

## Spine (Phila Pa 1976)

# CHANGES IN MUSCLE MORPHOLOGY IN FEMALE CHRONIC NECK PAIN PATIENTS USING MAGNETIC RESONANCE IMAGING

--Manuscript Draft--

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Dear reviewers,

Thank you for the useful comments, critical advices and additional references you have provided to this article. Your feedback has been incorporated into the manuscript and changes are listed in the text below. Adjustments to the original text are indicated in bold.

## REVIEWER #1

### Materials and Methods

1. *This study recruited only female subjects. Please describe the reason why this study looked at female subjects, or add some references related to this.*

The paragraph on page 17, lines 16 to 21, of the pdf document, namely

“Women (n = 117) aged between 18 and 65 years were recruited through various media channels, leaflets and posters distributed in Flanders (Belgium). Eligible candidates were attributed into three subgroups (45 CINP, 37 CWAD, 35 HC).”

is replaced by the following paragraph:

“Participants (n = 117) aged between 18 and 65 years were recruited through various media channels, leaflets and posters distributed in Flanders (Belgium). **Previous research implies the use of a gender-specific model as necessary in the study of neck related disorders as differences in muscle morphology between men and women are assumed.** <sup>16,36,37</sup> **In addition, female sex seems to predict a poor outcome after a whiplash accident, causing women to develop chronic whiplash complaints more often.** <sup>38,39</sup> Eligible candidates were attributed into three subgroups (45 CINP, 37 CWAD, 35 HC).”

References referring to:

<sup>16</sup> Ulbrich EJ, Aeberhard R, Wetli S, et al. Cervical muscle area measurements in whiplash patients: Acute, 3, and 6 months of follow-up. *J Magn Reson Imaging* 2012;36:1413–20.

<sup>36</sup> Vasavada AN, Danaraj J, Siegmund GP. Head and neck anthropometry, vertebral geometry and neck strength in height-matched men and women. *J Biomech* 2008;41:114–21.

<sup>37</sup> Snodgrass SJ, Croker C, Yerrapothu M, et al. Cervical muscle volume in individuals with idiopathic neck pain compared to asymptomatic controls: A cross-sectional magnetic resonance imaging study. *Musculoskelet Sci Pract* 2019;44:102050.

<sup>38</sup> Côté P, Cassidy JD, Carroll L, et al. A systematic review of the prognosis of acute whiplash and a new conceptual framework to synthesize the literature. *Spine (Phila Pa 1976)* 2001;26:445–58.

<sup>39</sup> Walton DM, Macdermid JC, Giorgianni AA, et al. Risk factors for persistent problems following acute whiplash injury: Update of a systematic review and meta-analysis. *J Orthop Sports Phys Ther* 2013;43:31–43.

2. *Did you define any specific posture or instructions for setting a posture when acquiring MRI scans? Posture might have an influence on cross section area. If not, please indicate this as a limitation in the discussion section.*

The section on MRI measurements (p. 19, lines 9 to 23) is supplemented with a sentence about the specific posture of the subjects.

“Magnetic resonance images were acquired with a Siemens (Berlin, Germany) Magnetom Trio Tim Syngo MR B17 scanner with a 3T magnet. Parameters of the two point Dixon scan consisted of 6.59 ms repetition time, 2.45 ms echo time 1 and 3.675 ms echo time 2. Voxel size was 0,7 x 0,7 x 3 mm and field of view 320 x 340 mm. The thickness of the 40 slices was 3 mm. **Subjects were placed supine in the scanner, with the head in a neutral position in order to minimize differences in posture that might affect the results.** Magnetic resonance imaging data were calculated blind to the subject’s NP status.”

#### Discussion

3. *Related to the above comment 1, sex might influence FI, CSA and mCSA. Please discuss this in the discussion section.*

In the section ‘Materials and Methods’, the gender difference in muscle morphology is already mentioned (see answer to comment 1). This has been briefly supplemented in the discussion as follows:

“Some strengths and limitations were present in this study. A strength was that assessors were blinded to the different study groups, so potential detection bias was precluded. **Moreover, gender was excluded as confounding factor, as only women were included in this study. However, the results can therefore not be generalized to a broader population including men.** The use of MRI was also seen as an added value as it considered to be the gold standard for measuring muscle morphology, but again some considerations should be observed. ...”

4. *Age might also influence FI, CSA and mCSA. Please discuss about the effect of age in this study.*

The following section was added to the discussion, before addressing the strengths and limitations (p. 24).

“**Consideration should also be given to natural muscle degeneration with aging. Thus, denervation of motor neurons could result in a secondary decrease in lean muscle mass<sup>59-62</sup> and atrophy of the neck muscles may occur from the fourth decade of life with an acceleration after the age of fifty.<sup>63,64</sup> Since this age-related increase in FI and decrease in CSA are reported later in life<sup>65,66</sup>, and the age of the study subjects was relatively young, age did not seem to have a causal influence on the reported results.**”

References referring to:

- <sup>59</sup> Delmonico MJ, Harris TB, Visser M, et al. Longitudinal study of muscle strength, quality, and adipose tissue infiltration. *Am J Clin Nutr* 2009;90:1579–85.
- <sup>60</sup> Marcus R, Addison O, Kidde J, et al. Skeletal muscle fat infiltration: impact of age, inactivity, and exercise. *J Nutr Heal Aging* 2010;14:362–6.
- <sup>61</sup> Fortin M, Videman T, Gibbons LE, et al. Paraspinal muscle morphology and composition: A 15-yr longitudinal magnetic resonance imaging study. *Med Sci Sports Exerc* 2014;46:893–901.
- <sup>62</sup> Crawford RJ, Filli L, Elliott JM, et al. Age- and level-dependence of fatty infiltration in lumbar paravertebral muscles of healthy volunteers. *Am J Neuroradiol* 2016;37:742–8.
- <sup>63</sup> Lexell J, Taylor CC, Sjöström M. What is the cause of the ageing atrophy?. Total number, size and proportion of different fiber types studied in whole vastus lateralis muscle from 15- to 83-year-old men. *J Neurol Sci* 1988;84:275–94.
- <sup>64</sup> Frontera WR, Hughes VA, Fielding RA, et al. Aging of skeletal muscle: A 12-yr longitudinal study. *J Appl Physiol* 2000;88:1321–6.
- <sup>65</sup> Cohn SH, Vartsky D, Yasumura S. Compartmental body composition based on total-body nitrogen, potassium, and calcium. *Am J Physiol - Endocrinol Metab*;2. Epub ahead of print 1980. DOI: 10.1152/ajpendo.1980.239.6.e524.
- <sup>66</sup> Waters D, Baumgartner R, Garry P. Sarcopenia: current perspectives. *J Nutr Heal Aging* 2000;4:133–9.

5. *Regarding pain duration, did you looked at differences of FI, CSA and mCSA between short and long duration groups?*

This study presented the interaction between pain duration and muscle morphology for the included groups, but did not report the specific impact of short versus long pain duration by using a cut-off value. However, by only including neck pain patients who experience pain for at least three months, a distinction, but no comparison, was made between (sub)acute and chronic pain.

“A significant group-specific association was found between pain duration and both CSA and mCSA of the Trap (respectively  $P < .001$ , [.3003-.5386 mm<sup>2</sup>] and  $P < .001$ , [.2497-.4563 mm<sup>2</sup>]) and LCa (respectively  $P < .001$ , [.0487-.1977 mm<sup>2</sup>] and  $P = .002$ , [.0416-.1782 mm<sup>2</sup>]) muscles in the CWAD group. Additionally, there was a significant overall relationship between pain duration ( $\geq 3$  months) and CSA of the SeCa ( $P = .017$ , [.0217-.3247 mm<sup>2</sup>]). (Table 4)”

## Tables

6. *In Table 2, "m" for Pain duration might be month. Please check this.*

The list of abbreviations corresponding to Table 2 was completed according to the alphabetical order with “m, months”.

“CWAD, Chronic Whiplash Associated Disorder; CINP, Chronic Idiopathic Neck Pain; HC, Healthy Control; SD, standard deviation; y, years; kg, kilogram; **m, months**; m<sup>2</sup>, square meter; NDI, Neck Disability Index; m, meter; TSK, Tampa Scale for Kinesiophobia; NA, not applicable.”

7. *Table 3 does not contain FI, CSA and mCSA for all muscles you investigated in this study. Please provide information about all items for all muscles. Or, please provide a description why Table 3 does not indicate all items.*

Only those regions of interest for which a significant between-group difference was found have been included in the Table 3 in order to limit the extent of the table. In the caption of Table 3 it was added that only the significant results were shown.

**“Only the ROIs for which a significant between-group difference was found are shown in the table.**

ROI, Region of Interest; AIC, Akaike’s Information Criterion; LS, Levator Scapulae; Longi, Longus Capitis + Colli; SeCa, Semispinalis Capitis; SpCa, Splenius Capitis; SCM, Sternocleidomastoideus; Trap, Trapezius; LCa, Longus Capitis; LCo, Longus Colli; Mult, Multifidus; OCInf, Obliquus Capitis Inferior; FI, fat infiltration; CSA, cross-sectional area; mCSA, muscle cross-sectional area; NA, not applicable;

\* significance  $P < .05$ .”

8. *Figures seems to be box plots. To make sure, please provide meanings of "x", box (length of the box) and bar in the box for readers.*

The figures have been reworked (see new PDF document) to make them easier to interpret. Figures 1 to 3 show the estimated marginal means for fat infiltration, cross-sectional area and muscle cross-sectional area respectively, with age integrated as a confounding factor. Error bars represents plus and minus one standard error.

9. *Figure (A) and (B) don't have figures.*

The illustrations belonging to figure (A) and (B) were added in the new PDF document.

10. *Some symbols in the figures are not displayed correctly.*

The figures were provided with the required symbols to make interpretation possible.

## REVIEWER #2

The authors have provided a population-based cross-sectional study that aimed to gain a better understanding of changes in muscle morphology (as they define to mean, muscle fatty infiltration (FI) and cross-sectional area) in 118 female participants with chronic neck pain; traumatic ( $n = 37$ ) and non-traumatic ( $n = 45$ ) in origin and 35 healthy controls. Overall, a lovely paper. This reviewer has provided some suggestions below with hopes this helps the authors in revising their manuscript.

The paper is well-written, adds to the literature (from a replication standpoint), and the references mostly capture the available literature. The methods, while appropriate, do not reflect current clinical practice in that it is highly unlikely a clinician (radiologist) would manually segment muscles in post-hoc fashion. Furthermore, while the use of fat/water DIXON keeps with current thought on how to quantify FI (across the body, not just the cervical region), it is not known if such a method has been implemented/uptaken in clinical practice. In short, the use of Fat/Water DIXON (in Siemens environment, mDIXON with Philips, IDEAL with GE, etc) is unique to research efforts in controlled environments.

The findings from this cross-sectional study show strikingly similar results to the available literature demonstrating larger magnitudes of FI in some muscles traversing the cervical region in participants with chronic whiplash compared to idiopathic neck pain and healthy controls. While this does align with previous findings, there was no clear association between pain duration, kinesiophobia, and disability, which could reflect the low-resolution nature of such self-reported scales (TSK, NDI), which of course is NOT a deal-breaker, but it would be good to offer some insight into how AIM 2 of this paper is challenged (e.g. investigating possible associations between muscle morphologic changes and symptom duration, kinesiophobia and disability). For example, it is difficult to argue that using such scales will adequately provide 'true' measures of cognition or irrational fear (TSKinesiophobia) and neck related interference (NDI). This reviewer doesn't believe the response items for the TSK represent true irrational fears, which is the definition of a phobia. Equally, this reviewer does not believe the 10-items on the NDI adequately capture true pain-related disability on a patient-by-patient basis. For example, not everyone drives and there is only one question re: pain intensity. This is NOT an attempt to slight the TSK or NDI as they are widely used (and accepted) across clinical practice, worldwide. However, it is an opportunity to offer some insight into the challenges of showing strong associations based on what people circle on a self-reported questionnaire that they may not even understand OR believe in the provided options to circle.

#### MINOR points

1. Line 28 pg 16 of annotated .pdf - 'did not had' should be 'did not have'. Same for line 30-31 - 'did not had' should be 'did not have'

The text has been adapted in line with this comment.

"Healthy controls needed to be pain-free on the day of testing (verbal numbering rating scale [VNRS] <sup>40</sup> score <2/10), **did not have** history of neck-shoulder-arm pain (VNRS-score ≥2/10) <sup>40</sup> for >8 successive days during the last year, **did not have** medical consultation for neck-shoulder-arm pain during the last year, **did not have** a whiplash in the past and scored <8/50 on the Neck Disability Index (NDI)."

2. Line 43-44 pg 16 RE: the WAD II A to C modified QTF. The reference (#36) represents a "Masters Class" narrative from 2004. This reviewer would recommend referencing the original QTF as, to my knowledge, the proposed new classification has never been implemented and replaced the original and well-cited QTF from 1995 (Spitzer et al). The

*referenced paper from 2005 has a total of 76 cites, whereas the original QTF has been cited nearly 2,300 times (Source, Google Scholar). Suggest, removing the 2005 reference and replacing with the 1995 original QTF manuscript.*

The reference in the manuscript is replaced by the original reference on Quebec Task Force of Spitzer et al. .

“For inclusion in the CWAD group, patients needed to be classifiable as WAD II A to C on the modified Quebec Task Force Classification. <sup>41</sup>”

Reference referring to:

<sup>41</sup> Spitzer W, Skovron M, Salmi L, et al. Scientific monograph of Quebec Task Force on whiplash associated disorders: redefining “whiplash” and its management. *Spine (Phila Pa 1976)* 1995;20:1–73.

3. *Ln 28 pg 17 - suggest adding another reference re: the NPRS - Walton et al., A reconceptualization of the pain numeric rating scale: Anchors and clinically important differences. J Hand Ther 2018.*

Thank you for this interesting addition. The reference is added to the manuscript on the indicated line, as well as on Ln 24 pg 17 as shown below.

“Pain intensity on the day of testing was assessed using the VNRS-11. A score between 0, reflecting “no pain at all” and 10, meaning “the worst pain imaginable” was obtained verbally. This scale is a useful and valid tool for measuring pain. <sup>40,42</sup>”

“Healthy controls needed to be pain-free on the day of testing (verbal numbering rating scale [VNRS] score <2/10) <sup>40</sup>, did not have history of neck-shoulder-arm pain (VNRS-score ≥2/10) <sup>40</sup> for >8 successive days during the last year, ...”

Reference referring to:

<sup>40</sup> Walton DM, Elliott JM, Salim S, et al. A reconceptualization of the pain numeric rating scale: Anchors and clinically important differences. *J Hand Ther* 2018;31:179–83.

4. *Ln 44 pg 17 - RE: the NDI - perhaps this should read - 'this questionnaire is considered a reliable and valid way to capture neck-related interference in daily life' as neck pain-related disability is far more complex and not adequately captured in 10 questions, of which only 1 question relates to pain intensity. Don't get me wrong, I use and like the NDI, but I believe we too easily suggest it accurately measures neck-pain related disability.*

The sentence has been modified according to your justified comment. So,

"This questionnaire is considered a valid way to measure neck-pain related disability. <sup>39,40</sup>"

is replaced by:



“This questionnaire is considered a **reliable and** valid way to **capture neck-related interference in daily life.** <sup>44,45</sup>”

5. *RE: the Imaging Protocol. Were the in-phase and opposed-phase images used to quantify FI?*

The original sentence relating to this question on page 18, line 28 has been modified to clarify that in-phase and opposed-phase images were used to quantify fat infiltration.

“Using the two point Dixon scan, **in-phase (water)** and **opposed-phase (fat)** images were obtained.”

6. *Is it truly a cross-sectional area? On grounds each imaged slice has a thickness, therefore, it is 3D. Should this not be a series of volumetric measures vs. the 2D CSA?*

Each slice indeed has a thickness, although this was not taken into account in the calculation of the CSA, where the number of voxels was multiplied by the size of the voxel. However, 2D images should have a certain volume due to an increased sensitivity to the slicing profile and this finding has been added to the discussion.

“The use of MRI was also seen as an added value as it considered to be the gold standard for measuring muscle morphology, but again some considerations should be observed. **Despite the fact that interpreting mCSA is an enhancement over previous studies in which only CSA was reported, it seems that 2D images may have partial volumes due to an increased sensitivity to the radio frequency slicing profile. Measuring muscle morphology based on 3D volume could be more precise for this purpose.** <sup>12</sup> The applied mCSA calculation also fails to account for material that is invisible on MRI. The use of already existing, more sophisticated MRI applications is therefore recommended for subsequent research. <sup>67</sup> In parallel, the manual segmenting the muscle or more time-efficient convolutional neural network segmenting may also help obtain more valid morphology values in the future. <sup>14,68</sup> This in turns could lead to its implementation in clinical practice, complementary to radiology, in quantifying muscle composition. <sup>68</sup> To assess the prognostic or diagnostic value of MRI findings, studies should monitor people over time and gain access to MRI findings related to important patient outcomes.”

Reference referring to:

<sup>12</sup> Elliott JM, Pedler AR, Jull GA, et al. Differential changes in muscle composition exist in traumatic and nontraumatic neck pain. *Spine (Phila Pa 1976)* 2014;39:39–47.

7. *The CSA x (1-FI) is great...but do we truly know that doing so provides an accurate measure of fat-free contractile elements, on grounds some tissue contain a significant quantity of MR invisible material that is not measured using MR-based methods leading to natural disagreement between different sequences...and this could explain the discrepant findings in the literature as the authors point out from recent SRs. In short, those SR's included papers using completely different methodologies.*

Besides emphasizing the difference in research methodologies applied in the studies referred to (see question 8), another point was added to the discussion concerning the inability to measure invisible material by means of T1-weighted images.

“The use of MRI was also seen as an added value as it considered to be the gold standard for measuring muscle morphology, but again some considerations should be observed. Despite the fact that interpreting mCSA is an enhancement over previous studies in which only CSA was reported, it seems that 2D images may have partial volumes due to an increased sensitivity to the radio frequency slicing profile. Measuring muscle morphology based on 3D volume could be more precise for this purpose.<sup>12</sup> **The applied mCSA calculation also fails to account for material that is invisible on MRI. The use of already existing, more sophisticated MRI applications is therefore recommended for subsequent research.**<sup>67</sup> In parallel, the manual segmenting the muscle or more time-efficient convolutional neural network segmenting may also help obtain more valid morphology values in the future.<sup>14,68</sup> This in turns could lead to its implementation in clinical practice, complementary to radiology, in quantifying muscle composition.<sup>68</sup> To assess the prognostic or diagnostic value of MRI findings, studies should monitor people over time and gain access to MRI findings related to important patient outcomes.”

Reference referring to:

<sup>67</sup> Reeder SB, Hu HH, Sirlin CB. Proton density fat-fraction: A standardized mr-based biomarker of tissue fat concentration. *J Magn Reson Imaging* 2012;36:1011–4.

8. *Ln 44-45 page 21. The authors state "However, more recent research did not support the assumption of increased FI as a feature of CWAD. 23,24". Rather than state 'recent research' it would be good if the authors stated 'two recent SR, relying on studies using different measurement techniques, did not support...' This then reinforces their next sentence that future studies should use consistent outcome measures across larger cohorts). Others have already done this.*

The original sentence has been changed following the suggestion.

**“Two other, more recent systematic reviews relying on studies using different measurement techniques, did not support the assumption of increased FI as a feature of CWAD.<sup>23,24</sup>”**

9. *I don't think it's accurate to opine 'Muscle atrophy may therefore only be present in specific muscles in CINP, which 'can be explained' by avoidance behaviour due to pain-related fear in these patients.' Atrophic signaling pathways are very complex and most certainly cannot be uniquely tied to responses on a 17-item scale measuring 'fear-avoidance' behaviour or a 10-item scale measuring neck related interference. It is far more complex. Would suggest softening this sentence.*

As a result of the comment, the sentence in question has been substituted.

**“The assumption could be made that muscle atrophy is only present in specific muscles in CINP, which could possibly be explained by avoidance behavior as a result of pain-related anxiety in these patients.”**

10. *The authors could add a reference to the sentence on pg 23 (of .pdf) lines 6-12 when detailing future segmentation of muscles...this would highlight the rapidly evolving world of Artificial Intelligence with use of neural networks. A reference to consider is from Weber et al, here <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6538618/> This then provides opportunity to translate to clinical practice if/when standard of care imaging is ordered as AI algorithms could enhance, NOT replace, radiology practice to include muscle (and other soft-tissue) composition quantification.*

The reference have been included in the sentence. Furthermore, an addition has been made to the original sentence to accommodate your further comment regarding clinical practice.

“In the future, segmenting the muscle may help obtain more valid morphology values.<sup>14”</sup>

is replaced by:

**“In parallel, manual segmenting the muscle or more time-efficient convolutional neural network segmenting may also help obtain more valid morphology values in the future.<sup>14,68</sup> This in turns could lead to its implementation in clinical practice, complementary to radiology, in quantifying muscle composition.<sup>68</sup> To assess the prognostic or diagnostic value of MRI findings, studies should monitor people over time and gain access to MRI findings related to important patient outcomes.”**

Reference referring to:

<sup>68</sup>Weber KA, Smith AC, Wasielewski M, et al. Deep Learning Convolutional Neural Networks for the Automatic Quantification of Muscle Fat Infiltration Following Whiplash Injury. *Sci Rep* 2019;9:1–8.

11. *Ln 26-28 on page 23. The reference (54) is more than appropriate but this reference measured one muscle in the lower extremity and made no attempt to relate muscle composition to function of the lower extremity plantar flexors. Consider adding another reference to square up the available literature...where consistency was found between a small number of participants with chronic WAD and motor incomplete SCI compared to healthy controls. Smith et al., <https://www.ncbi.nlm.nih.gov/pubmed/27630770>*

Thanks for this useful addition. The proposed article have been added as a reference to the specific paragraph.

“Because of the cross-sectional design of this study, no comparisons according to muscle morphology were made pre- and post-injury. Also, because only chronic patients were included, it was not possible to differentiate between acute and chronic changes in muscle morphology. No reference scan of more distant muscles was made to exclude a general change in muscle morphology. This way, it would be possible to determine whether local or global factors influence changes in muscle morphology. This way, it would be possible to determine whether local or global factors influence changes in muscle morphology.<sup>28,69”</sup>

Reference referring to:

<sup>69</sup> Smith A, Parrish T, Hoggarth M, et al. Potential associations between chronic whiplash and incomplete spinal cord injury. *Spinal Cord Ser Cases* 2015;2:15024.

**Changes in muscle morphology in female chronic neck pain patients using magnetic resonance imaging**

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## ABSTRACT

**Study Design.** Population based cross-sectional study

**Objective.** The aim of this study was to gain a better understanding of changes in muscle morphology in patients with chronic idiopathic neck pain (CINP) and whiplash associated disorder (CWAD).

**Summary of Background Data.** Worldwide, neck pain is a common health problem with high socio-economic burden. A high percentage of these patients evolves towards chronic symptoms. Efficacy of treatments for these complaints remains variable. In current literature, changes in muscle morphology (muscle fatty infiltration [FI] and cross-sectional area [CSA]) have been reported in neck pain patients, both CWAD and CINP. However, no strong conclusions could be made.

**Methods.** In this study, magnetic resonance imaging was used to obtain data on muscle morphology from 14 cervical flexor and extensor muscles in 118 female subjects with neck pain (CWAD = 37; CINP = 45) and healthy controls (HC = 35).

**Results.** The CWAD group had a significantly larger muscle FI in some extensor (Semispinalis and Splenius Capitis, Trapezius, Obliquus Capitis Inferior) and flexor (Sternocleidomastoid) muscles compared to the CINP and/or HC group. A significant larger (muscle) CSA was found in some extensor (Levator Scapulae, Semispinalis Capitis, Trapezius) and flexor (Longus Colli, Longus Capitis, Sternocleidomastoid) muscles in the HC group compared to the CINP and/or CWAD group. No clear associations were found between group differences and factors as pain duration, kinesiophobia and disability.

**Conclusions.** The results in this study suggest changes in muscle morphology in both neck pain cohorts. These results show some similarities with earlier findings in this research domain. Further studies based on controlled longitudinal designs are needed to facilitate data compilation, to draw stronger conclusions and to integrate them into the treatment of chronic neck pain patients.

**Key Words.** chronic pain, idiopathic neck pain, whiplash, muscle morphology, fat infiltration, cross-sectional area, magnetic resonance imaging

**Level of Evidence Level:** 4

## KEY POINTS

- Significant larger MRI fatty infiltration was found in both flexor (Sternocleidomastoid) and extensor (Semispinalis and Splenius Capitis, Trapezius, Obliquus Capitis Inferior) muscles in CWAD patients compared to the CINP patients and/or healthy controls.
- A significant larger (muscle) MRI cross-sectional area was found in some flexor (Longus Colli, Longus Capitis, Sternocleidomastoid) and extensor (Levator Scapulae, Semispinalis Capitis, Trapezius) muscles in healthy controls compared to neck pain patients.
- No clear associations were found between group differences and pain duration, kinesiophobia, and disability.
- This data suggests that fat infiltration could be a specific characteristic of a whiplash associated disorder.



# **MINI ABSTRACT**

Cervical muscle morphology was examined using MRI in women with chronic idiopathic neck pain and whiplash associated disorder. Fat infiltration was significantly larger in specific muscles in the whiplash group compared to idiopathic neck pain and controls. No consistent results were found for (muscle) cross-sectional area.

# INTRODUCTION

Neck pain (NP) is a common disability worldwide, whereby the development of chronic complaints entails a high socio-economic burden.<sup>1-4</sup> Chronic NP ( $\geq 3$  months), may be caused by a serious disease or traumatic condition. In most cases, however, there is no discernible cause for the pain, hence defined as chronic idiopathic neck pain (CINP).<sup>5,6</sup>

Chronic whiplash associated disorder (CWAD), due to an acceleration-deceleration trauma, is also a common form of chronic NP. As in CINP, the exact pathophysiology of CWAD is not yet known. The current lack of detectable tissue damage raises questions about its role in the clinical context of CWAD. After all, no valid diagnostic test is available to assess the clinical relevance of muscle lesions.<sup>7</sup>

Over the past years, research has been published on changes in muscle morphology using magnetic resonance imaging (MRI) in CWAD and CINP compared to healthy controls (HC).<sup>8-21</sup> However, systematic reviews<sup>22-24</sup> on this subject reported divergent results for changes in muscle fat infiltration (FI) and cross-sectional area (CSA).<sup>10,12,14-18,20</sup> An increase in muscle FI in extensor and flexor muscles in CWAD, but not in CINP is suggested.<sup>8-11,13,14,17-19,21</sup> This difference might be explained by an initial, trauma-induced mild spinal cord lesion, which could be a cause of increased FI.<sup>25-29</sup> However, no firm conclusions could be drawn about muscle morphologic changes on MRI in chronic NP.<sup>22-24</sup> Psychological factors such as fear of movement and disuse play an additional role in changes in muscle morphology.<sup>11,21,30-33</sup> So, kinesiophobia can lead to the idea that physical activity will exacerbate pain, which can increase disability and have an impact on muscle properties.<sup>34,35</sup>

The first aim of this study was to gain a better understanding of changes in muscle morphology in CINP and CWAD patients. The second aim was to investigate possible associations between muscle morphologic changes and symptom duration, kinesiophobia and disability. The proposed research

questions may lead to a better understanding of the pathophysiology and the integration of possible new insights with regard to the treatment of chronic NP.

## MATERIALS AND METHODS

### Subjects

Participants (n = 117) aged between 18 and 65 years were recruited through various media channels, leaflets and posters distributed in Flanders (Belgium). Previous research implies the use of a gender-specific model as necessary in the study of neck related disorders as differences in muscle morphology between men and women are assumed.<sup>16,36,37</sup> In addition, female sex seems to predict a poor outcome after a whiplash accident, causing women to develop chronic whiplash complaints more often.<sup>38,39</sup>

Eligible candidates were attributed into three subgroups (45 CINP, 37 CWAD, 35 HC).

Healthy controls needed to be pain-free on the day of testing (verbal numbering rating scale [VNRS] score <2/10)<sup>40</sup>, did not have history of neck-shoulder-arm pain (VNRS-score ≥2/10)<sup>40</sup> for >8 successive days during the last year, did not have medical consultation for neck-shoulder-arm pain during the last year, did not have a whiplash in the past and scored <8/50 on the Neck Disability Index (NDI).

Chronic NP was defined as persistent NP for at least three months with an average pain VNRS-score ≥2/10. Mild or moderate to severe pain-related disability (NDI score ≥10) needed to be present in this patient population. Medication intake had to be stable at least four weeks before study participation. Patients were allocated to the CINP group if they were suffering from non-specific NP. For inclusion in the CWAD group, patients needed to be classifiable as WAD II A to C on the modified Quebec Task Force Classification.<sup>41</sup>

General exclusion criteria were the presence of major depression or psychiatric illness; neurologic, metabolic, and cardiovascular disorders; fibromyalgia; chronic fatigue syndrome; inflammatory

conditions; and neck or shoulder girdle surgery in the past. Women who were pregnant or had given birth in the past year were also excluded.

Participants were asked not to make strenuous physical effort, to abstain from alcohol, caffeine, and nicotine on the day of study participation and to stop taking non-opioid analgesics 48 hours before testing.

All subjects provided written informed consent prior to study participation. Approval was granted by the local human ethical committee of Ghent University (EC2013/1053).

## Questionnaires

### *Verbal Numeric Rating Scale*

Pain intensity on the day of testing was assessed using the VNRS-11. A score between 0, reflecting “no pain at all” and 10, meaning “the worst pain imaginable” was obtained verbally. This scale is a useful and valid tool for measuring pain.<sup>40,42</sup>

### *Neck Disability Index*

In the Dutch NDI, subjects score their degree of disability. Ten items concerning pain intensity, personal care, lifting, reading, headaches, concentration, work, driving, sleeping, and recreation are questioned. Each item is scored on a 6 point Likert scale, ranging from 0 (no disability) to 5 (total disability). A total score is determined by the sum of all scores on the questions, whereby a higher score indicates more self-reported disability.<sup>43</sup> This questionnaire is considered a reliable and valid way to capture neck-related interference in daily life.<sup>44,45</sup>

### *Tampa Scale of Kinesiophobia (TSK)*

The Dutch TSK consists of 17 items that question symptoms of kinesiophobia. Scores range from 1 (completely disagree) to 4 (completely agree). After reversing the scores on questions 4, 8, 12 and 16, a

total score is obtained by adding the scores for each question. A higher score indicates more severe

kinesiophobia.<sup>46</sup> The Dutch TSK is a valid and reliable tool for measuring fear of movement and fear of

(re)injury.<sup>47,48</sup>

#### **MRI measurements**

Magnetic resonance images were acquired with a Siemens (Berlin, Germany) Magnetom Trio Tim Syngo

MR B17 scanner with a 3T magnet. Parameters of the two point Dixon scan consisted of 6.59 ms

repetition time, 2.45 ms echo time 1 and 3.675 ms echo time 2. Voxel size was 0,7 x 0,7 x 3 mm and field

of view 320 x 340 mm. The thickness of the 40 slices was 3 mm. Subjects were placed supine in the

scanner with the head in a neutral position in order to minimize differences in posture that might affect

the results. Magnetic resonance imaging data were calculated blind to the subject's NP status.

#### *Imaging protocol*

Using the two point Dixon scan, in-phase (water) and opposed-phase (fat) images were obtained.

Defined regions of interest (ROIs) were manually identified across each included bilateral muscle on

these axial water and fat images. Regions of interest were established using images obtained from

different cervical levels: C0-C1 (top dens axis), and the upper part of the corpus of C2, C3, C4 and C5 for

the flexors and extensors. (Table 1)

The CSA of the included muscles was determined as the number of voxels in the corresponding ROI on a

T1-weighted image, multiplied by the size of the voxel. Then, the mean and standard deviation of both

fat and water slices were calculated. The signal intensities for fat (Sifat) and water (Slwater) were also

acquired via the ROIs. Muscle FI was calculated by the formula:  $Sifat \times 100 / (Sifat + Slwater)$ .

To calculate lean muscle CSA (mCSA), the following formula was applied:  $CSA \times (1 - FI)$ . In this formula, the fat signals on T1-weighted images were subtracted from the total CSA, representing the fat-free contractile elements of the muscle.

#### **Statistical Analysis**

All statistical analyses were performed using SPSS version 25. The normal distribution of age, body mass index (BMI), pain duration, NDI-score and TSK-score within each relevant group was checked using histograms, QQ-plots and by conducting a Shapiro-Wilk test. Because all continuous variables were not normally distributed, groups were compared using a non-parametric Kruskal-Wallis test. If findings were significant, a multi-comparison corrected post-hoc Mann-Whitney U test was performed. Because of its significance, only age was included as a covariate in further analyses.

Correlations between left and right, and between different levels were visually inspected with scatter plots, and investigated with Pearson correlations coefficients for all ROIs for FI, CSA and mCSA. Also, the correlation between BMI and FI, CSA and mCSA was examined this way. A Pearson correlation coefficient lower than .4 was considered a small correlation.

A random intercept model was estimated including group and level as main effects and group x level as interaction to compare FI, CSA and mCSA across the different groups (CWAD, CINP and HC) and different levels, including age as a variable of no interest. Group differences were analyzed by means of a Bonferroni correction post-hoc analysis to ensure a Family-wise error rate of .05.

To assess the association between muscle morphology and clinical factors, a random intercept model, including a variable of interest x group interaction, was constructed to examine between-group differences. Main effects were analyzed in case of insignificant interactions. Analyses were performed at a significance level ( $\alpha$ ) of .05.

## RESULTS

Demographic data for each group are presented in Table 2. Participants in the CINP ( $P<.001$ ) and CWAD ( $P<.001$ ) group were significantly older than in the HC group. The CWAD group had a significant higher ( $P=.001$ ) score on the NDI compared to the CINP group. No significant between-groups differences were found for BMI, pain duration and TSK. The correlation coefficient between muscle morphology and BMI was mostly small ( $<.4$ ).

### Fat Infiltration

When comparing CWAD with the HC group, FI in the CWAD group was significantly larger in four out of fourteen ROIs (C2: SeCa, SpCa, Trap; C3: SCM, Trap). For CWAD versus CINP, FI in the CWAD group was significantly larger in four out of fourteen ROIs (C2: SpCa, OCInf; C3: SCM, Trap). No significant differences in FI were found between HC and the CINP. (Table 3 and Figure 1)

### (Muscle) Cross-Sectional Area

The CSA of the HC group was significantly larger compared to the CWAD group in four out of fourteen ROIs (C2: SeCa; C4: LS, SCM, SeCa; C5: LS, SCM, Trap). In three ROIs (C1: LCa; C3: LCo; C5: Trap) the CSA of the HC group was significantly larger than these in the CINP group. For CWAD versus CINP, compared with the CINP group, the CSA of the CWAD group was significantly larger in two ROIs (C1: LCa; C4: LCo). By contrast, the CSA was significantly smaller in the CWAD group in one ROI (C5: LS) when comparing the CINP group and CWAD group. (Table 3 and Figure 2)

Similar results were obtained for mCSA, whereby in three out of fourteen ROIs (C2: SeCa; C4: LS and C5: LS, SCM) a significantly larger mCSA was identified in HC compared to CWAD. A significant larger mCSA was found in two ROIs (C3: LCo; C5 Trap) for HC compared to CINP. The CWAD group had significantly

1 larger mCSA in two ROIs (C1: LCo; C4: Longi) and significantly smaller mCSA in one ROI (C2: SeCa)  
 2 compared to the CINP group. (Table 3 and Figure 3)

### 3 **Associations with changes in muscle morphology**

4 A significant group-specific association was found between pain duration and both CSA and mCSA of the  
 5 Trap (respectively  $P < .001$ ,  $[-.3003 \text{--} .5386 \text{ mm}^2]$  and  $P < .001$ ,  $[-.2497 \text{--} .4563 \text{ mm}^2]$ ) and LCa (respectively  
 6  $P < .001$ ,  $[-.0487 \text{--} .1977 \text{ mm}^2]$  and  $P = .002$ ,  $[-.0416 \text{--} .1782 \text{ mm}^2]$ ) muscles in the CWAD group. Additionally,  
 7 there was a significant overall relationship between pain duration ( $\geq 3$  months) and CSA of the SeCa  
 8 ( $P = .017$ ,  $[-.0217 \text{--} .3247 \text{ mm}^2]$ ). (Table 4)

9 A significant group-specific association was found for the TSK score and FI of the SeCa in CINP ( $P = .044$ ,  
 10  $[-.0082 \text{--} .6088 \text{ mm}^2]$ ). For CWAD, a significant association between TSK score and CSA of the Multif was  
 11 found ( $P = .042$ ,  $[-.0825 \text{--} 4.3478 \text{ mm}^2]$ ). (Table 5)

12 No association between NDI and muscle morphology was found in both CINP and CWAD.

### 13 **DISCUSSION**

14 To date, results regarding changes in cervical muscle morphology of chronic NP patients are diverse.

15 Therefore, this study further investigated changes in muscle morphology in CWAD and CINP. A significant  
 16 increase in FI in specific muscles, possibly related to previous trauma, was found in CWAD compared to  
 17 HC and CINP. Besides injury, several explanatory models for this increase have been proposed: central  
 18 (e.g. chronic denervation)<sup>26,49,50</sup> and peripheral mechanisms (e.g. muscle strain)<sup>29</sup>, post-traumatic stress  
 19 symptoms<sup>15</sup>, increased inflammatory biomarkers<sup>51</sup>, fiber-specific differences in lipid content<sup>52,53</sup> and  
 20 functional muscle changes.<sup>54</sup> However, the exact underlying process remains unknown. Interestingly,  
 21 one systematic review also suggested an increased FI in cervical flexors and extensors.<sup>22</sup> **Two other,**  
 22 **more recent systematic reviews relying on studies using different measurement techniques, did not**



support the assumption of increased FI as a feature of CWAD.<sup>23,24</sup> No solid conclusions could be drawn in all these studies due to high heterogeneity in measurement techniques<sup>22,23</sup> and study sample, and small study cohorts.<sup>22–24</sup> Future longitudinal studies would benefit from consistent outcome measures and homogeneous, sufficiently large study cohorts.

This study showed no significant increased CSA or mCSA in CINP compared to HC. Instead, higher CSA and mCSA measures were found in HC and CWAD for the deeper flexor muscles and Trap. Previous literature presented similar findings, with an increased CSA of superficial, but not deep neck flexors.<sup>10,12,55</sup> The assumption could be made that muscle atrophy is only present in specific muscles in CINP, which could possibly be explained by avoidance behavior as a result of pain-related anxiety in these patients. However, this hypothesis was not reinforced within the results as the TSK score did not appear to play a pronounced role in between group differences for (m)CSA, indicating that kinesiophobia was no specific feature of CINP. Ris et al. confirmed this since they found a high TSK score in both traumatic and non-traumatic NP patients.<sup>56</sup> Furthermore, deep flexors atrophy may be part of a disturbed motor control strategy in which decreased activity of these muscles is associated with increased activity of the superficial flexors.<sup>54,57</sup> So, SCM pseudohypertrophy, and consequently an increased mCSA of this muscle would be expected, but this could not be confirmed in this study.

Previous systematic reviews identified inconsistent findings regarding (m)CSA of cervical muscles in CINP or CWAD.<sup>22–24</sup> Increased CSA as general finding in CWAD could be explained by increased FI<sup>22</sup>, but this study showed no significant increase in (m)CSA, indicating that FI as an indicator for increased CSA might not be valid. Also, not all former studies took muscle fat ratios into account, which may give a distorted picture since fat content is likely to alter and extend the musculo-fascial borders.<sup>20</sup>

For the CWAD group, results showed a plausible group-specific association between pain duration and changes in CSA (SeCa, Trap, LCa) and mCSA (Trap and LCa), possibly explained by the occurrence of

passive coping strategies affecting muscle morphology. Moreover, previous literature showed that a higher score on the TSK was related to longer NP duration.<sup>58</sup>

No association was found in between-group differences of (m)CSA and NDI scores in both NP groups. A study of Elliott et al. revealed that the manifestation of muscle FI occurs soon after whiplash, but only in those with higher disability.<sup>11,14,51</sup> However, no link was found between disability and CSA in WAD, which is confirmed by the current results.<sup>20</sup>

Consideration should also be given to natural muscle degeneration with aging. Thus, denervation of motor neurons could result in a secondary decrease in lean muscle mass<sup>59–62</sup> and atrophy of the neck muscles may occur from the fourth decade of life with an acceleration after the age of fifty.<sup>63,64</sup> Since this age-related increase in FI and decrease in CSA are reported later in life<sup>65,66</sup>, and the age of the study subjects was relatively young, age did not seem to have a causal influence on the reported results.

Some strengths and limitations were present in this study. A strength was that assessors were blinded to the different study groups, so potential detection bias was precluded. Moreover, gender was excluded as confounding factor, as only women were included in this study. However, the results can therefore not be generalized to a broader population including men. The use of MRI was also seen as an added value as it considered to be the gold standard for measuring muscle morphology, but again some considerations should be observed. Despite the fact that interpreting mCSA is an enhancement over previous studies in which only CSA was reported, it seems that 2D images may have partial volumes due to an increased sensitivity to the radio frequency slicing profile. Measuring muscle morphology based on 3D volume could be more precise for this purpose.<sup>12</sup> The applied mCSA calculation also fails to account for material that is invisible on MRI. The use of already existing, more sophisticated MRI applications is therefore recommended for subsequent research.<sup>67</sup> In parallel, manual segmenting the muscle or more time-efficient convolutional neural network segmenting may also help obtain more valid morphology

values in the future.<sup>14,68</sup> This in turns could lead to its implementation in clinical practice, complementary to radiology, in quantifying muscle composition.<sup>68</sup> To assess the prognostic or diagnostic value of MRI findings, studies should monitor people over time and gain access to MRI findings related to important patient outcomes.

Because of the cross-sectional design of this study, no comparisons according to muscle morphology were made pre- and post-injury. Also, because only chronic patients were included, it was not possible to differentiate between acute and chronic changes in muscle morphology. No reference scan of more distant muscles was made to exclude a general change in muscle morphology. This way, it would be possible to determine whether local or global factors influence changes in muscle morphology.<sup>28,69</sup> Furthermore, previous therapies regarding muscle strengthening were not questioned but these could possibly have an impact on changes in muscle morphology.

In conclusion, the results in this MRI-based study suggest changes in muscle morphology in both NP cohorts. While FI seemed to be a specific characteristic of the whiplash population, changes in CSA are mainly found in the CINP cohort, showing some similarities with earlier findings in this research domain. Further studies based on controlled longitudinal designs are needed to facilitate data pooling and to make stronger conclusions. This way, the impact of changes in muscle morphology on chronic NP could be demonstrated and this in turn could lead to a treatment optimization.

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TABLES

Table 1. Regions of Interest.						
		C1	C2	C3	C4	C5
flexors	LCa	x				x
	LCo			x		x
	SCM			x	x	x
extensors	Trap		x	x	x	x
	SpCa		x	x	x	
	LSc			x	x	x
	SeCa	x	x	x	x	
	Sp					x
	Mult			x		
	Longi				x	
	Group					x
	RCPMaj	x				
	OCSup	x				
	OCInf		x			

LCa, Longus Capitis; LCo, Longus Colli; SCM, Sternocleido-mastoid; Trap,Trapezius; SpCa, Splenius Capitis; LSc, Levator Scapulae; SeCa, Semispinalis Capitis; Sp, Spinalis; Mult, Multifidus; Longi, Longissimus Cervicis + Longissimus Capitis; Group, Splenius Cervicis + SpCa + SeCa + Longissimus Cervicis; RCPMaj, Rectus Capitis Posterior Major; OCSup, Obliquus Capitis Superior; OCInf, Obliquus Capitis Inferior.

**Table 2. Demographics (Mean (SD) [Range]) for CWAD, CINP and pain-free HC group.**

<b>Descriptive</b>	<b>CWAD</b>	<b>CINP</b>	<b>HC</b>
<b>n</b>	37	45	35
<b>Age (y)</b>	36.8 (11.6) [21-59]	39.0 (12.5) [18-63]	31.2 (12.9) [18-62]
<b>BMI (kg/m<sup>2</sup>)</b>	22.5 (3.7) [16.7-32.1]	22.8 (2.8) [18.3-29.1]	21.7 (2.0) [18.1-26.8]
<b>NDI (/50)</b>	22.4 (6.7) [10-37]	16.9 (5.1) [10-27]	NA
<b>Pain Duration (m)</b>	85.3 (87.3) [3-444]	91.9 (87.6) [4-300]	NA
<b>TSK (/68)</b>	34.5 (6.3) [21-47]	34.3 (5.3) [27-45]	NA

CWAD, Chronic Whiplash Associated Disorder; CINP, Chronic Idiopathic Neck Pain; HC, Healthy Control; SD, standard deviation; y, years; kg, kilogram; **m, months**; m<sup>2</sup>, square meter; NDI, Neck Disability Index; m, meter; TSK, Tampa Scale for Kinesiophobia; NA, not applicable.

**Table 3. Between-group differences for FI, CSA and mCSA per level**

ROI		AIC	Group	Level	Group x Level	Age
<b>LS</b>	CSA	698.951	.013*	< .001*	.003*	.751
	mCSA	571.139	.015*	< .001*	.007*	.466
<b>Longi</b>	CSA	17.097	.006*	NA	NA	.345
	mCSA	-26.105	.009*	NA	NA	.586
<b>SeCa</b>	FI	-1735.718	.203	< .001*	.026*	.001*
	CSA	1209.233	.350	< .001*	< .001*	.244
	mCSA	1021.731	.137	< .001*	< .001*	.977
<b>SpCa</b>	FI	-1749.872	.005*	< .001*	.026*	< .000*
<b>SCM</b>	FI	-1459.655	.032*	< .001*	.001*	.120
	CSA	588.551	.169	< .001*	< .001*	.242
	mCSA	513.034	.050	< .001*	< .001*	.157
<b>Trap</b>	FI	-1614.950	.104	< .001*	< .001*	.035*
	CSA	1061.249	.024*	< .001*	.011*	.021*
	mCSA	922.420	.027*	< .001*	.048*	.027*
<b>LCa</b>	CSA	-70.728	.071	< .001*	.020*	.650
	mCSA	-122.443	.097	< .001*	.064	.532
<b>LCo</b>	CSA	-97.674	.338	< .001*	.040*	.019*
	mCSA	-174.028	.416	< .001*	.040*	.002*
<b>Mult</b>	CSA	169.813	.047*	NA	NA	.743
<b>OCInf</b>	FI	-533.559	.001*	NA	NA	.093

Only the ROIs for which a significant between-group difference was found are shown in the table. ROI, Region of Interest; AIC, Akaike's Information Criterion; LS, Levator Scapulae; Longi, Longus Capitis + Colli; SeCa, Semispinalis Capitis; SpCa, Splenius Capitis; SCM, Sternocleidomastoideus; Trap, Trapezius; LCa, Longus Capitis; LCo, Longus Colli; Mult, Multifidus; OCInf, Obliquus Capitis Inferior; FI, fat infiltration; CSA, cross-sectional area; mCSA, muscle cross-sectional area; NA, not applicable;

\* significance  $P < .05$ .

**Table 4. Association between pain duration and between-group differences in muscle morphology**

ROI		AIC	Group* $\beta \pm SD$ (P)	Pain duration $\beta \pm SD$ (P)	Group* x Pain duration $\beta \pm SD$ (P)
SeCa	CSA	923.912		.002 $\pm$ .001 (.017)	
	mCSA				
Trap	CSA	814.539	.422 $\pm$ .101 (<.001)	.004 $\pm$ .001 (<.001)	-.004 $\pm$ .001 (<.001)
	mCSA	724.182	.357 $\pm$ .087 (<.001)	.004 $\pm$ .001 (<.001)	-.004 $\pm$ .001 (<.001)
LCa	CSA	84.953	.005 $\pm$ .063 (.940)	.001 $\pm$ .000 (.018)	-.001 $\pm$ .001 (.023)
	mCSA	50.673	.017 $\pm$ .057 (.771)	.001 $\pm$ .000 (.025)	-.001 $\pm$ .000 (.023)

\*Chronic whiplash associated disorder is the reference group; ROI, Region of Interest; AIC, Akaike's Information Criterion; Longi, Longus Colli + Longus Capitis; SeCa, Semispinalis Capitis; Trap, Trapezius; LCa, Longus Capitis; FI, fat infiltration; CSA, cross-sectional area; mCSA, muscle cross-sectional area; P, significance < .05.

**Table 5. Association between TSK and between-group differences in muscle morphology**

ROI		AIC	Group* $\beta \pm SD$ (P)	TSK $\beta \pm SD$ (P)	Group* x TSK $\beta \pm SD$ (P)
SeCa	FI	-1031.305	-.157 $\pm$ .069 (.028)	-.001 $\pm$ .001 (.400)	.004 $\pm$ .002 (.028)
Mult	CSA	119.190		.002 $\pm$ .011 (.008)	

\*Chronic whiplash associated disorder is the reference group; ROI, Region of Interest; AIC, Akaike's Information Criterion; TSK, Tampa Scale Kinesiophobia; SeCa, Semispinalis Capitis; FI, fat infiltration; CSA, cross-sectional area; P, significance < .05.



LEGENDS

FIGURE 1




-  Healthy Controls
-  Chronic Idiopathic Neck Pain
-  Chronic Whiplash Associated Disorder

Figure 1 displays estimated marginal means for fat infiltration with age as a confounder. Error bars representing + and – 1 standard error.

- \* Chronic Whiplash Associated Disorder significant larger than Healthy Controls
- ◆ Chronic Whiplash Associated Disorder significant larger than Chronic Idiopathic Neck Pain

LEGENDS

FIGURE 2




-  Healthy Controls
-  Chronic Idiopathic Neck Pain
-  Chronic Whiplash Associated Disorder

Figure 2 displays estimated marginal means for cross-sectional area with age as a confounder. Error bars representing + and – 1 standard error.

- \* Healthy Controls significant larger than Chronic Whiplash Associated Disorder
- ◆ Healthy Controls significant larger than Chronic Idiopathic Neck Pain
- ◇ Chronic Whiplash associated disorder significant larger than Chronic Idiopathic Neck Pain
- ▣ Chronic Idiopathic Neck Pain significant larger than Chronic Whiplash Associated Disorder

LEGENDS

FIGURE 3




-  Healthy Controls
-  Chronic Idiopathic Neck Pain
-  Chronic Whiplash Associated Disorder

Figure 3 displays estimated marginal means for muscle cross-sectional area with age as a confounder. Error bars representing + and – 1 standard error.

- \* Healthy Controls significant larger than Chronic Whiplash Associated Disorder
- ◆ Healthy Controls significant larger than Chronic Idiopathic Neck Pain
- ◇ Chronic Whiplash associated disorder significant larger than Chronic Idiopathic Neck Pain
- ▣ Chronic Idiopathic Neck Pain significant larger than Chronic Whiplash Associated Disorder

Figure 1. Between group differences for fat infiltration

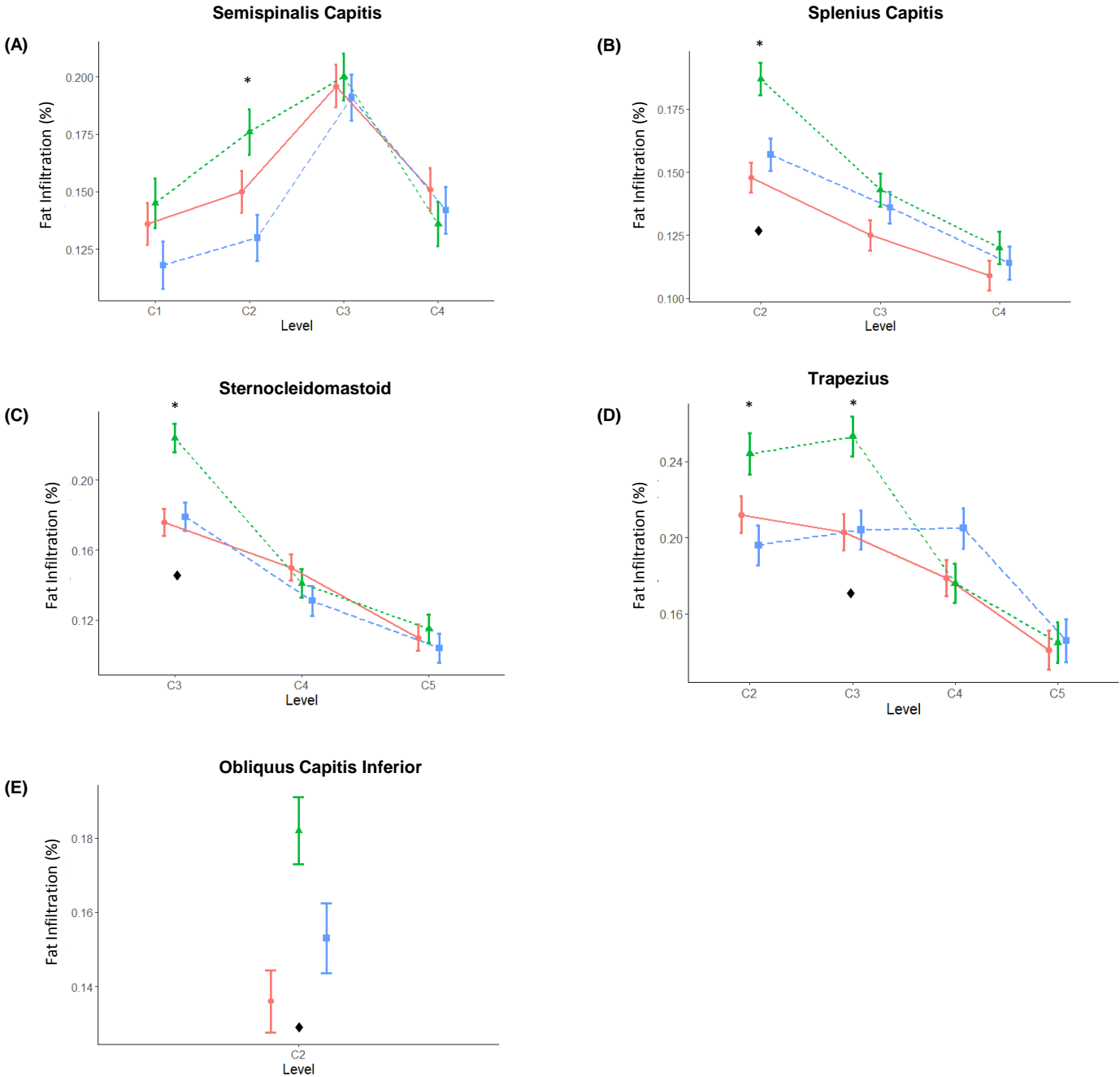
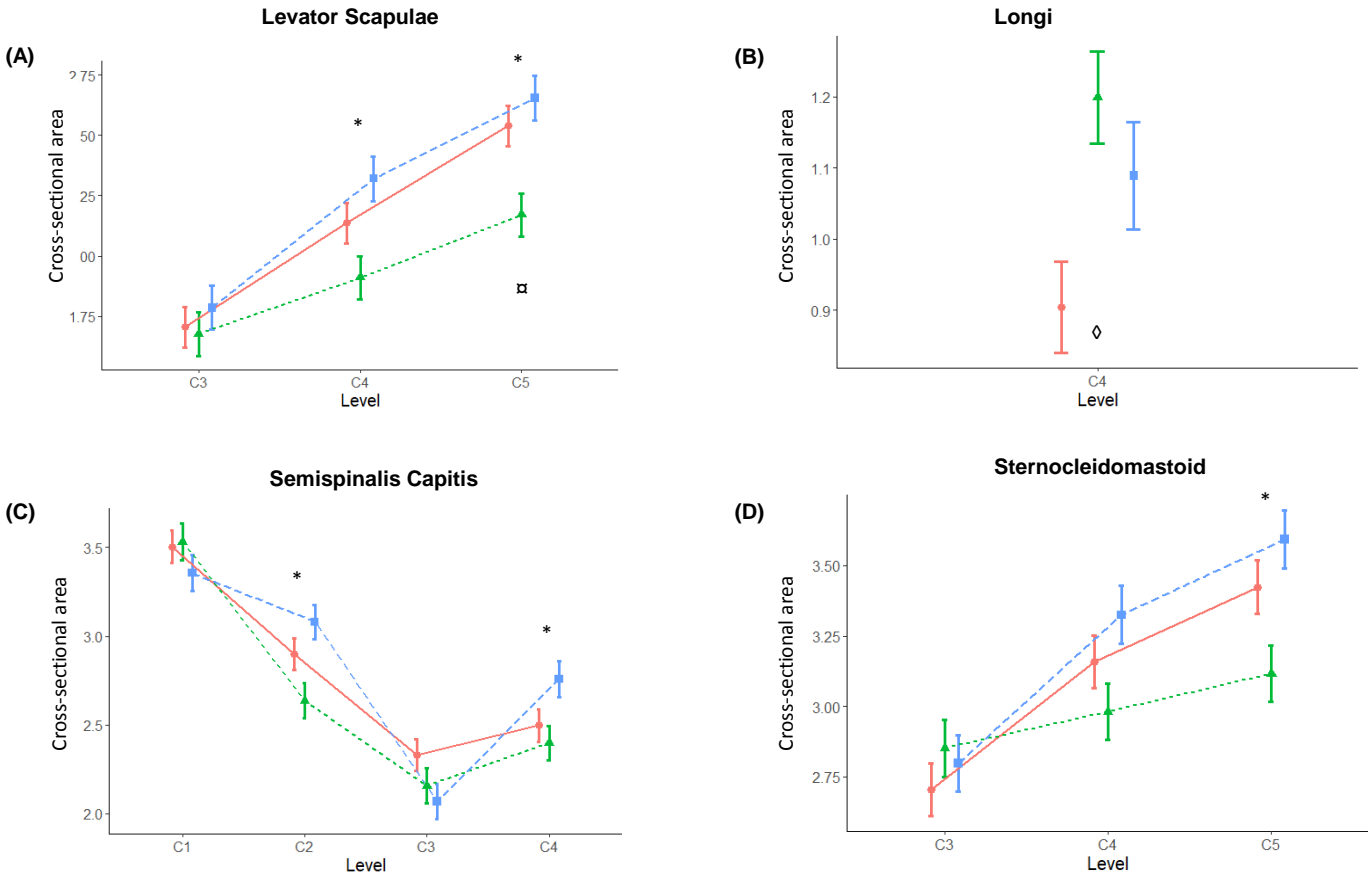


Figure 2. Between group differences for cross-sectional area



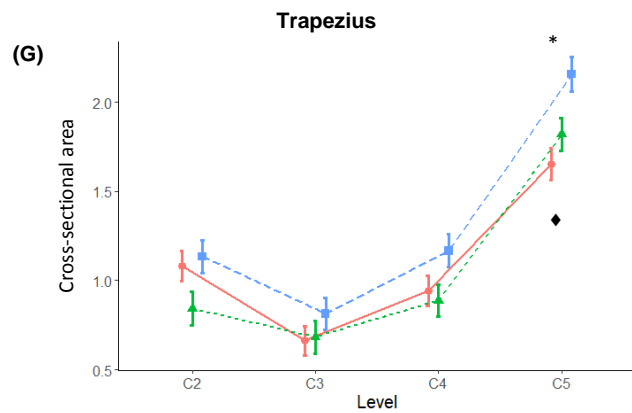
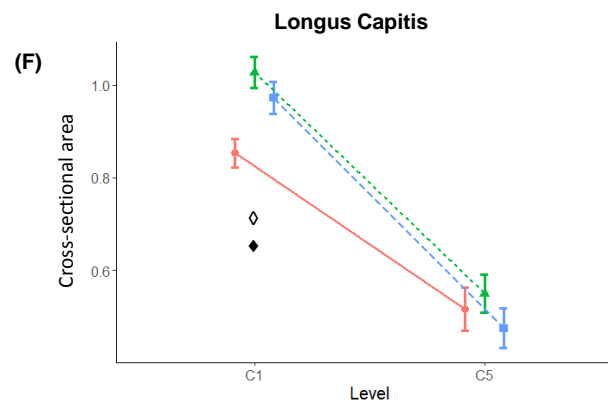
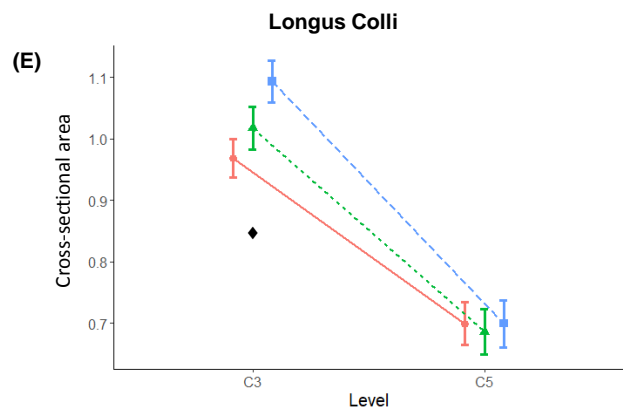


Figure 3. Between group differences for muscle cross-sectional area

