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**EXPERIMENTAL CERVICAL INTERSPINOUS LIGAMENT PAIN ALTERED CERVICAL JOINT MOTION DURING DYNAMIC EXTENSION MOVEMENT**Ning Qu<sup>1</sup>, Rene Lindstrøm<sup>1</sup>, Thomas Graven-Nielsen<sup>2</sup>, Rogerio Pessoto Hirata<sup>1\*</sup><sup>1</sup> SMI, Department of Health and Science Technology, Faculty of Medicine, Aalborg University, Denmark<sup>2</sup> Center for Neuroplasticity and Pain (CNAP), SMI, Department of Health and Science Technology, Faculty of Medicine, Aalborg University, Denmark**Original paper for: Clinical Biomechanics****Number of text pages: 13****Number of Figures and tables: 5****Total words of abstracts: 249****Total words: 3397****Key words:** Neck pain, Interspinous ligament, Cervical joint motion, Video-fluoroscopy, Flexion, Extension, Anti-directional motion, Pro-directional motion**Acknowledgement:** NQ has been awarded a scholarship provided by the China Scholarship Council (CSC NO.201506170031) to pursue his PhD study at Aalborg University. TGN is a part of Center for Neuroplasticity and Pain (CNAP) which is supported by the Danish National Research Foundation (DNRF121).**\*Corresponding author:**

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**Abstract**

*Background:* Although the cervical interspinous ligament is a potential source of neck pain, the effects on cervical joint motion and pressure pain sensitivity has never been investigated. The understanding of the relationship will broaden our understanding of cervical biomechanics and improve diagnosis and treatment of neck pain.

*Methods:* Fluoroscopy videos of cervical flexion and extension movements and pressure pain thresholds over bilateral C2/C3 and C5/C6 facet joints were collected in fifteen healthy subjects before and after injections of hypertonic and isotonic saline in C4/C5 ISL. The videos were divided into 10 even epochs and the motion of individual joints during each epoch was extracted. Joint motion parameters including anti-directional motion, pro-directional motion, total joint motion and joint motion variability were extracted across epochs. Joint motion parameters and PPTs were compared before and after injection of hypertonic and isotonic saline separately.

*Findings:* Compared with baselines: hypertonic saline injection 1) decreased anti-directional motion and joint motion variability at C4/C5 ( $P < 0.05$ ) and increased at C2/C3 ( $P < 0.05$ ) during extension; 2) increased total joint motion of C0/C1 during first half range ( $P < 0.05$ ) and decreased during second half range of extension, and total joint motion of C2/C3 increased during second half range of extension ( $P < 0.05$ ) and; 3) increased pressure pain thresholds over left C2/C3 facet joint ( $P < 0.01$ ).

*Interpretation:* The cervical interspinous ligament pain redistributed anti-directional motion between C4/C5 and C2/C3 during dynamic extension and decreased pressure pain sensitivity over the left C2/C3 facet joint.

## 1. Introduction

Cervical interspinous ligament (ISL) is a posterior element of the neck, which exists between spinous processes of two adjacent cervical vertebrae and prevents the corresponding joint from hyperflexion<sup>1</sup>. Cervical ISL injury was demonstrated in 56.1% of patients with cervical spine traumas<sup>2</sup>, and potentially became the source of neck pain<sup>3</sup>.

Patients suffering from neck pain are associated with decreased range of motion, large joint position sense errors and decreased smoothness of movement which indicate a poor neuromuscular control of the neck movements<sup>4,5</sup>. Furthermore, patients with neck pain commonly conducts neck movements with a stiffer motor control strategy compared with healthy subjects<sup>6</sup>. Since cervical ISL is crucial for cervical muscle coordination and contributes to the dynamic joint stability<sup>7,8</sup>, it is possible that pain in cervical ISL leads to restriction of cervical joint motion during dynamic neck movements.

Cervical ISL contributes to the sensorimotor control of cervical joints during the entire range of neck movements<sup>9,10</sup>, however, previous studies mostly assessed cervical joint motion at end-static ranges of neck movements<sup>11,12</sup>. Recently, with quantitative video-fluoroscopy, the neck movements are able to be tracked in real time<sup>13</sup>. The cervical joint motion was revealed to contain motion along with the primary direction (pro-directional motion) and motion opposite to the primary direction (anti-directional motion) during cervical flexion and extension movements<sup>14</sup>. Wang et al. (2018) further showed that the cervical joint motion pattern during cervical flexion and extension movements was repeatable within and between days in healthy subjects<sup>15</sup>. Our previous work showed that experimental multifidus muscle pain redistributed the anti-directional motion between joints and experimental trapezius muscle pain decreased the overall anti-directional and pro-directional motion during cervical extension compared with before pain<sup>16</sup>. Additionally, the smoothness of joint motion indicated as joint motion variability decreased during cervical extension

by experimental trapezius muscle pain compared with before pain<sup>16</sup>. However, the effect of cervical ISL pain (both in patients and experimental pain models of healthy populations) on cervical joint motion during dynamic neck movements has never been investigated. Experimental pain models were extensively applied to explore the cause-effect relationship between pain and sensory/motor alterations without the confounding factors usually found in patients<sup>17-19</sup>.

Assessment of pressure pain thresholds (PPTs) was widely used to quantify sensory deficit during experimental neck pain and indicated the underlying mechanisms of different pain conditions<sup>20, 21</sup>. Decreased PPTs were normally found over the injection site or the referred pain areas and reflects the sensitization of peripheral nociceptors<sup>22</sup>. With respect to areas out of the injection site, PPTs were related with central sensitization which reflected the balance between enhanced descending inhibitory and facilitatory mechanisms<sup>23</sup>. Diverse findings of PPTs were demonstrated over areas out of the injection site when experimental pain was induced in different structures<sup>24-26</sup>. However, experimental pain induced in tendon or ligament was prone to decrease PPTs over the areas out of the injection site<sup>26</sup>.

The current study aimed to investigate the effects of hypertonic saline induced interspinous ligament pain on cervical joint motion during cervical flexion and extension and PPTs over cervical facet joints. It was hypothesized that experimental interspinous ligament pain will decrease anti-directional, pro-directional motion and joint motion variability during cervical flexion and extension and decrease PPTs over cervical facet joints compared with before pain.

## **2. Methods**

### *2.1 Subject*

Eleven male and four female healthy participants without neck pain for the last 3 months were recruited (Mean and standard deviation (SD), age: 27.4 years (SD 6.5), height: 173.7

cm (SD 11.5) and weight: 73.6 kg (SD 11.8). Participants were excluded if they had: (1) Cervical trauma or surgery, (2) Cervical musculoskeletal diseases, (3) Psychosocial profile that would affect responsiveness to pain, (4) Lack of ability to cooperate and (5) Possibility of pregnancy. The study was approved by North Denmark Region ethics committee (N20140004) and written consent forms were provided by all participants.

## *2.2 Experimental protocol*

This was a repeated-measures design study with two experimental sessions separated by an interval of at least one week. Hypertonic saline and isotonic saline were randomly injected in the cervical interspinous ligament across the two sessions. In each session, fluoroscopy videos of cervical flexion and extension movements were recorded and PPTs over cervical facet joints were assessed before and after the injection. Pain intensity, pain duration and pain distribution were obtained after the injection.

## *2.3 Experimental neck pain*

Sterile hypertonic saline (0.2 ml, 5.8%) and isotonic saline (0.2 ml, 0.9%) were injected into C4/C5 interspinous ligament with 27G needle and a tuberculin syringe. The injection procedure was guided by real-time ultrasound imaging<sup>27</sup>. The skin was cleaned by alcohol wipes before injections.

A 10-cm visual analogue scale (VAS) anchored with 'no pain' at 0 cm and 'maximum pain' at 10 cm was used to record pain intensity every minute after injections until the pain disappeared. Pain distribution was drawn on a body chart at the end of each session. Peak VAS score, pain duration and pain distribution (VistaMetrix v.1.38.0; SkillCrest, LLC, Tucson, AZ, USA) in arbitrary units (a.u.) were extracted for further analysis.

#### *2.4 Pressure pain thresholds (PPTs)*

The PPTs were measured over bilateral C2/C3 and C5/C6 cervical facet joints by a handheld digital algometer (Algometer, Somedic Production AB, Sollentuna, Sweden) with a 1-cm<sup>2</sup> round rubber tip when the subject lay prone on a bench and totally relaxed the neck. Application of pressure increased at a rate of 30kPa/s. Subjects pressed a button once the pressure stimulation elicited detectable pain. An average of three measurements determined PPT at each site.

#### *2.5 Fluoroscopic records and extraction of kinematic data*

A previously-published method was applied to record cervical flexion and extension movements<sup>14-16, 28</sup>. Participants were seated in a wooden chair with restriction of their trunk and wore custom glasses with four steel balls (external markers represented the occiput). Cervical flexion and extension movements were recorded by a Video-fluoroscope system (Philips BV Libra, 2006, Netherland) from neutral position (self-determined) to the maximal range position (the farthest position participants could achieve). Speed training of neck movements was performed before formal recordings to avoid blur videos. Visual instruction of a straight line was provided during movements to reduce out-of-plane rotations.

Eleven images representing 10 even epochs of each cervical flexion and extension video was selected and marked via a custom Matlab program (The MathWorks, Inc., Natick, Massachusetts, USA). The marking process was previously published with low marking errors and good reliability<sup>29</sup>. The landmarks of cervical vertebrae were identified and used to calculate cervical joint motion according to modified method initiated by Frobin et al<sup>30</sup>. The motion of individual joints (C0/C1, C2/C3, C3/C4, C4/C5, C5/C6 and C6/C7) during each epoch (1<sup>st</sup> epoch, 2<sup>nd</sup> epoch...10<sup>th</sup> epoch) was extracted. The motion opposite to the primary direction was defined as anti-



directional motion, the motion was otherwise defined as pro-directional motion<sup>14</sup>. Anti- and pro-directional motions of individual joints were extracted across epochs. Total joint motion was the sum of anti- and pro-directional motions. Joint motion variability was extracted as the variance of individual joint motions across epochs. The total joint motion was further extracted during the first half range (1<sup>st</sup> to 5<sup>th</sup> epochs) and the second half range (6<sup>th</sup> to 10<sup>th</sup> epochs) of cervical flexion and extension movements.

## 2.6 Statistical Analyses

Results are reported as mean and standard deviations (SD) in the text and mean and standard error (SE) in the figures. SPSS (IBM Statistics 24) was used to conduct statistical analysis. The data was tested for normality by the Shapiro Wilk test before comparison and was generally normally distributed ( $P > 0.05$ ). The homogeneity of variance between paired conditions was tested by Mauchly's test and the Greenhouse-Geisser correction was applied when the paired conditions did not meet the homogeneity.

To compare pain characteristics induced by hypertonic and isotonic saline, the pain intensity after injection for hypertonic and isotonic saline was analyzed by a two-way analysis of variance (ANOVA) with Saline (hypertonic, isotonic) and Time after injection (0 min, 1 min...12 mins) as repeated measures. The peak VAS score, pain duration and pain distribution were compared for hypertonic and isotonic saline injection by paired t-test.

To assess different effects of hypertonic and isotonic saline on cervical joint motion and PPTs, baselines of the two sessions were firstly compared by two-way ANOVA with Joint (C0/C1, C2/C3, C3/C4, C4/C5, C5/C6 and C6/C7) and Saline (hypertonic, isotonic) as repeated measures. No statistical difference was found between two baselines of PPTs and cervical joint motion parameters, therefore, the cervical joint motion parameters and PPTs were further analyzed

separately for hypertonic and isotonic saline during cervical flexion and extension movements. A two-way ANOVA was applied to analyze pro-directional motion, anti-directional motion, total joint motion and joint motion variability with Joint (C0/C1, C2/C3, C3/C4, C4/C5, C5/C6 and C6/C7) and Time (before injection, after injection) as repeated measures. In addition, a three-way ANOVA was applied to total joint motion during half ranges of flexion and extension movements with Joint (C0/C1, C2/C3, C3/C4, C4/C5, C5/C6 and C6/C7), Time (before injection, after injection) and Range (first half, second half) as repeated measures. The PPTs were analyzed by a two-way ANOVA with Site (right C2/C3, left C2/C3, right C5/C6 and left C5/C6) and Time (before injection, after injection) as repeated measures. Each ANOVA P-value was corrected by multiplying the total number of ANOVAs. Post hoc test with Bonferroni correction was performed when significant main effects or interactions were found. Statistical significance was accepted at value of  $P < 0.05$ .

### 3. Results

#### 3.1 Pain intensity, duration and distribution

The normality of the data was confirmed. Compared with isotonic saline, injection of hypertonic saline showed a higher peak VAS score (Fig. 1A, Hypertonic: 5.0 cm (SD 2.2), Isotonic: 0.9 cm (SD 1.2),  $t_{(14)} = 7.34$ ,  $P < 0.001$ ) and higher average pain intensity from immediate time after injection to 10 minutes after injection ( $P < 0.05$ ). Injections of hypertonic saline showed a longer duration (Fig. 1B, Hypertonic: 7.8 min (SD 3.2), Isotonic: 1.7 min (SD 2.6),  $t_{(14)} = 6.45$ ,  $P < 0.001$ ) and a larger pain distribution (Fig. 1C, Hypertonic: 3.5 a.u. (SD 3.0), Fig. 1B, Isotonic: 0.7 a.u. (SD 1.7),  $t_{(14)} = 2.87$ ,  $P = 0.012$ ) compared with isotonic saline injections.

#### 3.2 Pressure pain thresholds (PPTs)

The PPTs over cervical facet joints before and after injections of hypertonic saline and isotonic saline are shown in Fig. 2A and Fig. 2B. The normality of the data was confirmed ( $P > 0.05$ ). Significant interaction between Site and Time was found before and after hypertonic saline injection (RM-ANOVA:  $F_{(3,42)} = 3.694$ ,  $P = 0.038$ ) and the assumption of homogeneity was met ( $P = 0.408$ ). Post hoc analysis revealed PPTs over the left C2/C3 facet joint was higher after injection compared to before injection (Bonferroni:  $P = 0.014$ ).

### 3.3 Pro-directional motion and anti-directional motion

Pro-directional motion and anti-directional motion before and after hypertonic saline injection is shown in Fig. 3. The normality of the data was confirmed ( $P > 0.05$ ). Significant interaction between Joint and Time was found in anti-directional motion of cervical extension movement before and after hypertonic saline injection ( $F_{(6,84)} = 4.791$ ,  $P = 0.002$ ) and the assumption of homogeneity was met ( $P = 0.155$ ). Post hoc analysis revealed that the C2/C3 anti-directional motion increased (Bonferroni:  $P = 0.0001$ ) and C4/C5 anti-directional motion decreased (Bonferroni:  $P = 0.005$ ) after injection compared to before injection. No significance was found in pro-directional motion. No significance was found in pro-directional motion and anti-directional motion of cervical flexion. For Isotonic saline injection, no significance was found. Pro-directional motion and anti-directional motion before and after isotonic saline injection could be found in supplementary Fig. 1.

### 3.4 Total joint motion

The normality and homogeneity of the data were confirmed ( $P > 0.05$ ). However, no significant difference was found for Time, Joint and interaction between Time and Joint at any joint motion before and after hypertonic and isotonic saline injection (supplementary Fig. 2).

### 3.5 Joint motion variability

The normality of the data was confirmed ( $P > 0.05$ ). Significant interaction between Time and Joint was found in cervical extension movement before and after hypertonic saline injection ( $F_{(6,84)} = 3.537$ ,  $P = 0.014$ ) and the assumption of homogeneity was met ( $P = 0.06$ ). Post hoc analysis revealed that the C2/C3 motion variability increased (Bonferroni:  $P = 0.014$ ) and C4/C5 motion variability decreased (Bonferroni:  $P = 0.021$ ) after injection compared to before injection (Fig. 4).

### 3.6 Total joint motion during half ranges of flexion and extension

The joint motion during half ranges of flexion and extension movements before and after hypertonic and isotonic saline injection was shown in Fig. 5. The normality of the data was confirmed ( $P > 0.05$ ). Significant interaction effect between Joint, Time and Range was found in cervical extension movement before and after hypertonic saline injection ( $F_{(6,84)} = 4.401$ ,  $P = 0.0026$ ) and the assumption of homogeneity was met ( $P = 0.767$ ). Post hoc analysis revealed that during first half range, the C0/C1 motion (Bonferroni:  $P = 0.003$ ) increased compared to before injection. During second half range, the C0/C1 motion decreased (Bonferroni:  $P = 0.021$ ) and C2/C3 motion decreased (Bonferroni:  $P = 0.004$ ) compared to before injection.

## 4. Discussion

The C4/C5 interspinous ligament pain induced by hypertonic saline injection decreased both anti-directional motion and joint motion variability at C4/C5 and increased at C2/C3 compared to before injection conditions. Meanwhile, total joint motion of C0/C1 and C2/C3 was redistributed during the second half range of cervical extension, and total joint motion of C0/C1 was

redistributed between the first and the second half ranges of extension. In addition, PPTs over left C2/C3 facet joint increased after hypertonic saline injection compared to before injection.

#### *4.1 Pain intensity, duration and distribution*

Injection of hypertonic saline into the cervical interspinous ligament showed higher peak pain VAS, longer pain duration and larger pain distribution compared to the isotonic saline injection. Since similar volumes of hypertonic and isotonic saline were used, the distinctions may result from the higher saline concentration in the hypertonic solution (5.8% vs 0.9%). Furthermore, it is documented that pain intensity is correlated with the saline concentration<sup>31</sup>. Indeed, the peak pain VAS (5 cm) in the present study is higher than the previous study (around 4 cm), using similar volume (0.2 ml) but lower concentration (5%) of hypertonic saline injected into the lumbar interspinous ligament<sup>27</sup>. Another possible explanation is that cervical interspinous ligament contains higher nociceptor density than lumbar interspinous ligament<sup>32</sup> since experimental pain sensation results from the membrane depolarization of nociceptors after hypertonic saline injection<sup>33</sup>. The shorter pain duration (7.8 mins) was found compared with hypertonic saline injection in the lumbar interspinous ligament (10.7 mins)<sup>27</sup>. It may be explained by the rich vascularity of the neck structures around the cervical interspinous ligament which may increase the process of absorbing or dissolving the bolus of saline<sup>34</sup>. In the present study, most subjects showed localized pain distribution following both injections in cervical interspinous ligament. The localized anatomical morphology of the cervical interspinous ligament may account for this finding, since the ligament lies between two adjacent spinous process<sup>35</sup>.

#### *4.2 Pressure pain thresholds (PPTs)*

Contrary to our hypothesis, increased PPTs were found over left C2/C3 facet joint following the injection of hypertonic saline compared to before injection condition. The pressure hypoalgesia indicated the potential role of conditioned pain modulation which reflects the descending noxious inhibitory control mechanisms<sup>36</sup>. The finding is in accordance with previous studies where pressure hypoalgesia during pain conditions was found at areas outside the pain site<sup>37, 38</sup>. However, previous studies also simultaneously showed pressure hyperalgesia and hypoalgesia at different areas during the comparable experimental pain<sup>24, 25</sup>. The enhanced descending inhibitory and facilitatory mechanisms were activated simultaneously by experimental pain and the balance between them determined the alterations of PPTs over areas outside the pain site<sup>39</sup>. Therefore, the pressure hypoalgesia over left C2/C3 facet joint during pain may result from the predomination of the enhanced descending inhibitory mechanism in the present study<sup>24, 25</sup>. In addition, the inherent difference of sensitivity between human areas should also be considered<sup>32</sup>. Schomacher et al. (2013) demonstrated that the C2/C3 facet joint was more sensitive to mechanical pressure stimulation than C5/C6 facet joint in healthy controls<sup>40</sup>. This may explain why there were only findings over C2/C3 facet joint without changes over C5/C6 facet joint in the present study.

#### *4.3 Cervical joint kinematics*

The effect of cervical interspinous ligament pain on cervical joint motion during dynamic flexion and extension movements was investigated for the first time. The high repeatability of individual cervical joint motion analysis used in this study during flexion and extension movements was established previously<sup>15</sup>.

Hypertonic saline injection in the C4/C5 interspinous ligament decreased C4/C5 anti-directional motion and increased C2/C3 anti-directional motion during extension movement. Wang et al. have reported that anti-directional motion is a common sign of healthy cervical joints and is

equal to approximately 40% of the pro-directional motion during either flexion or extension movements<sup>14</sup>. The alteration in anti-directional motion may be a biomechanical marker which reflects the fine motor control on individual cervical joints under different pathological conditions.

The present findings are in concurrence with previous studies that patients with neck pain are commonly associated with altered motor control patterns<sup>41-45</sup>. Decreased anti-directional motion and joint motion variability at C4/C5 may indicate a local stiffing strategy of the joint during cervical extension, which concurs with previous studies where patients with neck pain showed stiffer and more rigid movements compared with healthy controls<sup>6, 45</sup>. The stiffing strategy was supposed to avoid movements which may cause pain or further damage and keep dynamic stability of the neck during pain conditions<sup>46</sup>. Patients with neck pain are normally present with increased activity in superficial cervical muscles and decreased activity in deep cervical muscles compared to healthy subjects<sup>47-50</sup>. Deep cervical muscles are crucial to the fine control of individual joints<sup>16, 51</sup>. The muscular co-contraction of agonist and antagonist or the cooperation of deep and superficial muscles determine the proper cervical joint motion during dynamic neck movements<sup>52</sup>. Moreover, ligaments are involved in the ligamento-muscular reflex and pain induced in the ligaments may activate the associated muscle activity<sup>53, 54</sup>. Even though this study did not measure muscle activity during the task, decreased anti-directional motion and joint motion variability at C4/C5 may be a result from activating deep cervical muscles by pain stimulation in the interspinous ligament<sup>55</sup>. Consequently, the redistribution of anti-directional motion between C4/C5 and C2/C3 could be a compensative response to the experimental pain. Such a compensative mechanism is a common way for the neck to maintain the motor outputs during pathologic conditions<sup>52, 56</sup>. Previous studies also showed that the decreased motion contribution at C5/C6 was compensated by C3/C4 during cervical lateral bending in patients with disc herniation<sup>57</sup>.

Motion redistribution was found between joints (C0/C1 and C2/C3) and between half ranges (C0/C1) during extension movement, this together with findings in anti-directional motion and joint motion variability, indicated cervical joint motion during dynamic neck movements is more sensitive to neck pain compared with motion parameters measured at static and end range positions<sup>58, 59</sup>.

Interestingly, the interspinous ligament pain only affected cervical joint motion during extension movement without any changes found during flexion movement. First, the anterior and posterior cervical structures generated different resistances during cervical flexion and extension. These differences in resistance demand different motor control strategies to conduct cervical flexion and extension movements<sup>60</sup>. Second, the cervical joint motion depends on proper co-contraction between agonist and antagonist muscles<sup>52</sup>. Cheng et al. showed that the co-contraction patterns of cervical muscles during cervical flexion and extension were different, which indicated different motor demands<sup>61, 62</sup>. The previous study also indicated the cervical extensors were more activated than cervical flexor during both cervical flexion and extension<sup>61</sup>.

#### *4.4 Clinical implication*

The present results highlighted that pain induced in the cervical interspinous ligament altered the motor control strategy during the entire cervical range of motion. The findings challenge previous notions that the cervical interspinous ligament merely contributes to the restriction of cervical flexion at the end of the motion. The widely applied flexion-extension radiographs in the clinical practice provide less diagnostic value in recognizing the pain sources. The present findings provided evidence in support of the possibility of investigating cervical joint motion during dynamic neck movements to detect motor impairments related with interspinous ligament pain. Cervical ligaments were most likely to be one of pain sources in chronic whiplash neck pain.



patients<sup>3</sup>. The current results may help clinician to recognize ligament pain in the acute phase, design target treatments and prevent the pain becoming chronic.

#### 4.5 Limitation

In the current study, some limitations should be considered. First, the marking error is the largest error, however, the reproducibility of the marking procedure has been published with good reliability and low marking errors<sup>29</sup>. Second, the results are limited to a young and healthy population although degeneration of the neck is more severe in older subjects<sup>63</sup>. Therefore, further research needs to investigate the effects of degeneration on cervical joint motion during cervical flexion and extension in older adults. Third, the gender was not balanced in the study. Since the gender could be a potential factor affecting the cervical joint motion<sup>64</sup>, further studies should take gender balance into consideration when designing studies. Fourth, the cervical joint motion has not been examined when the pain disappeared in the study. It will be of interest to check whether the altered motion pattern can return to baseline when the induced pain is gone. Lastly, cervical joint motion is three-dimensional. The motion in sagittal plane is accompanied by motions in the frontal and transversal planes<sup>65</sup>. The further studies need to investigate the pain effects on motion in the frontal and transversal planes or investigate the pain effects on motion in the three planes simultaneously.

#### 5. Conclusion

Cervical interspinous ligament pain induced by hypertonic saline altered cervical joint motion during dynamic extension movement and altered pressure pain sensitivity in the neck. The interspinous ligament pain redistributed anti-directional motion between C4/C5 and C2/C3 during cervical extension. The present study highlighted the value of the dynamic characteristics of neck

movements and the possibility of investigating cervical joint motion during dynamic neck movements to detect impairments associated with neck pain. Nevertheless, even localized noxious provocations can affect joint function, also at a distance from the painful structure, illustrating that the widespread functional effects of neck pain in patients may be difficult to localize to the source of pain.

ACCEPTED MANUSCRIPT

**Reference**

1. Hartman RA, Tisherman RE, Wang C, et al. Mechanical role of the posterior column components in the cervical spine. *European Spine Journal*. 2016;25:2129-2138.
2. Song K, Lee K. The significance of soft tissue injury in lower cervical spine trauma. *Neurosurgery Quarterly*. 2008;18:28-33.
3. Siegmund GP, Winkelstein BA, Ivancic PC, Svensson MY, Vasavada A. The anatomy and biomechanics of acute and chronic whiplash injury. *Traffic injury prevention*. 2009;10:101-112.
4. Sjolander P, Michaelson P, Jaric S, Djupsjobacka M. Sensorimotor disturbances in chronic neck pain--range of motion, peak velocity, smoothness of movement, and repositioning acuity. *Man Ther*. 2008;13:122-131.
5. Paulus I, Brumagne S. Altered interpretation of neck proprioceptive signals in persons with subclinical recurrent neck pain. *J Rehabil Med*. 2008;40:426-432.
6. Meisingset I, Woodhouse A, Stensdotter A, et al. Evidence for a general stiffening motor control pattern in neck pain: A cross sectional study. *BMC musculoskeletal disorders*. 2015;16:56.
7. Jiang H, Russell G, Raso VJ, Moreau MJ, Hill DL, Bagnall KM. The nature and distribution of the innervation of human supraspinal and interspinal ligaments. *Spine (Phila Pa 1976)*. 1995;20:869-876.
8. Panjabi MM. The stabilizing system of the spine. part I. function, dysfunction, adaptation, and enhancement. *J Spinal Disord*. 1992;5:383-383.

9. Malmström E, Karlberg M, Fransson P, Lindbladh J, Magnusson M. Cervical proprioception is sufficient for head orientation after bilateral vestibular loss. *Eur J Appl Physiol.* 2009;107:73-81.
10. Nagai T, Clark NC, Abt JP, et al. The effect of target position on the accuracy of cervical-spine-rotation active joint-position sense. *J Sport Rehab.* 2016;25:58-63.
11. Oh JJ, Asha SE. Utility of flexion–extension radiography for the detection of ligamentous cervical spine injury and its current role in the clearance of the cervical spine. *Emergency Medicine Australasia.* 2016;28:216-223.
12. Oh JJ, Asha SE, Curtis K. Diagnostic accuracy of flexion-extension radiography for the detection of ligamentous cervical spine injury following a normal cervical spine computed tomography. *Emergency Medicine Australasia.* 2016;28:450-455.
13. Bifulco P, Cesarelli M, Romano M, Fratini A, Sansone M. Measurement of intervertebral cervical motion by means of dynamic x-ray image processing and data interpolation. *Journal of Biomedical Imaging.* 2013;2013:21.
14. Wang X, Lindstroem R, Plochanski M, Østergaard LR, Graven-Nielsen T. Cervical flexion and extension includes anti-directional cervical joint motion in healthy adults. *The Spine Journal.* 2017.
15. Wang X, Lindstroem R, Plochanski M, Østergaard LR, Graven-Nielsen T. Repeatability of cervical joint flexion and extension within and between days. *Journal of Manipulative & Physiological Therapeutics.* 2018;41:10-18.
16. Qu N, Lindstrøm R, Hirata RP, Graven-Nielsen T. Origin of neck pain and direction of movement influence dynamic cervical joint motion and pressure pain sensitivity. *Clin Biomech.* 2018.

17. Graven-Nielsen T, Svensson P, Arendt-Nielsen L. Effects of experimental muscle pain on muscle activity and co-ordination during static and dynamic motor function. *Electroencephalography and Clinical Neurophysiology/Electromyography and Motor Control*. 1997;105:156-164.
18. Graven-Nielsen T, Babenko V, Svensson P, Arendt-Nielsen L. Experimentally induced muscle pain induces hypoalgesia in heterotopic deep tissues, but not in homotopic deep tissues. *Brain Res*. 1998;787:203-210.
19. Christensen SW, Hirata RP, Graven-Nielsen T. The effect of experimental neck pain on pressure pain sensitivity and axioscapular motor control. *The Journal of Pain*. 2015;16:367-379.
20. Walton D, MacDermid J, Nielson W, Teasell R, Chiasson M, Brown L. Reliability, standard error, and minimum detectable change of clinical pressure pain threshold testing in people with and without acute neck pain. *journal of orthopaedic & sports physical therapy*. 2011;41:644-650.
21. Kinser AM, Sands WA, Stone MH. Reliability and validity of a pressure algometer. *The Journal of Strength & Conditioning Research*. 2009;23:312-314.
22. Staahl C, Drewes AM. Experimental human pain models: A review of standardised methods for preclinical testing of analgesics. *Basic & clinical pharmacology & toxicology*. 2004;95:97-111.
23. Ge H, Madeleine P, Wang K, Arendt-Nielsen L. Hypoalgesia to pressure pain in referred pain areas triggered by spatial summation of experimental muscle pain from unilateral or bilateral trapezius muscles. *European Journal of Pain*. 2003;7:531-537.
24. Izumi M, Petersen KK, Arendt-Nielsen L, Graven-Nielsen T. Pain referral and regional deep tissue hyperalgesia in experimental human hip pain models. *PAIN®*. 2014;155:792-800.

25. Palsson TS, Graven-Nielsen T. Experimental pelvic pain facilitates pain provocation tests and causes regional hyperalgesia. *PAIN®*. 2012;153:2233-2240.
26. Graven-Nielsen T. Fundamentals of muscle pain, referred pain, and deep tissue hyperalgesia. *Scand J Rheumatol*. 2006;35:1-43.
27. Tsao H, Tucker KJ, Coppieters MW, Hodges PW. Experimentally induced low back pain from hypertonic saline injections into lumbar interspinous ligament and erector spinae muscle. *Pain*. 2010;150:167-172.
28. Wang X, Lindstroem R, Carstens NPB, Graven-Nielsen T. Cervical spine reposition errors after cervical flexion and extension. *BMC musculoskeletal disorders*. 2017;18:102.
29. Plochanski M, Lindstroem R, Lindstroem CF, Østergaard LR. Motion analysis of the cervical spine during extension and flexion: Reliability of the vertebral marking procedure. *Med Eng Phys*. 2018.
30. Frobin W, Leivseth G, Biggemann M, Brinckmann P. Sagittal plane segmental motion of the cervical spine. A new precision measurement protocol and normal motion data of healthy adults. *Clin Biomech*. 2002;17:21-31.
31. Graven-Nielsen T, Arendt-Nielsen L, Svensson P, Jensen TS. Experimental muscle pain: A quantitative study of local and referred pain in humans following injection of hypertonic saline. *J Musculoskeletal Pain*. 1997;5:49-69.
32. Keating L, Lubke C, Powell V, Young T, Souvlis T, Jull G. Mid-thoracic tenderness: A comparison of pressure pain threshold between spinal regions, in asymptomatic subjects. *Man Ther*. 2001;6:34-39.

33. Paintal A. Functional analysis of group III afferent fibres of mammalian muscles. *J Physiol (Lond)*. 1960;152:250-270.
34. Clark CR, Benzel EC. *The Cervical Spine*. Lippincott Williams & Wilkins, 2005.
35. Yoganandan N, Kumaresan S, Pintar FA. Geometric and mechanical properties of human cervical spine ligaments. *J Biomech Eng*. 2000;122:623-629.
36. Yarnitsky D. Conditioned pain modulation (the diffuse noxious inhibitory control-like effect): Its relevance for acute and chronic pain states. *Curr Opin Anaesthesiol*. 2010;23:611-615.
37. Gibson W, Arendt-Nielsen L, Graven-Nielsen T. Referred pain and hyperalgesia in human tendon and muscle belly tissue. *Pain*. 2006;120:113-123.
38. Slaterl H, Gibsonl W, Graven-Nielsenl T. Sensory responses to mechanically and chemically induced tendon pain in healthy subjects. *European Journal of Pain*. 2011;15:146-152.
39. Ge H, Madeleine P, Wang K, Arendt-Nielsen L. Hypoalgesia to pressure pain in referred pain areas triggered by spatial summation of experimental muscle pain from unilateral or bilateral trapezius muscles. *European Journal of Pain*. 2003;7:531-537.
40. Schomacher J, Boudreau SA, Petzke F, Falla D. Localized pressure pain sensitivity is associated with lower activation of the semispinalis cervicis muscle in patients with chronic neck pain. *Clin J Pain*. 2013;29:898-906.
41. Bahat HS, Weiss PL, Laufer Y. The effect of neck pain on cervical kinematics, as assessed in a virtual environment. *Arch Phys Med Rehabil*. 2010;91:1884-1890.

42. Elsig S, Luomajoki H, Sattelmayer M, Taeymans J, Tal-Akabi A, Hilfiker R. Sensorimotor tests, such as movement control and laterality judgment accuracy, in persons with recurrent neck pain and controls. A case-control study. *Man Ther.* 2014;19:555-561.
43. Woodhouse A, Stavadahl Ø, Vasseljen O. Irregular head movement patterns in whiplash patients during a trajectory task. *Experimental brain research.* 2010;201:261-270.
44. Tsang SM, Szeto GP, Lee RY. Movement coordination and differential kinematics of the cervical and thoracic spines in people with chronic neck pain. *Clin Biomech (Bristol, Avon).* 2013;28:610-617.
45. Woodhouse A, Vasseljen O. Altered motor control patterns in whiplash and chronic neck pain. *BMC musculoskeletal disorders.* 2008;9:90.
46. Hodges PW, Tucker K. Moving differently in pain: A new theory to explain the adaptation to pain. *Pain.* 2011;152:S90-8.
47. Johnston V, Jull G, Souvlis T, Jimmieson NL. Neck movement and muscle activity characteristics in female office workers with neck pain. *Spine (Phila Pa 1976).* 2008;33:555-563.
48. Falla DL, Jull GA, Hodges PW. Patients with neck pain demonstrate reduced electromyographic activity of the deep cervical flexor muscles during performance of the craniocervical flexion test. *Spine.* 2004;29:2108-2114.
49. Lindstrøm R, Schomacher J, Farina D, Rechter L, Falla D. Association between neck muscle coactivation, pain, and strength in women with neck pain. *Man Ther.* 2011;16:80-86.



50. O'Leary S, Cagnie B, Reeve A, Jull G, Elliott JM. Is there altered activity of the extensor muscles in chronic mechanical neck pain? A functional magnetic resonance imaging study. *Arch Phys Med Rehabil.* 2011;92:929-934.
51. Blouin JS, Siegmund GP, Carpenter MG, Inglis JT. Neural control of superficial and deep neck muscles in humans. *J Neurophysiol.* 2007;98:920-928.
52. Falla D, Farina D, Dahl MK, Graven-Nielsen T. Muscle pain induces task-dependent changes in cervical agonist/antagonist activity. *J Appl Physiol (1985).* 2007;102:601-609.
53. Hendershot B, Bazrgari B, Muslim K, Toosizadeh N, Nussbaum MA, Madigan ML. Disturbance and recovery of trunk stiffness and reflexive muscle responses following prolonged trunk flexion: Influences of flexion angle and duration. *Clin Biomech.* 2011;26:250-256.
54. Dyhre-Poulsen P, Krogsgaard MR. Muscular reflexes elicited by electrical stimulation of the anterior cruciate ligament in humans. *J Appl Physiol.* 2000;89:2191-2195.
55. Solomonow M, Zhou B, Harris M, Lu Y, Baratta RV. The ligamento-muscular stabilizing system of the spine. *Spine.* 1998;23:2552-2562.
56. Schwab JS, Diangelo DJ, Foley KT. Motion compensation associated with single-level cervical fusion: Where does the lost motion go? *Spine (Phila Pa 1976).* 2006;31:2439-2448.
57. Lan HH, Chen H, Kuo L, You J, Li W, Wu S. The shift of segmental contribution ratio in patients with herniated disc during cervical lateral bending. *BMC musculoskeletal disorders.* 2014;15:273.

58. Anderst WJ, Donaldson WF, Lee JY, Kang JD. Continuous cervical spine kinematics during in vivo dynamic flexion-extension. *The Spine Journal*. 2014;14:1221-1227.
59. Wu SK, Kuo LC, Lan HC, Tsai SW, Su FC. Segmental percentage contributions of cervical spine during different motion ranges of flexion and extension. *J Spinal Disord Tech*. 2010;23:278-284.
60. Hsu WH, Chen YL, Lui TN, et al. Comparison of the kinematic features between the in vivo active and passive flexion-extension of the subaxial cervical spine and their biomechanical implications. *Spine (Phila Pa 1976)*. 2011;36:630-638.
61. Cheng C, Lin K, Wang J. Co-contraction of cervical muscles during sagittal and coronal neck motions at different movement speeds. *Eur J Appl Physiol*. 2008;103:647.
62. Cheng C, Cheng HK, Chen CP, et al. Altered co-contraction of cervical muscles in young adults with chronic neck pain during voluntary neck motions. *Journal of physical therapy science*. 2014;26:587-590.
63. Miyazaki M, Woo HS, Yoon SH, et al. Kinematic analysis of the relationship between the grade of disc degeneration and motion unit of the cervical spine. 2007:248-249.
64. Stemper BD, Yoganandan N, Pintar FA. Gender dependent cervical spine segmental kinematics during whiplash. *J Biomech*. 2003;36:1281-1289.
65. Anderst WJ, Donaldson III WF, Lee JY, Kang JD. Three-dimensional intervertebral kinematics in the healthy young adult cervical spine during dynamic functional loading. *J Biomech*. 2015;48:1286-1293.

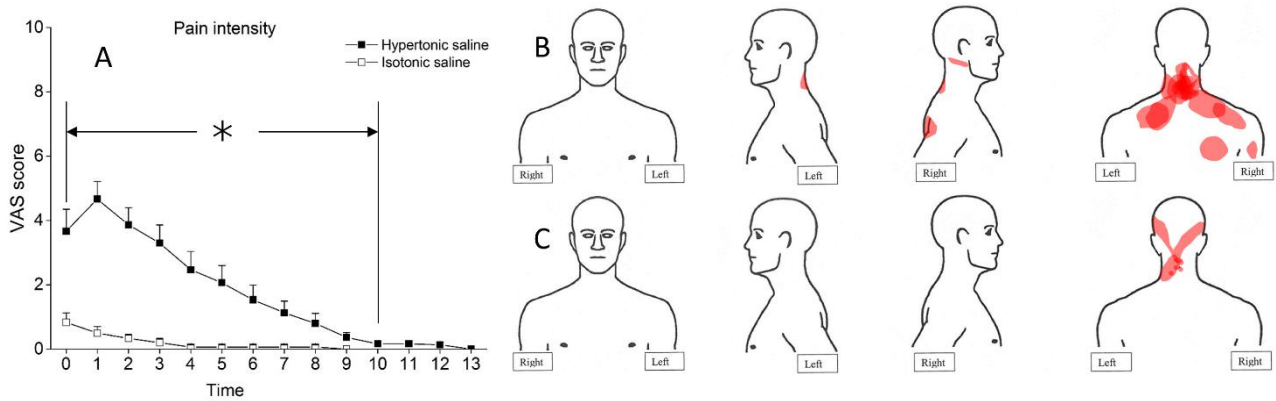


Fig.1. Pain distribution followed injection of hypertonic saline (**A**) and isotonic saline (**B**) in C4/C5 interspinous ligament. Low transparency in color indicates the area is more frequently marked by the subjects. **C**: Visual analogue scale (VAS) score (mean $\pm$  SE) against time followed the injection of hypertonic saline and isotonic saline. Significant differences in pain intensity between hypertonic and isotonic saline injections: \*  $P < 0.05$ .

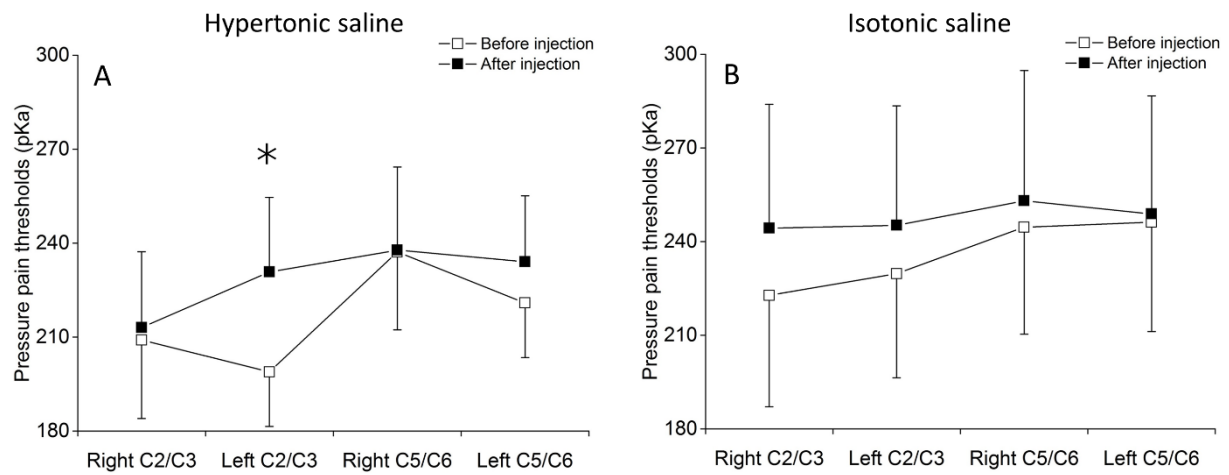


Fig.2. Mean and SE of pressure pain thresholds over bilateral C2/C3 and C5/C6 facet joints before and after hypertonic (A) and isotonic (B) saline injection. Significant differences after injection compared with before injection: \*  $P < 0.05$ .

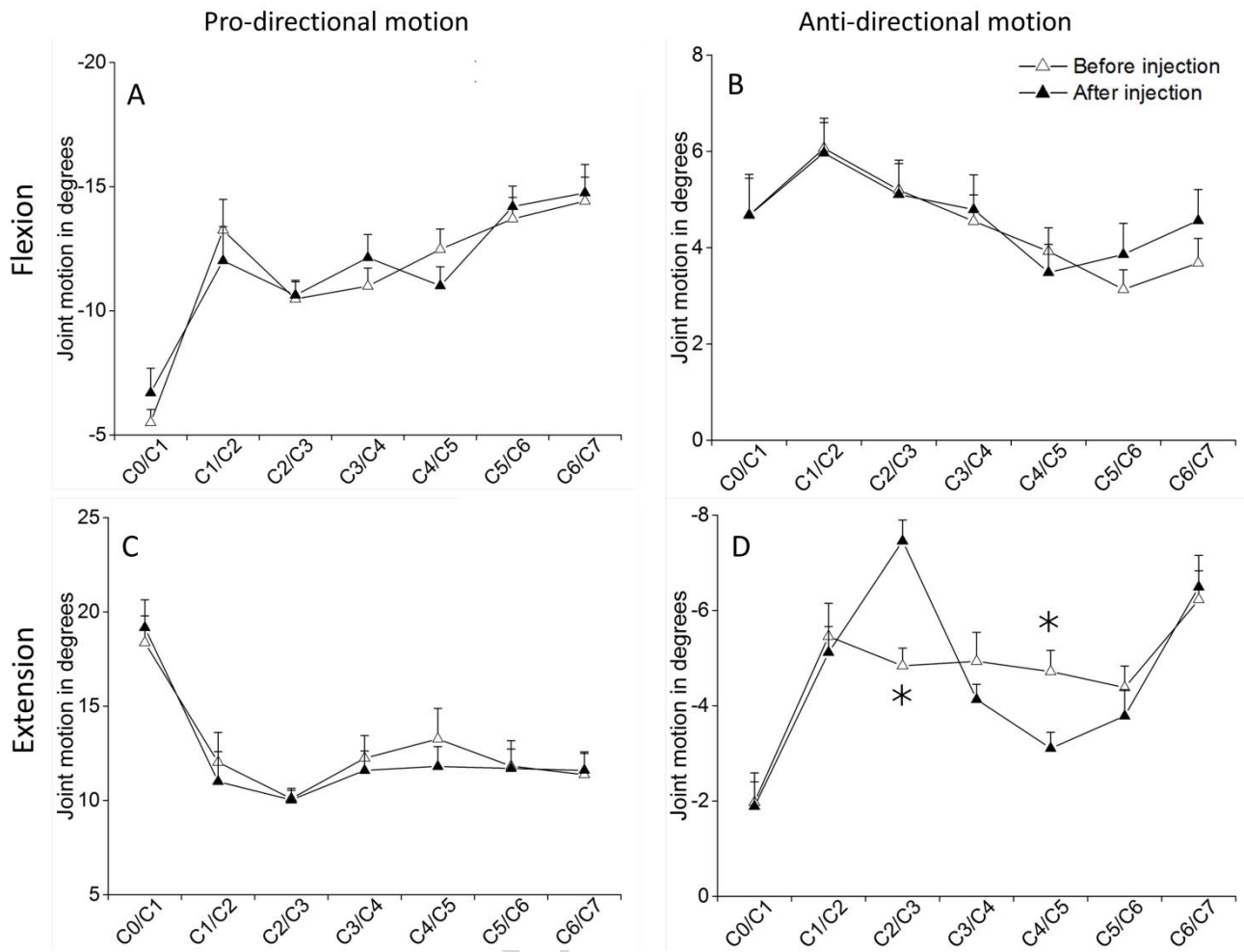


Fig.3. Mean and SE of pro-directional motion and anti-directional motion of cervical flexion and extension before and after hypertonic saline injection. **A:** Pro-directional motion during cervical flexion; **B:** Anti-directional motion during cervical flexion; **C:** Pro-directional motion during cervical extension; **D:** Anti-directional motion during cervical extension. Significant differences after injection compared with before injection: \*  $P < 0.05$ .

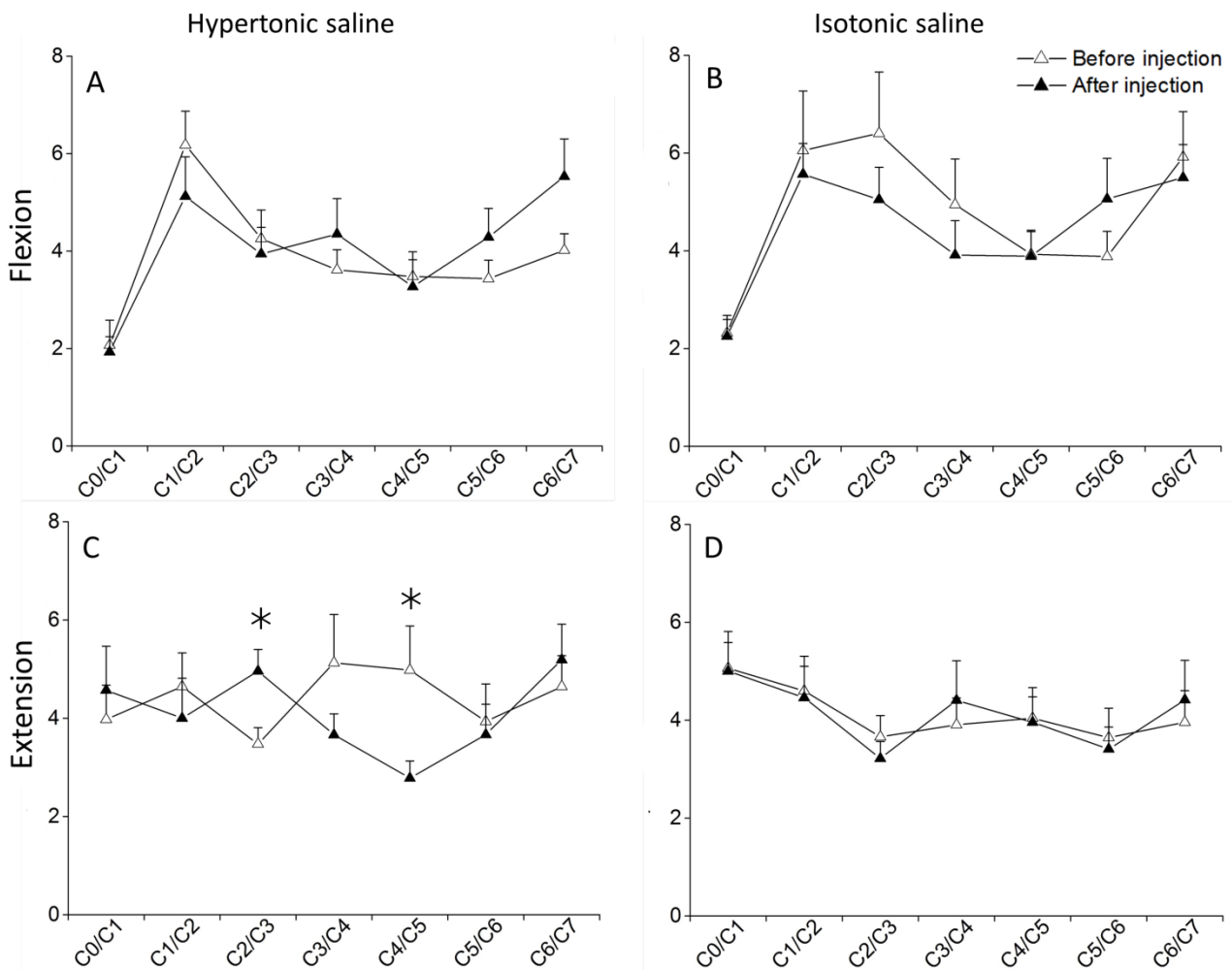


Fig.4. Mean and SE of joint motion variability of cervical flexion and extension before and after hypertonic and isotonic saline injection. **A:** Flexion before and after hypertonic saline injection; **B:** Flexion before and after isotonic saline injection; **C:** Extension before and after hypertonic saline injection; **D:** Extension before and after isotonic saline injection. Significant differences after injection compared with before injection: \*  $P < 0.05$ .

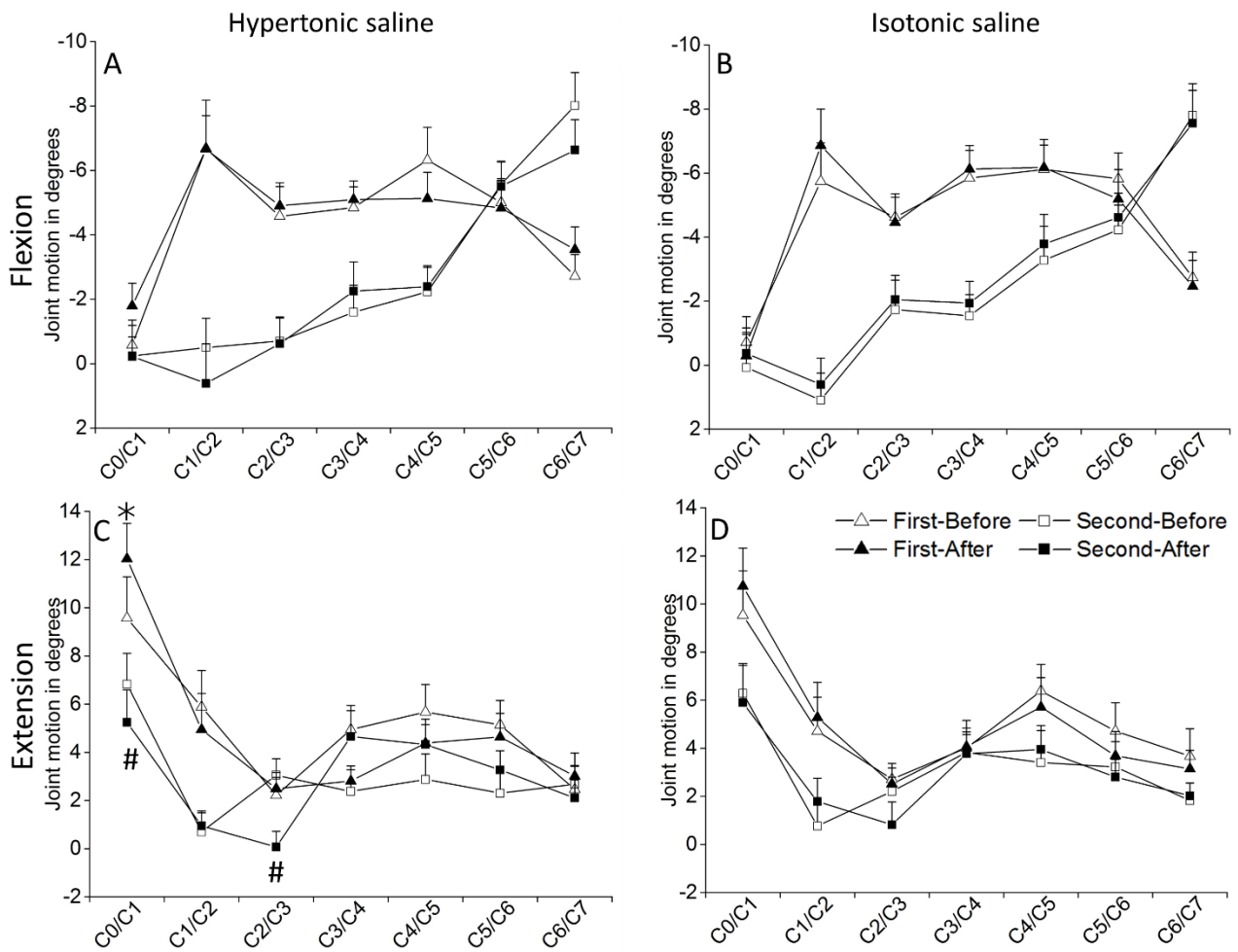


Fig.5. Total joint motion during half ranges (first half, second half) of cervical flexion and extension before and after hypertonic and isotonic saline injection. **A:** Flexion before and after hypertonic saline injection; **B:** Flexion before and after isotonic saline injection; **C:** Extension before and after hypertonic saline injection; **D:** Extension before and after isotonic saline injection. Significant differences during first half range (\*  $P < 0.05$ ) and during second half range (#  $P < 0.05$ ) are illustrated.

**Highlights:**

- Interspinous ligament pain altered cervical joint motion pattern during extension
- Anti-directional motion was redistributed from C4/C5 to C2/C3 during extension
- Interspinous ligament pain increased pressure pain threshold over left C2/C3 facet joint

ACCEPTED MANUSCRIPT