

# Influence of tibial plateau levelling osteotomy on the tensile forces sustained by ligaments in cranial cruciate ligament-intact canine stifles: An ex vivo pilot study

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## Abstract

**Background:** Tibial plateau levelling osteotomy (TPLO) changes the anatomical tibial conformation and might alter the positional relationship of the ligaments comprising the stifle joint. As a result, it is expected to affect the tensile force of the ligaments. However, studies analyzing the details of the effect of osteotomy are limited.

**Objectives:** To evaluate the influence of TPLO on the tensile force on the stifle ligaments in the intact canine stifle using a six-degree-of-freedom (6-DOF) robotic testing system.

**Methods:** Eight stifles were categorised into the reference group and nine stifles into the TPLO group. The stifles were then analysed using a 6-DOF robotic joint biomechanical testing system. The stifles were applied 30 N at cranial, caudal, and compression loads and 1 Nm at the internal and external torque loads (the load applied to the tibia relative to the femur) on extension, at 135° and 120°, respectively. The tensile force placed on the cranial cruciate ligament (CrCL), the caudal cruciate ligament, the medial collateral ligament, lateral collateral ligament and the total tensile force placed on the four ligaments was calculated under each load.

**Results:** For the caudal load applied to the tibia relative to the femur, the CrCL tensile force in the TPLO group was lower than that in the reference group at 120° ( $p = 0.02$ ). The CrCL tensile force in the TPLO group was lower than that in the reference group at 120° ( $p < 0.01$ ) for the compression load. Regarding the cranial, internal, and external load, the CrCL tensile force remains unchanged between both groups at each angle.

**Conclusions:** TPLO reduces CrCL tensile force during compression and caudal force application. TPLO may reduce tensile forces contributing to CrCL rupture.

## KEYWORDS

biomechanics, cranial cruciate ligament disease, six-degree-of-freedom robotic testing system, tibial plateau levelling osteotomy

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## 1 | INTRODUCTION

Cranial cruciate ligament (CrCL) rupture is a common cause of hind limb lameness in dogs. The static stabilisers that constitutes the stifle joint includes joint capsule, ligaments and menisci. In particular, the ligaments serving as the primary stabilisers at the stifle joint are the CrCL, the caudal cruciate ligament (CaCL), the medial collateral ligament (MCL) and the lateral collateral ligament (LCL) (Arnoczky & Marshall, 1977; Vasseur & Arnoczky, 1981). Rupture of the CrCL in dogs is mediated by factors that induce chronic ligament degeneration, called 'CrCL disease' (Hayashi et al., 2004). This disease indicates a pathological condition that transitions from chronic degeneration to microinjury, partial ligament rupture and complete macroscopic rupture with corresponding stifle instability (Hayashi et al., 2004).

Tibial plateau levelling osteotomy (TPLO) can provide early functional restoration following rupture of the CrCL (Bergh et al., 2014; Krotscheck et al., 2016). This method involves an osteotomy and rotation of the tibial plateau to a desired tibial plateau angle (TPA) of 6.5° (Slocum & Slocum, 1993; Warzee et al., 2001). TPLO neutralises cranial tibial thrust (CrTT) and prevents cranial displacement of the tibia in the stance phase (Slocum & Slocum, 1993; Warzee et al., 2001). Hulse et al. (2010) evaluated the articular cartilage on arthroscopy after TPLO and reported that the extent of articular cartilage injury after osteotomy depends on the degree of damage of the CrCL. The authors stated that in particular, if the function of the CrCL is preserved, the articular cartilage is normal or nearly normal and partial tears of the CrCL are suppressed (Hulse et al., 2010). Furthermore, TPLO inhibited degeneration of the CrCL in an experimental CrCL degeneration model (Shimada et al., 2022). These protective effects of the TPLO on CrCL may be caused by biomechanical effects. In addition, TPLO changes the anatomical tibial conformation and might alter the positional relationship of the ligaments comprising the stifle joint. Most of the *ex vivo* biomechanical studies related to TPLO report the kinematics of the stifle (Kanno et al., 2014; Kanno et al., 2012; Kim et al., 2009; Shimada et al., 2020; Warzee et al., 2001), and only a few reports have included a discussion of the kinetics of the stifle (Haynes et al., 2015; Warzee et al., 2001). The authors of the latter reports have generally focused on the CrCL and CaCL during the axial loading test, and they have not evaluated the collateral ligaments. Moreover, many biomechanical reports on stifle joints have focused on axial loading and the craniocaudal drawer test (Kanno et al., 2014; Kanno et al., 2012; Kim et al., 2009; Haynes et al., 2015; Warzee et al., 2001). However, joints are expected to receive various loads in daily life. Biomechanical studies in various conditions are crucial to understand the surgery relevant to this context. Therefore, the current study was conducted under the hypothesis that TPLO decreases the tensile force on the CrCL. To clarify these aspects, the tensile forces of ligaments during motion of normal and TPLO-treated stifles with a preserved CrCL were analysed. For these analyses, each stifle received cranial, caudal, compression, internal rotational and external rotational loads using a robotic system for joint biomechanical tests.

## 2 | MATERIALS AND METHODS

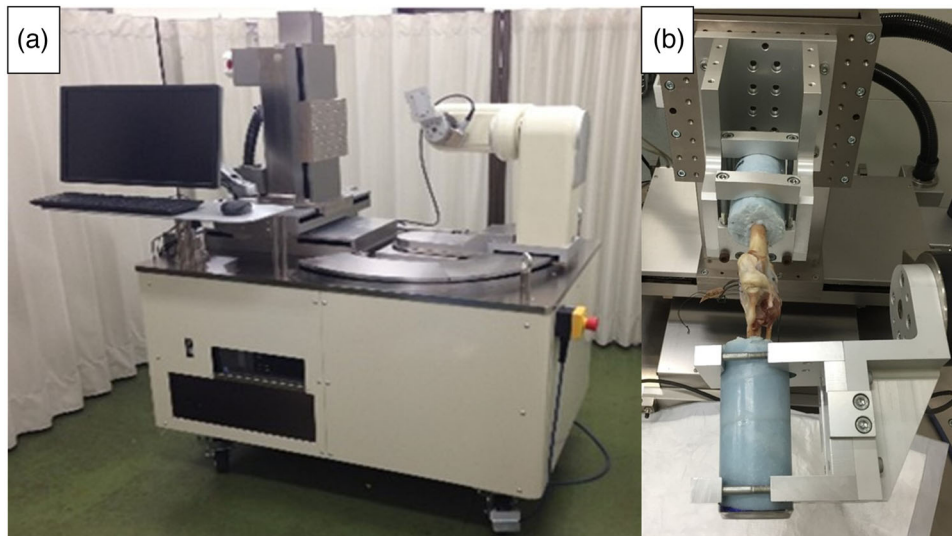
### 2.1 | Animals

The left stifle joints used in this study were obtained from 17 healthy mature adult Beagle dogs, after the joints were used for surgical practice by veterinary students. The right stifle joint could not be used in this study since it was used for orthopaedic surgical practice. Therefore, the left stifle joint was used instead of paired stifles. Owing to the limited number of stifle joints sampled in this study, the sample size was not calculated. This study was approved by the Animal Experiment Committee and Bioethics Committee of our university (approval number: 28S-57). A general physical examination and blood test, including complete blood count and serum chemistry, confirmed each dog as being healthy. In addition, an orthopaedic examination was performed before euthanasia, and mediolateral and craniocaudal radiographic images of all stifles were obtained to evaluate pathology and rule out any other orthopaedic disease. The stifles were randomised into two groups: those who did not undergo TPLO (reference group) and those who did (TPLO group).

### 2.2 | Specimen preparation

All soft tissues, except the cruciate ligaments, the collateral ligaments, the menisci, the joint capsule, the patella and the patellar ligament, were removed from the stifle joint. After that, a bone–ligament model was created, as described by Shimada et al. (2020). Since it is difficult to visually confirm complete transection of the cruciate ligaments that are intra-articular, the cruciate ligaments were checked for complete transection using nylon sutures. After stifle arthrotomy, nylon sutures were placed around the CrCL and CaCL, and the joint capsule was sutured to avoid the impact of arthrotomy; these served as a mark when the cruciate ligaments were cut. Since the collateral ligaments are located outside the joint, the MCL and LCL were visually assessed to confirm complete transection. A mediolateral radiograph of each stifle was obtained. The TPA was measured according to the method described by Warzee et al. (2001). A craniocaudal radiograph was also obtained to check for deformities, such as varus or valgus.

In the TPLO group, surgery was performed according to the method described by Slocum and Slocum (1993). Based on a report by Warzee et al., the target TPA was adjusted by 6.5° (Warzee et al., 2001). After the osteotomy, the tibia was fixed using a 2.4-mm TPLO locking compression plate (Johnson & Johnson, New Brunswick, NJ). After fixation, radiographs were obtained again, and the postoperative TPA was measured. The TPA was determined by a single observer. The proximal femur and distal tibia were fixed using dental resin (GC OSTRON II; GC Corporation, Tokyo, Japan) and a cylindrical paper tube, as described previously (Shimada et al., 2020). A Kirschner wire was inserted along the femoral and tibial axes as a landmark, and the resin was fixed in a tube with a diameter of 50 mm so that the bone axis was positioned at



**FIGURE 1** (a) The testing system used in this study consisted of a 6-degree-of-freedom (6-DOF) manipulator with a 6-DOF universal force/moment sensor. (b) The photo of the stifle joint installed in the robotic system. The femoral side of the robot controls the three translations (medial–lateral, cranial–caudal, proximal–distal) and the tibial side of the robot controls the three rotational movements (flexion–extension, internal–external, varus–valgus)

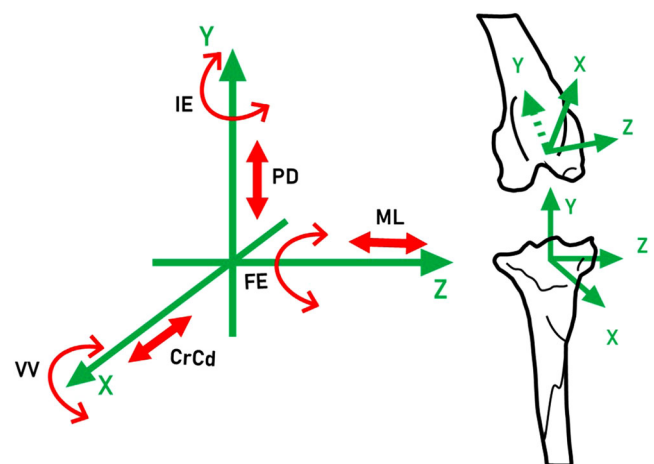
the centre of the tube. Thus, the bone axis was positioned at the centre of the robot's grasping device. The specimens were then wrapped with gauze soaked in lactated Ringer's solution and cryopreserved at  $-20^{\circ}\text{C}$ . They were thawed at  $4^{\circ}\text{C}$  for 24 h to facilitate examination and testing by the robotic system.

### 2.3 | Six-degree-of-freedom robotic testing system

A six-degree-of-freedom (6-DOF) robotic testing system developed by Fujie et al. (1993, 2004, 1996, 1995) was used for testing (Figure 1a). This system enables simulation of physiological stifle joint motions that are controlled with respect to either position or force, and it is also possible to reproduce the recorded stifle joint motions accurately. With this function, the system can not only measure joint laxity but can also calculate the tensile forces of ligaments by using the principle of superposition (Fujie et al., 1995). The joint coordinate system described by Grood and Suntay (1983) was used for simulating the physiological stifle joint motion. This coordinate system can be defined in three rotation axes (flexion–extension [FE], internal–external [IE], varus–valgus [VV]) and three translational movements (medial–lateral [ML], cranial–caudal [CrCd] and proximal–distal [PD]) (Figure 2). In the robotic system, the FE axis (Z-axis) is defined using the insertion of the MCL and LCL of the femur, and the IE rotation axis (Y-axis) is defined as an anatomical axis of the femur. The VV axis (X-axis) is defined as the line perpendicular to the FE and IE rotation axes.

### 2.4 | Initial testing to determine test conditions

Before this study, experiments were performed using two test normal stifle joint specimens. When force was applied in the CrCd direction,



**FIGURE 2** The canine tibial femoral joint was used to illustrate the stifle joint coordinate system. The coordinate system consists of three rotational axes (FE, flexion–extension; IE, internal–external; VV, varus–valgus) and three translations (ML, medial–lateral; CrCd, cranial–caudal and PD, proximal–distal). The FE axis (Z-axis) is defined using the insertion of the MCL and LCL of the femur, and the IE axis (Y-axis) is defined as an anatomical axis of the femur. The VV axis (X-axis) is defined as the line perpendicular to the FE and IE axes

the displacement became smaller (approximately 30 N), and the stress–displacement curve became similar to that of the plateau. Similarly, when the torque was applied in the IE direction, the displacement became smaller (approximately 1 Nm), and the stress–displacement curve became similar to that of the plateau. For the compression test, the compression load was chosen to replicate the peak vertical force of the hind limbs in the standing position, which is approximately 30% bodyweight; since the specimen weighed approximately 10 kg, 30 N was chosen.

## 2.5 | Test condition

A device made to hold the 50-mm diameter tube was held in place by two screws in each of the two fixtures in two places (Figure 1b). At this time, the insertions of the MCL and LCL were marked with a surgical pen because the coordinate system was determined on the basis of these marks. After the stifle joint was fixed to the system, the flexion–extension DOF was controlled by position control. In a pilot study with normal stifles, when the joint angle was <90° range of motion during the IE rotation, some specimens would contact the limits of the robotic arm at an applied torque of 1 Nm. Therefore, the stifles were tested on maximum extension, at 135°, which is generally considered the angle during standing, and at 120° corresponding to pre-paw strike (Tashman et al., 2004). To extend the stifle joint, an extension force corresponding to 0.5 Nm of torque was applied to the stifle while maintaining the other five DOFs at 0 N (CrCd, PD, and ML) and 0 Nm (VV and IE) by force control. This state was defined as the ‘maximum extension position,’ and the flexion–extension angle was determined using a clear plastic manual goniometer with 1-degree graduation. The mean maximum extension of the reference group was  $153 \pm 2.3^\circ$ ; therefore, 153° was used as the extension value for the TPLO group. Measurements were obtained under CrCd load, proximal compression load and IE rotational torque from stifles at maximum extension, 135° and 120°.

CrCd drawer loads, as for the cranial drawer test, of up to 30N were applied to the stifles. In the axial rotation test, 1 Nm of IE torque was applied to the stifle joints. In proximal compression load, to mimic the positive CrTT test, 30 N of proximal force was applied to the stifle joint.

In the CrCd loading test, CrCd loads up to 30 N were applied to the stifles while maintaining the flexion angle and keeping the other 4-DOFs (PD, ML, IE and VV) load/torque at 0. In the proximal compression test, 30 N of proximal force was applied to the stifle joints while maintaining the flexion angle and keeping the other 4-DOFs (CrCd, ML, IE, VV) load/torque at 0. In the IE rotation test, 1 Nm of IE torque was applied to the stifle joints while maintaining the flexion angle and keeping the other 4-DOFs (CrCd, PD, ML, VV) load/torque at 0. Each test was carried out three times to minimise the effects of deformation due to creep, and the data from the third test were used. All the tests were conducted in a fixed order (Figure 3).

## 2.6 | Calculation of tensile force

The tensile force on each ligament was calculated using the principle of superposition devised by Fujie et al. (1995). First, in both groups, the motion in response to the applied loads in each test was recorded as the intact stifles motion. In addition, the output of the three forces from a 6-DOF universal force/moment sensor (Figure 1) at this time was recorded as  $f_x$ ,  $f_y$  and  $f_z$ . After transection of the target ligament, the recorded intact stifles motion was reproduced, and the output of the three forces of the sensor at that time was recorded as  $f_x'$ ,  $f_y'$  and  $f_z'$ . The tensile force ( $F$ ) generated in the ligament was calculated using the following formula (Fujie et al., 1995).

The  $f_x$ ,  $f_y$  and  $f_z$  are computer-generated values, and each ligament tensile force is calculated after the transection of the target ligament.

$$\text{Tensile force } (F) = \sqrt{(f_x - f_x')^2 + (f_y - f_y')^2 + (f_z - f_z')^2}.$$

A similar motion was applied in the order of the following tests 1–5: test 1, intact; test 2, test after cutting the CrCL; test 3, test after cutting the CaCL; test 4, test after cutting the MCL and test 5, test after cutting the LCL. Based on the outputs recorded in tests 1–5, the tensile forces of CrCL, CaCL, MCL and LCL from the outputs of tests 1 and 2, tests 2 and 3, tests 3 and 4 and tests 4 and 5, respectively, were calculated. From these results, the sum of each ligament was calculated. All transections of the ligaments were performed while the stifle was still in the robotic system.

## 2.7 | Statistical analysis

Statistical processing was performed using SAS software Ver 9.3 (SAS 2011) (SAS Institute Inc., Cary, NC). A two-sample *t*-test was performed to compare age, body weight and TPA between groups. Since the tensile force of ligaments was not normally distributed without logarithmic transformation, the values obtained in this study were logarithmically transformed and used for statistical analysis. Statistical analyses were carried out with repeated-measures analysis of variance using the Proc Mixed procedure of the SAS software. The normality of the residuals was also assessed. The linear model included the fixed effects of angle, ligament, treatment, and treatment group as well as their interaction. The effects of the groups in individual dogs were included as random effects. Multiple comparisons of least-square means were adjusted using the Tukey–Kramer test.

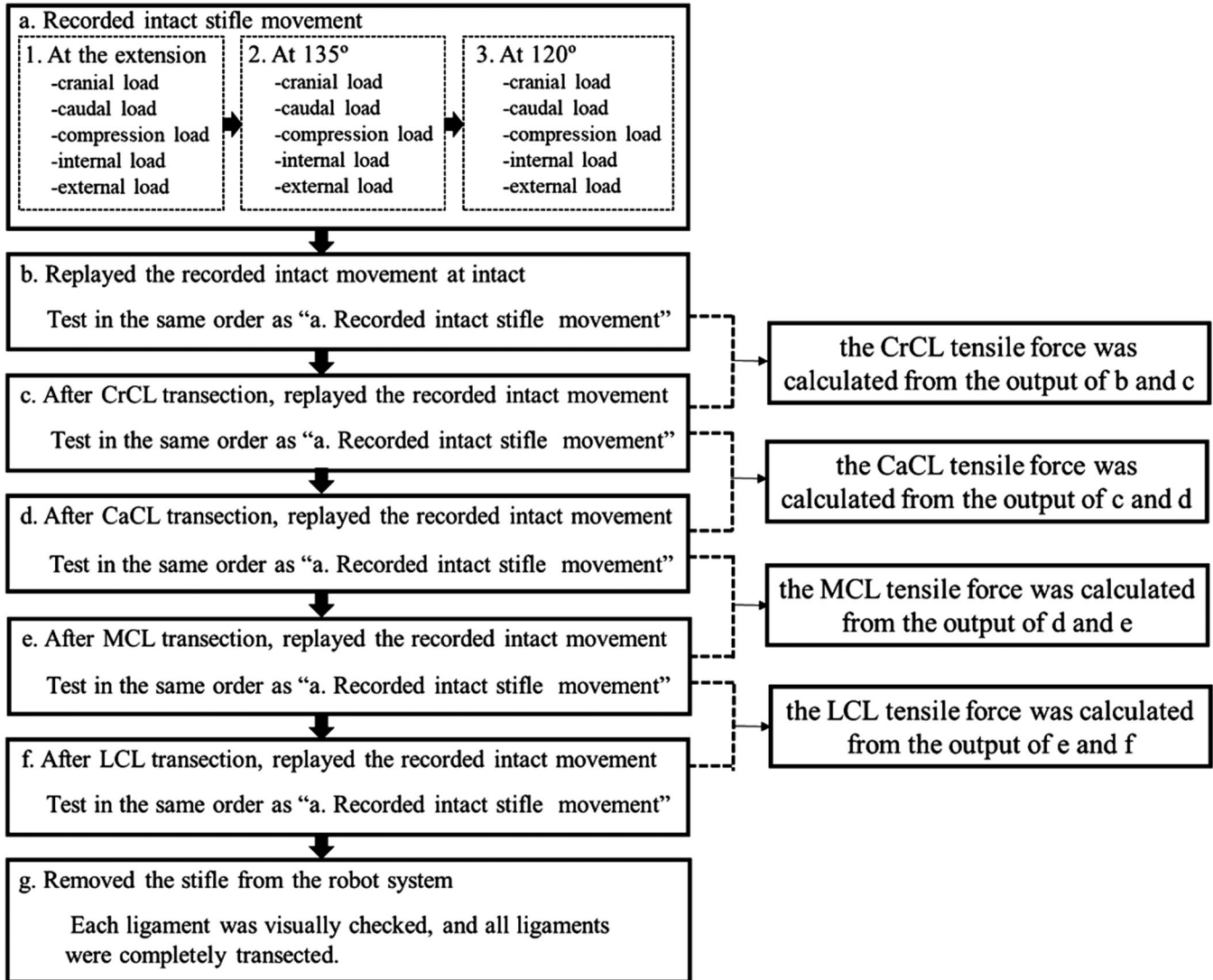
## 3 | RESULTS

### 3.1 | Animals

No orthopaedic disease was found in any dog. In all stifle joints, radiographic findings suggestive of osteoarthritis, such as osteophytes, fat pad signs, and bone deformities, were not observed. The stifles had no ligament damage on macroscopic assessment. The reference group included stifles from eight dogs (seven male and one female; age  $21.1 \pm 6.7$  months; body weight  $11.0 \pm 1.0$  kg), and the TPLO group included stifles from nine dogs (four male and five female; age  $14.8 \pm 3.4$  months; body weight  $10.8 \pm 1.8$  kg). Significant differences were found with respect to age but not body weight between groups (age:  $p = 0.01$ , body weight:  $p = 0.60$ ).

The TPA was  $31.6^\circ (\pm 2.7^\circ)$  in the reference group and  $30.8^\circ (\pm 2.5^\circ)$  in the TPLO group before the operation. There were no significant differences in preoperative TPA between the groups (preoperative TPA:  $p = 0.21$ ). The TPA was adjusted by  $6.4^\circ (\pm 2.8^\circ)$  after the operation in the TPLO group.

After the test, the stifle joint was removed from the robot, and each ligament was visually checked. All ligaments were wholly transected.



**FIGURE 3** The diagram contains the order in the test

### 3.2 | Tensile force in response to 30 N of cranial load on the tibia relative to the femur

In both the reference and TPLO groups, the CrCL tensile force was higher than the CaCL (reference:  $p < 0.01$  on maximum extension,  $p < 0.01$  at 135°,  $p < 0.001$  at 120°; TPLO:  $p < 0.01$  on maximum extension,  $p < 0.01$  at 135°,  $p < 0.01$  at 120°), MCL (reference:  $p < 0.01$  on maximum extension,  $p < 0.01$  at 135°,  $p < 0.01$  at 120°; TPLO:  $p < 0.01$  on maximum extension,  $p < 0.01$  at 135°,  $p < 0.01$  at 120°) and LCL (reference:  $p < 0.01$  on maximum extension,  $p < 0.01$  at 135°,  $p < 0.01$  at 120°; TPLO:  $p < 0.01$  on maximum extension,  $p < 0.01$  at 135°,  $p < 0.01$  at 120°) tensile forces. In addition, the CrCL tensile force accounted for most of the tensile force on the ligaments (reference: 65.9% on maximum extension, 79.8% at 135°, 77.9% at 120°; TPLO: 77.8% on maximum extension, 76.7% at 135°, 70.1% at 120°). The tensile force on each ligament did not change with TPLO (Table 1 and Figure 4a).

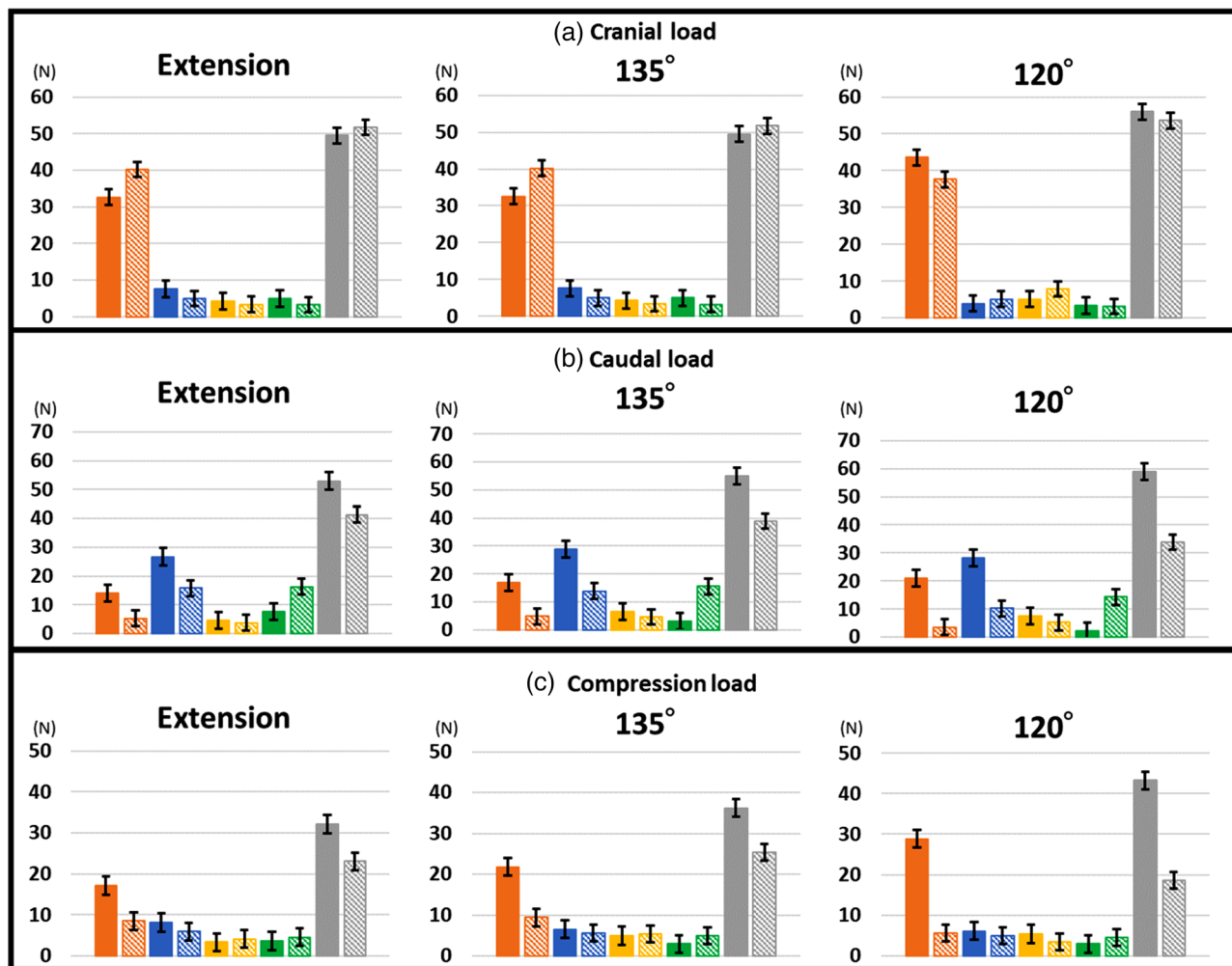
### 3.3 | Tensile force in response to 30 N of caudal load on the tibia relative to the femur

In the reference group, the CaCL tensile force was higher than the MCL ( $p < 0.01$  on maximum extension,  $p < 0.01$  at 135°,  $p < 0.01$  at 120°) and LCL ( $p = 0.01$  on maximum extension,  $p < 0.01$  at 135°,  $p < 0.01$  at 120°) tensile forces. Moreover, the CrCL tensile force was higher than the LCL tensile force at 120° and 135° ( $p = 0.87$  on maximum extension,  $p < 0.01$  at 135°,  $p < 0.01$  at 120°) in the reference group. The CrCL tensile force was lower in the TPLO group than in the reference group ( $p = 0.49$  on maximum extension,  $p = 0.23$  at 135°,  $p = 0.02$  at 120°). The CaCL tensile force was lower in the TPLO group than in the reference group at 120° ( $p = 1.00$  on maximum extension,  $p = 0.72$  at 135°,  $p = 0.08$  at 120°). The LCL tensile force was higher in the TPLO group than in the reference group at 135° and 120° ( $p = 1.00$  on maximum extension,  $p < 0.01$  at 135°,  $p < 0.01$  at 120°) (Table 2 and Figure 4b).

**TABLE 1** Least squares mean of tensile force in response to 30 N of cranial force on the tibia relative to the femur

Least squares mean of tensile force in response to 30 N of cranial force (N)									
Category	Maximum extension			135°			120°		
	Estimate (± SD)	LN (± SD)		Estimate (± SD)	LN (± SD)		Estimate (± SD)	LN (± SD)	
R-CrCL	32.6 ± 2.2	3.48 ± 0.25	A	39.0 ± 2.2	3.65 ± 0.25	A	43.6 ± 2.2	3.75 ± 0.25	A
R-CaCL	7.59 ± 2.2	1.69 ± 0.25	B	2.99 ± 2.2	0.73 ± 0.25	B	3.94 ± 2.2	1.07 ± 0.25	B
R-MCL	4.24 ± 2.2	1.36 ± 0.25	B	4.40 ± 2.2	1.10 ± 0.25	B	5.09 ± 2.2	1.18 ± 0.25	B
R-LCL	5.00 ± 2.2	1.52 ± 0.25	B	2.59 ± 2.2	0.50 ± 0.25	B	3.40 ± 2.2	0.94 ± 0.25	B
R-Total	49.5 ± 2.2	3.89 ± 0.25	A	48.9 ± 2.2	3.87 ± 0.25	A	56.0 ± 2.2	4.00 ± 0.25	A
T-CrCL	40.2 ± 2.1	3.69 ± 0.24	A	41.4 ± 2.1	3.72 ± 0.24	A	37.6 ± 2.2	3.64 ± 0.25	A
T-CaCL	4.94 ± 2.1	1.42 ± 0.24	B	5.11 ± 2.1	1.41 ± 0.24	B	5.11 ± 2.2	1.45 ± 0.25	B
T-MCL	3.40 ± 2.1	1.04 ± 0.24	B	3.98 ± 2.1	0.64 ± 0.24	B	7.87 ± 2.2	1.49 ± 0.25	B
T-LCL	3.23 ± 2.1	0.84 ± 0.24	B	3.54 ± 2.1	0.88 ± 0.24	B	3.12 ± 2.2	0.80 ± 0.25	B
T-Total	51.7 ± 2.1	3.94 ± 0.24	A	54.0 ± 2.1	3.98 ± 0.24	A	53.6 ± 2.2	3.99 ± 0.25	A

Note: Means of logarithms were compared. The same letters within a column are not significantly different. Letters cannot be compared between columns. LN, logarithm natural; R, reference; T, tibial plateau levelling osteotomy; CrCL, cranial cruciate ligament; CaCL, caudal cruciate ligament; MCL, medial collateral ligament; LCL, lateral collateral ligament.



**FIGURE 4** The least squares mean and SD of tensile force at cranial, caudal, and compression loads are shown. (a) Cranial load; (b) caudal load; (c) compression load. Solid bar, reference group; striped bar, tibial plateau levelling osteotomy group; orange colour, cranial cruciate ligament; blue colour, caudal cruciate ligament; yellow colour, medial collateral ligament; green colour, lateral collateral ligament; grey colour, total

**TABLE 2** Least squares mean of tensile force in response to 30 N of caudal force on the tibia relative to the femur

Least squares mean of tensile force in response to 30 N of caudal force (N)									
Category	Maximum extension			135°			120°		
	Estimate (± SD)	LN (± SD)		Estimate (± SD)	LN (± SD)		Estimate (± SD)	LN (± SD)	
R-CrCL	14.1 ± 3.0	2.56 ± 0.27	BCD	16.8 ± 3.0	2.62 ± 0.27	ABC	20.9 ± 3.0	2.77 ± 0.27	ABCD
R-CaCL	26.7 ± 3.0	3.26 ± 0.27	AB	28.8 ± 3.0	3.29 ± 0.27	ABC	28.2 ± 3.0	3.24 ± 0.27	ABC
R-MCL	4.59 ± 3.0	1.35 ± 0.27	CD	6.46 ± 3.0	1.49 ± 0.27	DE	7.66 ± 3.0	1.59 ± 0.27	DEF
R-LCL	7.64 ± 3.0	1.71 ± 0.27	CD	2.98 ± 3.0	0.60 ± 0.27	E	2.26 ± 3.0	0.43 ± 0.27	F
R-Total	53.0 ± 3.0	3.96 ± 0.27	A	55.0 ± 3.0	3.98 ± 0.27	A	59.0 ± 3.0	4.03 ± 0.27	A
T-CrCL	5.33 ± 2.8	1.49 ± 0.25	CD	4.81 ± 2.8	1.41 ± 0.25	DE	3.52 ± 3.0	1.18 ± 0.27	EF
T-CaCL	15.8 ± 2.8	2.65 ± 0.25	ABC	13.9 ± 2.8	2.33 ± 0.25	CDE	10.3 ± 3.0	1.85 ± 0.27	CDEF
T-MCL	3.76 ± 2.8	1.02 ± 0.25	D	4.64 ± 2.8	1.16 ± 0.25	DE	5.30 ± 3.0	1.39 ± 0.27	DEF
T-LCL	16.3 ± 2.8	2.62 ± 0.25	ABC	15.6 ± 2.8	2.35 ± 0.25	BCD	14.3 ± 3.0	2.25 ± 0.27	BCDE
T-Total	41.3 ± 2.8	3.72 ± 0.25	AB	38.9 ± 2.8	3.65 ± 0.25	AB	33.9 ± 3.0	3.46 ± 0.27	AB

Note: Means of logarithms were compared. The same letters within a column are not significantly different. Letters cannot be compared between columns. LN, logarithm natural; R, reference; T, tibial plateau levelling osteotomy; CrCL, cranial cruciate ligament; CaCL, caudal cruciate ligament; MCL, medial collateral ligament; LCL, lateral collateral ligament.

**TABLE 3** Least squares mean of tensile force in response to 30 N of proximal compression force on the tibia relative to the femur

Least squares mean of tensile force in response to 30 N of compression force (N)									
Category	Maximum extension			135°			120°		
	Estimate (± SD)	LN (± SD)		Estimate (± SD)	LN (± SD)		Estimate (± SD)	LN (± SD)	
R-CrCL	17.2 ± 2.9	2.75 ± 0.27	ABC	21.8 ± 2.9	2.94 ± 0.27	A	28.9 ± 2.9	3.25 ± 0.27	A
R-CaCL	8.10 ± 2.9	1.72 ± 0.27	BCD	6.53 ± 2.9	1.45 ± 0.27	B	6.13 ± 2.9	1.53 ± 0.27	BC
R-MCL	3.34 ± 2.9	1.02 ± 0.27	D	4.91 ± 2.9	1.23 ± 0.27	B	5.39 ± 2.9	1.24 ± 0.27	C
R-LCL	3.64 ± 2.9	1.13 ± 0.27	D	2.95 ± 2.9	0.86 ± 0.27	B	2.93 ± 2.9	0.84 ± 0.27	C
R-Total	32.2 ± 2.9	3.38 ± 0.27	A	36.2 ± 2.9	3.49 ± 0.27	A	43.3 ± 2.9	3.69 ± 0.27	A
T-CrCL	8.48 ± 2.7	1.97 ± 0.26	ABCD	9.38 ± 2.7	2.06 ± 0.26	AB	5.58 ± 2.7	1.48 ± 0.26	C
T-CaCL	5.92 ± 2.7	1.58 ± 0.26	CD	5.60 ± 2.7	1.50 ± 0.26	B	5.01 ± 2.7	1.36 ± 0.26	C
T-MCL	4.14 ± 2.7	1.02 ± 0.26	D	5.43 ± 2.7	1.00 ± 0.26	B	3.40 ± 2.7	0.89 ± 0.26	C
T-LCL	4.56 ± 2.7	1.30 ± 0.26	D	5.00 ± 2.7	1.36 ± 0.26	B	4.57 ± 2.7	1.11 ± 0.26	C
T-Total	23.1 ± 2.7	3.05 ± 0.26	AB	25.4 ± 2.7	3.11 ± 0.26	A	18.6 ± 2.7	2.84 ± 0.26	AB

Note: Means of logarithms were compared. The same letters within a column are not significantly different. Letters cannot be compared between columns. LN, logarithm natural; R, reference; T, tibial plateau levelling osteotomy; CrCL, cranial cruciate ligament; CaCL, caudal cruciate ligament; MCL, medial collateral ligament; LCL, lateral collateral ligament.

### 3.4 | Tensile force in response to 30 N of proximal compression load on the tibia relative to the femur

In the reference group, the CrCL tensile force was higher than the tensile force on other ligaments (maximum extension:  $p = 0.26$  vs. CaCL,  $p < 0.01$  vs. MCL,  $p < 0.01$  vs. LCL; 135°:  $p < 0.01$  vs. CaCL,  $p < 0.01$  vs. MCL,  $p < 0.01$  vs. LCL; 120°:  $p < 0.01$  vs. CaCL,  $p < 0.01$  vs. MCL,  $p < 0.01$  vs. LCL). The CrCL tensile force in the TPLO group was lower than that in the reference group at 120° ( $p = 0.96$  on maximum extension,  $p = 0.87$  at 135°,  $p < 0.01$  at 120°) (Table 3 and Figure 4c).

### 3.5 | Tensile force in response to 1 Nm of internal rotational torque on the tibia relative to the femur

In the reference group, the CrCL tensile force was higher than the tensile force on other ligaments at each angle (maximum extension:  $p = 0.46$  vs. CaCL,  $p < 0.01$  vs. MCL,  $p < 0.01$  vs. LCL; 135°:  $p < 0.01$  vs. CaCL,  $p = 0.15$  vs. MCL,  $p < 0.01$  vs. LCL; 120°:  $p < 0.01$  vs. CaCL,  $p = 0.87$  vs. MCL,  $p = 0.05$  vs. LCL). In the TPLO group, the CrCL tensile force was higher than the tensile force on other ligaments at each angle, (maximum extension:  $p < 0.01$  vs. CaCL,  $p < 0.01$  vs. MCL,

**TABLE 4** Least squares mean of tensile force in response to 1 Nm of internal torque on the tibia relative to the femur

Least squares mean of tensile force in response to 1 Nm of internal torque (N)									
Category	Maximum extension			135°			120°		
	Estimate (± SD)	LN (± SD)		Estimate (± SD)	LN (± SD)		Estimate (± SD)	LN (± SD)	
R-CrCL	53.5 ± 3.5	3.97 ± 0.18	AB	56.3 ± 3.5	3.99 ± 0.18	ABC	48.4 ± 3.5	3.81 ± 0.18	ABC
R-CaCL	26.0 ± 3.5	3.23 ± 0.18	BC	11.9 ± 3.5	2.42 ± 0.18	EF	11.9 ± 3.5	2.29 ± 0.18	E
R-MCL	18.8 ± 3.5	2.80 ± 0.18	CD	23.8 ± 3.5	3.12 ± 0.18	CDE	26.4 ± 3.5	3.22 ± 0.18	CD
R-LCL	13.6 ± 3.5	2.54 ± 0.18	CD	17.4 ± 3.5	2.77 ± 0.18	CDE	19.0 ± 3.5	2.83 ± 0.18	D
R-Total	111 ± 3.5	4.71 ± 0.18	A	109 ± 3.5	4.68 ± 0.18	A	106 ± 3.5	4.65 ± 0.18	A
T-CrCL	48.6 ± 3.3	3.87 ± 0.17	AB	41.8 ± 3.3	3.70 ± 0.17	BCD	30.4 ± 3.2	3.30 ± 0.17	BCD
T-CaCL	8.30 ± 3.3	1.86 ± 0.17	D	7.57 ± 3.3	1.66 ± 0.17	F	7.89 ± 3.2	1.73 ± 0.17	E
T-MCL	15.6 ± 3.3	2.71 ± 0.17	CD	15.2 ± 3.3	2.64 ± 0.17	DE	16.2 ± 3.2	2.63 ± 0.17	DE
T-LCL	14.3 ± 3.3	2.46 ± 0.17	CD	14.2 ± 3.3	2.55 ± 0.17	EF	14.0 ± 3.2	2.48 ± 0.17	DE
T-Total	86.8 ± 3.3	4.46 ± 0.17	A	78.8 ± 3.3	4.36 ± 0.17	AB	68.5 ± 3.2	4.21 ± 0.17	AB

Note: Means of logarithms were compared. The same letters within a column are not significantly different. Letters cannot be compared between columns. LN, logarithm natural; R, reference; T, tibial plateau levelling osteotomy; CrCL, cranial cruciate ligament; CaCL, caudal cruciate ligament; MCL, medial collateral ligament; LCL, lateral collateral ligament.

$p < 0.01$  vs. LCL; 135°:  $p < 0.01$  vs. CaCL,  $p < 0.01$  vs. MCL,  $p < 0.01$  vs. LCL; 120°:  $p < 0.01$  vs. CaCL,  $p = 0.56$  vs. MCL,  $p = 0.17$  vs. LCL). The CaCL tensile force was higher in the reference group than in the TPLO group on maximum extension ( $p < 0.01$  on maximum extension,  $p = 0.32$  at 135°,  $p = 0.90$  at 120°) (Table 4 and Figure 5b).

### 3.6 | Tensile force in response to 1 Nm of external rotational torque on the tibia relative to the femur

In the reference group, the CaCL tensile force was lower than the tensile force on other ligaments (maximum extension:  $p < 0.01$  vs. CrCL,  $p < 0.01$  vs. MCL,  $p < 0.01$  vs. LCL; 135°:  $p < 0.01$  vs. CrCL,  $p < 0.01$  vs. MCL,  $p < 0.01$  vs. LCL; 120°:  $p = 0.18$  vs. CrCL,  $p < 0.01$  vs. MCL,  $p < 0.01$  vs. LCL). In the TPLO group, the CaCL tensile force was lower than the tensile force on other ligaments (maximum extension:  $p < 0.01$  vs. CrCL,  $p < 0.01$  vs. MCL,  $p < 0.01$  vs. LCL; 135°:  $p < 0.01$  vs. CrCL,  $p < 0.01$  vs. MCL,  $p < 0.01$  vs. LCL; 120°:  $p = 1.00$  vs. CrCL,  $p < 0.01$  vs. MCL,  $p < 0.01$  vs. LCL). The tensile force on each ligament did not change with TPLO (Table 5 and Figure 5b).

## 4 | DISCUSSION

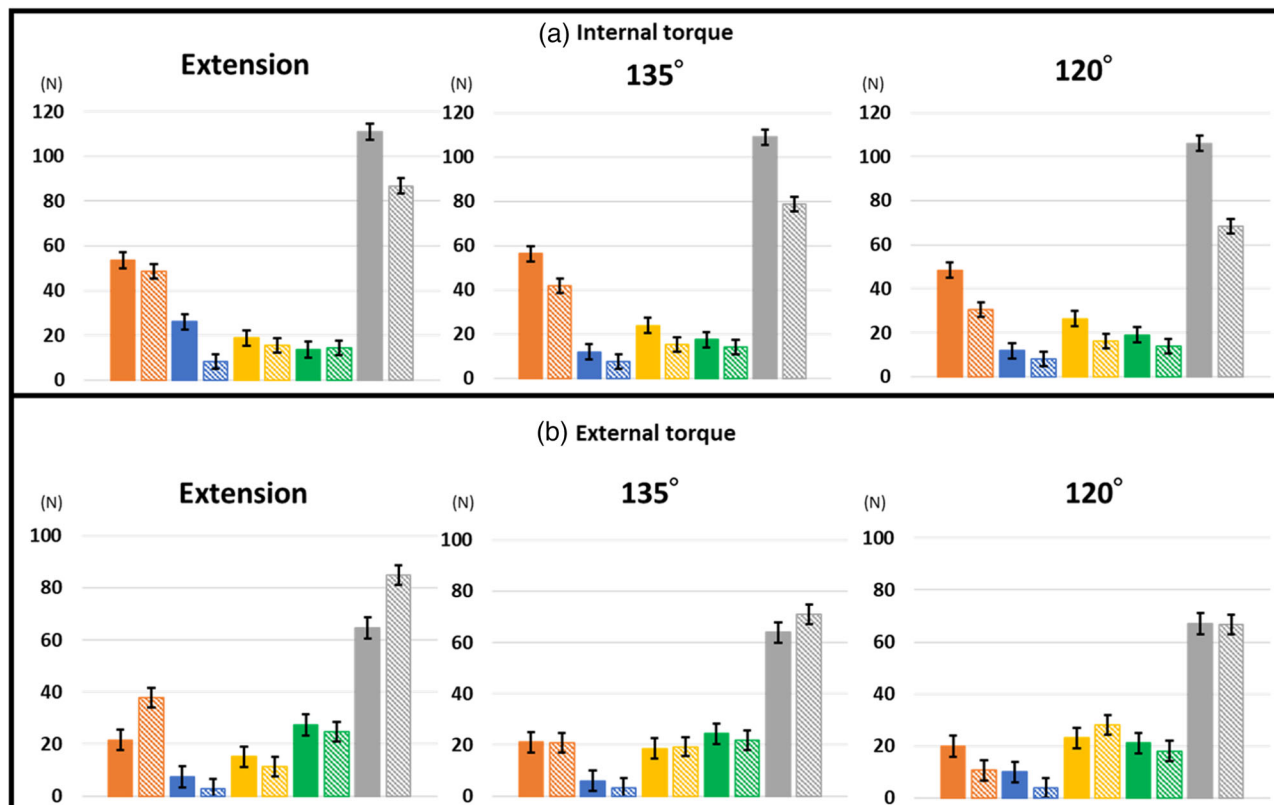
In this study, we investigated the tensile force placed on the stifle ligaments with TPLO, which has not been clarified to date. We found that the CrCL tensile force was reduced after TPLO under the caudal load and the compression load at 120°. In other words, the mechanical load on the CrCL decreased with TPLO.

A previous paper using a similar robotic system showed that the CrCL tensile force increases in an increased TPA model (Ichinohe et al., 2021). Increased TPA has been reported to promote CrCL degeneration in a canine model (Ichinohe et al., 2015). In other words, the CrTT

generated during compression may be involved in the development of disease with chronic progressive ligament degeneration. In this study, the tensile force on the CrCL under all loads as well as compressive load was relatively large in the reference group. Therefore, considering this result, the CrCL might be subjected to a variety of physical loads in addition to compressive loads. The purpose of TPLO is to reduce the TPA and neutralise the CrTT (Warzee et al., 2001). In a previous ex vivo study, the authors showed that the reduced TPA following TPLO decreased the strain placed on the CrCL during stifle compression (Haynes et al., 2015). The results of compression load in this study confirmed these previous results, as we identified a decreased tensile force in the CrCL following TPLO. In other words, TPLO has a protective effect on the mechanical load of the CrCL when a compression force is applied. The results of caudal load in this study indicate that the CrCL tensile force is reduced with TPLO at each joint angle. TPLO changes the anatomical tibial conformation and might change the positional relationship of the ligaments composing the stifle joint. As a result, the tensile force placed on the MCL and LCL might change as the position of the collateral ligaments attachment change, and the contact between the CrCL and CaCL might change with TPLO. Therefore, the CrCL tensile force might be decreased under a caudal force. In the present study, the tensile force on the CrCL was reduced not only in the compression load but also in the caudal load. Therefore, TPLO is expected to have a biomechanical protective effect against the degeneration of the CrCL.

The CaCL tensile force under internal rotational torque and caudal load decreased with TPLO, and the LCL tensile force under caudal load increased with TPLO. TPLO might have an effect on other stabilisers by altering the tibial anatomical morphology. In particular, the LCL is attached to the fibular head and may have limited displacement associated with TPLO when compared with the MCL. As a result, it may have had less laxity associated with TPLO than MCL had as well as compensatory control over other lax ligaments. The total tensile forces of the tested ligaments decreased with TPLO (Figure 5a). It is possible that





**FIGURE 5** The least squares mean and SD of tensile force at internal and external torque are shown. (a) Internal torque; (b) external torque. Solid bar, reference group; striped bar, tibial plateau levelling osteotomy group; orange colour, cranial cruciate ligament; blue colour, caudal cruciate ligament; yellow colour, medial collateral ligament; green colour, lateral collateral ligament; grey colour, total

the load compensated by each ligament shifted to the load on other soft tissues, such as the meniscus or meniscofemoral ligament, with TPLO. To clarify this, it is necessary to determine the functions of these tissues and the extent to which they contribute to stifle joint stability. In addition, interpretation of the results for ligaments other than CrCL is limited because the order of resection was not random in this study.

There are some limitations in this study. First, each group differed in age and sex. A recent review of etiopathogenetic factors reported that the main predisposing factors for rupture of the CrCL include an age between 2 and 10 years, having been neutered or spayed, and being large and/or overweight (Spinella et al., 2021). Notably, the population in this study is at little risk of having these predisposing factors. A previous report showed that degeneration of CrCL occurs by the age of 5 years in dogs weighing more than 15 kg (Vasseur et al., 1985). Hence, we considered the risk posed by the age differences to be minimal because the dogs used in this study were 15 kg or less and were all aged <2.5 years. The effect of sex on the tensile force on ligaments is unknown because previous reports do not indicate the effect of sex on the biomechanical properties in dogs. Second, we used only the stifles of Beagle dogs. Since this study analysed the biomechanical properties that occur in the stifles of Beagle dogs, similar evaluations in other breeds with different bone morphologies are warranted. Also, we only used the left side in our study and could not completely rule out the risk of potential differences in results due to laterality-based differ-

ences. Other limitations include the fact that the dynamic stabiliser, namely, the quadriceps muscle, was removed in this study, which may differ from the range of motion that occurs in vivo. Since each ligament tensile force may depend on the treatment order, a randomised order would be required. However, with the specimen mounted on the robot, it was difficult to transect the CaCL prior to the CrCL without damaging other intra-articular structures. Also, each time each ligament is cut, there is a risk of damage to the joint. Therefore, the interpretation of ligaments other than CrCL, in this case, is limited. However, the tests were conducted in a certain order to ensure that the experiments could be conducted with a limited number of samples. Also, since previous reports have indicated that freezing and thawing have little effect on the biomechanical characteristics of the patellar tendon (Suto et al., 2012), the effects of freezing and thawing are expected to be small in our results. However, the effects of these factors on other soft tissues such as the menisci and joint capsule remain unknown. The varus–valgus rotation and medial–lateral movement experiments were not conducted in this study because of the expected long duration of the experiments and the risk of specimen degradation.

This study's results indicate that TPLO causes changes in the tensile force on each ligament along with changes in the anatomy. In particular, it was found to reduce the mechanical load of the CrCL under compressive and caudal loads and may have a protective effect on the CrCL.

**TABLE 5** Least squares mean of tensile force in response to 1 N m of external torque on the tibia relative to the femur

Least squares mean of tensile force in response to 1 Nm of external torque (N)									
Category	Maximum extension			135°			120°		
	Estimate (± SD)	LN (± SD)		Estimate (± SD)	LN (± SD)		Estimate (± SD)	LN (± SD)	
R-CrCL	21.6 ± 4.0	2.91 ± 0.20	CD	21.1 ± 4.0	2.79 ± 0.20	C	19.9 ± 4.0	2.46 ± 0.20	BC
R-CaCL	7.41 ± 4.0	1.39 ± 0.20	E	5.89 ± 4.0	1.14 ± 0.20	B	10.0 ± 4.0	1.58 ± 0.20	D
R-MCL	15.1 ± 4.0	2.51 ± 0.20	D	18.5 ± 4.0	2.75 ± 0.20	C	23.1 ± 4.0	3.02 ± 0.20	BC
R-LCL	27.3 ± 4.0	3.18 ± 0.20	BC	24.4 ± 4.0	3.06 ± 0.20	C	21.1 ± 4.0	2.89 ± 0.20	BC
R-Total	64.6 ± 4.0	4.14 ± 0.20	AB	64.0 ± 4.0	4.12 ± 0.20	AB	67.1 ± 4.0	4.13 ± 0.20	A
T-CrCL	37.8 ± 3.8	3.65 ± 0.19	ABC	20.7 ± 3.8	3.00 ± 0.19	C	10.6 ± 3.8	1.99 ± 0.19	C
T-CaCL	2.88 ± 3.8	1.22 ± 0.19	E	3.33 ± 3.8	1.33 ± 0.19	D	3.90 ± 3.8	1.65 ± 0.19	D
T-MCL	11.4 ± 3.8	2.51 ± 0.19	D	19.3 ± 3.8	2.85 ± 0.19	C	28.1 ± 3.8	3.31 ± 0.19	AB
T-LCL	24.7 ± 3.8	3.26 ± 0.19	BC	21.7 ± 3.8	3.12 ± 0.19	BC	18.1 ± 3.8	2.90 ± 0.19	AB
T-Total	84.9 ± 3.8	4.42 ± 0.19	A	70.9 ± 3.8	4.24 ± 0.19	A	66.8 ± 3.8	4.19 ± 0.19	A

Note: Means of logarithms were compared. The same letters within a column are not significantly different. Letters cannot be compared between columns. LN, logarithm natural; R, reference; T, tibial plateau levelling osteotomy; CrCL, cranial cruciate ligament; CaCL, caudal cruciate ligament; MCL, medial collateral ligament; LCL, lateral collateral ligament.

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## ETHICS STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. This study was approved by the Animal Experiment and Bioethics Committee of Nippon Veterinary and Life Science University (approval number: 28S-57).

## AUTHOR CONTRIBUTIONS

Masakazu Shimada: conceptualisation; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; visualisation; writing – original draft; writing – review & editing. Tetsuya Takagi: conceptualisation; data curation; formal analysis; investigation; methodology; visualisation. Nobuo Kanno: conceptualisation; data curation; formal analysis; funding acquisition; investigation; methodology; resources; supervision. Satoshi Yamakawa: conceptualisation; data curation; formal analysis; investigation; methodology; supervision; visualisation. Hiromichi Fujie: conceptualisation; funding acquisition; methodology; resources; software; supervision. Yasushi Hara: conceptualisation; funding acquisition; methodology; resources; supervision.

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## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## PEER REVIEW

The peer review history for this article is available at <https://publons.com/publon/10.1002/vms3.889>.

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