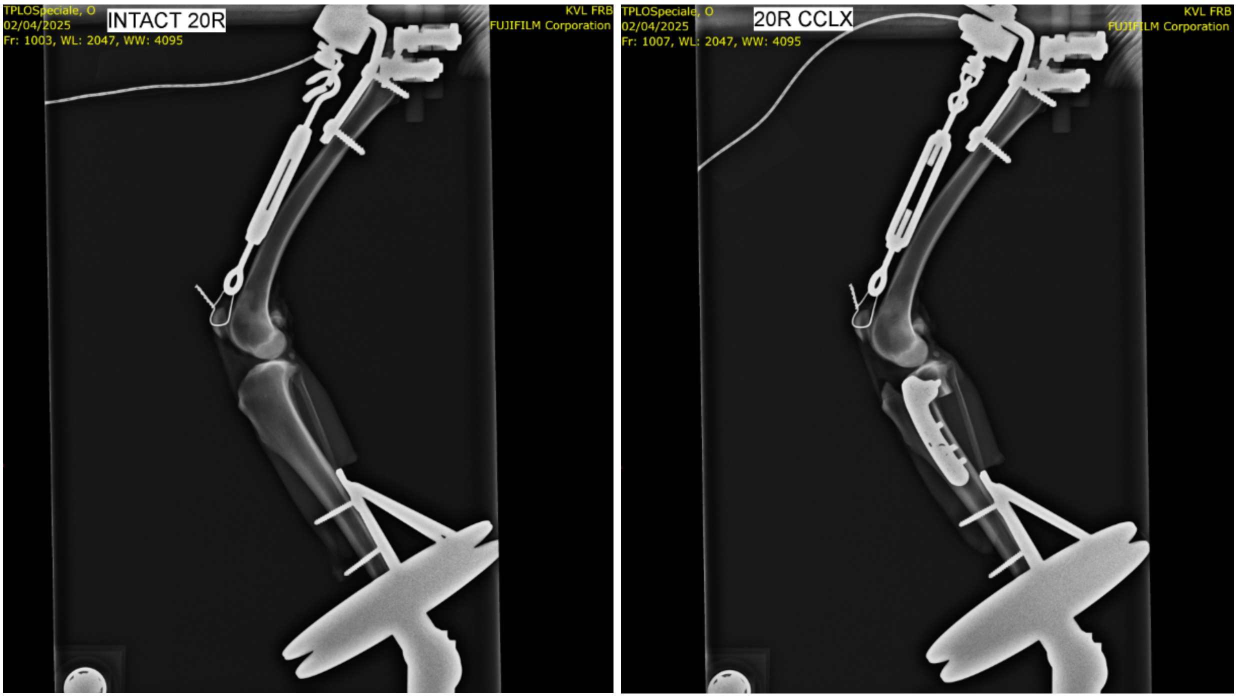
UNIVERSITY OF COPENHAGEN
FACULTY OF HEALTH AND MEDICAL SCIENCES

Rotational Stability of the Canine Stifle Joint Following Tibial Plateau Leveling Osteotomy (TPLO)

A Cadaveric Limb Press Model Study



Master's Thesis in Veterinary Medicine

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FRONT PAGE PICTURES Laterolateral radiographic views of a pelvic limb attached to the limp press model with an intact stifle joint (left picture) and medial collateral ligament transection and tibial plateau leveling osteotomy with cranial cruciate ligament rupture (right picture). The radiographs are taken at The University Hospital for Companion Animals by Holmbjerg ML & Tvedsborg CK with supervisor Miles JE.

Preface and Acknowledgments

This master's thesis was conducted at The University Hospital of Companion Animals at The University of Copenhagen and is the final part of the master's degree in Veterinary Medicine, thereby earning the authors of this thesis the title of *Cand.med.vet*. This study investigated the rotational stability of the canine stifle joint following tibial plateau leveling osteotomy (TPLO). The goal was to investigate which, if any part, of the TPLO procedure could cause rotational instability in the stifle joint. The authors of this thesis wanted to write about an orthopedic subject because of their interests in orthopedics and a desire for a hands-on thesis where they could develop their skills as veterinarians. The study design and purpose of this project was developed in a constructive meeting with supervisor James E. Miles and co-supervisor Michelle B. M. Nielsen, who in collaboration with the authors built and constructed the limb press model used in this study. The authors of this master's thesis wish to send a special thanks and sincerest admiration and appreciation for supervisor James E. Miles and co-supervisor Michelle B. M. Nielsen. This thesis would not have been possible without the incredible knowledge, support and brainstorming that you provided. The authors are eternally grateful for this collaboration. Additionally, a big thanks is in order to Conny Sørensen Due, Emilie Josephine E. M. Bjerril and Sophia Leonhardt Boetius from The Imaging Diagnostic Department of The University Hospital for Companion Animals for assisting in performing radiographs of the cadaveric limbs. A big thanks is also in order for the blacksmith at The Department of Plant and Environmental Sciences at The Faculty of Science at The University of Copenhagen who helped design and develop the metal plate for the limb press model. Sincere thanks and gratitude goes to Dansk Kennel Club (DKK) for funding this thesis, enabling the authors to further educate themselves and the field of veterinary medicine. Lastly, a most heartfelt thanks to those who choose to donate their beloved pets to further scientific reasons and education. The selfless and generous donation the owners have made, has had a tremendous impact throughout the entirety of this education, and we thank you for trusting us with your family members after they have crossed the rainbow bridge.

The authors hope that this master's thesis can inspire and educate all personnel involved in the field of veterinary medicine when deciding, planning and performing TPLO for cranial cruciate ligament rupture.

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Abbreviations

BCS	Body condition score
CaCL	Caudal cruciate ligament
CCL	Cranial cruciate ligament
CCLR	Cranial cruciate ligament rupture
CCLX	Medial collateral ligament transection and tibial plateau leveling osteotomy with cranial cruciate ligament transection
CTT	Cranial tibial thrust
FA	Functional axis
INTACT	Intact stifle joint
LCL	Lateral collateral ligament
MCL	Medial collateral ligament
MCLX	Medial collateral ligament transection
OA	Osteoarthritis
PSP	Pivot shift phenomenon
TCT	Tibial compression test
TPA	Tibial plateau angle
TPLO	Tibial plateau leveling osteotomy
TPLOX	Medial collateral ligament transection and tibial plateau leveling osteotomy

Abstract

Cranial cruciate ligament rupture (CCLR) is one of the most frequent orthopedic disorders of the canine stifle joint affecting joint stability. For dogs with CCLR, tibial plateau leveling osteotomy (TPLO) is currently recognized as one of the most effective surgical interventions for restoring normal stifle joint function. This study was designed to investigate the rotational stability of the canine stifle joint following TPLO, utilizing 10 canine cadaver pelvic limbs. The stifles underwent preoperative planning and postoperative radiographs. Each limb was placed in a custom-made limb press model, with the stifle joint flexed at 135° and the femur angled at 70° under an axial load of 30% body mass. A turnbuckle was applied between the patella and a load cell to replicate and measure quadriceps muscle force. Two load cells were attached to each side of the limb press model where one observer observed the torque, and two observers measured the rotation. Pre and postoperative tibial plateau angle (TPA) were measured and noted, as well as the length of the medial collateral ligament (MCL) pre and post transection. Each limb was tested under four conditions in this exact order: (I) Intact stifle joint (INTACT), (II) Medial collateral ligament transection (MCLX), (III) Medial collateral ligament transection and tibial plateau leveling osteotomy (TPLOX) and (IV) Medial collateral ligament transection and tibial plateau leveling osteotomy with cranial cruciate ligament transection (CCLX), with the external and internal rotation being tested twice for each condition. Data from this study indicated that neither the transection of the distal part of MCL nor the length of the MCL influenced the rotational stability of the stifle joint ($P > 0.99$, $P = 0.30$). However, the TPLO procedure significantly increased the rotational range compared to the INTACT stifle ($P < 0.001$). In particular, the internal range of rotation increased significantly compared to the external rotation, potentially contributing to the development of pivot shift postoperatively - though this was not tested. A significant negative correlation was revealed between the postoperative TPA and the rotational range of the stifle, indicating that a low postoperative TPA increased the rotational range and conversely a greater postoperative TPA decreased the rotational range ($P < 0.001$). CCLR did not affect the rotational stability of the already TPLO-operated stifle ($P > 0.99$). Due to the study design, it was not possible to examine if CCLR itself caused a greater or lesser rotational stability than the TPLO-operated stifle. In conclusion, this study reveals that although the TPLO procedure (TPLOX) significantly increases the rotational instability compared to the INTACT stifle, it cannot be attributed to the transection of the distal part of MCL, suggesting other factors may contribute to the increased rotational instability following TPLO.

Resume

Kranial korsbåndsruptur (CCLR) er en af de hyppigste ortopædiske lidelser i hundes knæled, som påvirker ledstabiliteten. For hunde med CCLR, betragtes tibial plateau leveling osteotomi (TPLO) i øjeblikket som en af de mest effektive kirurgiske metoder til at genoprette normal funktion i knæleddet. Formålet med dette studie var at undersøge den rotationelle stabilitet i hundens knæled efter TPLO-operationen, ved brug af 10 kadaver bagben fra hunde. Alle knæled gennemgik præoperativ planlægning og røntgen. Hvert bagben blev placeret i en specialbygget limb press model med knæleddet flekteret til 135° og femur vinklet til 70°, under en aksial belastning svarende til 30% af kropsvægten. Et spændespænde var placeret mellem patella og en vejecelle for at efterligne og måle quadriceps muskelkraft. To vejeceller var tilkoblet hver side af limb press modellen, hvor én observatør observerede drejningsmomentet og to observatører målte rotationen af benet. Den præ- og postoperative tibial plateau vinkel (TPA) blev målt og noteret, samt længden af det mediale kollaterale ligament (MCL) før og efter overskæring. Hvert bagben blev testet under fire forskellige forhold i følgende rækkefølge: (I) Intakt knæled (INTACT), (II) Medial kollateral ligament overskæring (MCLX), (III) Medial kollateral ligament overskæring og tibial plateau leveling osteotomi (TPLOX) og (IV) Medial kollateral ligament overskæring og tibial plateau leveling osteotomi med kranial korsbåndsruptur (CCLX), hvor både ekstern og intern rotation blev testet to gange for hvert forhold. Data fra studiet indikerede, at hverken overskæring af den distale del af MCL eller længden af MCL påvirkede den rotationelle stabilitet af knæleddet ($P > 0.99$, $P = 30$). TPLO-proceduren medførte en signifikant øget rotationel drejning sammenlignet med INTACT knæleddene ($P < 0.001$). Især den interne rotation steg signifikant sammenlignet med den eksterne rotation, hvilket potentielt kan bidrage til udviklingen af pivot shift postoperativt - dette er dog ikke undersøgt. Dette studie fandt en signifikant negativ korrelation mellem den postoperative TPA og den rotationelle drejning, hvilket indikerede, at en lav postoperativ TPA øgede den rotationelle drejning, mens en højere postoperativ TPA mindskede den rotationelle drejning ($P < 0.001$). Resultaterne viste, at CCLR ikke påvirkede den rotationelle stabilitet af det allerede TPLO-opererede knæled ($P > 0.99$). Grundet studiets design var det ikke muligt at undersøge om CCLR i sig selv forårsagede en større eller mindre rotational stabilitet sammenlignet med et TPLO-opereret knæled. Afslutningsvis konkluderer dette studie, at selvom TPLO-proceduren (TPLOX) signifikant øger den rotationelle instabilitet sammenlignet med INTACT knæleddet, kan instabiliteten ikke tilskrives overskæringen af den distale del af MCL, hvilket antyder at andre faktorer må være medvirkende til den øgede rotationsinstabilitet efter TPLO.

1. Introduction

Cranial cruciate ligament rupture (CCLR) is one of the most common orthopedic disorders affecting the canine stifle joint, with significant implications for joint stability, mobility and long-term quality of life (Bergh et al., 2014; Fossum et al., 2018; Rafla et al. 2025). Although the exact pathogenesis of CCLR remains incompletely understood, it is widely accepted that the condition arises primarily as a consequence of chronic, progressive degeneration of the cranial cruciate ligament (CCL), and rarely from isolated traumatic events caused by hyperextension and internal rotation of the pelvic limb (Fossum et al., 2018; Muir, 2017; Rafla et al. 2025). This degenerative process weakens the structural integrity of the CCL, which makes it susceptible to partial or complete rupture under normal physiological loading (Fossum et al., 2018; Muir, 2018; Rafla et al., 2025; Vasseur et al., 1985). The resulting joint instability initiates a cascade of pathological changes including meniscal damage, synovitis, osteoarthritis (OA) and lameness, affecting limb function and canine welfare (Bergh et al., 2014; Fung et al., 2023).

To address the resulting joint instability, several surgical techniques have been developed, among which tibial plateau leveling osteotomy (TPLO) has demonstrated to be effective in restoring normal stifle joint function (Bergh et al., 2014; Nanda & Hans, 2019). Despite its clinical success, in vitro studies have demonstrated that TPLO does not fully restore the initial biomechanics of the intact stifle joint (Brown et al., 2014, Shimada et al., 2020; Warzee et al., 2001). Studies investigating the postoperative complications of TPLO have also identified a phenomenon referred to as *pivot shift* causing instability in the stifle joint (Gatineau et al., 2011; Knight et al., 2017). The biomechanical changes of this phenomenon have led to further research into factors contributing to remaining instability following TPLO. In this context, this present study used a limb press model to investigate the rotational stability of the canine stifle joint in intact and postoperative TPLO conditions, with particular focus on the transection performed on the medial collateral ligament (MCL) as part of the TPLO procedure.

Given the high prevalence of CCLR and its significant impact on canine welfare, the condition remains a major subject of clinical and biomechanical research, with the overall aim of improving surgical intervention and long-term outcomes. The use of a limb press model provides a valuable and ethical methodological alternative, enabling detailed investigation of the joint mechanics under controlled conditions, without subjecting live animals to invasive procedures. This approach does not only enhance the scientific understanding of the postoperative stifle joint stability but is also aligned with the principles of animal welfare and the development of more effective and ethical treatment strategies for dogs affected by CCLR (Hoffmann et al., 2011; Hubrecht & Carter, 2019; Kneifel et al., 2018; Kowaleski et al., 2005; Lechner et al., 2020; Warzee et al., 2001).

2. Background

2.1 Cranial Cruciate Ligament (CCL)

The canine stifle joint has two cruciate ligaments: the CCL and the caudal cruciate ligament (CaCL) (Figure 1). The CCL functions as a primary stabilizer of the canine stifle joint, limiting excessive internal rotation, hyperextension and cranial tibial translation in relation to the femur. The prevention of cranial tibial translation is crucial during weight-bearing, as the contraction of the quadriceps muscle generates a cranially directed force on the tibia, influencing stifle joint stability (Arnoczky & Marshall, 1977; Slocum & Slocum, 1993). The CCL has two bands: a craniomedial band, which is taut in flexion and extension phases, and a caudolateral band, which is taut in extension and loose in flexion. When the stifle joint is flexed, the CCL and CaCL twist on each other, restricting the degree of internal tibial rotation relative to the femur. Additionally, the interaction between the CCL and CaCL during flexion contributes to a degree of angular deformity stability, thereby providing structural support to the flexed stifle joint and reducing mediolateral instability (Arnoczky & Marshall, 1977).

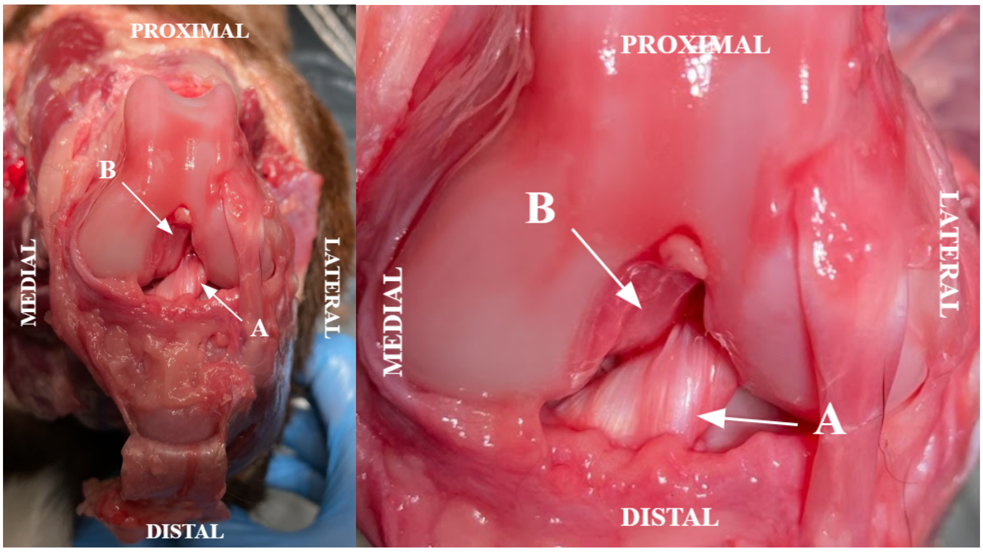

FIGURE 1 Craniocaudal view of a canine stifle joint. A: Cranial cruciate ligament (CCL), B: Caudal cruciate ligament (CaCL) (left picture). Close-up of the CCL (A) and the CaCL (B) (right picture). The pictures are taken at The University Hospital for Companion Animals by Holmbjerg ML & Tvedsborg CK.

Stifle joint instability, which consequently leads to pelvic limb lameness in dogs, is most frequently associated with CCLR, resulting in a significant craniocaudal translation of the tibia and axial rotational instability, compared to that of normal stifle joint (Tinga et al., 2018). The majority of CCLR occurs due to chronic degenerative processes of the ligament and are associated with age-related changes (especially in larger dogs), increased body size, conformational abnormalities, and immune-mediated arthropathies (Fossum et al., 2018; Muir, 2017; Rafla et al., 2025; Vasseur et al., 1985). Furthermore, a tibial plateau angle (TPA) greater than 35° has been implicated as a predisposing factor in CCLR, as an excessive TPA induces chronic biomechanical stress on the ligament, ultimately resulting in progressive structural deterioration and mechanical failure (Ichinohe et al., 2021). The degenerative process most commonly affects both pelvic limbs and therefore a high percentage of dogs develop bilateral CCLR over time. Dogs with increased risk of CCLR includes Rottweiler, Newfoundland, Labrador retriever, Brazilian Fila, Akita, Saint Bernard, German Shorthaired Pointer, Chow Chow, Bullmastiff, Bulldog, Boxer, American Cocker Spaniel and American Staffordshire Terrier (Nečas et al., 2000; Rafla et al., 2025).

Cranial drawer is a test used when performing an orthopedic examination for CCLR (Fossum et al., 2018; Muir, 2017; Slocum & Slocum, 1993). The term is used when describing an excessive cranial translation of the tibia relative to the femur when the femur is stabilized (Arnoczky & Marshall, 1977; Fossum et al., 2018). Cranial tibial thrust (CTT) is a biomechanical force that translates tibia cranial relative to the femur during weight-bearing and can be tested using the tibial compression test (TCT). In a healthy canine stifle joint, the CTT is counteracted by the intact CCL (Johnson et al., 2011; Muir, 2017; Slocum & Slocum, 1993). If the TCT is positive, the test creates a cranial translation of the tibial tuberosity relative to the femur when the hock is flexed and the gastrocnemius muscle is contracted, confirming a partial or complete CCLR (Fossum et al., 2018; Johnson et al., 2011; Slocum & Slocum, 1993).

2.2 Medial Collateral Ligament (MCL)

The function of both the lateral collateral ligament (LCL) and the MCL is to prevent excessive axial tibial rotation from causing damage to the stifle joint and structures related to the stifle joint (Vasseur & Arnoczky, 1981). Specifically, the LCL and MCL prevent excessive internal rotation when the stifle is extended and limit excessive external rotation throughout both extension and flexion (Arnoczky & Marshall, 1977; Vasseur & Arnoczky, 1981). The MCL is pictured in Figure 2.

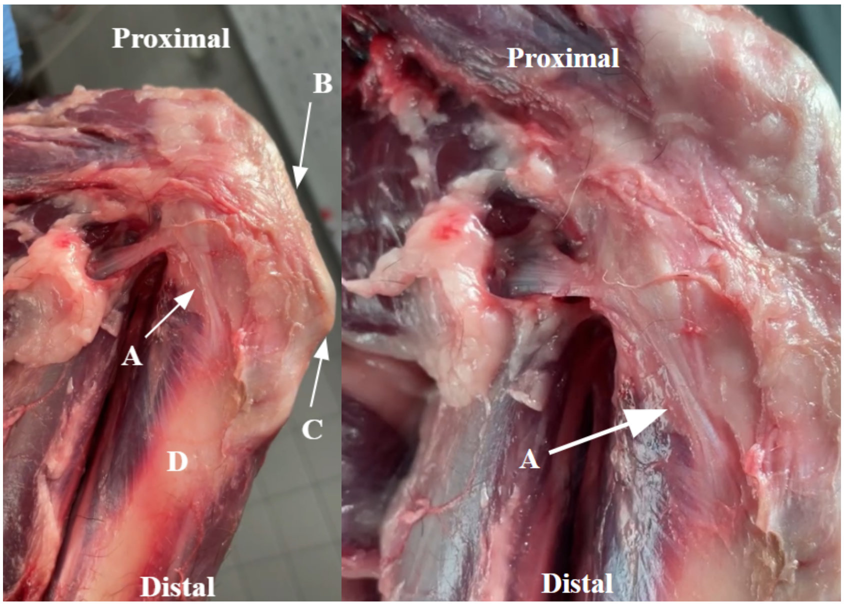

FIGURE 2 Laterolateral view of a canine stifle joint. A: Medial collateral ligament (MCL), B: Patellar ligament, C: Patella, D: Tibia. Close-up of the MCL (right picture). The pictures are taken at The University Hospital for Companion Animals by Holmbjerg ML & Tvedsborg CK.

The MCL originates from the medial femoral epicondyle and extends distally over the femorotibial joint capsule with the largest insertion site being on the proximal medial tibia located between 8.2-15.5% of the total length of tibia (Palierne et al., 2022; Vasseur & Arnoczky, 1981). The MCL adheres strongly to the medial meniscus and is considered a potential important contributor in maintaining rotational stability of the stifle joint (Palierne et al., 2022). A serous bursa is placed between the MCL and the proximal part of the tibia, enabling the MCL to glide over the medial tibial condyle during flexion, extension and tibial rotation, thereby contributing to joint mobility and minimizing mechanical stress (Palierne et al., 2022; Vasseur & Arnoczky, 1981). A study by Vasseur & Arnoczky (1981) investigated the roles of the collateral ligaments in maintaining rotational stability of the stifle joint. They found that transection of the MCL resulted in a moderate increase in the internal tibial rotation when the pelvic limb was extended, whereas no significant change was observed during flexion. Conversely, external tibial rotation was only mildly affected during extension, but showed a moderate increase during flexion following MCL transection (Vasseur & Arnoczky, 1981).

2.3 The Menisci of the Canine Stifle Joint

The canine stifle joint has two fibrocartilaginous structures: the lateral and medial menisci, which serves multiple essential functions including shock absorption, load distribution, congruity enhancement and stabilization of the joint (Flo, 1993). The menisci are of particular interest in relation to CCLR due to their role in maintaining joint stability. Meniscal damage is a common clinical finding in dogs with CCLR and are typically diagnosed through arthroscopy or arthrotomy. If damage to the meniscus is left untreated, it can lead to persistent lameness, pain and long-term joint dysfunction (Flo, 1993; Fung et al., 2023; Hayes et al., 2010; Nečas & Zatloukal, 2002).

The prevalence of medial meniscal tears in dogs with CCLR ranges from 10-70%. The medial meniscus is more frequently damaged than the lateral meniscus, mainly due to its limited mobility with a markedly higher incidence of medial meniscal tears in cases of complete CCLR compared to partial CCLR (Flo, 1993; Fung et al., 2023; Hayes et al., 2010; Ralphs & Whitney, 2002). This incidence is believed to be the effect of repetitive CTT during weight-bearing in the CCLR stifle, causing the caudal aspect of the medial femoral condyle to apply pressure on the caudal horn of the medial meniscus, leading to damage and degeneration (Flo, 1993; Nečas & Zatloukal, 2002; Ralphs & Whitney, 2002). In contrast, the lateral meniscus has an increased mobility, which enables it to distribute the load more effectively from the caudal femoral condyle during CTT (Flo, 1993). Risk factors for meniscal injury include body condition score (BCS), age, chronicity of lameness, complete CCLR and severe OA changes (Fung et al., 2023; Hayes et al., 2010; Nečas & Zatloukal, 2002). A retrospective study by Fung et al. (2023) reported that among 479 canine stifles that underwent surgery for CCLR, 51% had partial meniscectomy, 28% had a hemi-meniscectomy and 21% had a complete meniscectomy. Kim et al. (2012) demonstrated that medial hemi-meniscectomy may predispose the stifle joint to persistent femorotibial subluxation following TPLO, highlighting the stabilizing effect of the medial meniscus to the stifle joint even after surgical intervention.

2.4 Tibial Plateau Leveling Osteotomy (TPLO)

The current treatment of choice for CCLR in dogs weighing > 15 kilogram (kg) is surgical intervention, with TPLO being the preferred technique for restoring long-term functional joint stability and regaining full efficacy of the affected pelvic limb postoperatively (Bergh et al., 2014; Nanda & Hans, 2019; Slocum & Slocum, 1993; Von Pfeil et al., 2018). The TPLO procedure involves rotation of the tibial plateau to reduce the TPA, thereby theoretically neutralizing the CTT (Figure 3) (Johnson et al. 2011; Nanda & Hans, 2019; Slocum & Slocum, 1993; Shahar et al., 2006). The pre and postoperative TPA is measured based on three lines on laterolateral radiographs of the pelvic limb with the hock and stifle flexed at 90°. The first line is drawn from the intercondylar eminence to the center of the talocrural joint, a second line is drawn from the cranial to the caudal border of the tibial plateau and the third line is placed perpendicular to the intersection of the first two lines. The angle between the second and third line defines the TPA (Appendix 2: Figure 8) (Fossum et al., 2018; Volz et al., 2025). Most existing research predominantly indicates that the ideal postoperative TPA should be between 5-6.5° to effectively reduce CTT (Nanda & Hans, 2019; Volz et al., 2024; Warzee et al., 2001). However, a recent study by Bester et al. (2025) indicated that the ideal postoperative TPA was closer to 10° to better replicate the biomechanics of the intact CCL stifle joint rather than a TPA between 5-6.5°.

While cranial drawer test can be used to diagnose CCLR, it is important to note that the test is not a reliable indicator of surgical success following TPLO, as it tests for passive force and therefore does not replicate the biomechanics of the stifle joint at full range of motion during weight-bearing. Instead, TPLO functions by altering the biomechanics to neutralize the CTT in the postoperative stifle joint (Cook, 2010; Slocum & Slocum, 1993).

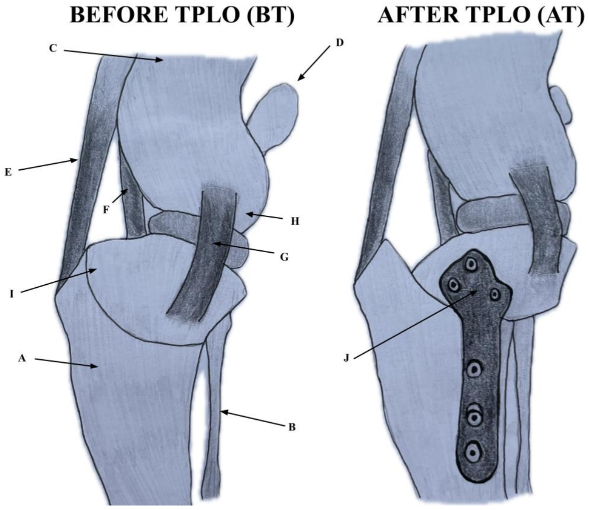

FIGURE 3 A: Tibia, B: Fibula, C: Femur, D: Fabella, E: Patella ligament, F: Lateral collateral ligament, G: Medial collateral ligament, H: Medial meniscus, I: Circular cut, J: Tibial plateau leveling osteotomy (TPLO) plate. (BT) The stifle joint before TPLO and where the circular cut is placed (I). (AT) the stifle joint following TPLO, the bone-fragment is moved and secured with a TPLO plate (J). The drawing is illustrated by Holmbjerg ML, with inspiration from illustrations by Slocum & Slocum (1993) with changes based on TPLO plate type and radial cut position.

2.5 Postoperatively TPLO Stifle Instability

While TPLO remains a preferred surgical technique for restoring function in CCLR stifles, in vitro studies by Warzee et al. (2001), Brown et al. (2014) and Shimada et al. (2020) have found that the procedure does not fully restore the initial biomechanical function of a CCL-intact stifle joint (Bergh et al., 2014; Von Pfeil et al., 2018).

One particular factor influencing the function of postoperative stifle joints is the TPA achieved following osteotomy and realignment of the tibial plateau. The degree of postoperative TPA has shown to affect the stifle joint biomechanics and the load on the remaining ligaments potentially contributing to stifle joint instability when compared to the CCL-intact stifle (Brown et al., 2014; Shahar et al., 2006; Shimada et al., 2020). One clinically relevant manifestation of this instability is the pivot shift phenomenon (PSP), which has been reported in 3.1% canine stifle joints following TPLO in a retrospective study including 476 dogs (Gatineau et al., 2011). PSP is characterized by a sudden cranial subluxation of the tibial plateau combined with an internal tibial rotation of the stifle joint, in response to a sudden lateral deviation during weight-bearing (Fossum et al., 2018; Gatineau et al., 2011; Knight et al., 2017). This dynamic instability is a postoperative complication of TPLO and has been identified in larger breeds such as Labrador retrievers, Golden retrievers and Bernese Mountain dogs (Gatineau et al., 2011). The etiology of PSP is widely regarded as multifactorial, and may involve tibial torsion, excessive internal tibial rotation, angular limb deformities and medial meniscal surgical interventions, such as meniscectomy or meniscus release (Bergh & Peirone, 2012; Gatineau et al., 2011; Knight et al., 2017). While CTT can still be detected at some point following TPLO, internal tibial rotation has also been shown to persist postoperatively (Johnson et al., 2011; Warzee et al., 2001). Shimada et al. (2020) found that the TPLO procedure increased the flexion of the femorotibial joint, thereby reducing the stabilization provided by the MCL and LCL, resulting in an increased rotational instability of the stifle following TPLO. The TPLO procedure creates a curvilinear osteotomy using a saw blade that can transect or disrupt the tibial insertion site of the MCL. The osteotomy can lead to a shortening between the MCL's femoral and tibial insertion sites, thereby altering its anatomical orientation and potentially affecting the functional stability of the stifle joint following TPLO (Palierne et al., 2022; Palierne et al., 2023; Shimada et al., 2020; Slocum & Slocum, 1993). In a study by Palierne et al. (2023), the relevance between the placement and size of the saw blade used for the osteotomy when performing TPLO on the postoperative stifle instability was evaluated. The findings demonstrated that while the placement and size of the saw blade significantly influenced the preservation of the MCL, partial loss of the MCL's tibial insertion site did not significantly affect postoperative stifle joint stability. Due to the strong anatomical association between the MCL and the medial meniscus, damage to the medial meniscus may compromise its attachment to the MCL. This disruption may contribute to the etiology of PSP by altering stifle joint stability and biomechanics (Gatineau et al., 2011; Palierne et al., 2022).

2.6 The Use of Limb Press Models in Veterinary Research

A limb press model is an in vitro biomechanical testing device, designed to simulate physiological loading conditions of an animal's limb, particularly joints, such as the stifle, under controlled conditions. The limb press model provides a reproducible environment for investigating joint stability and biomechanical outcomes of various surgical interventions. In the context of CCLR, limb press models have been essential for our understanding of the stifle joint function in both intact CCL and CCLR conditions (Hoffmann et al., 2011; Kneifel et al., 2018; Kowaleski et al., 2005; Lechner et al., 2020; Warzee et al., 2001).

The limb press model offers several main advantages, particularly in terms of ethical and methodological considerations. One of its primary advantages is the ability to conduct realistic biomechanical assessments without the use of live animals, aligning with the principles of the 3 R's (Replacement, Reduction and Refinement) in animal research (Hubrecht & Carter, 2019). When utilizing cadaveric limbs, it is possible for researchers to replicate in vivo joint mechanics and loading patterns while minimizing ethical concerns associated with testing on live animals. Previous studies have demonstrated the efficacy of limb press models in investigating CCL function and evaluating surgical stabilization techniques (Hoffmann et al. 2011; Kneifel et al., 2018; Kowaleski et al., 2005; Lechner et al. 2020, Warzee et al., 2001). Hoffmann et al. (2011) confirmed that the CCL plays a critical role in neutralizing CTT using a limb press model. Similarly, Warzee et al. (2001) and Lechner et al. (2020) utilized limb press models to assess postoperative stifle joint mechanics following TPLO and other stabilizing procedures in both canine and feline subjects. Their findings support the models' ability to replicate clinically relevant joint forces and movements, thereby allowing detailed investigations into postoperative stability. Despite these advantages, limb press models are not without limitations. In vitro testing lacks the neuromuscular and biological responses that occur in vivo, such as muscle tone, healing process and long-term adaptation. Therefore, limb press models must be interpreted within the context of these limitations.

3. Aim and Objectives

The objective of this study was to assess the rotational stability of the canine stifle joint under four different conditions, with a particular focus on determining whether an increased instability in the postoperative stifle was the result of the transection of the MCL at the site of the osteotomy for the TPLO procedure, or the TPLO itself. A limb press model was utilized to assess the rotational stability. This study hypothesised that; (1) the rotational range of the canine stifle joint would increase after transection of the MCL, (2) the rotational range of the stifle joint following TPLO would increase compared to the intact stifle joint and (3) the rotational range would increase following TPLO and CCLR compared to the isolated TPLO stifle joint.

4. Materials and Methods

4.1 Study Population

In this study, 20 dogs met the inclusion criteria with 10 legs being randomly selected using an online service (Sealed Envelope Ltd. 2024) with one pelvic limb per dog being included with an even distribution of right and left pelvic limbs. BCS, neutered status and age was not recorded in this study. The inclusion criteria for the study population were based on a body mass ranging from 20-40 kg, as well as the presence of a negative TCT and a negative cranial drawer motion in both pelvic limbs. All dogs used were euthanized for reasons unrelated to this study and donated to scientific research by the owners and approved by the local committee at the Institute of Clinical Veterinary Medicine at The University of Copenhagen with the ethical approval number 2024-44. The dogs selected were weighed before amputation of both pelvic limbs. The tibial length was measured from the proximal tibial intercondylar eminence to the center of the talus.

4.2 Study Design and Specimen Preparation

Following amputation, the limbs were stored at -22° Celsius (C), and subsequently thawed at 4°C for 24-72 hours prior to testing. Radiographs were taken prior to dissection, see section "4.3 *TPLO preoperative planning*" for further details. Muscular tissue dissection was performed while ensuring the preservation of the joint capsule, the collateral ligaments and the patellar ligament. Musculature surrounding the femur was removed, while tibial musculature was largely preserved. A medial arthrotomy was performed with a craniomedial incision proximal to the patella and distal to the tibial crest through the subcutaneous tissue, medial parapatellar retinaculum and joint capsule, to confirm the integrity of the CCL (Fossum et al., 2018). To facilitate controlled rotation and loading adjustments, a hole was drilled through the patella for attachment of a turnbuckle. To ensure anatomical alignment within the limb press model, the tibia was amputated 40 millimetres (mm) proximal to the malleolus, compensating for the natural distance between the amputation site and the malleolar region when attached to the metal plate of the limb press model (Figure 4).

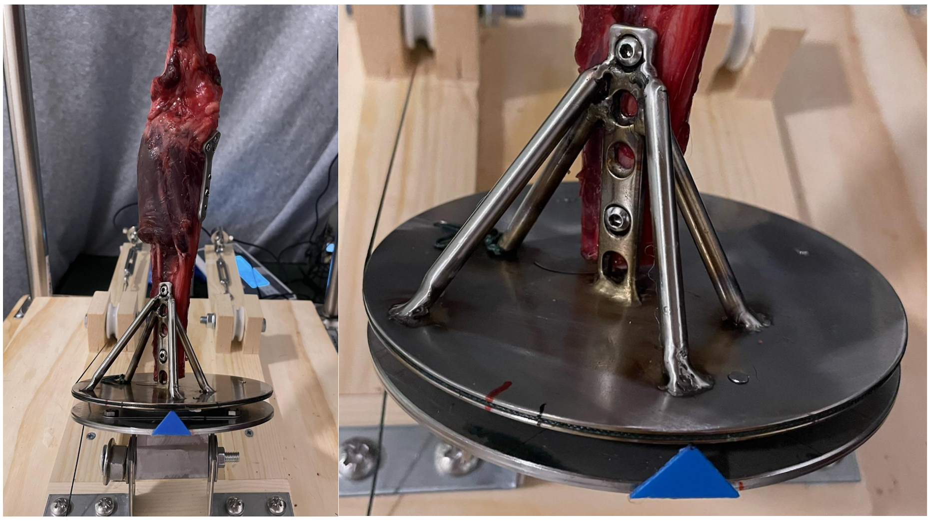

FIGURE 4 Caudocranial view of a pelvic limb attached to the limb press model. The picture shows the metal plate attached distally to the limb press model with the distal part of a specimen's tibia attached proximally to the plate (left picture). The blue triangle functions as a tool to assess change in neutral position for each tested condition marked with a permanent marker. The black line marks the neutral position for the intact stifle joint (right picture). The red line marks the neutral position for the medial collateral ligament transection and tibial plateau leveling osteotomy stifle joint, indicating an external shift of the neutral position (right picture). The pictures are taken at The University Hospital for Companion Animals by Holmbjerg ML & Tvedsborg CK, with the metal plate developed by the blacksmith at the Department of Plant and Environmental Sciences at The Faculty of Science at The University of Copenhagen.

During preoperative TPLO planning, a new landmark for measuring the functional axis (FA) was established by determining the angle between the distal tibial tuberosity and the FA. This approach ensured the highest possible accuracy in postoperative TPA measurements following tibiotarsal joint removal. A 3.5 mm wide hole was drilled through fovea capitis laterally, exiting at trochanter major. A 4.5 mm wide bone plate bent at 90° was positioned along the proximal cranial border of the femur and secured with 4.5 mm screws. A load cell (Model:DYMH-103, up to 100 kg load) designed to simulate quadriceps muscle forces was attached proximally to the top of the bone plate and distally to the turnbuckle fixed to the tibia, due to quadriceps influence on stifle joint stability in vivo (Arnoczky & Marshall, 1977; Slocum & Slocum, 1993).

The specimen was secured onto the limb press model (Figure 5) in a horizontal position using a 3 mm wide pin, inserted through fovea capitis and trochanter major. The femoral pin was fixed to an external fixator, ensuring joint capsule alignment in a horizontal orientation. The caudal aspect of the tibia was secured to the metal plate of the model using 3.5 mm screws. The metal plate functioned as the distal attachment point of the tibia to the limb press model. The metal plate had a groove in the middle of the plate, enabling a string to go around, allowing external and internal rotation of the stifle joint. The quadriceps turnbuckle was adjusted to set the stifle joint angle to 135°, while the base plate was modified to achieve a femoral angle of 70°. A strain force equivalent to 30% of the specimen's body mass was applied at the top of the limb press model, replicating physiological loading conditions (Shimada et al., 2022; Warzee et al., 2001). Throughout the experiment, soft tissue was kept moist by periodically applying a saline solution.

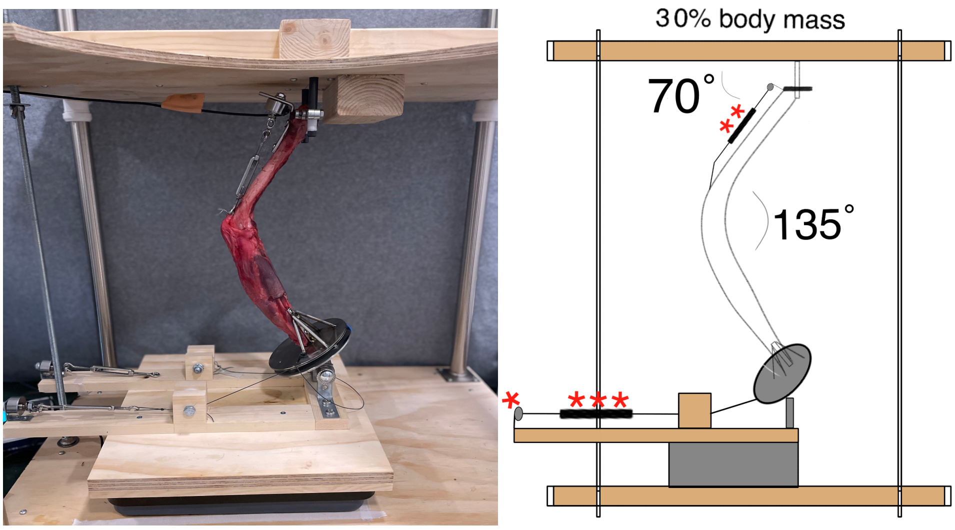

FIGURE 5 Experimental setup. Picture (left) and schematic illustration (right) of the limb press model. A load cell measuring torque was placed at the anterior part of the limb press model (*). The quadriceps load cell was attached proximally to femur and connected to patella by a turnbuckle (**). The femur condyles were attached to external fixators proximal to the model. The distal part of tibia was attached to the rotating metal plate placed distally on the model. A string was placed in the groove of the metal plate and connected to a turnbuckle to replicate either external or internal torque (***). The schematic illustration was illustrated by Tvedsborg CK. using 2021-2025 Sketchbook, Inc. with inspiration from the limb press model illustration by Warzee et al. (2001) with changes based on a custom-made design by Holmbjerg ML, Miles JE, Nielsen MBM and Tvedsborg CK.

4.3 TPLO Preoperative Planning

The TPLO procedure was performed by a single experienced surgeon (Miles JE) assisted by Holmbjerg ML, Tvedsborg CK and co-supervisor (Nielsen MBM), following the standardized technique described by Fossum et al. (2018) and Slocum & Slocum (1993). A jig was not used in this study when performing the TPLO due to the surgeon's (Miles JE) extensive experience performing TPLO without the use of a jig. On the day of testing, preoperative radiographic planning for TPLO was performed using mediolateral radiographic views of the pelvic limb with a 25 mm marker. The radiographs were taken with the femorotibial and tibiotarsal joints positioned at 90° prior to limb dissection. Preoperative TPLO planning was performed in an orthopedic preoperative planning software (vPOP^{PRO} version 3.3.2 [283], VetSOS Education Ltd., veterinary preoperative orthopedic planning software). The target postoperative TPA was set to 5° (Nanda & Hans, 2019; Volz et al., 2024; Warzee et al., 2001). How the TPA was measured is described in section "2.4 *Tibial plateau leveling osteotomy (TPLO)*".

4.4 Testing Conditions

Axial tibial rotation was assessed under four experimental testing conditions in this exact order: (I) Intact stifle joint (INTACT), (II) Medial collateral ligament transection (MCLX), (III) Medial collateral ligament transection and tibial plateau leveling osteotomy (TPLOX) and (IV) Medial collateral ligament transection and tibial plateau leveling osteotomy with cranial cruciate ligament transection (CCLX) (Figure 6).

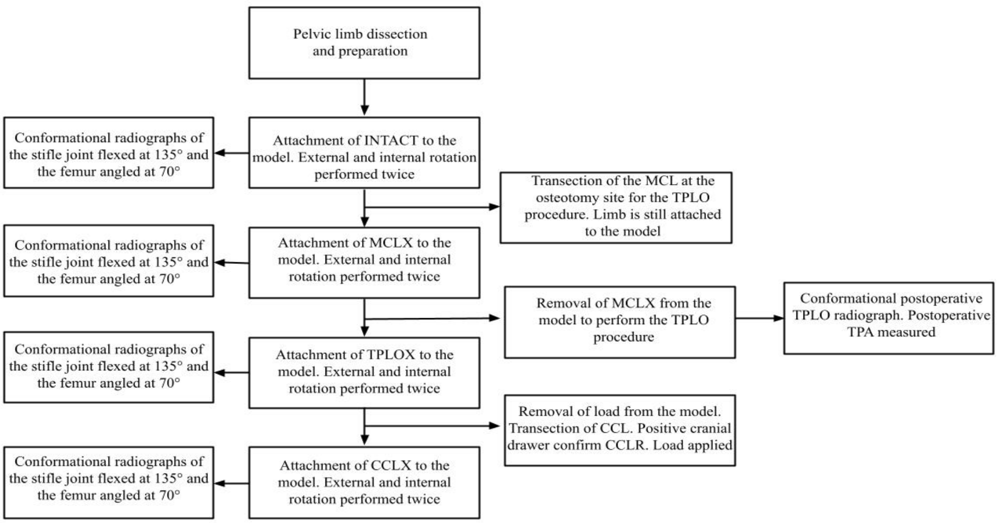

FIGURE 6 Flow diagram illustrating the experimental testing order. The flow diagram was illustrated by Tvedsborg CK, inspired by the flow diagram pictured in Shimada et al. (2022) with changes based on the present study's design and methodology.

Abbreviations: MCL, Medial collateral ligament; TPLO, Tibial plateau leveling osteotomy; TPA, Tibial plateau angle; CCL, Cranial cruciate ligament; CCLR, Cranial cruciate ligament rupture; INTACT, Intact stifle joint; MCLX, Medial collateral ligament transection; TPLOX, Medial collateral ligament transection and tibial plateau leveling osteotomy; CCLX, Medial collateral ligament transection and tibial plateau leveling osteotomy with cranial cruciate ligament transection.

The neutral position (Figure 4) of the INTACT limb was identified and marked with a permanent marker on the metal plate before the specimen was subjected to external and internal torque testing. The same procedure was repeated for the three remaining conditions: (II) MCLX, (III) TPLOX and (IV) CCLX.

Following the initial assessment, the MCL was transected at the osteotomy site for the TPLO procedure measured beforehand using an orthopedic preoperative planning software (vPOP^{PRO} version 3.3.2 [283], VetSOS Education Ltd., veterinary preoperative orthopedic planning software). The length of the transected MCL segment (from the joint surface of tibia to the tibial transection site) and the total length of the MCL was recorded. Radiographs were taken to verify the correct alignment of the stifle joint at 135° and femur at 70° to replicate the stance phase and ensure consistency of joint angles throughout the four testing conditions (Figure 7) (Shimada et al., 2022; Warzee et al., 2001).

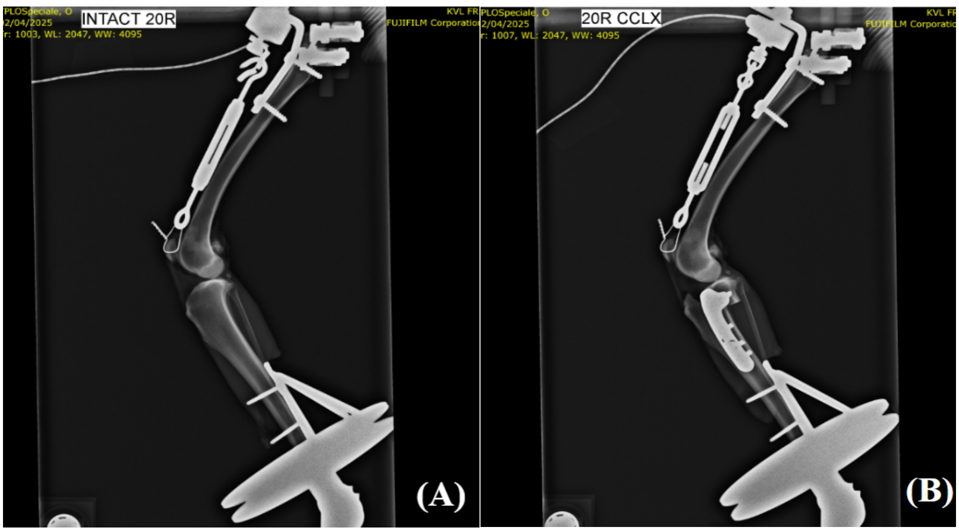

FIGURE 7 Laterolateral radiographic views of a pelvic limb attached to the limb press model. (A) Intact stifle joint; (B) Medial collateral ligament transection and tibial plateau leveling osteotomy with cranial cruciate ligament transection. The radiographs are taken at The University Hospital for Companion Animals by Holmbjerg ML & Tvedsborg CK with supervisor Miles JE.

The specimen was then removed from the limb press model and TPLO was performed (TPLOX) as described by Fossum et al. (2018) and Slocum & Slocum (1993) by an experienced surgeon (Miles JE), the co-supervisor (Nielsen MBM) and the authors (Holmbjerg ML & Tvedsborg CK). The osteotomies were secured with a 3.5 mm small locking TPLO plate from Veterinary Orthopedic Implants (VOI) (Movora "Global Partner in Veterinary Medtech & Orthopedics", 2025). Postoperative TPLO radiographs were taken to measure the postoperative TPA. The specimen was subsequently secured to the limb press model, and new radiographs were taken to verify that the stifle joint was flexed at 135° and the femur angled at 70°. The strain from the top of the limb press model was removed and the CCL was transected (CCLX), still attached to the limb press model, and CCLR was confirmed by a positive cranial drawer motion performed by the surgeon (Miles JE). Strain from the top of the limp press model was reapplied and new radiographs were taken.

The rotational range was measured in mm and subsequently converted to degrees using the arcsine function $\theta(°) = 2 * arcsin(\frac{c}{2r}) * (\frac{180}{\pi})$, with *r* being the 60 mm radius of the metal plate and *c* being the measured length from the neutral position of the metal plate (blue arrow as pictured in Figure 4) to the point of rotation on the metal plate when the external/internal torque was reached. The equation above is derived from the knowledge of fundamental trigonometric principles in circle geometry.

4.5 Laxity Testing of The Stifle Joints Anatomical Structures

To evaluate the potential effects of repeated axial tibial rotation on joint stabilizing structures, the specimen was tested in five cycles of controlled internal and external axial tibial rotation, replicating the rotational forces applied to the tested specimens in this study. This procedure aimed to determine whether repeated manipulation of the stifle joint would result in increased joint laxity over time and thereby increased joint instability. This test was performed on a single specimen meeting the inclusion criteria prior to testing of the 10 limbs included in the final data. To ensure there was little to no variability between the values recorded for the 10 limbs included, the external and internal rotation was examined twice for each condition and the mean value included.

4.6 Data Collection

The internal and external torque load was measured by two load cells (Model: DYMH-103, up to 100 kg load) anchored to the cranial part of the limb press model in relation to the specimen. A string placed in the groove of the metal plate was then attached to one of the load cells and the torque was measured. A third load cell was attached to the proximal part of the femur to represent the axial load during testing by replicating quadriceps muscle force. The torque was measured with hardware (DFRduino Boards UNO R3) calibrated to a software program (Arduino IDE version 2.3.4) on a computer by an experienced observer (Miles JE). Each load cell was calibrated each day of testing with 294.45 Newton (N) (30 kg). The torque for each specimen was calculated before the experiment using the equation: *(bodyweight (N) * 0.007 Nm*[1]*)/0.06 m*[2] (Meise et al., 2021).

4.7 Statistical Analysis

To get an overview of the data, a series of diagrams and plots were created in Excel (Microsoft Corporation, 2018) prior to statistical analysis in RStudio 2024.12.1 (R Core Team, 2021). The package *readxl* was used to import the data from excel to R (Wickham & Bryan, 2025). A Shapiro-Wilks test was performed to confirm normal distribution of the data and subsequently on the residuals from the repeated measures ANOVA. The packages *lme4* and *lmertTest* were used to perform one-way repeated measures ANOVA on the residual values (Bates et al., 2015; Kuznetsova et al., 2017). A one-way repeated measures ANOVA was performed to analyze if there were any statistically significant differences in rotation between the four conditions: (I) INTACT, (II) MCLX, (III) TPLOX and (IV) CCLX, using the *ez* package (Lawrence, 2016). Post-hoc analysis was performed using the *emmeans* package with a subsequent paired comparison in combination with Bonferroni correction to identify which, or if any, condition(s) had statistically significant difference(s) in the rotational range (Lenth, 2025). Lastly, a Pearson's correlation test was performed to investigate if there was a potential correlation between (1) the length of the MCL and the rotational range of the INTACT and MCLX condition and (2) the postoperative TPA and the rotational range of the TPLOX condition. The statistical significance level in this study was set to a P-value <0.05. ChatGPT was used for statistical advice and interpretation of results but was continuously examined thoroughly and

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[1] Newton meters (Nm)

[2] Radius of metal plate in meters (m)

critically to ensure the integrity of the results. For more information about the use of ChatGPT see section *"9. Disclosures"*.

5. Results

5.1 Study Population

A total of 20 dogs met the inclusion criteria and were selected for this study. Of those 20 dogs, 13 were female and six males. Due to technical errors in the initial phase of the project, three specimens were excluded and replaced with three new specimens. The following 10 dogs are the ones included in the final study. Of the 10 dogs included seven were female and three males. The represented breeds included: German shepherd (n = 1), Labrador retriever (n = 5), American Staffordshire Terrier (n = 1), mixed breed (n = 1) and Golden retriever (n = 2). The mean body mass of the selected dogs was 30.5 kg (range 24.2-36.6 kg) with a mean tibial length of 193.6 mm (range 171.4-215.9 mm). The mean preoperative TPA ranged from 20.3-30.5° (mean ± standard deviation (SD); 24.7 ± 3.0°) and the mean postoperative TPA ranged from 2.8-9.9° (mean ± SD; 5.5 ± 2.1°). The mean total length of MCL ranged from 25.7-38.6 mm (mean ± SD; 32.4 ± 4.1 mm) and the length of MCL preserved following osteotomy for TPLO ranged from 18.1-23.8 mm (mean ± SD; 20.2 ± 1.9 mm) and in percentage 48.4-78.2% (mean ± SD 63.1 ± 9.8%).

For limb press model validation, a right pelvic limb of a female Labrador retriever (29.1 kg) with a negative TCT and cranial drawer motion was used to assess potential increases in stifle joint laxity following repeated internal and external tibial rotation (Table 1). The specimen was prepared according to the standardized protocol outlined above and subjected to external and internal rotation five times. The results from the trial revealed a rotational range ranging from 18.33-22.24° with a mean ± SD of 20.73 ± 1.57°. These results revealed little variation with no wild outliers, suggesting that the repeated external and internal rotation of the stifle joint did not cause iatrogenic laxity of joint structures, suggesting that the repeated rotation of the included 10 pelvic limbs would not affect their rotational stifle joint stability.

TABLE 1 Limb press model validation. Repeated internal and external rotation measured in angle of rotation (°) of an intact stifle joint. Range: The sum of the internal and external values.

External (°)	Internal (°)	Range (°)
7.86	14.38	22.24
7.86	14.18	22.04
7.57	10.76	18.33
7.08	13.40	20.48
7.76	12.81	20.57

5.2 Assessment of Rotation

The results of the one-way repeated measures ANOVA revealed a significant difference in the rotational range across the four different conditions ($P < 0.001$). The residuals values from the one-way repeated measures ANOVA revealed a normal distribution. The mean values and SD for the rotational range of the four conditions are summarized in Table 2. The full dataset is included in the appendix (Appendix 1: Table 8).

TABLE 2 Limp press model results. Overview of the mean and standard deviation (SD) values of the rotational range for each of the four tested conditions: INTACT, MCLX, TPLOX and CCLX.

Condition	Mean ± SD (°)
INTACT	25.02 ± 3.12
MCLX	25.28 ± 2.94
TPLOX	39.15 ± 8.19
CCLX	40.38 ± 8.82

Abbreviations: INTACT, Intact stifle joint; MCLX, Medial collateral ligament transection; TPLOX, Medial collateral ligament transection and tibial plateau leveling osteotomy; CCLX, Medial collateral ligament transection and tibial plateau leveling osteotomy with cranial cruciate ligament transection.

Post hoc test comparisons are summarized in Table 3, following one-way repeated measures ANOVA, to distinguish between the four conditions and reveal which condition(s) either had a significant or insignificant increase or decrease in the rotational range. The results revealed a significant difference when comparing joint stability between the testing conditions: INTACT vs TPLOX, INTACT vs CCLX, MCLX vs TPLOX and MCLX vs CCLX (one-way repeated measures ANOVA, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$, as shown in Table 3). There was no significant difference in the rotational range between the INTACT vs MCLX and the TPLOX vs CCLX study groups (one-way repeated measures ANOVA, $P < 0.99$; $P > 0.99$).

TABLE 3 Overview of P-values from post-hoc test with Bonferroni correction when analysing the rotational range between all four tested conditions: INTACT, MCLX, TPLOX and CCLX. Estimated pairwise difference (Estimate), standard errors (SE), t-ratio and P-value are given for each comparison.

Condition			Estimate (°)	SE (°)	t-ratio	P-value
INTACT	vs	MCLX	-0.26	0.44	-0.59	> 0.99
INTACT	vs	TPLOX	-14.14	1.88	-7.51	< 0.001
INTACT	vs	CCLX	-15.36	2.40	-6.41	< 0.001
MCLX	vs	TPLOX	-13.88	1.80	-7.70	< 0.001
MCLX	vs	CCLX	-15.10	2.25	-6.72	< 0.001
TPLO	vs	CCLX	1.22	1.51	0.81	> 0.99

Abbreviations: INTACT, Intact stifle joint; MCLX, Medial collateral ligament transection; TPLOX, Medial collateral ligament transection and tibial plateau leveling osteotomy; CCLX, Medial collateral ligament transection and tibial plateau leveling osteotomy with cranial cruciate ligament transection.

The results did not reveal a difference between the transection of the MCL at the osteotomy site for the TPLO procedure and an increased joint instability between the INTACT and MCLX conditions (one-way repeated measures ANOVA, $P > 0.99$). Similarly, the length of the MCL from the joint surface to the osteotomy site did not show a significant influence on the rotational range of the stifle joint (correlation test, $P = 0.30$) (Table 4). Additionally, the postoperative TPA in the TPLOX study group was investigated to assess a potential influence on the observed increased rotational range when comparing the preoperative TPA in the INTACT study group. The postoperative TPA measured in this study ranged from 2.8-9.9° (mean ± SD; 5.5 ± 2.1°). A correlation test revealed a significant negative correlation (correlation test, -0.81) between the postoperative TPA and an increased rotational range (correlation test, $P < 0.001$), suggesting that the postoperative TPA has a significant influence on the rotational range following TPLO. The negative correlation implies that the greater postoperative TPA, the lesser rotational range following TPLO and conversely, the lesser postoperative TPA, the greater rotational range following TPLO (Table 4).

TABLE 4 Pearson's correlation test results. P-values and correlation values when testing (1) the residual length of the medial collateral ligament following tibial plateau leveling osteotomy (TPLO) in relation to the rotational range and (2) the postoperative tibial plateau angle following TPLO in relation to the rotational range.

Pearson's Correlation Test	P-Value	Correlation
Length of MCL	0.30	0.24
Postoperative TPA	< 0.001	-0.81

Abbreviations: MCL, Medial collateral ligament; TPLO, Tibial plateau leveling osteotomy; TPA, Tibial plateau angle.

Significant differences were observed in rotational range between the following conditions comparisons: INTACT vs TPLOX, INTACT vs CCLX, MCLX vs TPLOX and MCLX vs CCLX, as illustrated in Table 3. When analysing internal and external rotation independently, only the internal rotation showed a consistent statistically significant difference across the conditions. In contrast, isolated analysis of external rotation revealed a significant difference solely between the TPLO and CCLX conditions (Table 5 and 6). These findings suggest that the overall significant differences in rotational range between the conditions were primarily caused by an increase in internal rotation. Although external rotation was significantly different in the TPLOX vs CCLX comparison, it did not contribute to a significant difference in the total rotational range as shown in Table 3.

TABLE 5 Post hoc test with Bonferroni correction. P-values when external and internal rotation was analysed separately between all four testing conditions: INTACT, MCLX, TPLOX and CCLX.

Condition			P-values, Internal	P-values, External
INTACT	VS	MCLX	> 0.99	0.29
INTACT	VS	TPLO	0.001	0.53
INTACT	VS	CCLX	0.002	0.06
MCLX	VS	TPLO	< 0.001	> 0.99
MCLX	VS	CCLX	0.002	0.12
TPLO	VS	CCLX	> 0.99	0.02

Abbreviations: INTACT, Intact stifle joint; MCLX, Medial collateral ligament transection; TPLOX, Medial collateral ligament transection and tibial plateau leveling osteotomy; CCLX, Medial collateral ligament transection and tibial plateau leveling osteotomy with cranial cruciate ligament transection

TABLE 6 Limp press model results. Overview of the mean and standard deviation (SD) values of the internal and external rotational range for each of the four testing conditions: INTACT, MCLX, TPLOX and CCLX.

Condition	Mean ± SD (°), Internal	Mean ± SD (°), External
INTACT	15.35 ± 2.66	9.58 ± 1.75
MCLX	14.07 ± 2.30	10.51 ± 1.82
TPLO	26.95 ± 8.94	11.71 ± 2.92
CCLX	26.46 ± 8.25	13.95 ± 4.30

Abbreviations: INTACT, Intact stifle joint; MCLX, Medial collateral ligament transection; TPLOX, Medial collateral ligament transection and tibial plateau leveling osteotomy; CCLX, Medial collateral ligament transection and tibial plateau leveling osteotomy with cranial cruciate ligament transection

Neutral position was marked and evaluated between all four conditions. The neutral position marked in the beginning of testing the INTACT limb was referred to as the "original" neutral. If a shift in the neutral position had occurred, the new neutral position would be marked using a different color permanent marker on the metal plate and referred to as the "new" neutral (Figure 4). The distance between the original neutral and the new neutral was measured and noted. The new neutral was then used as the new starting point when measuring the external and internal torque for the current condition. It was also noted if no shift in the neutral position had occurred between the four testing conditions. The neutral position on the metal plate generally differed between the MCLX, TPLOX and CCLX conditions. The values from these observations are summarized in table 7.

TABLE 7 Mean and standard deviation (SD) for the neutral position for the four tested conditions in this study: INTACT, MCLX, TPLOX and CCLX. One of 10 specimens was excluded from this data due to technical errors in the initial phase of the project. Positive values indicate a new internal rotational landmark. Negative values indicate a new external rotational landmark.

Condition	Mean ± SD (°)
INTACT	0 ± 0
MCLX	0.43 ± 1.30
TPLO	6.26 ± 3.84
CCLX	7.16 ± 3.79

Abbreviations: INTACT, Intact stifle joint; MCLX, Medial collateral ligament transection; TPLOX, Medial collateral ligament transection and tibial plateau leveling osteotomy; CCLX, Medial collateral ligament transection and tibial plateau leveling osteotomy with cranial cruciate ligament transection.

Explanation: MCLX: 0.43 ±1.30 = 1.73 & -0.87 (internal, positive value & external, negative value indicating a shift of neutral position in the given direction).

6. Discussion

The findings of this study provide new insights into postoperative rotational stability of the canine stifle joint following TPLO. Among the three hypotheses investigated, only the hypothesis stating that the TPLO procedure increases postoperative rotational instability of the stifle joint was accepted.

The hypotheses suggesting that transection of the distal MCL would cause greater rotational instability following TPLO, and that TPLO combined with CCLR would result in greater instability compared to a TPLO stifle without CCLR, were rejected.

Most notably, in contrast to this study's initial hypothesis, the results demonstrated that the transection of the distal part of MCL (MCLX) did not result in a significant increase in rotational range when compared to the INTACT stifle. This suggests that the distal part of MCL may not play a primary role in preventing rotational forces in the context of TPLO-induced biomechanical changes. Similarly, the measured distance from the joint surface to the MCL transection site of the osteotomy site did not show a significant correlation with an increased rotational range when 63.1 ± 9.8% of the MCL from the joint surface was preserved. These results are in strong agreement with a study by Palierne et al. (2023) which similarly suggested that factors other than the preservation of the MCL's tibial insertion site may contribute to postoperative stifle joint instability following TPLO. Additional factors responsible for postoperative stifle joint instability may include medial meniscal damage due to the strong anatomical relation between the medial meniscus and the MCL (Palierne et al., 2022). While the present study did not investigate the medial meniscal's anatomical relation to the MCL nor perform a medial meniscectomy due to no orthopedic pathology in the included specimens, it is important to consider, since dogs with CCLR in vivo frequently present with concurrent medial meniscal injuries (Flo, 1993; Fung et al., 2023; Palierne et al., 2022; Ralphs & Whitney, 2002). Additionally, Gatineau et al. (2011) investigated complications associated with TPLO and found that performing a concurrent medial meniscectomy was a risk factor for developing pivot shift following TPLO. Based on the research by Kim et al. (2012) and Gatineau et al. (2011) it cannot be excluded that a medial meniscectomy could cause a greater rotational instability, potentially causing PSP in the postoperative stifle joint. If the medial meniscal integrity has an influence on the postoperative stifle joint stability, future studies should investigate the specific role of surgical interventions on the medial meniscus in relation to rotational stability following TPLO.

The findings of the present study demonstrated that the TPLO procedure significantly increased the rotational range of the stifle joint when comparing the INTACT and TPLOX stifle, indicating a measurable loss of rotational stability following TPLO, which Shimada et al. (2020) also demonstrated in an in vitro study. Notably, the data suggested that the TPLO procedure had a greater significant effect on the internal rotation than the external rotation when comparing the INTACT and TPLOX stifle, as summarized in Table 5. This observation is of particular interest, as excessive internal rotation has been theorized to be either the primary or contributing factor in the development of PSP following TPLO (Bergh & Peirone., 2012; Gatineau et al., 2011; Knight et al., 2017). Despite this association, the prevalence of pivot shift in a retrospective study from 2011 was relatively low with 3.1% reported cases, equating to approximately one in 32 dogs developing PSP (Gatineau et al., 2011). This implies that the 10 pelvic limbs investigated in this study would be unlikely to develop PSP following TPLO, suggesting that while internal rotation was consistently greater than the external rotation across all specimens following TPLO, it may not independently cause PSP in vivo. Other potential risk factors such as tibial torsion, angular limb deformities and medial meniscal surgical interventions require further investigation before definitive conclusions can be made regarding the etiology of PSP. It was not possible to replicate PSP when utilizing a limb press model, as the phenomenon is observed during the walking-phase and this study can therefore only speculate on the clinical relevance of the findings. Although the etiology of PSP is widely regarded as multifactorial, the increased internal rotation caused by the TPLO procedure itself appears less likely as a contributing factor to PSP's development. Continued investigation regarding the etiology of PSP following TPLO is essential due to its impact on canine welfare (Bergh & Peirone, 2012; Gatineau et al., 2011; Knight et al., 2017).

The target postoperative TPA for this study was set at 5°, based on current research suggesting this angle as being the most effective at neutralizing the CTT (Nanda & Hans, 2019; 1998; Volz et al., 2024; Warzee et al., 2001). While aiming for a 5° TPA is generally beneficial, recent studies have demonstrated that a TPA lower than 5° almost eliminates craniocaudal stifle stability, but increases the caudal load and is therefore a risk factor for CaCL damage (Shahar et al., 2006; Volz et al., 2024). Furthermore, a study by Bester et al. (2025) demonstrated that a postoperative TPA closer to 10° effectively reduced the strain on the patellar ligament by replicating the patellar ligament strain of the CCL-intact stifle joint, potentially eliminating the rotational forces responsible for femorotibial subluxation following TPLO. This study revealed a significant negative correlation between the postoperative TPA (mean ± SD; 5.5 ± 2.1°) and the rotational range of the TPLOX stifle (mean ± SD; 39.15 ± 8.19°). This observation suggests that an increase of the postoperative TPA correspondingly reduces the postoperative rotational range, and conversely, a lower postoperative TPA increases the rotational range. Given that a greater internal rotation has been associated with PSP, this finding may contribute to improving the optimal postoperative TPA in combination with existing research (Gatineau et al., 2011). However, it is important to recognize that individual patient factors, such as breed, size, neuter status and preoperative TPA, may influence the optimal postoperative angle

(Brown et al., 2014). Therefore, surgical planning should be tailored to each dog's specific anatomy and biomechanical needs.

There were no significant differences in the rotational range between the TPLOX and the CCLX conditions. This finding suggests that the TPLO procedure did not improve the rotational stability of the CCLX stifle, indicating that while in theory the CTT is neutralized and the biomechanics of the stifle improved, it does not fully return the stifle to the biomechanics of the INTACT stifle. However, it is worth noting, that the present study did not investigate the rotational stability of an isolated CCLR stifle without prior TPLO performed, and it is therefore not possible to conclude if the findings replicate in vivo conditions, when debating just how much the TPLO improves the rotational stability of an isolated CCLR stifle joint.

A shift in the neutral rotational position was observed across all specimens during testing of the four conditions. The most notable shift in neutral position occurred following TPLO (TPLOX) with a mean of 6.26°, predominantly shifting to an internal direction of rotation. This consistent internal shift in direction may suggest a biomechanical change induced by the TPLO procedure itself or as a result from the detachment and reattachment of the specimens between the MCLX and TPLOX conditions. While the limb press model allows for a controlled and reproducible testing environment, it is not possible to determine if the shift in the neutral rotational position has any clinical relevance for the patients, assuming the shift results from the TPLO procedure. The internal shift of the neutral position cannot be excluded as a potential cause of PSP, but has not been investigated. Further research is needed to determine if the shift in the neutral position affects the biomechanics of the stifle joint following TPLO and its impact on canine welfare.

This study focused on assessing the rotational stability of the canine stifle joint using a limb press model. As demonstrated, limb press models allow for reproducible and consistent simulation of weight-bearing conditions for pelvic limbs, thereby enabling precise evaluation of rotational stifle joint stability following TPLO. When utilizing cadavers, limb press models allow for an ethical alternative to in vivo experimentation, thereby aligning with the principles of the 3 R's (Hubrecht & Carter, 2019). The knowledge obtained in this study can be utilized in future in vitro and in vivo studies, examining the clinical consequences of biomechanical changes following TPLO. Further research assessing the consequences of a medial meniscectomy combined with TPLO and its potential effect on rotational stability and development of PSP should be investigated.

Previous studies have investigated the impact and biomechanical consequences of CCLR on rotational stability in canine stifle joints, both in vitro and in vivo, revealing a consistent increase in internal tibial rotation (Arnoczky & Marshall, 1977; Shimada et al., 2020; Tinga et al., 2018; Winters, et al., 2025). In vitro studies, such as Arnoczky & Marshall (1977), reported a mean internal tibial rotation of 45° following CCLR in cadaveric limbs when the stifle was flexed at 90°, while a study by Winters et al. (2025) found a lower mean of internal tibial rotation at 33°. These studies utilized canine cadaveric limbs that lacked replication of critical dynamic stabilizers like the quadriceps muscle and the patellar ligament, which are essential during weight-bearing. In contrast, the present study utilized a limb press model designed to replicate the force vectors of both the quadriceps muscle and the patellar ligament. This implementation allowed for investigation of rotational stability in an isolated stifle joint under more physiological loading conditions in the stance phase of gait. Under these conditions, a mean internal tibial rotation of 24.46° was observed in the CCLX stifle, which is a value notably lower than previously reported by Arnoczky & Marshall (1977) and Winters et al. (2025). The reduced degree of internal rotation observed in the present study may also reflect other model refinements, such as exclusion of the talocrural joint, implementation of the TPLO procedure and the stifle flexed to replicate the stance phase. In addition to these findings, the in vivo fluoroscopic study by Tinga et al. (2018) further highlights the complexity of CCLR instability. Using a 3D-to-2D image registration, they demonstrated that dogs with CCLR exhibit consistent CTT and increased internal tibial rotation during the stance phase. This supports the importance of incorporating physiological loading and dynamic stabilising structures when investigating biomechanical alterations and surgical interventions for CCLR in vitro to demonstrate in vivo conditions most accurately.

The present study has several limitations. The pelvic limbs included did not indicate signs of orthopedic pathology. This could potentially lead to a misinterpretation of the effect of TPLO on the rotational stability of the stifle joint, when the pathological and inflammatory process of a partial or complete CCLR stifle is not considered (Kim et al., 2012). Additionally, BCS, neuter status and age was not recorded for this study, which may represent unaccounted variables influencing the TPA and rotational stability. In particular, increased BCS causes joint stress and inflammation and has been implicated in the progression of CCLR, altered limb biomechanics such as CTT and potentially persistent joint instability following TPLO due to continued excessive joint loading (Rafla et al., 2025; Taylor-Brown et al., 2015). It cannot be excluded that accounting for BCS could have influenced the data. Future studies should include these parameters to better understand their potential contribution to postoperative stifle joint stability and long-term clinical outcomes following TPLO. In the present study, assessment of CTT following TPLO was not possible due to the removal of the tibiotarsal joint. This modification of the pelvic limb was implemented to solely analyse the biomechanics of the stifle joint when attached to the limb press model and is described under section "4. *Materials and Methods"*. It was necessary to remove the talocrural joint of the tibia to ensure that the rotation of the stifle joint was not influenced by the movement of the talocrural joint. Based on existing literature, it is assumed that the CTT theoretically would be eliminated, despite the inability to perform a negative TCT following TPLO (Slocum & Slocum, 1993). The limb press model does not fully replicate all the physiological forces acting on the stifle joint in vivo, as the only forces included were the quadriceps muscle and the patellar ligament. The dynamic stabilization forces of other muscles and tendons can therefore not be assessed in the context of the rotational stifle joint stability in this study (Cook, 2010; Nanda & Hans, 2019).

7. Conclusion

Contrary to this study's initial hypothesis, the results suggest that neither the transection of the distal part of the MCL at the osteotomy site for the TPLO procedure nor the length of the MCL significantly influences the rotational range of the canine stifle joint. These parameters appear to have a limited effect on postoperative stifle joint stability in the context of TPLO. In contrast, the TPLO procedure itself resulted in a significant increase in the rotational range compared to the CCL-intact stifle (INTACT), indicating that the TPLO procedure (TPLOX) does not fully restore initial stifle joint biomechanics, potentially contributing to postoperative rotational instability. Importantly, a significant negative correlation was revealed between the postoperative TPA and the degree of rotational range following TPLO, suggesting that a low TPA may be associated with greater rotational instability of the stifle joint. This finding indicates the need for further research of the optimal postoperative TPA while aiming to minimize the risk of rotational instability and development of PSP following TPLO. The findings of the present study revealed that CCLR (CCLX) did not affect the rotational range of the already TPLO-operated stifle joint (TPLOX). While it was not possible to conclude if the TPLO procedure would have an influence on the rotational stability of the isolated CCLR stifle joint, the present study did not reveal a significant greater or lesser rotational stability between the TPLOX and CCLX stifle joints.

8. Perspectives

The continued development of limp press models and integration of more dynamic systems holds great potential for advancing veterinary knowledge for joint biomechanics. Additionally, expanding the use of limb press models to study implant designs and their effect on joint biomechanics may provide additional knowledge for improving surgical outcomes, canine welfare and evidence based clinical decision making. Moreover, the implementation of more dynamic limb press models could enable in vitro assessment of stabilizing extracapsular implants or sutures as a treatment option for pivot shift. The limb press model allows the combination of in vitro research with evidence based clinical practice in veterinary orthopedics to improve long-term clinical outcome and canine welfare. As more knowledge is gained, such models will play a critical role in optimizing orthopedic clinical outcomes and elevating the standard of care in veterinary medicine.

From an ethical standpoint and to serve the best interests of both veterinarians, patients and owners, it is important to deepen our understanding to improve the surgical treatment options for CCLR, to reduce the risk of postoperative complications for the patients and owners' economy. Animal welfare remains the authors' top priority, who recognizes the ethical limitations associated with conducting in vivo studies.

9. Disclosures

The authors of this study acknowledge their use of ChatGPT for research purposes, editing of the authors own phrases and help with creating and interpreting statistical results. The authors of this study have consistently been critical towards the results of ChatGPT and extensively edited phrases to ensure the accuracy of this study. Every article and content from ChatGPT have been thoroughly and critically examined by the authors to ensure the quality of the work and the integrity of this study.

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Appendix

Appendix 1

TABLE 8 An overview of the rotational range in degree of rotation (°) of the 10 specimens and the results when tested under four different conditions: INTACT, MCLX, TPLOX and CCLX.

	INTACT	**MCLX**	**TPLOX**	**CCLX**
1R	21.79	23.86	36.57	37.55
2L	27.65	28.34	52.54	53.90
3L	25.57	24.53	35.87	45.66
6R	26.77	29.83	45.75	48.84
11L	27.72	27.64	39.03	35.02
14L	29.91	27.24	42.74	36.61
15L	21.63	22.14	31.15	35.19
17R	23.79	22.73	34.22	35.09
19R	26.15	26.44	47.26	50.63
20R	19.88	20.52	25.41	26.26

Abbreviations: INTACT, Intact stifle joint; MCLX, medial collateral ligament transection; TPLOX, medial collateral ligament transection and tibial plateau leveling osteotomy; CCLX, medial collateral ligament transection and tibial plateau leveling osteotomy with cranial cruciate ligament transection.

Appendix 2

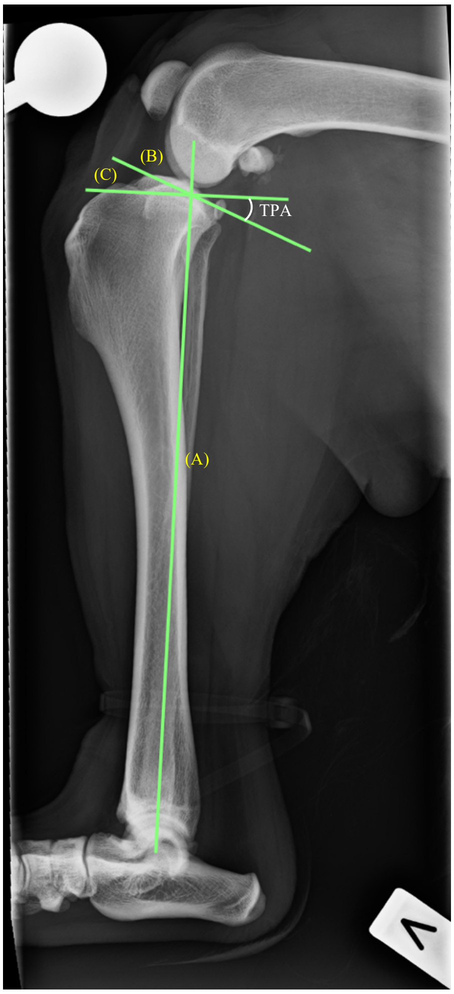

Figure 8 Laterolateral radiograph of a pelvic limb for preoperative tibial plateau leveling osteotomy planning to measure tibial plateau angle (TPA). The stifle and hock are flexed at 90°. (A) Line drawn from intercondylar eminence to center of talocrural joint; (B) Cranial to caudal border of tibial plateau; (C) Perpendicular line to the intersection between (A) and (B). The TPA (white angle line) is the angle between (B) and (C). The radiograph is taken by The Imaging Diagnostic Department at The University Hospital for Companion Animals by Connie S. Due. The lines are drawn by Holmbjerg ML, Miles JE and Tvedsborg CK in an orthopedic preoperative planning software (vPOP^{PRO} version 3.3.2 [283], VetSOS Education Ltd., veterinary preoperative orthopedic planning software).