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In vitro upper cervical spine kinematics: Rotation with combined movements and its variation after alar ligament transection

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ABSTRACT

Previous studies indicate that maximum upper cervical axial rotation occurs only through a combination of transverse, frontal, and sagittal plane motions. This study explores the relationship between transection of the alar ligament and combined upper cervical axial rotation movements. Ten cryopreserved upper cervical spines were manually mobilized in bilateral axial rotation and two different motion combinations with simultaneous motion in the three anatomical planes: rotation in extension (extension + axial rotation + contralateral lateral bending) and rotation in flexion (flexion + axial rotation + ipsilateral lateral bending). These three motions were performed before and after right alar ligament transection. The occiput-axis axial rotation was measured using an optical motion capture system while measuring the applied load. With intact alar ligament, the axial rotation in flexion showed the lowest range of motion (right, R: 9.81 ± 3.89°; left, L: 15.54 ± 5.89°). Similar results were found between the other two mobilizations: axial rotation (R: 33.87 ± 6.64°; L: 27.99 ± 6.90°) and rotation in extension (R: 35.15 ± 5.97°; L: 28.96 ± 6.47°). After right alar ligament transection, rotation in flexion (particularly in left rotation) showed the largest increase in motion: rotation in flexion (R: 13.78 ± 9.63°; L: 23.04 ± 5.59°), rotation in extension (R: 36.39 ± 7.10°; L: 31.71 ± 7.67°), and axial rotation (R: 38.50 ± 9.47°; L: 31.59 ± 6.55°). Different combinations of movements should be evaluated when analyzing the maximum axial rotation of the upper cervical spine, as axial rotation alone and rotation in extension showed a larger range of motion than rotation in flexion. After unilateral alar ligament injury, rotation to the non-injured side in flexion demonstrates the most movement increase.

1. Introduction

The upper cervical spine (UCS) is comprised of the occipital-atlas (C0-C1) and atlas-axis (C1-C2) spinal segments. These segments possess the greatest axial rotation of any segments in the spine (Lummel et al., 2012). Sixty percent of the cervical axial rotation occurs at the UCS due to the lack of intervertebral discs, the horizontal nature of its joints, and the presence of specialized muscles and ligaments (Kang et al., 2019; Morishita et al., 2009). Most UCS axial rotation occurs in C1-C2 (Salem et al., 2013). UCS axial rotation is mainly restrained by the alar ligaments' bone-ligament-bone system (Crisco et al., 1991). However, other capsules, ligaments, and soft tissues are also involved in the stability (Brolin and Halldin, 2004). The alar ligaments join the odontoid process of C2 and the occipital bone medially and closely to the atlanto-

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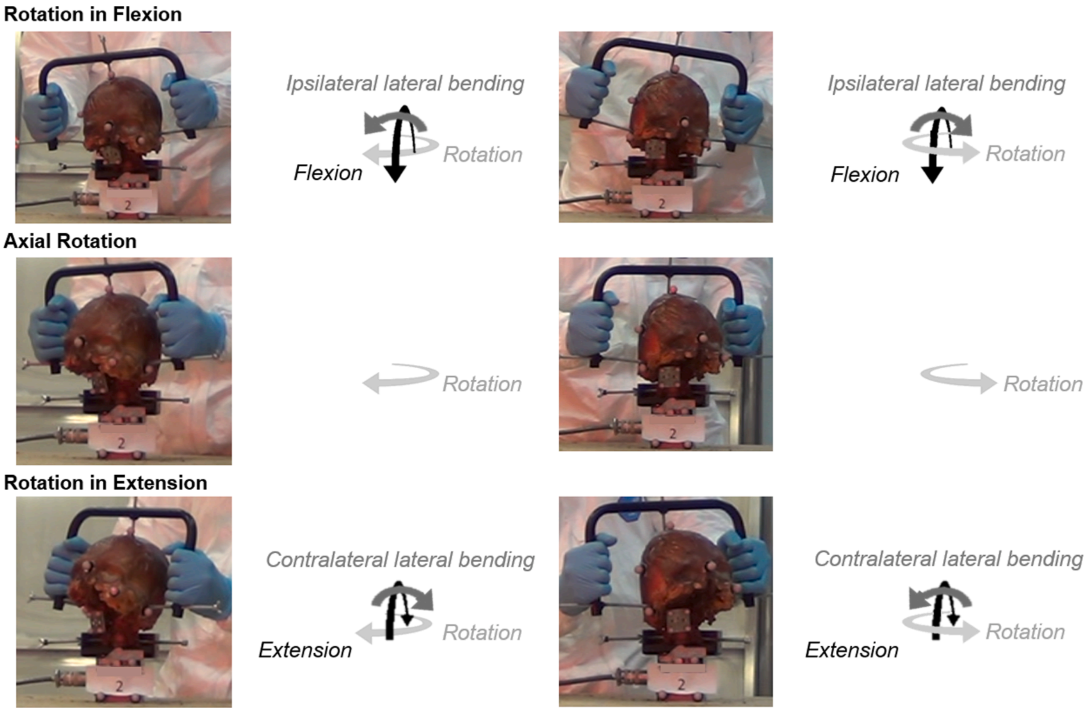

Fig. 1. The three movements studied: rotation in flexion, axial rotation, and rotation in extension. The sensors to track the motion (grey spheres) were attached to the cranium and the load cell.

occipital joints (Osmotherly et al., 2013) with various fiber orientations (Osmotherly et al., 2011). Apart from being the primary stabilizer in UCS axial rotation, the alar ligaments also provide stabilization during lateral bending. In the sagittal plane, alar ligaments are believed to limit excessive motion into flexion (Panjabi et al., 1998), extension (Ishak et al., 2020), or both movements (Panjabi et al., 1988).

The testing of UCS rotation range of motion (ROM) is used clinically for detecting potential instabilities via the rotation stress test (Osmotherly et al., 2012) and also for diagnosing restricted motions frequently associated to cervicogenic headache (Ogince et al., 2007). During the rotation stress test the axis is stabilized and occiput and atlas are rotated in both directions. This test has been shown to increase alar ligament length in vivo (Osmotherly et al., 2012) and detect increases in motion following alar ligament transection in vitro (Hidalgo-García et al., 2020). The rotation stress test is performed with the UCS in flexion, neutral, and extended positions. Laxity in all three positions is considered a positive finding. If the rotation stress test results in a larger amount of rotation and a reduced feeling of tissue resistance, UCS instability is suspected and a medical referral for further investigation is required. However, the threshold for diagnosing a positive rotation stress test in any of these positions is controversial (Osmotherly et al., 2012).

Normal UCS axial rotation values are highly variable within the literature. Total axial rotation ROM at C1-C2 is estimated between 28.4° (Panjabi et al., 1991) and 47° (Werne, 1959) during cadaveric examinations, with most in vivo studies reporting values between 36 and 41° (Anderst et al., 2017; Dvorak et al., 1988; Kang et al., 2019; Penning and Wilmink, 1987; Salem et al., 2013; Zhao et al., 2013). However, Osmotherly et al. (2013) concluded that the ROM of UCS axial rotation with C2 stabilization is 21° or less in participants with intact alar ligaments. This value represents half of the generally accepted 40-45° presented during unilateral UCS axial rotation (Ishii et al., 2004). By contrast, the ROM of UCS axial rotation measured by magnetic resonance (MR) by Osmotherly et al. (2013) was 10.58°.

Boszczyk et al. (2012) determined that the isolated biomechanics of C1-C2 was not enough to explain the maximal UCS axial rotation ROM (40° in their mathematical model with intact alar ligaments). Their study emphasized the importance of coupled motions associated with UCS axial rotation as a potential explanation for the maximal C1-C2 axial rotation (Boszczyk et al., 2012). The most frequent coupled movements associated with UCS axial rotation and least tightening of the alar ligaments are contralateral lateral bending and extension (Anderst et al., 2017; Dugailly et al., 2010; Ishii et al., 2004; Kang et al., 2019; Salem et al., 2013; Zhao et al., 2013; Zhou et al., 2020). According to Dvorak and Panjabi (1987), maximal tension of the right alar ligament is produced by a combined movement of contralateral axial rotation, contralateral lateral bending, and flexion. Therefore, the tightening of alar ligaments may impact the available ROM in the different combinations of UCS axial rotation. It may be possible that UCS rotation is misdiagnosed as restricted if only tested using a combination that tightens the alar ligaments.

This study seeks to analyze the effect of different movement combinations (rotation in flexion, rotation in extension, and axial rotation) on UCS axial rotation ROM. All movements were tested in vitro before and after unilateral transection of the alar ligament. We hypothesize that (a) UCS axial rotation in the extension combination is larger than the flexion combination, and (b) following alar ligament dissection the axial rotation ROM in the flexion combination would increase more than in the extension combination.

2. Methods

2.1. Sample

Ten head-UCS (C0-C2) specimens (9 men/1 woman; 74 ± 8 years, range 63–85 years, 169 ± 5.14 cm, 75.4 ± 10.77 kg) from cryopreserved cadavers were studied. All the specimens were visually examined to ensure that they were free of anatomical abnormalities and surgeries. This sample set was from donors of the Universitat Internacional de Catalunya. The procedure described below was approved by the Research Ethics Committee from UIC-Barcelona (Ref. CBAS-2017-03).

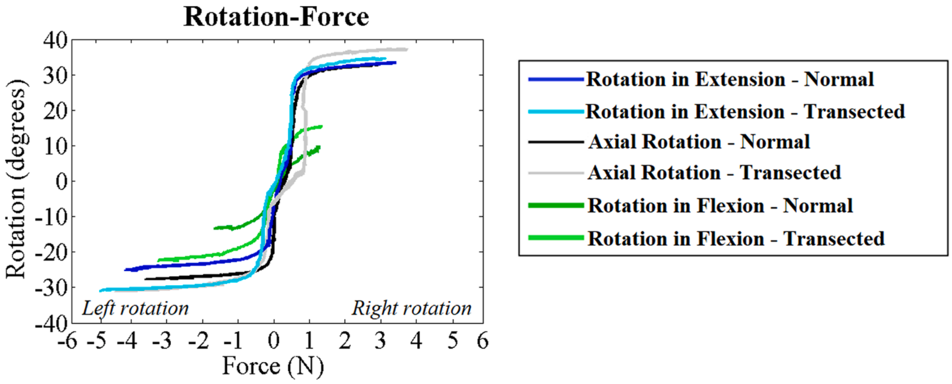

Fig. 2. Average force and rotation for axial rotation, rotation in flexion, and rotation in extension, with and without right alar ligament transection.

2.2. *Anatomical procedure*

The C0-C2 samples were obtained from head-cervical spine specimens disarticulated between C2 and C3. The skull was prepared with a posterior wedge cut as previously described by Dvorak et al. (1988). Care was taken to preserve all ligaments, and the integrity of axis and atlas. The full procedure is available in: Hidalgo-García et al. (2020).

Specimens were stored at −14 °C and thawed to room temperature 24 h prior to the tests. Dehydration of the specimens was prevented to ensure their mechanical properties with the test room maintained at a room temperature between 17.0 and 17.8°C and humidity between 47 and 52%.

2.3. *Biomechanical procedure*

Specimens were fixed on a 6-axis load cell (capacity of 56 Nm; MC3A Force and Torque Sensor, AMTI, MA, USA) to track the applied torque. The three anatomical planes (transverse, frontal, and sagittal) were aligned with the axes of the load cell. An optical motion capture system of 4 cameras (Vicon, TS series, Oxford, UK) was synchronized to the load cell and tracked how the head was moved. A metallic U-form handlebar was attached to the head to move the head without blocking camera view of the optical markers. This handlebar was fixed in 3 points: bilaterally to the auditory canals and on the top of the skull (Fig. 1).

Six retroreflective spherical markers were placed directly on the head to track its motion. Four markers were glued to the load cell where C2 was attached (Fig. 1), since the available surface of C2 was too small to directly accommodate the markers. Motion was quantified using the local coordinate systems of the head and C2. 3D coordinates of anatomical landmarks were measured with a FaroArm (FARO Technologies, Lake Mary, FL, USA) using the right and left external auditory meati and left infraorbital foraminae of the skull and the anterior and posterior lower central points and both sides on the transverse process of C2. By also measuring the Vicon markers, the tracked motion was analyzed using the skull and C2 coordinate systems as references. The required matrix transformations have been previously described by Shaw et al. (2009). The axis of motion for axial rotation pointed downwards; right rotation was defined as positive and left rotation as negative. All the rotation values provided herein are direct measurements on this axis.

Each specimen was moved in right and left rotation in:

1. Axial rotation,
2. Rotation in extension: extension + axial rotation + contralateral lateral bending (motion in the three anatomical planes at the same time),
3. Rotation in flexion: flexion + axial rotation + ipsilateral lateral bending (motion in the three anatomical planes at the same time).

The same order (1–2–3) was followed in the mobilization of all the specimens. Prior to quantifying these mobilizations (1–2–3), the specimens were pre-conditioned: the head was moved three times (full-ROM) in flexion–extension, then three times in lateral bending, and, lastly, two times in axial rotation. Then axial rotation, rotation in extension, and rotation in flexion were performed and measured. These three movements are displayed in Fig. 1. Next, the same measurements were performed in the same sequence (1–2–3) after right alar ligament transection. The neutral position of the head was ensured prior to each mobilization by two red-light lasers. One horizontal laser was aligned with the anatomical references of the Frankfurt plane (infraorbital foramen and external auditory meatus; Moorrees and Kean, 1958) and one vertical laser ensured that the central line chin-nose-forehead was vertically oriented.

All mobilizations were performed by the same researcher with more than 15 years of experience in manual therapy. The researcher was blinded to the ROM and load applied. All movements were manually performed until a marked resistance was perceived by the tester. The rate in all the mobilizations was between 2.5 and 4.5°/s, which is between the recommended range of 0.5–5.0°/s (Wilke et al., 1998).

The applied torques are also reported as forces in Newtons. The values in Newtons represent the total magnitude, i.e., the load from both hands in the transverse plane to generate the measured torque for the axial rotation. The values in Newtons were obtained from the measured torque (by the load cell, in Newton-meters) divided by the length of the lever arm: 0.15 m. When comparing maximum ROM between different test conditions, these values were achieved with different loads. To determine how axial rotation differs with equal loads, we selected 1 N (0.15 Nm) and 2 N (0.30 Nm) to compare the axial rotation with the same applied torque.

2.4. *Statistical analysis*

Data was analyzed using SPSS statistical software (IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.). The ROM at different loads, the maximal applied load, and the maximal ROM were analyzed. The difference between the intact and transected alar ligament specimens was compared using the Wilcoxon test, and the differences between the three movements (axial rotation, rotation in flexion, and rotation in extension) were analyzed with a two-way repeated measures ANOVA test. An ANOVA test was used to compare the main interaction effect of time (before and after) and group (intact and transected alar ligament) (Potvin and Schutz, 2000). The Bonferroni test was used when interactions were identified. Shapiro-Wilk test was used to check the normal distribution of the sample. The level of significance was set at alpha = 0.05.

Table 1
Rotation values in degrees of the 10 specimens for 1 N, 2 N and maximum force at the end of the range of motion (*ROM Max*) for the movements of rotation in flexion (*Rot Flex*), axial rotation (*Rot*), and rotation in extension (*Rot Ext*), before (normal configuration) and after alar ligament transection (*normal/transected*).

Test		Right axial rotation (normal/transected)				Left axial rotation (normal/transected)			
		1 N (deg)	2 N (deg)	F Max (N)	ROM Max (deg)	1 N (deg)	2 N (deg)	F Max (N)	ROM Max (deg)
1	Rot Flex			0.22/0.52	3.61/8.04	15.93/23.94		1.90/1.15	20.86/24.55
	Rot	26.14/33.90	30.04/38.06	2.15/2.50	30.77/39.42	33.59/35.21	36.00/37.09	2.91/2.30	38.01/41.06
	Rot Ext	23.82/32.34	28.53/35.29	5.73/4.46	34.13/39.35	32.00/33.34	34.94/35.53	3.15/6.43	37.66/40.11
2	Rot Flex	3.90/–		1.28/0.97	11.98/10.53	–/31.32		0.18/1.61	23.72/33.20
	Rot	5.66/26.26	32.63/38.73	2.52/4.32	33.53/44.63	38.49/37.77	–/41.83	1.31/3.53	38.99/43.79
	Rot Ext	0.29/–	13.26/18.49	5.50/3.48	35.11/29.72	32.26/26.12	36.71/30.53	3.46/5.65	41.22/44.07
3	Rot Flex	0.57/1.19	2.20/3.81	3.28/2.89	4.63/4.48	4.78/6.52	–/8.78	1.78/4.37	5.43/14.11
	Rot	0.01/15.70	7.46/20.21	3.45/5.59	24.84/30.71	13.61/13.21	16.39/19.63	2.46/7.81	17.56/29.56
	Rot Ext	19.87/4.44	24.65/20.71	2.41/3.96	25.84/27.39	21.28/21.35	–/25.96	1.60/4.07	22.05/30.08
4	Rot Flex			0.77/0.29	9.11/8.19	9.60/11.08		1.41/1.88	11.29/16.79
	Rot	38.00/47.92	42.72/50.29	3.79/2.84	43.85/52.57	15.64/13.38	20.71/19.68	4.34/6.20	25.38/25.43
	Rot Ext	33.63/40.21	40.42/45.77	2.64/2.68	42.44/48.80	13.14/11.18	19.92/16.68	6.56/5.18	28.99/24.96
5	Rot Flex	10.15/–		1.21/0.61	11.52/7.77	9.62/14.66	–/17.35	1.96/3.32	12.15/19.17
	Rot	21.31/16.00	22.06/17.45	3.14/3.66	24.60/17.97	12.23/20.24	14.54/24.53	5.28/5.00	21.15/28.01
	Rot Ext	11.64/13.24	19.53/24.87	5.05/5.48	26.60/25.78	4.84/5.73	11.21/10.76	6.61/4.62	21.42/17.72
6	Rot Flex	–/10.80		0.40/1.00	7.06/10.91	9.64/10.29	–/15.88	1.06/5.42	9.78/22.10
	Rot	26.66/27.15	29.24/31.35	2.83/4.19	30.49/35.35	15.10/12.63	18.79/15.49	4.57/8.27	22.15/22.73
	Rot Ext	24.93/30.30	28.93/34.25	3.68/3.60	32.01/36.65	15.70/11.66	18.99/17.86	4.59/8.38	23.01/25.65
7	Rot Flex	10.64/18.07		1.38/1.46	12.62/19.04	14.40/19.38		1.19/1.83	15.91/21.95
	Rot	22.70/32.97	30.96/37.56	4.04/4.17	36.90/42.78	21.54/27.48	26.30/32.46	2.92/2.06	28.38/32.76
	Rot Ext	32.90/35.39	36.29/39.03	2.93/2.86	38.84/41.12	17.83/25.66	25.04/33.70	3.12/2.92	28.68/36.37
8	Rot Flex	7.04/6.48		1.74/1.67	14.71/9.48	7.67/19.96	12.99/23.32	2.50/4.45	15.30/27.11
	Rot	30.00/32.39	34.34/36.54	2.85/3.59	36.51/40.38	23.20/22.64	26.53/26.63	4.79/5.62	30.76/32.09
	Rot Ext	34.94/38.89	37.37/41.47	2.99/2.02	41.03/41.79	25.88/29.08	27.91/30.96	4.65/4.32	31.48/33.46
9	Rot Flex	5.94/10.80	–/30.72	1.69/3.06	8.42/35.92	5.29/5.58	12.49/11.64	3.67/6.89	19.37/27.78
	Rot	16.11/25.67	31.13/37.58	5.47/4.00	43.48/45.52	18.86/25.70	23.67/29.33	4.21/3.78	28.04/32.37
	Rot Ext	34.67/30.30	39.36/35.95	2.99/2.15	41.95/36.09	16.78/24.67	20.92/28.12	4.91/3.87	27.53/32.22
10	Rot Flex	12.00/13.46	–/21.37	1.31/2.32	14.45/23.39	16.70/15.60	20.33/20.02	2.49/3.44	21.61/23.68
	Rot	24.27/26.42	30.08/31.91	3.55/2.92	33.74/35.65	23.44/22.43	25.98/25.22	5.46/4.01	29.48/28.12
	Rot Ext	28.83/34.96	32.75/36.73	2.32/2.37	33.55/37.20	24.88/22.43	26.66/26.86	2.74/6.16	27.56/32.46

Abbreviations: deg: degrees; F: force; N: Newtons; Max: Maximal; ROM: Range of motion.

Table 2
Mean and standard deviation of all the specimens (10) for the three movements: rotation in flexion, axial rotation, and rotation in extension, before (normal configuration) and after unilateral alar ligament transection.

	1 N 0.15 Nm Mean (SD) (deg)	2 N 0.30 Nm Mean (SD) (deg)	F Max Mean (SD) (N)	T Max Mean (SD) (Nm)	ROM Max Mean (SD) (deg)	1 N 0.15 Nm Mean (SD) (deg)	2 N 0.30 Nm Mean (SD) (deg)	F max Mean (SD) (N)	T Max Mean (SD) (Nm)	ROM Max Mean (SD) (deg)
		Right rotation in flexion					**Left rotation in flexion**			
Normal	7.18 (4.08)	–	1.33 (0.85)	0.20 (0.13)	9.81 (3.89)	10.40 (4.38)	15.27 (4.39)	1.81 (0.95)	0.27 (0.14)	15.54 (5.89)
Transected	10.13 (5.80)	18.63 (13.66)	1.48 (0.99)	0.22 (0.15)	13.78 (9.63)	15.83 (8.04)	16.16 (5.34)	3.44 (1.87)	0.52 (0.28)	23.04 (5.59)
P-value	0,080	–	0,683		0,285	**0,021**[*]	1,000	**0,013**[*]		**0,005**[*]
		Right axial rotation					**Left axial rotation**			
Normal	21.09 (11.27)	29.07 (9.14)	3.38 (0.94)	0.51 (0.14)	33.87 (6.64)	21.57 (8.64)	23.21 (6.51)	3.82 (1.36)	0.57 (0.20)	27.99 (6.90)
Transected	28.44 (9.31)	33.97 (9.49)	3.78 (0.90)	0.57 (0.13)	38.50 (9.47)	23.07 (8.81)	27.19 (8.21)	4.86 (2.13)	0.73 (0.32)	31.59 (6.55)
P-value	**0,017**[*]	**0,017**[*]	0,333		**0,037**[*]	0,646	0,173	0,285		**0,017**[*]
		Right rotation in extension					**Left rotation in extension**			
Normal	24.55 (11.35)	30.11 (8.94)	3.62 (1.31)	0.54 (0.20)	35.15 (5.97)	20.46 (8.58)	24.70 (8.02)	4.14 (1.63)	0.62 (0.24)	28.96 (6.47)
Transected	28.90 (12.07)	33.26 (8.99)	3.31 (1.11)	0.50 (0.17)	36.39 (7.10)	21.12 (8.81)	25.70 (8.06)	5.16 (1.57)	0.77 (0.23)	31.71 (7.67)
P-value	0,260	**0,022**[*]	0,285		0,386	0,721	0,678	0,203		0,093

Abbreviations: N: Newtons; F: Force; T: Torque; Max: Maximal; ROM: Range of motion; SD: Standard Deviation.

[*] Statistical significance (p = 0.05, Wilcoxon test).

3. Results

The right and left axial rotations were quantified for the three mobilizations: axial rotation, rotation in extension, and rotation in flexion. The following two sections describe the results before and after unilateral alar ligament transection.

3.1. *Configuration: intact alar ligaments (before transection)*

The rotation in flexion showed less than half of the axial rotation measured in the other two motions: 9.81 ± 3.89° (0.20 ± 0.13 Nm) to the right side and 15.54 ± 5.89° (0.27 ± 0.14 Nm) to the left side (Fig. 2). No statistical difference was detected between axial rotation and axial rotation in extension (right: p = 0.194; left: p = 0.451): axial rotation was 33.87 ± 6.64° (right; 0.51 ± 0.14 Nm) and 27.99 ± 6.90° (left; 0.57 ± 0.20 Nm), and rotation in extension showed 35.15 ± 5.97° (right; 0.54 ± 0.20 Nm) and 28.96 ± 6.47° (left; 0.62 ± 0.24 Nm) (Table 1).

Rotation in flexion showed the lowest axial rotation, and the lowest average loads were also observed in the rotation in flexion (p-values in Table 3). Some specimens, such as tests #4 and #5, received more load without showing a larger ROM: the individual response of each

Table 3
P-values from Bonferroni test between rotation (Rot.) in flexion, rotation in extension, and axial rotation, for normal (N, without alar ligament transection) and with unilateral alar ligament transection (T), for right (R) and left (L) sides. As Table 1 and Table 2, results are provided for 1 N (0.15 Nm) and 2 N (0.30 Nm) of applied force, maximum force (F Max) and maximum ROM. Bold values represent that there is a statistical significant difference between the two compared movements.

Test		Side	1 N 0.15 Nm	2 N 0.30 Nm	Load Max	ROM Max
Rot. in Flexion – Axial Rot.	N	R	**0.043**	–	**0.001**	**0.001**
		L	**0.002**	0.148	**0.001**	**0.001**
	T	R	**0.001**	0.167	**0.001**	**0.001**
		L	**0.006**	0.110	0.166	**0.001**
Rot. in Extension – Axial Rot.	N	R	0.583	–	1.000	0.194
		L	1.000	1.000	1.000	0.451
	T	R	1.000	1.000	0.900	0.961
		L	1.000	1.000	1.000	1.000
Rot. in Flexion – Rot. in Extension	N	R	**0.044**	–	**0.012**	**0.001**
		L	**0.025**	0.186	**0.008**	**0.001**
	T	R	**0.013**	0.228	**0.031**	**0.001**
		L	0.254	0.314	0.157	**0.003**

specimen can be seen in Table 2 and Fig. 3. Fig. 3 is presented with the three motions separated in Appendix A.

The axial rotations in the three movements (axial rotation, in flexion, and in extension) were compared with 1 N (0.15 Nm). With the same load, rotation in flexion still showed the lowest axial rotation (right: 7.18 ± 4.08°; left: 10.40 ± 4.38°; p-values in Table 3), which was close to half of the other two movements (axial rotation, right: 21.09 ± 11.27°, left: 21.57 ± 8.64°; in extension, right: 24.55 ± 11.35°, left: 20.46 ± 8.58°). The same comparison for 2 N (0.30 Nm) is shown in Table 2 (only between axial rotation alone and rotation in extension as the maximum force in rotation in flexion is lower than 2 N).

3.2. *Configuration: right alar ligament transected*

Right alar ligament transection increased the axial rotation ROM (from the neutral position) in both directions (right: $p = 0.037$; left: $p = 0.017$) (Hidalgo-García et al., 2020). An increased ROM was also observed in the two combinations (rotation in flexion and rotation in extension), although it was only statistically significant in left rotation in flexion; before transection: 15.54 ± 5.89° (0.27 ± 0.14 Nm), after transection: 23.04 ± 5.59° (0.52 ± 0.28 Nm; $p = 0.005$; Table 1). The lowest increase was observed in right rotation in extension: from 35.15 ± 5.97° (0.54 ± 0.20 Nm) to 36.39 ± 7.10° (0.50 ± 0.17 Nm) after alar ligament transection ($p = 0.386$), being slightly lower than the increase for the left side: from 28.96 ± 6.47° (0.62 ± 0.24 Nm) to 31.71 ± 7.67° (0.77 ± 0.23 Nm; $p = 0.093$).

For the three movements (axial rotation, rotation in flexion, and rotation in extension), a higher ROM after right alar ligament transection was always observed when comparing the axial rotation for 1 N and 2 N. However, the increase was only significant in left rotation in flexion with 1 N ($p = 0.021$) and right rotation in extension with 2 N ($p = 0.022$; Table 2).

4. Discussion

The combined movement of rotation in flexion showed a reduced axial rotation ROM in comparison to axial rotation and rotation in extension; and after right alar ligament transection, rotation in flexion showed the largest ROM increase in axial rotation (left greater than right). In contrast, the rotation in extension combination showed the smallest increase of ROM. To our knowledge, this is the first in vitro study, with C2 fixation, to describe axial rotation alone and two 3D

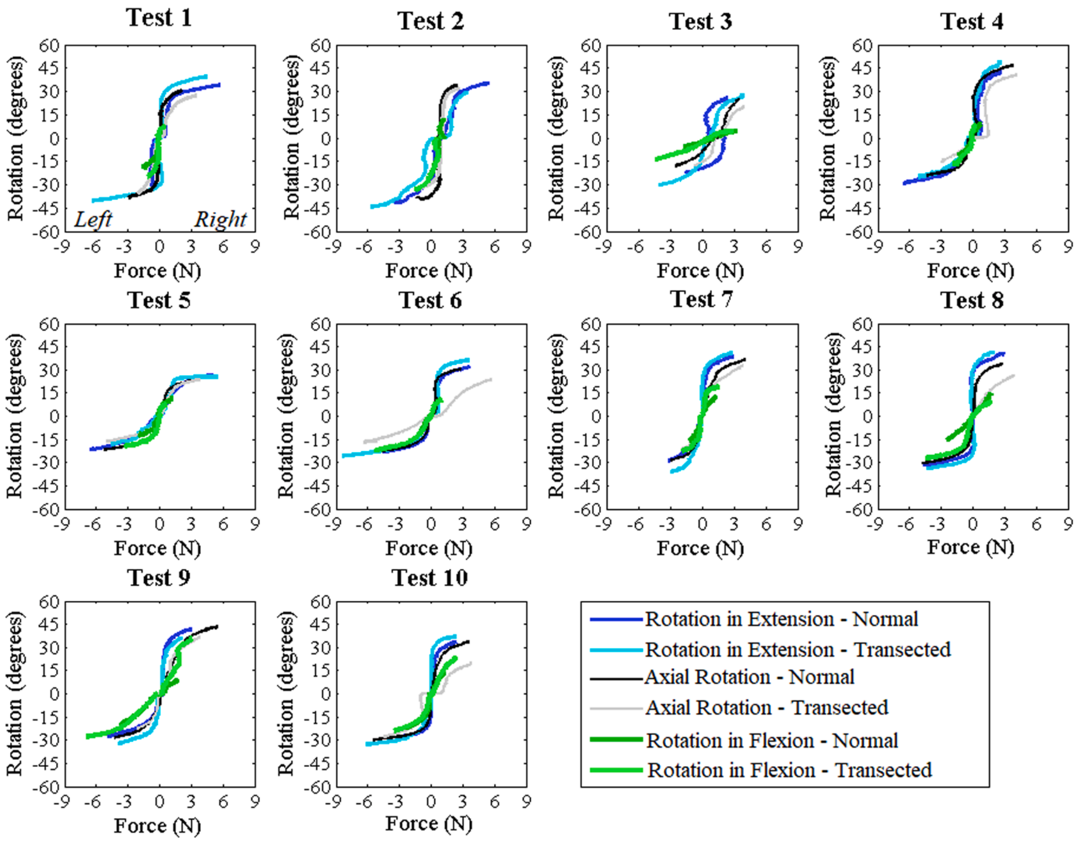

Fig. 3. Forces required for axial rotation, rotation in flexion, and rotation in extension during the right (positive) and left (negative) full range of motion in the 10 specimens.

combinations of movement during upper cervical spine axial rotation. The C0-C2 specimens were moved without and with right alar ligament transection, and the motion and load required to generate the movement were continuously measured throughout the full ROM.

4.1. Configuration: normal (before alar ligament transection)

The UCS axial rotation, considering both sides (right and left), was 61.91 ± 10.97° in our sample (Hidalgo-García et al., 2020). Total axial rotation (right and left) results are lower than the previous reported 92.2° in vitro study of Panjabi et al. (1988). However, Panjabi et al. (1988) applied stepwise loading, which increases the ROM in comparison with continuous loading (Goertzen et al., 2004). In addition, the 1.5 Nm load used within their study was applied to the full cervical spine (C0–C7) which is not comparable to this study. Two additional studies have reported closer amounts of C0–C2 axial bilateral rotation to our results using a testing apparatus with continuous loading: Tisherman et al. (2020) (68.13°, 0.75 Nm in C0-C3 specimens) and Takigawa et al. (2012) (62.3°, 2.0 Nm in C0–C2 specimens).

The C0-C2 total left to right ROM with rotation in extension combination was 64.11 ± 10.47° in our sample. This is the most frequent coupled movement associated with UCS axial rotation (Anderst et al., 2017; Ishii et al., 2004; Kang et al., 2019; Salem et al., 2013; Zhao et al., 2013). According to Osmotherly et al. (2013), the tension developed within the alar ligaments contributes to the coupling of lateral bending and axial rotation of the craniovertebral segments. Extension and contralateral lateral bending would minimize the tension to the alar ligament and allow more axial rotation ROM (Dvorak and Panjabi, 1987); although this mechanism may not be present in all cadavers. In our sample, the increase in axial rotation ROM with the rotation in extension combination was not statistically significant, specimen #3 (left side) showed a 25.6% increase with less mobilization load in the rotation in extension combination while other specimens (e.g., #9 right side) showed more ROM with axial rotation. Therefore, the difference in axial rotation ROM between axial rotation and rotation in extension combination could be specimen specific as morphological variations are frequent in the UCS (Cattrysse et al., 2011).

UCS ROM in the rotation in flexion combination in both directions was 25.35 ± 8.12° in our sample. The reduction of axial rotation ROM with this combination is statistically significant and viewed in all the specimens. According to Dvorak and Panjabi (1987), this combined movement would maximally tighten the contralateral alar ligament. The fact that the force at end range was lower in this combined movement than in axial rotation and the rotation in extension combination could be related to premature alar tightening, offering resistance earlier. In fact, when measuring for 1 N and 2 N in axial rotation, the average axial rotation ROM with this combined movement was always lower than in axial rotation or rotation in extension.

4.2. Configuration: right alar ligament transected

The transection of the right alar ligament increased left axial rotation ROM more than right axial rotation for all movements. This increase supports the findings of Dvorak et al. (1988), although our findings also demonstrated an increase in both directions as predicted by Crisco et al. (1991). Changes in axial rotation due to alar ligament transection are also discussed in Hidalgo-García et al. (2020). Dvorak and Panjabi (1987) concluded that the combination of flexion-left lateral bending-left axial rotation would cause the most tightening of the right alar ligament. In our study, this combination showed the maximal average increase after right alar ligament transection (48.3%; $p = 0.005$), higher than flexion-right lateral bending rotation-right axial rotation (29.7%; $p = 0.285$), axial rotation alone to the left (12.9%; $p = 0.017$), and axial rotation alone to the right (11.9%; $p = 0.037$). Interestingly, the combinations in extension showed less average increase after right alar ligament transection with extension-left lateral bending-right axial rotation showing the lowest increase (3.5%; $p > 0.05$).

The results of this study suggest that the UCS ROM in rotation with flexion combinations will generate more tension in the alar ligaments and less motion than axial rotation and rotation in extension. These findings may have important clinical implications since the most validated test for measuring C1-C2 axial rotation uses rotation from an end range position of cervical flexion (Ogince et al., 2007). Moreover, considering the clinical implications for the rotation stress test, although lateral bending as used in our study is not intended during the performance of rotation stress test, it would be reasonable to expect a larger physiological ROM in an extended position in normal conditions and a larger increase in ROM in a flexed position with instability. However, evaluation of UCS axial rotation should be interpreted as specimen specific and should be tested using different combinations of movement since morphological variability is frequently present (Beyer et al., 2016).

5. Limitations

The present study has several limitations. An in vitro study is not comparable to in vivo studies. However, in vitro conditions allowed the fixation of the C2 vertebra as a reference point for measuring UCS ROM. In this study, the mobilization force was applied manually, simulating a clinical and physiological procedure. Our study could not guarantee that rotation occurred in the pure transverse plane during axial rotation alone. In fact, our study measured an average of 4.6° in the sagittal plane and 5.7° in the frontal plane. These motions out of the transverse plane also occurred in prior literature where the specimens were moved by a machine and in other in vivo studies due to the intersegmental coupled motions (Dugailly et al., 2010; Ishii et al., 2004; Salem et al., 2013; Zhou et al., 2020). Dugailly et al. (2014) showed an intra-operator maximal variability up to 3° in a similar 3D in vitro mobilization of the upper cervical spine simulating a clinical procedure (Dugailly et al., 2014). Although the literature identifies a wide variability in the amount of UCS axial rotation and inter-individual anatomical variations are also likely to lead to differences in results (Hidalgo-García et al., 2021), we recognize that intra-operator reproducibility could partly explain the large asymmetry found among our specimens. Furthermore, the dissected structures may influence the UCS motion in the different planes of movement (Beyer et al., 2020; Lenz et al., 2012). The small sample size should also be considered a limitation. Lastly, age-related degenerative changes could influence UCS ROM, since ROM is expected to reduce with age due to changes in joints and ligaments orientation, and articular surface areas (Cattrysse et al., 2011).

6. Conclusions

With the alar ligaments intact, combined motion of rotation in flexion demonstrates the lowest range of motion compared to axial rotation and rotation in extension in both directions. When evaluating upper cervical rotation ROM, axial rotation or rotation in extension may provide the greatest mobility. Unilateral alar ligament transection demonstrates the greatest increase of range of motion during contralateral rotation in flexion, followed by axial rotation, with the least amount of increase in rotation in extension which may correlate with clinical findings during the rotation stress test.

CRediT authorship contribution statement

Ana I Lorente: Methodology, Software, Data curation, Validation, Writing – original draft, Writing – review & editing. **César Hidalgo-García:** Project administration, Conceptualization, Methodology, Formal analysis, Writing – original draft preparation, Writing – review & editing. **Pablo Fanlo-Mazas:** Visualization, Investigation, Writing – review & editing. **Jacobo Rodríguez-Sanz:** Supervision, Writing – review & editing. **Carlos López-de-Celis:** Supervision, Writing – review & editing. **John Krauss:** Writing- review & editing. **Mario Maza-Frechín:**

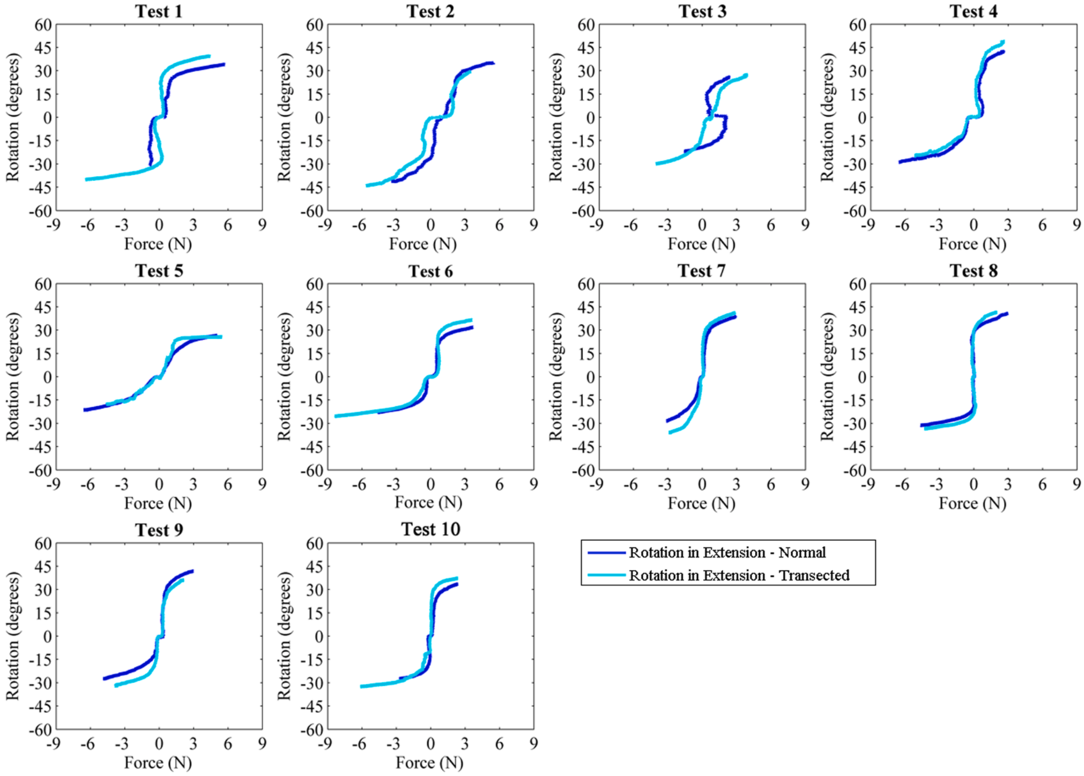

Fig. A1. Forces required for rotation in extension during the right (positive) and left (negative) full range of motion in the 10 specimens.

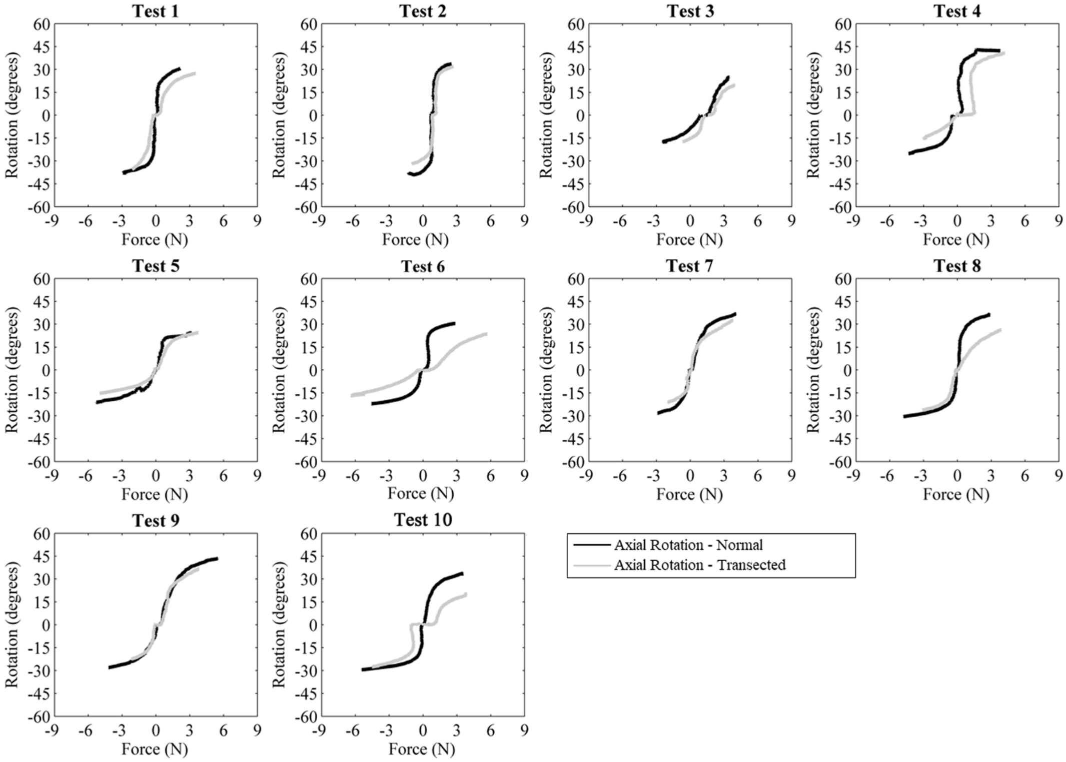

Fig. A2. Forces required for axial rotation during the right (positive) and left (negative) full range of motion in the 10 specimens.

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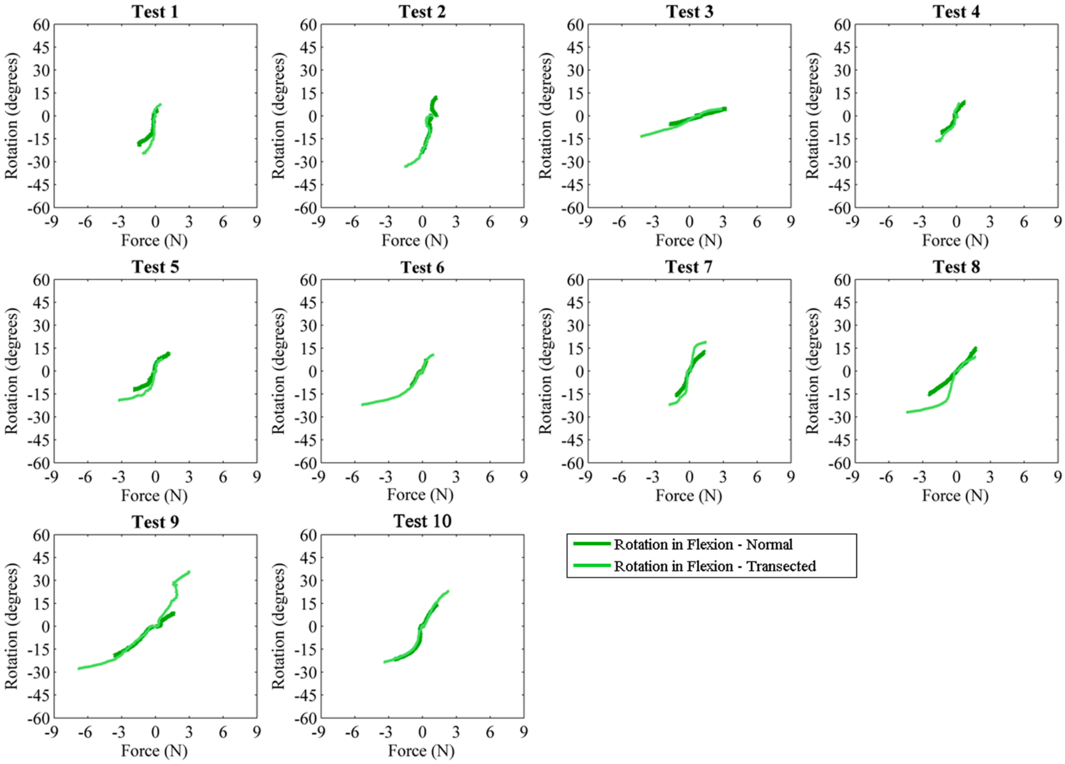

Fig. A3. Forces required for rotation in flexion during the right (positive) and left (negative) full range of motion in the 10 specimens.

Table A1
Rotation values in degrees of the 10 specimens for 0.15 Nm, 0.30 Nm and maximum torque *(T. Max)* at the end of the range of motion (*ROM Max*) for the movements of rotation in flexion (*Rot Flex*), axial rotation (*Rot*), and rotation in extension (*Rot Ext*), before (normal configuration) and after alar ligament transection (*normal/transected*).

Test		Right axial rotation (normal/transected)				Left axial rotation (normal/transected)			
		0.15 Nm (deg)	0.30 Nm (deg)	T Max (Nm)	ROM Max (deg)	0.15 Nm (deg)	0.30 Nm (deg)	T Max (Nm)	ROM Max (deg)
1	Rot Flex			0.03/0.08	3.61/8.04	15.93/23.94		0.28/0.17	20.86/24.55
	Rot	26.14/33.90	30.04/38.06	0.32/0.37	30.77/39.42	33.59/35.21	36.00/37.09	0.44/0.35	38.01/41.06
	Rot Ext	23.82/32.34	28.53/35.29	0.86/0.67	34.13/39.35	32.00/33.34	34.94/35.53	0.47/0.96	37.66/40.11
2	Rot Flex	3.90/–		0.19/0.15	11.98/10.53	–/31.32		0.03/0.24	23.72/33.20
	Rot	5.66/26.26	32.63/38.73	0.38/0.65	33.53/44.63	38.49/37.77	–/41.83	0.20/0.53	38.99/43.79
	Rot Ext	0.29/–	13.26/18.49	0.83/0.52	35.11/29.72	32.26/26.12	36.71/30.53	0.52/0.85	41.22/44.07
3	Rot Flex	0.57/1.19	2.20/3.81	0.49/0.43	4.63/4.48	4.78/6.52	–/8.78	0.27/0.65	5.43/14.11
	Rot	0.01/15.70	7.46/20.21	0.52/0.84	24.84/30.71	13.61/13.21	16.39/19.63	0.37/1.17	17.56/29.56
	Rot Ext	19.87/4.44	24.65/20.71	0.36/0.59	25.84/27.39	21.28/21.35	–/25.96	0.24/0.61	22.05/30.08
4	Rot Flex			0.12/0.04	9.11/8.19	9.60/11.08		0.21/0.28	11.29/16.79
	Rot	38.00/47.92	42.72/50.29	0.57/0.43	43.85/52.57	15.64/13.38	20.71/19.68	0.65/0.93	25.38/25.43
	Rot Ext	33.63/40.21	40.42/45.77	0.40/0.40	42.44/48.80	13.14/11.18	19.92/16.68	0.98/0.78	28.99/24.96
5	Rot Flex	10.15/–		0.18/0.09	11.52/7.77	9.62/14.66	–/17.35	0.29/0.50	12.15/19.17
	Rot	21.31/16.00	22.06/17.45	0.47/0.55	24.60/17.97	12.23/20.24	14.54/24.53	0.79/0.75	21.15/28.01
	Rot Ext	11.64/13.24	19.53/24.87	0.76/0.82	26.60/25.78	4.84/5.73	11.21/10.76	0.99/0.69	21.42/17.72
6	Rot Flex	–/10.80		0.06/0.15	7.06/10.91	9.64/10.29	–/15.88	0.16/0.81	9.78/22.10
	Rot	26.66/27.15	29.24/31.35	0.42/0.63	30.49/35.35	15.10/12.63	18.79/15.49	0.68/1.24	22.15/22.73
	Rot Ext	24.93/30.30	28.93/34.25	0.55/0.54	32.01/36.65	15.70/11.66	18.99/17.86	0.69/1.26	23.01/25.65
7	Rot Flex	10.64/18.07		0.21/0.22	12.62/19.04	14.40/19.38		0.18/0.27	15.91/21.95
	Rot	22.70/32.97	30.96/37.56	0.61/0.63	36.90/42.78	21.54/27.48	26.30/32.46	0.44/0.31	28.38/32.76
	Rot Ext	32.90/35.39	36.29/39.03	0.44/0.43	38.84/41.12	17.83/25.66	25.04/33.70	0.47/0.44	28.68/36.37
8	Rot Flex	7.04/6.48		0.26/0.25	14.71/9.48	7.67/19.96	12.99/23.32	0.37/0.67	15.30/27.11
	Rot	30.00/32.39	34.34/36.54	0.43/0.54	36.51/40.38	23.20/22.64	26.53/26.63	0.72/0.84	30.76/32.09
	Rot Ext	34.94/38.89	37.37/41.47	0.45/0.30	41.03/41.79	25.88/29.08	27.91/30.96	0.70/0.65	31.48/33.46
9	Rot Flex	5.94/10.80	–/30.72	0.25/0.46	8.42/35.92	5.29/5.58	12.49/11.64	0.55/1.03	19.37/27.78
	Rot	16.11/25.67	31.13/37.58	0.82/0.60	43.48/45.52	18.86/25.70	23.67/29.33	0.63/0.57	28.04/32.37
	Rot Ext	34.67/30.30	39.36/35.95	0.45/0.32	41.95/36.09	16.78/24.67	20.92/28.12	0.74/0.58	27.53/32.22
10	Rot Flex	12.00/13.46	–/21.37	0.20/0.35	14.45/23.39	16.70/15.60	20.33/20.02	0.37/0.52	21.61/23.68
	Rot	24.27/26.42	30.08/31.91	0.53/0.44	33.74/35.65	23.44/22.43	25.98/25.22	0.82/0.60	29.48/28.12
	Rot Ext	28.83/34.96	32.75/36.73	0.35/0.36	33.55/37.20	24.88/22.43	26.66/26.86	0.41/0.92	27.56/32.46

Abbreviations: deg: degrees; T: torque; Nm: Newton-meter; Max: Maximal; ROM: Range of motion.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A

See Figs. A1–A3 and Table A1.

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