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Christensen, Steffan Wittrup McPhee; Bellosta-López, Pablo; Doménech-García, Víctor; Herrero, Pablo; Palsson, Thorvaldur Skuli

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CHANGES IN PAIN SENSITIVITY AND CONDITIONED PAIN MODULATION DURING RECOVERY FROM WHIPLASH ASSOCIATED DISORDERS

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Steffan Wittrup McPhee Christensen[a,b], PhD; Pablo Bellosta-López[c*], MSc; Víctor Doménech-García[c], PhD; Pablo Herrero[c,d], PhD; Thorvaldur Skuli Palsson[a], PhD.

[a]*Department of Health Science and Technology, Faculty of Medicine, Aalborg University, Aalborg, Denmark.*

[b]*Department of Physiotherapy, University College of Northern Denmark, Aalborg, Denmark.*

[c]*Universidad San Jorge. Campus Universitario, Autov. A23 km 299, 50830. Villanueva de Gállego, Zaragoza, España.*

[d]*Universidad de Zaragoza. Departamento de Fisiatría y Enfermería. Facultad de Ciencias de la Salud. C/ Domingo Miral s/n, CP 50009 Zaragoza, España.*

***Corresponding author:** Pablo Bellosta-López, Universidad San Jorge. Campus Universitario, Autov. A23, Km 299, 50830 Villanueva de Gállego, Zaragoza, Spain. Tel.: (+34) 976 060 100 Fax.: 976 077 581. Email: pbellosta@usj.es

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ABSTRACT

Objectives: To investigate the pain-sensory profile of patients with whiplash-associated disorders (WAD) prior and post 2-weeks of standardized rehabilitation and after at 6-months follow-up.

Methods: Twenty-two WAD-participants (Grade-II; 14 women) and 22 sex-and age-matched healthy controls were enrolled. Pressure pain thresholds (PPTs) were assessed at local and distal muscles. Conditioned pain modulation (CPM) of PPTs was assessed using cuff-pressure around the upper-arm. Referred area of pain following supra-threshold pressure stimulation of the infraspinatus muscle was recorded on a body chart. Psychometric variables (Pain intensity, area of perceived pain, pain catastrophizing, kinesiophobia, sleep problems, depression level) were assessed. WAD-group additionally completed the Neck Disability Index (NDI).

Results: The WAD-group demonstrated lower local PPTs compared to controls at all timepoints ($P<0.05$) and lower distal PPTs at baseline and at 2 weeks when compared to 6-months (within-group) ($P<0.05$). The WAD-group had a reduced CPM response and larger induced referred pain areas compared to controls ($P<0.05$), while no within-group changes were observed at any time point. The WAD-group reported higher pain intensity and perceived area of pain compared to controls at all timepoints ($P<0.05$) and a mean NDI-score of 41% at baseline, 16% at 2-weeks, and 4% at 6-months. Furthermore, the WAD-group reported improvements in all other psychometric variables ($P<0.05$), although only pain catastrophizing levels were comparable to controls at 2-weeks.

Discussion: PPTs but not CPM improved in the WAD-group and were comparable to controls following 2-weeks following standardized rehabilitation, indicating that normalization of CPM may not be required to recover from WAD.
Keywords: whiplash, pain sensitivity, conditioned pain modulation, rehabilitation, psychometric characteristics.

INTRODUCTION

Whiplash-associated disorders (WAD) are commonly diagnosed following a motor vehicle accident (1, 2). Of those diagnosed with WAD, up to 55% may still experience symptoms such as neck pain and stiffness, dizziness, and/or sleep problems several years after the incident (3-6). However, despite significant advances in the understanding and management of WAD, there seems to be no reduction in the proportion of people developing persistent symptoms (7).

WAD is a complex and multifactorial condition with a great need for understanding potential underlying mechanisms (8). Tissue damage may explain some WAD-symptoms, although a direct causal link cannot be made in the majority of cases (4, 9). Instead, a variety of additional factors such as heightened pain sensitivity, psychological and emotional factors have been suggested as contributing factors for the development and maintenance of persistent symptoms (9-12). In line with this, a range of studies has demonstrated associations between ongoing symptoms in WAD-populations and psychometric variables such as catastrophizing thoughts (13, 14), fear of movement (15), depression (16) and sleep problems (17-19).

Findings of increased pain sensitivity such as widespread reductions in pressure pain thresholds (PPTs) are a common feature of persistent painful conditions (20, 21), including WAD populations (22-24). Furthermore, expanded spatial distribution of the perceived area of pain has been shown in those with WAD following experimental muscle pain when compared to a healthy pain-free population, which was attributed to facilitated central pain mechanisms (25). Similarly, conditioned pain modulation (CPM), a proxy of endogenous pain modulation, has been shown to be less efficient in ongoing WAD compared with healthy controls (16). Interestingly, changes in the pain-sensory profile seem to emerge already in acute WAD and more so in those presenting

with greatest symptom intensity (26, 27), indicating that the severity of symptoms may be associated to larger changes in the pain-sensory profile.

Evidence suggests that removing the locus of nociceptive activity, such as in total knee replacement for osteoarthritis, may, to some degree, normalize the pain-sensory profile (28). As removing the locus of nociception in WAD may not be possible, it is of great interest to investigate whether successful WAD rehabilitation also can reverse or normalize the pain-sensory profile and to what extent either possibility is relevant for pain and functional recovery.

This study aimed to investigate the pain-sensory profile of a group with WAD before and after 2-weeks of standardized rehabilitation compared to a healthy pain-free control-group. It was hypothesized that the WAD-group would display reduced local and distal PPTs and CPM response compared to healthy pain-free controls, but these would normalize following rehabilitation. A secondary aim was to investigate psychometric characteristics of the participants. Here, it was hypothesized that improvements in self-reported variables (pain intensity, area of perceived pain, disability, pain catastrophizing, kinesiophobia, depression, and sleep problems) would be observed over time for the WAD-group.

MATERIALS AND METHODS

Participants

Participants suffering from WAD were recruited for this prospective, observational, case-control study. The study was conducted at the WAD rehabilitation center (*Trauma Aragón, Zaragoza, Spain*), from May 2016 to April 2019. All WAD participants were referred to the center for rehabilitation by their insurance company within 2-3 weeks after being involved in a motor vehicle accident. The inclusion criteria for this study were age between 18 and 50 years old, ability to read and speak Spanish, and a

diagnosis of WAD grade-II, defined as neck complaints and musculoskeletal signs such as decreased range of motion or muscle soreness (29). All clinical assessments were made by the same medical doctor , who had 7 years of experience in the WAD rehabilitation unit. Decreased range of motion was recorded if participants could not reach 90º of active rotation and reported self-perceived reduction in range of motion compared to before the motor vehicle accident. Exclusion criteria were the presence of any ongoing painful condition other than WAD, neck or back pain in the 6 months prior to the motor vehicle accident, pain related to a specific pathology such as spinal stenosis, fracture, nerve lesions, etc., previous history of surgery on the neck, spine or shoulder area, signs of radiculopathy, pregnancy or lack of ability to cooperate. Sex and age-matched healthy pain-free controls were recruited from the local community throughout flyers, announcements on the university website and social media accounts. The exclusion criteria for pain-free healthy controls were similar to those of the WAD-participants with the addition that any current or recent use of analgesic and/or other medication was not allowed.

For power calculations, G*Power (*v3.1.9.2, Heinrich-Heine-University, Dusseldorf, Germany*) was used to determine the feasible sample size for a mixed model ANOVA with two groups (WAD, Control) participating in three test sessions. A power of 80% and an alpha level of 0.05 was required to detect the minimal change of 47.2 kPa (partial η^2=0.05) for PPT in the neck area (30). Based on the requirements, a total of 34 participants (17 per group) were needed for participation. Dropouts of approximately 20% were assumed (31), and therefore, twenty-two participants were recruited for each group. All participants provided informed consent before being enrolled in the study.

The project was approved by the local Ethics Committee (C.P. - C.I. PI16/0132) and conducted in accordance with the Helsinki Declaration. The study was registered at ClinicalTrial.gov (NCT03784196) and reported following the STROBE statement for observational studies.

Protocol

The study consisted of three experimental sessions: 1) A baseline-session prior to starting a 2-week standardized WAD-rehabilitation provided at the rehabilitation center; 2) A follow-up session after the WAD-group had completed their 2-weeks standardized rehabilitation (1 session on all weekdays for 2 weeks, 10 in total) and 3) a follow-up session at 6-month (Fig 1). An identical protocol was used on all sessions, which consisted of 1) Filling out questionnaires, 2) Recording of pain intensity and symptom location, and 3) Assessing pain sensitivity. Prior (>1hr) to the first experimental session, all participants participated in a short introduction session where they were informed of what participation in the project required (i.e., filling out questionnaires and assessing pain sensitivity). A single assessor (PBL) trained in the assessment methods performed all procedures at all time points.

The protocol was identical for all participants except for the rehabilitation intervention and filling out the Neck Disability Index (NDI) questionnaire, which was only done by the WAD-group.

Treatment intervention

In the period between the motor vehicle accident and the baseline assessment, before starting the standardized rehabilitation, participants did receive any other intervention except for any recommendations regarding over-the-counter analgesics they may have received at the emergency department.

At the WAD rehabilitation center, the WAD-group received ten sessions of standardized physiotherapy-guided rehabilitation delivered by the staff at the rehabilitation center over a fourteen-day period, which is considered standard intervention by insurance companies in Spain. In line with standard procedures at the rehabilitation center, each session lasted 30-min and included e.g. pain neuroscience education, motor control exercises, manual therapy, and transcutaneous electrical nerve stimulation (Supplementary Table 1, Supplemental Digital Content 1, http://links.lww.com/CJP/A811). Each participant received an individualized approach determined by the clinical staff at the rehabilitation center using the modalities mentioned above.

No changes were made to the standardized physiotherapy intervention during this study, and participants were asked to inform if they had received other types of interventions (e.g., chiropractic care, acupuncture, etc.) in the interim between the 2-week assessment and the 6-month follow up.

Pressure pain sensitivity

PPTs were assessed using a handheld pressure algometer (*Somedic, Hörby, Sweden*) mounted with a $1cm^2$ probe. The pressure was gradually increased at the stimulation site with a ramp of 30kPa/s. PPT was defined as the point in time where the applied stimuli went from being a pressure to first becoming painful. Here, the participants pushed a button that stopped the stimulation and recorded the exact pressure at that time point (24). PPTs were recorded bilaterally over three muscles: 1) Splenius capitis muscle, between the lateral border of the upper trapezius and the posterior border of the sternocleidomastoid muscles, at the level of C3 (24, 32, 33); 2) Upper trapezius muscle, at the midline between C7 and the acromion (34-36); 3) Gastrocnemius muscle, on the distal third on a line connecting the popliteal line with the calcaneus (37, 38) (Figure 2).

The splenius capitis and upper trapezius sites were chosen to assess pain sensitivity in the neck region, while the gastrocnemius site was selected as a control point in order to detect the potential presence of facilitated central pain mechanisms (20, 37). Measurements were conducted with participants lying in a prone position. Splenius capitis and upper trapezius muscles were chosen to evaluate pressure sensitivity in the neck/shoulder area, while gastrocnemius muscle was selected as a control point distant to the painful area (37). The order of which side PPT was tested first (left or right) was randomized before the first session, and the order was kept for each participant in the subsequent sessions. Assessments started at one site on one side, with the next recording conducted on the contralateral side before moving on to the next site. Two rounds of PPTs were recorded for each site, with an interval of 30 seconds before re-assessing the same site again. The average value of each site was extracted for analysis. Besides, participants indicated the side of the body they perceived to be most affected. The data were analyzed accordingly. If neither side was perceived as being most symptomatic, data from the dominant side were used to represent the most symptomatic side in the data analysis.

Conditioned pain modulation

CPM was assessed with participants in the same position as for PPTs, with the addition of an inflatable pressure-cuff (*Model DS54, Welch Allyn, NY, USA*) mounted on their non-dominant arm (Figure 2). The pressure-cuff was inflated until participants indicated a score of 7 on the 11-point NRS. The mmHg value reached (i.e., pressure pain tolerance (PPTol)) was recorded to allow for between-session comparison of the required pressure. With the cuff inflated, a 30 second period passed before PPTs were re-assessed as described above on the contralateral side to the cuff (16, 39). The cuff was deflated once all PPT recordings were conducted. A CPM value was calculated by

subtracting baseline PPTs without painful stimuli from PPTs recorded while the conditioning stimulus was maintained., resulting in a positive value indicating an inhibitory response and a negative value indicating a facilitatory response (40).

Pressure-induced referred pain

The area of referred pain following a painful stimulus has been suggested to be a feasible biomarker to investigate the sensitivity of central pain mechanisms (37), where larger areas are suggested to reflect facilitated central pain mechanisms (38, 41). In the current study, referred pain following a painful pressure stimulation was assessed by applying a supra-threshold (120% PPT) pressure stimulation over the infraspinatus muscle on the dominant side. The stimulation site was located by finding the equidistant point between the medial point of the spine-, the inferior angle- and the midpoint of the medial border of the scapula (37, 38) (Fig. 2). A baseline PPT recording was made, and the supra-threshold pressure stimulation was applied at the infraspinatus muscle site for 60 seconds (37, 38). Immediately following the pressure stimulation, the size of the pain area was recorded by asking participants to draw any pain and referral pain patterns on the electronic body chart.

Psychometric variables

At the beginning of each experimental session, if they had any pain, participants were asked to draw in the area of perceived pain on an electronic body chart (*Navigate Pain v1.0, Aalborg University, Aalborg, Denmark*) (42). Any potential area of pain was drawn separately on the anterior and posterior view of the body chart. The total area of perceived pain, indicated in colored pixels, was summarized by adding the anterior and posterior views, and the combined number was used for further analysis (38).

Neck pain intensity at rest was rated on an 11-point numeric rating scale (NRS; 0 = no pain, 10 = the worst imaginable pain) (43).

Perceived disability was assessed using the NDI in the WAD-group (44). The NDI consists of 10 dimensions measured on a 6-point scale from 0 (no disability) to 5 (full disability). The sum of these 10 dimensions is expressed as a percentage, where a higher score indicates higher disability levels (45). A percentage score of <8% was considered recovered, between 10-28% mild symptoms, and >30% moderate to severe symptoms (46, 47).

The Pain Catastrophizing Scale (PCS) was used to quantify the perceived painful experience with particular emphasis on catastrophizing thoughts (48). It comprises 13 items measured on a 5-point scale from 0 (not at all) to 4 (all the time) where higher scores indicating higher levels of pain catastrophizing (49).

The short version of the Tampa Scale of Kinesiophobia (TSK-11) was used to screen for fear of movement and re-injury (50). TSK-11 includes 11 items scored from 1 (strongly disagree) to 4 (strongly agree). The total score is calculated with higher scores indicating greater pain-related kinesiophobia (51).

The Beck Depression Inventory (BDI) was used to assess possible levels of depression (52). It consists of 21 items scored from 0 to 3, where higher scores indicate higher levels of depression (53).

The Medical Outcomes Study Sleep Scale (MOS-Sleep) was used to assess potential sleep disturbance (54). MOS-Sleep consists of 12 items, and quality of sleep is assessed through the Sleep Problem Index (SPI), with scores range from 0 to 100, where higher scores indicate worse sleep quality (54).

These questionnaires have previously been shown valid and reliable to assess disability (55), catastrophizing thoughts (56), kinesiophobia (57), depression (58), and sleep (59) in populations with neck pain. If participants were unable to attend the 6-month follow-up in person, the questionnaires were completed via telephone.

Statistical analysis

Statistical analysis was performed using SPSS v.25 (*IBM, Chicago, IL, USA*). A $P<0.05$ was accepted as a significant difference between compared variables. Data distribution was assessed using the Shapiro-Wilk test and expressed as median and interquartile ranges (IQR) or mean and standard deviations (SD) depending on the distribution of data. In the case of missing data, an intention-to-treat analysis was conducted, where the last recorded value was carried forward. Additionally, data from dropouts in each group at baseline and two-weeks were compared to those who completed the study, using either independent t-test or the Mann-Whitney U test.

PPT data were analysed using a mixed-model repeated-measures analysis of variance (RM-ANOVA) with *site* (splenius capitis, upper trapezius and gastrocnemius muscle), *side* (most symptomatic/dominant, less symptomatic/non-dominant), and *time* (Baseline, 2-weeks, 6-months) as within factors and *group* (WAD, Controls) as between factor.

For CPM data, a mixed-model ANOVA was likewise performed with *site* (splenius capitis, upper trapezius, and gastrocnemius muscle), and *time* (Baseline, 2-weeks, 6-months) as within factors and *group* (WAD, Controls) as between factor.

Infraspinatus muscle site and PPTol were investigated for interactions using an RM-ANOVA with *time* (Baseline, 2-weeks, 6-months) as within factor and *group* (WAD, Controls) as between factor. When indicated, a Bonferroni test was used to correct for pairwise comparisons post hoc.

For the area of perceived pain and pressure-induced referred pain, NRS, NDI, PCS, TSK-11, BDI, and MOS-Sleep changes over time (baseline, 2-weeks, 6-months) were investigated using a Friedman's ANOVA with Dunn's test as a post-hoc test. In

addition, between-group differences were investigated for each timepoint using a Mann-Whitney U test.

Inter-relationships among the pain sensory profile measures at each time point were explored using a Pearson correlation coefficient.

RESULTS

All WAD participants started their rehabilitation on within 11 to 21 days following the motor vehicle accident, which is considered the acute phase (26). 2-weeks and 6-month follow-up was conducted after 24 to 37 and 178 to 206 days following the motor vehicle accident, respectively. All participants completed the baseline and 2-week assessment, while seven participants did not attend (five WAD-participants and two controls) the 6-months follow-up. All seven participants were invited to complete the questionnaires by telephone, which was done by all but two from the WAD-group. When comparing data from baseline and 2-weeks (within-group) for those who dropped-out (WAD: n=5; Controls: n=2) and those who completed the entire study (WAD: n=17; Control: n=20), no significant difference was seen for any of the variables of interest in this study. Only one WAD-participant, who still experienced pain and disability at 6-months, reported having received three sessions of chiropractic care in the interim between the 2-week assessment and the 6-month follow up. For demographic details of participants, see table 1.

Pain-sensory profile

For PPTs, significant between- and within-group differences were indicated (RM-ANOVA: $F[4,84] = 2.9$; $P=0.025$). The post-hoc tests showed that at baseline, the WAD-group displayed lower bilateral PPTs at the splenius- and upper trapezius sites compared to controls ($P=0.001$), as well as compared to all other timepoints ($P<0.001$). Furthermore, the WAD-group displayed lower PPT at the upper trapezius site on the

most symptomatic side when compared to the less symptomatic side at baseline (P<0.001). For the PPTs at the gastrocnemius site, no between-group differences were observed. However, the WAD-group displayed bilaterally lower PPTs at the gastrocnemius site at baseline (P<0.001) and at 2-weeks (P=0.039), compared to 6-months (Figure 3). A between-group difference for PPTs at the infraspinatus site on the dominant side was indicated (RM-ANOVA: F[2,84] = 7.0; P=0.002) with the post-hoc test revealing that displayed lower PPTs in the WAD-group compared to the control-group at all time-points (P=0.011, Table 2).

For the CPM responses, a between-group differences were found (RM-ANOVA: F[1,42] = 24.5). The post-hoc test showed an impaired CPM response for the WAD-group compared to the control-group at all timepoints (Figure 4). No significant within-group differences were found.

For PPTol, between-group differences were seen over time (RM-ANOVA: F[2,84] = 16.6; P<0.001) with the WAD-group displaying lower PPTol compared to the control-group at all timepoints (P<0.001), as well as a within-group difference when comparing baseline to 2-weeks (P=0.001) and 6-month (P<0.001, Table 2).

The WAD-group reported expanded pressure-induced referred pain areas when compared to the control-group at baseline (P=0.028) and 6-month (P=0.037), but not at 2-weeks (P=0.051). No significant change was seen in either group over time found for the area of pressure-induced referred pain (Table 2).

The analysis of inter-relationships among the pain sensory profile measures revealed a positive correlation between recordings at all PPT sites (i.e. bilateral splenius capitis, upper trapezius, gastrocnemius, and infraspinatus muscles) at all time points indicating that higher PPTs at one site were related to higher recordings at other sites throughout the study (Supplementary Table 2, Supplemental Digital Content 2,

http://links.lww.com/CJP/A812). Additionally, a significant positive correlation between PPTs on the most symptomatic side and PPTol was observed at all time points indicating that higher PPTs were related to higher PPTol throughout the study (Supplementary Table 3, Supplemental Digital Content 3, http://links.lww.com/CJP/A813).

Psychometric variables

At baseline, when compared with the control-group, the WAD-group reported significantly higher pain intensity, a larger area of perceived pain, higher levels of pain catastrophizing and kinesiophobia, sleep problems, and higher levels of depression ($P<0.001$). These group differences remained significant at 2-weeks and 6-months for all questionnaires except the PCS (Table 3). No significant within-group changes were observed for the control-group for any of the questionnaires, whereas a significant improvement was seen for all questionnaire data in the WAD-group (Table 3 and Figure 5).

According to the preestablished classification of NDI scores, 27% of WAD-group was fully recovered at 2-weeks, 46% reported mild symptoms, and 27% moderate to severe symptoms. At 6-months follow-up 68% was fully recovered while the remaining 32% reported only mild symptoms.

DISCUSSION

This study shows that participants with WAD displayed increased pain sensitivity and impaired CPM response before a standardized rehabilitation compared to a healthy pain-free control-group. However, a novel finding of the current study is that over time, the pressure pain sensitivity for the WAD-group approximated that of the control-group, along with a reduction of perceived pain intensity and disability. The expanded referred area of pain following supra-threshold pressure stimulation reduced over time, whereas

the impaired CPM response in the WAD-group seen at baseline did not improve. Over time, the WAD-group displayed a reduction in pain catastrophizing, kinesiophobia, depression, and sleep problems, although only pain catastrophizing reached the level of what was observed in the control-group.

Pain-sensory profile

The WAD-group displayed lower PPTs in and around area but not in remote areas when compared to the control-group. In contrast, previous studies have demonstrated reduced PPTs at remote non-painful sites in WAD populations, which is considered a sign of facilitated central pain mechanisms (22, 26, 60). However, the current results are in line with those of Sterling, Jull (47) where, based on the NDI score at 6-months, only those with moderate/severe symptoms (NDI>30) had reduced widespread PPTs at baseline while this was only true for local sites for those with mild symptoms (NDI:10-28) or who had recovered (NDI<8). Furthermore, in line with the current the previous study found the PPTs of those in the mild and recovered groups (based on the NDI) to be comparable to those in a healthy pain-free control-group at follow-up (47). This normalization of local PPTs was suggested to reflect the healing of underlying soft-tissue injury rather than altered central pain mechanisms (47). It is possible that the lower local PPTs seen in the current study simply reflect ongoing local nociception which normalizes over time, in line with tissue healing. However, soft-tissue damage cannot be the only explanation for the current findings considering the likely involvement of central pain mechanisms, as seen in area of referred pain following pressure stimulation and reduced efficiency of the CPM system despite recovery (Table 2).

The expanded area of referred pain following a painful stimulus seen in the WAD-group in line with previous findings (25, 61) and is possibly an indication of an

increased sensitivity of central pain mechanisms (37, 62). It has been suggested that supra-threshold stimuli may be better suited than PPTs to determine differences in pain mechanisms (63), which could be the case in the current study. Here, however, the WAD-group showed larger areas of referred pain than the control-group, which remained unchanged throughout the recovery process while PPTs normalized. This could suggest that that PPTs may be feasible to monitor the presence of ongoing nociception but less sensitive to determine potential sensitization of central pain mechanisms in recovered-mild WAD-participants. The same may apply for the reduced CPM response observed in the WAD-group when compared to the control-group. While the findings of an impaired CPM response in the current study are in line with previous findings in both WAD-populations (27, 64, 65) and other clinical pain conditions (21, 66-68), this response did not resolve over time as pain intensity and disability diminished. However, recent findings in recurring spinal pain indicate that the impaired CPM response seems to be stable despite fluctuations in pain (40), which may indicate a delay in the normalization of CPM following pain resolution as compared with other measures such as PPTs. In summary, these current findings may suggest that CPM and area of perceived pain following a painful stimulus may need considerable time following pain resolution to normalize. Also, a CPM response may not be an appropriate biomarker for recovery as previously suggested (69), as such response may potentially be driven by other underlying mechanisms than pain alone (21, 70) with is supported by the considerable variability reported for CPM (71). With this in mind, static measures such as PPT, which have been suggested to be more stable over time (71), may be better suited to monitor the recovery process in whiplash-participants.

Psychometric variables

The pain intensity, area of perceived pain and disability improved significantly throughout the study for the WAD-group. Based on the NDI score alone, all the included participants could be considered having only mild disability or being fully recovered at 6 months when using the definition (NDI<8) provided by Sterling et al. (47). Furthermore, when considering current clinical prediction rules for the prognosis of WAD (72, 73), it is not surprising that the included WAD-population in the current study all recovered, although this is not reflected in CPM response.

Psychometric variables, such as catastrophizing and depression, have been suggested to impact both pain experience and sensitization of central pain mechanisms (21, 70, 74). In the present study, the WAD-group performed unfavorably on TSK, BDI, and MOOS-sleep when compared to the control-group at all timepoints, whereas the PCS normalized over time. This is in line with previous reports from acute WAD-populations where higher levels of pain catastrophizing, kinesiophobia, depression, and sleep problems were demonstrated (15, 18, 39, 75). Furthermore, all of these variables improved significantly following rehabilitation, similar to previous findings (14, 76-78) and without know the pre-injury values for these variable it is not clear if there is room for further improvement to scores in line what was observed for controls.

Interestingly, despite these favorable outcomes in psychometric variables, only the PPT responses followed a similar trajectory whilst the CPM response did not. A lack of association between a CPM response and psychometric factors has previously been reported by Nahman-Averbuch, Nir (70), although the authors did report that certain psychological factors do seem to correlate with modality-specific (i.e., electrical, heat & pressure stimulation) CPM. However, as pain catastrophizing for the WAD-group was comparable to the control-group at the 6-month follow-up while the area of referred

pain following painful stimulation remained facilitated, such a relationship cannot be inferred from the current data. Future studies that are appropriately designed to investigate such associations are needed to investigate whether a relationship exists between changes in psychometric and psychophysical variables in WAD-populations.

Limitations and methodological considerations

In the current study all WAD participants were referred to the rehabilitation by an insurance company and while there is literature to suggest that compensation claims may delay recovery (79) the evidence on the topic is not clear (80). Although it is impossible to assess if any potential compensation claims have had any detrimental effect on the speed of recovery in the current study, this seems unlikely when considering the large and clinically important improvements in both pain and disability.

The current study did not monitor if and what medication the WAD-participants might have consumed prior to and during the study and as analgesic medication is widely used in WAD (81), it is therefore unclear if this could have influenced the results.

Another important limitation that should be considering is the CPM protocol used in the current study. Here, the CPM protocol followed a parallel design, where the test stimulus (i.e., PPTs) was applied at the same time as the conditioning stimulus (i.e., pressure-cuff). Although the CPM response is not considered to be a reflection of distraction from the original painful stimulus (82), it is possible that the participants directed their attention towards the conditioning stimulus rather than the PPT being recorded. Therefore, future studies could consider a sequential design for such analyses. Additionally, it is important to acknowledge that CPM responses are highly variable (71), which may indicate that the study was potentially underpowered to detect any

change in CPM on a group level, which should be taken into consideration in future studies.

The study was designed to detect between- and within-group differences, the sample size was underpowered to perform a stratified analysis between recovered and non-recovered WAD-participants at 2-week or 6-months. A larger sample and a longer follow-up period would have enabled an investigation of whether changes in CPM response occur slower than changes in pain and disability in WAD-participants or if complete pain recovery only occurs in those who demonstrate normalization of the pain-sensory profile.

Conclusion

This is the first study to investigate the temporal course of CPM response in WAD-participants during and after a rehabilitation period. Although the findings indicate a normalization of some psychophysical and psychometric variables, it is unclear how or if these are related. CPM response was significantly impaired in the WAD-participants and remained unchanged despite significant improvements in pain, disability, and PPTs. Such findings could indicate that normalization of CPM may not be required for WAD-recovery. Future studies with larger sample sizes and longer follow-up are warranted to investigate whether this can affect the clinical trajectory of WAD-participants.

Author's contribution

SWMC and TSP drafted the initial protocol with contributions from all authors. PBL was in charge of planning and executing data collection in association with VDG and PH. PBL conducted the statistical analysis with contributions from all authors. SWMC and PBL wrote the first draft of the manuscript. All authors discussed and contributed to the final version of the manuscript.

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Figure 1. Schematic overview of the study design and timeline with assessments at baseline, 2 weeks, and 6 months for the two groups (WAD, Control). Only the WAD-group participated in the 10 rehabilitation sessions while the control-group received no other intervention/contact time outside the three experimental assessments.

Figure 2. Bilateral assessment sites of pressure pain threshold over the splenius capitis, upper trapezius, and gastrocnemius muscles (□,■). Pressure cuff placed over the nondominant arm. CPM assessment sites contralateral to the conditioning stimulus (■). Supra-threshold pressure stimulation over the infraspinatus muscle on the dominant arm (❋).

Figure 3. Pressure pain thresholds in WAD-participants (n=22) and controls (n=22) at baseline, 2-week and 6-month patients. WAD: Whiplash associated disorders; MS side: Most symptomatic or dominant side; LS side: Less symptomatic or non-dominant side; kPa: kilopascal. * Significant between-group difference (P<0.05). # Significant within-group difference compared to baseline (P<0.05). ‡ Significant within-group difference compared to the LS side (P<0.05). Values are the mean ± SD. Number of days following the motor vehicle accident and baseline was 11-21, for 2-weeks follow-up 24-37 and 6-month follow-up 178-206 days.

Figure 4. Conditioned pain modulation (CPM) in WAD-participants (n=22) and controls (n=22) at baseline, 2-week and 6-month patients. WAD: Whiplash associated disorders; kPa: kilopascal. * Significant between-group difference (P<0.05). Values are the mean ± SD. Positive values indicate an inhibitory response, and negative values indicate a facilitatory response. Number of days following the motor vehicle accident and baseline was 11-21, for 2-weeks follow-up 24-37 and 6-month follow-up 178-206 days.

Figure 5. Area of perceived pain for the WAD-group (n=22) at baseline, 2-weeks, and 6-months. Median [IQR 25-75]. Total area of perceived pain (i.e., sum of anterior and posterior views) expressed in pixels: a = 13029 [7302-18980]; b = 1964 [985-6022]; c = 0 [0-3303]. WAD: Whiplash associated disorders. * Significant between-group difference (P<0.05). # Significant within-group difference compared to baseline (P<0.05). Number of days following the motor vehicle accident and baseline was 11-21, for 2-weeks follow-up 24-37 and 6-month follow-up 178-206 days.

Table 1. Characteristics of participants and periods of assessment.

	WAD	Control
Age (years)	30.6 (7.4)	30.5 (7.4)
Female (n, %)	14 (64%)	14 (64%)
Time period between MVA and baseline assessment (days)	15 (4)	-
Time period between baseline assessment and 2-weeks assessment (days)	17 (2)	17 (2)
Time period between 2-weeks and 6-months assessments (days)	165 (7)	170 (6)

Mean ± SD (N=44: 22 WAD, 22 Control). Whiplash associated disorders (WAD); Motor Vehicle Accident (MVA). No WAD participant lost consciousness during/immediately after the MVA but all reported pain within the following 24hr. Baseline assessment corresponds to the time of inclusion in the study.

Table 2. Questionnaire scores for WAD-participants and controls at baseline, 2-week and 6-month: Results from between and within-group comparisons.

		BASELINE	2-WEEK	6-MONTH
NRS	WAD	7.5 [7.0-8.0]	3.0 [2.0-5.0] [#]	0.0 [0.0-2.0] [# ‡]
(0-10)	Control	0 [0-0] [*]	0 [0-0] [*]	0 [0-0] [*]
NDI	WAD	36 [34-48]	11 [6-28] [#]	2 [0-12] [# ‡]
(0-100)	Control	-	-	-
PCS	WAD	18 [13-26]	10 [4-18] [#]	9 [4-16] [#]
(0-52)	Control	10 [5-14] [*]	9 [6-16]	11 [4-16]
TSK-11	WAD	32 [26-35]	22 [19-25] [#]	20 [16-25] [#]
(11-44)	Control	16 [14-20] [*]	16 [14-21] [*]	16 [14-19] [*]
BDI	WAD	7 [5-11]	6 [4-10]	4 [0-10] [#]
(0-62)	Control	2 [0-3] [*]	2 [0-3] [*]	1 [0-4] [*]
MOS-Sleep	WAD	50 [45-61]	45 [28-51] [#]	29 [22-45] [#]
(0-100)	Control	20 [13-28] [*]	18 [13-29] [*]	18 [13-31] [*]

Median [IQR 25-75] (N=44: 22 WAD, 22 Control). Whiplash associated disorders (WAD); Numeric Rating Scale (NRS); Neck Disability Index (NDI); Pain Catastrophizing Scale (PCS); Shortened version of Tampa Scale of Kinesiophobia (TSK-11); Beck Depression Inventory (BDI); Medical Outcomes Study Sleep Scale (MOS-Sleep). [*] Significant between-group difference ($P<0.05$). [#] Significant within-group difference compared to baseline ($P<0.05$). [‡] Significant within-group difference compared to 2-week ($P<0.05$). Number of days following the motor vehicle accident and baseline was 11-21, for 2-weeks follow-up 24-37 and 6-month follow-up 178-206 days.

Table 3. Pain sensitivity values for WAD-participants and control-group at baseline, 2-week, and 6-month: Results from between and within-group comparisons

Procedure	Variable	Group	BASELINE	2-WEEK	6-MONTH
CPM	PPTol 7/10 NRS (mmHg)	WAD	117 ± 39	137 ± 49[#]	148 ± 47[#]
		Control	227 ± 62[*]	219 ± 62[*]	215 ± 62[*]
STPS	IS PPT (kPa)	WAD	214 ± 149	285 ± 173[#]	292 ± 174[#]
		Control	329 ± 137[*]	335 ± 152	339 ± 126
	RPPS area (pixels)	WAD	2830 [734-7792]	1655 [258-5159]	2070 [410-5232]
		Control	585 [289-1811][*]	608 [357-1737]	557 [367-1767][*]

Mean ± SD or median [IQR 25-75] (N=44: 22 WAD, 22 Control). Pressure Pain Threshold (PPT); Conditioned Pain Modulation (CPM); Pressure Pain Tolerance (PPTol); Supra-Threshold Pain Stimulus (STPS); Referred Pain evoked by supra-threshold Pressure Stimulation (RPPS); Numeric Rating Scale (NRS); Infraspinatus muscle (IS); kilopascal (kPa). *Significant between-group difference ($P<0.05$). [#] Significant within-group difference compared to baseline ($P<0.05$). Number of days following the motor vehicle accident and baseline was 11-21, for 2-weeks follow-up 24-37 and 6-month follow-up 178-206 days.

Supplementary Table 1. Description of interventions within the 30-minute standardized physiotherapy rehabilitation.

Intervention	Description
Pain Neuroscience Education	The pain neuroscience education was based on work by David S. Butler & G. Lorimer Moseley[1]. The aim was to provide an understanding of pain to support self-management.
Motor Control Exercises	Motor Control Exercises were based on the recommendations by Gwendolen Jull and Michelle Sterling[2]. The exercises could include active neck range of motion in all directions, , cranio-cervical flexion, and exercises targeting muscles involved in scapular motion.
Manual Therapy	If indicated, manual Therapy could include techniques for joint mobilizations for the upper, mid, and lower cervical spine[3] as well as soft tissue mobilizations for the upper trapezius, sternocleidomastoid and suboccipital muscles.
Transcutaneous Electrical Nerve Stimulation	Transcutaneous Electrical Nerve Stimulation was applied during the last 10 minutes of treatment sessions to induce a short-term analgesic effect[4]. Adhesive silicone electrodes were placed bilaterally on each side of the upper trapezius, one electrode over the C7 spinous process and the other electrode over the scapula, approximately on the supraspinatus fossa after which the patient was placed in a supine position. The intensity was raised according to the tolerance and comfort of each participant.

[1] Butler DS, Moseley GL 2003 Explain Pain Second edition (2013) Noigroup Publications, Adelaide. (Translated into Spanish)

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Table S2. Correlations between pressure pain thresholds at baseline, 2-weeks, and 6-months for the WAD-group (n=22).

BASELINE	SPL MS	UT MS	GN MS	IS	SPL LS	UT LS	GN LS
SPL MS	1	,82**	,53*	,82**	,90**	,81**	,57**
UT MS		1	,65**	,91**	,84**	,95**	,71**
GN MS			1	,73**	,71**	,68**	,93**
IS				1	,90**	,90**	,76**
SPL LS					1	,88**	,71**
UT LS						1	,70**
GN LS							1

2-WEEKS	SPL MS	UT MS	GN MS	IS	SPL LS	UT LS	GN LS
SPL MS	1	,90**	,80**	,78**	,94**	,73**	,84**
UT MS		1	,81**	,90**	,88**	,82**	,86**
GN MS			1	,74**	,77**	,64**	,94**
IS				1	,86**	,90**	,72**
SPL LS					1	,81**	,80**
UT LS						1	,60**
GN LS							1

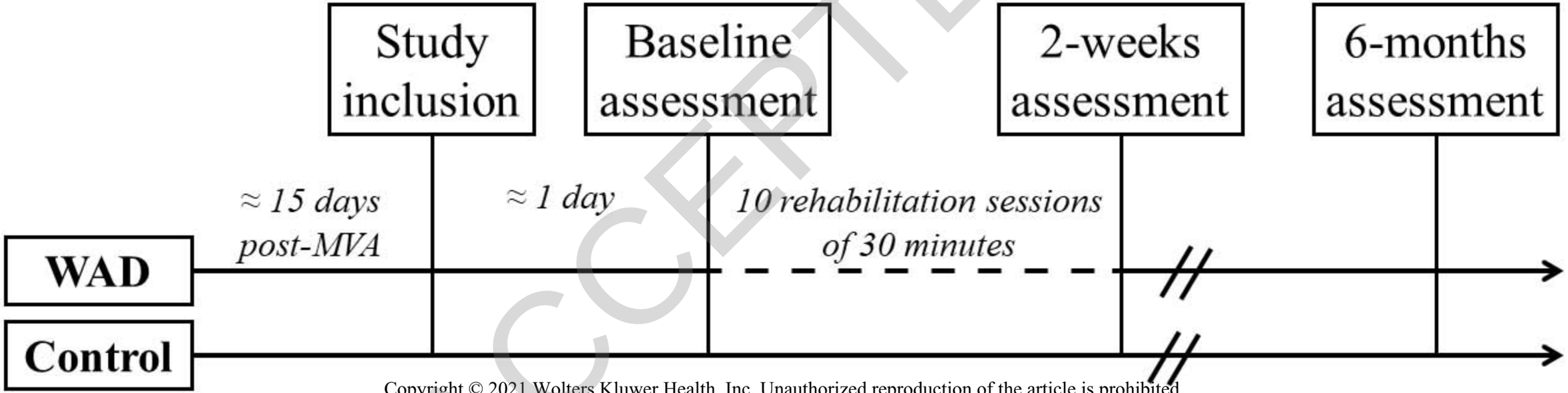
6-MONTHS	SPL MS	UT MS	GN MS	IS	SPL LS	UT LS	GN LS
SPL MS	1	,84**	,50*	,73**	,87**	,82**	,61**
UT MS		1	,65**	,92**	,80**	,93**	,77**
GN MS			1	,76**	,70**	,59**	,95**
IS				1	,73**	,89**	,83**
SPL LS					1	,80**	,80**
UT LS						1	,72**
GN LS							1

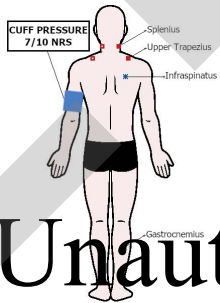
Whiplash associated disorders (WAD); MS side: Most symptomatic or dominant side; LS side: Less symptomatic or non-dominant side; SPL: Splenius capitis muscle; UT: Upper trapezius muscle; GN: Gastrocnemius muscle; IS: Infraspinatus muscle. Pearson correlation's coefficients; ** = p<0.01; * = p< 0.5. Number of days following the motor vehicle accident and baseline was 11-21, for 2-weeks follow-up 24-37 and 6-month follow-up 178-206 days.

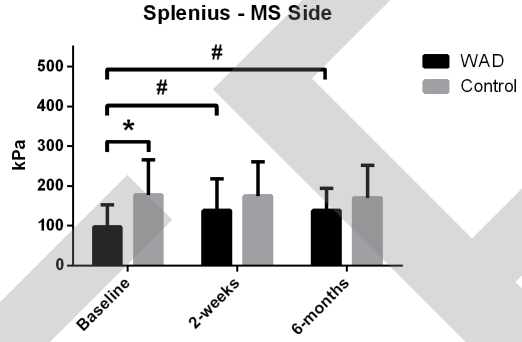
Table S3. Correlations between pressure pain thresholds, conditioned pain modulation, pressure pain tolerance and referred pain evoked by supra-threshold pressure stimulation at baseline, 2-weeks, and 6-months for the WAD-group (n=22).

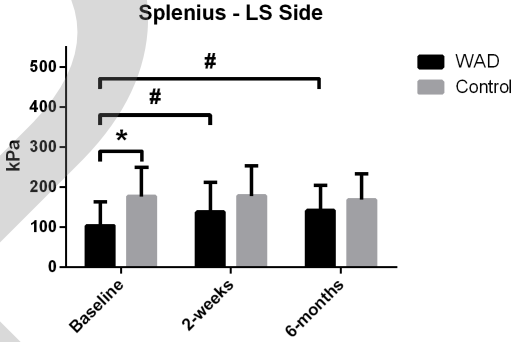
	BASELINE						**2-WEEKS**						**6-MONTHS**					
Baseline	CMP SPLMS	CPM UTMS	CPM GNMS	PPTol	RPPS area		**2-weeks**	CMP SPLMS	CPM UTMS	CPM GNMS	PPTol	RPPS area	**6-months**	CMP SPLMS	CPM UTMS	CPM GNMS	PPTol	RPPS area
PPT SPLMS	,08	-,25	,29	,67**	-,34		PPT SPLMS	-,15	-,18	,06	,61**	-,32	PPT SPLMS	,38	,24	,53*	,57**	-,23
PPT UTMS	,42	-,20	,08	,54**	-,40		PPT UTMS	,13	,03	,17	,59**	-,28	PPT UTMS	,53*	,18	,66**	,56**	-,24
PPT GNMS	,33	,12	-,25	,49*	-,38		PPT GNMS	-,18	,07	-,11	,50*	-,36	PPT GNMS	,39	,22	,16	,26	-,16
CMP SPLMS	1	,33	,05	,09	-,18		CMP SPLMS	1	,30	,22	-,14	,08	CMP SPLMS	1	,44*	,44*	-,09	-,22
CPM UTMS		1	,07	,06	,03		CPM UTMS		1	-,06	-,58**	,12	CPM UTMS		1	,30	-,14	-,33
CPM GNMS			1	,10	,01		CPM GNMS			1	,12	,19	CPM GNMS			1	,35	-,05
PPTol				1	-,29		PPTol				1	-,29	PPTol				1	-,29
RPPS area					1		RPPS area					1	RPPS area					1

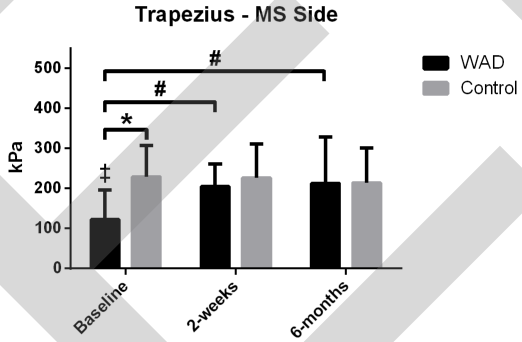
Whiplash associated disorders (WAD); MS side: Most symptomatic or dominant side; LS side: Less symptomatic or non-dominant side; SPL: Splenius capitis muscle; UT: Upper trapezius muscle; GN: Gastrocnemius muscle; IS: Infraspinatus muscle; PPT: Pressure Pain Threshold; CPM: Conditioned Pain Modulation; PPTol: Pressure Pain Tolerance; RPPS: Referred Pain evoked by supra-threshold Pressure Stimulation. Pearson correlation's coefficients; ** = p<0.01; * = p< 0.5. Number of days following the motor vehicle accident and baseline was 11-21, for 2-weeks follow-up 24-37 and 6-month follow-up 178-206 days.

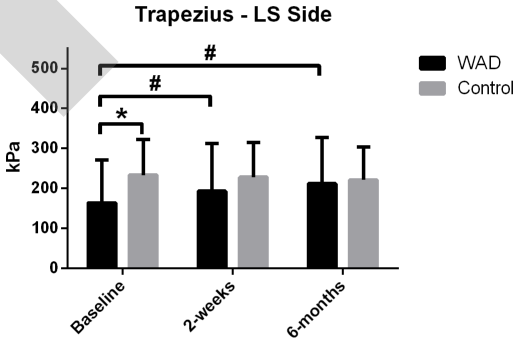


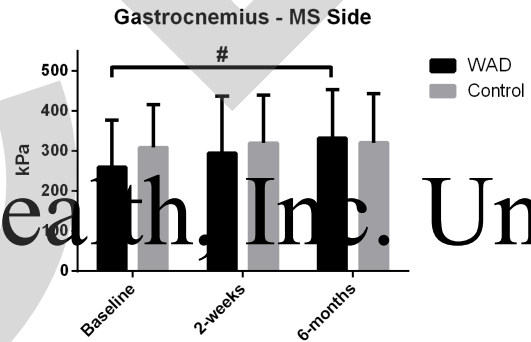


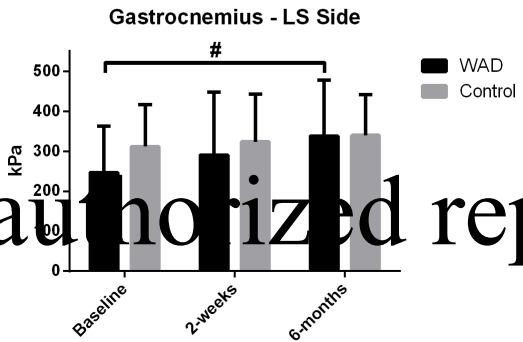


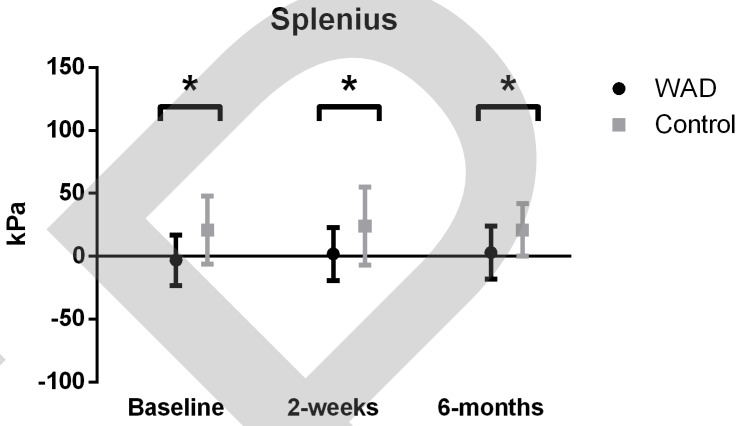


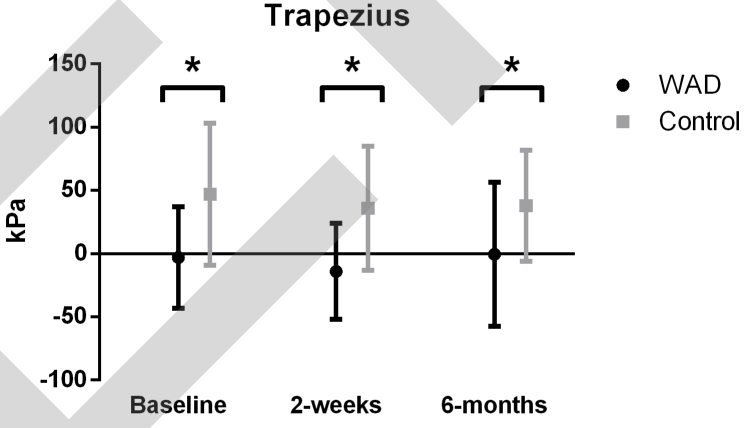


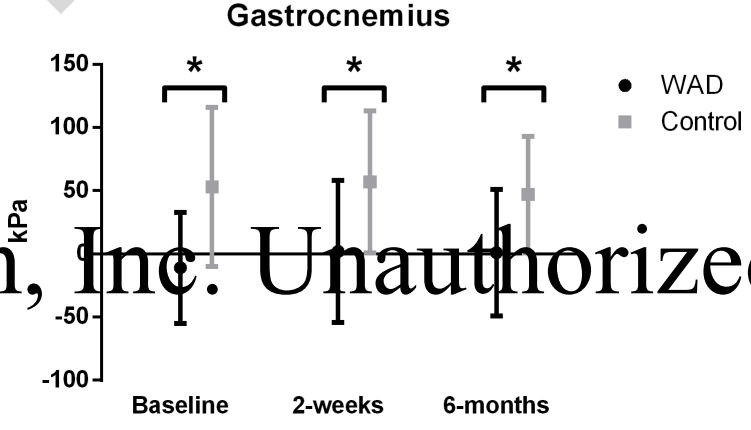


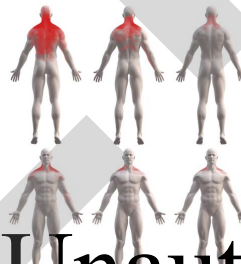












BASELINE	2-WEEKS	6-MONTHS
pixels: 3,029*	1,964'*	0'*
[7,302-18,980]	[985-6,022]	[0-3,303]

Inc. Unauthoriz