CHANGES IN DORSAL NECK MUSCLE FUNCTION IN INDIVIDUALS WITH CHRONIC WHIPLASH-ASSOCIATED DISORDERS: A REAL-TIME ULTRASOUND CASE-CONTROL STUDY

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Changes in dorsal neck muscle function in individuals with chronic whiplash-associated disorders: a real-time ultrasound case-control study

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ABSTRACT

Impaired neck muscle function leads to disability in individuals with chronic whiplash-associated disorder (WAD), but diagnostic tools are lacking. In this study, deformations and deformation rates were investigated in five dorsal neck muscles during 10 arm elevations by ultrasonography with speckle tracking analyses. Forty individuals with chronic WAD (28 women and 12 men; mean age 37 years) and 40 healthy controls matched for age and sex were included. The WAD group showed higher deformation rates in the multifidus muscle during the first ($p < 0.04$) and tenth (only women; $p < 0.01$) arm elevations compared with the control group. Linear relationships between the neck muscles for the deformation rate (controls; $R^2 = 0.24 – 0.82$, WAD; $R^2 = 0.05 – 0.74$) and deformation of the deepest muscles (controls; $R^2 = 0.61 – 0.32$, WAD; $R^2 = 0.15 – 0.01$) were stronger for women in the control group versus women with WAD, indicating there is altered interplay between dorsal neck muscles in chronic WAD.

Keywords: Whiplash injury; ultrasonography; neck muscles; spine

INTRODUCTION

The annual incidence of whiplash injury is 200-300 per 100 000 in the general population (Holm et al. 2008, Styrke et al. 2012) and is defined as a sudden acceleration-deceleration movement of the head that can cause both joints and soft tissue injuries in the neck (Siegmund et al. 2009). Despite decades of research, the mechanisms responsible for the burden in chronic whiplash-associated disorders (WAD) are not well understood and diagnostic tools are lacking. The neck muscles surrounding the cervical spine and the deep neck muscle layers play an important role in maintaining a stable base for the postural control of the neck (Mayoux-Benhamou et al. 1994, Panjabi 1992). Impaired ventral neck muscle function has been reported in chronic neck pain (Falla et al. 2004, Falla et al. 2011, Jull et al. 2004) and WAD (Cagnie et al. 2010, Elliott et al. 2010, Jull et al. 2004, Peterson et al. 2015a, Sterling et al. 2003), but the function of the neck extensor muscles in WAD are not as well studied. Elliott et al. (2008a) demonstrated fatty infiltrate in the extensor muscle in WAD using magnetic resonance imaging (MRI). Functional MRI studies have reported altered neck muscle activity after induced pain in healthy individuals (Cagnie et al. 2011) and in individuals with neck pain (O'Leary et al. 2011). Electromyography (EMG) studies have shown that not all dorsal muscles act synergistically as extensors; the splenius capitis muscle can activate during neck flexion in some healthy individuals (Keshner et al. 1989, Siegmund et al. 2007) and higher levels of extensor muscle co-activation in neck pain have been reported (Lindstrom et al. 2011). Surface EMG has demonstrated increased activity in superficial neck extensors in WAD (Bexander et al. 2012, Juul-Kristensen et al. 2013), but when controlling for differences in movement velocity, no differences were found between WAD and healthy controls (Vikne et al. 2013). Small invasive EMG studies have reported decreased and less defined activity in the deep semispinalis cervicis muscle in WAD (Schomacher et al. 2012), and the multifidus muscle was affected by eye movement

(Bexander et al. 2012). Both the expensive MRI and invasive EMG are difficult to apply in routine clinical practice and MRI does not provide information on the interplay between muscle layers. Real-time ultrasound imaging measures the mechanical function in the muscle, the elongation and shortening of the muscle (i.e., deformation) and how fast the deformation occurs (i.e., deformation rate). This method has been used to detect altered ventral neck muscle function in individuals with chronic WAD (Peterson et al. 2015b) and altered muscle function in the semispinalis capitis muscle after decompression surgery for cervical disc disease (Peolsson et al. 2015). The strength of real-time ultrasound is that it can non-invasively evaluate both superficial and deep muscle function simultaneously. To the best of our knowledge, this method has not been applied to investigate dorsal neck muscle function in WAD.

The aim of the present study was two-fold: to compare mechanical neck muscle function, deformation, and deformation rate in five dorsal neck muscles in individuals with chronic WAD versus healthy controls during repetitive arm elevation, and to investigate if the interplay in deformation and deformation rate between these muscles differs between the WAD and control groups.

METHODS

Participants

Forty individuals (28 women and 12 men; mean age 37 years, SD 11.2) with chronic WAD, including 29 individuals with WAD grade II (neck pain and musculoskeletal signs) and 11 individuals with WAD grade III (neck pain plus neurological signs), were included in the study. The participants were recruited consecutively for ultrasound investigation among patients enrolled in a randomized controlled trial (Peolsson et al. 2013). Inclusion criteria were persistent symptoms associated with a whiplash injury 6 months to 3 years prior to study entry; WAD grade II or III; age 18 – 63 years; persistent neck pain rated greater than 20 mm and/or neck disability index (NDI) (Vernon and Mior 1991) greater than 20%; dominant neck pain on the right side of the neck; and right-handedness. Exclusion criteria were signs of traumatic brain injury at the time of whiplash injury; known or suspected serious pathology; previous fracture or luxation in the cervical spine; contraindication to exercise; neuromuscular diseases; rheumatologic disease; previous neck pain causing more than 1 month of sick leave in the year before the whiplash injury; severe mental illness; current alcohol or drug abuse; or an inability to understand spoken and written Swedish.

For comparison, 40 healthy controls matched for age and sex (mean age 37 years, SD 11.4) were recruited from university staff, hospital staff, and acquaintances. Exclusion criteria were present or past neck problems; trauma to the neck or head, including whiplash injury; neck or low back pain; rheumatologic or neurological disease; or generalized myalgia.

The baseline characteristics of the participants are provided in Table 1. The study was approved by the Regional Ethics Review Board and was conducted according to the Declaration of Helsinki. Written informed consent was obtained from all participants. The study had no adverse effects.

Ultrasound measurement

To evaluate the dorsal neck muscles we used a B-mode, 2-D ultrasound Vivid-i scanner (GE Healthcare, Horten, Norway) and hand-held 12 MHz linear array transducer (12L-RS, footprint 39 mm, GE Healthcare, Horten, Norway) with high frame rate (235 frames/s). Five dorsal neck muscles (upper trapezius, splenius capitis, semispinalis capitis, semispinalis cervicis, and multifidus/rotatores) (Fig. 1a and 1b) were recorded during 10 repetitive arm elevations (Fig. 1c and 1d). Measurements were made from the ultrasound movies during the first and tenth arm elevations at the level of the 4^{th} cervical vertebrae, which was identified by palpation of the C4 spinous process.

Speckle tracking

Real-time ultrasound imaging of skeletal muscle is able to detect the unique speckle pattern in muscles and can be post-processed analyzed using the speckle tracking method based on an algorithm developed by Kanade-Lucas-Tomasi (Lucas and Kanade 1981, Tomasi and Kanade1991) and Farron et al. (2009). A region of interest (ROI) was manually placed in the recorded muscle images and made it possible to track the unique speckle pattern frame by frame through the ultrasound video sequence. Each ROI consisted of a large number of measuring points and was placed in the first frame in the "video" sequence, following the frame to frame deformation throughout the ultrasound imaging. When the speckle pattern changes length during muscle activity, so does the length of the ROI. This provides measurement of *muscle deformation* (elongation or shortening) and was calculated as the percentage change in the ROI from the first original frame length of the ROI (expressed as % deformation). The *muscle deformation rate* was expressed as the deformation per time unit (% deformation/s).

Three rectangular ROIs (each 10 x 3.3 mm) were manually placed longitudinal to the muscle fibers in each muscle; together, the three ROIs covered 30 mm of the muscle of interest. The areas under the deformation curves (Fig. 1e) were calculated to measure the muscle deformation. The trapezoidal rule was used as a basis for evaluating the area (Equation 1), where A is the area, t is time between samples, and y_n is the present ROI position at sample point n. The equation was modified to handle intersections with the 0% line. To estimate additional sample points, linear interpolation was used with adjusted t-values at intersections with the 0% line. Thus, the area under and the area above the 0% line could be separated.

$$A = \frac{t}{2}(y_1+2y_2+2y_3+..+2y_{n-2}+2y_{n-1}+y_n) \qquad \text{(Equation 1)}$$

The speckle tracking analysis method has been shown to have excellent test-retest reliability (ICC 0.71-0.99) (Peolsson et al. 2015), and the magnitude of muscle deformation was positively related to force measurements and progressive electrical stimulation (Lopata et al 2010).

Other measurements

Before ultrasound measurements, all participants completed a set of questionnaires asking about age, gender, average pain intensity over the prior week (visual analogue scale [VAS], 0 = no pain, 100 = worst imaginable pain) (Carlsson 1983), and neck disability (NDI, 0% = no disability, 100% = highest score for disability) (MacDermid et al. 2009). Other baseline characteristics, such as WAD grade, body mass index (BMI), neck fatigue (Borg CR-10 scale, 0 = no fatigue, 10 = extremely strong fatigue) (Borg 1990), and activity level (activity index, 1 = inactivity, 2 = low activity, 3 = moderate activity, 4 = high activity) (Kallings et al. 2008), were also recorded (Table 1).

Test procedure

The test procedure was designed to evaluate neck muscle function during repeated arm elevation. In our clinical experience, increased pain is commonly described in patients with WAD after arm lifts, and activity-related increases in pain have been reported (Sullivan et al. 2010). The participants stood in an upright and comfortable position, their feet behind a line marked on the floor, and performed 10 arm elevations from 0 to 90 degrees. Two qualified physiotherapists performed the ultrasound test; one completed the ultrasound examination and the other instructed the participants and assisted the ultrasound examiner. The ultrasound transducer was placed on the right side of the neck at the level of the 4th cervical vertebrae and adjusted for clear ultrasound imaging of the five dorsal neck muscles and then, the test was performed. (Fig. 1c and 1d).

Ultrasound analyses

The post-processing speckle tracking methodology was provided with a program designed in-house for Matlab 2014a (The Mathworks Inc, Natick, MA, USA). The area was based on the curve of the changes in deformation and calculated to evaluate muscle deformation (Fig. 1e). The deformation rate, is negative during shortening and positive during elongation, and was expressed as the root mean square (RMS), which gives information on the local muscle velocity of deformation. An independent researcher coded all ultrasound images with a number to ensure that the person with three years of experience in speckle tracking analysis was blinded to group affiliation during post-processing analysis.

Statistical analyses

Statistical analyses were performed using SPSS software (IBM SPSS, Statistics for windows, Version 22.0, Armonk, NY). The participants' demographic characteristics were compared between groups using the two-tailed unpaired Students t-test for parametric data. For non-parametric demographic data the chi-squared test was used, and physical activity levels and neck muscle fatigue were analyzed using the Mann Whitney U test (Field, 2009). Deformation and deformation rate were normally distributed and parametric statistical tests were applied. Six outliers (defined as deviating more than three times the interquartile range) were detected in deformation, three in the WAD group (two men and one woman) and three in the control group (all men). Seven outliers were identified in deformation rate (one in the WAD group and six controls, all men). These outliers were excluded because they impacted the results of the analysis and statistics derived from data that include outliers may be misleading. A mixed design analysis of variance (ANOVA) with Bonferroni correction was used to evaluate between-subject factor of group (two levels: WAD and controls) and within group factor of deformation and deformation rate (five levels, one for each muscle), and the analyses were adjusted for the duration of each arm elevation and sex (Field, 2009). The assumptions of variance were violated (Levene´s test $p < 0.05$) in deformation (first arm elevation) and deformation rate (tenth arm elevation) therefore, the data were log^{10} transformed (Field, 2009).

To evaluate the interplay between muscles, the statistical analyses were performed as follows. The first step was simple linear regression to investigate the relationships between pairs of muscles in individuals in the WAD and control groups regarding the total deformation area and the deformation rate (RMS). As men had much greater variation in deformation and deformation rate with many outliers, only women were further analyzed regarding the linear relationship between muscles and multivariate analyses. Adjusted R^2 values were reported and the strength of the linear relationship reported as follows: weak, 0.1 – 0.3; moderate, 0.31 – 0.6; strong, 0.61 – 0.9 (Dancey and Reidy 2014). The second step was multivariate analysis able to analyze the highly correlated five muscle layers simultaneously (Simca 13.0) (Eriksson 2013). Principal component analysis (PCA) is designed to extract systematic variation in the dataset and was utilized to obtain an overview of the data. Partial least squares discriminant analysis (PLS-DA) is a regression extension of PCA that was used to analyze the differences between the WAD and control groups, and orthogonal partial least squares discriminant analysis (OPLS-DA) is able to improve interpretability (Eriksson 2013). The strength in the multivariate statistics is the possibility to identify underlying patterns in large datasets from complex neck muscle interaction data. The data analyzed with the multivariate techniques comprised 40 variables, including the total deformation area, elongation and shortening area, and deformation rate from the five dorsal neck muscles during the first and tenth arm elevations (4 deformation variables x 5 muscles x 2 arm elevations = 40 variables). All possible two-way combinations of interaction terms, including quadratic terms, between these 40 variables were evaluated, resulting in a total of 820 variables. Each variable was mean centered and scaled to unit variance prior to calculating the interaction between the variables. The interaction can be defined as the element-wise multiplication (Equation 2) between variables a and b; $\bar{x}_a$ and $\bar{x}_b$ are the means of the two variables a and b and s_a and s_b are the standard deviations. The quadratic terms were logarithmically transformed with base 10 prior to further analysis.

$$i_{ab} = \frac{a-\bar{x}_a}{s_a} \circ \frac{a-\bar{x}_b}{s_b} \qquad \text{(Equation 2)}$$

The explained variance in the X-matrix (deformation and deformation rate variables) was determined by $R^2\mathbf{X}$, the explained variance in the Y-matrix (control or WAD) by $R^2\mathbf{Y}$, and the predictive explained variance in **Y** by $Q^2\mathbf{Y}$. The maximum values is 1.0 and denotes a perfect model.

Statistical significance was set at $P \leq 0.05$.

RESULTS

Comparisons of deformation in the WAD and control groups

We found no significant group by deformation interaction effect (F = 0.2 to 2.0, p > .10) or differences between groups (F = 0.02 to 0.55, p > 0.46), but a significant group by sex interaction effect for both the first (F = 3.9, p < 0.01) and tenth (F = 2.7, p < 0.04) arm elevations. Men in the control group had greater deformation in all muscles (F = 11.3 to 35.5, p < 0.001) compared to women, but this difference was not seen in the WAD group (F = 1.3 to 0.39, p > 0.25). (Supplementary Table 1).

Comparisons deformation rate in the WAD and control groups

We found a significant group by deformation rate interaction effect for the first ($F = 3.3$, $p < .04$) and tenth ($F = 5.9$, $p < .01$) arm elevation and a significant main effect between the sexes ($F = 25.0$ to 19.1, $p < .001$). The WAD group had higher deformation rates in the multifidus muscle during the first and tenth (only women) arm elevations compared to the control group. Men had a higher deformation rate in all muscles compared to women in both the WAD ($F = 5.6$ to 7.4, $p < .03$) and control groups ($F = 18.9$ to 19.3, $p < .001$). (Supplementary Table 2).

Interplay in deformation between pairs of muscles in women in the WAD and control groups

Women in the WAD group exhibited moderate relationships between more pairs of muscles during the first arm elevation compared to controls ($R^2 = 0.32$ to 0.50). Compared to the WAD group, controls had stronger positive linear relationships between the two deepest neck muscles (semispinalis cervicis and multifidus) during the first ($R^2 = 0.61$ vs. $R^2 = 0.15$) and tenth arm elevations ($R^2 = 0.32$ vs. $R^2 = 0.01$) (Table 2a).

Interplay in deformation rate between pairs of muscles in women in the WAD and control groups

In the control group, we found stronger positive relationships between the muscles during both the first and tenth arm elevations ($R^2 = 0.24$ to 0.76) compared to the WAD group ($R^2 = 0.05$ to 0.74), except for trapezius/semispinalis cervicis and semispinalis capitis/semispinalis cervicis during the tenth arm elevation (Table 2b). In the control group, the deep multifidus muscle had a stronger relationship with the other four investigated muscles than in the WAD group during both the first ($R^2 = 0.24$ to 0.56 vs. 0.05 to 0.41) and tenth arm elevations ($R^2 = 0.49$ to 0.68 vs. 0.07 to 0.64) (Fig. 2).

Multivariate analyses of deformation and deformation rate

PCA was used to compress the 40 deformation area and deformation rate variables into a model with four components and an R^2X of 0.585. The score plot of the two first components (Fig. 3a), explaining 35.0% and 8.7%, respectively, is an overview in which each dot corresponds to one person. Several outliers (i.e., dots outside the elliptical 95% confidence region) were seen, predominantly among males (colored in blue) with only one female outlier. Moreover, males (blue dots) were essentially positioned on the right side of plot, whereas females were on the left. No differences between individuals with WAD and controls were detected in any of the four PCA components when the color was set according to group status (WAD/control).

Similar to the first model, PCA modelling of the complete data set with interaction terms (40 + 820 variables) revealed that men exhibited a much larger variation (not shown) with many severe outliers. Consequently, the more homogenous female group was analyzed using PLS-DA to find underlying structures that could separate patients from controls. Four out of 56 individuals were considered to be outliers (three WAD and one control) and were removed from further modelling. Only a weak relationship was found (R^2Y 0.36 and $Q^2Y = -0.22$) between the deformation areas/rates and their interactions. Any attempt to add model components resulted in further worsening of the prediction efficiency Q^2Y.

Guided by the results of the univariate analysis of the deformation rate in the first and tenth elevations, in which the WAD group had significantly higher rates for the multifidus muscle, attempts were made to only include deformation rates and their interactions in the subsequent OPLS-DA modelling, which only included females and excluded the same individuals as the previous model. The best model only included strain rate deformations, as no interaction terms improved the model. The final model using the ten deformation rate variables (for the five muscles and first and tenth elevations) had an R^2Y of 0.468 and Q^2Y of 0.158 using one predictive and three orthogonal components. The loading plot (Fig. 3b) shows the relationship between the deformation rate variables and their impact on group status (WAD/control) y. The main contributors to separating WAD from controls are the multifidus deformation rates for the first and tenth elevations. The cross-validated score plot (Fig. 3c) did not exhibit a clear separation between patients and controls, but WAD (in red) resided primarily on the right side of the plot.

DISCUSSION

The main result from this study was a significant difference in deformation rate with a higher rate in the deepest multifidus muscle in individuals with WAD compared to healthy controls during the first and tenth (only women) arm elevations. There were also differences in deformation and the deformation rate for women regarding the interplay between muscle pairs. Regarding deformation, we identified a stronger positive linear relationship between the two deepest muscles in women in the control group compared to women in the WAD group. During the first and tenth arm elevations, women in the control group had a stronger positive linear relationship between the deformation rates of most of the investigated dorsal neck muscles compared to women in the WAD group. Men had much greater variations in mechanical neck muscle function but subgroup analyses of men were not possible due to too few men being included in the study. We found no other differences in interactions between muscles.

The deformation rate measures the rate during shortening and elongation deformation and was higher in the multifidus muscle in the WAD group. We speculate that a higher deformation rate indicates a muscle with less smooth activation, and that higher and oscillating rates may indicate ineffective muscle function. The interplay between the multifidus and the other four neck muscles had a weaker relationship in women in the WAD group, indicating an irregular interplay between the dorsal neck muscles, especially between superficial and deep neck muscles. Other studies have reported impaired neck muscle function in the deepest muscles; reduced activity in the semispinalis cervicis after induced pain in healthy subjects (Cagnie et al. 2011), altered activity in the multifidus during neck rotation in combination with eye movement in WAD subjects and authors speculated it could indicate less muscular support to stabilize the cervical spine (Bexander et al. 2012), and that higher amount of fatty infiltrate in the multifidus in women with WAD compared to women with insidious-onset neck pain (Elliott et al. 2008b) may be the results of structural damage, nerve injury or disuse of the muscle.

There were no differences between the WAD and control groups in the deformation of the five dorsal muscles. Large variation between individuals was observed with large standard deviations in deformation group data (Supplementary file 1). For women, a strong positive linear relationship was found between the deepest neck muscles (semispinalis cervicis and multifidus) in the control group, but not in the WAD group. This finding is in accordance with an ultrasound study of the interplay between ventral neck muscles (Peterson et al. 2015a) in which an individual muscle pattern was obtained with a stronger linear relationship between ventral neck muscles in healthy controls compared to individuals with WAD. The neck muscle layers support the cervical spine and the deep neck muscles in particular are involved in the intersegmental motion that is very important for postural control (Panjabi 1992, Mayoux-Benhamou et al. 1994). Increased motion in the lower cervical spine was observed among significantly more women with chronic WAD (Kristjansen et al. 2003) than non-traumatic neck pain, and reduced head steadiness was related to increased neck pain and dizziness in individuals with chronic WAD (Woodhouse et al. 2010). Moreover, Elliott et al.

revealed a larger cross-sectional area in the multifidus muscles in women with chronic WAD than healthy controls (Elliot et al. 2008a) and non-specific neck pain (Elliot et al. 2008b), which reflects the higher amount of fatty infiltrate in the extensor neck muscles in WAD. Speculation as to the cause of fatty infiltrate in neck muscles in WAD involves a persistent inflammatory response to injured neck tissues, minor nerve injury, and/or disuse of the muscles. The increased cross-sectional area in the multifidus muscle (Elliott et al. 2008a) may also indicate overuse of the multifidus muscle with weaker interplay with the other neck muscles and higher deformation rate. The direct attachment of the multifidus to cervical facet capsules (Andersson et al. 2005) may be a source of increased neck pain in WAD, especially if the muscle has a higher deformation rate and weaker interplay with the other neck muscle layers. The present study investigated mechanical neck muscle function during a postural task in which the deep neck muscles are assumed to maintain a stable cervical spine (Panjabi 1992, Peterson 2004), and it seems reasonable that these muscles and the deep ventral muscles (Peterson et al. 2015a) need to be activated to support the cervical spine for postural control. The present study demonstrated altered function in the deep dorsal neck muscles in women with chronic WAD, which may decrease muscular support in the maintenance of a stable cervical spine and explain the persistent pain and disability in WAD. The findings are important for diagnosis and the development of exercise programs for individuals with chronic WAD.

The higher values and greater variations in both deformation and deformation rate among men indicate different mechanical muscle function compared to women, but only 12 men were included in the study, which is not enough for subgroup analyses of men. Men with WAD and healthy men also had a larger spread in interactions between ventral neck muscles compared to women (Peterson et al. 2015b). The differences in muscle characteristics, with larger muscles size and greater strength in men compared to women (Cote 2012), may explain the greater variations in interactions between dorsal neck muscles. To date, few studies investigating neck extensor muscles have included men. Only women have been investigated for fatty infiltrate (Elliott et al. 2008a) and superficial (Juul-Kristensen et al. 2013) and deep neck muscle activation (Schomacher et al. 2012). Some small studies have had an unequal distribution of men and women in the control group compared to the intervention group (Bexander et al. 2012, O'Leary et al. 2011), and small sample sizes in studies with more equal gender distribution (Cagnie et al. 2011, Vikne et al. 2013) make it difficult to detect differences between males and females. To the best of our knowledge, no analyses of sex have been reported. Further studies are needed to increase our knowledge about neck muscle function in men with chronic WAD.

Multivariate PCA and OPLSA-DA analyses allow the simultaneous investigation of many variables despite multicollinearity and the detection of underlying structures in the data set to find models (Eriksson 2013). In this study the analyses were applied to investigate deformation rate, total deformation area, and the elongation and shortening area in the five dorsal neck muscles. For women, the final model had limited predictive value but showed that the deformation rate in the multifidus muscle separates individuals with WAD and controls.

Studies of the neuromuscular control in the cervical spine have revealed a complex muscle activation pattern. Healthy individuals exhibit different preferred activation of the splenius as a neck extensor or flexor (Blouin et al. 2007, Keshner et al. 1989, Siegmund et al. 2007), synchronized neuronal drive between deep and superficial muscles (Blouin et al. 2007), and different activation strategies among individuals during a reflexive task (Siegmund et al. 2007). Individuals with WAD had less defined activity in the deep semispinalis capitis (Schomacher et al. 2012) and higher activity in the superficial neck extensor has been reported (Juul-Kristensen et al. 2013), but when controlling for movement velocity, the acceleration or deceleration peak in the splenius was not different from that of healthy controls (Vikne et al.2013). The different methods make it challenging to interpret results from different studies. EMG measures the muscle action potentials when the muscle fibers are neurologically activated, whereas ultrasound measures the mechanical muscle function. Different EMG tests result in different neck muscle activation patterns; if the head is firmly clamped and force applied to the head, preferred activation was seen in the neck muscles except for the splenius capitis (Keshner et al. 1989, Siegmund et al. 2007), whereas more individual muscle responses occur during reflexive movements (Siegmund et al. 2007). Taken together, the results of the present study suggest altered dorsal neck muscle function in women with chronic WAD in accordance with earlier observations of structural (Elliott et al. 2008a) and muscular impairment (Bexander et al. 2012, Cagnie et al. 2011) in these muscles.

Limitations

The present study has several limitations. Ultrasound measurements of local deformation in muscles have been validated successfully against force measurements (Lopata et al. 2010), but more studies are required to validate the method. The probe placement could also have been limited due to the lack of significant anatomical landmarks, though the C4 spinous process and vertebral column were used as reference points. In addition, men had greater deformation and higher deformation rates compared to women, with several severe outliers that excluded the men from further analyses. Thus, men likely have different mechanical muscle functions than women and studies including more men are warranted.

CONCLUSION

In conclusion, we showed that individuals with WAD have higher deformation rates in the multifidus muscle compared to healthy controls. In addition, the interplay between the deep dorsal neck muscles was weaker in women with WAD. The altered function of deep dorsal neck muscles in women with chronic WAD may decrease muscular support in the maintenance of a stable cervical spine and cause persistent pain and disability. To the best of our knowledge, no studies have previously investigated the deep dorsal neck muscles non-invasively during real-time motion in WAD. The results provide important information that can be used in the assessment and diagnosis of patients following whiplash trauma.

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Additional Information

The authors declare no competing financial interests.

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Table 1. *Baseline characteristics of participants in the study.*

	WAD (n=40)	Healthy controls (n=40)	*P*-value
Female, n (%)	28 (70%)	28 (70%)	1.0
WAD grade II/III, n	29/11	0	
Age, years	37.4 (11.2)	37.4 (11.4)	1.0
Injury duration[a], months	21.2 (8.5)	0	
BMI male, kg/m^2	25.0 (4.8)	26.3 (3.6)	0.48
BMI female, kg/m^2	24.5 (6.4)	22.5 (2.5)	0.14
Physical activity level[b], median (range)	2.0 (2.0 - 3.75)	3.5 (4.0 - 4.0)	0.001
Neck Disability Index [c]	32.4 (13.9)	1.4 (1.8)	< 0.001
Pain previous week [d] (VAS)	45.9 (18.7)	1.1 (2.2)	< 0.001
Fatigue[e] (Borg), median (range)	3.3 (2.0 - 5.0)	0.1 (0.0 - 0.0)	< 0.001

a) Months since whiplash injury, range 6 to 36 months.

b) Physical activity level over the last year; 1 = inactivity, 2 = low activity, 3 = moderate activity, 4 = high activity.

c) 0-100%, higher scores represent higher disability.

d) Average pain in the prior week, range 0-100 mm, higher rating represents higher pain intensity.

e) CR 1-10; anchored with 1 = no fatigue, 10 = extremely strong fatigue.

Data are given as mean (SD) unless otherwise noted. BMI, body mass index; VAS, visual analog scale.

Table 2a. *Linear relationships in deformation between muscle pairs for women.*

First arm elevation women												Tenth arm elevation women											
Control group						WAD group						Control group						WAD group					
TR		.32	.03	.05	-.04	**TR**		.33	.07	.32	.13	**TR**		.24	.37	-.01	-.05	**TR**		.50	.22	.17	.14
SP	.32		.02	.07	.06	**SP**	.33		.28	.36	.16	**SP**	.24		.22	.16	.02	**SP**	.50		.11	.07	-.08
Scap	.03	.02		.07	.24	**Scap**	.06	.28		.02	.47	**Scap**	.37	.22		.04	.06	**Scap**	.22	.11		.40	.08
Scerv	.05	.07	.07		.61	**Scerv**	.32	.36	.02		.15	**Scerv**	-.01	.16	.04		.32	**Scerv**	.17	.07	.40		.01
MF	-.04	.06	.24	.61		**MF**	.13	.16	.47	.15		**MF**	-.05	.02	.06	.32		**MF**	.14	-.08	.08	.01	
	TR	**SP**	**Scap**	**Scerv**	**MF**		**TR**	**SP**	**Scap**	**Scerv**	**MF**		**TR**	**SP**	**Scap**	**Scerv**	**MF**		**TR**	**SP**	**Scap**	**Scerv**	**MF**

Data are adjusted R^2 values. Grey shading indicates a moderate relationship (R^2 = 0.30 – 0.60; $p < 0.02$) and black shading a strong relationship (R^2 = 0.61; $p < 0.001$) between muscle pairs. Abbreviations: TR, trapezius; SP, splenius capitis; Scap, semispinalis capitis; Scerv, semispinalis cervicis; MF, multifidus/rotatores.

Table 2b. *Linear relationships in deformation rate between muscle pairs for women.*

First arm elevation women												Tenth arm elevation women											
Control group						WAD group						Control group						WAD group					
TR		.76	.73	.28	.24	TR		.61	.28	.24	.07	TR		.71	.61	.24	.49	TR		.63	.21	.31	.11
SP	.76		.82	.55	.33	SP	.61		.52	.37	.05	SP	.71		.66	.64	.68	SP	.63		.44	.45	.07
Scap	.73	.82		.57	.38	Scap	.28	.52		.33	.17	Scap	.61	.66		.49	.58	Scap	.21	.44		.74	.43
Scerv	.28	.55	.57		.56	Scerv	.24	.37	.33		.41	Scerv	.24	.64	.49		.65	Scerv	.31	.45	.74		.64
MF	.24	.33	.38	.56		MF	.07	.05	.17	.41		MF	.49	.68	.58	.65		MF	.11	.07	.43	.64	
	TR	SP	Scap	Scerv	MF		TR	SP	Scap	Scerv	MF		TR	SP	Scap	Scerv	MF		TR	SP	Scap	Scerv	MF

Data are adjusted R^2 values. Grey shading indicates a moderate relationship ($R^2 = 0.30 – 0.60$; $p < 0.02$) and black shading a strong relationship ($R^2 = 0.61 – 0.82$; $p < 0.001$) between muscle pairs. Abbreviations: TR, trapezius; SP, splenius capitis; Scap, semispinalis capitis; Scerv, semispinalis cervicis; MF, multifidus/rotatores.

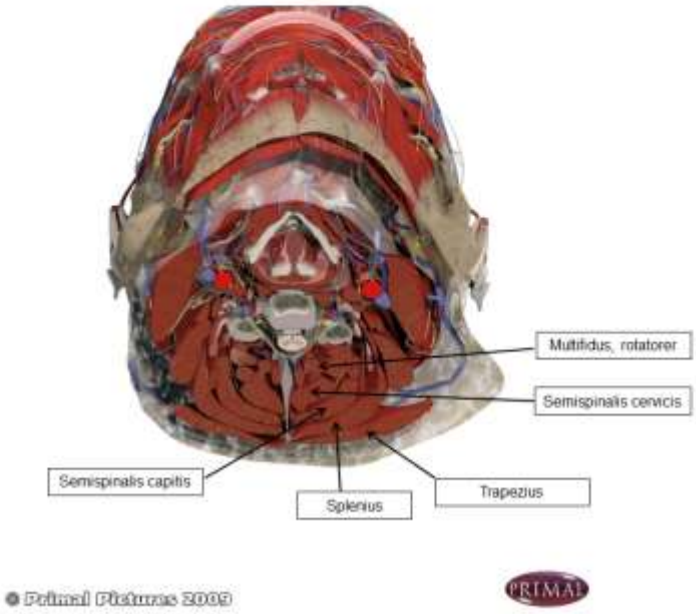

Fig. 1a **Dorsal neck muscles**

The five dorsal neck muscles of interest were the upper trapezius, splenius capitis, semispinalis capitis, semispinalis cervicis, and multifidus/rotatores (credit: Primal Pictures Ltd, 19 November 2015).

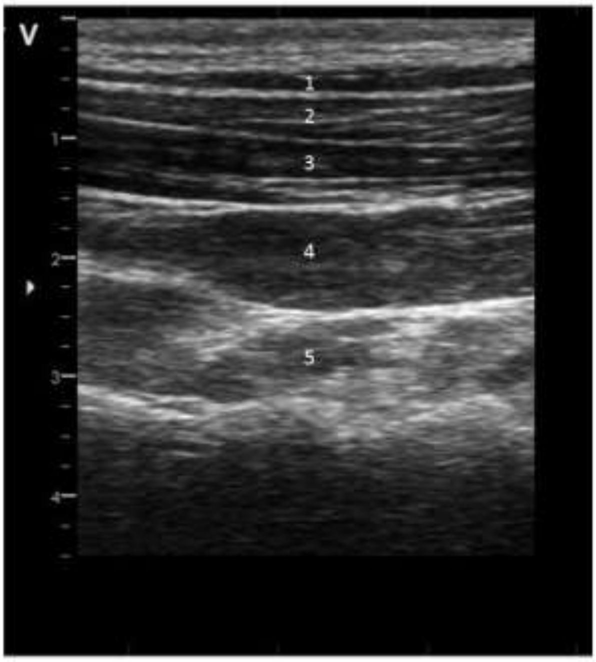

Fig. 1b **Ultrasound image of the dorsal neck muscles**

The image shows a longitudinal ultrasound B-mode projection. The specific muscles are numbered as follows: 1, trapezius; 2, splenius; 3, semispinalis capitis; 4, semispinalis cervicis, 5, multifidus/rotatores.

Fig.1c **Ultrasound imaging of dorsal neck muscles**

The test was standardized as follows. A custom-made contact switch was fastened to the participant’s right hip and right wrist, allowing synchronized data between arm movement and ultrasound measurements. The right arm was raised to an adjustable horizontal bar fixed at 90 degree arm elevation, which was measured by a goniometer when the index finger touched the bar. Each participant was instructed to hold their head steady and look at the bar during the test. The participants held a 0.5 kg (women) or 1 kg (men) weight in their right hand and were told to move their arm in a smooth motion and raise it with the first beat, and then lower it to the switch contact in the next beat. A metronome was set at 40 beats per minute to maintain a steady pace. Each participant practiced the test with the left arm to familiarize themselves with the test.

Fig 1d. **Position of the ultrasound transducer**

The ultrasound transducer was positioned on the right side of the neck. First, the transducer was positioned in a transverse orientation at the level of the 4th cervical vertebrae for identification of neck muscle layers and bony landmarks. Next, the transducer was rotated 90° to the longitudinal position to ensure an optimal image plane for the dorsal neck muscles.

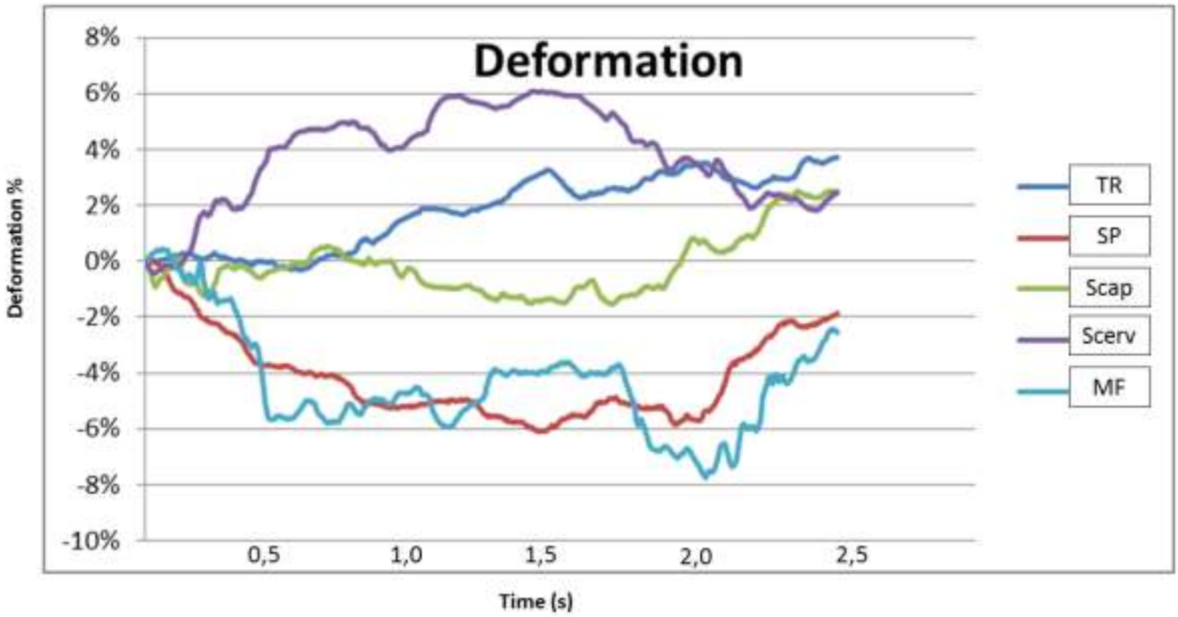

Fig. 1e. **Muscle deformation areas**

Different muscle deformation areas in five dorsal neck muscles during one arm elevation in a single participant. Each line denotes the changes in the ROI (deformation %) in one muscle during one arm elevation. The negative values (area below zero) denote muscle shortening and the positive values (area above zero) denote muscle elongation. The total area (sum of negative and positive areas) denotes the total muscle deformation. When the line crosses the 0% line, the muscle switches from shortening to elongation, or vice versa. Abbreviations: TR, trapezius; SP, splenius capitis; Scap, semispinalis capitis; Scerv, semispinalis cervicis; MF, multifidus/rotatores

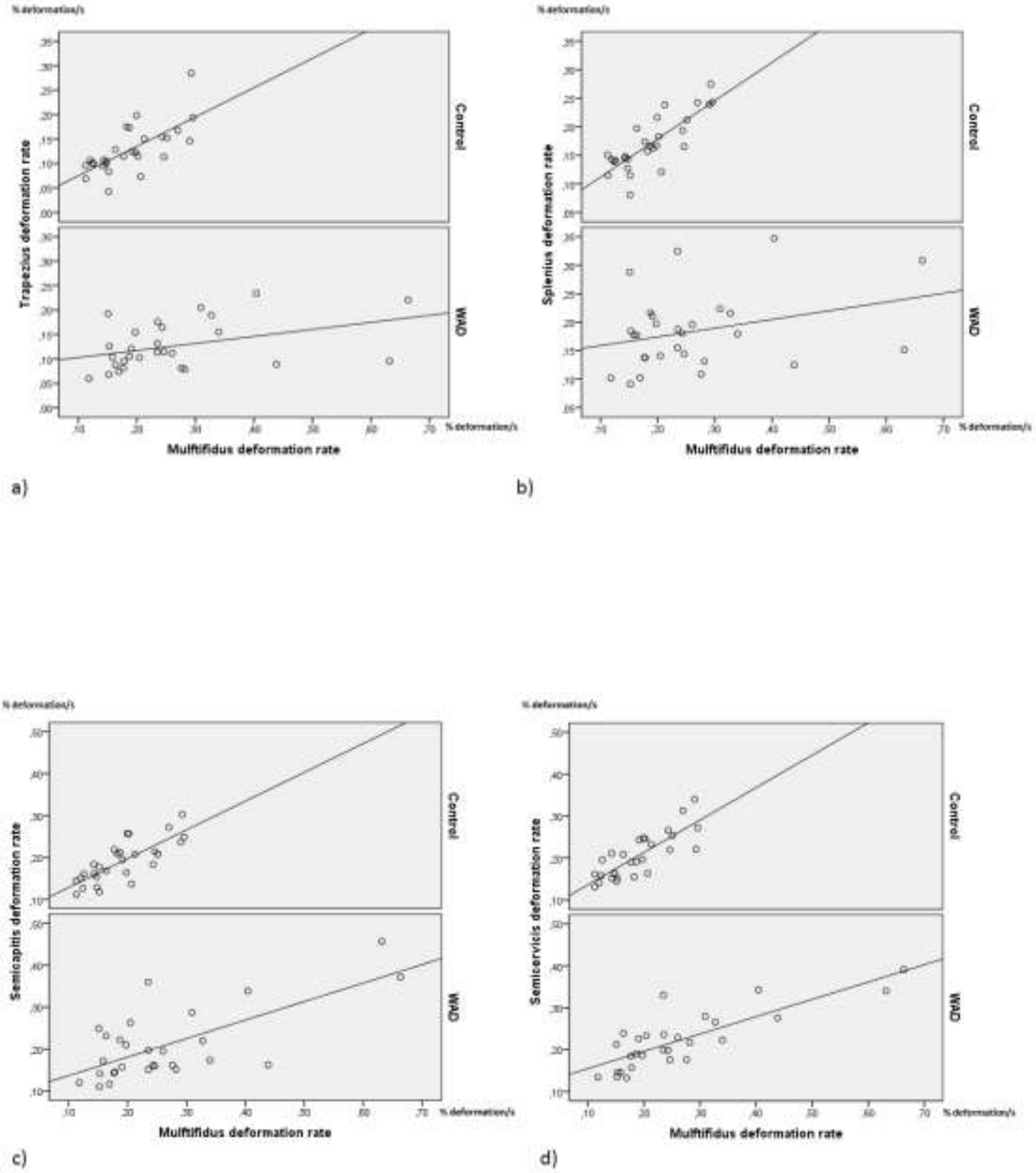

Fig. 2 a-d **Linear relationships between the deformation rates of muscle pairs in women**

Linear relationships between the deformation rates of muscle pairs in women based on the tenth arm elevation. a) Controls (top panel) exhibited a moderate relationship between the trapezius and multifidus (MF) muscles ($R^2 = 0.49$) compared to a weak relationship in individuals with WAD (bottom panel) ($R^2 = 0.11$). b) Controls (top panel) exhibited a strong relationship between the splenius and MF muscles ($R^2 = 0.68$) compared to a weak relationship in individuals with WAD (bottom panel) ($R^2 = 0.07$). c) Controls (top panel) and individuals with WAD (bottom panel) exhibited a moderate relationship between the semispinalis capitis and MF muscles (controls, $R^2 = 0.58$; WAD, $R^2 = 0.42$). d) Controls (top panel) and individuals with WAD (bottom panel) exhibited a strong relationship between the semispinalis cervicis and MF muscles (controls, $R^2 = 0.65$; WAD, $R^2 = 0.64$).

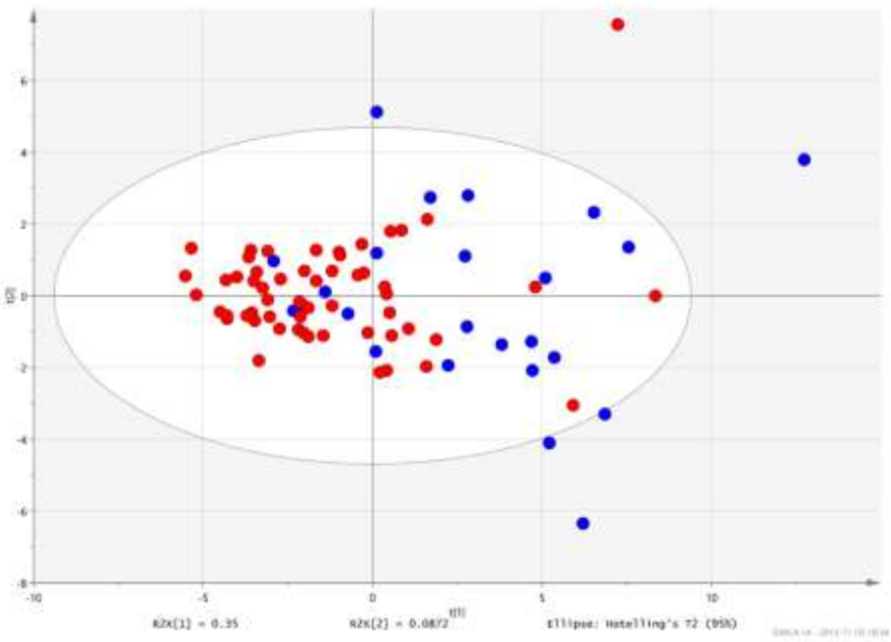

Fig. 3. a **Score plot**

Score plot for the first two components of the PCA model created for the 40 deformation and deformation rate variables. The plot shows score values for the principal component t[1] on the x-axis and the second component t[2]on the y-axis. A score value, which does not have unit, gives the position of a certain observation on the drawn principal component. Observations of similar properties, as given by the measured variables, obtain comparable score values. Men (blue) had a more heterogenous spread and more outliers than women (red). Also, they tend to be situated more to the right, which indicates differences between men and women.

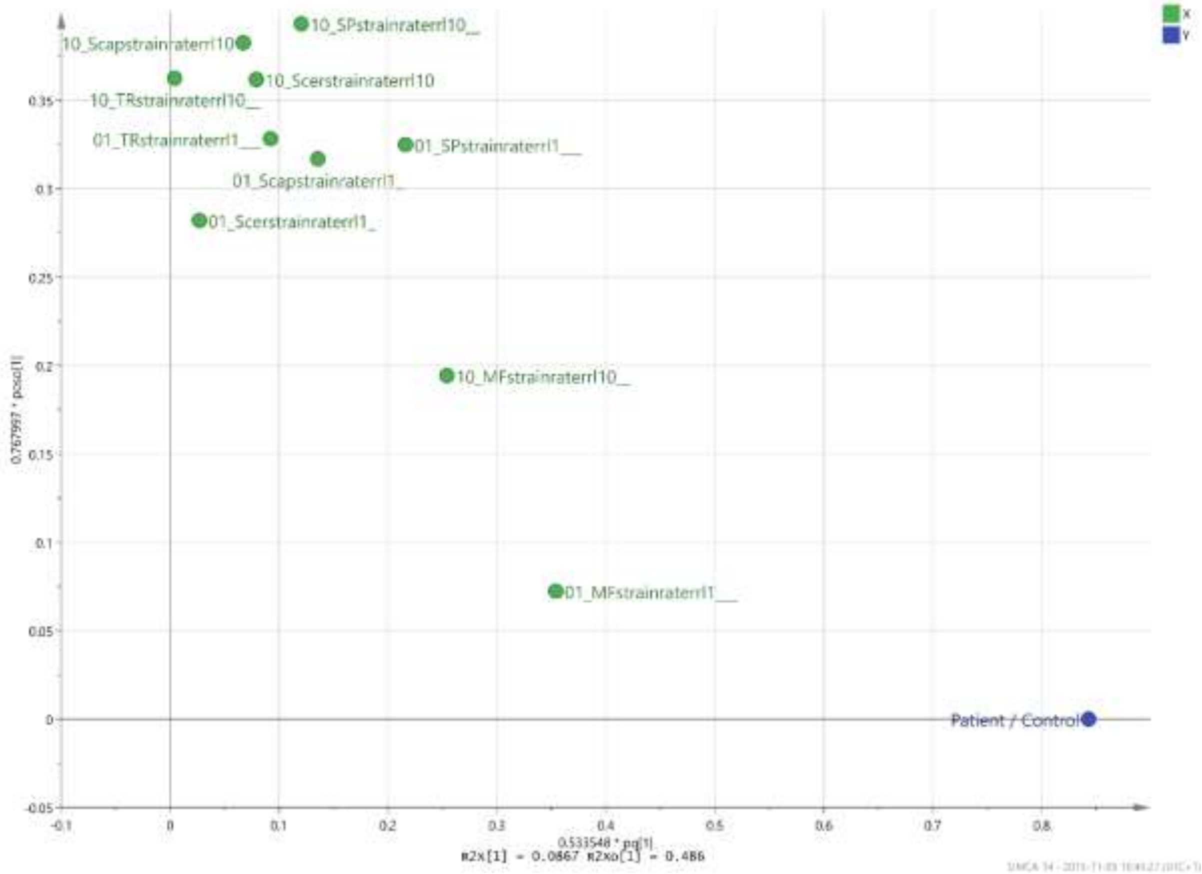

Fig. 3. b **Loading plot**

Loading plot for the OPLS-DA model, which separates individuals with WAD from controls. The variables positioned closest to Y (WAD/control) are the deformation rates for the multifidus muscle at the first and tenth arm elevations. This plot shows the loading vectors of the model; the correlated (important) loading is shown on the x-axis and the orthogonal loading on the y-axis. Loading values are without unit, and express the importance of each variable in the model. There is one loading value for each of the variables that are included in the model. Loading values with higher values are more important than those that are close to zero. Abbreviations: strainrate, deformation rate; 01and rrl1, first arm elevation; 10 and rrl10, tenth arm elevation; TR, trapezius; SP, splenius capitis; Scap, semispinalis capitis; Scerv, semispinalis cervicis; MF, multifidus/rotatores. Example: 01_MFstrainraterrl1, multifidus deformation rate during first arm elevation.

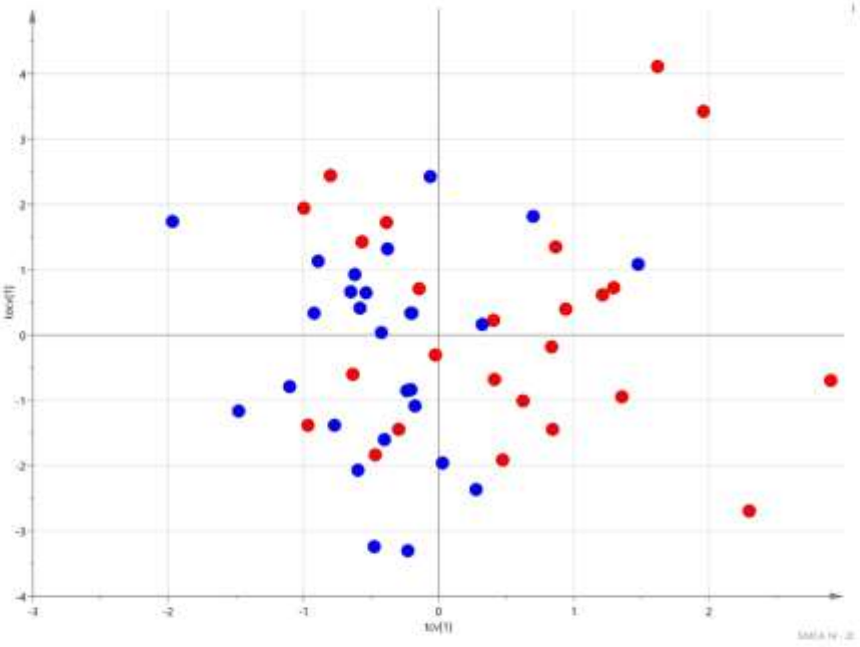

Fig. 3. c **Cross-validated score plot**

Cross-validated score plot for the OPLS-DA model in which ten deformation rate variables were used to separate patients from controls. The plot shows score values for the principal component t[1] on the x-axis and the second component t[2]on the y-axis. A score value, which does not have unit, gives the position of a certain observation on the drawn principal component. Observations of similar properties, as given by the measured variables, obtain comparable score values. The plot does not show a clear separation, but individuals with WAD (red) are mainly positioned to the right, which indicates differences between the control (blue) and WAD groups (red

Supplement file 1. Muscle deformation (% change in length) at the first (1st) and tenth (10th) arm elevation in the five dorsal neck muscles in the WAD and control groups, including total deformation area, elongation and shortening area with outliers excluded.

		1st arm elevation				10th arm elevation			
		n	Control	n	WAD	n	Control	n	WAD
Whole group	**Test time†**	38	2.38 (0.33)	40	2.42 (0.37)	39	2.39 (0.35)	37	2.48 (0.52)
Total area	TR	38	6.7 (4.5)	40	5.8 (3.2)	39	5.1 (3.0)	37	5.2 (3.0)
	SP	38	7.8 (5.2)	40	9.1 (5.1)	39	7.7 (5.1)	37	8.2 (5.5)
	Scap	38	9.0 (5.4)	40	10.5 (6.4)	39	9.1 (6.2)	37	10.3 (6.7)
	Scerv	38	11.2 (5.9)	40	11.8 (6.8)	39	9.8 (5.3)	37	10.6 (5.9)
	MF	38	14.2 (10.7)	40	12.0 (5.6)	39	9.7 (5.4)	37	9.9 (4.7)
Elongation	TR	38	3.3 (3.6)	40	3.1 (1.9)	39	2.6 (2.0)	37	2.6 (2.0)
	SP	38	3.2 (2.7)	40	3.8 (2.6)	39	3.0 (2.1)	37	3.6 (3.2)
	Scap	38	4.6 (3.6)	40	4.8 (3.8)	39	4.0 (3.6)	37	4.5 (4.4)
	Scerv	38	4.4 (3.3)	40	4.5 (3.9)	39	4.6 (4.4)	37	4.0 (2.1)
	MF	38	5.4 (5.2)	40	4.6 (3.1)	39	2.8 (2.0)	37	2.6 (2.1)
Shortening	TR	38	3.3 (3.0)	40	2.7 (2.3)	39	2.5 (2.2)	37	2.6 (1.9)
	SP	38	4.7 (4.1)	40	5.7 (5.6)	39	4.6 (4.6)	37	4.6 (3.3)
	Scap	38	4.8 (3.6)	40	5.6 (4.9)	39	5.1 (4.3)	37	5.7 (4.3)
	Scerv	38	6.8 (4.5)	40	7.3 (4.8)	39	5.2 (4.4)	37	6.6 (4.7)
	MF	38	8.9 (7.8)	40	7.3 (4.5)	39	6.9 (4.7)	37	7.3 (5.0)
Women	**Test time†**	28	2.41 (0.37)	28	2.44 (0.35)	28	2.37 (0.36)	27	2.42 (0.44)
Total area	TR	28	6.0 (4.3)*	28	5.7 (3.3)	28	4.4 (2.6)*	27	5.3 (3.3)
	SP	28	6.2 (3.2)*	28	8.0 (3.7)	28	5.8 (3.2)*	27	8.4 (5.2)
	Scap	28	8.2 (4.4)*	28	9.1 (5.1)	28	6.7 (3.1)*	27	9.4 (6.4)
	Scerv	28	10.5 (6.0)*	28	12.4 (7.1)	28	8.5 (5.3)*	27	10.1 (5.3)
	MF	28	11.7 (8.2)*	28	11.4 (5.4)	28	7.5 (3.8)*	27	9.8 (4.9)
Elongation	TR	28	2.9 (2.8)	28	3.2 (2.0)	28	2.2 (1.9)	27	2.7 (2.3)
	SP	28	2.6 (2.2)	28	3.8 (2.6)	28	2.6 (1.8)	27	3.8 (3.1)
	Scap	28	4.0 (3.4)	28	4.3 (3.2)	28	2.7 (2.2)	27	4.0 (4.5)
	Scerv	28	4.2 (3.2)	28	4.8 (4.1)	28	4.4 (3.6)	27	3.8 (2.7)
	MF	28	5.0 (5.6)	28	4.5 (3.2)	28	2.4 (1.9)	27	2.9 (2.3)
Shortening	TR	28	3.0 (2.8)	28	2.5 (2.0)	28	2.2 (2.1)	27	2.5 (2.0)
	SP	28	4.2 (4.0)	28	5.3 (5.2)	28	3.2 (2.6)	27	4.6 (2.9)
	Scap	28	4.2 (2.7)	28	4.8 (4.2)	28	4.0 (3.2)	27	5.4 (4.0)
	Scerv	28	6.3 (4.4)	28	7.6 (4.3)	28	4.1 (3.5)	27	6.4 (5.0)
	MF	28	6.7 (3.5)	28	6.9 (4.1)	28	5.1 (3.6)	27	6.9 (5.0)
Men	**Test time†**	10	2.31 (0.18)	12	2.36 (0.43)	11	2.42 (0.32)	10	2.64 (0.73)
Total area	TR	10	8.5 (4.8)*	12	6.0 (3.3)	11	7.1 (3.2)*	10	4.8 (2.3)
	SP	10	12.2 (7.3)*	12	11.8 (6.9)	11	12.3 (6.1)*	10	7.7 (6.4)
	Scap	10	13.1 (6.6)*	12	13.6 (8.1)	11	15.2 (7.9)*	10	12.6 (7.4)
	Scerv	10	13.2 (5.6)*	12	10.4 (6.1)	11	12.9 (4.3)*	10	11.9 (7.3)
	MF	10	21.5 (14.0)*	12	13.4 (5.9)	11	15.2 (5.1)*	10	10.2 (4.9)
Elongation	TR	10	4.5 (5.3)	12	2.7 (1.6)	11	3.6 (1.9)	10	2.1 (1.3)
	SP	10	4.6 (3.8)	12	4.1 (2.8)	11	4.0 (2.7)	10	3.2 (3.4)
	Scap	10	6.4 (3.8)	12	6.0 (5.0)	11	7.4 (4.3)	10	5.9 (4.0)
	Scerv	10	4.8 (3.8)	12	3.6 (3.2)	11	5.0 (6.0)	10	4.6 (4.1)
	MF	10	6.4 (3.9)	12	5.0 (2.8)	11	3.8 (2.0)	10	1.7 (1.3)
Shortening	TR	10	4.0 (3.6)	12	3.3 (2.7)	11	3.5 (2.5)	10	2.7 (1.6)
	SP	10	6.1 (4.3)	12	6.8 (6.6)	11	8.3 (6.6)	10	4.4 (4.3)
	Scap	10	6.7 (5.2)	12	7.6 (5.9)	11	7.8 (5.1)	10	6.6 (5.2)
	Scerv	10	8.4 (4.7)	12	6.8 (6.0)	11	7.9 (5.1)	10	7.3 (5.2)
	MF	10	15.1 (12.5)	12	8.4 (5.5)	11	11.4 (4.0)	10	8.5 (5.2)

Data are means ± SD. Muscle deformation (% change in length) at the first (1st) and tenth (10th) arm elevation in the five dorsal neck muscles in the WAD and control groups. Total area represents both elongation and shortening during arm elevation. †Test time in seconds for the first and tenth arm elevation. There were significant differences in deformation total area between women and men in the control group at the first and tenth arm elevations.

* (p > 0.001).

Supplementary file 2. Muscle deformation rate (% deformation/s) during the first (1st) and tenth (10th) arm elevation in the five dorsal neck muscles in the WAD and control groups with outliers excluded.

		1st arm elevation				10th arm elevation			
		n	**Control**	**n**	**WAD**	**n**	**Control**	**n**	**WAD**
Whole group	**TR**	35	0.15 (0.06)	38	0.15 (0.06)	36	0.14 (0.05)	40	0.14 (0.05)
	SP	35	0.19 (0.07)	38	0.19 (0.06)	36	0.20 (0.07)	40	0.20 (0.08)
	Scap	35	0.20 (0.06)	38	0.21 (0.07)	36	0.21 (0.07)	40	0.22 (0.08)
	Scerv	35	0.23 (0.06)	38	0.23 (0.06)	36	0.22 (0.06)	40	0.24 (0.08)
	MF	35	0.22 (0.08)	38	0.27 (0.10)	36	0.21 (0.08)	40	0.28 (0.15)
Women	**TR**	28	0.13 (0.05)	28	0.13 (0.03)	28	0.13 (0.05)	28	0.13 (0.05)
	SP	28	0.16 (0.05)	28	0.18 (0.06)	28	0.17 (0.05)	28	0.18 (0.07)
	Scap	28	0.18 (0.05)	28	0.20 (0.07)	28	0.19 (0.05)	28	0.21 (0.09)
	Scerv	28	0.20 (0.05)	28	0.21 (0.05)	28	0.20 (0.05)	28	0.22 (0.07)
	MF	28	0.20 (0.06)	28	0.24 (0.09)*	28	0.19 (0.06)	28	0.26 (0.13)*
Men	**TR**	7	0.21 (0.04)*	10	0.19 (0.07)*	8	0.18 (0.04)*	12	0.16 (0.04)*
	SP	7	0.28 (0.06)*	10	0.22 (0.05)*	8	0.28 (0.07)*	12	0.24 (0.10)*
	Scap	7	0.28 (0.06)*	10	0.24 (0.04)*	8	0.28 (0.09)*	12	0.23 (0.05)*
	Scerv	7	0.29 (0.05)*	10	0.27 (0.08)*	8	0.27 (0.06)*	12	0.27 (0.09)*
	MF	7	0.31 (0.09)*	10	0.33 (0.11)*	8	0.28 (0.09)*	12	0.34 (0.16)*

Data are mean ± SD. Muscle deformation rate (% deformation/s) during the first (1st) and tenth (10th) arm elevation in the five dorsal neck muscles in the WAD and control groups. Abbreviations; TR, trapezius; SP, splenius capitis; Scap, semispinalis capitis; Scerv, semispinalis cervicis; MF, multifidus/rotatores. There were significant differences in deformation rate between women and men in both group. There were significant differences between the WAD and control group in the multifidus muscle during the first and tenth (only between women) arm elevations.

*$p < 0.05$