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Evaluation of Meniscal Load and Load Distribution in the Canine Stifle after Tibial Plateau Levelling Osteotomy with Postoperative Tibia Plateau Angles of 6 and 1 Degrees

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Abstract

Objective The aim of the study was to investigate the kinetic and kinematic changes in the stifle after a tibial plateau levelling osteotomy (TPLO) with a postoperative tibia plateau angle (TPA) of either 6 or 1 degrees.

Study Design Biomechanical *ex vivo* study using seven unpaired canine cadaver hindlimbs from adult Retrievers.

Hinge plates were applied and a sham TPLO surgery was performed. Motion sensors were fixed to the tibia and the femur for kinematic data acquisition. Pressure mapping sensors were placed between femur and both menisci. Thirty per cent bodyweight was applied to the limbs with the stifle in 135 degrees of extension. Each knee was tested with intact cranial cruciate ligament (CCL), deficient CCL, 6 degrees TPLO and 1degree TPLO.

Results Transection of the CCL altered kinematics and kinetics. However, comparing the intact with both TPLO set-ups, no changes in kinematics were detected. After 1 degree TPLO, a significant reduction in the force acting on both menisci was detected ($p = 0.006$).

Conclusion Tibial plateau levelling osteotomy restores stifle kinematics and meniscal kinetics after transection of the CCL *ex vivo*. The contact force on both menisci is reduced significantly after TPLO with a TPA of 1 degree. Increased stifle flexion might lead to caudal tibial motion.

Keywords
- stifle
- cranial cruciate ligament
- kinetics
- TPLO
- biomechanics
- tibia plateau angle

Introduction

Rupture of the cranial cruciate ligament (CCL) is one of the most common causes for hindlimb lameness in dogs.[1] The CCL is a main stabilizer of the canine stifle, as it neutralizes cranial tibial thrust and internal rotation of the tibia.[2,3] Disruption of the CCL has been shown to result in pain, lameness, development of osteoarthritis and often in secondary damage to the medial meniscus.[4–9] Consequently, many different surgical techniques have been developed to reestablish normal stifle kinematics. The concept of dynamic stabilization by various types of corrective

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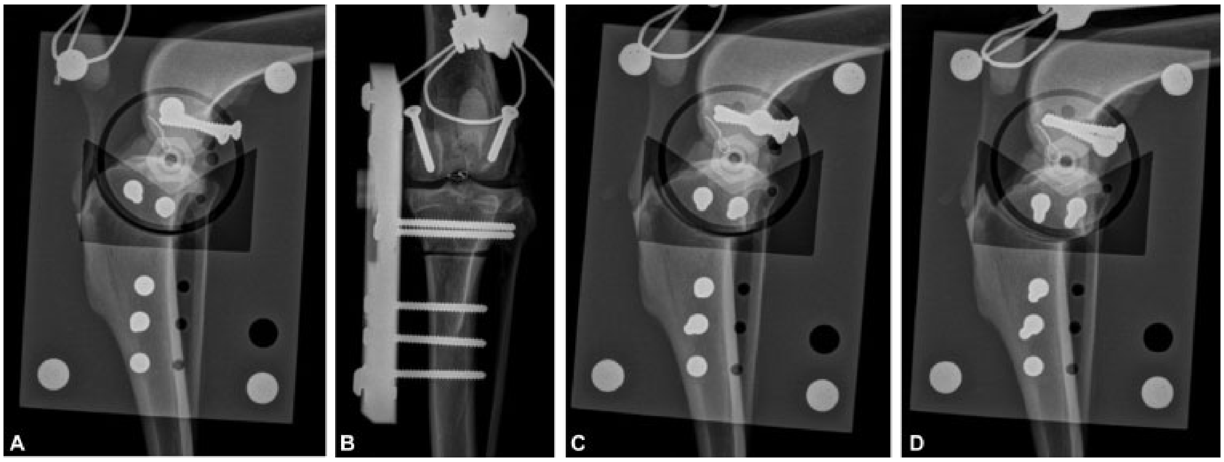

Fig. 1 Radiographs of a right stifle before (**A**) medio/lateral and (**B**) cranio/caudal view; medial to the left) and after rotation of the tibia plateau to 6 (**C**) and 1 degrees (**D**).

osteotomies is commonly accepted today. For many years, tibial plateau levelling osteotomy (TPLO) has probably been the most common technique applied in larger dogs by specialized veterinary surgeons.[10] By altering the tibia plateau slope, biomechanics in the stifle change, eliminating the cranial tibial thrust.[11] Nevertheless, internal rotation of the tibia is not prevented by this type of stabilization.[12] Tibial plateau levelling osteotomy has been described to fully stabilize the CCL-insufficient stifle *ex vivo*,[11,13–15] whereas Kim and colleagues showed in 2012 that more than 30% of the dogs treated still suffer from cranial tibial subluxation after TPLO *in vivo*.[16] A recent study suggested that following TPLO, dogs exhibited less cranial subluxation of the tibia if the postoperative tibia plateau angle (TPA) was close to 0 degree compared with dogs with a higher postoperative TPA. The authors did not describe caudal subluxation either.[17] These findings stand in contrast to other recent works suggesting that a modification of the TPLO may lead to caudal subluxation of the tibia.[18]

Meniscal kinematics and kinetics have been addressed in earlier studies, but the influence of postoperative TPA on the menisci has not been investigated at all. Further research is required to determine if the recommendation to aim for a postoperative TPA of 6 degrees should be changed. The objective of the present study was to compare the kinetic differences in the stifle and kinematic differences of the medial and lateral menisci after TPLO with TPA of 6 degrees (6 degrees TPLO) and 1 degree (1 degree TPLO) simultaneously. We hypothesized that stifle kinetics and kinematics would change significantly after transection of the CCL and 6 degrees TPLO treatment. We specifically expected a 1 degree TPLO to restore the kinetics and kinematics more efficiently.

Materials and Methods

Specimen Preparation

Seven pairs of hindlimbs from adult Retriever cadavers (bodyweight: 25–40 kg) that had died or were euthanatized for unrelated reasons were disarticulated at the coxofemoral level. The exclusion of stifle and tarsal joint pathologies was based on orthogonal radiographs and orthopaedic examination of the cadavers. The limbs were equally and randomly divided into two groups. The contralateral limbs of the investigated ones were used in a different study. All muscles proximal of the hock joint were dissected while preserving the stifle and tarsal joints. The proximal femur was embedded in polymethylmethacrylate (RENCAST FC 53, Huntsman Advanced Materials, Germany) to allow a fixation in an adjustable mounting bracket that enabled the adjustment of hip joint angles and femoral torsion. Custom-made aluminium TPLO hinge plates were placed—fluoroscopically guided—on three left and four right limbs and fixated with five to six cortical screws. A radial osteotomy centred on the midpoint between the medial and lateral intercondylar tubercles—as described for TPLO—was performed using the plate as a saw guide.[19] With the hinge plate in position, the plateau could be adjusted at desired TPA (unaltered, 6 and 1 degrees TPA; ►**Fig. 1**).

A 1.5 mm braided stainless steel cable was passed through a 2 mm tunnel drilled through the widest part of the patella. The cable was secured with two cable clamps. Another 2.0 mm cable was passed through a 2.5 mm transversal drill hole in the tip of the calcaneus and secured as a loop.

Two 3.5 mm cortical bone screws were inserted in the femoral articular surface of the femorofabellar joint. The specimens were then stored at –20°C covered in physiological saline-soaked towels in vacuum bags. Prior to testing, the limbs were thawed at room temperature.

To secure the ultrasound motion sensors, one Schanz screw with a 3.2 mm shaft diameter was inserted in the distal femoral diaphysis and another one in the proximal tibia. Pressure mapping sensors (detailed below) were placed between the femoral condyles and the corresponding medial and lateral meniscus, held in place by suturing and gluing their sensor-free peripheral part to the joint capsule and collateral ligaments.

Stifle kinetics were continuously recorded with an I-Scan system, the K-Scan 4041 Sensor (Tekscan Inc., South Boston, Massachusetts, United States). The sensing region of this sensor is 31.5 × 12.7 mm including 90 sensels with a thickness of 0.2mm. The recorded parameters on the menisci (separately and together) were contact area, peak pressure, mean contact pressure, peak pressure location and contact force. The contact force in relation to the applied load acting on both menisci was calculated by dividing the contact force by the applied force $\left(\frac{\text{contact force}}{\text{30\% body weight}}\right)$. This parameter will be referred to as contact force ratio (CFR) in the following. The average pressure recorded across the contact area was defined as mean pressure, whereas peak contact pressure represented the highest pressure measured. Pressure location was defined as the distance from the caudal meniscal boarder to the peak pressure recording sensel. For each stifle, a new sensor was used and calibrated before use—according to the producer's guidelines. Stifle kinematics were measured using the CMS20BI ultrasound system (Zebris Medical GmbH, Isny, Germany). Muscle forces of the quadriceps and gastrocnemius muscles were simulated using steel cables and turnbuckles. Weight bearing was simulated by applying 30% of the specific bodyweight[11] with a material testing machine (Model Z010, Zwick & Roell GmbH & Co. KG, Ulm, Germany).

With the sensor in place, the patellar cable was fixed to the proximal femur potting with a custom-made low-profile turnbuckle to simulate the quadriceps muscle. The calcaneal cable was connected to the fabellar screws with a turnbuckle to simulate the gastrocnemius muscle.

Testing Protocol

The limbs were mounted in the testing apparatus with the sensors in place. The turnbuckles were adjusted to maintain the stifle at a 135 degrees and the tarsal joint at a 140 degrees angle under load. Torsion of the femur was still possible during the whole test. Testing was started with the pressure on both menisci as equal as possible with a preload of 10 N. Four tests were performed in the following order: intact-CCL, 6 degrees TPLO, 1 degree TPLO and deficient-CCL (►**Fig. 2**). Therefore, the CCL was transected after the first test. Then, the tibial plateau was rotated to achieve a TPA of 6 degrees for the second and a 1 degree TPA for the third test. Finally, the plateau was repositioned in its original position and the test simulating a ruptured CCL was executed.

Statistical Analysis

Homogeneity of variances was checked with Levene's test. Univariate analysis of variance (ANOVA) was performed using IBM SPSS statistics 25.0 (IBM, Armonk, New York, United States). Contact area, contact pressure, peak pressure, centre of force, and contact force were analysed for the medial, lateral and both menisci combined in all four set-ups. Tukey tests were performed for paired comparison if ANOVA indicated significant differences. For the cranial, medial, and proximal translation as well as flexion, adduction, and internal rotation of the tibia, no homogeneity of variances was found, so Welch's ANOVA was used. Games-Howell tests were applied for paired comparison if Welch's-ANOVA indicated significant differences. Statistical significance was accepted at $p < 0.05$.

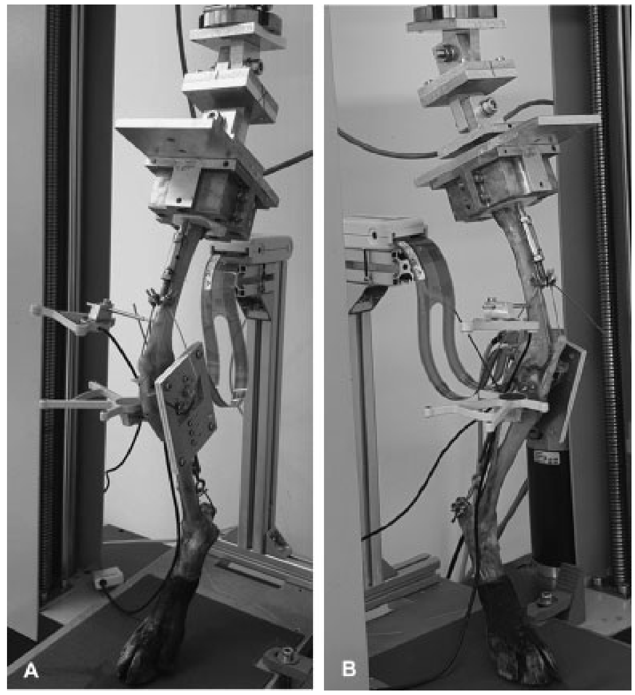

Fig. 2 Specimen ready for testing, with the sensors in place. (**A**) Medial and (**B**) lateral view.

Results

Tibia plateau angles were 21.3 ± 1.9 degrees (intact and deficient), 6.2 ± 1.2 degrees (6 degrees TPLO) and 1.6 ± 0.9 degrees (1 degree TPLO) before and after rotating the plateau respectively. The median bodyweight of the dogs was 31.5 ± 4.1 kg. The stifle joint angle (135.3 degrees [95% CI −134.6–136.0]) did not significantly vary between the four tests.

A mean of 12.2 mm cranial tibial motion (positive value) was recorded after transecting the CCL in comparison to all other groups. Stifles with intact CCL had a mean caudal motion (negative value) of the tibia by (−)1.3 mm when the axial load increased from 10 N to 30% bodyweight. This was also observed in the 6 degrees TPLO ([−]1.4) and 1 degree TPLO ([−]1.5). Additionally, a significantly increased internal rotation of 7.8 degrees and medial subluxation of 4.8 mm was detected in CCL insufficient tests. No significant changes between the intact and both TPLO set-ups for these parameters were detected. Consequently, transection of the CCL strongly altered stifle kinematics, but TPLO, irrespective of the postoperative TPA (1 or 6 degrees), seemed to restore normal *in vitro* level kinematics (►**Table 1**).

In the stifle, the contact force relative to the applied axial force CFR acting on both menisci was significantly higher in the intact CCL (4.9), 6 degrees TPLO (4.4) and 1 degree TPLO (4.0) than in the insufficient CCL (3.2) setting. Furthermore, the menisci in the 1 degree TPLO received significantly less

Table 1 Kinematic variables (mean [95% confidence interval]) of the knee joint before and after surgery

Variable	Intact CCL	6 degrees TPLO	1 degree TPLO	Insufficient CCL
Cranial subluxation under load (mm)	−1.3 (−2.2–−0.4) $P_{\text{intact–deficient}} < 0.001$	−1.4 (−2.5–−0.3) $P_{\text{6 degrees- deficient}} < 0.001$	−1.5 (−2.3–0.8) $P_{\text{1 degree deficient}} < 0.001$	12.2 (10.2–14.3)
Medial subluxation under load (mm)	0.2 (−0.1–0.5)	0.2 (−0.1–0.5)	0.1 (−0.2–0.4) $P_{\text{1 degree- deficient}} = 0.049$	4.8 (1.8–7.8)
Internal rotation under load (degree)	−0.4 (−1.1–0.3) $P_{\text{intact–deficient}} = 0.017$	0.4 (0–0.8) $P_{\text{6 degrees- deficient}} = 0.028$	0.5 (−0.1–1.0) $P_{\text{1 degree- deficient}} = 0.028$	7.9 (3.4–12.3)

Abbreviations: ANOVA, analysis of variance; CCL, cranial cruciate ligament; TPLO, tibial plateau levelling osteotomy.
Note: Variables with significant difference indicated by Welch's ANOVA.

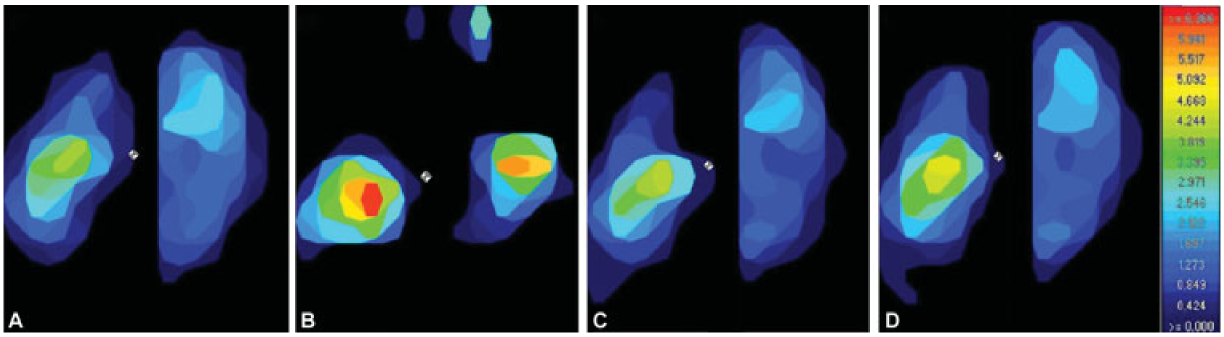

Fig. 3 Pressure distribution: (**A**) intact CCL, (**B**) deficient CCL, (**C**) TPLO 6 and (**D**) TPLO 1 degrees. The medial meniscus is on the left. The top of the picture represents caudal. centre of force: (exemplary data, scale in MPa).

CFR than with an intact CCL. This was observed also in the lateral meniscus. In contrast, the intact–insufficient comparison was only significantly different for the medial meniscus.

The mean contact area decreased in both menisci, while peak pressure increased significantly in the CCL-insufficient stifle (►**Fig. 3**, ►**Table 2**).

Discussion

This study aimed at determining the kinematic and kinetic changes in the canine stifle at 135 degrees extension after cutting the CCL and stabilization with TPLO establishing a postoperative TPA of either 1 or 6 degrees. Our set-up allowed monitoring of stifle kinematics and kinetics at a defined stifle joint angle while applying a load of 30% of the bodyweight. This was accomplished by continuous measurement of the stifle flexion with a Zebris sensor. Joint angles at which maximum vertical forces occur appear to differ between dog breeds.[20] Consequently, we only used limbs from Retriever breeds, which most likely experience peak vertical forces during trot at around 135 degrees stifle extension.[21] With our custom-made TPLO hinge plate, the TPA could be easily and precisely adjusted to simulate a TPLO at postoperative TPA of 6 or 1 degrees without dismounting the limbs from the actuator. The use of hinge plates has also proven to be reliable in earlier studies.[11,15,22] The I-Scan system was previously successfully used in other studies[15,23–27]. Their research group was able to show impressively how femorotibial contact mechanics change after meniscal surgery or damage and how TPLO and other techniques influence stifle kinetics and kinematics.[15,23–26,28] Nevertheless, the influence of postoperative TPA on the meniscal load was not investigated.

Due to the nature of *in vitro* studies, our results have to be interpreted with due care. We tested stifle kinetics and meniscal kinematics at one defined angle of flexion. Therefore, our tests represent one stage of the stance-phase and leave out the swing-phase completely. We chose the stage when maximal ground reaction force occurs. To place and secure the I-Scan sensor, wide parts of the joint capsule had to be transected, reducing the stabilizing effect of the joint capsule.[29] Inserting a sensor in the joint space might also interfere with joint mechanics.[30] However, as these alterations were the same for all set-ups, the comparison of different set-ups should still provide meaningful information. Since the single-use I-Scan sensors employed produced unreliable readings as soon as kinking occurred, we used a new sensor for each limb. In addition to the effects of quadriceps and gastrocnemius muscle on stifle biomechanics, hamstring muscles also influence the stability of the stifle after CCL rupture by working as an agonist to the CCL. Nevertheless, Kanno and colleagues could not show that simulation of the semitendinosus muscle in a similar set-up is able to compensate the transection of the CCL.[31] As a result, in our simplified biomechanical model, we did not include hamstring muscles similar to other authors.[11,15,32] But still, this has to be taken into account when interpreting our results.

As reported in earlier studies, cutting the CCL alters stifle kinematics and meniscal kinetics significantly.[11,15,32–34]

Table 2 Kinetic variables (mean (95% confidence interval)(of the stifle before and after surgery

Variable	Intact CCL	6 degrees TPLO	1 degree TPLO	Insufficient CCL
CFR both menisci	4.9 (4.8–5.1) $P_{\text{intact–deficient}} < 0.001$	4.4 (4.0–4.8) $P_{\text{6 degrees- deficient}} = 0.001$	4.0 (3.5–4.4) $P_{\text{1 degree intact}} = 0.006$ $P_{\text{1 degree deficient}} = 0.044$	3.2 (2.6–3.9)
CFR medial meniscus	2.6 (2.3–2.8) $P_{\text{intact–deficient}} = 0.046$	2.3 (1.9–2.6)	2.0 (1.5–2.5)	1.9 (1.2–2.5)
CFR lateral meniscus	2.4 (2.2–2.5) $P_{\text{intact–deficient}} < 0.001$	2.1 (1.9–2.4) $P_{\text{6 degrees deficient}} = 0.001$	2.0 (1.7–2.3) $P_{\text{1 degree intact}} = 0.048$ $P_{\text{1 degree deficient}} = 0.001$	1.4 (1.1–1.6)
Mean contact area in mm^2 Both menisci	376.4 (333.1–419.7) $P_{\text{intact–deficient}} < 0.001$	344.8 (314.6–374.9) $P_{\text{6 degrees deficient}} < 0.001$	334.2 (305.5–363.0) $P_{\text{1 degree deficient}} < 0.001$	233.0 (193.3–272.7)
Mean contact area in mm^2 Medial meniscus	184.3 (163.6–205.0) $P_{\text{intact–deficient}} < 0.001$	168.5 (152.1–184.9) $P_{\text{6 degrees deficient}} < 0.001$	159.3 (140.8–177.8) $P_{\text{1 degree deficient}} = 0.002$	112.7 (95% CI 89.4–136.1)
Mean contact area in mm^2 Lateral meniscus	192.4 (166.3–218.5) $P_{\text{intact–deficient}} < 0.001$	176.3 (157.2–195.4) $P_{\text{6 degrees deficient}} = 0.001$	174.8 (154.0–195.6) $_{-1 \text{ degrees deficient}} = 0.001$	120.4 (101.1–139.6)
Peak pressure in MPa Both menisci	3.1 (2.4–3.8) $P_{\text{intact–deficient}} = 0.030$	3.2 (2.1–4.2) $P_{\text{6 degrees deficient}} = 0.043$	3.0 (2.1–4.0) $P_{\text{1 degree deficient}} = 0.025$	4.6 (3.9–5.3)
Peak pressure in MPa Medial meniscus	3.0 (2.2–3.8)	3.1 (1.9–4.2)	2.9 (1.7–4.0)	4.2 (2.9–5.5)
Peak pressure in MPa Lateral meniscus	2.2 (1.9–2.6)	2.2 (1.9–2.5)	2.2 (1.8–2.5)	3.0 (2.1–3.9)

Abbreviations: ANOVA, analysis of variance; CCL, cranial cruciate ligament; CFR, contact force ratio; TPLO, tibial plateau levelling osteotomy.
Note: Variables with significant differences indicated by ANOVA.

Kinematics reached normal values after TPLO with 6 and 1 degrees TPA. Kinetic data between the intact and 6 degrees TPLO showed no significant changes, but a significant reduction in load on the menisci was measured after 1 degree TPLO. So, the assumption that kinetics after 1 degree TPLO might be more normal has to be rejected. In short, we expected TPLO with 1 degree TPLO to turn kinetics back to normal, but in contrast we found TPLO with 6 degrees produces parameters which allude to a more normal meniscal load.

As demonstrated previously, the peak pressure location in menisci and the contact area changed significantly after transection of the CCL.[15] Our data suggested a decrease in the contact area after TPLO, but this effect did not seem significant statistically. Kim and colleagues reported that the peak pressure location moved caudal in CCL-insufficient stifles and remained at a caudal location after TPLO.[15] These differences in the results might be due to different methods of data acquisition. Whereas they defined peak pressure location as the distance of the sensor recording the highest pressure from the caudal margin of the tibia, we determined peak pressure location in relation to the most caudal edge of the menisci. Both menisci are mobile on the tibia plateau and change their position with changing flexion angles and rotation of the stifle.[27] With our experimental set-up, we were not able to analyse meniscal movements during the tests. Further studies are required to determine the effect of meniscal movement on the pressure distribution.

We decided to compare the CFR to account for the diverse bodyweights in our test group of dogs. As a result, we observed a reduction in load after transection of the CCL and after TPLO. The data presented by Kim and colleagues already showed the reduction in contact force, but these results were not significant. This might be due to not accounting for the difference in applied force to the limb or by only testing TPLO with 6 degrees TPA[15]. There are different possibilities to explain these findings. For example, if not every contact point between femur and tibia was covered by our sensors. But the sensor was in all patients larger than the menisci and we had no additional load recorded on our sensors indicating load on other areas. Another explanation would be, less quadriceps pull is necessary after TPLO to keep the stifle in extension, or the force is shifted to the caudal cruciate ligament. A reduction in muscle force appears unlikely since the tensile force of the quadriceps muscle remains unchanged following TPLO.[35,36] An increased load on the caudal ligament could be explained by the occurrence of caudal tibial thrust *in vivo*.[16,18,34] In addition, biomechanical studies demonstrated caudal subluxation after TPLO.[11,14] Hulse and colleagues examined intra-articular effects of TPLO and found no evidence in stifles with partially ruptured CCL for changes in the caudal cruciate ligament.[37] But in cases of complete CCL rupture, more than half of the dogs had altered caudal ligaments and some even showed total disruptions.[37] Intriguingly, these findings are in contrast with *in vivo* studies that failed to document caudal tibial motion after TPLO.[17,38] In the present study, we did record caudal tibial motion after TPLO but the effect was not significant. We even observed a slight caudal motion of the tibia after applying axial force in the intact CCL set-up with the stifle in 135 degrees.

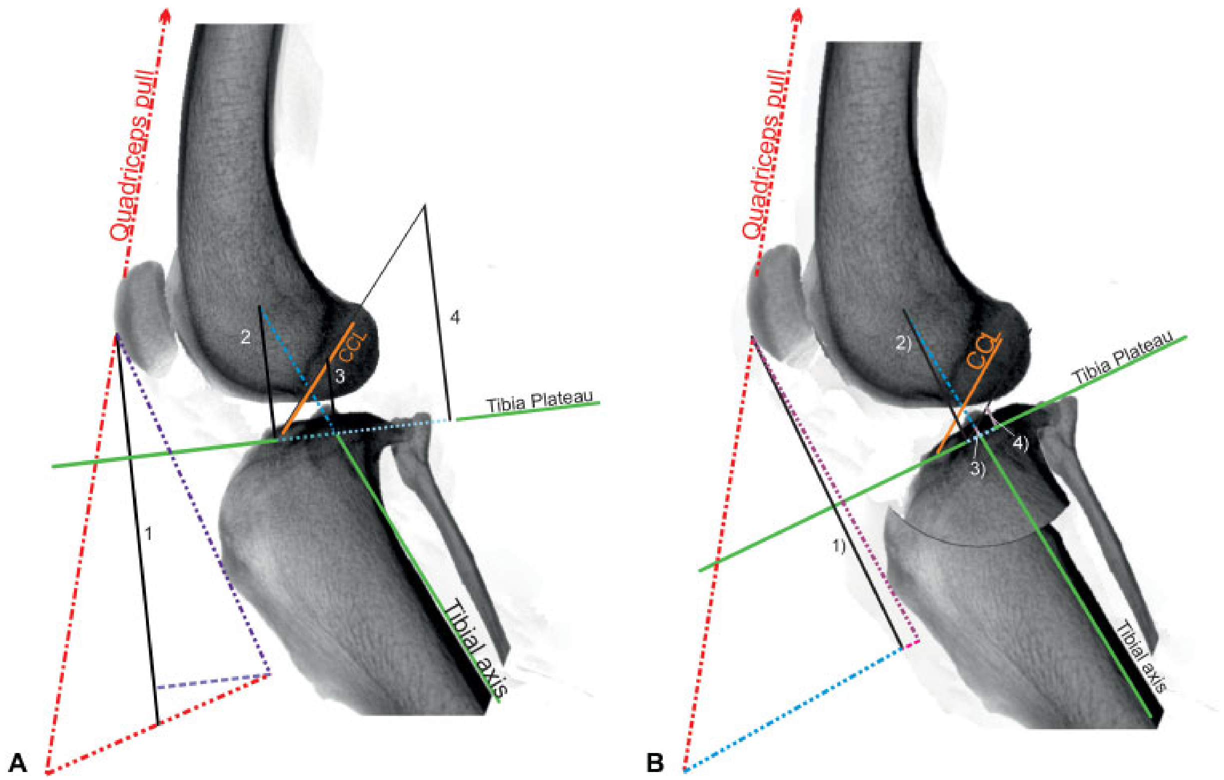

Fig. 4 (**A,B**) Stifle in 135 degrees flexion. Forces for intact CCL and 6 degrees TPLO. Quadriceps pull is ~3 times GRF.[35] (1) Quadriceps force perpendicular to the plateau (intact CCL: 2.7 GRF; TPLO: 2.3 GRF). (2) Ground reaction force perpendicular to the plateau (intact CCL: 0.9 GRF; TPLO: 1 GRF). (3) Perpendicular to the plateau orientated force created by GRF CTT action on the CCL to the plateau (intact CCL: 0.5 GRF; TPLO: 0.1 GRF). (4) Perpendicular orientated force created by GRF and quad CTT action on the CCL to the plateau (intact CCL: 1.4 GR (= 0.5 GRF + 0.9 GRF); TPLO: 0.2 GRF (= 0.1 GRF + 0.1 GRF). Dotted line (■ ■ ■) Cranial tibial thrust created by GRF and quad force (intact CCL: 1.2 GRF; TPLO: 0.2 GRF). Dashed line (■ ■) Cranial tibial thrust created by quad force (intact CCL: 0.8 GRF TPLO: 0.1 GRF). Line (■ ■ ■) patellar ligament pull (intact CCL: 2.6 GRF, TPLO: 2.4 GRF). Line (■ ■ ■ ■) Retropatellar force (intact CCL: 1.6 GRF, TPLO: 1.9 GRF. CCL, cranial cruciate ligament; CTT, cranial tibial thrust; GRF, ground reaction force; TPLO, tibial plateau levelling osteotomy.

Another possible explanation is that by applying the parallelogram of forces to the CCL, its orientation from proximal and caudal to distal and cranial will transform cranial tibial motion into a compressive force on the menisci. To visualize this explanatory model, we included graphics (►**Fig. 4A,B**). ►**Fig. 4A** combines the rationales of TPLO and TPA in one drawing. Furthermore, the forces generated by the CCL by counteracting the CTT are included. To demonstrate the changes after TPLO, ►**Fig. 4B** was added. The force generated by the CCL marked '3' and '4' are notably decreased while simulation of TPLO. Warzee and colleagues demonstrated that the cranial tibial thrust will be neutralized by TPLO; therefore, the compressive force created by the CCL also will be eliminated.[11] This interpretation is supported by *in vitro* analyses of the strain in the CCL under axial load of the stifle with different TPA, which showed decreasing strain with decreasing TPA.[22] Moreover, the quadriceps force also creates cranial tibial thrust,[39] which has to be compensated by the CCL. This force will also be reduced after TPLO, because the patellar ligament angle will be close to 90 degrees after TPLO at a 135 degrees stifle angle.[40] But as soon as caudal tibial motion occurs (over-correction of the TPA below 0 degree or the patellar ligament angle, below 90 degrees), strain in the caudal cruciate ligament will probably generate compressive forces in both menisci in the same fashion. This might always happen when the stifle is in a more flexed position. Therefore, stress in the caudal cruciate ligament occurs in the later stance phase when the stifle is more strongly flexed.[41] Considering the simplified geometric model, we created, the compressive force created by the CCL would be reduced by ~86%.

To overcome the unavoidable problems of static models commonly used in veterinary medicine, a robotic-based model as described by Beveridge and colleagues and Kanno and colleagues could be adapted.[42,43] To the authors knowledge, the study from Kanno and colleagues was the only adaptation of a robotic model to the canine stifle. The biggest limitation of this study is the absence of muscle forces. To include muscle forces in dynamic biomechanical models, more *in vivo* studies are necessary.

Conclusion

Tibial plateau levelling osteotomy restored stifle kinematics and meniscal kinetics after transection of the CCL *ex vivo* in the present study. Tibial plateau levelling osteotomy reduced the contact force on both menisci in comparison to CCL intact stifles, but only with a TPA of 1 degree, this finding was significant. No changes of peak pressure and peak pressure location occurred in any of the TPLO set-ups. Increased stifle flexion might lead to caudal tibial motion and therefore could produce effects not addressed in this study.

Authors' Contributions

J.M.S., A.M.-L. and P.A. contributed to conception of the study, study design, data analysis and interpretation. M.G. and J.M.S. additionally contributed to data acquisition and data analysis. All authors also drafted, revised and approved the submitted manuscript.

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Conflict of Interest

None declared.

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