

INTRODUCTION

1
2 Neck pain is a prevalent musculoskeletal condition, with annual and lifetime
3 prevalence rates reaching 37% and nearly 50%, respectively [1–4]. Its multifactorial
4 etiology includes physical, emotional, and psychological factors [5–7]. Persistent or
5 recurrent symptoms are common [8], and full recovery is achieved in only about one-
6 third of cases [9,10].

7 Among physical contributors, motor impairments are frequently reported in individuals
8 with neck pain [11]. These include increased co-contraction [12], decreased
9 directional activation [13], reduced deep muscle activity [13], delayed muscle
10 activation [14] or relaxation [15], and limited recruitment adaptability [16]. In addition
11 to coordination deficits, decreased strength [17], endurance [18] or force steadiness
12 [19] have also been reported. Although these impairments vary across individuals
13 [20–22], their identification is considered key to tailoring rehabilitation strategies
14 [23,24].

15 Clinical assessment of cervical extensor endurance is commonly performed using
16 tests that require maintaining the head against gravity while attempting to keep it
17 stable [25], with performance typically quantified as the time the position can be
18 maintained. Nevertheless, no standardized protocol currently exists, and multiple test
19 variants have been reported, differing in execution (e.g., different cranio-cervical
20 postures [18,26], use of additional load [27], or dynamometer-based setups [28]) and
21 termination criteria (e.g., fixed time limits [29], pain onset [18], perceived fatigue [30],
22 or loss of postural control [31]). Consequently, test performance represents a
23 composite outcome influenced by several factors beyond muscular endurance
24 capacity itself.

25 In addition to test-related factors, endurance performance may also be influenced by
26 individual characteristics, including anthropometric parameters (e.g., neck length or
27 head circumference [29]) and contextual factors [32,33] such as motivation, pain
28 intensity, or pain-related apprehension. Variability across these factors likely
29 contributes to the heterogeneity observed in cervical extensor endurance
30 performance across studies between individuals with and without neck pain [26,34].

31 Cranio-cervical position during the test is frequently monitored across protocols and
32 used as a criterion for test termination [26,27,29,34–40]. Sebastian et al. [41] suggest
33 that progressive cranio-cervical extension during the test might reflect a relative
34 weakness or altered function of the deep cervical extensors. This hypothesis was
35 informed by experimental studies using muscle functional magnetic resonance
36 imaging (mfMRI), which reported task-related changes in cervical extensor muscle
37 behavior influenced by pain [42] or cranio-cervical posture [43] during submaximal
38 isometric extension tasks. However, these findings have not been consistently
39 observed across studies, as some investigations using different imaging approaches
40 have reported no significant between-group differences in the mechanical behavior of
41 deep cervical extensors under similar submaximal conditions [44].

42 To date, the hypothesis that differential involvement of deep and superficial cervical
43 extensors underlies performance during clinical endurance testing has not been
44 directly verified. Although individuals with neck pain have been shown to present
45 reduced cross-sectional area of deep cervical extensor muscles, these morphological
46 differences were not associated with the final cranio-cervical position reached during
47 the endurance test, and only moderate associations have been reported with overall
48 endurance time [37,39]. Moreover, available evidence regarding activation of

49 superficial cervical extensors during endurance testing is limited. The only
50 electromyographic study conducted under gravity endurance conditions reported no
51 between-group differences in muscle fatigability [27].

52 To our knowledge, the specific muscle contributions during cervical endurance tests
53 remain unclear. mfMRI offers a non-invasive and reliable approach to assess
54 exercise-induced changes in muscle activity based on T2 relaxation time shifts [45],
55 allowing simultaneous investigation of deep and superficial cervical muscles [46–48].
56 This study aimed to address this gap by using mfMRI to compare muscle recruitment
57 in individuals with and without neck pain in response to a standardized cervical
58 extensor endurance test. Based on prior experimental work, we hypothesized that
59 cervical muscle activation during the endurance task might differ between individuals
60 with and without neck pain, potentially involving differential contributions of superficial
61 and deep extensor muscles.

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MATERIALS AND METHODS

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Study design

This cross-sectional experimental study was conducted at the “X”, with blinding of the investigator to group allocation. The protocol was approved by the Health Research Ethics Committee of the “X” (B707221000028) and registered at ClinicalTrials.gov (NCT06595927).

Participants

Two groups of participants were recruited between November 2024 and January 2025 on a voluntary basis through advertisements. Following recruitment, participants were screened based on eligibility criteria. All participants provided informed written consent.

Participants in the “neck pain group” were aged between 18 and 65 years and presented with persistent or recurrent chronic idiopathic or traumatic neck pain, a Neck Disability Index (NDI) score $\geq 8/50$, and an average daily Numeric Pain Rating Scale (NPRS) score over the past few days $< 7/10$. Exclusion criteria included fibromyalgia, cervical radiculopathy, cervical spine surgery, or contraindications to Magnetic Resonance Imaging (MRI).

Participants in the “healthy control group” were recruited to ensure a comparable distribution of age (± 5 years) and sex, with NDI score $\leq 3/50$, no low back pain in the past 7 days, no cervical treatment in the previous 12 months, and no MRI contraindication.

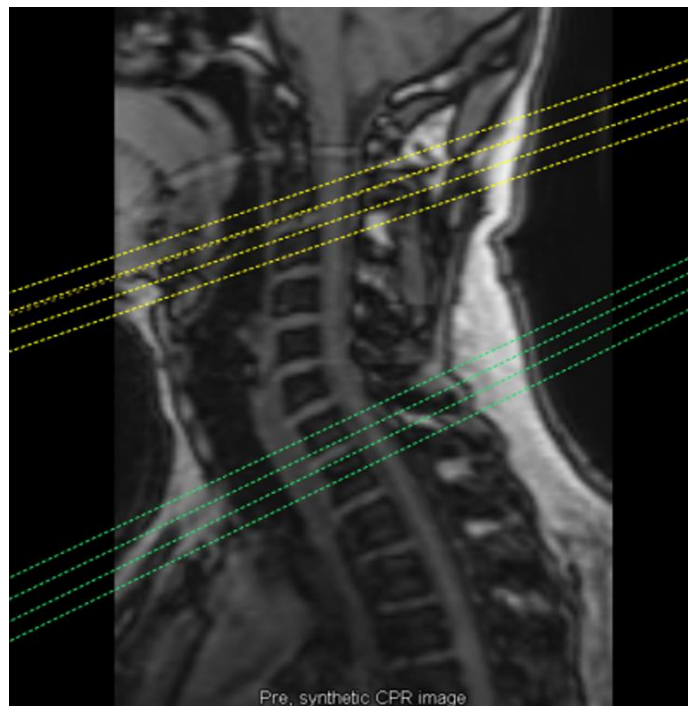
94 Experimental procedure

95 All participants underwent a baseline cervical MRI scan, a standardized 3-minute
96 cervical extensor endurance test, and a post-test scan within 2 minutes. The study
97 was completed in February 2025.

98 mfMRI

99 MRI was performed on a 3-Tesla magnet (Magnetom VIDA, syngo MR XA20,
100 Siemens AG, Erlangen, Germany). Participants lay supine with their head placed on
101 a Biomatrix Head/Neck (20 channels coil) combined with a spine (32 channels coil) in
102 a neutral position. Imaging procedures were identical for both the first and second
103 MRI scans.

104 A coronal *Auto Align Spine Scout* was performed, allowing the automatic detection of
105 intervertebral discs and ensuring
106 the consistency of slice prescription
107 across scans. Axial images parallel
108 to the intervertebral discs, with a
109 slice thickness of 5 mm, were
110 acquired at two cervical levels
111 (C2/C3 and C7/T1). At each level,
112 four contiguous axial slices were
113 obtained using a disc-centered
114 acquisition. See Figure 1.



115 *Figure 1: MRI slices obtained at C2/C3 (yellow) and C7/T1 (green) levels.*

116 A spin echo sequence was used to calculate T2 values: repetition time of 2500
117 milliseconds, echo times 10 to 161.6 milliseconds (in 10.1 millisecond increments
118 across 16 echoes), field of view of 256 x 256 mm, matrix 128 x 128, voxel size of 2 x
119 2 x 5 mm, 4 slices with no gap, and bandwidth of 215 Hz/Px. The Acquisition Time
120 was 4:34 per sequence, resulting in 11 minutes of total scan time

121 Cervical extensor endurance test

122 Participants performed the endurance test while maintaining a standardized cranio-
123 cervical flexion position throughout the procedure, as described below. Participants
124 lay prone on a two-section massage table, with the C7/T1 junction aligned with the
125 gap between sections. The head was initially supported, and a rigid strap secured the
126 thorax at T4 level to limit upper thoracic extension. An inertial sensor (part of an
127 Inertial Measurement Unit, consisting of a 3-axis accelerometer, gyroscope and
128 magnetometer) was placed above the external occipital protuberance and held by a
129 frontal strap to monitor head and neck position. A horizontal height gauge was
130 positioned in front of the head, aligned with the apex of thoracic kyphosis. A
131 reference block, matching the sensor's thickness, was placed at the thoracic apex to
132 ensure alignment with the occiput. See Figure 2 for the positioning of the participants
133 and materials.



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135 *Figure 2: Participant and material setup.*

136 Familiarization: the participant's head was passively placed so that the occiput and
137 thoracic apex contacted the horizontal gauge, with full cranio-cervical flexion. The
138 participant actively held this position for 5 seconds. This was repeated once after a
139 30-second rest. After a second rest and a reminder of the instructions, the test
140 began.

141 A 10-second verbal countdown was given to the participant to indicate when the
142 endurance test would officially begin and when they should independently hold the
143 pre-established position, previously maintained by the experimenter. Once the
144 experimenter released the participant's head, the head section of the table was fully
145 lowered for the entire test duration.

146 After a 10-second countdown, the examiner released the head and lowered the head
147 section of the table. The participant was asked to maintain the test position for 3
148 minutes. Deviations >5 mm or $>5^\circ$ triggered an audible alert, and the examiner
149 provided verbal guidance to return to the target position. The test was terminated if
150 the participant voluntarily stopped or completed the 3-minute period.

151 No motivational encouragement was given during the task, aside from corrections to
152 help maintain the position.

153 The reproducibility of the specific cervical extensor endurance test protocol used in
154 the present study has not been previously established. However, prior studies using
155 related endurance test variants have reported acceptable intra-rater [26,35], inter-
156 rater [35,40,41], and test–retest reliability [36,37], albeit with substantial
157 measurement error and considerable variability depending on testing conditions and
158 population studied.

159 Outcome measurement

160 After data acquisition, T2 maps were generated using the MapIt inline function.
161 Generated series were transferred to Syngovia (VB60 version) for manual
162 processing. Regions of interest were drawn on the axial slices for nine pairs of
163 cervical muscles at two cervical levels (see Figure 3; Figure 4.a). At C2/C3 level, the
164 following muscles were investigated: multifidus, semispinalis cervicis, obliquus capitis
165 inferior, semispinalis capitis, splenius capitis, spinalis capitis, upper trapezius,
166 sternocleidomastoid, longus colli, and longus capitis muscles (See Appendices A and
167 B). At C7/T1 level, regions of interest were drawn for the levator scapulae, multifidus,
168 semispinalis cervicis, upper trapezius, and anterior scalene muscles (See
169 Appendices C and D).

170 Considering the boundaries of some adjacent muscles could not be consistently
171 distinguished on axial imaging, the following groupings were applied : (1) longus colli
172 and longus capitis muscles were combined as one ROI at the C2/C3 level; (2)
173 multifidus and semispinalis cervicis muscles were grouped as one ROI at both levels;

174 (3) semispinalis capitis, splenius capitis, spinalis capitis, and upper trapezius muscles
175 were grouped as one ROI at C2/C3 level, corresponding to the “superficial extensors”
176 group. In contrast, at the C7/T1 level, the upper trapezius exhibited distinct
177 anatomical borders and could therefore be segmented and analyzed as an individual
178 muscle. ROIs for each muscle or muscle groups are shown in the Appendices A-D.



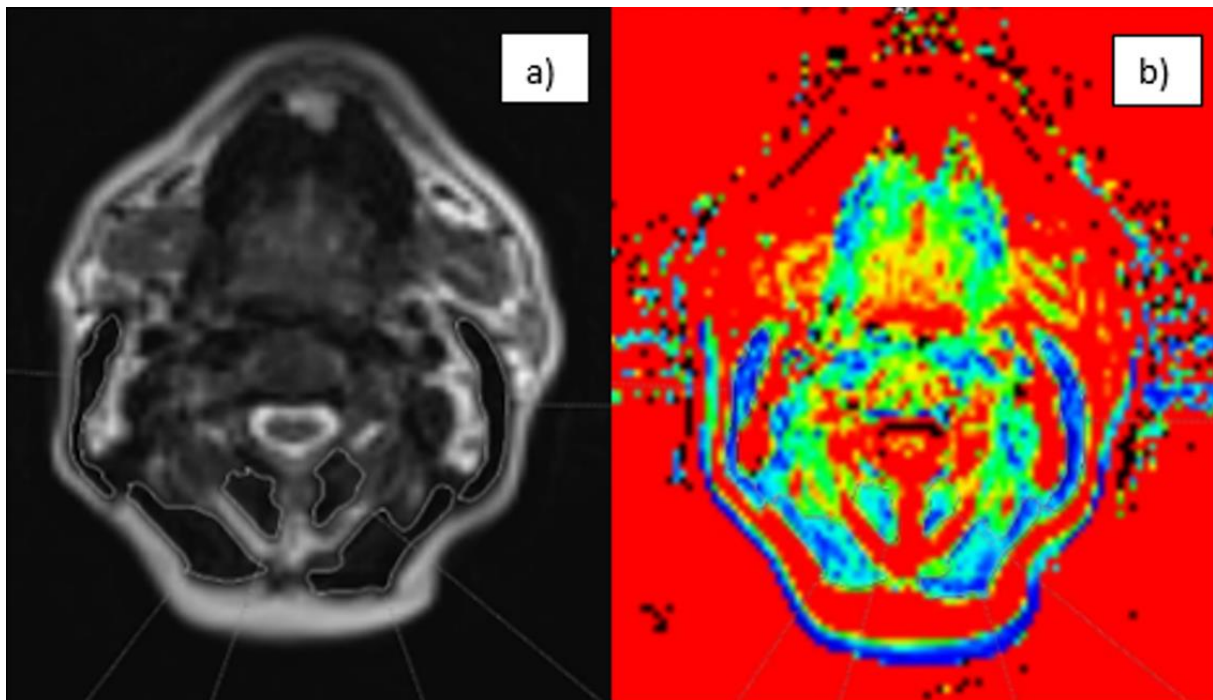
179

180 *Figure 3: Cervical muscles assessed at C2/3 and C7/T1 levels.*

181 When drawing each ROI, care was taken to exclude non-muscular tissues such as
182 fat, fascia, and vascular structures, which present markedly higher T2 values and
183 distinct signal characteristics on T2 maps. Muscle borders were identified using axial
184 MRI anatomical references (MRI atlases and published cervical muscle maps [49]).

185 Sixteen echoes were used for T2 calculations, applying a simplex algorithm to fit the
186 values from a specific slice in a T2 image (see Figure 4.b) volume to the exponential
187 $S_n = S_0 \exp(-TE_n/T_2)$ ($n=1:16$). T2 values (in milliseconds) and their standard
188 deviations were extracted for each ROI, separately for the left and right sides at
189 baseline and post-test. For statistical analyses, left and right values were averaged
190 per muscle and time point. This averaging strategy was selected because the

191 endurance task required symmetric bilateral activation, and no systematic side-to-
192 side asymmetry was expected in the absence of unilateral pain and because side-to-
193 side differences were not a primary outcome. Group-level means and standard
194 deviations were then computed. T2 shifts were calculated as the difference between
195 post-test and baseline values.



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197 *Figure 4: Example of MRI images at C2/C3 level*

198 Moreover, the level of perceived pain was assessed immediately before and after the
199 cervical extensor endurance test using the 0-10 Numeric Pain Rating Scale.

200 Perceived effort during the test was also evaluated immediately afterward using the
201 Modified Borg scale (Borg CR10 scale) [40], in order to capture the participants'
202 subjective experience of task intensity and exertion.

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205 Statistical analysis

206 Statistical analyses were conducted using R (Version 4.3.0, R Foundation for
207 Statistical Computing). The distribution of quantitative variables was assessed using
208 histograms, quantile-quantile plots, and Shapiro-Wilk tests. Descriptive statistics were
209 presented as mean \pm standard deviation for normally distributed variables, or as
210 medians [interquartile range] otherwise.

211 Between-group comparisons of baseline demographic and clinical characteristics
212 (i.e., age, sex, Neck Disability Index scores), as well as between-group comparisons
213 of perceived effort (Borg scale) and pain intensity (before and after the endurance
214 test), were performed using independent samples *t*-tests for normally distributed
215 variables, or Mann-Whitney *U* tests when normality assumptions were not satisfied.
216 The significance threshold for these comparisons was set at $p < 0.05$.

217 To assess the effects of time (baseline vs post-test), group (NP vs HC), and their
218 interaction on T2 values for each muscle, linear mixed-effects models were fitted,
219 including random intercepts for participant ID to account for repeated measurements
220 within subjects. Time, group, and their interaction were specified as fixed effects, and
221 an unstructured covariance matrix was used to model the within-subject correlation
222 between repeated measurements. Model residuals were assessed graphically and
223 tested for normality. When normality assumptions were not met, T2 values were log-
224 transformed prior to model fitting using a natural logarithm (\ln), and model residuals
225 were subsequently re-evaluated to confirm the validity of parametric assumptions. To
226 account for multiple comparisons across the nine muscles analyzed, a Bonferroni-
227 corrected significance level was applied (adjusted $\alpha = 0.0056$), which was the only
228 threshold used to determine statistical significance for the linear mixed-effects

229 models. P-values were obtained from the models and interpreted using this adjusted
230 significance threshold. Age and sex were not included as covariates in the primary
231 models, as groups were comparable at baseline and the inclusion of additional
232 covariates was considered likely to over parameterize the models given the sample
233 size and the exploratory nature of the analyses.

234 Intra-group pre-post changes were quantified descriptively using Cohen's *d* effect
235 sizes, calculated from group-level means and standard deviations of T2 shifts for
236 each muscle. No formal hypothesis testing was conducted for intra-group
237 comparisons to limit multiple testing.

238 An a priori sample size calculation was not performed due to the exploratory nature
239 of this cross-sectional study, the absence of previous data using a comparable
240 cervical extensor endurance protocol, and the assessment of multiple muscle-specific
241 outcomes across two cervical levels, which precluded the definition of a single
242 primary outcome. Effect sizes were therefore reported alongside statistical results to
243 support interpretation.

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RESULTS

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Participants

Thirty participants were recruited, but one was excluded due to recent low back pain, leaving twenty-nine participants who completed the study (22 females, 7 males), comprising 13 healthy controls and 16 individuals with neck pain. Demographic and clinical characteristics are presented in Table 1.

mfMRI results

All participants completed the endurance test and successfully maintained the test position for 3 minutes. T2 values for the anterior scalene in one participant and for the multifidus/semispinalis cervicis at C2/C3 in another participant were excluded due to, respectively, incorrect slice acquisition and insufficient image quality.

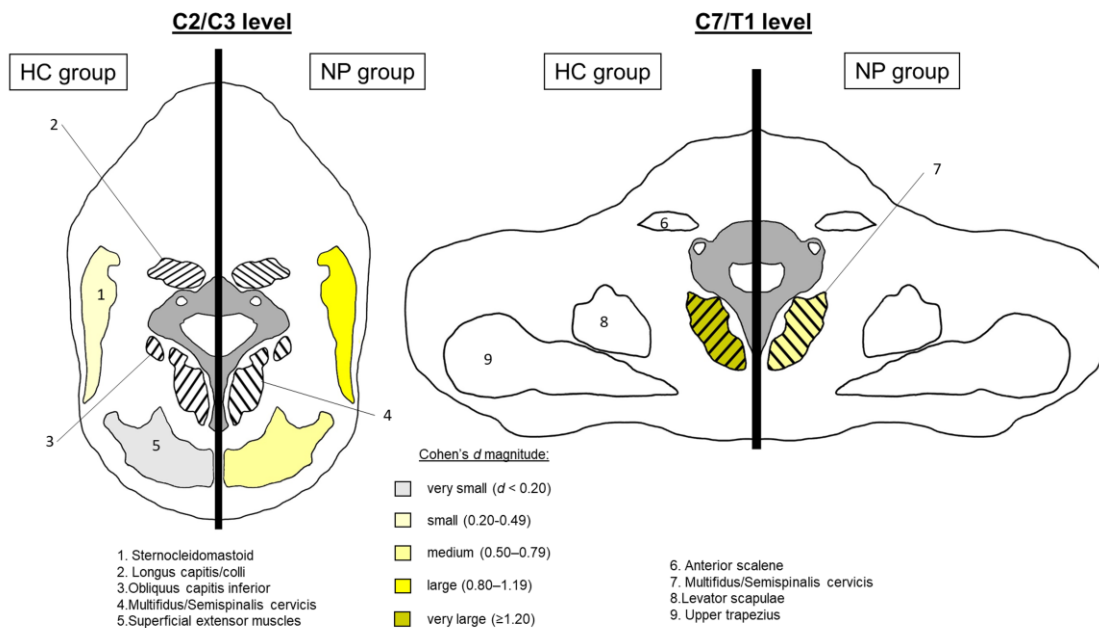
T2 values, linear mixed-effects model results and intra-group effect sizes for all muscles are presented in Table B. No significant group differences were observed at baseline across cervical levels, indicating comparable resting T2 values between healthy controls and participants with neck pain prior to the endurance test.

For the superficial extensor muscles at C2/C3 level, no significant main effect of group was observed. A time x group interaction ($p = 0.023$) was observed, although this effect did not remain significant after Bonferroni correction. A main effect of time ($p = 0.019$) was also observed, but this effect did not remain significant after correction for multiple comparisons. T2 shifts were numerically larger in the neck pain group (Cohen's $d = 0.73$) than in controls, whose values remained relatively stable.

272 For the sternocleidomastoid muscle, no significant group effect or time x group
 273 interaction was found. A highly significant main effect of time was observed ($p =$
 274 0.0005) across groups. Mean T2 shifts were larger in the neck pain group than in
 275 controls, with within-group effect sizes of Cohen's $d = 0.93$ and 0.49 , respectively.

276 For the multifidus/semispinalis cervicis at C7/T1, no significant group effect or time x
 277 group interaction was detected. A highly significant main effect of time was observed
 278 ($p = 0.0004$) with increased T2 values in both groups. The absolute T2 shift was
 279 larger in the neck pain group than in controls, whereas the within-group effect size
 280 was larger in controls (Cohen's $d = 1.23$) than in the neck pain group (Cohen's $d =$
 281 0.72).

282 No other significant effects were observed for remaining muscles. Between-group
 283 comparisons of T2 shifts and within-group changes are illustrated in Figure 5 and
 284 further detailed in Appendix E.



286 **Figure 5:** T2 shifts in cervical muscles: comparison between groups

287 Perceived effort and pain intensity

288 Perceived effort was significantly higher ($p = 0.0032$) in the neck pain group (median
289 Borg score = 6 [5–6.25]) than in the control group (median score = 3 [2-5]). Post-test
290 pain intensity was also significantly higher ($p = 0.003$) in the neck pain group (3 [2.75-
291 4]) compared to the healthy control group (0 [0-3]).

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DISCUSSION

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This study investigated cervical muscle recruitment in response to a clinical endurance task using mfMRI. All participants completed the 3-minute endurance test. Results showed task-related muscle recruitment in both groups, with differences in the magnitude and variability of responses between individuals with and without chronic neck pain. While both groups activated lower deep cervical extensors and sternocleidomastoid over time, individuals with neck pain showed patterns suggestive of group-dependent changes for the superficial extensor muscles at C2/C3 level. However, these effects did not survive Bonferroni correction and should therefore be interpreted with caution. Individuals with neck pain also reported significantly higher perceived effort and post-test pain.

Although widely used, the cervical extensor endurance test remains incompletely characterized in terms of underlying muscle behavior. To our knowledge, this is the first mfMRI study assessing recruitment in response to a continuous 3-minute clinical task. Previous research has primarily relied on electromyographic parameters of fatigue rather than recruitment [33,34]. Only one prior study, by O’Leary et al. [43], explored cervical extensor activation in response to a 60-second isometric contraction at 20% MVC. While their protocol differed from ours, some comparisons are informative.

At the C2/C3 level, O’Leary et al. reported no significant between-group difference in deep extensor activity, regardless of cranio-cervical position, which aligns with our findings. For superficial extensor muscles, they observed a trend toward increased splenius capitis activity in the neck pain group under neutral cranio-cervical position. Similarly, we observed moderate T2 increases of superficial extensor muscles in both

329 groups, with smaller responses observed in controls; however, this pattern did not
330 survive Bonferroni correction. In addition, sternocleidomastoid activation increased
331 over time in both groups, reflecting a task-related response, although larger within-
332 group effect sizes were observed in individuals with neck pain. Together, these
333 findings may indicate differences in the magnitude and coordination of superficial
334 muscle involvement during the task, rather than a systematic impairment of deep
335 muscle recruitment. Such patterns could reflect adaptive or compensatory motor
336 strategies characterized by altered or more variable involvement of superficial flexor
337 and extensor muscles; however, this differs from compensatory mechanisms
338 previously associated with deep muscle dysfunction [20], as deep extensor activation
339 at this level remained comparable between groups.

340 Several mechanisms may underlie these findings. First, the magnitude or variability
341 of sternocleidomastoid and superficial extensor involvement observed in individuals
342 with neck pain may reflect altered flexor-extensor coordination. This may be
343 influenced by the requirement to maintain sustained cranio-cervical flexion during the
344 task, and is consistent with previously described mechanisms of increased antagonist
345 involvement and co-activation between these muscle groups [12,50]. Second,
346 individuals with neck pain may overestimate submaximal force requirements [51],
347 which could lead to unnecessary engagement of superficial extensors to ensure task
348 completion.

349 At C7/T1, both groups showed a significant increase in deep extensor activation
350 following the endurance task, with slightly higher T2 shifts in controls. This contrasts
351 with findings by O'Leary et al. [43], who reported reduced deep and superficial
352 extensor activity in individuals with neck pain in response to a low-load isometric

353 contraction performed in neutral cranio-cervical position. In the present study,
354 differences in task characteristics, including the sustained nature of the endurance
355 task performed against gravity, may partly explain the preserved deep extensor
356 activation observed across both groups. Notably, marked activation of deep
357 extensors at the C7/T1 level was observed not only in healthy controls, but also in
358 individuals with neck pain, despite common observations of reduced deep extensor
359 function in this population [13,52]. This finding suggests that reduced deep extensor
360 activation may not be a systematic feature of neck pain and could depend on task
361 demands and context. In line with this interpretation, Schomacher et al.[13] reported
362 enhanced motor recruitment of impaired deep extensor muscles with increasing
363 contraction intensity. Together, these observations suggest that notable deep
364 extensor activation can occur during demanding endurance tasks, even in individuals
365 with neck pain. Although cranio-cervical posture at test termination was not assessed
366 in the present study, these results challenge the view that deep extensor function is
367 systematically altered in this population, as previously suggested based on postural
368 observations during the cervical extensor endurance test [41]. In a complementary
369 manner, Kahlaee et al. [39] reported that individuals with neck pain did not terminate
370 the endurance test more frequently in cranio-cervical extension despite presenting
371 reduced deep muscle cross-sectional area.

372 Our results can also be interpreted in light of broader literature describing motor
373 adaptations in individuals with neck pain across a variety of tasks. A recent
374 systematic review reported that differences in cervical extensor muscle recruitment
375 are observed in approximately 50% of the comparisons, reflecting substantial inter-
376 individual variability [53]. When present, these changes tend to manifest as increased
377 activation of superficial muscles, whereas alterations in deep muscle recruitment are

378 more frequently characterized by reduced activity. In the present study, the tendency
379 toward greater involvement of superficial muscles is consistent with this pattern,
380 whereas the preserved activation of deep extensors contrasts with the average trend
381 reported for the deep muscles. This discrepancy may be interpreted within the
382 redistribution model of motor adaptation proposed by Hodges and Tucker [54], which
383 suggests that individuals may adopt different motor strategies to preserve function in
384 the presence of pain. Such redistribution may manifest as increased, decreased, or
385 redistributed activity depending on the individual's psychological context [33,55],
386 functional disability [56] and neck pain intensity during contractions [14,55]. The
387 marked inter-individual variability observed particularly in the neck pain group in the
388 present study is consistent with this model and underscores the relevance of
389 individualized assessments.

390 Although all participants completed the endurance test, individuals with neck pain
391 appeared to adopt different neuromuscular strategies, characterized by a trend
392 toward greater involvement of some superficial muscles and higher perceived effort.
393 Together with preserved deep extensor activation, these findings provide novel
394 insight into the distinct contribution of deep and superficial cervical muscles during a
395 clinical endurance task, while remaining largely subclinical. Our findings suggest that
396 increasing task difficulty (e.g., adding load or duration) may help accentuate these
397 differences in motor strategies and perceived effort, in line with previous studies
398 showing that fatigue-related neuromuscular responses in individuals with neck pain
399 become more apparent at higher levels of mechanical demand and are closely
400 associated with perceived effort [57,58]. Future studies should build on these findings
401 by adjusting the execution criteria of the cervical extensor endurance test in order to
402 enhance perceived effort and differential neuromuscular activation between groups,

403 and by establishing termination criteria that are meaningfully associated with
404 objective neuromuscular indicators of fatigue or reduced endurance. Such an
405 approach may help refine the test protocol and contribute to improve the metrological
406 properties of cervical extensor endurance testing.

407 Study limitations

408 This study has several limitations. First, the sample size was modest; larger studies
409 are needed to confirm and expand upon these findings. In addition, no a priori
410 sample size calculation was performed due to the exploratory nature of the study,
411 which may have limited the ability to detect small between-group differences.
412 Second, our sample included individuals with idiopathic and traumatic neck pain,
413 introducing heterogeneity that may have increased variability. In addition, participants
414 with neck pain reported relatively low pain intensity at baseline, which may have
415 limited the magnitude of between-group differences and hindered firm conclusions
416 regarding neck extensor muscle activity during the endurance task. Although men
417 and women were included in both groups and no statistically significant between-
418 group differences were observed for sex distribution, the overall sample comprised
419 relatively few male participants, and group sizes were unequal. This imbalance may
420 have contributed to increased variability in the mfMRI responses and should be
421 considered when interpreting the findings. Third, manual segmentation of small
422 cervical muscles using anatomical atlases, although carefully performed, remains
423 technically challenging. Muscle fat infiltration, especially in whiplash-associated
424 disorders [59], may elevate T2 values independent of activation, though previous
425 studies have shown high reliability of T2 shift measurements [60]. Fourth, while
426 mfMRI offers valuable insight into recruitment patterns, the physiological basis of T2

427 shifts remains incompletely understood [47], and inter-individual variability in T2
428 response [61] may influence comparisons. Finally, the cervical extensor endurance
429 test was limited to three minutes. Although a longer endurance test might have
430 accentuated group differences, using a fixed 3-minute protocol allowed standardized
431 comparisons. It is also worth noting that endurance capacity is influenced by factors
432 [32], such as fiber type composition, psychological state, motivation, pain, and fear of
433 movement, all of which may vary across individuals and impact motor strategies.

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CONCLUSIONS

This study provides new insights into cervical muscle recruitment in response to an extensor endurance task by distinguishing the responses of deep and superficial muscles in individuals with and without neck pain. The findings indicate more variable involvement of some superficial muscles and higher perceived effort in individuals with neck pain, despite preserved deep extensor activation. These observations are consistent with the individual- and task-related variability of neuromuscular adaptations to neck pain. Although these results were largely subclinical and not directly translatable to clinical decision-making, they contribute to a better understanding of cervical muscle behavior during extensor endurance testing and support the need for future studies aimed at refining task demands and execution or termination criteria to determine whether neuromuscular differences can be amplified and translated into clinically meaningful indicators during endurance testing.

468 **DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN**
469 **THE WRITING PROCESS.**

470 During the preparation of this work the authors used ChatGPT in order to improve
471 readability and language. After using this tool/service, the authors reviewed and
472 edited the content as needed and take full responsibility for the content of the
473 publication.

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TABLES

490 Table 1: Demographic and clinical characteristics of the participants.

	Healthy Control Group (n = 13)	Neck Pain Group (n = 16)	p-value
Sex (M/F)	3 / 10	4 / 12	0.93
Age (years), median [P25–P75]	47 [24–58]	44.5 [23.8–60.3]	0.79
NDI (%), median [P25–P75]	0 [0–2]	28 [20.5–34.5]	< 0.0001
NPRS (0–10), median [P25–P75]	0	1 [0–2.3]	0.0020
History of whiplash, n (%)	0 (0%)	10 (62.5%)	< 0.0001
Symptom duration (months), median [P25–P75]	–	48 [36–51]	–

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492 *Caption:* **M** = male; **F**= female; **NDI** = Neck Disability Index; **NPRS** = Numeric Pain
493 Rating Scale

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503 Table 2: Summary of T2 values, linear mixed-effects model results, and intra-group effect sizes (Cohen's *d*) for all muscles investigated

Muscle	Level	Group (HC n= 13; NP n= 16)	T2 values (mean (SD), ms)			Linear mixed-effects model			Cohen's <i>d</i>
			Baseline	Post-test	T2 shift	TIME (<i>F</i> , <i>p</i> -values)	GROUP (<i>F</i> , <i>p</i> -values)	TIME x GROUP (<i>F</i> , <i>p</i> -values)	
Sternocleidomastoid †	C2/C3	HC	31.53 (2.61)	32.61 (2.93)	1.08 (2.17)	15.94, 0.0005 (**)	0.28, 0.60	1.48, 0.23	0.49 0.93
		NP	30.42 (3.54)	32.51 (3.76)	2.09 (2.24)				
Longus colli/longus capitis	C2/C3	HC	35.44 (4.69)	36.78 (4.27)	1.34 (2.15)	3.50, 0.072	0.38, 0.54	0.51, 0.48	0.62 0.20
		NP	36.86 (4.88)	37.47 (5.06)	0.61 (3.06)				
Obliquus capitis inferior	C2/C3	HC	38.37 (7.71)	37.87 (6.33)	-0.49 (4.74)	0.33, 0.57	0.03, 0.86	0.01, 0.99	0.26 0.29
		NP	38.01 (5.52)	37.5 (4.47)	-0.51 (4.68)				
Multifidus/semispinalis cervicis	C2/C3	HC	44.48 (5.82)	45.95 (5.27)	1.47 (3.61)	1.28, 0.27	0.39, 0.54	0.57, 0.46	-0.19 0.06
		NP	43.87 (5.28)	44.13 (4.72)	0.26 (4.62)				
Superficial extensor muscles †	C2/C3	HC	40.49 (3.35)	40.4 (3.29)	-0.09 (1.76)	6.25, 0.019 (*)	0.04, 0.85	5.85, 0.023 (*)	-0.051 0.73
		NP	39.62 (4.26)	41.82 (4.47)	2.2 (3.01)				
Anterior scalene	C7/T1	HC	29.61 (2.9)	29.83 (3.21)	0.22 (1.79)	1.55, 0.23	0.34, 0.57	0.57, 0.46	0.47 0.30
		NP	30.38 (6.65)	31.38 (7.12)	1 (3.32)				
Multifidus/semispinalis cervicis †	C7/T1	HC	39.93 (3.51)	41.68 (3.43)	1.75 (1.42)	15.97, 0.0004 (**)	0.33, 0.57	0.72, 0.40	1.23 0.72
		NP	40.43 (4.92)	43.21 (4.85)	2.78 (3.87)				
Levator scapulae	C7/T1	HC	35.89 (4.8)	36.86 (5.72)	0.97 (2.09)	0.67, 0.42	0.01, 0.96	0.25, 0.62	-0.12 0.01
		NP	36.18 (5.48)	36.3 (4.69)	0.12 (3.99)				
Upper trapezius	C7/T1	HC	36.62 (3.15)	36.22 (5.18)	-0.4 (2.67)	0.51, 0.48	1.42, 0.24	3.23, 0.08	0.42 0.32
		NP	34.29 (4.67)	35.2 (3.41)	0.91 (2.75)				

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505 *Caption:* **HC** = healthy controls; **NP** = neck pain participants.

506 T2 values (mean (SD)) are presented at baseline and post-test for each group, along with T2
507 shifts (post-test minus baseline). Results of linear mixed-effects models (fixed effects of time,
508 group, and time × group interaction) are reported as *F* and p-values for each muscle. Intra-
509 group effect sizes (Cohen's *d*) are provided descriptively for all muscles to quantify the
510 magnitude of pre-post T2 changes within each group. Statistical significance for fixed effects
511 was determined using Bonferroni correction to adjust for multiple comparisons across muscles
512 (adjusted $\alpha = 0.0056$). In the table, p-values < 0.05 are indicated in bold with (*), and p-values
513 < 0.0056 are indicated in bold with (**). Muscles for which T2 values were log-transformed
514 prior to model fitting due to non-normal residual distribution are indicated with †.

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CAPTIONS AND ILLUSTRATIONS

530 Figure 1: Illustration of the anatomical slices used for MRI acquisition. Axial T2-
531 weighted images were obtained at two cervical levels: C2/C3 (highlighted in yellow)
532 and C7/T1 (highlighted in green). These levels were selected to represent,
533 respectively, the upper and lower cervical regions, allowing separate analysis of deep
534 and superficial extensor muscles.

535 Figure 2: Illustration of participant positioning and material setup. (1) Inertial sensor
536 placed at the occipital apex; (2) Reference block placed at the thoracic apex. The
537 yellow lines indicate the matched height between the inertial sensor and the
538 reference block, used to ensure accurate alignment of the occipital and thoracic
539 apices with the horizontal arm of the height gauge.

540 Figure 3: Representation of the nine pairs of cervical muscles assessed at both
541 C2/C3 and C7/T1 levels. Muscles are numbered as follows: 1) sternocleidomastoid,
542 2) longus colli/longus capitis, 3) obliquus capitis inferior, 4) multifidus/semispinalis
543 cervicis, 5) superficial extensor muscles, 6) anterior scalene, 7)
544 multifidus/semispinalis cervicis, 8) levator scapulae, 9) upper trapezius. Cervical
545 flexor muscles are in purple and cervical extensor muscles are in green. Deep
546 muscles are in dashed. The obliquus capitis inferior, although anatomically located
547 between C1 and C2, is included in the illustration as an extrapolated representation
548 at the C2/C3 level to provide a comprehensive overview of all muscles analyzed.

549 Figure 4: Example of MRI images: a) Axial image of C2/C3 level, used for the ROI's
550 delimitation; b) T2 images of C2/C3 level, used for T2 values calculation.

551 Figure 5: Illustration of linear mixed-effects model results for the nine pairs of cervical
552 muscles assessed at both C2/C3 and C7/T1 levels, comparing T2 shifts between

553 healthy controls and individuals with neck pain. Muscles are numbered as follows: 1)
554 sternocleidomastoid, 2) longus colli/longus capitis, 3) obliquus capitis inferior, 4)
555 multifidus/semispinalis cervicis, 5) superficial extensor muscles, 6) anterior scalene,
556 7) multifidus/semispinalis cervicis, 8) levator scapulae, 9) upper trapezius. Deep
557 muscles are dashed. Muscles showing a significant main effect of time in the linear
558 mixed-effects model are highlighted in color, indicating increased activation in
559 response to the endurance task. The intensity of the yellow shading reflects the
560 magnitude of within-group effect sizes (Cohen's d): very small ($d < 0.20$), small
561 ($0.20-0.49$), medium ($0.50-0.79$), large ($0.80-1.19$), and very large (≥ 1.20), with the
562 corresponding legend included in the figure. The obliquus capitis inferior, although
563 anatomically located between C1 and C2, is included in the illustration as an
564 extrapolated representation at the C2/C3 level to provide a comprehensive overview
565 of all muscles analyzed.

566 Color should be used for Figure 1, 3, 4.a, 4.b and 5.

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