance of sporting, social and recreational activities. As well as the obvious lack of mobility, OA has been found to have more systemic effects on wellbeing, including negative impacts on sleep quality [Sezgin et al., 2017], mental health and general quality of life [Affleck et al., 1999]

In terms of the burden to society as a whole, osteoarthritis has a huge economic cost, which as the population ages is likely to increase further. In terms of overall cost, the economic burden of osteoarthritis is difficult to quantify, with one study from 1997 putting it at between 1 and 2.5% of GDP in western countries (USA, Canada, UK, France and Australia) [March and Bachmeier, 1997]. Much of this comes from the direct costs of treating the disease. Joint replacement surgery alone costs the UK over 1 billion in 2010 [Chen et al., 2012], with estimates of $42 billion in the US a year later [Murphy and Helmick, 2012]. These direct costs are compounded by indirect costs such as absenteeism and social welfare payments. When these are included the total estimated costs to the UK in 2002 were estimated to be up to 3.2 billion [Hiligsmann et al., 2013].

### 2.2.3 Risk Factors

Because of the complex nature of OA, it is often very difficult to link the development of the disease to any particular cause, with more often it being a combination of both systemic and local factors [Heidari, 2011]. Systemic factors can include age; gender; ethnicity; congenital conditions; diet and genetics, with the latter of these attracting the most interest in recent years with the rapid advances in genotyping technologies [Zhang and Jordan, 2010]. Results from a number of recent studies have shown that to some degree predisposition to OA can be inherited and even prevalence of OA in certain joints can be an inherited trait, suggesting a genetic component. Most of the data for this comes from familial or twin studies in which identical and non-identical twins are tracked over a period of time. One study looked at the relative genetic and environmental contributors to hand and knee OA in female twins aged 48-70, estimating the proportion of genetic variance in the development of osteoarthritis to be above 50% [Spector et al., 1996]. This would suggest genetic variations may play a large role in predisposing patients to the disease.

Local risk factors are also involved in the initiation and/or development of the disease. The
majority of these are biomechanical factors which either act to increase or alter the loading going through a particular joint, causing changes to the local biomechanical environment. Obesity is an obvious example of where external changes can directly influence internal joint loading. Increasing the weight going through load bearing joints (in particular the knee) accelerates cartilage degeneration and leads to osteoarthritic changes [Reyes et al., 2016]. Another factor which influences the biomechanical environment that the joint is exposed to is the occupation of the patient and/or the level of physical activity the patient undergoes. Repetitive use of joints in the workplace has been shown to increase the incidence of OA. Jobs with increased rates of OA range from farming [Croft et al., 1992] to cotton milling [Lawrence, 1961] alongside any job requiring repeated thumb and forefinger gripping [Hadler et al., 1978] or kneeling and squatting [Coggon et al., 2000]. Physical activity or sport can have similar effects, overloading or overusing the joint, with football players, for example, being shown to have elevated risks of OA [Kujala et al., 1995].

Because of the mechanical nature of osteoarthritis, internal biomechanical variations, as well as environmental variations, can be important factors. Joint alignment is known to be important in determining the prevalence of OA, particularly of the load bearing joints in the hip and knee. Knee alignment, in particular, is a key factor in determining knee joint loads and any shift from neutral alignment leads to malaligned knees with higher risks both of developing OA and of it progressing faster. Studies have shown that patients with knee OA saw a much greater acceleration of structural damage when it was coupled with abnormal alignments and that the deterioration was focussed in the condyle under the greatest compressive stress [Sharma et al., 2001]. Varus alignment of the knee was associated with a 4 times increase in the odds of progression of medial OA and valgus misalignment was responsible for a 5 times increase in the odds of progression of lateral OA.

2.2.4 Pathophysiology

Osteoarthritis is a complex, multi-factorial disease which has both mechanical and biological effects on the whole synovial joint. Generally speaking, it is characterised by severe damage to load bearing regions of articular cartilage, excess bone formation at the edges of the joints (osteophytosis), alterations to the morphology and structure of the subchondral bone, synovitis and often inflammation or thickening of the joint capsule itself. These changes can be seen in
Figure 2.2 which compares the morphology and anatomy of a healthy joint with that of one after osteoarthritic changes have taken place. Two main types of OA exist, which are differentiated by the mechanism of pathogenesis: primary and secondary. Primary OA usually develops over a long period of time due to the biomechanics and forces acting on the joint, although the actual cause of initiation is relatively unknown. Secondary OA develops as a result of another injury such as a tear of the anterior cruciate ligament (ACL), which alters the motion of the joint and leads to irregular biomechanics [Samson et al., 2007]. Of these, primary OA is the most commonly seen and due to the nature of the disease presents frequently in more elderly patients. As such it will be the focus of this section.

Figure 2.2: Frontal cross section of the knee showing healthy anatomy on the left alongside osteoarthritic changes on the right

Cartilage has been considered as having the most important role in the pathogenesis of OA, primarily because of the gross cartilage changes present in advanced phases of the disease. Cartilage is an avascular tissue that covers the surfaces of joints, providing a smooth surface with a low coefficient of friction over which joints can articulate. Structurally it is composed of four layers, as seen in Figure 2.3. Deep, middle and superficial zones consisting of noncalcified cartilage lie on top of a layer of calcified cartilage, separated by a tide mark. The main structure of the cartilage consists of an extracellular matrix containing water, collagen and proteoglycans [Goldring and Marcu, 2009]. The turnover and production of this matrix is
governed by chondrocytes, the density and orientation of which depends on the layer of cartilage being looked at. In osteoarthritic pathology, a failure of the chondrocytes to properly regulate the production and resorption of the extracellular matrix leads to an increase in both anabolic and catabolic processes. In the early stages, increasing matrix synthesis and an increase in the number and activity of chondrocytes are able to maintain cartilage integrity [Goldring and Goldring, 2007]. Over time, however, the slow turnover rate of matrix components leads to destructive loss of cartilage integrity and osteoarthritic changes. Initially, these changes are isolated to the more superficial zones, with fibrillation and fissuring of these layers leading to a reduction in cartilage thickness. As this progresses and the deeper zones become affected, the process accelerates and leads to extreme cartilage destruction and eventually exposure of the underlying subchondral bone [Man and Mologhianu, 2014].

There are a number of factors that influence the behaviour of the chondrocytes, both mechanical and biochemical [Goldring et al., 2011]. Traditionally it has been postulated that mechanical stimuli drive this process, at least in the early stages of the disease. This can be either through mechanotransduction of chondrocytes themselves or via the direct ‘wear and tear’ of the articular surface in response to mechanical loading. The second of these mechanisms has been studied extensively and has been the traditional approach to explaining disease initiation and propagation. In this hypothesis, focal or cyclic stresses on the cartilage surface can cause fissures and cause wear particulates to be removed from the articular surface and absorbed into the synovial fluid. As well as surface cartilage damage, the abnormal loading on the joint triggers remodelling in the underlying bone. The combination of cartilage loss and bone reshaping exacerbates joint misalignment and focal stresses leading to accelerated joint damage [Felson, 2013].

Work on the mechanotransductive capacities of chondrocytes themselves has shown that they are acutely sensitive to changing mechanical loads and could also play a key role in disease pathogenesis [Lee et al., 2005, Honda et al., 2000, Sanchez-Adams et al., 2014, Ashwell et al., 2013]. In general, physiological dynamic loading has been shown to have a positive effect on matrix synthesis, suggesting a protective response to mechanical loading [Sanchez-Adams et al., 2014]. Super-physical loading, however, has been shown to have a catabolic impact on matrix homoeostasis, suggesting possible pathological mechanisms. Increasing the compressive force on individual chondrocytes has been shown to reduce aggrecan and collagen II production,
two of the key proteins involved in the formation of the ECM [Leipzig and Athanasiou, 2008]. Further studies have corroborated this on a tissue level, showing that biomechanical stresses downregulated aggrecan production whilst increasing the expression levels of catabolic enzymes such as MMP1 and MMP13 [Liu et al., 2016].

Changes in the subchondral bone itself are known to occur in osteoarthritis, however, whether they precede or follow on from destructive cartilage loss is not yet clear [Felson and Neogi, 2004]. Like cortical and trabecular bone, the properties of subchondral bone are altered through cellular modelling and remodelling [Goldring and Goldring, 2010]. During osteoarthritis, the homoeostasis of subchondral bone remodelling is altered leading to sclerosis and the development of bone marrow lesions. These are followed by more extreme structural changes, with bony cysts and an increase in subchondral bone thickness being followed by osteophyte formation at the joint margins [Man and Mologhianu, 2014]. In later stages of the disease, the process of remodelling begins to accelerate surrounding regions of severe cartilage loss. Synovial fluid comes into contact with the subchondral bone and is thought to lead to an increase in the production of bony cysts as well as extensive bone sclerosis [Li et al., 2013].

The synovial membrane itself is also known be involved in disease pathogenesis, but once again whether the effect it has is primary or secondary to other degenerative changes in the joint is not entirely clear [Sutton et al., 2009]. The fibrillation of superficial cartilage layers caused by proteolytic matrix degradation produces wear particulates that enter the synovial fluid. The
influx of foreign tissue triggers the activation of an immune response leading to inflammation of the synovial membrane. These inflammatory agents eventually diffuse through the synovial fluid into the cartilage and accelerate the osteoarthritic process by increasing chondrocyte activation. This, in turn, produces more wear particles and produces a vicious cycle which accelerates disease progression. Inflammation of the tissue leads to the production of large quantities of pro-inflammatory cytokines which are thought to play a key role in the pain associated with late stage disease [Man and Mologhianu, 2014]. Inflammatory pathogenic pathways have also been postulated not just to be the result of biomechanically induced cartilage changes, but as initiators of disease in their own right [Berenbaum, 2013]. As such, the role of inflammation and mechanics is the subject of much debate in the literature.

Recent studies have proposed that primary OA is actually a group of pathologically different disease phenotypes. In one study, for example, metabolic OA, inflammatory OA, mechanical OA and bone related OA are all proposed as distinct phenotypes [DellIsola et al., 2016]. This classification of the disease may help to overcome some of the current misunderstandings as to the exact disease pathology.

### 2.2.5 Diagnosis and Treatments

Because of the complex nature of the disease, OA can be difficult to diagnose in its early stages. The most common symptom is joint pain, which worsens when the joint is put under strain, such as during exercise. It is not uncommon for a reduction in the range of motion of the joint, joint instability, joint effusion or tenderness to present, however, joint pain is normally exhibited alongside these. Because of the issues in correlating radiographic measures to the stage of disease, physical examinations by experienced clinicians are often used to make a diagnosis. Radiography is normally used to rule out other conditions and confirm the diagnosis of the physician [Skou et al., 2014]. Once diagnosed with the disease there are normally four treatment options available: nonpharmacologic, pharmacologic, alternative and surgical [Sinusas, 2012]. Patients with early stage disease are often symptomless and so diagnosis and treatment are very difficult, but for patients with symptomatic but non-debilitating forms of the disease, treatments will usually start with the safest options and progress through to more invasive options such as surgery.
Non-pharmacologic treatment often involves redistributing the loading at the knee in order to reduce the rate of progression of the disease and reduce the associated symptomatic pain. Perhaps the simplest method of achieving this is through the use of weight loss regimes. Losing weight, particularly in obese patients, significantly reduces knee loading and can have benefits to the long term prospects of OA sufferers [Christensen et al., 2007, Riddle and Stratford, 2013, Atukorala et al., 2016, Runhaar et al., 2016]. A recent long term study by Gersing et al (2017), for example, showed that patients with radiographic knee OA who lost just 5% of body weight over a period of 48 months had significantly reduced cartilage degeneration. This reduction increased even further in patients who managed to lose 10% of body weight [Gersing et al., 2017]. Muscle strengthening regimes have also been shown to reduce overall loading at the knee [Vahtrik et al., 2014]. Studies have shown significant improvements in arthritic symptoms when patients have been put on muscle strengthening regimes for extended periods of time, and thus it is a viable option for patients with less destructive OA to delay or remove the need for more invasive therapies [Thomas et al., 2002].

As well as reducing overall loading, several non-invasive methods have been developed that focus specifically on reducing medial loading. The most prevalent of these is the use of wedged insoles, which have been shown to reduce the peak external knee adduction moment in biomechanical studies [Bennell et al., 2011, Sawada et al., 2016]. Valgus knee braces have also been shown to reduce knee adduction moments, and using both techniques in parallel has shown to provide more benefits than individually [Moyer et al., 2017]. Although there are debates as to what degree these techniques reduce the rate of onset of radiographic disease [Campos et al., 2015], studies have shown that they reduce pain and increase the overall quality of life and mobility for patients [Hsieh and Lee, 2016]. Other nonpharmacologic options can include aquatic exercise [Wang et al., 2007, Mattos et al., 2016, Bartels et al., 2016] , walking aids [Kemp et al., 2008] or directed patellar taping [Warden et al., 2008].

Pharmacologic and alternative treatments are a secondary option for those patients who do not respond well to non-pharmacologic measures or who are waiting for surgery. Traditionally these have tended to focus on relieving symptoms of the disease rather than slowing down or reversing the condition. These will normally be either non-steroidal anti-inflammatory drugs such as ibuprofen to treat inflammation and joint stiffness or opioids used to treat pain symptoms. In either case, they are usually a precursor to surgical interventions [Hochberg et al., 2012]. In
recent years the focus has shifted towards disease-modifying drugs that can support anabolic events in the joint or inhibit catabolic pathways [Filardo et al., 2016]. Recombinant human fibroblast growth factor 18, a protein that plays a role in bone development and homoeostasis, has been shown to reduce secondary osteoarthritis by promoting chondrogenesis and matrix production in a rat model of the disease [Kon et al., 2012]. Clinical human trials have shown that the protein could reduce the loss of cartilage, however, this effect was isolated to the lateral compartment. The reasons for this are still under investigation [Lohmander et al., 2014]. With the knowledge that inflammatory pathways may play a large role in OA pathogenesis (particularly secondary OA), inflammatory mediators have also been proposed as disease modifying therapeutics. In animal models of secondary OA, injections of anti-inflammatory mediators (IL-1Ra) significantly reduced cartilage loss and markers of synovitis. Preliminary trials with human secondary OA patients have shown promising results [Olson et al., 2014], however, the ability to prevent primary disease has not been established. The injection of corticosteroids, mesenchymal stem cells and hyaluronic acid have also been proposed but none have yet shown clear disease modifying properties in humans [Filardo et al., 2016].

Because of the lack of truly disease modifying therapeutics, surgical options are often required for those patients whose symptoms cannot be controlled. The most common surgical methods are total joint replacements, whereby the articulating surface or entire joint is removed and replaced with a prosthetic version. This is a good option for those who have failed to respond to nonsurgical treatments and are suffering from a reduced quality of life because of the disease [Mandl, 2013]. Treatment with total knee replacements followed by a series of nonsurgical remedies has been shown to reduce pain and increase function in patients [Skou et al., 2014]. High tibial osteotomies have also been used to slow down disease progression. The tibia of the patient is realigned to adjust loading from the affected side of the knee to the contralateral compartment [Coventry, 1965]. Biomechanical studies post-operatively have demonstrated its ability to shift load onto the medial side of the joint via a reduction in knee adduction moment [Lee et al., 2017]. This technique has the benefit of retaining the original joint structure and reducing the need for invasive joint arthroplasty. Studies of the long term success of these techniques have found that they are able to delay the need for joint replacements by 5 to 10 years for 85% of patients and more than 15 years for 60% of patients [Gomoll, 2011]. They have proved particularly useful for younger patients who would otherwise face the need to have multiple joint replacements due to implant failure [Ekeland et al., 2017]. Another less
invasive technique, is unicompartmental knee arthroplasty, whereby only one half of the knee joint is replaced. This preserves the intact side of the knee (often lateral) and has been shown to be more successful than HTO techniques in delaying the need for total joint arthroplasty and reducing long term symptoms [Petersen and Metzlaff, 2016, Stukenborg-Colsman et al., 2001].

Of course, all surgical options are expensive, invasive and come with an element of risk to the patient so great efforts are being made to either develop new modalities, to diagnose the disease earlier or to develop truly disease modifying therapies. Some effort has gone into trying to develop techniques to bridge the gap between more conservative treatments and surgical joint replacement for patients with unicompartmental OA. One such method uses an implantable, spring-based prosthesis to alleviate loading on the medial side of the knee without the need for invasive arthroplasty [Clifford et al., 2013]. Although short term results appear quite promising [Madonna et al., 2016], data from long term studies are not yet available. Novel approaches such as these will be required to reduce the requirement for more invasive surgical techniques.
2.3 Animal Models of OA

2.3.1 Overview

The nature of primary OA in humans, with the long asymptomatic stage, makes studies of the initiation and early stages of the disease very difficult. By the time of diagnosis, most patients are in the latter stages, with severe tissue damage. For this reason, animal models are very often used to study the pathogenesis of the disease and for measuring the potential benefit of novel therapeutic or interventional methods of treatment.

2.3.2 Choice of Animal

When choosing to use an animal model as an analogue to human disease it is important to consider how to best replicate the pathophysiology. One of the important decisions is which species of animal to be used, with each having their own sets of advantages and disadvantages. Small animals (mice, rats, guinea pigs and rabbits) have been frequently used due to their combination of low cost and the rapid onset and progression of cartilage lesions after the onset of OA [McCoy, 2015]. Mice, as the smallest of these animals, are the least expensive and so are often used in animal models reported in the literature. One issue with the use of mice is that there are significant anatomical differences between cartilage in mice and humans. These include significantly thinner cartilage, a relatively thicker layer of calcified cartilage and an absence of chondrocyte zones [Glasson et al., 2010, McNulty et al., 2012]. The scale of the animal also makes induced surgical models of the disease very technically difficult, although intra-articular surgical models have been produced [Glasson et al., 2007, Kamekura et al., 2005]. The main benefit with murine models of the disease is the ease and availability of genetically modified animals. The ability to investigate the effect of knocking out certain genes on the development of the disease has the potential to highlight disease mechanisms and pathways for treatment that would otherwise be very difficult to detect [McCoy, 2015]. The fact that mice develop OA spontaneously also makes them ideal candidates for spontaneous disease models.

Rats share many of the benefits with mice when it comes to the low cost of housing and rapid generation of OA. The main difference in comparison is that genetic knock-out models are significantly less easily available and so rats are not suitable for studying genetic disease
pathways [McCoy, 2015]. Another difference is that the rat has been shown not to develop spontaneous OA. When using these animals therefore, a consideration needs to be made as to whether they can be used to accurately model human joints, where the development of spontaneous OA is known to occur in a sizable subset of the population. The advantage of this, however, is that specific causes of osteoarthritis can be studied independently, to evaluate whether they can lead to osteoarthritic development even in joints which do not have any significant predisposition to disease. Another advantage of this in models of induced disease (either surgically, mechanically or chemically) is that arthritic free controls are more easily available and so the effect of the induction method can be more clearly established. The other benefit of the rat is that it has much thicker cartilage than the mouse that resembles better the human tissue. This allows for the creation of full thickness cartilage defects that would be impossible in murine models [Gregory et al., 2012].

Guinea pigs have been used as models of OA for a long time, primarily because the size of the animal allows for easier collection of joint serum biomarkers that are difficult to obtain in smaller animals [McCoy, 2015]. Certain strains of the animal are also known to develop OA naturally, making them a potentially good model of spontaneous disease [Bendele and Hulman, 1988]. The cost and size of the animal relative to the smaller rodents, however, mean that it is less frequently used. The rabbit has similar issues with regard to its size and cost of housing, as well as significant biomechanical differences with human subjects. Compared to the other animals mentioned, rabbits have significantly increased knee flexion [Proffen et al., 2012] and tend to preferentially load the lateral side of their joint (compared to the medial for humans and most animals) [Teeple et al., 2013]. This means they tend to develop osteoarthritic changes on the lateral condyle first. Rabbit cartilage has also been shown to exhibit spontaneous healing [Cook et al., 2014], a property that could be detrimental to the ability to register the impact of therapeutic treatments and something which is not seen in human subjects. Larger animals such as dogs, sheep, and horses have also been used, but the size and cost of the animals means they are normally reserved for the pre-clinical evaluation of treatments rather than early stage pathogenic studies [McCoy, 2015].
2.3.3 Spontaneous Models of OA

Spontaneous models of OA are, in theory, the closest equivalent to human primary disease, with OA progressing naturally over an extended period of time. In mice, for example, several strains have been identified that develop OA naturally with little or no intervention. STR/ort mice have been reported to develop osteoarthritic lesions early in their lifespan which mimic human OA in many ways, including proteoglycan loss, cartilage fibrillation, extracellular matrix degradation, osteophyte formation and subchondral sclerosis [Staines et al., 2017]. The guinea pig is also widely used as a model of primary OA, with the Dunkin-Hartley strain receiving significant attention due to the fact that osteoarthritic changes occur without intervention. Early studies demonstrated that without intervention the animals began to develop signs of OA within 3 months of birth, with acute cartilage degeneration seen in both the medial and femoral condyles of the knee after 18 months [Bendele and Hulman, 1988]. Further studies, looking at the histopathology of the joints in more detail at similar time points found that the structural and biomechanical changes correlated well with those seen in clinical studies of human patients [Jimenez et al., 1997]. The fact that these models develop over a long period of time suggests that they may represent well the slow, time dependent disease profile in human primary OA. Recent studies, however, have suggested that there are secondary mechanisms present in a number of these spontaneous models. In the Dunkin-Hartley guinea pigs for instance, the increased laxity in the joint that leads to the osteoarthritic changes may be more reflective of the increases in joint laxity seen after ACL injuries than that present in primary patients. The low level of repeatability as well as the uncontrolled time span also need to be considered when using these models [Little and Smith, 2008].

2.3.4 Genetic Models

Genetic murine models of the disease have been used to study the pathogenesis of OA. Genetic mutations made to the animals are intended to either accelerate disease progression or protect the animal from structural joint changes. In this way, they are useful both for improving the understanding of pathophysiological mechanisms and for evaluating potential therapeutic pathways [Kuyimu et al., 2016]. Knockout mice lacking the gene responsible for specific collagen production (collagen type IX alpha 1), for example, have been shown to develop osteoarthritic
joint changes at a significantly faster rate than wild type littermates, helping to elucidate the potential role of collagen deficit in osteoarthritic pathophysiology [Hu et al., 2006]. The use of other genetic knockouts has also led to new understandings of the role of proteases in the development of the disease, potentially leading the way to novel protease-blocking therapeutics [Miller et al., 2013]. The utility of these models for evaluating specific disease pathways is unquestionable, however other models are probably required to provide a more systemic analysis of the disease. Genetic models tend to evaluate the effect of knocking out one specific gene but are unable to take into consideration other contributing genes that participate in disease pathogenesis [Kuyinu et al., 2016].

2.3.5 Non-Invasive Mechanical Models

Non-invasive mechanical overload models have been developed as an alternative to surgical models of the disease to reduce the effects of possible inflammatory agents and to remove the reliance on surgeon skill to produce repeatable results. These have tended to be carried out on mice, where the small size makes high-quality surgery very difficult. The earliest models were based on intra-articular tibial fracture, where the flexed knee of a mouse is fixed on a cradle with an indenter used to create a closed joint fracture [Furman et al., 2007]. These models represent well the pathogenic changes seen after acute injury such as a motor accident but may not represent well the pathophysiology of most patients [Christiansen et al., 2015]. Direct joint loading has also been used to produce osteoarthritic changes without the need for fracture. An axial load is applied to the stifle which produces a displacement of the femur relative to the tibia [Poulet et al., 2011]. This load can either be applied cyclically as a model of how chronic joint overuse can lead to OA or it can be applied as a single load to rupture the anterior cruciate ligament [Kuyinu et al., 2016]. Although far less frequently, the technique has been used in rats to create ACL tears without the need for invasive surgery, however, the effect of cyclic loading has yet to be investigated [Maerz et al., 2015].

2.3.6 Surgical Models

Surgical models of OA are widespread in the literature, with a vast number of alternative approaches having been adopted. The most commonly used models are ACL transection (ACLT),
2.3. Animal Models of OA

MMT (medial meniscal tear) and osteotomy. Surgical models tend to be very reliable, reproducible and generally produce rapid osteoarthritic changes, making them ideal for high throughput studies [Kuyinu et al., 2016]. ACL transection was one of the first used surgical disease models [Pond and Nuki, 1973]. It has been utilised in a number of animal models to produce instability based lesions that mimic disease progression in human subjects following injury [Hayami et al., 2006, Murata et al., 2017, Maerz et al., 2016, Wen et al., 2016]. Laxity in the knee joint leads to a change in the biomechanics, causing localised cartilage wear and eventually OA [Marijnissen et al., 2002]. This has been used frequently in canine models where meniscal tearing and cartilage damage indicative of OA have been shown to develop around 48 weeks postoperatively [Pond and Nuki, 1973]. Some ACL models have shown that load bearing on the joint is diminished after surgery, with the animal preferentially loading the other limbs. This has the potential to slow down the progression of OA in the limb that underwent surgery, limiting the repeatability of the model [Sniekers et al., 2008]. It is also common for the tissue surrounding the ACL to be damaged during the surgical procedure or for unwanted inflammatory responses to take place in the joint post-surgery. Secondary damage to the menisci has been shown to occur in up to 74% of subjects [Lafeber and Mastbergen, 2009]. Injuries and pathologies that occur as a result of the surgical procedure itself and not the development and progression of OA are detrimental to any animal model as they make determining whether the joint damage seen is a result of OA or other inflammatory processes very difficult.

Partial or full meniscectomies are another widely used surgical method for initiating OA in animals, with the procedure being first demonstrated in 1936 [King, 1936]. Localised areas of the cartilage are exposed to large stresses after mechanical failure of the meniscal structures and these stresses lead to cartilage degradation and eventually OA [Moskowitz et al., 1973]. Studies on rabbits showed how a partial meniscectomy could lead to degenerative cartilage changes in the knee after as little as 3 weeks [Moskowitz et al., 1973]. As with the ACLT model mentioned above, studies have found that preferential loading of the non-affected limbs is common after meniscal surgery, potentially slowing down or retarding the rate of osteoarthritic damage. The intra-articular nature of the surgery also gives rise to the fear that injury due to surgical error or inflammation of the joint may alter the progression of the disease. It could be said that due to the fact that both these models reflect the development of OA after the pre-existing injury they are better models of secondary than primary OA [Kuyinu et al., 2016].
Osteotomy based techniques, despite being less widely used, have also been shown to be effective in producing osteoarthritis-like changes. The technique normally involves the use of a wedge osteotomy to perform a re-angulation of either the femur or tibia to illicit an increase in unicompartmental joint loading [Wei et al., 1998]. Despite never having been used in small rodent studies, a number of studies have used this principal to induce arthritic changes in the lateral knee of rabbits [Reimann, 1973, Lovsz et al., 1995]. A 30-degree varus tibial angulation was shown to lead to histological cartilage changes that were reflective of those seen in the early stages of human OA. A similar technique was used to accelerate the progression of OA in a strain of guinea pigs known to develop the disease spontaneously [Wei et al., 1998]. The study found a decrease in the medial subchondral bone thickness and an increase in osteophyte formation after 9 months in the osteotomized guinea pigs as compared to the control subjects. The benefit of this model as compared to the other surgical techniques is that it avoids intra-articular surgery and the possible inflammatory effects that may occur. This makes it potentially a better model of the mechanical disease mechanisms that are thought to lead to human primary OA [Lovsz et al., 1995]. Over 80% of patients suffering from radiographic OA have some degree of knee misalignment [Felson, 2013] and so this model may have a better chance than most to replicate these conditions. The major issues with the model are the complexity of surgery (particularly for small rodents) and the slow rate of osteoarthritic progression. This, however, could prove to be a benefit in studies focusing on early stage pathogenesis as it will increase the usable study time.

2.3.7 Disease Outcome Measures

Histopathology has long been the gold standard for assessing the progression of OA in animal models [Rutgers et al., 2010]. Histological sections, in combination with specific immunohistochemical staining, can be used to measure and classify the progression of OA, either quantitatively or observationally. Early studies evaluated the progression of disease by classifying the histological damage into four groups depending on the scale of disease. Grade 0 indicated healthy cartilage, grade 1 indicated superficial cartilage damage, grade 2 indicated fibrillation extending into deep cartilage and grade 3 indicated significant cartilage loss and subchondral bone exposure [Kuyimnu et al., 2016]. The issue with this system was that it was prone to individual observation, where one investigator might grade the sample differently to another. This
of course made inter study comparisons very difficult. In order to account for that, a number of point-based systems have been developed since. Mankin [Mankin et al., 1971] or modified-Mankin systems are widely used and more recently a new standardised OARSI scoring system was developed to improve repeatability [Gerwin et al., 2010].

An issue with histology is that it is a post-mortem procedure and so cannot be used to evaluate the progression of disease through a long term trial without requiring a large number of animals to be sacrificed at each time point. For this reason, biological markers from serum, urine or synovial fluids have regularly been used in animal models. They can not only be used to monitor disease progression but can also add to the understanding of pathophysiological processes throughout the disease process. The most commonly used biomarkers are urinary C-telopeptide of type II collagen (CTX-II - a marker of collagen disruption) and serum cartilage oligomeric matrix protein (COMP-a marker of matrix disruption). These are both markers of cartilage turnover and have been shown to correlate strongly with histological OA in animal models of the disease [Hayami et al., 2004]. One limitation in the use of biomarkers to monitor disease progression is that in general, they are more useful when taken directly from synovial fluids [McCoy, 2015]. Once they enter the bloodstream the relative increase in the marker is diluted significantly and the levels can easily be influenced by other systemic conditions that have nothing to do with the joint that is being investigated. In small animals particularly, obtaining synovial fluid is very difficult and this dramatically limits their potential utility [Kuyinu et al., 2016]. Recently, methods to quantify the presence of CTX-II particles in small quantities of synovial fluids using magnetically labelled antibodies have been presented [Yarmola et al., 2016, Yarmola et al., 2017]. These could potentially lead the way to more frequent measurement of synovial fluid biomarker concentrations in small animal studies.

Serial imaging can also be used to study the progression of disease through long term studies, although the application of these techniques to rodent models of disease is limited. Micro computed tomography (micro-CT) techniques have been used to evaluate the progression of induced arthritis in rats [Barck et al., 2004, Piscaer et al., 2008, Botter et al., 2008]. It has been used successfully to evaluate structural changes in subchondral bone [Moodie et al., 2011, de Visser et al., 2017], however its low sensitivity for soft tissue means characterizing cartilage degeneration has proved more difficult [Tremoleda et al., 2011]. The use of contrast agents has been shown to aid in enhancing the contrast of OA and allow for direct imaging of articular
cartilage [Roemer et al., 2005, Palmer et al., 2006], although the use of these methods *in vivo* has been limited. Optical coherence tomography (OCT) has also been used to evaluate the presence of OA at serial time points in a chemically induced model of knee OA. Structural changes to the cartilage-bone interface detected using the imaging modality correlated well to histological scoring [Adams et al., 2006]. The fact that these methods have not been used more frequently is likely due to the expense and complications involved in imaging small animals *in vivo*. In order to obtain accurate imaging the motion of the animal must be minimized, requiring the use of powerful anaesthetic agents to immobilize the animal and reduce respiratory based motion artifacts. The dose of radiation the animals are exposed to is also a factor for consideration in x-ray based methods, with obvious implications for animal welfare, especially where animals will be kept long term [Tremoleda et al., 2011].

Gait analysis and biomechanics have also been postulated as good measures of disease progression, and an outline of their use is presented in the section below.
2.4 Rodent Gait and Biomechanics

2.4.1 Overview

Patients suffering from knee osteoarthritis often suffer from changes in the way in which they walk, both subtle and chronic, and animals have been shown to display similar gait changes when suffering from the disease. Typically gait is believed to alter for two main reasons: pain avoidance and joint dysfunction, with a significant overlap between the two making evaluating the cause of pain particularly difficult. Pain avoiding gait changes often occur in the early stages of the symptomatic disease, where the patient or animal makes changes to their gait to alleviate pain which occurs during certain motions. These gait changes, when amplified over long periods of time can lead to degenerative atrophy of muscles surrounding the affected joint, altering the internal biomechanics and switching the cause of gait change from pain avoidance to joint dysfunction. Gait changes due to joint dysfunction can also be induced by the degenerative changes occurring during OA progression. Cartilage loss and osteophyte production change the internal mechanical environment of the joint and fundamentally alter the way in which the joint articulates. In animal models of the disease, investigating these gait changes can be used both as a noninvasive measure of disease progression and as a method to investigate disease pathology and how joint pain and dysfunction relate to one another.

2.4.2 Overview of Terminology

Before evaluating the different ways in which rodent gait can be evaluated, it is important to be familiar with the nomenclature and definitions used to define it. In gait analysis, a single gait cycle (or stride) can be defined as the period between when the foot makes initial contact with the ground (heel-strike) and when the foot next makes contact with the ground. During normal gait, this cycle repeats itself continuously. Within this gait cycle, there are two clearly defined phases: the stance phase and the swing phase, the relationship between which is often very important in understanding abnormal gait pathologies. The stance defines the period during the gait cycle when the foot is in contact with the ground, starting with the heel-strike, progressing through mid-stance and ending when the foot leaves contact with the ground (toe-off). The swing phase defines the exact opposite, the period during which the foot is not in
contact with the ground. Starting with toe-off, it progresses through mid-swing and terminates at the heel-strike commencing the following gait cycle. By dividing the gait cycle up in such ways, quantitative measures of gait can be compared at either specific points in the cycle or during specific phases. Figure 2.4 demonstrates the gait cycle pictographically, highlighting the phases of gait as well as key time points during the cycle.

![Gait Cycle Diagram](image)

**Figure 2.4:** Figure showing the phases of gait for the right leg of the rat with phases of gait labelled underneath

### 2.4.3 Spatiotemporal Gait Measurements

Spatiotemporal gait parameters define the way in which an animal’s paws move with respect to space and time and are often key measures in determining pathological gait changes [Lakes and Allen, 2016]. Spatial parameters can include stride length, step length, step width and foot splay. In certain rodent models paw print characterization may also be used, particularly in peripheral nerve studies where the distribution of toes and length of paw prints are used as functional indices of both peripheral and central nerve damage [Deumens et al., 2007, Bozkurt et al., 2008, Miyagi et al., 2013]. Temporal parameters commonly include simple measures such as stance time, swing time and stride time, however, these can be combined to provide more complex measures such as limb duty factor, temporal symmetry and limb phase [Jacobs et al., 2014]. These are described in the equations below:

\[
\text{Limb duty factor} = \frac{\text{stance time of limb}}{\text{swing time of limb}}
\]
Temporal symmetry = \( \frac{\text{time of right foot strike} - \text{time of left foot strike}}{\text{stride time}} \)

Limb phase = \( \frac{\text{time of left fore foot strike} - \text{time of left hind foot strike}}{\text{swing time}} \)

A number of different techniques have been developed for quantifying these spatiotemporal parameters. Perhaps one of the simplest is via the use of an inkpad, designed in such a way that when the animal walks over it the impression left by its paws can be used to evaluate print characteristics. This technique has been used to investigate the symmetry of rat gait after the development of antigen-induced inflammatory knee arthritis [Boettger et al., 2009]. It was found that affected animals displayed asymmetrical gait indicated via a reduction in step length and changes in paw angle that were proposed to be pain-related gait modifications. Several other studies have used ink print techniques to investigate the effect of induced osteoarthritis on paw print characteristics with varying results. Reductions in stride length were seen in both genetic models of mouse OA [Daans et al., 2011] as well as an induced inflammatory model of rat arthritis [Bonnet et al., 2015]. Further studies used ink based analyses to correlate gait changes in an over-exercise model of rat OA with histological levels of cartilage damage in the knee [Beckett et al., 2012]. Unlike the previous studies mentioned, they found no difference in stride lengths between healthy and osteoarthritic animals but did find a strong correlation between paw angle and the histological grades of OA pathology in the knee.

Despite their success in determining distinct gait changes in pathological limbs, there are significant limitations with ink based methods as described above. The first is that without some form of videography, gait speed is very hard to measure and it is known that this is correlated very highly with almost all gait parameters. The second is that the amount of ink on the paws of animals can be inconsistent and this can lead to inaccuracies in the quantification of paw characteristics [Lakes and Allen, 2016]. To overcome these issues, a number of more modern videography techniques have been developed that can measure spatiotemporal gait parameters. CatWalk (Noldus, Netherlands) and DigiGait (Mouse Specific, USA) are two commercial systems that allow these measurements to be carried out. The CatWalk system measures spatiotemporal gait parameters alongside paw print characteristics for animals traversing over a glass walkway. When the paw of the animal touches down onto the glass, a light source beneath the walkway reflects off the paw and is captured by a high-speed camera underneath the platform [Vrinten and Hamers, 2003]. This system has been used frequently since its inception.
in studies of nervous system damage in rats [Bozkurt et al., 2008, Kappos et al., 2017] and more recently has been adopted as a tool to quantify gait changes after the induction of osteoarthritic changes [Ferreira-Gomes et al., 2008, Ferland et al., 2011, Fu et al., 2012, Ishikawa et al., 2014, Ades et al., 2014, Ades et al., 2017, Miyamoto et al., 2017]. Many of these studies use spatiotemporal parameters as a measure of pain to investigate pain-related behaviour in osteoarthritic animals. Ferland et al (2011) compared the pain response in two models of rat knee OA, an ACLT induced model and a chemically induced model (via injection of monoiodoacetate) [Ferland et al., 2011]. They found significant differences in gait adaptions between the models, with no alterations discovered in the surgically induced animals compared with significant pain-related gait changes in the chemically induced animals. These included an increased swing phase duration, slower swing speed and smaller duty factor in affected limbs. The study also discovered that pain relieving medication could reverse these gait changes, suggesting they were pain-related rather than driven by biomechanical joint changes post-OA.

The DigiGait system operates similarly to the one above, with the notable difference being that the animals run on a treadmill to enable the control of gait speed. It uses a clear treadmill with a camera directed upwards towards the treadmill surface to capture the paw when it makes contact with the belt [Lakes and Allen, 2016]. Although the system has not been used in rat osteoarthritic studies, it has been used in a number of studies of inflammatory arthritis [Berryman et al., 2009] as well as in mouse models of the disease [Poulet et al., 2014, Poulet et al., 2015].

Other studies utilising custom walkways have sought to discover gait changes associated with MMT models of OA. Allen et al (2012) discovered that significant gait asymmetry started to develop 9-12 weeks post-surgically in MMT animals, with a reduction in stance time on the operated limbs. Further studies, however, showed that the same gait changes could be seen in animals that had undergone medial cruciate ligament transection and had no sign of histological OA [Kloefkorn et al., 2015]. This highlights the need to be careful in assuming that gait changes seen post-surgically are directly related to osteoarthritic changes in the joint and to consider whether other factors may be the primary cause of behavioural changes.
2.4.4 Kinematics

Joint kinematics refers to the relative position of bony body positions during gait and most frequently are defined in terms of three joint angles (flexion, adduction, rotation) and translation [Lakes and Allen, 2016]. In situations where spatiotemporal parameters alone don’t give a good measure of pathological changes, joint kinematics (motion) may need to be included in order to better characterise the gait changes. In studies of nerve damage, for example, it has been shown that the combination of kinematic and spatiotemporal parameters is far better at differentiating between pathological and healthy rats than spatiotemporal parameters alone [Eftaxiopoulou et al., 2014]. Most studies of rodent kinematics have relied on reflective skin based markers to track the motion of animals running either on a treadmill or overground walkway. Reflective markers are attached to the lateral side of the hindlimb (normally under anaesthesia), which are detected by high-speed optical motion capture cameras as the animal moves [Webb et al., 2011]. Two-dimensional motion analysis is possible via the use of a single high-speed camera to detect flexion angles in the sagittal plane. More frequently, however, several cameras are used to triangulate the position of the joints in three dimensions to provide more detailed kinematic outputs [Eftaxiopoulou et al., 2014, Costa et al., 2011, Webb et al., 2011]. When considering the use of skin marker based kinematics as a measure of gait changes in rats, one of the key considerations is whether to use treadmill based techniques [Couto et al., 2008, Thota et al., 2005] or those using an overground walkway [Kloos et al., 2005, Kunkel-Bagden et al., 1993]. Treadmill based techniques allow for the speed to be fixed, which allows for many consecutive strides to be measured at the same controlled walking velocity. The difficulty with the approach is that it requires intensive training before the animals can run consistently, particularly at higher speeds, and it has been shown that even with training around half of rats still will not run effectively [Berryman et al., 2009]. Overground walkway techniques allow the animals to walk at a self-selected speed through an enclosed tunnel, normally with perspex walls to allow for marker visualisation. These techniques can be far less stressful for the animals involved which should facilitate the adoption of more natural gait and they may also be very useful in studies where the self-selected gait speed is a parameter in and of itself [Jacobs et al., 2014]. However, the fact that they don’t allow for the speed to be controlled could reduce the power of the technique to detect subtle changes, particularly as gait speed is known to influence kinematic parameters significantly [Costa et al., 2011]. Studies comparing
the two methods have found very little difference in velocity controlled kinematics and as such the decision over which to use will be dictated by the experiment in question [Pereira et al., 2006].

The main problem with marker based techniques as a whole is skin artefact error, in which the bony landmark moves relative to the skin based marker that is supposed to track its position. This is also a limitation in human studies, but in rodents, the errors incurred are far higher and can significantly reduce data reliability [Filipe et al., 2006]. This source of error has been found to be significantly larger for knee based markers than for those at the hip or ankle, presumably because of the large volume of soft tissue sitting just lateral of the tibia and femur in rats. Triangulation of the knee position by using the hip and ankle locations alongside knowledge of the femoral and tibial segment lengths has been shown to improve kinematic accuracy, but significant errors still persist [Bauman and Chang, 2010]. In recent years markerless techniques have been investigated to improve kinematic accuracy. The fact that they don’t rely on skin based markers means they can alleviate any issues with skin artefact error and in addition, they remove the need for the animal handling/anaesthesia that is required to apply skin based markers. High speed radiographic techniques are one markerless option that has been used for the quantification of lower limb kinematics in rats [Mattucci et al., 2017, Bauman and Chang, 2010, Haase et al., 2013, Bonnan et al., 2016, Gravel et al., 2010] and studies comparing the technique with marker based systems have shown significant improvements in kinematic outputs [Bauman and Chang, 2010]. These techniques normally use an x-ray emitter positioned on the sagittal plane of the animal in combination with a high-speed radiographic camera to record the motion. Bony landmarks corresponding to the ankle, knee and hip are identified from the x-ray films and used to compute the knee joint angles. For most studies, only one x-ray emitter and detector are used in order to simplify the process, reduce the x-ray dose that the animal receives and reduce operational costs [Lakes and Allen, 2016]. This is useful for looking at flexion angles which can be estimated to be planar (occur only in the lateral plane), but for looking at more complex motion three-dimensional motion analysis is required. By using a pair of x-ray emitters and receivers instead of just one, Bonnan et al (2016) were able to triangulate the position of joints in three dimensions to characterise more fully the motion of the rat forelimb during overground walking. This allowed them to investigate rotation and abduction of the joints as well as just flexion [Bonnan et al., 2016]. This makes it significantly more viable as an alternative to 3-D optical marker based systems and over time the adoption
of such systems will probably increase.

Although neither of these techniques has been used in studies of osteoarthritis, radiographic techniques have been used to study the effect of an antigen induced model of inflammatory osteoarthritis on knee kinematics of the rat hind-limb [Boettger et al., 2011]. It was found that the knee range of motion decreased significantly in the inflamed knee compared to both controls and the ipsilateral limb. It also reported that the use of pain relief normalised the range of motion suggesting that the kinematic changes detected may be an indication of pain in arthritic limbs.

2.4.5 Ground Reaction Forces

Ground reaction forces are often used in rodent studies to measure the loading profile of the hind and forelimbs throughout gait. The measurements are usually split into three components: vertical, propulsion and mediolateral depending on the direction of the ground reaction force being measured [Howard et al., 2000]. The component acting in the vertical component is the largest and is used to represent the weight loading of limbs throughout gait. As such it is often used as a measure of impairment, either pain-based or biomechanically driven. Braking/propulsive forces occur in a direction parallel to that of travel, with the relative amount of braking and propulsion often used as a measure of pathological gait changes. During healthy gait, the hindlimb provides a braking force just after heel strike to slow the motion of the limb and as the gait cycle evolves this transforms into a propulsive force to provide forward motion. The mediolateral force is often smaller and can change significantly through the gait cycle. At the beginning of the cycle, as weight is shifted from the left to right leg, the ground reaction force acts towards the midline of the animal to stabilise motion for a short period. When the weight is shifted back to the contralateral side of the animal the force increases again to accelerate weight transfer to the unloaded limb [Jacobs et al., 2014].

Most studies of ground reaction forces in rats require the use of instrumented force plates [Webb et al., 2011] which can detect the ground reaction forces in all three axes. Despite the use of ground reaction force measurements for other purposes [Webb et al., 2011, Pardes et al., 2016, Roemhildt et al., 2010], there has only been one thorough study of ground reaction forces in osteoarthritis. It showed that in rat limbs with ACLT induced OA, vertical and
propulsive ground reaction forces decrease post-surgically compared to both contralateral limbs and controls [Allen et al., 2012]. One limitation with ground reaction force measurements is that in order to collect data the animal must place its foot correctly on the force plate, with one foot making contact at a time in order to get accurate GRF data for each limb. This can often be difficult to achieve, especially in animals with varying gait speeds, and can add significantly to the experimental time required for data collection [Lakes and Allen, 2016]. In large animals and human studies, force instrumented treadmills have been used to allow the calculation of ground reaction forces at controlled speeds and split belt treadmills have been utilised to enable the collection of right and left leg loading simultaneously [Riley et al., 2007, Kram et al., 1998, Weishaupt et al., 2004, Bockstahler et al., 2007]. Developing such systems for rodents, however, would likely be very difficult and no such system exists in the literature. One system which may overcome some of the complications with ground reaction force measurements in rodents is the use of an instrumented wheel [Roach et al., 2012]. A standard mouse wheel was modified to include a force sensitive rung which was capable of quantifying the normal and tangential forces applied by each individual paw. The benefit of this system compared to the use of an overground runway is that the force is measured every time the animal’s paws make contact with the running surface rather than only when they hit a specific spot on a runway. This has the potential to dramatically reduce experimental time and improve the consistency of results. The forces measured using the system compared favourably to those calculated using instrumented force plates and creative methods such of these will probably need to be developed for GRF measurement to be used more frequently in rodent studies.

2.4.6 Musculoskeletal Models

Experimental data collection alone, as described above, has been shown to be a useful tool for differentiating pathologic and healthy gait. Despite this, it has several limitations when it comes to fully characterising dynamic movement such as gait. Important parameters such as muscle force generation and internal joint loading are very rarely measurable through direct experimentation, especially in small rodents. Musculoskeletal modelling involves a suite of in silico tools that provide a computational framework to enable the calculation of internal joint forces and muscle forces using in vivo kinematics and GRF in combination with an anatomical model [Delp et al., 2007]. The simplest models perform simple inverse dynamics simulations,
where the external joint forces and moments are calculated using linear equations of motion. A simplified two-dimensional example can be seen in Figure 2.5 with the equations of motions defined below:

\[
\sum F_x = ma_x = R_{xp} - R_{xd}
\]

\[
\sum F_y = ma_y = R_{yp} - R_{yd} - mg
\]

\[
\sum M = I\alpha = M_p + M_d + R_{yd} d_d \cos \theta - R_{xd} d_d \sin \theta - R_{xp} d_p \cos \theta + R_{yp} d_p \sin \theta
\]

These equations can be rearranged such that the mass (m), linear accelerations (a_x and a_y), segment angle (\(\theta\)), angular acceleration (\(\alpha\)), distal joint reaction forces (\(R_{xd}\) and \(R_{yd}\)), distal moments \(M_d\), inertial moment (I) and the location of the centre of mass \(d_d\) and \(d_p\) can be used to calculate the proximal joint forces (\(R_{xp}\) and \(R_{yp}\)) and moment \(M_p\). In three dimensions the procedure is exactly the same except equations need to be resolved in three axis. For the foot segment, distal forces and moments are derived from in vivo ground reaction force measurements.

Moving on from inverse dynamics, static optimisation can be used to deduce the contribution of muscle and internal joint contact forces to the external forces and moments shown above. This is a more complex procedure as the equations of motion are no longer deterministic, because of the large number of muscle forces contributing to external joint forces there are more unknowns than knowns. In order to arrive at a deterministic solution optimisation methods must be used to find a solution to the contribution of muscle forces that minimise or maximise some objective function. In many studies the muscle forces are optimised such that the sum of muscle stresses is equal to zero [Crowninshield and Brand, 1981], making the assumption that the body activates muscles in such a way to optimise overall efficiency.

OpenSim [Delp et al., 2007], an open source software for performing these biomechanical analyses, can take kinematic and GRF inputs in combination with a pre-built anatomical model and perform inverse dynamics and static optimisation to evaluate internal biomechanics. Traditionally this has been used for human subjects [Pizzolato et al., 2017, Xu et al., 2015], however recent efforts have been made to adapt the approach for use in rats [Johnson et al., 2008]. Johnson et al (2008) developed a musculoskeletal model of the rat hindlimb in OpenSim. Joint
centres of rotation, muscle insertion points and segmental parameters were calculated via cadaveric studies to produce an accurate anatomical model of the rat hindlimb. The model has the potential, when combined with in vivo kinematic and GRF data, to enable the comparison of internal joint biomechanics in vivo between pathological and healthy rats for a number of pathologies where biomechanics play a key role. Johnson et al (2012) used the model in combination with in vivo kinematics and GRFs of rats to evaluate muscle activity after spinal cord injury and whether it could be used as a marker of recovery [Johnson et al., 2012]. They discovered muscle activity recovered in a phased approach post-injury, but that full recovery was not reached even after 14 days. This study highlighted the discriminative power of these methods. Further studies using the model in silico investigated the effect of functional electrical stimulation on muscle forces in the rat hindlimb [Johnson et al., 2011].

Despite the availability of the model described above, musculoskeletal modelling has been used very rarely in rodents and never in studies of OA. In the only other example of rat muscu-
loskeletal modelling, Bennet et al (2012) used a two-dimensional inverse dynamics model for investigating the effects of peripheral nerve damage on joint moments and powers. Kinematics and GRFs collected in vivo were combined with a simple segmental model of the hindlimb to calculate the forces and moments acting on the ankle, knee and hip joints. It was found that joint power decreased in the injured limb for animals with peripheral nerve damage and a corresponding increase in joint power was seen for the contralateral limb. This study highlighted the utility of biomechanical analyses in identifying mechanisms of pathogenesis and recovery in rodent models of disease, but to date is the only such study [Bennett et al., 2012]
Chapter 3

Gait Analysis and Musculoskeletal Modeling Methods

All in-vivo data collection was carried out either by the author alone or in combination with Ibidumo Igah. All data was processed by the author alone.

3.1 Chapter Summary

This chapter summarizes the methodologies relating to in vivo kinematic and kinetic data capture as well as the in silico musculoskeletal modeling that was used in all three experimental chapters. Because it is used throughout the thesis it was thought best to detail the methodology here such that it can be used as a reference. Methodologies specific to Chapters 4, 5 and 6 can be found within the specific chapters.

3.2 In Vivo Data Collection

3.2.1 Overground Walkway

In order to collect kinematic data alongside ground reaction force data, an overground runway was designed to house an AMTI hex6x forceplate (AMTI Inc., MA, USA). The initial dimen-
3.2. In Vivo Data Collection

Figure 3.1: Solidworks model showing initial design for overground walkway

sioned design of the walkway can be seen in Figure 3.1 with the final constructed walkway shown in Figure 3.2. The base of the walkway was constructed from a 1.5m length of MDF, from which a hole was cut in the middle for the forceplate to be mounted alongside two moveable polyethylene plastic inserts which would allow for lateral movement of the forceplate if required. This allowed the forceplate to either be placed in the center of the walkway or to be offset such that only the right or left hindlimb could be placed on it. Cutouts were made in the base of the walkway for the forceplate cables to be passed through and connected to the Vicon Nexus hub. The runway was surrounded by a 900m tall perspex frame to restrain the animals’ motion whilst allowing for visualization.

3.2.2 Animal Preparation and Training

Naïve rats placed directly on the walkway are very unlikely to traverse it continuously at a speed suitable for acquiring repeatable data. Food rewards at either end of the runway act as an incentive for the animals to traverse, however rats are inherently suspicious of unfamiliar foods, scents and environments so a period of acclimatization and training was required. In the studies reported in this thesis, the acclimatization time prior to data capture was three weeks.
Figure 3.2: Image showing walkway setup. Top: Side on View. Overground walkway is positioned side on to the cameras and supported at either end by a polypropylene container. Three Vicon T-40 Cameras are positioned facing the centre of the runway at approximately 30 degrees offset with each other. An optical camera is positioned facing the centre of the walkway to capture real time video. Bottom: View from above showing location of force plate relative to perspex containment.
3.2. In Vivo Data Collection

Animals unable to traverse the runway either independently or with food rewards at the end of this period were culled using an appropriate schedule 1 method. For the first week, the animals were acclimatized both to the researcher carrying out the experiment and to the food used as a reward. Handling each animal for a period of 10 minutes twice daily for seven days was seen to familiarize most animals with the researcher, although longer may be needed for more nervous rats. To familiarize them, the researcher’s hand was placed in the cage and the animal was allowed to approach in its own time. After several sessions it was noticed that the animals approached readily, whereas at the beginning, the animals were seen to approach nervously, if at all. To avoid cross-contamination of scents, gloves were changed when handling animals from different cages as unfamiliar smells can increase stress levels and nervousness. Throughout this first week an appropriate food reward (that will be used on the walkway) was placed in the animals’ cages twice daily to familiarize them. In this study, quartered Cheerios (Nestlé, Vevey, Switzerland) were used, but other cereals or fruit will likely also be appropriate. After the first week the animals were placed on a restricted diet for two weeks such that they were only given their maintenance energy requirements (114kcal/kg$^{0.75}$). This was to improve the effectiveness of food rewards when placed on the walkway. For the first 2-3 days of this period, the animals were placed in the walkway twice daily for 5 minutes and allowed to roam freely so that they could become accustomed to the environment. The walkway was wiped down with an ethanol spray between animals to avoid cross-contamination of scents. After 2-3 days (or when the animal started to appear less nervous in the walkway), the food reward was placed at either end of the walkway prior to placing the animal inside. This helped to accustom the animals to eating inside the walkway and taught them that food rewards are placed at either end. After 3-4 days, or once the animal was eating the rewards consistently, rewards were placed at one end of the walkway only prior to placing the animal inside. Once the animal had eaten the reward from one side, another was placed at the far end. This process was repeated twice daily for the remaining training time until the animals traversed the walkway consistently, at which time they were considered ready for data collection to take place.
Figure 3.3: One complete gait cycle showing, from top to bottom, the rat during toe-off, mid-swing, toe-down, mid-stance and toe-off. Red arrow represents ground the ground reaction force vector and the red dots represent the location of the skin markers on the right hindlimb.

3.2.3 Three-Dimensional Kinematics and Ground Reaction Force Measurement

Before attempting to capture kinematics, 5mm reflective markers were applied to the skin of the rat at the location of five bony landmarks. The animals were anaesthetized prior to data
3.2. In Vivo Data Collection

In Vivo Data Collection using inhalation anaesthesia (isoflurane 2% in O₂). Under anaesthesia the animals were shaved liberally around both hindlimbs and ink marks were applied to the skin at the palpated locations of the 5th metatarsal, lateral malleolus, lateral femoral epicondyle, greater trochantar and iliac crest [Eftaxiopoulou et al., 2014]. The animals were then woken from anaesthesia and allowed a minimum of 24 hours to recover from the effects. On the day of data collection, the animals were restrained by one researcher, whilst another applied 3mm reflective markers to the pre-marked skin locations using veterinary glue (3M Vetbond Tissue Adhesive, 3M, Minneapolis, US). The animal was then placed in the walkway and either allowed to run freely or incentivized to traverse via the use of food rewards as described earlier.

Three-dimensional kinematics were measured using a Vicon motion capture system (Oxford Metrics Group, Oxford, UK). Three Vicon T-40 infrared cameras were placed on the lateral side of the runway to measure the location of the reflective markers placed on the animal. A ring of infra-red 120Hz strobe lights surround each of these cameras and the reflection of these lights is recorded from each camera. In order to optimize the chance that at least two cameras would detect each marker (the minimum needed for 3D triangulation) the cameras were placed at an angle of approximately 30° from one another. This can be seen in Figure 3.2. As well as the infra-red cameras, an optical camera is positioned lateral to the forceplate in order to visualize the animal in the recordings.

![Figure 3.4: Capture volume as seen in the Vicon Nexus software. The position of the three infra-red cameras (1, 4 and 5) as well as the optical camera (1) and forceplate (1) can be seen](image)

As well as kinematics, ground reaction forces were measured using the AMTI He6x6 force-plate
Chapter 3. Gait Analysis and Musculoskeletal Modeling Methods

The surface of the force-plate, on which the animal places its limbs, contains a strain-gauge force transducer in all four corners. These measurements combined allow the device to output three orthogonal forces \( (F_x, F_y, F_z) \) as well as three moments \( (M_x, M_y, M_z) \). These measurements are fed into the Vicon motion capture system in real time to allow the ground reaction forces to be combined and synchronized with the kinematic data.

Figure 3.3 shows the data captured by the Vicon motion capture system at five stages of the gait cycle in a rat. The ground reaction force vectors for the fore and hindlimb as well as measured hindlimb marker positions are highlighted in red. Figure 3.4 shows the Vicon capture volume containing the position of the three infrared cameras, the optical camera and the forceplate relative to each other.

3.2.4 Data Processing

After collecting the data it was saved and imported into the Vicon Nexus Software. Where gaps existed in the marker kinematics (due to the cameras not registering a specific marker during a frame), the gaps were filled using a spline fill. The times corresponding to toe-down and toe-off were marked manually using the Nexus software, utilizing force-plate data where available to pinpoint the duration of contact between the foot and ground. The ground reaction forces and kinematics were filtered using a 15hz butterworth filter before being exported as a standard motion capture format (c3d).

3.3 Biomechanical Modeling

3.3.1 OpenSim Model

An existing OpenSim model of the rat hindlimb [Johnson et al., 2008] was used to translate the kinematic and ground reaction force data collected \textit{in vivo} into joint angles, moments and forces. The model contains five bodies (spine, hip, femur, tibia and foot) and contains 13 degrees of freedom. As well as the five bodies, the model contains 38 actuators in the form of muscles and 7 wrapping objects to constrain muscle geometry. Prior to performing musculoskeletal modeling using OpenSim, a custom MATLAB routine was used to convert the c3d file output
from the Vicon Nexus software into two separate files for compatibility with OpenSim: A .trc file containing the three dimensional marker trajectories and a .mot file containing the ground reaction force data.

### 3.3.2 Scaling

Before calculating any output data, the model was scaled so that the weight of segments and size of animal more closely matched the one being evaluated. The scale tool provided in OpenSim uses a static trial (subject standing still) to scale the generic model provided to match the size of a subject. When using animals, the ability to record a static trial is constrained by the fact that the animal cannot be told to stand still. Because of this the first five frames after toe-down were used in place of a static trial as this position best matched the position of the generic model. When scaling the model the first stage is to alter the size of the body segments in the model using average marker data provided in the static trial, minimizing the distances between the experimental and virtual marker pairings. As an example, the foot is scaled based on the distance between the experimental positions of the toe and ankle marker. User defined weightings can be provided to increase the relative importance of minimizing specific marker mismatches and in this study the knee was given a weighting of 0.5 compared to 1 for all other markers. This was to minimize errors caused by incorrect placement or dynamic movement of the knee marker. After scaling the segment lengths, the weight of each segment is scaled linearly by comparing the mass of the generic model to the mass of the animal being experimented upon.

### 3.3.3 Inverse Kinematics

Once a scaled model was produced, inverse kinematics was used to determine joint angles and motion during the provided gait cycle. The position of the animal during each frame was calculated using a global least squares optimization to minimize the distance between virtual and experimental markers as well as the distance between the experimentally derived joint centers and those produced via the inverse kinematics. This derived motion was then used to calculate joint angles for each frame of the trial. The optimization function is shown in Equation 3.1 below:
\[ E = \sum_{i=1}^{\text{markers}} w_i (x_{i}^{\text{subject}} - x_{i}^{\text{model}})^2 + \sum_{j=1}^{\text{coordinates}} \omega_j (\Omega_{j}^{\text{subject}} - \Omega_{j}^{\text{model}})^2 \] (3.1)

(E is the least squared error, \( x \) is the three dimensional marker location, \( \Omega \) is the three dimensional coordinate location, \( w \) and \( \omega \) are the relevant weighting factors)

Figure 3.5: OpenSim model of the rat right hindlimb shown from three orthogonal orientations. Segments (white), muscles (red), wrapping objects(blue) and markers (pink) can all be seen

3.3.4 Inverse Dynamics

Inverse dynamics was used to take the optimized motion derived from the inverse kinematics, in combination with external ground reaction forces and calculate external joint forces and moments. The general principle defining this step is outlined in Chapter 2 together with the equation of motions that are used.

3.3.5 Static Optimization

Static optimization was then used to resolve the external moments and forces at each joint into muscle forces and activations, considering frames sequentially one at a time. Because the motion is treated as a series of static frames, most dynamic musculo-tendon dynamics were ignored in order to simplify the process and reduce computation time. Moment calculations were carried out at each joint creating a series of indeterminate equations with the known variables being the external moments and muscle moment arms and the unknown variables being the muscle
forces. This indeterminate equation was solved at each joint using an optimization function, which minimizes the sum of muscle activations squared as seen in Equation 3.2 below:

\[ C = \sum_{i=1}^{n} (a_i)^2 \] (3.2)

\( C \) is the cost function, \( a_i \) is the activation of the \( i^{th} \) muscle and \( n \) is the number of muscle actuators acting on the joint.

### 3.3.6 Joint Reaction Analysis

Joint force analysis was then performed to calculate the resultant internal forces and moments at the joint caused by all loads acting on the joint. Force and moment equilibrium equations at each joint incorporating muscle forces were used to perform the calculations. To calculate the medial and lateral joint contact forces a probe was used to calculate the scaled distance between the knee joint centre of rotation and the centre of the medial condyle (\( d_m \)), which was assumed to be equal to the distance to the lateral condyle. The medial and lateral compressive forces were then calculated by taking moments about the medial and lateral condyles using Equations 3.3 and 3.4.

\[ F_{medial} = \frac{F_{knee}}{2} - \frac{M_x}{d_m} \] (3.3)

\[ F_{lateral} = F_{knee} - F_{medial} \] (3.4)

\( F_{medial} \) is the force acting on the medial condyle, \( F_{lateral} \) is the force acting on the lateral condyle, \( F_{knee} \) is the total knee joint contact force and \( M_x \) is the internal knee joint moment in the frontal plane.

The next chapter provides an analysis of the \textit{in vivo} results collected using the methodologies described here alongside an evaluation of potential sources of experimental error and how they effect key model outputs.
Chapter 4

Errors in Musculoskeletal Modeling of the Rat Hind Limb

Parts of this Chapter are in the process of being drafted as a paper prior to submission

4.1 Chapter Summary

Gait analysis and musculoskeletal modeling can be incredibly important techniques in attempting to understand the link between biomechanics and musculoskeletal diseases such as osteoarthritis. In the previous chapter, the methodologies used for evaluating rat biomechanics via musculoskeletal modeling were detailed. This chapter expands upon this, evaluating the errors implicit in this type of modeling both in general and specifically in rats. It was found that errors caused by misplacement of markers had moderate effects on all model outputs, but had the largest effects on hip flexion moments. It was also found that errors in the placement of markers at the knee joint were highest and had the largest effects on model outputs. Suggestions were made detailing how these errors could be reduced in-vivo including the use of long-term marking and the use of a single experienced experimenter during longitudinal studies. Errors in muscle geometries were also seen to cause large variations in model outputs, specifically in muscle forces with some peak muscle forces varying by up to 100%. As well as informing future studies in this evolving field this chapter should put into context the results seen in Chapters 5 and 6, where musculoskeletal modeling is used to evaluate changes in hindlimb biomechanics.
4.2 Introduction

Gait analysis and musculoskeletal modeling in rodents has increased dramatically in its use over the past several years. Understanding how a given intervention impacts upon the kinematics and internal kinetics of animals has been used in studies of nerve injury [Eftaxiopoulou et al., 2014, Hansen et al., 2016], neurological disorder [Johnson et al., 2012, Wang et al., 2008] and more direct musculoskeletal conditions such as OA [Jacobs et al., 2017, Maerz et al., 2015]. Most of these studies focus on the use of optical motion based kinematic analyses, whereby the kinematics of the animal during gait are measured via the observation of reflective markers attached to the skin of the animal meant to represent the underlying skeletal anatomy. In recent years these methods have been advanced to begin allowing the calculation of internal joint kinetics via the use of musculoskeletal modeling. Despite the increasing use of these methods, there has been fairly little analysis of potential sources of error, and how these compare to those found in similar human studies. The one source of error that has received a lot of attention (in both human and animal studies) is the skin artifact error, the difference in location between skin based markers, and the underlying bony geometry that they are supposed to correspond to. These errors have been documented in human musculoskeletal studies previously, with studies evaluating the magnitude of these errors on knee joint rotations by comparing the joint rotation obtained using skin based markers with that obtained by surgically embedded bone markers [Lafortune et al., 1993, Benoit et al., 2006]. Average angulation errors of 4.4° were found to occur between the knee flexion angle obtained via the two methodologies, along with tibial translation errors of up to 13.0mm. Even for the foot (where you would expect less skin artifact error because of the decrease in soft tissue between the skin and bony landmark) significant translational errors have been found between the position of skin based markers and that of the underlying bone geometry [Shultz et al., 2011].

In animal studies these sources of error can sometimes be far larger, particularly in rodents where the greater quantity of soft tissue surrounding the lower limb makes accurate palpation of the bony landmarks more difficult. As well as this, the movement of the skin marker relative to the bone during gait leads to further inaccuracies, meaning even with good marker placement the skin markers can fail to accurately represent the actual bone position. Previous studies have used high speed x-ray videography to track the underlying joint motion and compare it to that obtained using an optical skin based marker system such as the one used in this study. It
was found that kinematics derived from the skin based methods varied significantly from those captured using the x-ray videography, with errors as high as 31 degrees in the hip and 31 degrees in the knee [Bauman and Chang, 2010]. Slightly smaller errors were detected in the ankle. The knee joint is known to be the least accurate, primarily because of the large concentration of muscles that sit adjacent to it. For this reason triangulation of the knee joint marker has been proposed as a method to reduce skin artifact errors [Filipe et al., 2006]. This method uses the hip and ankle position, as well as knowledge about the tibial and femoral segments to triangulate the position of the knee in 3D. When this technique was used, despite reducing the magnitude of errors there were still significant differences found between the skin and x-ray based techniques [Bauman and Chang, 2010]. Global optimization (such as inverse kinematics) is another technique used to reduce skin artifact errors by optimizing the joint position such that the distance between the joint markers and the skin based markers are minimized whilst maintaining consistent segment lengths (often from a scaled model) and staying within certain motion constraints like the range of joint angle [Lu and O’Connor, 1999]. When used in the analysis of rodent gait these methods have produced more accurate kinematics than either traditional skin based marker techniques or those using knee joint triangulation [Joao et al., 2014].

As well as marker errors, anatomical errors in the model are possible. These can occur either when the type of animal being used doesn’t match that used to develop the model (eg different strain of animal), with scaling errors where the model incorrectly assumes that the anatomy scales linearly, or when significant inter-subject variabilities are present. One study attempted to quantify the location of a variety of muscle insertion points for use in a musculoskeletal model of the rat hind limb based on CT imaging [Johnson et al., 2008]. They found that the muscle insertion points of certain muscles in female Sprague-Dawley rats could vary by over 20mm, even between weight, age and gender matched animals. A recent study applied this model of the rat hindlimb to a mouse by altering the geometry (muscle insertion points, segment parameters etc.) [Charles et al., 2016]. They performed sensitivity analyses on the model in silico to evaluate how sensitive the muscle moment arms were to the insertion and origin points of the muscles, discovering that moving the insertion points by 0.5mm could have large effects on both the quantity of the moment arm and how that moment arm responded to changing joint flexion. Despite the utility of these techniques in understanding the fundamental sensitivity of the model to changing geometry, they do little to elucidate the effect that changing
the model geometry has on functional model outputs such as joint contact forces. This study presents the first thorough sensitivity analysis for an animal musculoskeletal model using \textit{in vivo} data alongside an analysis of potential sources of error and an attempt to quantify these where possible. This will lead to a greater appreciation of how variations in both the model geometry and the skin marker placement affect important output functions and lead to an understanding of the limitations of these methods.
Chapter 4. Errors in Musculoskeletal Modeling of the Rat Hind Limb

4.3 Methods

4.3.1 Animal Ethics

This study was performed under full institutional and departmental license with ethic committee and Home Office approval. Euthanasia was performed under license by overdose intraperitoneal (IP) injection of pentobarbitone. Animals were provided food and water ad libidum.

4.3.2 Error Quantification

Marker Position Error

The error caused by inconsistency in the positioning of the skin based markers through the palpation of bony landmarks was evaluated using three male cadaveric Sprague-Dawley rats (475g). Two researchers took place in the study so that the difference in consistency between experienced and inexperienced experimenters could be evaluated. The first researcher was experienced in rat hindlimb anatomy, palpation of bony landmarks, and the placement of skin markers for the purpose of motion capture (in this study the author assumed the role of an experienced researcher). The second researcher had an understanding of rat anatomy, but had not attempted either the palpation of bony landmarks in the hindlimb or the application of skin based markers previously. Before commencing the study the process of palpation and marker application was explained to the inexperienced researcher and was demonstrated 3 times. They were then given a chance to apply the markers and be given corrections/tips on how to palpate the correct bony landmarks more accurately. After this had been completed, both researchers took it in turns to apply the markers to the 5th metatarsal (foot), lateral malleolus (ankle), lateral epicondyle (knee), greater trochanter (hip) and articular rim (hip). The relative position of the applied markers was triangulated using 8 black ink marks that had been applied to the animal prior to the study to enable a comparison to be made between the position of each marker from one trial to the next (Figure 4.1). Briefly, the 2D position of each marker in the sagittal plane was triangulated by measuring the distance from the marker to 2 dots positioned on the same segment as the related bony landmark. This was then translated to a position in the local co-ordinate frame so that comparisons could be made. Each participant was asked
to repeat placing the markers 5 times to evaluate their consistency, with a gap of 1 hour between each trial to attempt to reduce the chance that the researcher could remember where the markers had been placed previously.

Figure 4.1: Method for triangulating the palpated positions at which markers were attached. The distance of the marker from two black dots placed on attaching segments was used to determine the location of the marker in a local co-ordinate frame determined via the two marks

The maximum within-participant variation of the marker positions was calculated for both the inexperienced and experienced researchers alongside the maximum intra-participant difference between the two participants. For the purposes of sensitivity analyses the variation in marker position was defined by the maximum within-participant variations of marker positions for the expert.
Inconsistent marker placement was seen for both researchers, although it was higher for the inexperienced operator for all markers. This suggests that the level of experience can play a large role in reducing marker placement variation. Even for the experienced researcher, however, significant errors exist, particularly for the knee marker. Perhaps surprisingly, there are still significant errors for the ankle and foot, despite the lack of soft tissue overlaying these joints. For the expert investigator the variations in marker placement are fairly small (<1mm), but for the novice investigator these are much higher, demonstrating the importance of having experienced researchers, not just for increased accuracy of marker placement, but also for increased consistency. Variations between the researchers were seen to be higher than the variation between trials when only one researcher was considered indicating that the use of multiple investigators may not be ideal. This study did not look at the variation between different expert researchers, and so it may be possible that the use of multiple, highly skilled operators would overcome these errors. The conclusion, however, must be that unless there is a specific reason otherwise, the use of an individual operator throughout the study is essential to maintain consistency between trials.

**Muscle Geometry Error**

The error in the location of the origin and insertion points of all 38 muscles was estimated using data from a previous study [Johnson et al., 2008] (Table 4.1 and 4.2). The standard deviation in the insertion or origin points of the muscles between weight matched animals in that study was seen to be roughly similar to the breadth of the muscle at the respective insertion point,
with an average of 2.2mm. As this is likely to be larger when considering animals that are not
weight or gender matched, these muscle breadths were considered to be conservative estimates
of inter-subject variation of muscle insertion and origin points in this study. The animals used in
the study highlighted were also smaller than the animals used in this study (300g vs 475g) and
so the absolute errors are likely to be smaller than those which would be found experimentally.
The breadth of each muscle at the insertion and origin points in the local x, y and z axis was
documented, with the possible error for each location being taken as the magnitude of the x, y
and z breadths.
Table 4.1: Muscle Origin Morphology [Johnson et al., 2008]

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### Table 4.2: Muscle Insertion Morphology [Johnson et al., 2008]

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<td>Foot</td>
<td>1.36</td>
<td>0.16</td>
<td>0.68</td>
</tr>
<tr>
<td>FDL</td>
<td>Flexor digitalis longus</td>
<td>Other</td>
<td>Foot</td>
<td>1.37</td>
<td>0.17</td>
<td>0.67</td>
</tr>
<tr>
<td>FHL</td>
<td>Flexor hallucis longus</td>
<td>Other</td>
<td>Foot</td>
<td>1.36</td>
<td>0.16</td>
<td>0.68</td>
</tr>
<tr>
<td>Per</td>
<td>Peronei</td>
<td>Other</td>
<td>Foot</td>
<td>0.34</td>
<td>0.00</td>
<td>0.82</td>
</tr>
</tbody>
</table>
Segment Mass Error

Two groups of three male cadaveric Sprague-Dawley rats were used. The first group of rats had an overall body mass of 250g (249-251g) and the second group a body mass of 475g (473-476g). These animal masses were intended to match roughly the weight of animals at the beginning and end of the longitudinal OA study presented in Chapter 6. The foot, shank, thigh, hip and spine segments were dissected and weighed for each animal to evaluate the segment masses. Relative segment masses were calculated by dividing the mass of each segment by the overall mass of the animal. The difference in relative segment masses between the 250g and 475g animals was used to evaluate the effect of scaling errors, whereby the assumption that segment masses scale uniformly with overall body mass may not be correct.

Figure 4.3 shows errors that could be caused by both natural variation in segment masses within animals of the same weight and also by errors that could occur during the scaling process incorrectly assuming that the distribution of segment masses between animals of different weights is equal. Figure 4.3 (left) shows the mass of the foot, tibia, femur hip and spine from dissected cadaveric rats weighing 250g and 475g. It is clear that variations in the distribution of these masses occur, even within animals of the same weight, as demonstrated by the standard deviations presented, and that these could potentially induce errors into the modeling process when a generic anatomy is assumed for all animals. Figure 4.3 (right) shows changes that occur in the distribution of segment masses between different sized animals. A series of t-tests were carried out to evaluate the difference in the relative mass of the foot, tibia and femur in both sizes of animal. It was found that the foot (p=.012) and femoral segments (p=.029) were significantly larger as a fraction of total body mass in smaller animals. The hip, spine and tibia on the other hand were seen to be relatively lighter in smaller animals, but this did not reach the level of significance in this study (p>0.05). This trend might suggest that when the animals grow, instead of adding mass uniformly (as is assumed by linear scaling methods), the majority of the increased mass is focused around the abdomen and pelvis rather than in the lower limbs.
4.3 Methods

Figure 4.3: (Left): Mass of foot, tibia, femur, hip and spine segments for 250g (n=3) and 475g (n=3) rats. (Right): % difference in relative mass of the foot, tibia, femur, hip and spine segments of 250g (n=3) and 475g (n=3) rats. Relative mass is defined as the mass of the segment relative to body mass.

4.3.3 In Vivo Gait Analysis and Musculoskeletal Modeling

In vivo gait analysis was carried out using the overground walkway as described in Chapter 3 for one male Sprague Dawley rat (475g) running at 34cm s\(^{-1}\). The kinematic and ground reaction force data was processed using Vicon Nexus and prepared as described in Chapter 3 so that it could be used as input data for an existing musculoskeletal model of the rat hindlimb [Johnson et al., 2008] in OpenSim [Delp et al., 2007]. Inverse kinematics, inverse dynamics, static optimization and joint reaction analyses were carried out to evaluate the baseline joint flexion angles, joint flexion moments, muscle forces and joint contact forces respectively.

4.3.4 Sensitivity Analysis

Multi-variate sensitivity analysis was carried out on 86 variables simultaneously using a custom Matlab routine: 38 muscle insertion points, 38 muscle origin points, 5 skin based marker positions, and 5 segment masses. An elementary effects method [Morris, 1991, Sin and Gernaey, 2009] was used to evaluate the percentage change in joint flexion angles, flexion moments, joint contact forces (JCF) and muscle forces caused by variations in skin marker positions, muscle geometries and segment masses. The input variables were initially set to a random value within a predefined range and then one at a time are varied by the defined step size, \( \delta \), in a random direction such that they remain within the predefined sample space. Step sizes for each input variable were calculated by dividing the maximum error in that input variable as determined...
experimentally elsewhere in the study, $\epsilon_i$ by 10. The range or sample space for each input variable, $\epsilon_i$, is taken from the experimental evaluation of marker placement, muscle geometry and segment mass errors described elsewhere in this chapter. After each complete cycle the elementary effect for each variable is calculated by dividing the percentage change in output variable before and after the selected input variable was changed by the step size for that variable. This can be seen in Equation 4.1. After this, the variables are again set to a random position and the cycle repeats. In total n=10 cycles were used for this study in order to fully explore the variable sample space. After completing the analysis the mean elementary effect $\mu^*$ for each variable was quantified using the absolute value of the elementary effect as seen in Equation 4.2.

$$d_i(x) = \frac{y(x_1, x_2, ..., x_i - 1, x_i + \delta_i, x_i + 1, ..., x_n) - y(x)}{\delta_i \ast y(x)}$$ (4.1)

$$\mu^* = \frac{1}{n} \sum_{j=1}^{n} |d_i(X^j)|$$ (4.2)

In this way, $\mu^*$ represents the percentage change in an output variable, $y(x)$, caused by adjusting the input variable by its step size, $\delta_i$. This value was then multiplied by 10 to evaluate the percentage change in an output variable caused by adjusting the input variable by its experimentally calculated error, $\epsilon_i$, giving a better representation of maximum errors in the output variable that might be encountered in vivo.

Changes to the muscle attachment points and segment masses were made by altering the anatomical dataset provided with the OpenSim model. Variations to the marker attachment points were made by changing the marker locations in the C3D file outputted by Vicon Nexus prior to being used as an input to the musculoskeletal model. One animal and one trial were used for this study and all variable changes were applied to post-capture data.
4.4 Results

4.4.1 Baseline Data

*Summary:* Trends in kinematic and inverse dynamic model outputs match well with those found in the literature using other methodologies. Direct validation of static optimization outputs was not possible due to a lack of available data in the literature.

Prior to performing sensitivity analyses, baseline data was collected and compared to the literature to validate that the methodology was producing reliable results. The joint flexion angles, flexion moments and contact forces for the hip knee and ankle are shown in Figure 4.4 below. Where possible data from other techniques used in the literature was used to validate model outputs and is presented in graphical form alongside the baseline data. When considering joint kinematics, the ankle and knee go through flexion at the beginning of the stance phase as the toe makes contact with the ground, followed by extension as the leg extends ready to push off. The knee and ankle then go through a period of flexion as the leg swings forward and then extend again prior to toe down. The hip flexes slightly at the beginning of stance, before extending during the stance phase. After toe off the hip enters a period of flexion as the hind limb moves towards the body during the swing phase. These trends match well with those displayed from the literature, despite differences in the absolute angles. Joint moments also follow trends set out in the literature. When looking at joint flexion moments, the ankle produces an extension moment throughout stance, peaking at about 30% of the way through the stance phase, while the hip produces an initial extension moment followed by a flexion moment as the hip translates cranially through the line of action of the GRF. The knee undergoes an initial flexion moment, which changes to a net extension moment at about 50% of the way through the stance phase. Joint contact forces appear to peak at around 25% of stance for all three joints, with the highest maximum forces seen in the hip joint, followed by the ankle. Patterns of muscle force activation are also presented and differ depending on the muscle group. Quadriceps and Gluteal forces peak at the start and end of stance phase respectively, while hamstring forces appear to have two peak activation points. These occur during early and late stance, with muscle activation throughout the stance phase.
Figure 4.4: Baseline data showing flexion angles, flexion moments, joint contact forces and muscle forces for 1 rat running at 50cm/s. Comparisons to literature data are provided where available.
4.4 Results

4.4.2 Sensitivity Analysis

Effect of Marker Position Error on In-Vivo Joint Kinematics and Kinetics

Summary: Errors in palpation of the knee joint cause the largest variations in all model outputs except muscle forces. Marker position errors have largest effects on hip flexion moments. Ankle flexion moments and joint contact forces do not appear sensitive to marker palpation errors.

Figure 4.5 shows the potential variations in the ankle, knee and hip flexion angles, flexion moments and joint contact forces caused by measured errors in the locations of the foot, ankle, knee, hip and pelvis markers (from expert). It can be seen that all output variables are more affected by inconsistent palpation of the knee joint marker than all other markers. When comparing the errors induced in the ankle, knee and hip output variables it can be seen that there are varying results. Knee flexion angles and joint contact forces are more affected by variations in all five markers than those at the ankle or hip. When looking at joint flexion moments, however, this trend is reversed, with the hip being affected more than the knee or ankle. For all markers and all joints, the errors induced in joint flexion moments are higher than those in joint angles or contact forces, with the hip joint moment experiencing variations of almost 20%.

The sensitivity of muscle forces to marker position errors is shown in Figure 4.6, with the quadriceps being most sensitive to errors in all markers. As before, all muscle forces were most sensitive to changes in knee marker position, driven by the difficulty in being able to consistently palpate this landmark. When considering calf and quadriceps forces, however, the percentage variation caused by inconsistency in ankle marker palpation was higher than that of the knee. For hamstring and gluteal forces this trend is reversed. This could be explained by considering the anatomical geometry of the muscles shown in Table 2.2. The gluteal muscles insert into the femur, far away from the ankle and so the fact it is less sensitive to changes in ankle location might be expected. The calf and quadriceps muscles on the other hand insert into the foot and tibia respectively, much closer to the ankle and so, particularly in the case of the calves, it is easy to understand why they may be more sensitive to variations in the ankle marker position.
Figure 4.5: Variations in the ankle, knee and hip flexion angles, flexion moments and joint contact forces caused by measured errors in the locations of the foot, ankle, knee, hip and pelvis markers.

Figure 4.6: Variations in the hamstring, calf, quadriceps and gluteal force caused measured errors in the locations of the foot, ankle, knee, hip and pelvis markers.
Effect of Muscle Geometry Errors on In-Vivo Joint Kinetics

Summary: Muscle geometry errors have moderate effects on knee and hip joint contact forces (up to 15%) and large effects on some muscle forces (up to 100%).

The sensitivity of joint contact forces to the location of muscle insertion and origin points is shown in Figures 4.7, 4.8 and 4.9. It can be seen that neither ankle, knee or hip joint contact forces were particularly sensitive to variations in individual muscle geometries, with the largest variations being in the hip force. In general, joint forces were more sensitive to changes in the geometry of muscles that were acting around that joint and to muscles whose insertion or origin points were closer to the joint centre of rotation. For example the ankle joint was most sensitive to variations in soleus insertion and origin locations, the knee was most sensitive to variations in the semimembranosus and vastus lateralis, and the hip was most sensitive to variations in the biceps femoris anterior.

Figure 4.7: Variations in average ankle joint contact force during stance phase caused by measured errors in muscle insertion and origin points

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1As the muscle geometries are only used in calculating static optimization based metrics, analysis of potential errors was limited to joint contact forces and muscle forces. Joint kinematics and external moments were not considered as changes in muscle geometries would have no effect.
Figure 4.8: Variations in average knee joint contact force during stance phase caused by measured errors in muscle insertion and origin points.

Figure 4.9: Variations in average hip joint contact force during stance phase caused by measured errors in muscle insertion and origin points.

Figure 4.10: Variations in average calf force during stance phase caused by measured errors in muscle insertion and origin points.
4.4. Results

Figure 4.11: Variations in average quadriceps force during stance phase caused by measured errors in muscle insertion and origin points

Figure 4.12: Variations in average hamstring force during stance phase caused by measured errors in muscle insertion and origin points

Figure 4.13: Variations in average gluteal force during stance phase caused by measured errors in muscle insertion and origin points
Effect of Segment Mass Errors on In-Vivo Joint Kinetics

Summary: Errors in segment masses have small effects on all model outputs\(^2\)

The sensitivity of joint flexion moments, joint contact forces and muscle forces to changes in segment masses was considered and can be seen in Figures 4.14 and 4.15. In general it can be seen that the knee flexion moment is most sensitive to changes in the foot and tibia masses, whereas the hip moment is most sensitive to changes in the femur mass. When considering joint contact forces and muscle forces (Figure 4.15) it can be seen that the model is again fairly insensitive to subtle changes. All output variables showed very little sensitivity to errors in hip or spine masses.

![Figure 4.14: Variations in average ankle, knee and hip joint flexion moments (top) and joint contact forces (below) during stance phase caused by measured errors in foot, tibia, femur, hip and spine mass for 250g animal](image)

\(^2\)As the segment masses are only used in calculating inverse dynamic and static optimization based metrics, analysis of potential errors was limited to flexion moments, joint contact forces and muscle forces. Joint kinematics were not considered as changes in segment masses would have no effect
Figure 4.15: Variations in average calf, quadricep, hamstring and gluteal forces during stance phase caused by measured errors in foot, tibia, femur, hip and spine mass for 250g animal
4.5 Discussion

This study has looked to characterize and evaluate the use of an existing musculoskeletal model of the rat hindlimb. The first step in this was to evaluate whether the outputs of the model matched those found in previous studies in rats, or would be expected based on studies in other animals. When looking at joint kinematics the trends for ankle, knee and hip joint angles follow roughly those seen both in the literature [Bauman and Chang, 2010, Pereira et al., 2006] and in previous treadmill based studies within the group [Eftaxiopoulou et al., 2014]. Joint moments derived from inverse dynamics were also seen to follow trends set out in the literature for both the ankle and hip. Bennett et al (2012), however, showed a knee extension moment throughout stance, similar to that of the ankle, which was not seen in this study. The findings here, however, correlate well with canine studies where a more upright posture is present [Reglety, 2011]. Joint contact forces for the ankle, knee and hip peak at around 30% of stance before decreasing, driven mainly by similar patterns in the raw ground reaction force data. As of yet, there have been no measurements of rat joint forces in the literature and so comparisons are difficult to make. As modeling of in vivo muscle forces has not been carried out in rats it is difficult to compare the results here with those seen elsewhere. A number of studies, however, have used electromyography (EMG) to study the activation of various hindlimb muscles during gait and these should act as a good measure to validate the results seen here. Quadriceps forces follow those shown in EMG studies, with the majority of activation during the initial 2/3 of stance, tailing off as the animal begins to move cranially and prepare for toe off [Gillis and Biewener, 2001, Gillis and Blob, 2001]. Hamstring forces as a whole have not been studied, however there have been studies looking at EMG activation of the biceps femoris. These suggest that biceps femoris activation occurs during the first half of gait, peaking just after toe-off [Gillis and Biewener, 2001]. In this study hamstring forces (of which biceps femoris muscles are major components) appear to be active throughout the stance phase, with peaks both at the beginning and end of stance. When looking at biceps femoris forces separately, however, the pattern of force activation follows that seen in the literature with a peak during early stance, tailing off towards toe-off as can be seen in Figure 4.16. This highlights how looking at individual forces can often be more beneficial than looking at the combined effect of muscle groups such as the hamstrings or quadriceps. Gluteal forces have also not been studied in rats, but studies in cats report strong gluteal activation throughout stance as seen in this study [Yakovenko et al., 2002]
4.5. Discussion

As well as evaluating the effectiveness of a musculoskeletal model to determine key biomechanical parameters during overground motion, this study has sought to analyze potential sources of error in rodent gait analyses that as of yet have not been fully elucidated. By evaluating the magnitude of these potential errors, and carrying out global sensitivity analyses, it has been possible to provide estimates of the magnitude of variations in potential output variables caused by the three types of error considered: joint marker placement, muscle geometry and segment masses. In general, variations in segment masses had very little impact on model outputs, meaning that the use of subject specific data is likely unnecessary and that the use of linear scaling methods to determine the weight of varying sized animals can be assumed without major consequence. If trying to reduce these errors is a priority then regression analyses would need to be carried out for a wider range of animal masses to evaluate the true relationship between the five segment masses and the overall animal mass. One limitation to the sensitivity analysis carried out on segment masses in this study was that when segment masses were altered the overall mass of the animal varied as well. Of course in reality the mass of the animal is constant and increases in the mass of one segment, for example, must be accompanied by an equivalent mass decrease in one or more other segments. Because of the small changes in mass being investigated its unlikely this would have a major impact, however it is something to be considered when evaluating the findings.

Errors caused by variations in the palpation of joint marker positions are far larger. In general, model outputs seem to be affected more by variations in the knee marker position than the other

![Figure 4.16: Biceps femoris muscle force over stance phase](image-url)
four markers, perhaps due to the difficulty in palpation. Both expert and novice experimenters found consistent location of the femoral condyle to be challenging. This pattern has also been seen in studies looking at the dynamic skin artifact error during gait, where the movement of the knee marker relative to the underlying anatomy has the largest net effect on joint kinematics [Filipe et al., 2006]. The magnitude of joint flexion angle variations caused by the palpation errors here are smaller than those caused by dynamic skin artifact errors, with maximum errors of $10^\circ$ and $15^\circ$ at the hip and knee respectively reported [Bauman and Chang, 2010]. It is possible that the use of globally optimized kinematics used in this study helps to reduce the effect of raw errors in marker position and could explain the reduced errors as compared to studies evaluating the errors during gait itself. Previous studies have suggested that the use of such methods could have similar effects, reducing the variation in flexion angles caused by skin artifact errors [Joao et al., 2014]. Either way, it should be considered that in reality both sources of error could be operating simultaneously, with errors in joint palpation accentuating any dynamic errors during gait itself. This suggests that both estimates of the potential effects of error are likely to be best case scenarios, and should be considered as such.

One interesting point when considering the sensitivity of model outputs to errors in marker locations is that joints seem to be sensitive to the position of markers that would not be thought to have an impact. For example the knee joint flexion is sensitive to changes in the foot marker despite the fact that traditionally the knee position would be calculated solely using the three connected marker positions (ankle, knee and hip). This is similar to sensitivity studies in human subjects where the hip angle has been seen to be sensitive to changes in the ankle and foot marker positions [Habachi et al., 2015]. It would be interesting in future studies to compare the sensitivity of a global kinematic approach such as the one used here with a more traditional approach based on the sequential calculation of joint angles.

For all markers, the sensitivity of flexion moments seems to increase as you move to more proximal joints (the hip is more sensitive than the knee, which is more sensitive than the ankle). This could be due to the way in which inverse dynamic calculations are carried out, with forces and moments being calculated firstly at the ankle, then the knee and then the hip. In this way the calculation of the hip moments incorporate all errors at the knee and ankle, whereas the knee only incorporates errors at the ankle. When looking at the joint contact forces, however, this trend is reversed, with variations in marker placement having larger effects on the
knee than the hip or ankle. This is again potentially an effect of how the model calculates these metrics. The flexion moment, as described above, is calculated via a simple inverse dynamics process, whereas the joint contact forces require optimization subject to an objective function in order to be calculated. This optimization takes into account all of the muscles surrounding the joint. Because more muscles surround the knee than the ankle or hip, the calculation of its joint contact force is more complicated which may lead to increased errors.

The scale of variations in output variables caused by marker palpation errors mean that consideration should be given to how these could be reduced. Previous methods to reduce variation in marker placement, at least within the same animal, have used tattoo systems to make permanent marks on the animal at the palpated bony landmarks where the reflective markers are to be applied [Perrot et al., 2009, Thota et al., 2005]. This allows for consistent marker placement during longitudinal studies. The use of such methods should be considered carefully, particularly when gait analyses take place over varying time intervals among aging animals. If the animal increases in size it might be natural for the point on the skin that corresponds to a certain anatomical landmark to translate. The use of tattoo based methods would potentially ignore this translation and lead to erroneous results. Despite this, they should be strongly considered based on the errors seen in this study. Of course, the use of markerless motion capture would eliminate these errors all together and recent studies have used several such methods with great effect [Bauman and Chang, 2010, Bonnan et al., 2016]. The expense and complication of these methods make them unsuitable for all studies, but the analysis presented here has shown that if the aim is to detect subtle changes in output parameters they may be necessary.

Errors in muscle geometries were also large, but overcoming these is significantly more challenging. Because of the relatively large variations found in the insertion and origin locations of certain muscles (as outlined in tables 2.1 and 2.2) the relative importance of variations in these muscles increases when the magnitude of errors are taken into account. This shows how important it is to take into account the magnitude or errors found experimentally and incorporate them into the sensitivity analysis. It may be that the model is incredibly sensitive to changes in a certain input parameter, but if the experimental error of that input parameter is negligible then it may end up having less of an impact on model outputs than another input parameter which is less sensitive but has much larger experimental variation. The effect of variations
in individual muscle geometries is isolated to static optimization methods and so only has an impact on joint contact and muscle forces. For studies in which these are not necessary, inverse dynamics approaches are far simpler and alleviate the need for complex muscle geometries. As yet there have been no studies using in silico musculoskeletal modeling to evaluate joint contact or muscle forces, and so the analysis presented here may have less relevance than that focusing on joint palpation errors. With the development of musculoskeletal rodent models over previous years [Johnson et al., 2008, Johnson et al., 2011, Charles et al., 2016] it is expected that the use of these kind of studies will increase. The data presented here suggests that extreme caution should be used when attempting these methods, particularly when trying to evaluate individual muscle forces, which can display extreme sensitivity to muscle geometry errors.

One limitation of this study is the method of sensitivity analysis chosen. The elementary effects method is very good at providing an estimate as to the sensitivity of a model to large numbers of input parameters, however, it does not provide as thorough an exploration of the sample space as Monte-Carlo based techniques. In this case, however, it seems to have provided a reasonable overview of the model’s sensitivity and produced results which would not be considered unreasonable. The second limitation is that only one trial for one subject was used for the sensitivity analysis. This, however, is a common practice for sensitivity analyses and has been used previously in determining the sensitivity of other musculoskeletal models [Habachi et al., 2015]. Despite the limitations, this study has provided an insight into the utility of musculoskeletal modeling in rodents whilst simultaneously allowed for a greater appreciation of the errors and uncertainties associated with the methods.

After evaluating the errors involved in musculoskeletal modeling of the rat hindlimb and attempting to quantify the effect of these, the next Chapter proceeds to use the techniques to further evaluate the shifts that occur in rodent biomechanics when changing gait speed or inclination. This was both to develop an understanding of rodent gait more systemically and to evaluate whether the model is capable of discerning between healthy and atypical rodent gait in preparation for the pathological OA study presented in Chapter 6.
Chapter 5

Effect of Speed and Inclination on Biomechanics of Rodent Gait

*Parts of this Chapter are in the process of being drafted as a paper prior to submission*

5.1 Chapter Summary

This chapter uses the musculoskeletal model, on which sensitivity analysis was performed in the previous chapter, to evaluate the effect of gait speed and inclination on the biomechanics of rodent gait. The intention was to find out what combination of speed/inclination could be used to overload the medial side of the knee joint in order to accelerate the uniarticular OA induced via the surgical model outlined in Chapter 6. It was found that increasing speed and inclination had significant effects on kinematic, inverse dynamic and static optimization model outputs. Of particular interest, increasing the inclination appeared to increase overall knee joint loading, whereas increasing speed seemed to increase medial knee joint loading preferentially.
### 5.2 Introduction

Exercise regimes have been shown to be very important in the initiation, propagation and/or treatment of disease in a number of different animal models. In rodent models of osteoarthritis for example, studies have demonstrated that exercise can be used to dramatically alter the profile of disease progression and that the type and duration of exercise are key parameters. Very few papers have used exercise as the sole method of generating OA, most likely due to the time it takes to train the animals alongside the experimental time required to exercise the animals to the level required. Pap et al showed clear evidence that excessive exercise alone could lead to degenerative joint changes and that the magnitude of the joint changes were correlated with the level of exercise [Pap et al., 1998]. Histological evaluation of the cartilage alongside MMP-13 immuno-staining showed mild osteoarthritic changes in animals undergoing a moderate exercise regime and moderate osteoarthritis in those undergoing an extreme regime. More recent studies have focussed on the use of exercise to accelerate the progression of other surgical models. Appleton et al used anterior cruciate ligament transection plus partial menisectomy to induce OA in the knee of male rats and then used forced mobilization to accelerate the disease. They found significantly higher rates of OA in the samples that were forced to mobilize post-surgically [Appleton et al., 2007], although this may be an increased inflammatory response.

Despite the deleterious effect of exercise, mild exercise can in fact have beneficial consequences. Galois et al [Galois et al., 2003] were the first to demonstrate that moderate levels of exercise can actually be beneficial to rats after the induction of osteoarthritis, correlating with results of studies in human subjects [Manninen et al., 2001]. They determined that treadmill running at 30 cm/s for 30 minutes for a total distance of 15km over 28 days reduced histological lesion sizes. Further studies expanded on the dose-response properties of mechanical loading, suggesting that while mild and moderate exercise regimes can have beneficial consequences, severe exercise has extreme effects on OA progression, increasing lesion sizes and subchondral bone sclerosis significantly [Galois et al., 2004]. Joint loading can not only reduce the rate of osteoarthritic progression, but also increase the rate of cartilage repair. Takashi et al (2014) evaluated the effect of external knee loading on cartilage repair in rats and found that animals subjected to external loading repaired cartilage defects more effectively than those without [Takahashi et al., 2014]. This has been backed up by a number of more recent studies exploring the positive effects of exercise in preventing or slowing the progression of post-traumatic OA.
[Iijima et al., 2017, Rios et al., 2017, Allen et al., 2017] as well as reducing the burden of pain for animals who are afflicted with knee OA [Cormier et al., 2017]

Despite the knowledge that exercise has a large role to play in both the development and recovery from musculoskeletal disease in rats, there has been little work looking at what kind of exercise has the most pronounced effect. Studies using varying exercise regimes have usually focussed on the duration of exercise as the differentiating factor rather than the speed or inclination under which that exercise is carried out. The effect of walking speed on rodent gait parameters during overground running has been studied previously. Increased treadmill speed in studies of treadmill based rat gait has shown that although the pattern of joint kinematics does not shift substantially, there are significant changes to the maximum and range of hip, knee and ankle flexion angles [Costa et al., 2011]. In other quadripeds, velocity has been shown to be significantly correlated with hindlimb ground reaction forces. Hindlimb braking forces were seen to increase as walking velocity increased suggesting the hind limb is being used to control or prevent over-speeding of the body when running at high speeds [McLaughlin et al., 1996].

Inclination is another way in which the exercise regime could be altered to affect the loading profile on the limbs. In studies other quadrupedal animals such as horses, inclination has shown to play a key role in joint loading and the distribution of forces between the hind and fore limb [Dutto et al., 2004]. Running on an inclined surface was shown to increase hindlimb ground reaction forces and decrease those for the forelimb proportionately. A study by Crook et al sought to evaluate how the musculoskeletal system of horses adapts to positive and negative slopes, with obvious consequences for thoroughbred horse racing. They evaluated the electromyographic (EMG) intensity of six key muscles in the horse as it was made to walk on a treadmill at various speeds and inclinations: gluteus medius (GM), biceps femoris (BF), vastus lateralis (VL), gastrocnemius lateralis (GL) and extensor digitorum longus (EDL). It was found that increasing gait speed increased the EMG intensity for the GM, BF and GL regardless of the inclination. Increasing inclination provoked an increase in all six muscles, whilst running on a decline reduced the EMG intensity of GM and BF significantly [Crook et al., 2010]. This has been backed up in further studies suggesting that the effect of inclination on EMG based muscle activities were greater for inclined walking, with small decreases in the activity of the GM and BF seen during declined treadmill gait [Takahashi et al., 2014].
To date there has been very little research on how varying the exercise regime experienced actually affects the biomechanics of rats and how it can be used to influence the development of biomechanical disease/injury. Although EMG data has been used to evaluate muscle forces, it does not allow for the evaluation of joint moments or forces which are essential for developing a complete understanding of pathological gait variations. In this study we sought to investigate the effect that changing gait speed and inclination had on the kinematics and kinetics of rats. This will provide researchers with a better understanding of how exercise regimes can be altered to provoke specific pathological changes in the lower limb.
5.3 Methodolgy

5.3.1 In Vivo Data Collection

Twelve male Sprague-Dawley rats (400-450g) were used for this experiment. All animals were trained for two weeks as per the methods reported in Chapter 3, with 3 animals being culled at the end of this timepoint as they were still unable to traverse the walkway successfully. Kinematic and ground reaction force data was collected for each of the remaining animals using the methods set out in Chapter 3. For the first day, data was collected for each animal running on the flat walkway as well as on a 10 degree incline and decline. Animals were asked to traverse the walkway until 20 samples had been collected (at varying speeds) or until the animal would no longer traverse the walkway unaided. Two more sessions were carried out on subsequent days where the animals were made to run across level terrain in order to collect as wide a range of gait speeds as possible. Again the sessions continued until a minimum of 20 samples had been collected or the animals refused to traverse the walkway. The number of data samples collected for each animal at the various inclinations can be found in Appendix B.

Effect of Speed

To evaluate the correlation between gait variables and walking speed, the gait of one animal walking over multiple speeds during the three sessions on level terrain was evaluated. Correlations between gait variables and speed were tested using Spearman’s test via a custom Matlab routine.

Effect of Gait Type

For animals running on level terrain, each gait cycle analyzed was labeled as a distinct gait type depending on the speed: walking (30-50cms\(^{-1}\)), trotting (50-70cms\(^{-1}\)) or cantering (70-90cms\(^{-1}\)) [Whishaw and Kolb, 2004]. For each animal, 3 recorded gait cycles were selected for each gait type where the animal made clean contact on the forceplate with its right fore and hind limbs. If three samples could not be found of an animal walking at a certain speed then it was excluded from that group. Visual checks were carried out to ensure that the gait
type was correctly labelled as either walking or trotting. Average ground reaction forces, flexion angles, joint moments, joint contact forces and muscle forces were determined for each animal by averaging the 3 trials. Where statistical comparisons were made between the 3 groups a separate ANOVA analysis was performed using a custom MATLAB routine to evaluate the interaction effect (tests for normality were carried out beforehand to ensure the data was suitable for the statistical tests). Where post-hoc tests are referenced, they were carried out using Bonferroni corrections.

Effect of Inclination

Three samples were chosen at random for each animal at each inclination. Only trials where walking speeds were between 50-60cm/s were selected to avoid variability induced by varying gait speed. A custom MATLAB routine was used to check for significance between the three groups for each variable using a separate ANOVA (with tests for normality being carried out beforehand). Where post-hoc tests are mentioned, Bonferroni corrections were used to account for multiple testing errors.
5.4 Results

5.4.1 Summary Statistics

Summary: Variation in speed as well as maximum speed is higher in animals walking on level terrain than either inclined or declined

Table 5.1 shows the average variation of speed of all 9 subjects as well as the percentage of samples that fall into the three distinct gait types described earlier. It can be seen that during both inclined and declined motion, the range of walking speeds as well as the average speed is slower than for animals traversing on level terrain. This results in animals adopting faster gait profiles more often when walking on flat terrain and fairly rarely on inclined or declined surfaces. This is particularly evident when looking at the percentage of the time animals adopted cantering gait, falling from 13.0% on level terrain to 4.10% and 5.01% on inclined and declined terrain respectively.

Table 5.1: Summary table showing variation of speed characteristics with inclination

<table>
<thead>
<tr>
<th></th>
<th>Uphill</th>
<th>Flat</th>
<th>Downhill</th>
</tr>
</thead>
<tbody>
<tr>
<td>Max Speed (cm/s)</td>
<td>79.5</td>
<td>104</td>
<td>75.9</td>
</tr>
<tr>
<td>Min Speed (cm/s)</td>
<td>26.9</td>
<td>16.1</td>
<td>27.6</td>
</tr>
<tr>
<td>Mean Speed (cm/s)</td>
<td>48.0</td>
<td>56.2</td>
<td>49.3</td>
</tr>
<tr>
<td>Walking (%)</td>
<td>48.1</td>
<td>27.3</td>
<td>45.5</td>
</tr>
<tr>
<td>Trotting (%)</td>
<td>39.0</td>
<td>50.8</td>
<td>45.2</td>
</tr>
<tr>
<td>Cantering (%)</td>
<td>4.10</td>
<td>13.0</td>
<td>5.01</td>
</tr>
</tbody>
</table>

5.4.2 Effect of Speed

Effect of Speed on Ground Reaction Forces

Summary: Increasing speed increases hindlimb vertical GRF’s forelimb braking GRF’s and hindlimb propulsive GRF’s

Figure 5.1 shows the effect of gait speed on vertical ground reaction forces. Both hindlimb and forelimb peak vertical ground reaction forces showed significant positive correlation with gait
Figure 5.1: Effect of gait type on ground reaction forces for animals walking (n=8), trotting (n=9) and cantering (n=7) +/-SEM. * represents points where an ANOVA indicates a significant interaction effect occurs. B1: Peak braking force 0-50% gait; P1: Peak propulsive force 0-50% gait; B2: Peak braking force 50-100% gait; P2: Peak propulsive force 50-100% gait

speed ($R^2=.285$, $p=.002$; $R^2=.526$, $p=.000$). There was also seen to be a significant interaction between gait type and peak hindlimb forces ($p=.002$), but not those of the forelimb ($p=.060$). Hindlimb forces during cantering were significantly higher than those during walking. Analyses of braking forces can also be seen in Figure 5.1, with positive forces representing braking forces directed towards the direction of motion and negative forces representing propulsive forces directed away from the direction of motion. Both limbs go through a period of braking to stabilize the leg as it touches down, followed by a period of propulsion as the animal attempts to transfer its weight forward in the direction of motion. Peak braking and propulsive forces for both limbs are labeled as B1, B2, P1 and P2 respectively. Neither forelimb nor hindlimb peak braking forces were seen to correlate significantly with gait speed ($R^2=.1225$, $p=.054$; $R^2=.001$, $p=.888$). Analysis of the effect of gait type on braking forces however showed significant interaction effects with both the peak forelimb braking force (B1) and the peak hindlimb propulsive force (P2) ($p=.049$; $p=.037$). Animals walking at a canter used their forelimb proportionally more for
braking than animals walking with slower gait profiles, whilst they produced more propulsive force from the hindlimb. Forelimb forces were fairly similar between trotting and walking animals, but walking animals were seen to have lower peak propulsive forces in the hindlimb than those trotting. When considering medio-lateral forces, lateral forces (acting away from the body) are present almost throughout gait for both limbs, with the locations of the maximum and minimum forces for each limb displayed (for the hindlimb minimum forces were calculated excluding the last 10% of the gait cycle). Maximum forelimb forces were not seen to correlate significantly with speed (R²=.000, p=.932), but hindlimb forces showed a significant positive correlation (R²=.265, p=.004). When comparing the differences between walking, trotting and cantering, it was seen that no significant differences occurred in either limb (p>0.05).

**Effect of Speed on Joint Kinematics**

*Summary: Cantering animals have a more extended ankle and a more flexed knee and hip at mid stance*

Figure 5.2 shows the effect of speed on ankle joint angles, with decreasing angles indicating increased flexion and increasing angles indicating increased extension of the joint. In general the ankle goes through flexion followed by extension in both stance and swing. Peak flexion during swing and stance phases (F1 and F2) as well as peak extension (E1) are highlighted on the figures. Average ankle angles during this cycle were shown to be positively correlated with speed (R²=.174, p=.018). The first and second peak flexion angles are similar between all gait types (p=.413), although appear to occur earlier during the gait cycle for cantering animals. The peak extension angle, however, varies significantly between gait types (p=0.046), with cantering animals seeming to extend the angle a lot more than those using slower gait profiles prior to toe-off. Knee flexion followed a similar trend to the ankle, with flexion followed by extension in both the stance and swing phases. Peak flexions (F1 and F2) during the stance and swing phases are separated by peak extension (E1) at the stance-swing transition. Average knee angles were seen to be negatively correlated with speed throughout the gait cycle as a whole (R²=.384, p<.001). Analysis of the waveform as a whole suggests that as animals move into faster gait methods, they extend their knees more during both the stance and swing phase, with significant interaction effects of gait type on joint angles found at F1 (p=0.002), F2 (p=0.014) and E1 (p=0.047). Hip joint angles follow a slightly different pattern to those at the
knee or ankle, with a period of extension during the swing phase leading to the peak extension angle (E1) and a period of flexion during stance (Figure ??). Like at the knee, average hip angles during gait were seen to have a significant negative correlation with speed ($R^2 = .244$, $p = .006$). Analysis of the effect of gait type on hip joint flexion showed it to have a significant interaction effect with the peak extension moment at E1 ($p = .022$). In general the gait types adopted during faster gait resulted in increased hip flexion throughout the gait cycle.

![Figure 5.2: (Top): Effect of gait type on joint flexion angles for animals walking (n=8), trotting (n=9) and cantering (n=7) +/-SEM. represents points where an ANOVA indicates a significant interaction effect occurs. F1: Minimum flexion angle 0-50% gait; E1: Maximum flexion angle 10-90% gait; F2: Minimum flexion angle 50-100% gait.](image)

**Effect of Speed on Joint Moments**

*Summary: Speed increases knee flexion and adduction moments as well as adduction moments at the ankle*

Figure 5.3 shows the effect of inclination and speed on joint flexion moments. Peak ankle joint flexion moments were seen to have a positive correlation with maximum ankle flexion moments during the stance phase ($R^2 = .262, p = .005$). No significant interaction effect was found between
gait type and the peak flexion moment (p=.051), although average peak flexion moments on the canter were higher than those walking. It can also be seen from the graph that the peak flexion moment occurs later during the stance when compared to walking or trotting animals. Maximum knee flexion moments correlated strongly with gait speed ($R^2=.806, p=.000$) and a
significant interaction effect was found between gait type and peak flexion moments \((p=.000)\). Peak extension moments at the start of stance, however, were not significantly affected by gait style \((p=.168)\). Unlike at the knee and ankle, peak flexion moments were not seen to correlate with gait speed \((R^2=.003,p=.751)\). Gait type was found to have no effect on peak extension moments \((p=.188)\), but did have a significant effect on peak flexion moments \((p=.047)\). Walking animals were found to have lower peak flexion moments during early stance than those trotting or cantering.

There was no correlation found between gait speed and peak adduction moments \((R^2=.092, p=.102)\), and gait type was also seen to have no significant interaction \((p=.105)\). Gait type was, however, seen to have a significant effect on minimum adduction moments \((p=.001)\), with cantering animals maintaining higher adduction moments through the second half of the stance phase compared to those walking or trotting. In general the waveforms of animals walking at these slower gait types were fairly similar. Peak knee adduction moments showed a strong positive correlation with gait speed \((R^2=.641,p=.000)\), with gait type having a significant effect on peak moments \((p=.000)\). Post-hoc analyses showed significant differences between animals when walking, trotting and galloping \((p<0.05)\), with galloping animals displaying peak moments almost twice as large as those when walking \((8.7\text{Nmm/BW} \text{ vs } 4.5\text{Nmm/BW})\). Maximum hip adduction moments, unlike at the knee and ankle, showed no correlation with speed \((R^2=.009,p=.781)\) and gait type was shown to have no significant effect on peak moments \((p=.769)\). Observation of the waveforms suggest that there may be differences in late stance, but these were not tested statistically.

**Effect of Speed on Joint Contact Forces**

*Summary:* Speed increases ankle and hip joint contact forces. Overall joint contact forces at the knee are not effected significantly by speed, but speed appeared to increase medial side loading and reduce lateral side loading at the knee joint.

Analysis of the effect of gait speed on joint contact forces can be seen in Figure 5.4. Peak ankle forces were seen to correlate strongly with gait speed \((R^2=.628,p=.000)\), with increasing speed leading to increased angles. A similar trend appears when looking at the peak force of animals walking, trotting and cantering, with significant differences found between the gait
Figure 5.4: Effect of gait type on joint contact forces for animals walking (n=8), trotting (n=9) and cantering (n=7) +/-SEM. * represents points where an ANOVA indicates a significant interaction effect occurs.

types (p=.041). Peak total joint contact forces were not seen to correlate with gait speed ($R^2=.020$, $p=.518$), however peak medial and lateral forces showed strong correlation. Peak medial forces increased with speed ($R^2=.563$, $p=.000$), whilst peak lateral forces decreased with speed ($R^2=.332$, $p=.003$). Gait type was not seen to have any significant interaction effect with total contact forces, but both medial and lateral forces were significantly affected ($p=.000$, $p=.020$). When cantering, animals experienced greater medial knee forces than trotting or walking animals for the majority of stance, with a corresponding decrease in lateral forces. Hip
forces showed significant positive correlation with gait speed ($R^2=.561$, $p=.001$), with cantering animals experiencing greater peak forces than those walking or trotting ($p=.004$).

### Effect of Speed on Muscle Forces

**Summary:** Increasing speed increases maximum gluteal forces, but reduces forces generated by the hamstring and calf muscles.

![Graphs showing muscle forces for different gaits](image)

Figure 5.5: Effect of gait type on muscle forces for animals walking (n=8), trotting (n=9) and cantering (n=7) +/-SEM. * represents points where an ANOVA indicates a significant interaction effect occurs.

The effect of gait speed on muscle forces can be seen in Figure ???. Gluteal forces were seen to increase with increasing gait speed ($R^2=.489$, $p=.000$). Gait type seemed to have a distinct effect on gluteal forces ($p=.001$), with cantering animals having larger peak forces than those walking or trotting and those peak forces occurring much earlier during the stance phase ($p=.048$). Peak forces for cantering animals occurred 42% of the way through the gait cycle compared to 53% for trotting and 77% for walking animals. Hamstring forces showed no correlation with gait.
speed \((R^2=.041, p=.278)\), but were significantly affected by gait type \((p=.049)\). Cantering and walking animals had very similar waveforms, but trotting animals appear to have higher peak forces in early stance. Peak quadriceps forces also showed no significant correlation \((R^2=.187, p=.058)\), but clear changes can be seen in the pattern and timing of activation between walking animals and those adopting faster gait techniques. Walking animals had later peak force activation than those trotting or cantering \((90\% \text{ vs } 23\% \text{ vs } 34\%)\) \((p=.020)\) although the magnitude of that peak force was pretty similar \((p=.162)\). Peak calf forces had no correlation with speed \((R^2=.023, p=.326)\), however cantering animals had a distinct pattern of activation compared to those walking or trotting, including significantly reduced peak forces \((p=.001)\).

### 5.4.3 Effect of Inclination

#### Effect of Inclination on Ground Reaction Forces

*Summary: Increasing inclination reduces forelimb vertical loading, while increasing propulsive forces for both the fore and hind limbs*

Figure 5.6 shows the effect of inclination on ground reaction forces. Forelimb vertical ground reaction forces in general appear to increase with decreasing inclination, with animals moving downhill having significantly larger peak forces than those moving on a inclined surface \((p=.032)\). Analyses of braking forces showed that inclination had a significant effect on braking forces, with significant interaction effects found at B1, P1, B2 and P2 \((p<0.001 \text{ for all})\). The braking force throughout gait seems to get larger as the inclination decreases, with animals walking on a decline using both limbs much more for braking, whereas animals walking on an incline use them much more for propulsion. Inclination had no significant interaction with lateral forces for any points of interest \((p>0.05)\).

#### Effect of Inclination on Joint Kinematics

*Summary: Inclination has no significant effect on joint flexion angles*

Figure 5.7 shows the effect of inclination on joint flexion angles, with decreasing angles indicating increased flexion and increasing angles indicating increased extension of the joint.
Figure 5.6: Effect of inclination on ground reaction forces for animals walking at -10° (n=9), 0° (n=9) or 10° (n=9) inclinations at 50-60cm/s +/-SEM. * represents points where an ANOVA indicates a significant interaction effect occurs.

Inclination had no significant effects on ankle, knee or hip angles (p=.192; p=.405; p=.886). Observationally, however, it appears that the peak ankle and knee extension angle occurs slightly later in animals walking uphill compared to those on level or declined ground.

**Effect of Inclination on Joint Moments**

*Summary:* Inclination has significant effects on ankle, knee and hip adduction and flexion moments throughout stance. Increasing the inclination increases adduction moments during early stance and abduction moments during late stance for all joints. Increasing inclination also increases ankle and hip flexion moments as well as knee joint extension moments.

Figure 5.8 shows the effect of inclination on joint flexion and adduction moments. Inclination was seen to have a significant interaction effect with the peak ankle flexion moments (p=.034), with increased inclination leading to an increased moment, with animals walking uphill having higher flexion moments than those walking on level or declined surfaces. Peak knee flexion
5.4. Results

Figure 5.7: Effect of inclination on joint flexion angles for animals walking at -10° (n=9), 0° (n=9) or 10° (n=9) inclinations at 50-60cm/s +/-SEM. * represents points where an ANOVA indicates a significant interaction effect. F1: Minimum flexion angle 0-50% gait; E1: Maximum flexion angle 10-90% gait; F2: Minimum flexion angle 50-100% gait

and extension moments were also significantly affected by inclination (p=.020, p=.000), with increased extension moments in animals running downhill throughout the entirety of the stance phase. Inclination was also seen to have a profound effect on peak extension (p=.002) and flexion (p=.000) moments at the hip, with peak flexion moments tending to increase with increasing inclination. Despite this, post-hoc analyses found no differences between declined and level motion (p>0.05), indicating that animals walking on an incline may be adopting specifically optimized gait patterns.

Both maximum (p=.005) and minimum (p=.002) ankle adduction moments were seen to be significantly affected by inclination. Animals walking on inclined and declined surfaces displayed higher peak adduction during the first half of gait compare to those on level surfaces. During the second half of gait, animals walking on flat and declined surfaces have similar waveforms, but on inclined surfaces the adduction moment decreases dramatically and becomes a net abduction moment as the ankle shifts inside the GRF line of action. As with the ankle,
Figure 5.8: Effect of inclination on joint flexion and adduction moments for animals walking at -10° (n=9), 0° (n=9) or 10° (n=9) inclinations at 50-60cm/s +/-SEM. * represents points where an ANOVA indicates a significant interaction effect occurs. The minimum adduction moment varied significantly between the inclinations (p=.008), with animals walking on an inclined surface experiencing large net abduction moments leading towards toe-off. Inclination was seen to have a significant effect on peak hip adduction moments during stance (p=.005), with animals walking on an inclined surface experiencing the highest
peak moments (12.4Nmm/BW) and animals walking on a declined surface experiencing the lowest (8.31 Nmm/BW). Peak moments also seem to occur later during the stance phase for rats walking uphill than for those on level or declined surfaces (27% vs 21%).

**Effect of Inclination on Joint Contact Forces**

*Summary: Increasing inclination increases overall joint contact forces at the knee as well as medial and lateral forces*

Analysis of the effect of inclination on ankle joint contact forces can be seen in Figure 5.9. Inclination, was not seen to have any effect on ankle (p=.444) or hip (p=.195) joint contact forces with similar waveforms seen for all three inclinations studied. Inclination did, however, have a significant effect on total knee joint forces (p=.001), as well as medial (p=.007) and lateral forces (p=.006). Animals walking on an incline had higher overall knee joint contact forces than animals walking on declined or level surfaces (p=.004) as well as higher medial (p=.001) and lateral forces (p=.002).

**Effect of Inclination on Muscle Forces**

*Summary: Increasing inclination increases hamstring forces whilst decreasing quadriceps forces*

Inclination had no effect on peak gluteal forces (p=.205) or calf forces (p=.560), however it is clear from the waveforms that the timing of peak forces is affected, with peak gluteal activation occurring later in stance for declined runners (p=.011). Hamstring (p=.0000) and quadriceps (p=.043) forces, however, were significantly affected by inclination, with increasing the inclination increasing hamstring activation and decreasing quadriceps activation.

**5.4.4 Summary Tables**

The tables displayed on the following pages display a tabulated summary of the results presented this far. Where significant interaction effects have been found between a variable and either gait type or inclination they have been highlighted in the tables.
Figure 5.9: Effect of inclination on joint contact forces for animals walking at -10° (n=9), 0° (n=9) or 10° (n=9) inclinations at 50-60cm/s +/-SEM. * represents points where an ANOVA indicates a significant interaction effect occurs.
5.4. Results

Figure 5.10: Effect of inclination on muscle forces for animals walking at -10° (n=9), 0° (n=9) or 10° (n=9) inclinations at 50-60cm/s +/-SEM. * represents points where an ANOVA indicates a significant interaction effect occurs.
Table 5.2: Summary table showing effect of gait type on output variables (SD). Shaded values depict where gait type has had significant (p<0.05) main effects on output parameters

<table>
<thead>
<tr>
<th>Walk</th>
<th>Trot</th>
<th>Canter</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vertical GRF (N/BW)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fore Max</td>
<td>0.517 (0.07)</td>
<td>0.563 (0.09)</td>
</tr>
<tr>
<td>Hind Max</td>
<td>0.584 (0.07)</td>
<td>0.686 (0.12)</td>
</tr>
<tr>
<td><strong>Braking Force (N/BW)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fore Max</td>
<td>0.073 (0.03)</td>
<td>0.064 (0.06)</td>
</tr>
<tr>
<td>Fore Min</td>
<td>-0.064 (0.02)</td>
<td>0.056 (0.04)</td>
</tr>
<tr>
<td>Hind Max</td>
<td>0.038 (0.03)</td>
<td>0.056 (0.04)</td>
</tr>
<tr>
<td>Hind Min</td>
<td>-0.073 (0.02)</td>
<td>-0.101 (0.05)</td>
</tr>
<tr>
<td><strong>Lateral Force (N/BW)</strong></td>
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<tr>
<td>Fore Max</td>
<td>0.056 (0.02)</td>
<td>0.052 (0.02)</td>
</tr>
<tr>
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<td>-0.008 (0.01)</td>
</tr>
<tr>
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<td>0.125 (0.04)</td>
</tr>
<tr>
<td>Hind Min</td>
<td>0.000 (0.00)</td>
<td>-0.005 (0.01)</td>
</tr>
<tr>
<td><strong>Ankle Angle (°)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F1</td>
<td>48.19 (6.17)</td>
<td>50.55 (5.63)</td>
</tr>
<tr>
<td>E1</td>
<td>78.20 (9.07)</td>
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</tr>
<tr>
<td>F2</td>
<td>36.70 (2.11)</td>
<td>38.41 (3.22)</td>
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<tr>
<td><strong>Knee Angle (°)</strong></td>
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<td></td>
</tr>
<tr>
<td>F1</td>
<td>72.21 (10.2)</td>
<td>62.30 (11.4)</td>
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<tr>
<td>E1</td>
<td>100.2 (10.1)</td>
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<tr>
<td>F2</td>
<td>54.45 (11.8)</td>
<td>45.96 (5.03)</td>
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<tr>
<td><strong>Hip Angle (°)</strong></td>
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</tr>
<tr>
<td>E1</td>
<td>63.26 (11.9)</td>
<td>55.79 (8.21)</td>
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<tr>
<td><strong>Peak Joint Moment (Nmm/BW)</strong></td>
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<td></td>
</tr>
<tr>
<td>Ankle Flx</td>
<td>12.34 (2.63)</td>
<td>13.64 (3.92)</td>
</tr>
<tr>
<td>Knee Ext</td>
<td>2.009 (2.69)</td>
<td>1.427 (2.52)</td>
</tr>
<tr>
<td>Knee Flx</td>
<td>8.491 (3.39)</td>
<td>10.64 (2.07)</td>
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<td>Hip Ext</td>
<td>5.491 (3.12)</td>
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<td>Hip Flx</td>
<td>11.35 (3.51)</td>
<td>16.32 (2.64)</td>
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<td>Ankle Abd</td>
<td>0.014 (0.91)</td>
<td>0.456 (0.54)</td>
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<td>Hip Add</td>
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<td>9.720 (1.82)</td>
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<td><strong>Peak JCF (N/BW)</strong></td>
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<tr>
<td>Ankle</td>
<td>0.588 (0.06)</td>
<td>0.685 (0.11)</td>
</tr>
<tr>
<td>Knee-Total</td>
<td>0.933 (0.21)</td>
<td>1.069 (0.30)</td>
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<td>Knee-Medial</td>
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<td>0.513 (0.16)</td>
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<td>Knee-Lateral</td>
<td>0.553 (0.29)</td>
<td>0.609 (0.17)</td>
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<tr>
<td>Hip</td>
<td>2.505 (0.41)</td>
<td>2.788 (0.64)</td>
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<tr>
<td><strong>Peak Muscle Force (N/BW)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hamstrings</td>
<td>1.225 (0.29)</td>
<td>1.517 (0.56)</td>
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<tr>
<td>Quadriceps</td>
<td>0.095 (0.03)</td>
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<tr>
<td>Gluteals</td>
<td>0.670 (0.50)</td>
<td>0.620 (0.49)</td>
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<tr>
<td>Calves</td>
<td>0.002 (0.00)</td>
<td>0.001 (0.00)</td>
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</tbody>
</table>
Table 5.3: Summary table showing effect of inclination on output variables (SD). Shaded values depict where inclination has had significant (p < 0.05) main effects on output parameters.

<table>
<thead>
<tr>
<th></th>
<th>Flat</th>
<th>Incline</th>
<th>Decline</th>
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</thead>
<tbody>
<tr>
<td><strong>Vertical GRF (N/BW)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fore Max</td>
<td>0.551 (0.09)</td>
<td>0.491 (0.06)</td>
<td>0.625 (0.05)</td>
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<tr>
<td>Hind Max</td>
<td>0.632 (0.08)</td>
<td>0.683 (0.10)</td>
<td>0.653 (0.06)</td>
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<tr>
<td><strong>Braking Force (N/BW)</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Fore Max</td>
<td>0.048 (0.02)</td>
<td>-0.041 (0.04)</td>
<td>0.147 (0.03)</td>
</tr>
<tr>
<td>Fore Min</td>
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<td>-0.082 (0.04)</td>
<td>0.138 (0.04)</td>
</tr>
<tr>
<td>Hind Max</td>
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<td>-0.063 (0.03)</td>
<td>0.122 (0.05)</td>
</tr>
<tr>
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<td>-0.200 (0.06)</td>
<td>-0.025 (0.02)</td>
</tr>
<tr>
<td><strong>Lateral GRF (N/BW)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fore Max</td>
<td>0.044 (0.02)</td>
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<td>0.062 (0.02)</td>
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<td>-0.003 (0.01)</td>
</tr>
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<td><strong>Ankle Angle (°)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F1</td>
<td>49.36 (4.16)</td>
<td>46.75 (4.51)</td>
<td>46.82 (5.59)</td>
</tr>
<tr>
<td>E1</td>
<td>76.96 (12.2)</td>
<td>86.38 (5.85)</td>
<td>81.00 (8.37)</td>
</tr>
<tr>
<td>F2</td>
<td>37.15 (2.67)</td>
<td>38.08 (2.37)</td>
<td>38.39 (1.96)</td>
</tr>
<tr>
<td><strong>Knee Angle (°)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F1</td>
<td>61.57 (12.7)</td>
<td>57.34 (5.63)</td>
<td>68.41 (22.5)</td>
</tr>
<tr>
<td>E1</td>
<td>86.52 (16.2)</td>
<td>88.64 (6.58)</td>
<td>92.76 (28.1)</td>
</tr>
<tr>
<td>F2</td>
<td>45.89 (5.95)</td>
<td>44.54 (6.65)</td>
<td>51.29 (10.1)</td>
</tr>
<tr>
<td><strong>Hip Angle (°)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E1</td>
<td>102.9 (9.90)</td>
<td>103.0 (8.50)</td>
<td>105.4 (13.2)</td>
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<td><strong>Peak Joint Moment (Nmm/BW)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ankle Flx</td>
<td>11.09 (2.06)</td>
<td>14.7 (1.21)</td>
<td>11.54 (3.09)</td>
</tr>
<tr>
<td>Knee Ext</td>
<td>1.612 (1.67)</td>
<td>8.035 (2.63)</td>
<td>2.680 (2.02)</td>
</tr>
<tr>
<td>Knee Flx</td>
<td>9.487 (1.61)</td>
<td>6.002 (1.43)</td>
<td>8.350 (2.11)</td>
</tr>
<tr>
<td>Hip Ext</td>
<td>6.886 (2.24)</td>
<td>3.088 (1.67)</td>
<td>7.753 (2.06)</td>
</tr>
<tr>
<td>Hip Flx</td>
<td>11.46 (3.72)</td>
<td>23.48 (5.31)</td>
<td>9.588 (4.92)</td>
</tr>
<tr>
<td>Ankle Abd</td>
<td>1.564 (0.51)</td>
<td>3.609 (2.46)</td>
<td>1.086 (0.41)</td>
</tr>
<tr>
<td>Ankle Add</td>
<td>0.928 (0.86)</td>
<td>3.761 (2.77)</td>
<td>2.297 (0.95)</td>
</tr>
<tr>
<td>Knee Abd</td>
<td>0.546 (0.93)</td>
<td>3.554 (2.29)</td>
<td>-0.125 (0.52)</td>
</tr>
<tr>
<td>Knee Add</td>
<td>4.070 (1.63)</td>
<td>5.385 (2.68)</td>
<td>4.462 (1.63)</td>
</tr>
<tr>
<td>Hip Add</td>
<td>8.285 (1.61)</td>
<td>12.44 (3.09)</td>
<td>9.980 (1.61)</td>
</tr>
<tr>
<td><strong>Peak JCF (N/BW)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ankle</td>
<td>0.632 (0.08)</td>
<td>0.684 (0.09)</td>
<td>0.652 (0.07)</td>
</tr>
<tr>
<td>Knee-Total</td>
<td>0.893 (0.14)</td>
<td>1.289 (0.18)</td>
<td>0.848 (0.12)</td>
</tr>
<tr>
<td>Knee-Medial</td>
<td>0.434 (0.09)</td>
<td>0.627 (0.22)</td>
<td>0.437 (0.17)</td>
</tr>
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<td>Knee-Lateral</td>
<td>0.518 (0.09)</td>
<td>0.726 (0.12)</td>
<td>0.467 (0.10)</td>
</tr>
<tr>
<td>Hip</td>
<td>2.465 (0.50)</td>
<td>3.061 (0.55)</td>
<td>2.584 (0.58)</td>
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<tr>
<td><strong>Peak Muscle Forces (N/BW)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hamstrings</td>
<td>1.279 (0.40)</td>
<td>2.451 (0.41)</td>
<td>1.081 (0.44)</td>
</tr>
<tr>
<td>Quadriceps</td>
<td>0.131 (0.06)</td>
<td>0.110 (0.05)</td>
<td>0.135 (0.03)</td>
</tr>
<tr>
<td>Gluteals</td>
<td>0.670 (0.50)</td>
<td>0.620 (0.49)</td>
<td>1.152 (0.61)</td>
</tr>
<tr>
<td>Calves</td>
<td>0.002 (0.00)</td>
<td>0.003 (0.00)</td>
<td>0.002 (0.00)</td>
</tr>
</tbody>
</table>
5.5 Discussion

5.5.1 Effect of Speed

*Increasing Speed:*

- Increases ankle extension as well as hip and knee flexion angles
- Increases knee flexion and adduction moments
- Increases ankle and hip JCF
- Increases medial knee JCF and reduces lateral knee JCF
- Increases gluteal forces whilst reducing hamstring and calf forces

In this study, the relationship between gait speed and inclination on the biomechanics of rat gait has been investigated. Understanding the role these play on rat kinetics and kinematics during gait will allow for a greater understanding of rodent motion in general and act as a reference for future researchers performing musculoskeletal modeling in rats. Gait speed and type (walking, trotting, cantering) were seen to have significant effects on almost all model outputs, highlighting the need for them to be considered carefully, particularly in overground studies where gait speed is more difficult to control. When looking at ground reaction forces the only major change was in hindlimb vertical loading, which increased with gait speed as expected. Joint angles, however, varied more with gait speed. For the hip and knee, the average joint angle tended more towards extension throughout gait, which is a similar trend to that seen in previous studies [Costa et al., 2011], whilst the ankle seemed to be more flexed at faster speeds. These kinematic changes lead to significant changes in the dynamic joint moments present at all three joints, with both ankle and knee flexion moments increasing with speed. The combination of changes in kinematics and joint moments suggest that the animal leans forward more during faster gait types, extending the hip and ankle as the knee moves cranially. This movement of the knee significantly increases the flexion moment experienced. Interestingly, the knee adduction moment is also significantly increased, suggesting that the foot is positioned more medial to the knee during faster motion, perhaps to allow for greater power generation from the hind limb. Gluteal and quadriceps forces also increase with speed,
whilst hamstring and calf forces either do not change or actually decrease. The same applies when looking at cantering rats vs those walking or trotting. This suggests a shift to reliance on the larger gluteal and quadriceps muscles for power generation during faster gait types as the animal starts to priorities speed over gait efficiency. Studies in dogs using EMG data to evaluate muscle activation during walking, trotting and cantering have also shown that gluteal and quadriceps activation increases in the transition between trotting and cantering [Deban et al., 2012].

5.5.2 Effect of Inclination

Increasing Inclination:

- Reduces forelimb vertical GRF
- Increases knee extension moments as well as ankle and hip flexion moments
- Increases overall knee joint loading
- Increases Hamstring loading

Inclination was also seen to have significant impacts on most model outputs. Unlike gait speed, however, there were no significant changes in joint kinematics so the majority of the kinetic changes seen within the joints must have been caused by changes in the ground reaction forces. As in previous studies of quadriceps and humans, the largest changes when switching from inclined to declined walking are in the braking forces. In this study we saw that braking forces in both limbs reduced with increasing inclination as has been shown previously in other animal models [Arnold et al., 2013, Lammers et al., 2006, Birn-Jeffery and Higham, 2014] as well as in human studies [Redfern and DiPasquale, 1997]. When walking on inclined surfaces, the rat has to push against gravity and so uses its limbs predominantly for propulsion as no braking is required. When moving downhill, however, the rat needs to use its limbs to control movement speed and so uses them much more for braking. It can be seen in this model that this alteration in the use of the limb has significant effects on internal joint kinetics, including joint moments, joint contact forces and muscle forces. Some of the largest changes were seen in joint flexion moments, where inclined motion seemed to produce significantly different waveforms
compared to flat or declined gait. Ankle and hip flexion moments as well as knee extension moments during the first half of gait were seen to be larger during inclined gait. This suggests that the line of action of the ground reaction force is directed more cranially as the animal pushes against the direction of motion to propel itself forward and up the slope. Increased knee extension moments during inclined running have also been seen in human studies [Redfern and DiPasquale, 1997]. Knee and ankle adduction moments are also markedly different for inclined motion, with increased increased joint abduction moments during the second half of gait for both joints. When looking at muscle forces the most significant changes can be seen in the hamstring forces, with increased force generation in inclined gait and decreased forces in declined gait compared to on level surfaces similar to that seen in horses [Crook et al., 2010]. Decreases in gluteal forces during downhill motion, as seen in studies in horses [Crook et al., 2010, Takahashi et al., 2014], were not seen in this study during the stance phase as a whole suggesting that gait adaptions to declined surfaces may vary between species.

5.5.3 Medial Joint Contact Forces and Relationship to OA

Part of the motivation for this study was to attempt to determine whether altering the exercise regime could be used to alleviate or accelerate the progression of OA via alterations in knee joint loading, specifically an increase in medial knee joint contact forces. Previous investigations looking at the effect of exercise on OA in rats have focused on exercise duration, finding that increasing the duration of exercise will increase the severity of OA [Galois et al., 2004, Yamaguchi et al., 2013]. The effect of varying speed, however, has never been investigated in rats. In this study gait speed was seen to be positively correlated with peak medial side forces, whilst a negative correlation was seen with lateral forces suggesting a redistribution of knee joint contact forces to the medial condyle. These results suggest that running the animals at faster speeds for a fixed period of time could be another viable method for increasing the medial knee JCF and initiating or accelerating OA progression. The fact that lateral forces are also decreased at these faster speeds would potentially make it preferable for inducing uni-articular OA representative of the human condition where medial side cartilage erosion occurs in the absence of significant lateral damage. Inclination was also seen to have an effect on knee joint loading in this study, with inclined motion inducing higher peak medial and lateral joint contact forces. This could be used in combination with increased gait speed to both increase loading on
the knee in general via inclination and increase medial loading specifically via increased speed.

Increasing knee adduction moments and medial joint contact forces have been shown to be precursors to osteoarthritis in humans and to have a strong correlation with each other. For this reason external knee adduction moments are often used as an analogue for medial joint contact forces in human clinical trials to remove the need to calculate the forces directly using complex static optimization techniques [Simic et al., 2011, Radzimski et al., 2012]. In order to delve further into this relationship and its applicability to rodent studies, Figure 5.11 below looks into the effect of external adduction moments on medial joint contact forces for different inclinations. It is clear that for all three inclinations there is a positive correlation between peak adduction moments and medial JCF’s, however the relationship between them seems to vary depending on the inclination. Linear regression analysis was carried out to determine the y-insertion point, gradient and correlation coefficient of the three correlations to explore these differences (Table 5.4). It can be seen that the correlation between knee adduction moments and medial JCF’s is quite weak even for flat terrain ($R^2=.454$, $p<.001$) suggesting that caution may be needed when trying to use the knee adduction moment as an analogue for the medial JCF. On a declined surface the correlation improves ($R^2=.627$, $p<.001$), but is still not as high as might be expected. In human studies looking at the relationship between these two measures, positive correlations have also been seen, but again these correlations were only moderate ($R^2=.560$) [Kutzner et al., 2013].

![Figure 5.11: Correlation between peak medial JCF’s and peak knee adduction moments](image)

Further differences can be noticed when looking at the gradient of the regression lines, with the gradient seemingly decreasing with increasing inclination. This suggests that the relationship between adduction moments and JCF’s may not be fixed during abnormal gaits, suggesting
Table 5.4: Results from linear regression analysis looking at relationship between peak medial JCF and external adduction moment.
a: gradient of slope
b: y-intercept
r²: Correlation coefficient

<table>
<thead>
<tr>
<th>Slope Type</th>
<th>a</th>
<th>b</th>
<th>r²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Declined</td>
<td>0.060</td>
<td>0.299</td>
<td>0.627</td>
</tr>
<tr>
<td>Flat</td>
<td>0.040</td>
<td>0.462</td>
<td>0.454</td>
</tr>
<tr>
<td>Inclined</td>
<td>0.028</td>
<td>0.595</td>
<td>0.423</td>
</tr>
</tbody>
</table>

that for studies in which the local knee joint biomechanics are changed such as surgical destabilization models, the use of adduction moments to compare between healthy and impaired animals may not be reasonable. These variances between gait type, as well as the lack of strong correlation between knee adduction moments and medial JCF’s mean that inverse dynamics techniques alone may not be reasonable to estimate the effect of either treatment or intervention on rodent medial JCF’s and that they should be calculated directly using static optimization methods such as those described in this thesis. Chapter 6 uses these techniques to evaluate the changes in joint loading induced via a novel musculoskeletal model of OA in rats. This is used to develop an understanding of how these quantifiable changes in joint kinetics effect disease development.
Chapter 6

Novel In-Vivo Biomechanical Model of Knee OA in Rats

*Parts of this Chapter have been drafted as a paper prior to submission* ¹

6.1 Chapter Summary

This chapter aimed to detail the development of a novel model of malalignment induced OA in rats. It was found that a surgical varus malalignment of the tibia induced histological osteoarthritic changes in the medial tibial compartments of rat knees 14 weeks post-surgically, potentially caused by quantifiably increased medial joint loading. The model was also validated using CTX-II, a serum marker of enzymatic cartilage degradation, which was seen to be up-regulated in animals with a varus tibial malalignment post-surgically. This novel model of the disease should help to better characterize the distinct pathophysiology of mechanical OA and lead to the development of phenotype specific treatment or rehabilitation options.

¹Britzman, D., Eftaxiopoulou, T., Igah, I., Macdonald, W., Bull, AMJ. (2017). Tibial Osteotomy as a Mechanical Model of Primary Osteoarthritis in Rats
6.2 Introduction

The background to this chapter has been reviewed in detail in Chapter 2, Section 2.2. The following introduction expands upon concepts from that section to put this Chapter in context.

Osteoarthritis is a complex, multi-factorial disease which has both mechanical and biological effects on the whole synovial joint. It is characterized by severe damage to load bearing regions of articular cartilage, excess bone formation at the edges of the joints (osteophytosis), alterations to the morphology and structure of the subchondral bone, synovitis and often inflammation or thickening of the joint capsule itself [Dieppe and Lohmander, 2005]. The disease is often separated into two distinct subtypes: primary and secondary, with secondary OA being defined as that occurring secondary to a known affliction such as an Anterior Cruciate Ligament (ACL) rupture. Primary OA occurs mainly in elderly patients, developing gradually over time, and represents 82% of OA patients [Kuyinu et al., 2016]. Because of the complex, multi-tissue nature of the disease it has proven hard to isolate the exact causes for these degenerative changes in primary OA; however it is likely to be a combination of many. Mechanical factors have long been known to play a role in the development of the disease [Baliunas et al., 2002, Thorp et al., 2006], and recent studies have proposed mechanical OA to be a distinct pathological phenotype of primary OA, often related to tibial malalignment [DellIsola et al., 2016]. Studies have also postulated a large inflammatory aspect to the disease [Berenbaum, 2013, Rahmati et al., 2016, Robinson et al., 2016]. It has been found that the progressive degeneration of the cartilage stimulates chondrocyte production, which helps to repair the tissue. When the process of cartilage destruction is faster than the attempted repair, further erosion and cracking of the cartilage ensues, leading to damage in deeper layers of the cartilage and even subchondral bone [Kuyinu et al., 2016]. This destruction of the cartilage is caused by enzymes called matrix metalloproteinases (MMP’s) which are produced by synovial cells and chondrocytes. Inhibition of these proteins is an area under investigation for the development of new anti-arthritic medications [Tchetverikov et al., 2005].

Because of the difficulty in recruiting patients during the early, asymptomatic phase of disease pathogenesis, a variety of animal models have been developed to attempt to model the disease. The most commonly used methods tend to be surgically induced either using ACL transection (ACLT) [Marijnissen et al., 2002, Pond and Nuki, 1973] or medial meniscal tear (MMT)
Both of these models involve surgical destabilization of the joint, leading to altered internal loading conditions in the joint and eventually OA. The issue with these models is that the intra-articular surgery itself can often lead to unintended damage to other important joint tissues, potentially triggering unwanted inflammatory responses [Lafeber and Mastbergen, 2009]. Pure primary models of OA have tended to rely either on genetically inducing biological changes to increase the incidence of disease or utilizing a small subset of animals that develop OA spontaneously such as the Hartley guinea pig [Bendele and Hulman, 1988]. Studies looking at the histopathology of the joint at 18 months and 3 years post surgically found biochemical changes which reflected reasonably well those present in human patients suggesting that they may hold translational benefits [Jimenez et al., 1997]. The downside, however, is that the long disease development time alongside relatively low incidence rates of the disease make them unsuitable for any large scale therapeutic studies [Little and Smith, 2008].

One methodology that has received fairly little interest of late is the tibial osteotomy model, whereby the tibia is reset at a varus (or valgus) angle to increase the mechanical loading on the medial (or lateral) side of the knee joint without the need for intra-articular surgery or destabilization of internal joint mechanics, potentially allowing it to be used as an induced model of mechanical disease. This methodology is potentially more analogous to mechanical OA phenotypes in humans, where alterations in external loading caused by misalignment of the tibia are known to lead to OA without any internal damage to the joint itself [Sharma, 2007]. This is backed up by the fact that surgical interventions to realign the tibia have been shown to reduce or inhibit the development of OA on the medial side of the knee [Benzakour et al., 2010]. Previous models showing the pathogenesis of OA in response to surgically induced tibial angulation have mainly focused on the rabbit, as its large size (relative to rodents) simplifies the surgical procedure. In these cases angles of up to 30 degrees valgus have been used to overload the lateral tibial compartment and have been shown to lead to mild degenerative changes in the cartilage suggestive of early stage OA 12 weeks post-surgically [Wu et al., 1990, Goodman et al., 1991] In studies on dogs even 60 weeks post-surgically no evidence of OA was reported. The lack of substantive interest in these models can be partly related to the species chosen. Practical considerations have led to the decision to use larger mammals, however, the biggest demand for models of OA is in rodents, due to their low price, space requirements and ease of access as well as the ease with which genetic knock-out models can be developed [Bendele, 2001].
The exact effect that changing biomechanics have on the medial loading are also unknown in these studies, making it hard to translate the results to a clinical context.

Evaluation of potential biological markers for OA is an ever expanding field with a number of prognostic and diagnostic markers currently under investigation [Bay-Jensen et al., 2016]. C-telopeptide of type II collagen (CTX-II) has perhaps had the most success as a diagnostic biomarker, having been shown to correlate strongly with OA progression in both human subjects [Sharif et al., 2007, Garnero et al., 2002] and in animal models of the disease [Duclos et al., 2010]. As articular cartilage is disrupted during the course of the disease, proteoglycans and type II collagen are deposited in the synovium, and increases in the level of this type II collagen can be measured via serum CTX-II levels [Duclos et al., 2010]. Cartilage oligomeric matrix protein (COMP), a marker of extracellular matrix breakdown, has also been proposed as a marker of disease progression in osteoarthritic patients although its utility as a pure marker of cartilage damage is more debatable. Studies in humans [Attur et al., 2013] and animals [Larsson et al., 2002] have shown upregulation of the cytokine in arthritic subjects, but the fact that COMP can be secreted by almost all synovial tissues means there is a lack of specificity as to where it originated [Di Cesare et al., 1997]. It is possible, as some have suggested, that serum COMP levels are affected more by pro-inflammatory responses in the synovium than by extracellular matrix breakdown in the cartilage [Zivanovi et al., 2011]. This may explain why the biomarker has been shown to correlate well with levels of rheumatoid arthritis, but not so well with radiographic levels of knee OA [Kim et al., 2016]. Markers of bone turnover like C-terminal type I collagen telopeptide (CTX-I) have also been proposed as diagnostic markers of OA [Bettica et al., 2002], with the theory being that they are able to monitor levels of subchondral bone resorption in progressive disease. Studies of their potential as diagnostic disease indicators, however, are limited.

In this chapter, a novel model of malalignment induced OA in rats is proposed based on a surgically induced varus tibial misalignment. A biomechanical model is used to evaluate the levels of knee joint loading pre and post surgery to quantify the specific alterations in local joint biomechanics that lead to medial knee OA. Serum levels of CTX-I, CTX-II and COMP are investigated post-surgically both to validate the presence of OA and to evaluate the potential use of these biomarkers as indicators of disease progression.
6.3 Methods

6.3.1 Animal Ethics and Approval

This study was performed under full institutional and departmental license with ethic committee and Home Office approval. Euthanasia was performed under license by overdose intraperitoneal (IP) injection of pentobarbitone. Animals were provided food and water ad libidum except where specific exemptions were approved under a home office license.

6.3.2 Animal Selection, Husbandry and Training

In total 22 animals were used (10 for the trial, 8 were culled post-training and 4 were culled post-surgically due to either sub-optimal osteotomies or surgical complications). 22 healthy male Sprague-Dawley rats (12 weeks old) were used in this study. The animals were placed in quarantine for 1 week prior to being handled, during which time cheerios (Nestlé, Vevey, Switzerland) were provided daily to familiarize the animals. The animals were then trained for 2 weeks to traverse a 1.5m long walkway (FIG) following the guidelines set out in Chapter 3. Throughout this time the animals were restricted to maintenance energy requirements to prevent excessive weight gain and to allow the cheerios to provide an incentive to train. Following this training, the animals were not subjected to the training regime again for the duration of the study. At the end of 2 weeks any animals which could not successfully traverse the runway were culled using an appropriate schedule 1 method (8/22 animals). The remaining 14 animals were allocated into two groups, one group of seven to undergo varus osteotomy, and one sham group of seven. Following surgical complications this was eventually reduced to five animals in both the osteotomized and sham groups. Animals were kept in groups of 2-3 in 1500cm$^2$ individually ventilated cages throughout the study, except for one week post-surgically where they were single caged to allow for effective recovery and monitoring.
6.3.3 Surgical Methods

**Osteotomy**

In order to increase the loading on the medial compartment of the knee, surgical osteotomies were performed on male Sprague-Dawley rats. Before commencing the surgery all equipment was autoclaved in order to ensure sterility. Although possible to perform the surgery with one person, in this study two were used, one as a non-sterile ‘nurse’ and the other as a sterile ‘surgeon’\(^2\). This not only reduces the duration of the surgery but also reduces the risk of infection by ensuring the surgeon stays sterile at all times. After checking that all the equipment was available the room was divided into a recovery and surgical area so that a sterile surgical field could be maintained throughout surgery. The animal was anesthetized in an inhalation chamber using 5% Isoflurane in O\(_2\) and then placed on a heating pad in the non-sterile area with anaesthesia maintained using an inhalation mask at 2% Isoflurane. Before preparing the animal for surgery, pre-emptive analgesia and antibiotics were administered subcutaneously as follows: 0.05mg/kg Buprenorphine and 10mg/kg Baytrill. The animal was clipped liberally around the right leg and then cleaned using clorhexidine applied to sterile gauze. The animal, heat pad and inhalation mask were then moved to the surgical table and final skin cleansing performed. At this point (or preferably before) the surgeon underwent a standard ‘gown-up’ procedure, first washing their hands and forearms before being handed a sterile gown and then sterile gloves. The surgeon is then deemed to be sterile and care must be taken to avoid touching any non-sterile items. If this happens then the surgeon changed into a new, sterile pair of gloves before continuing. After gowning up the surgeon prepared the operating site by using sterile drapes to cover the unprepared parts of the animal and all adjacent surfaces to avoid the risk of contamination. Sterile instruments were then placed in an instrument tray or on the sterile surface. This can be seen in Figure 6.1a, where the instruments are laid out on a sterile surface, and the multisaw and drill have been placed in their sterile draping ready for use by the surgeon. At this point a final check was made that the animal was adequately anesthetized by checking the withdrawal response to toe web pinch. Once the animal had been prepared an incision was made just lateral to the tibia running from anterior of the ankle to just anterior of the knee joint (Figure 6.1b). Blunt dissection techniques were used to expose the tibia being

\(^2\)In this study the role of surgeon and sterile nurse was alternated between the author of the thesis and Ibidumo Igah
careful to avoid excess tissue trauma and muscle damage. A surgical plate was inserted against
the lateral side of the tibia and aligned such that the top edge was as anterior on the tibia as
possible. At this point a hole was drilled through the top hole on the plate and through the
tibia, with an appropriately sized screw used to secure the plate in place to act as a guide for
the osteotomy. Using the middle of the plate as a guide, a surgical saw was used to cut half way
through the tibia perpendicular to the bone, running from the medial to lateral side. A incision
was made approximately 10mm below at an angle of 30 degrees such that it approached the first
incision. Using the saw, both incisions were advanced slowly, one at a time, until they meet,
and a triangular section of bone was removed. At this point the two sections of tibia should
be aligned to make sure that the osteotomy cut is smooth and that they fit at an appropriate
angle. If necessary the bone surfaces can be smoothed or adjusted using the saw, however
effort was made to ensure that this was not required as it can lead to unnecessary soft tissue
trauma. The plate was then removed from the animal and bent to an angle of 30 degrees using
two sterile needle holders, one holding each side of the plate. The angle was checked using a
goniometer. Once bent the plate was reattached to the bone using the hole previously drilled,
and the two halves of the tibia positioned such that they conform to the plate. The drill was
then be used to bore a hole through the bottom most hole on the plate and a screw inserted
to hold it in place. After this the remaining two holes were drilled, and screws placed through
them as described previously. All screws were then tightened to ensure the plate was stable
prior to closing up. Absorbable sutures were then used to secure the soft tissue underneath the
skin to avoid any dead spaces, with nylon sutures used to clean the wound before cleaning any
excess blood using sterile saline. The isoflurane was then turned off and the animal allowed to
regain consciousness on oxygen, before being placed in a heat box at 37 degrees for 30 minutes
post-surgery to allow full recovery. Post-operative analgesics and antibiotics were given for a
period of 5 days and then as appropriate. The animals were single-housed post-operatively for
a period of 10 days, during which daily pain scoring was carried out to monitor the wellbeing
of the animals. After this period any remaining sutures were removed and the animals were
placed back in their original caging. A schematic representation showing how the surgery was
performed is displayed in Figure 6.2.
Sham

Sham surgeries were used to produce animals with an osteotomy angle of 0 degrees, following much the same approach outlined for the osteotomy. The animals were prepared and the tibia was exposed as above and then the plate attached by aligning it with the tibia and drilling through all four holes before fixing with the screws. The surgical saw was then used to create an incision approximately 50% of the way through the bone making sure to reach the medullar cavity so the fracture healing response is similar to that for the osteotomies. After this the animals were closed up as before and allowed to recover in exactly the same way. A schematic representation of the surgical technique is displayed in Figure 6.3.

6.3.4 Histology

Sample Preparation

Animals were culled via schedule 1 methods appropriate for rats. Specifically an intraperitoneal injection of buprenorphine (1ml) was administered under anaesthesia followed by puncture of the femoral artery to confirm death. Both legs of the animals were removed and the knee exposed by removing soft tissue from the femur and tibia via blunt dissection. The metal plate used to fix the osteotomy was removed and the post-operative osteotomy angle measured using a goniometer for records. The tibia and femur were dissected approximately 10mm from the joint using a surgical saw to isolate the knee. The patella was then removed by cutting through the patella tendon with a surgical scalpel, sliding it under the patella and then slicing through the quadriceps tendon to remove it from the joint. Removal of the patella prior to fixation is not essential if it is thought the process may damage the specimen, however it allows easier access to the joint for histological chemicals and thus reduces histological processing times.

Histological Processing

After excision of the knees they were placed immediately in 100ml glass containers filled with 10% NBF (Sigma-Aldrich, St Louis, MO) and placed on a gentle roller in order to agitate the sample and allow proper fixation of the sample. The 10% NBF was changed once daily for 3
6.3. Methods

Figure 6.1: Figure showing individual stages in the surgical technique for inducing a tibial varus osteotomy. (a): Setup of equipment on sterile drape in preparation for surgery. (b): Incision made using scalpel on the frontal side of the shank to expose the tibia. (c): Exposed tibia. (d): Hole drilled for cranial screw placement adjacent to the insertion point of the patella tendon. (e): Screw inserted into the cranial hole in order to provide stability of the plate during sawing. (f): Wedge osteotomy created by removing triangular section of bone with the bone saw at the required osteotomy angle. (g) Frontal view of open wedge osteotomy. (h): Plate bent to appropriate angle using a pair of sterile pliers. (i): Plate reinserted onto the lateral side of the tibia and both parts of the tibia are pressed into the correct angle against the plate. Screws are then placed in the 2 cranial most holes to secure the plate. (j): Bottom 2 holes are drilled in the tibia through the holes in the plate. (k): Final two screws are inserted into the tibia to secure the osteotomy. (l): Biodegradable sutures used to secure the wound internally followed by application of mattress sutures on the exterior of the wound.
days, at which point the samples were placed in 10% formic acid (Sigma-Aldrich) in order for
decalcification to take place, once again on the roller for gentle agitation. This was changed once
daily for 10 days at which point the samples were cut in half along the medial-lateral axis to allow the
decalcifier to penetrate the inside of the joint adequately. Briefly the knee joint was placed posterior
side down and a scalpel was used to dissect the joint into medial and lateral sections along the
visible midline of the joint. If the sample is hard to cut it means that the sample has not
decalcified adequately and should be placed back in the formic acid, and changed daily until the
sample is ready to be cut. After separating the medial and lateral halves they were placed back
in 10% formic acid in the same container and left for a period of seven
days or until the samples had fully decalcified, changing daily as previously. Decalcification
was tested for by lightly bending the samples, if they were resistant to bending then they were
placed back in the formic acid for another 24 hours before being tested again. After testing for
Table 6.1: Histological protocol for processing of rat knees

<table>
<thead>
<tr>
<th>Step</th>
<th>Chemical</th>
<th>Duration</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fixation</td>
<td>10% NBF</td>
<td>48 hrs</td>
<td>Change every 24 hrs</td>
</tr>
<tr>
<td>Decalcification</td>
<td>10% Formic Acid</td>
<td>21 days</td>
<td>Change every 24 hrs</td>
</tr>
<tr>
<td>Dehydration</td>
<td>50% Ethanol</td>
<td>1 hr</td>
<td></td>
</tr>
<tr>
<td>Dehydration</td>
<td>60% Ethanol</td>
<td>1 hr</td>
<td></td>
</tr>
<tr>
<td>Dehydration</td>
<td>70% Ethanol</td>
<td>1 hr</td>
<td></td>
</tr>
<tr>
<td>Dehydration</td>
<td>80% Ethanol</td>
<td>1 hr</td>
<td></td>
</tr>
<tr>
<td>Dehydration</td>
<td>90% Ethanol</td>
<td>1 hr</td>
<td></td>
</tr>
<tr>
<td>Dehydration</td>
<td>100% Ethanol</td>
<td>1 hr</td>
<td></td>
</tr>
<tr>
<td>Clearing</td>
<td>Histoclear</td>
<td>Overnight</td>
<td></td>
</tr>
<tr>
<td>Clearing</td>
<td>Histoclear</td>
<td>2 hrs</td>
<td></td>
</tr>
<tr>
<td>Clearing</td>
<td>Histoclear</td>
<td>2 hrs</td>
<td></td>
</tr>
<tr>
<td>Infiltration</td>
<td>Paraffin Wax</td>
<td>2 hrs</td>
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</tr>
<tr>
<td>Infiltration</td>
<td>Paraffin Wax</td>
<td>2 hrs</td>
<td></td>
</tr>
<tr>
<td>Infiltration</td>
<td>Paraffin Wax</td>
<td>Overnight</td>
<td></td>
</tr>
<tr>
<td>Embedding</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sectioning</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

decalification the samples were dehydrated using a sequential series of ethanol dilutions, from 50% to 100%, leaving the samples for an hour in each dilution. It is important to make sure that the entire dehydration series is completed on the same day to avoid the samples being left overnight in ethanol and over-dehydrating. Once dehydrated the samples are placed in Histo-Clear II (National Diagnostics, Atlanta, US) to clear the samples overnight followed by two changes of two hours each the following day. The samples were then placed in paraffin wax using an embedding station and placed in an oven at 60 degrees for six hours, with the wax being changed every two hours. If possible, it is often preferable to leave the last change overnight as to ensure proper infiltration. The samples were then removed from the paraffin wax and placed into an appropriate size embedding cassette, before filling it with hot wax and allowing it to cool gradually. First on the cool side of the embedding station, then on a table or surface at room temperature and then onto the cold station before placing in the freezer overnight. The samples were then removed from the freezer and placed on the microtome. 10µm sections were taken every 100µm throughout the section and placed in a warm flotation
bath to remove creases. The sections were then placed on slides prior to staining and allowed to cool at room temperature briefly before being placed in a 60° oven to remove any paraffin wax attached to the slide. Staining with toluidine blue was then conducted using the protocol set out in Table 6.2. Once stained, sections were scored for lesion width, depth and area using OARSI guidelines, with a total cartilage damage score calculated by taking an average of the lesion width, depth and area scores [Gerwin et al., 2010].

Table 6.2: Toluidine blue staining protocol

<table>
<thead>
<tr>
<th>Solution</th>
<th>Duration (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histo-Clear II</td>
<td>4</td>
</tr>
<tr>
<td>Histo-Clear II</td>
<td>4</td>
</tr>
<tr>
<td>Histo-Clear II</td>
<td>4</td>
</tr>
<tr>
<td>100% Ethanol</td>
<td>2</td>
</tr>
<tr>
<td>95% Ethanol</td>
<td>1</td>
</tr>
<tr>
<td>95% Ethanol</td>
<td>1</td>
</tr>
<tr>
<td>Distilled Water</td>
<td>1</td>
</tr>
<tr>
<td>0.04% Toluidine blue*</td>
<td>5</td>
</tr>
<tr>
<td>Distilled Water</td>
<td>1</td>
</tr>
<tr>
<td>Warm Air</td>
<td>9</td>
</tr>
<tr>
<td>Histo-Clear II</td>
<td>1</td>
</tr>
</tbody>
</table>

### 6.3.5 Serum Analysis

Serum analysis was carried out to calculate the serum levels of C-terminal telopeptide of type I collagen (CTX-I, a marker of bone turnover); C-terminal telopeptide of type I collagen (CTX-II, a marker of cartilage turnover) and Cartilage oligomeric matrix protein (COMP, a marker of extracellular matrix degradation). Serum samples were collected from the animals following ASPA guidelines pre-operatively as well as 3,6,8,10 and 12 weeks post-operatively. 0.8ml of blood was collected from a peripheral vein using a 25G needle and placed in a 2ml Vacutainer for 30 minutes in order for the blood to clot. At this point the samples were spun on a centrifuge at 1200g for 15 minutes and the serum removed from the container using an appropriately sized pipette. The serum was separated into 50µl eppendorfs and frozen at -80°C until required. Enzyme based assays were used to evaluate the serum levels of CTX-I (RatLaps
TM, IDS, Boldon, UK), CTX-II (Serum Pre-Clinical CartiLaps EIA, IDS, Boldon, UK) and COMP (Animal COMP ELISA, IDS, Boldon, UK) as per the manufacturer’s guidance. Serum samples were collected from the sham and osteotomized animals following ASPA guidelines pre-operatively as well as 3,6,8,10 and 12 weeks post-operatively.

6.3.6 In Vivo Kinematics, Kinetics and Musculoskeletal Modeling

In vivo kinematics and ground reaction force measurements were collected from the animals pre-operatively and then at 10 weeks post operatively following the methods outlined in Chapter 3. An existing musculoskeletal model of the rat hindlimb [Johnson et al., 2008] coded within OpenSim [Delp et al., 2007] was used to determine the joint loading conditions post surgically. An extra fixed joint was created in the anterior portion of the tibia to replicate the point of osteotomy. This joint adducts about the tibia at a fixed angle which was determined using subject specific post-culling measurements of the osteotomy angle. Body segment parameters for the two tibial segments were found via a least squares optimization approach such that the peak knee joint reaction force with an osteotomy angle of 0 degrees was as close as possible to that of the existing model. A diagramatic illustration of 0, 15 and 30 degree osteotomy angles can be seen in Figure 6.4. Inverse kinematics, inverse dynamics, static optimization and joint reaction analyses were used in OpenSim as outlined in Chapter 3.

![Figure 6.4: Visualization of two-segment tibia model in OpenSim showing (from left to right) a representation of 0, 15 and 30° varus osteotomies](image)
6.3.7 Statistical Methods

Quantitative histology comparisons were generated using a one-way MANOVA to compare the difference in cartilage degeneration metrics between the eight groups shown in the qualitative histology images. Post-hoc univariate ANOVAs were carried out for each damage metric to evaluate the simple main effects where differences were detected. Statistical analyses of serum biomarker levels and biomechanical measures were completed using two-way mixed ANOVAs to consider both the effect of treatment group and of time, with each biomechanical parameter being considered separately. Post-hoc univariate ANOVAs were undertaken to investigate where statistically significant differences between the osteotomized and sham groups occurred. Where appropriate, tests for normality (Shapiro-Wilk) or sphericity (Machly) were conducted prior to each analysis. A Pearson’s correlation test was carried out to determine the relationship between selected important parameters and overall histological scores. For each parameter the correlation calculated taking into account both osteotomized and sham groups. All analyses were carried out using IBM SPSS Statistics for Windows, version 24 (IBM Corp., Armonk, N.Y., USA).
6.4 Results

6.4.1 Surgical Outcomes

Figure 6.5 shows the dissected limbs of a sham and osteotomized rat at 14 weeks post-surgery. The varus misalignment of the osteotomized rat (right) can be clearly seen whilst the tibia of the sham subject remains in normal alignment.

![Image: Left: Excised rat limb for a sham animal Right: Excised rat limb for osteotomized animal (angle = 30°)]

Figure 6.5: Left: Excised rat limb for a sham animal Right: Excised rat limb for osteotomized animal (angle = 30°)

6.4.2 Histological grading

Summary: Osteotomized group has significant cartilage degradation compared to sham animals

Histological analysis was carried out on osteotomized and sham animals. Quantitative histological results can be seen in Figure 6.6, with histological images of the medial and lateral anterior sections of the knee joint for both groups shown in 6.7 and Figure 6.8. Extensive cartilage
matrix loss can be seen in the ipsilateral knee of the osteotomized animals with severe lesions present. Sham animals display healthy cartilage, with a smooth lining of cartilage on both the femoral and tibial sides without fibrillation. A one-way MANOVA determined that the differences between the eight groups on lesion width (p<0.01), depth (p<0.01), area (p<0.01) and total cartilage score (p<0.01) were statistically significant. Post-hoc tests confirmed that for all four metrics the scores for the ipsilateral medial knee of the osteotomized animals were significantly higher than all other conditions (p<0.01 for all)

Figure 6.6: Bar charts showing lesion morphology in sham (n=5) and osteotomized (n=5) subjects for contralateral and ipsilateral limbs as per OARSI guidelines. Medial lesions are shown in black with lateral lesions in red. Bars plotted with +/-SD. Asteriks denote significant differences to the equivalent condyle on the sham subject at the 0.05 significance level
Figure 6.7: Representative toluidine blue stained sections of medial (left) and lateral (right) tibial plateaus for the contralateral and ipsilateral knees of the osteotomized animals with cartilage stained dark blue/purple. Osteoarthritic lesions present where the line of cartilage is disrupted. Site of severe lesions marked with red arrows.
Figure 6.8: Representative toluidine blue stained sections of medial (left) and lateral (right) tibial plateaus for the contralateral and ipsilateral knees of the osteotomized animals with cartilage stained dark blue/purple. Osteoarthritic lesions present where the line of cartilage is disrupted. Site of severe lesions marked with red arrows.
6.4. Results

6.4.3 Biomarker Analysis

Summary: Serum CTX-II levels increase post-operatively in the osteotomized group compared to the sham animals. COMP and CTX-I show no significant differences between the two groups.

Levels of CTX-I, CTX-II and COMP can be seen in Figure 6.9 for both the osteotomized and sham groups at several time intervals.

![Graph showing serum concentration changes](image)

Figure 6.9: Serum CTX-I (left), CTX-II (right) and COMP (bottom) levels over the course of the experiment for both sham (n=5) and osteotomized (n=5) animals. Samples taken pre-surgically, 3 and 6 weeks post-surgically and then every 2 weeks subsequently until 14 weeks. Points plotted +/-SE with * indicating a significant difference at p<0.05.

For control animals CTX-II levels appear to drop over time, eventually reaching a plateau at around 40% of their original level. Serum levels for the osteotomized group appear to follow the same trend for the first 3-6 weeks before jumping sharply between week 6 and 12 to 194%
of their original level and then dropping off in week 14. There was a statistically significant interaction between the type of surgery performed and the time on the relative CTX-II serum concentration ($p<0.001$). Post-hoc analysis of simple main effects revealed that the CTX-II level in the osteotomized groups was significantly higher than the sham subjects 8 and 10 weeks post-operatively ($p<0.01, p<0.01$). Levels of CTX-I or COMP did not show any significant interaction effect between the type of surgery performed and the relative serum biomarker level ($p>0.05$).

### 6.4.4 Effect of Osteotomy on Ground Reaction Forces

Summary: No differences in GRF between osteotomized and sham animals

The effect of the osteotomy on ground reaction forces was investigated both to see if there were any differences in the pattern of ground reaction forces caused by the variation in hindlimb anatomy in the osteotomized group as well as to see if there was any pain induced redistribution of loading away from the ipsilateral limb. Vertical, lateral and cranial ground reaction forces 12 weeks post-surgically can be seen in Figure 6.10, with no statistically significant differences found between the type of surgery performed and maximum hindlimb or forelimb forces in all orientations ($p>.05$).

![Ground Reaction Forces](image)

Figure 6.10: Ground reaction forces, kinematics, knee joint moments and knee joint contact forces 12 weeks post-surgically for sham ($n=5$) and osteotomy ($n=5$) animals. Error bars +/- SE. * denotes significant difference between sham and osteotomy samples at $p<0.05$.
6.4.5 Effect of Osteotomy on Knee Biomechanics

Summary: Osteotomized animals have increased medial knee joint loading alongside increased knee joint adduction moments 12 weeks post-surgically

Knee joint adduction moments, as well as medial and lateral forces were evaluated to determine whether the osteotomized animals had increased medial side loading as predicted and to quantify the scale of this increase. As can be seen in Figure 6.11, peak adduction moments (p=.011) and peak medial joint contact forces (p=.006) were higher in the osteotomized group post-surgery than in the sham subjects, while lateral joint contact forces showed no significant interaction effects (p>.05). Changes in kinematics and muscle forces can be found in Appendix B.

![Graphs showing knee joint biomechanics](image)

Figure 6.11: Knee joint biomechanics 12 weeks post-surgically for sham (n=5) and osteotomy (n=5) animals. Error bars +/-SE. * denotes significant difference between sham and osteotomy samples at p<0.05

6.4.6 Effect of Osteotomy Angle on OA Development

Summary: Increasing the osteotomy angle increases medial joint loading, leading to increases in histological OA and serum CTX-II levels
Figure 6.12 shows the correlation between the angle of osteotomy found post surgically, the medial JCF, histological levels of OA and the 10-week CTX-II serum levels. The angle of osteotomy was found to be significantly correlated with histology cartilage degradation ($R^2=.881, p=.001$) and 10-week CTX-II levels ($R^2=.809, p=.002$) as well as peak medial JCF’s found via musculoskeletal modelling ($R^2=.691, p=.010$).
6.5 Discussion

Evaluating the exact pathophysiology of OA is difficult for a number of reasons, however, findings from recent animal work have proposed a dual role for both local joint biomechanics and systemic inflammation type activities. Previous surgical models of the disease in rats have tended to involve intra-articular surgery which may lead to an increase in localized synovial inflammation and provide a misleading picture of how the disease initiates and spreads in primary disease patients. This thesis has proposed a model in rats which could help to isolate the affects of biomechanical changes, quantify them and evaluate the effect they have on disease progression. Histological analyses showed that inducing a varus osteotomy in the animals produced severe cartilage matrix loss in the medial ipsilateral knee which is not present in sham animals. Quantitative analysis of the histological sections further highlighted the osteoarthritic cartilage changes present in the ipsilateral limb of the osteotomized animal, with lesions found to be bigger, wider and deeper than in both the contralateral limb and the sham animal. The fact that the cartilage damage scores are not negligible for the sham animals and contralateral limbs is surprising, particular for the sham animals, as cartilage damage is not known to occur in healthy animals. It is possible that this damage could be a result of histological processing artifacts such as microtome blade damage or that minor cartilage disruption in the upper layers is present even in healthy animals. It is also possible that the surgery itself has led to minor degenerative changes in the sham animals (despite the lack of tibial angulation). Either way, the fact that the damage seen in the ipsilateral medial joint is far higher (particularly for cartilage area loss) suggests that the clinical relevance of the lesions in the sham and contralateral joints is limited. In future studies this could be addressed by the inclusion of non-sham controls.

The level of disease seems to be significantly greater than that reported in previous studies using similar methods in rabbits [Goodman et al., 1991, Wu et al., 1990], however, due to the different species and varying husbandry conditions it is hard to provide an accurate comparison of the two models. One potential reason for the increased rate of development could be the species of animal chosen, with smaller animals having thinner cartilage and thus developing more substantial lesions at an earlier time point. Compared to many ACLT models, the rate of disease progression appears to be fairly gentle, with histological changes seen in rats subjected to ACLT in as little as 1 week post surgically [Hayami et al., 2006], whereas serum biomarkers in this study seem to suggest that osteoarthritic changes commence later. One limitation of
this study is that, because of the lack of serial histology, it is difficult to say conclusively how the osteoarthritis developed. For example, it cannot be determined conclusively whether the lesions seen histologically at 14 weeks developed gradually over time, as in the case of human disease, or as the result of short term trauma potentially initiated by the surgery. The fact that sham subjects show little sign of similar cartilage damage, coupled with the gradual rise in CTX-II levels post-surgically suggest that the OA developed over the course of the study and is unlikely to be the result of extreme cartilage trauma. Further histological timepoints will need to be taken in future studies to evaluate whether this is the case. If so then this would be beneficial in giving the ability to study the early phase of the disease over a longer time period and evaluate early stage interventions for which there would not be adequate time in other surgical models.

The development and optimization of the musculoskeletal model reported on in this thesis allowed for the direct calculation of ground reaction forces, kinematics and internal loading after the surgery. By understanding the effect that the surgery had on internal joint loading in particular, the mechanical factors causing the osteoarthritic changes seen in the joint can be isolated and quantified. Ground reaction forces in the osteotomized and sham groups post surgically did not show any significant differences, which is an encouraging sign that the animal is loading its ipsilateral limb fully. This optimizes the chances of the animal developing osteoarthritis through biomechanical mechanisms and should allow for good repeatability of the model if the result can be sustained. In previous studies of OA in rats it has been seen that the vertical ground reaction force decreases relative to control subjects after the induction of disease [Allen et al., 2012], something which was not seen in this study. This reduction in GRF’s is thought to be a response to pain in the ipsilateral limb and has been suggested as a potential biomarker to evaluate disease progression. It is possible that the osteoarthritis produced via this model induces differing pain responses to those using intra-articular techniques, where increased joint inflammation may lead the animal to reduce loading on the affected joint. Analysis of knee joint contact forces showed that maximum medial joint loading and adduction moments were significantly higher in the osteotomized animals (p<0.05). Previous studies in humans have demonstrated the link between these two measures and the incidence of OA [Vanwanseele et al., 2010]. One benefit of this model is that the biomechanical alterations made to the local environment can be easily adjusted by either changing the angle or placement of the osteotomy. By either increasing the angle of osteotomy or creating the tibial wedge higher
up on the tibia the medial loading can be increased further, potentially inducing more rapid lesion development and cartilage degeneration. Adversely, reducing the angle could have the opposite effect, and potentially allow for easier studying of early stage disease and preventative therapies. This gives great flexibility in how the model can be used; future studies will hope to elucidate this property more thoroughly. A limitation of the study as presented here is that because the biomechanics were measured 12 weeks after the surgery, it is difficult to evaluate whether biomechanical alterations derive from the surgery or are responses to osteoarthritic development in the joint. Future studies will be required to evaluate whether the variations in kinematics and knee joint loading seen post surgically are time independent, or whether they worsen as OA develops.

CTX-II biomarker analysis has also served to validate the model further, with levels of the biomarker rising after 6 weeks for the osteotomized animals and falling consistently throughout the study for the sham animals. CTX-II is known to increase significantly in human OA patients and is one of the best options currently available for non-invasive diagnosis of early stage disease [Bai and Li, 2016]. In this study we were not able to evaluate how the timing of CTX-II changes corresponded to osteoarthritic changes as histology was done at 14 weeks only, however, a strong correlation between CTX-II levels and the level of histological cartilage degradation suggest that it may well be a good diagnostic and prognostic biomarker for OA in this model. This corresponds to work done previously which suggests that CTX-II can not only predict the presence of osteoarthritic changes, but also the severity of these changes. The fact that it falls sharply in the osteoarthritic animals towards the end of the study suggests that in the later stages of the disease, the biomarker may lose its ability to differentiate between healthy and diseased tissue as the serum level reverts back to normal. This has been shown in previous studies and highlights the difficulty in finding a consistent biomarker for osteoarthritis, where the disease evolves and changes rapidly, and different phases of the disease can have significantly different biological symptoms [Lotz et al., 2013]. Unlike CTX-II, serum levels of CTX-I did not change significantly during the time course of this project. Human studies looking at levels of serum markers in progressive osteoarthritic patients have shown a large upregulation of CTX-I [Rousseau and Delmas, 2007], however these studies have focused on late stage disease where bone turnover is likely to accelerate. Studies on rat CTX-I levels are rare, but studies have been carried out on models of post-traumatic OA in mice, appearing to show an upregulation of CTX-I similar to that in human patients [Khorasani et al., 2015]. Although this study cannot prove
conclusively that no changes in serum levels exist, there is no strong evidence of upregulation and this might suggest a different pathological mechanism of disease whereby subchondral bone loss does not play as major a role in early disease. Further studies will be required to investigate why this might be the case, including direct comparisons between this model and intra-articular models of post-traumatic disease. These could help to develop an understanding of phenotypic differences between mechanically induced primary and post-traumatic forms of the disease.

COMP levels were also seen not to change much over the timecourse of the project and did not vary significantly between osteoarthritic and sham animals. Rats suffering from collagen induced OA have been shown to have increased COMP expression in previous studies [Larsson et al., 2002], but studies of COMP levels in surgically induced models are rare despite there being strong evidence of its upregulation in human disease [Attur et al., 2013]. Interestingly some studies have suggested that COMP may be produced by all elements of the synovial joint instead of just the cartilage as was originally proposed [Di Cesare et al., 1997]. Inflammation in the synovium in particular has been shown to correlate more with serum COMP levels than cartilage damage, perhaps because the COMP can be deposited in the bloodstream directly by the synovium as supposed to the cartilage [Vilm et al., 2001]. Other studies have shown that an upregulation in COMP can be activated by the injection of proinflammatory cytokines [Zivanovic et al., 2011]. It may be the case, therefore, that in other animal models of the disease increased COMP levels have more to do with inflammatory responses to the disease than to direct cartilage damage. A reduction in synovial inflammatory response in this model may explain the lack of COMP upregulation, but future studies will be required to elucidate this point more fully.

The ability of the model to generate osteoarthritic changes without the need for intra-articular surgery has advantages. The problem with surgical models that operate on the joint itself is that it is unclear whether they are truly modeling the primary or post-traumatic form of the disease. Recent animal studies have started to show clear pathogenic differences between primary and post-traumatic forms of the disease, however, comparative studies of different model types within the same group are still very rare. A study by Wei et al (1998) showed significant differences in serum biomarker levels of several cytokines known to be related to the development of OA. Lubricin, a glycoprotein present in synovial fluid that aids lubrication, was shown to significantly decrease in animals with ACLT induced OA compared to those with spontaneous disease development and control subjects. It is known from genetic studies that a reduction in
lubricin levels significantly increases the rate of osteoarthritic development [Rhee et al., 2005] and it is known to decrease after traumatic knee injuries such as ACL rupture where a rapid inflammatory response is initiated in the affected knee [Elsaid et al., 2009]. Studies on synovitis post-injury have proposed lubricin as a key biomarker and the lack of a decrease in this marker in the spontaneous subjects could suggest that inflammatory responses occurring in the knee in post-traumatic disease models may not accurately reflect what happens in human subjects. Because of the overuse of these post-traumatic models relative to the incidence of primary and post-traumatic disease in humans, the role of inflammatory agents versus biomechanical changes may well be misunderstood in mechanically derived disease and intervention based on reducing or preventing this inflammatory element may not have the desired outcome. If it is true that the two diseases are fundamentally different in both origin and pathophysiology then it seems there is a need for two discrete sets of models. The model presented here has shown it is able to produce reliable, controllable, unicompartmental osteoarthritis without the need to open the joint capsule itself and run the risk of systemic inflammation, unnatural internal joint biomechanics or localized tissue damage.
Chapter 7

Conclusion

7.1 Summary of Findings

This thesis has undertaken a thorough investigation of lower limb kinetics in rats and, in the previous chapter, their relationship to osteoarthritis. The main findings of the thesis can thus be split into two main categories:

- How rodent biomechanics can best be measured and how they vary during different gait profiles
- How altering these local biomechanics can be used to induce osteoarthritic changes and the role of biomechanics in the disease

This thesis presents the first use of *in vivo* kinematics and GRF’s in rats to provide details of internal joint loading via an existing musculoskeletal model. GRF’s, kinematics, joint loading, and muscle forces were evaluated and compared to previous studies using alternative methods to validate the model. Overall, the model outputs matched closely those seen in the literature. Kinematic measures were compared to previous studies using similar techniques and the patterns of flexion and extension calculated by the inverse kinematics methods in OpenSim matched closely with those derived from other techniques. Joint moments were compared to a previous study using a simple inverse dynamics model and were seen again to follow similar trends, with subtle differences in some measures. Muscle forces were compared to EMG data...
collected either directly from rats or from other quadripeds in order to validate the model and again there was a good correlation between trends reported in this thesis via an in silico model and those found experimentally.

An evaluation of this musculoskeletal model was carried out in two parts. The first looked to evaluate the source of potential errors both within the model itself and in the methodologies that are required to collect the in vivo data. Previous studies have looked into the effect of skin artifact errors (the translation of skin markers to the underlying anatomy during motion) on kinematic measures, but this is the first study to have investigated the magnitude of scaling errors, errors in muscle geometry or marker placement errors and is also the first to have considered the effect of these errors on musculoskeletal models of rodents using sensitivity analyses. It was found that errors caused by misplacement of markers had moderate effects on all model outputs, but had the largest effects on hip flexion moments. It was also found that errors in the placement of markers at the knee joint were highest and had the largest effects on model outputs. Suggestions were made detailing how these errors could be reduced in-vivo including the use of long-term marking and the use of a single experienced experimenter during longitudinal studies. Errors in muscle geometries were also seen to cause large variations in model outputs, specifically in muscle forces with some peak muscle forces varying by up to 100%.

After evaluating the potential errors in techniques used to measure biomechanical outputs in rats, a study was conducted to evaluate whether this model could be used to detect the subtle gait changes induced by varying gait inclination and speed. The intention was to find out what combination of speed/inclination could be used to overload the medial side of the knee joint in order to accelerate the uniarticular OA induced via the surgical model outlined in Chapter 6. It was found that increasing speed and inclination had significant effects on kinematic, inverse dynamic and static optimization model outputs. Of particular interest, increasing the inclination appeared to increase overall knee joint loading, whereas increasing speed seemed to increase medial knee joint loading preferentially.

Following on from this, a novel model of malalignment induced knee OA was developed in rats, using a high tibial osteotomy to increase medial knee forces, inducing degenerative cartilage changes. This model was designed to better replicate the pathogenesis of mechanically induced human primary OA, where malaligned joints are often present prior to the development of
progressive disease. Medial cartilage degeneration consistent with OA was found in the knees of the operated animals, with the medial tibial plateau scoring significantly higher for depth, width and percentage area of lesion compared to sham subjects. Serum CTX-II levels as well were shown to be significantly higher in the HTO operated group and correlated well with histological grading. Analysis using the musculoskeletal model showed that medial compressive forces and adduction moments in the knee joint were significantly higher post-operatively in the ipsilateral limb, highlighting the relationship between these forces and the development of the disease. As far as the author is aware the study presented the first purely biomechanical surgical model of OA in rats, which could be more representative of the human mechanical OA than intra-articular techniques published previously. The potential avoidance of joint inflammation post-surgically, the maintenance of internal joint biomechanics and the ability to quantify the alterations in joint loading should make this a better candidate for translation to primary forms of the disease in humans.

7.2 Future Work and Applications

After summarizing the findings it is important to understand how they could be used in future to enable translational benefits to come from the research. In order to evaluate this, the chapter is split into two: one part looking at general biomechanics in rats and the other looking specifically at its role in OA.

7.2.1 Kinematic and Kinetic Measurement in Rats

This thesis has described in more detail than before some of the errors associated with measuring gait in rodents. The issues surrounding kinematic measurements are perhaps the most pressing and will need to be considered carefully if the field is to move forward and be trusted to detect acute physiological and behavioural gait variations. In this thesis, studies looking at the errors associated with placing skin markers on the animals showed that consistent marker placement was challenging for both novice and experienced researchers due to the large amount of soft tissue on the lower limb. The effect of these errors on kinematic outputs was also larger with maximum errors of $10^\circ$ and $15^\circ$ at the hip and knee respectively. In this study, global
optimization was used to evaluate joint kinematics and reduce the reliance on the accuracy of the knee joint marker in particular. Future work should aim to look at the difference in kinematic outputs between optimized, triangulated and raw kinematics in order to evaluate any potential weakness in the global optimization routines and to provide more evidence as to the potential magnitude of skin artifact errors. In human studies, the use of marker clusters has been shown to increase the accuracy of kinematic measurement and is now regularly used. Potential further work could look at developing small scale marker clusters for use on the rat to improve the accuracy of measurement. Using one marker per joint means that joint adduction and internal/external rotations must be estimated, as they were in this study via inverse kinematics. The use of clusters could overcome this problem as a cluster of 3 markers per joint would allow for deterministic measurements of joint kinematics in all six degrees of freedom (three translations and three rotations), although studies in humans have found that using four or more markers is even more effective at reducing errors [Cappozzo et al., 1997].

Limitations in the musculoskeletal model could also be addressed in future studies. One such limitation is the absence of patellofemoral structures at the knee and soft tissue structures such as ligaments. The absence of patellofemoral forces in particular is a significant limitation in the model at present, especially when using knee joint contact forces as key model outputs. It is likely that some of the load transmitted to the knee joint will actually be experienced as patellofemoral loading and this may act to reduce knee joint loads. In human studies the patellofemoral joint loads can be nearly as high as the tibio-femoral joint contact forces [Trepczynski et al., 2012]. Developing the model in this direction would require in-depth anatomical studies including the use of micro-imaging techniques such as micro-CT to determine the relative locations and orientations of the patellofemoral structures and soft tissues holding it in place. These studies would involve significant complications but are not impossible and should be considered if the model is to be used in future work.

Another area of development could be to attempt to develop similar techniques to the ones outlined in this thesis on mice. Mice are used far more frequently than rats in studies of almost all disease and as such the availability of methods to evaluate kinetic changes in mouse gait would be valuable. Their wide use is partly due to their reduced cost and housing space, but is also due to the fact that it is far easier to create genetic phenotypes that allow for the study of specific disease pathways. The use of skin based markers to evaluate mouse gait is very rare,
due to their small size, and so markerless kinematic methods may need to be considered if the field is to move in this direction. A recent study adapted the musculoskeletal model seen here for use in mice [Charles et al., 2016], although it has not been used for in vivo studies in the literature as of yet.

7.2.2 Osteoarthritis

There are two main areas where animal models can be used to provide translational benefits to the field of osteoarthritis. The first is in the development of new biomarkers to enable earlier detection of the disease, either biological or mechanical. The second is to develop a greater understanding of disease pathology to enable the development of more effective disease interventions. Future work using the techniques outlined in this thesis can help develop the field in both of these areas.

In terms of the development of biomarkers, future work can look at both the effectiveness of mechanical disease indicators such as medial joint contact forces, and the effectiveness or otherwise of biological markers in identifying the progression of mechanically driven disease. The musculoskeletal model could be used post-surgically at more frequent intervals to determine whether a correlation exists between in silico model outputs and histological levels of OA, with animals having to be culled at regular intervals post-operatively. Recent studies in humans with progressive OA have shown that mechanical biomarkers of OA have the potential to be used in the diagnosis of the disease [Mezghani et al., 2017]. and future work using the musculoskeletal modelling techniques described in this thesis could help understand if mechanical biomarkers could be a useful diagnostic measures of early disease as well. Much of the work on biomarkers of OA at the moment is focused on biological serum or urine markers. CTX-II and CTX-I are considered strong candidates and both were evaluated post-operatively in HTO operated rats. While serum CTX-II levels appeared to correlate strongly with disease progression, CTX-I levels did not. In humans urinary CTX-I is often used instead of serum and so future studies might include urine analysis to determine if any difference exists between the two sampling methods.

It is also possible that other markers of osteoarthritic progression, such as immunohistochemistry could be used in future studies to both validate the presence of OA and further elucidate
pathogenic disease pathways. Evaluation of proteoglycan levels and type II collagen have been used frequently in other studies of the disease and so would be potential candidates for future work [Wang et al., 2013]. Immunohistochemistry could also be used to look at the expression of MMPs to test enzymatic cartilage digestion and potentially provide insights into the mechanisms of cartilage loss seen in this model [Gepstein et al., 2002].

The model as described here clearly demonstrates the important role that biomechanics play on the generation of osteoarthritis in rats, but the study as presented cannot determine exactly what this role is. There is a large degree of disagreement in the literature as to what this role might be, with some studies proposing biomechanics to be the progenitor cause of OA [Felson, 2013], with others suggesting it may be a consequence of pre-existing inflammation in the joint or that once initiated, inflammation is the primary mediator of progressive disease [Berenbaum, 2013]. Now that the model presented here has been shown to develop mechanically driven OA, future studies can use it to investigate how the disease develops. Animals can be culled at regular timepoints post-surgically to evaluate disease progression more thoroughly through histological evaluation, with biological markers studied to look at specific disease mechanisms. In this study the blood serum levels of biomarkers were analyzed because of the ease with which samples could be taken from live animals. Analysis of synovial fluid samples would provide a more direct measure of changes going on in the joint, but extraction of samples from live rats is difficult and invasive. Collecting samples from freshly culled animals, however, is relatively straightforward and would be possible if animals were culled at regular timepoints anyway for histological evaluations. Analysis of these markers would also allow a future study into the pathophysiological differences between primary and secondary forms of the disease. By comparing the chronological order of joint damage seen through histology and synovial markers with that of a secondary model such as ACLT or MMT, phenotypic differences might be seen that suggest differing pathways for future therapeutic interventions.

It would also be interesting in the future to attempt to determine precisely how medial joint loading relates to the rate of OA progression, and whether or not this progression is dependent on mechanical stimuli once initiated. To achieve this, a surgical HTO could be performed on the animals as per the experiments in this thesis, followed later by another surgery to reset the limb to its healthy orientation. The timing of this resetting of the limb could be altered to evaluate whether there is a point past which disease development is unstoppable, even without
the increased medial loading. This might suggest that other factors, such as inflammation, become more important once the abnormal biomechanics have initiated the cascade of joint damage. Investigating the effect of altering the angle of osteotomy would also have value in future studies, as it would provide new insight as to the dose response of osteoarthritic joint changes with increases in medial knee joint loading. Reducing the angle of osteotomy should, in theory, reduce the medial JCF and slow the progression of OA. It would be interesting to see whether the joint is resistant to small increases in medial loading or whether it just slows down disease progression. If the latter is true then this could allow the model to be customized for different purposes, another advantage over traditional surgical models such as ACLT. Reduced angles of osteotomy could be used to study the early stages of disease, with a longer time period during which the disease would progress. Conversely, larger angles of osteotomy could be used in therapeutic trials where the fast generation of OA is required.

The use of exercise in combination with or without the HTO model would also be an avenue for future trials. The results in this thesis have shown that medial joint loading can be increased by running uphill at speed and it would be interesting to see if, in combination with the HTO model, this accelerated the disease more quickly than level running. Future studies could also use these findings to try to develop a non-invasive model of uni-articular OA where a specific exercise regime is used to overload the medial compartment of the knee. These studies would also improve upon the understanding of how exactly biomechanical stimuli are related to disease initiation and development.

Finally, the focus of this study has been on osteoarthritis in rats, with good reasons for using this particular animal outlined elsewhere in the thesis. As described previously, however, mice have significant benefits when it comes to developing genetic phenotypes. This is also the case in osteoarthritis, where genetic models are used frequently. It may be useful in future to adapt the surgical model demonstrated here in rats, for use in mice, allowing for more complex studies of whether certain genetic variations make the animals more or less susceptible to mechanically driven disease mechanisms. The difficulties in achieving this are mainly experimental, with the surgery becoming significantly more difficult in smaller animals. It is, however, reasonable that a skilled operator would be able to perform similar surgical interventions in the mouse and this should be an avenue for future study as it would expand the potential use and benefit of the model.
References


REFERENCES


[Hiligsmann et al., 2013] Hiligsmann, M., Cooper, C., Arden, N., Boers, M., Branco, J. C., Luisa Brandi, M., Bruyre, O., Guillemin, F., Hochberg, M. C., Hunter, D. J., Kanis, J. A.,


REFERENCES


REFERENCES


Appendix B: Home Office Project License

Details of the home office project license (PPL) used to undertake the *in vivo* experimentation in this thesis are outlined below:

PPL Title: Biomechanical *in vivo* models of osteoarthritis

PPL Number: 70/7599

PPL Holder: Dr Warren Macdonald
Appendix B: Animal Numbers

Table 7.1: Summary table showing number of gait cycles recorded for each animal

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<th>Downhill</th>
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Appendix C: Post-Operative Kinematics and Muscle Forces

Figure 7.1: Kinematics and muscle forces 12 weeks post-surgically for sham (n=5) and osteotomy (n=5) animals. Error bars +/-SE. * denotes significant difference between sham and osteotomy samples at p<0.05