**Exercise induced hypoalgesia is impaired in chronic Whiplash Associated Disorders (WAD)
with both aerobic and isometric exercise.**

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ABSTRACT

Objectives: Exercise induced hypoalgesia (EIH) can be impaired in patients with chronic pain and may be dependent on exercise type. Factors predictive of EIH are not known. This study aimed to: 1) compare EIH in participants with chronic WAD to asymptomatic controls, 2) determine if EIH differs between aerobic and isometric exercise, 3) determine predictors of EIH.

Methods: A pre-post study investigated the effect of single sessions of submaximal aerobic treadmill walking and isometric knee extension on EIH in 40 participants with chronic whiplash associated disorders (WAD) and 30 controls. Pressure pain thresholds were measured at the hand, cervical spine and tibialis anterior. Pain intensity, reported previous week physical activity, temporal summation and conditioned pain modulation (CPM) were measured at baseline.

Results: Participants with WAD demonstrated impaired EIH. Whilst, the WAD participants demonstrated a partial EIH response (EIH induced at the hand during exercise), no EIH response was found immediately after exercise. There was no difference in EIH between exercise types. In the WAD group, moderate physical activity levels predicted EIH at the hand and high physical activity levels predicted impaired EIH at the neck. More efficient CPM predicted EIH at the hand and less efficient CPM predicted impaired EIH at the neck. These associations were found for isometric exercise only.

Discussion: Individuals with chronic WAD have impaired EIH with both aerobic and isometric exercise. Higher levels of physical activity and less efficient CPM may be associated with impaired EIH. This may have implications for exercise prescription in this patient group.

Key words: exercise induced hypoalgesia, exercise, whiplash associated disorders

The authors declare no conflict of interest.

INTRODUCTION

Chronic whiplash-associated disorders (WAD) are defined as neck pain and other symptoms arising for an acceleration/deceleration injury to the neck, usually from a motor vehicle crash (MVC). They are characterized by widespread increased pain sensitivity to various stimuli (e.g. pressure, thermal, electrical) [1] indicative of a pro-nociceptive profile. Thus, interventions that reduce pain sensitivity may be beneficial for this condition. Exercise is one intervention that has consistently demonstrated capacity to reduce pain sensitivity in healthy individuals [2], with this phenomena termed exercise-induced hypoalgesia (EIH) [3].

Conflicting results have been demonstrated for EIH in chronic WAD. Van Oosterwijck and colleagues [4] showed that individuals with WAD and moderate pain and disability demonstrated hyperalgesia not hypoalgesia following a short bout (four minutes) of sub-maximal stationary cycling. More recently, a similar exercise protocol demonstrated neither hyper- nor hypoalgesia [5]. This result is consistent with a recent study showing that individuals with chronic WAD and milder pain and disability demonstrated EIH following a three-minute isometric wall squat but not following 30 minutes of sub-maximal stationary cycling [6]. It is unclear whether the discrepant results are due to the study samples investigated (mild versus moderate pain and disability), or differences in the types or duration of exercise performed. Thus, further investigation of EIH in people with chronic WAD is warranted.

Factors associated with or predictive of EIH are not clear, but their identification could assist with improved EIH activation. Recent studies have not found any association between other inhibitory pain modulation processes such as conditioned pain modulation (CPM) and EIH in people with chronic WAD [6] [5]. However, in other conditions including mixed chronic musculoskeletal pain and osteoarthritis, impaired EIH has been demonstrated to co-occur with impaired CPM, [7] [8] prompting further investigation of relationships between these endogenous processes.

Another measure of pro-nociception is temporal summation [9]. Increased temporal summation of pain has been found in patients with chronic musculoskeletal pain and high pain sensitivity [7]. Less temporal summation has been shown to be associated with greater EIH in healthy individuals [10]. We

are not aware of any studies investigating if temporal summation is predictive of EIH in chronic WAD. Physical activity levels may also influence pain modulation [11]. A recent study demonstrated that levels of physical activity differentially impacted pain inhibitory (CPM) and facilitatory processes (temporal summation) in older adults [11]. However, the relationship between physical activity and EIH has been mixed [12] [13]. Thus, further investigation of the relationship between CPM, temporal summation, physical activity and EIH is warranted.

This study aimed to: 1) compare EIH in chronic WAD and healthy controls with isometric and aerobic exercise, 2) determine whether there is a differential EIH response to exercise type in chronic WAD, and 3) determine if EIH is predicted by CPM, temporal summation, neck pain intensity or physical activity levels. We hypothesized that individuals with chronic WAD would demonstrate impaired EIH to both isometric and aerobic exercise, and that increased neck pain intensity, temporal summation of pain, impaired CPM and low physical activity levels would predict impaired EIH.

MATERIALS AND METHOD

Participants

Participants with WAD were recruited through referral from medical practitioners, physiotherapists, general advertising and from a patient database.

Inclusion criteria: 1) average neck pain ($\geq 4/10$) over the last 24 hours or moderate activity limitation because of pain (item eight of Short Form 36 survey); 2) involvement in a traumatic event (e.g. motor vehicle crash, boat crash, fall); 3) symptom duration ≥ 3 months and < 10 years; and 4) classifiable as WAD grade II (neck pain and impairment such as movement loss and/or tenderness) according to the Quebec Task Force classification system where grade 1 is neck pain but no physical impairment, grade III is neck pain and neurological deficit and grade IV is neck fracture or dislocation [14].

Exclusion criteria: 1) WAD Grade III or IV injury (neurological deficit, fracture or dislocation) [14]; 2) concussion or other head injury as a result of the traumatic event; 3) insufficient English language ability to complete questionnaires; 4) previous diagnosis of a neurological disorder (e.g. multiple sclerosis), inflammatory condition (e.g. rheumatoid arthritis), cardiovascular condition (e.g.

hypertension), metabolic disorder (e.g. diabetes), psychopathology (e.g. depression) or pregnancy; 5) unwilling to perform the prescribed exercises; 6) screened positive on the validated Physical Activity Readiness Questionnaire (PAR-Q) indicating a need to consult a medical doctor prior to participating in physical activity [15] unable to discontinue analgesic/anti-inflammatory medications for 48 hours prior to participation in the research sessions.

Asymptomatic controls were recruited from the local university campus through word-of-mouth, electronic advertising and posted advertisements, and were included if they reported no history of neck pain or other chronic pain conditions requiring treatment in the past 12 months. They were excluded on the same general health criteria as the participants with WAD. All participants were aged 18 – 65 years old and received a \$50 gift voucher for participating.

Procedure

Volunteers who met the inclusion criteria were invited to attend a single session to perform a submaximal aerobic treadmill exercise, and an isometric knee extension exercise. Participants were asked to refrain from analgesics for 48 hours prior to testing and to refrain from exercise, nicotine, alcohol and caffeine for 12 hours prior to testing. Participant information was provided and written informed consent obtained prior to completing the baseline questionnaires, the baseline physical measures and tests of endogenous analgesia.

After collection of baseline measures, participants sat quietly for 15 minutes prior to commencing exercise to avoid the testing protocols influencing exercise response [16]. This time was defined as the rest period. Following the rest period, pressure pain threshold (PPT) was measured at three sites (left tibialis anterior, right dorsal hand region and right C5/6 articular pillar) immediately prior to commencing exercise. Participants then completed either an aerobic or isometric exercise session, immediately followed by collection of post-exercise PPTs, before repeating this procedure with the alternate exercise after 15 minutes rest (Fig. 1). The order of exercises was randomized through block (size = 4 participants) randomization. Participants rested after the second exercise for 15 minutes, prior to having final PPTs measured (Fig. 1). Ethical clearance for this study was granted by the Griffith University Medical Research Ethics Committee (2016/141).

Baseline Measures

Baseline Questionnaires

Participants with WAD completed an intake questionnaire, which included the following: age, sex, level of education, traumatic event information and compensation status. The following measures were also collected for descriptive purposes: 1. Neck Disability Index (NDI), a valid and reliable measure of neck pain related disability [17]; 2. Pain Catastrophizing Scale (PCS), a 13 item questionnaire that measures catastrophic thinking about pain [18], 3. Tampa Scale of Kinesiophobia (TSK), a 17-item checklist that measures fear of movement/(re)injury [19], and 4. PCL-5 (PTSD Checklist for DSM-5), a 20-item posttraumatic stress disorder checklist [20]. Asymptomatic controls completed the intake questions that did not pertain to the injury, and the NDI.

Baseline Physical Measures

Visual Analogue Scale (VAS) (0-100mm) was used to measure average pain intensity over the past 24 hours, with 0 = no pain, and 100 = worst pain imaginable [21].

Physical activity levels during the past 7 days were measured with the long form of the International Physical Activity Questionnaire (IPAQ) [22]. Across various domains (transport, work, home and leisure time), the amount of time performing walking and moderate or vigorous PA is collected, together with time spent sitting. Guidelines provided by <https://sites.google.com/site/theipaq/home> were used for data processing and scoring of the questionnaire. IPAQ results were reported in categories (LOW, MODERATE or HIGH physical activity levels). Scoring: HIGH level equates to approximately one hour or more of physical activity per day of at least a moderate intensity level; MODERATE level equates to some activity, equivalent to global recommendations on physical activity for health [23], and equal to half an hour on most days of at least moderate intensity physical activity; and LOW level equates to not meeting any of the criteria for either MODERATE or HIGH levels of physical activity.

The IPAQ categories were used to predict EIH.

Height, weight, resting heart rate and resting blood pressure were also measured.

Baseline Conditioned Pain Modulation (CPM)

The participant's right hand was immersed in thermostatically controlled 5°C water, and their pain threshold was determined by asking them to verbally state when he/she began to feel any pain. The time in seconds, that passed between the immersion and verbal confirmation was recorded as the cold pressor pain threshold. Participants were instructed to keep the immersed hand in the cold water for as long as possible and withdraw it from the water when no longer tolerable. Participants were motivated through verbal encouragement to maintain immersion for the duration of the defined period of 120 seconds. Cold pressor pain tolerance was defined as the total time, in seconds, that the participant's hand was immersed. The peak pain intensity rating was recorded during this task. Single PPTs were measured over the muscle belly of the left tibialis anterior at the time of cold pain threshold occurring, and at 30, 60 and 90 seconds during immersion. The average PPT during immersion was labelled as 'Average PPT'. CPM was calculated by subtracting the average of the three baseline PPTs from the Average PPT (during immersion). Relative CPM was then calculated using the formula $[(PPT_{\text{Average}} - PPT_{\text{Baseline}}) / PPT_{\text{Baseline}}] * 100$. Relative CPM was calculated for utility in the prediction model for EIH.

Baseline Temporal Summation

Temporal summation was measured using a 256mN PinPrick stimulator (MRC Systems, Heidelberg, Germany) [24] at two body regions, the C5/C6 neck region whilst the participant lay prone, and also over the dorsum of the left hand between the 2nd and 3rd metacarpals, whilst the participant was sitting. Application to the neck or hand region was block (size = 4) randomized for each participant. Participants were initially asked to rate the perceived pain intensity of a single stimulus, and secondly, to rate the average pain intensity over a series of 10 stimuli using a '0-100' numerical rating scale [24]. The numerical rating score was defined as 0 = no pain, and 100 = worst pain imaginable. There was a 20 second interval between application of the single stimulus and the first of the ten repeated stimuli. The repeated stimuli were performed to the beat of a metronome at a rate of 1/sec within an

area of 1 cm². This procedure (i.e. single stimulus, then 20 second interval, followed by 10 repeated stimuli) was repeated three times, with 30 seconds interspersing each procedure. Temporal summation was examined using the Wind Up Ratio (WUR) [24], defined as the ratio of the pain rating from the 10 repeated stimuli to the pain rating from the single stimuli. WUR was calculated for each of the three repeated trials, and all WUR values were used in the prediction model.

Exercise Sessions

Aerobic exercise

Participants completed a standardised, submaximal treadmill walking test based on the Aerobic Power Index test [25]. The procedure was as follows:

1. Prior to starting the test, the rating of perceived exertion scale was explained to participants [26] and 75% of age-predicted maximum heart rate was calculated ($.75 \times (220 - \text{age in years})$).
2. The participant rated their current neck pain on an 11-point (0 to 10) numerical pain rating scale (NRS).
3. The participant started to walk (0% grade) at 5-7 km/hr or at a comfortable pace. If, following 4 minutes, attainment of 75% of age-predicted maximum heart rate had not been achieved, the grade was increased by 2%. The grade was increased by 2% every minute until 75% age-predicted maximum heart rate was achieved.
4. The participant continued to walk at this power output for a total duration of 30 minutes. The Aerobic Power Index test has been shown to be reliable for healthy populations and populations with chronic medical conditions, and enables completion of a standardised, 30minute aerobic exercise session [25].
5. PPTs over the dorsum of the right hand between the 2nd and 3rd metacarpals were measured 90 and 180 seconds after exercise initiation.
6. Heart rate, rating of perceived exertion and blood pressure were recorded every two minutes during the increase in power output and then once every 4 minutes until the end of the exercise session.

7. NRS and PPTs at the cervical spine and tibialis anterior were collected immediately following exercise.

Isometric exercise

Participants completed a standardised isometric contraction of quadriceps femoris using the Biodex dynamometer (Biodex Medical Systems, New York). The procedure was as follows:

1. The participant was seated comfortably in the chair and familiarised with the equipment and procedure. NRS was collected;
2. Maximum isometric knee extensor strength at 90° knee flexion was determined during a 15 sec contraction. Heart rate, blood pressure and perceived exertion were measured immediately post-exercise;
3. Participants rested for 1 minute. This procedure was repeated three times and the best value was used as the maximum voluntary contraction;
4. 20 and 25% of maximum voluntary contraction was calculated and following 10 minutes rest, participants were instructed to perform the contraction at 20-25% maximum voluntary contraction until exhaustion or a maximum of 3 minutes;
5. PPTs over the dorsum of the right hand between the 2nd and 3rd metacarpals were measured 90 and 180 seconds after exercise initiation.
6. Heart rate, blood pressure and perceived exertion were measured each minute.
7. NRS and PPTs at the cervical spine and tibialis anterior were collected immediately following exercise.

Primary Outcome Measure

Pressure pain thresholds (PPTs) were measured as per previously reported protocols [6]. A hand held pressure algometer (Somedic AB, Fastra, Sweden) with a probe diameter of 1cm² was used to measure PPTs over the C5/6 spinous process in the cervical spine, providing a measure of local pain sensitivity; over the muscle belly of the left tibialis anterior and the dorsum of the right hand between the 2nd and 3rd metacarpal regions, both uninjured areas providing a measure of remote pain

sensitivity. The cervical spine and tibialis anterior sites have previously been used in investigations of chronic WAD [27], whilst the hand region was chosen as a bodily region that could be accessed during exercise performance. The participants were asked to push a button when the sensation changed from one of pressure alone to one of pressure and pain. The assessor was able to observe the rate of application of pressure, but was blinded to actual readings through the use of tape. Triplicate readings were taken (20 sec interstimulus interval) at each site, saved to the algometer, and recovered at the end of the session for entry into an Excel spreadsheet. Measures at the cervical spine and tibialis anterior regions were taken prior to and immediately following the rest period and each exercise (Fig. 1). Hand measures were taken prior to and during exercise.

EIH was determined to have occurred with a significant positive change in PPTs, i.e. when post-exercise PPTs were greater than pre-exercise PPTs. Relative EIH was expressed as a percentage value as per the following formula: $[(PPT_{\text{During or Post-Exercise}} - PPT_{\text{Pre-Exercise}}) / PPT_{\text{Pre-Exercise}}] * 100$.

Data Analysis

Sample Size Determination

The study was powered to detect a statistically significant difference in EIH between healthy control participants and those with WAD. Sample size for the primary outcome was established *a priori* based on previous research demonstrating a significant difference in EIH between the two groups [4], (Cohen's $d=0.72$), power of 80% ($\beta = 0.20$) and significance set at $P < 0.05$ (two-tailed); establishing that a total of 32 participants were required in each group. The sample size in the WAD group was increased to 40, however, for use in the prediction of EIH. Assuming 10 cases per predictor are needed to investigate the effect of the four baseline predictors (pain intensity (VAS), physical activity, CPM and temporal summation), a total of 40 participants with chronic WAD were required.

Statistical Analyses

All data were initially inspected via scatterplots, boxplots and Shapiro-Wilk statistics to evaluate normality. Statistical analyses were performed in SPSS Statistics (Version 24; IBM, Armonk, NY, USA). Normally distributed data were reported as means and standard deviations (SD) or 95%

confidence intervals (95% CI) in the text. Otherwise, data were reported as median and interquartile range (IQR). Appropriate parametric or non-parametric analyses were undertaken for unadjusted baseline comparisons between WAD and control participants.

To examine the fidelity of the study (manipulation check), individual repeated measures ANOVAs, incorporating averages of the triplicate pre-intervention PPTs, were used to evaluate whether the 15-minute rest period employed between interventions (baseline, aerobic and isometric exercises) was effective in preventing sensitization at each body region (cervical spine, hand, tibialis anterior).

For analysis of baseline CPM, baseline TS, and EIH, multilevel modeling, using the linear mixed models (LMM) function in SPSS, was used. This technique has certain advantages over repeated measures ANOVA, including the ability to include data from patients with only one value of the repeated measure (e.g. has pre-score but not post-score) and the ability to use all trials of a measure (e.g. triplicate measures of pre-exercise PPTs) as opposed to using the average of the three trials. Random effects for participant (random intercept and/or random slope, where indicated) were used to account for the repeated responses from each participant. In addition, age and sex were considered potential confounders and, when significant on univariate testing ($p < .01$), were included in the multivariable model.

For baseline CPM, a random intercept LMM was indicated as the best fitting model. Average PPT during immersion and the average of the three baseline PPTs were used to examine whether the CPM response was different between the control and WAD groups. To examine temporal summation, WUR (i.e ratio of pain rating from 10 stimuli to the pain rating from a single stimulus) from all three trials per participant was used to examine the difference between Group (Controls and WAD) and within Body Region (cervical spine or hand). WUR could not be calculated when pain from the single stimuli was rated as zero (due to division by zero being impossible). The best fitting LMM for this model included a random slope. In addition to the LMM results for CPM and temporal summation, unadjusted results were also presented in tabular form. For temporal summation, averages across the three trials for the single stimulus, the 10 stimuli and the WUR were used in Table 3.

When comparing the effect of Exercise type on EIH between the Controls and WAD groups, individual random slope LMMs were constructed for each body region tested. The fixed effects of Time (pre/post), Exercise type (rest/aerobic/isometric) and study Group (Control/WAD) were included in the model. The interaction between Time, Group and Exercise type (Time*Group * Exercise) was examined for each region. This same model was then used to examine the relationship between Exercise type and EIH within the WAD group only. A Bonferroni correction for multiple comparisons was used when undertaking pairwise comparisons between the three exercise types. *P*-values less than .05 were considered significant for all the above analyses.

Lastly, individual random slope LMMs for each body region were used to examine predictors of EIH in WAD. Time was treated as a factor [categorical variable: Pre/During (hand) or Pre/Post (cervical and tibialis anterior)], and four baseline predictor variables were assessed for their association with EIH: initial neck pain (VAS), baseline previous week physical activity levels (IPAQ Category), relative CPM, and temporal summation (WUR). Age and sex were also assessed as potential confounders. Prior to univariate analysis a median split was used to dichotomise WUR (Hand) and WUR (Cervical) as <2 and ≥ 2 , given their non-normal distributions. Initial neck pain (VAS) and relative CPM (i.e. the change from pre-immersion PPT to PPT during immersion, expressed as a percentage) were entered into the model as continuous, normally distributed variables, with the 25th and 75th percentile values used as 'low' and 'high' values for assessing the relationship with EIH. IPAQ category was entered in as three categories (Low, Moderate, High). Exercise type (2 levels: aerobic or isometric) was also assessed as a potential moderating variable of the relationship between the predictors and EIH in each body region. A conservative cut-off of $p < .10$ was used in univariate testing for including predictors in the initial multivariable model. For each variable, its interaction term with Time, and the three-way interaction with Time and Exercise type were assessed concurrently. The final model was developed using forward selection, starting with age and sex, then adding each significant univariate predictor (along with its two- and three-way interaction) one at a time, based on the strength of its univariate association with EIH. Model fit was evaluated using deviance (-2 log likelihood ratio test), comparing the nested models as additional variables were added. Estimated marginal means (95% CIs) were reported for the final model.

RESULTS

Participant Characteristics

Forty WAD (age $\pm$ SD: 37.3y $\pm$ 13.6y; 70% female) and 30 healthy control participants (40.4y $\pm$ 14.3y; 78% female) volunteered for the study. The majority of participants were employed (Controls: 90%; WAD: 85%), although 28% of WAD participants reported reduced hours or not working as a result of their whiplash injuries. There was no significant difference in 'highest level of education' between the groups ($\chi^2 = 5.68$, $p = .13$). Of the 40 WAD participants, symptom duration ranged from 3 to 120 months (median = 28 months), 22 (55%) had submitted a third-party compensation claim, 15 (38%) reported taking non-steroidal anti-inflammatories and 20 (50%) reported taking over-the-counter analgesics.

The participants with WAD demonstrated moderate levels of pain (VAS $\geq 4/10$ [28] and disability (NDI $> 28\%$) [29]; mostly moderate levels of physical activity (IPAQ); with mild levels of posttraumatic stress (PCL-5 < 33) [30], kinesiophobia (TSK < 40) [19] and pain catastrophizing (PCS < 24) [31]. The proportion of WAD participants reporting low physical activity levels was higher than the healthy controls, however the difference in physical activity levels between the two group was not statistically different (Fischer's Exact Test $p=.096$) (Table 1).

Conditioned Pain Modulation

Both the healthy control and WAD groups took similar amounts of time to report their pain threshold (10 and nine seconds, respectively) and overall, 29 (97%) of the control and 30 (75%) of the WAD participants immersed their hand for the full 120 seconds (Table 2). After controlling for sex, an overall significant CPM response was found, such that PPTs were significantly higher during immersion in 5°C water compared to pre-immersion ($F_{1,68} = 22.79$, $p<.001$), however the magnitude of this CPM response was not statistically different between the Control and WAD groups (Group*Time interaction: $F_{1,68} = 0.24$, $p=.63$).

Temporal Summation

Data from the hand site for five controls and two WAD participants and from the cervical spine site for four controls and the same two WAD participants were removed from the analysis as these participants reported no pain with the single stimulus meaning that WUR could not be calculated. Those with WAD reported significantly higher pain ratings following both the single and repeated pinprick stimuli in both the hand and cervical spine regions (Table 3). After adjusting for age ($F_{(1,85)} = 5.23$, $p = .025$) and sex ($F_{(1,52)} = 5.07$, $p = .029$), there was no difference in WUR between the groups or regions (Region*Group $F_{(1,57)} = 2.14$, $p = .149$).

Manipulation Check

There were no significant differences in pre-rest or pre-exercise PPTs over time (baseline, pre-aerobic, pre-isometric exercise) in the cervical spine ($F_{2,138} = .59$, $p = .55$), hand ($F_{2,138} = 2.28$, $p = .11$), or tibialis anterior ($F_{2,138} = 1.02$, $p = .36$) regions, indicating that individuals were not becoming sensitized over time.

Exercise Performance

Three participants with WAD did not complete 30 mins of aerobic (treadmill) exercise due to exacerbation of hip (20 mins), back (15 mins) or ankle (5 mins) pain. However, their peak power data was recorded and entered for analysis. One individual did not complete the knee extension exercise as a result of a software problem. Both groups performed the aerobic exercise at a similar exercise HR, and also performed the isometric exercise at a similar perceived intensity (RPE) (Table 4). However, compared to WAD participants, control participants demonstrated significantly higher peak power for aerobic exercise and greater maximum voluntary capacity for the knee extension isometric exercise (Table 4). Control participants also demonstrated higher maximal systolic and diastolic blood pressure during isometric exercise. There was no significant change in neck pain during exercise for either group (Table 4).

The effect of exercise on changes in PPTs

Aim 1. Comparing EIH in participants with chronic WAD and healthy controls

After controlling for age and sex, there were significant Group*Time*Exercise interactions for the hand region ($F_{2,1080} = 4.06$, $p = .017$), cervical spine ($F_{2,1109} = 3.21$, $p = .041$) and tibialis anterior ($F_{2,1095} = 6.45$, $p = .002$). Post hoc testing demonstrated that, compared with the WAD group, the Control group showed a greater increase in PPTs during or following aerobic (all $p < .05$) and isometric exercise (all $p < .05$) in the hand region (Figure 2), cervical spine (Figure 3) and tibialis anterior (Figure 4). In addition, compared with WAD, the Control group demonstrated higher PPTs prior to both types of exercise in the hand region (aerobic: $p = .041$; isometric: $p = .037$) (Figure 2), and tibialis anterior (aerobic: $p = .026$; isometric: $p = .028$) (Figure 4). There were no significant group differences in cervical PPTs prior to exercise (aerobic: $p = .17$; isometric: $p = .11$) (Figure 3).

Aim 2. Comparing aerobic and isometric exercise in participants with WAD

Within the WAD group, PPT's were higher in the hand region during both isometric exercise (95%CI: 28.8, 55.9; $p < .001$) and aerobic exercise (95%CI: 33.0, 60.1; $p < .001$) relative to the rest period, indicating EIH. There was no difference in hand PPTs between aerobic and isometric exercise (95%CI: -9.4, 17.9; $p = .54$) during exercise. There was no significant change in cervical spine PPTs following aerobic (95%CI: -9.4, 15.6; $p = .62$) and isometric (95%CI: -2.1, 23.2; $p = .10$) exercise when compared to rest, or between isometric and aerobic (95%CI: -20.0, 5.2; $p = .25$) exercise, indicating impaired EIH. Furthermore, there was no significant change in tibialis anterior PPTs between rest and aerobic (95%CI: -23.1, 14.6; $p = .66$); rest and isometric (95%CI: -35.7, 2.3; $p = .08$), or between aerobic and isometric (95%CI: -31.5, 6.5; $p = .20$) exercise, indicating impaired EIH.

Aim 3. Predictors of EIH in chronic WAD

In the hand region, after controlling for age ($F_{1,39} = 4.76$, $p = .036$) and sex ($F_{1,39} = 6.41$, $p = .015$), both IPAQ category and relative CPM were associated with significant changes in PPTs from pre-exercise to during-exercise, with exercise type being a significant moderator of these relationships (IPAQ category*Time*Exercise: $F_{5,382} = 10.41$, $p < .001$; Relative CPM*Time*Exercise: $F_{2,389} = 21.35$, $p < .001$). Significant increases in PPTs from pre- to during-exercise (EIH) were found for aerobic exercise at all categories of IPAQ (Low, Moderate, High), but only with IPAQ Moderate for isometric

exercise. Whilst there was an increase in PPTs with isometric exercise at low and high IPAQ categories, these did not reach statistical significance (Table 5). In terms of relative CPM, statistically significant increases in PPTs over time were seen at both low (20%) and high (90%) values for aerobic exercise, however, for isometric exercise, a statistically significant increase in PPTs during exercise was only seen when relative CPM was large (Δ PPT = 0.90) (Table 5). WUR and VAS did not predict EIH.

. In the cervical spine region, after controlling for age ($F_{1,39} = 8.83$, $p = .005$; sex was n/s), exercise type was also found to be a significant modifier of the relationship of IPAQ category and relative CPM with change in PPTs from pre-exercise to post-exercise (IPAQ category*Time*Exercise: $F_{5,387} = 3.12$, $p = .009$; Relative CPM*Time*Exercise: $F_{2,386} = 9.53$, $p < .001$) (Table 5). In contrast to the results seen in the hand, there were no statistically significant changes in PPTs following aerobic exercise for any IPAQ category, however following isometric exercise, there was a statistically significant decrease in PPTs from pre- to post- exercise when IPAQ category was high ($F_{1,89} = 8.6$, $p = .004$) (Table 5). There was also no association between level of relative CPM and PPT change in the neck following aerobic exercise, however, for isometric exercise, PPTs were significantly lower post-isometric exercise when relative CPM was small (Table 5). WUR and VAS did not predict EIH.

Lastly at the tibialis anterior site, IPAQ category, relative CPM, VAS and WUR did not predict EIH for either aerobic or isometric exercise.

DISCUSSION

The results of this study showed that individuals with chronic WAD have impaired EIH compared to healthy asymptomatic controls. The participants with WAD demonstrated an apparently partial response, in that EIH was induced at a hand site during exercise but EIH did not occur at either the cervical spine or leg site immediately following exercise. In general, EIH responses in the WAD group were no different for isometric or aerobic exercise. However, in the WAD group, the following relationships between baseline factors and EIH were found only for isometric exercise: more efficient CPM predicted EIH at the hand site and less efficient CPM predicted impaired EIH at the neck;

moderate levels of previous week physical activity predicted EIH at the hand but high levels of previous week physical activity predicted impaired EIH (hyperalgesia) at the neck site. For aerobic exercise, significant EIH was seen at the hand site, regardless of the level of self-reported previous week physical activity or the efficiency of CPM.

Consistent with previous studies, the participants with WAD in our study reported mild to moderate levels of post-traumatic stress symptoms, pain catastrophizing and fear of movement, together with moderate levels of pain and disability, [32], and widespread sensitivity to pressure [1]. In addition, the WAD group demonstrated efficacious CPM, consistent with some previous studies [33] [5] [6], but in contrast to others [34] [35]. These differences may be due to the different CPM methodologies employed, including the use of repeated PPTs as both the test and conditioning stimulus [34] or not accounting for group baseline differences [35]. There was also no difference in temporal summation of pain between our WAD group and controls. Although the WAD group reported significantly higher pain intensity with both the single and repeated stimuli, there were no differences in WUR between the groups and WURs of our participants were similar to those previously reported in healthy controls [36]. This contrasts with previous reports of widespread temporal summation of pain in chronic WAD [37] [9] but is consistent with others where temporal summation was not found [5]. Again, these inconsistent findings may be related to methodological differences. We utilized pinprick stimulation according to the German Research Network on Neuropathic Pain protocol [38], whilst others have used electrical [37], pressure algometry [5] and cuff stimulation [9].

EIH responses in chronic WAD

The WAD group demonstrated EIH in the hand region during both exercise types, but EIH was not induced at the neck or leg site after either exercise type. PPTs were measured during exercise at the hand, as this site was easy to access whilst the participants were undertaking the exercise. In hindsight, it would have been ideal to also measure PPTs at the hand following the exercise to determine if the EIH was maintained. Irrespective of this, we found no EIH at the other two sites. We have interpreted these findings as a partial EIH response that is still clearly different from the asymptomatic controls where robust EIH was induced at all sites. EIH responses can vary depending

on body site tested and/or exercised. In people with widespread pain, impaired EIH has been found at sites both local and remote to the body part being exercised [39]. In patients with more localised conditions (for example localised shoulder pain) intact EIH was demonstrated with lower limb exercise but impaired EIH when the painful shoulder muscles were exercised [39]. Our results partially support this scenario, in that EIH was found at the hand with lower limb exercise, but we would also expect to see EIH at the neck and tibia anterior. Neck muscle activity would also be present with lower limb exercise and this may have prevented an EIH response at the neck. There was a trend for increased PPTs at tibia anterior and the lack of a significant result could be due to the sample size. Since it has been proposed that exercising non-painful body regions could be a way of harnessing pain relief for more localised conditions[40], further investigation of nuanced EIH responses at different sites and with exercise at different body areas is warranted.

In general, our results are consistent with previous studies demonstrating impaired EIH in various conditions including chronic WAD [4], general musculoskeletal pain [7], arthritis [41] and fibromyalgia [42].

Comparison of exercise types

We have previously found EIH at neck and leg sites with isometric but not aerobic exercise [6]. We did not replicate these findings in the present study and found no evidence of a differential effect of exercise type on EIH. There are differences between the two studies that could account for this.

Firstly, participants with WAD in our first study reported lower levels of pain ($2.9 \pm 1.8/10$ compared to 5.0 IQR 4.3-5.6) and disability (NDI: Median [IQR] 20% [16,31] compared to 37% [33,41]).

Patients reporting higher pain and disability may have less effective endogenous inhibition, reflected by less EIH, but there is surprisingly little evidence to support this tenet. Pain intensity has been shown to predict CPM but not EIH [7] and in the current study, we did not find that pain intensity was a predictor of EIH, suggesting differences in pain severity between the two studies does not explain the different results. Secondly, the isometric exercise used in both studies was different. In our first study, the isometric exercise was a 3-minute wall squat and in the current study we used a more controlled isometric exercise of resisted knee extension in sitting at 20-25% maximum voluntary

contraction using a Biodex dynamometer, also of 3-minutes duration. Perceived exertion was the same in both studies (RPE: Median [IQR] 5 [3,9] compared to 5 [3,7]) and recorded heart rate and blood pressure were similar. However, in the current study, blood pressure and peak force during the isometric exercise were lower in the WAD participants than in controls. Previous studies have shown a dose-response relationship between the force of isometric exercise contraction and blood pressure responses [43] [13] with greater blood pressure response associated with a greater reduction in pain following isometric exercise [43]. Higher blood pressure activates arterial baroreceptors resulting in increased supraspinal inhibition [44,45]. The lower muscle force and lower blood pressure response to the exercise may be one explanation for the impaired EIH seen in our WAD participants. Thus, these findings are consistent with an arterial baroreceptor inhibition mechanism for EIH [43].

Prediction of EIH

Self-reported high levels of previous week physical activity predicted impaired EIH (hyperalgesia) at the neck, and moderate levels of activity predicted EIH at the hand. The relationship between physical activity and EIH is not clear. Higher levels of moderate and vigorous physical activity have been found to be associated with a greater magnitude of EIH in older healthy adults [46], but no relationship with EIH in younger adults [47]. It has been suggested that a U-shaped relationship exists between activity and pain where too little and too much physical activity may lead to chronic pain [48]. Our results support a U-shaped relationship but only for isometric exercise: high levels of activity predicted impaired EIH, moderate levels predicted EIH, and low levels showed a trend for impaired EIH (although this was non-significant). Although our study used self-report data to estimate physical activity levels rather than a more objective measure such as an accelerometer, [49 2938] our findings could explain why some people with chronic WAD and other chronic pain conditions report increased pain with higher levels of physical activity and this may then ultimately lead to activity avoidance [50] [51]. Further investigation of the influence of the intensity of physical activity on EIH in chronic pain conditions should be explored as this may provide information on the optimal activity level to activate EIH and potentially decrease pain.

More efficient CPM predicted EIH at the hand site and less efficient CPM predicted impaired EIH (hyperalgesia) at the neck with both findings occurring with isometric exercise only. It is proposed that CPM and EIH share underlying mechanisms in both healthy [52] and clinical pain samples [7,8] and our findings would support this proposal. We did not find an association between CPM and EIH responses for aerobic exercise in the hand or neck, nor any relationship between CPM and EIH responses at the leg site, indicating that the relationship between these two inhibitory processes is complicated.

Contrary to our hypotheses, pain intensity did not predict EIH. Vaegter et al [7] found that patients with musculoskeletal pain and high pain sensitivity (widespread decreased pressure pain thresholds) showed more impaired EIH compared to those with low pain sensitivity suggesting that the central mechanisms underlying high pain sensitivity may also be related to impaired EIH [7]. Reported pain intensity between the high and low pain sensitivity groups were no different [7]. In another study of women with menstrual pain, pain intensity also did not predict EIH response [53]. Based on these results, mechanical pain sensitivity rather than reported pain intensity may be more related to EIH responses. Similar to EIH, widespread mechanical pain sensitivity is thought to reflect impaired endogenous inhibitory processes [54] and may be the reason for associations between these two measures.

WUR, a measure of temporal summation, also did not predict EIH. The high pain sensitivity group of Vaegter et al's study [7] also demonstrated facilitated temporal summation but whether this factor was a predictor of EIH was not investigated. These authors proposed three possible scenarios in relation to temporal summation. The high pain sensitivity group had facilitated temporal summation because of less robust CPM/EIH, whether they have less robust CPM/EIH because of facilitated temporal summation, or whether facilitated temporal summation and less robust CPM/EIH are independent. The lack of association between temporal summation of pain and EIH found in our study would suggest the latter to be the most likely scenario.

Study Limitations

As mentioned, PPTs were measured at different body sites during exercise (hand) and post-exercise (cervical and tibialis anterior regions). The hand site was chosen as a suitable site for measurement during exercise, as PPTs were accessible during both exercises, especially treadmill walking. The 3-minutes of isometric exercise also did not allow triplicate PPTs to be performed for multiple body sites during exercise. Also, because of EIH dissipating quickly post-exercise [55], we wanted to minimise the number of PPT measures performed post-exercise. Another limitation was the lack of assessor blinding to the group status of participants (WAD or control).

Conclusion

Our study demonstrated that participants with chronic WAD and moderate levels of pain and disability demonstrate impaired EIH to both aerobic and isometric exercise. Higher self-reported levels of physical activity and less efficient CPM predicted impaired EIH (hyperalgesia) with isometric exercise. However moderate levels of activity predicted EIH. The findings may have implications for the prescription of exercise in patients with chronic WAD.

References

Figure legends

Figure 1 Timeline of research protocol. Black arrows indicate that pain sensitivity (PPTs) was measured over the cervical spine and tibialis anterior immediately prior to and following rest/exercise. Dashed arrows indicate that PPTs were measured in the hand region prior to and following rest, and prior to and during exercise (i.e. not following exercise).

Figure 2. Estimated marginal mean and 95% confidence intervals for hand PPTs prior to and during exercise in healthy control (HC) and WAD participants

Figure 3: Estimated marginal mean and 95% confidence intervals for cervical spine PPTs prior to and following exercise in healthy control (HC) and WAD participants

Figure 4: Estimated marginal mean and 95% confidence intervals for tibialis anterior PPTs prior to and following exercise in healthy control (HC) and WAD participants

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Table 1: Baseline Physical Characteristics of Study Participants

Characteristic	HC (N = 30)	WAD (N = 40)
Pain rating (VAS /100mm)	-	50 (43, 56)
NDI (%)	-	37 (33, 41)
PCL-5 (0-80)	-	23 [18, 37]
TSK (17-68)	-	38 [30, 44]
PCS (0-52)	-	12 [7, 22]
IPAQ Category [†]		
LOW	1 (3.3)	7 (17.9)
MODERATE	12 (40.0)	18 (46.2)
HIGH	17 (56.7)	14 (35.9)

Legend: HC = Healthy Control; WAD = Whiplash-Associated Disorders; NDI = Neck Disability Index; PCL-5 = Posttraumatic Stress Disorder Checklist for DSM-5; TSK = Tampa Scale of Kinesiophobia; PCS = Pain Catastrophizing Scale; IPAQ = International Physical Activity Questionnaire; Results presented as Median [IQR] except Pain rating and NDI, presented as Mean (95%CI) and IPAQ Category, presented as n (%). [†]N = 39 for WAD

Table 2: Unadjusted Cold Pressor Test results during baseline Conditioned Pain Modulation testing, prior to exercise performance

Cold Pressor Test Results	Controls (N = 30)	WAD (N = 40)	<i>p</i> -value†
Pain Threshold (s)	10 [7, 12]	9 [6, 13]	.56
Maximum Pain Tolerance (s)	120 [120, 120]	120 [90, 120]	.06
Maximum Pain reported (NRS/10)	8.5 [7.0, 9.5]	9.0 [8.0, 10]	.20
Average baseline PPT pre-immersion (kPa)	454 [371, 592]	363 [267, 451]	.004*
Average PPT during immersion (kPa)	572 [287, 780]	482 [325, 698]	.07
Relative CPM response (%)	30 (15, 46)	41 (24, 58)	.26

Legend: CPM = Conditioned Pain Modulation; HC = Healthy Control; WAD = Whiplash-Associated Disorder; NRS = Numerical Rating Scale; PPT = Pressure Pain Threshold; kPa = kilopascal. Results presented as Median [Interquartile Range]. * $p \leq .05$. †*P*-value associated with Mann-Whitney U test.

Table 3: Unadjusted participant pain ratings (/100) for 1 and 10 pinpricks, and Wind Up Ratio (WUR), for the hand and cervical spine regions during baseline Temporal Summation testing, prior to exercise performance

Region	No. of pinpricks	HC (N = 30)	WAD (N = 40)	p-value†
Hand	1	5 [2, 10]	9 [3, 20]	.04*
Hand	10	8 [5, 26]	18 [8, 40]	.04*
WUR (Hand) ¹	10/1	2.0 [1.2, 3.5]	2.0 [1.4, 2.8]	.98
Cervical	1	7 [4, 18]	20 [5, 40]	.03*
Cervical	10	14 [7, 33]	46 [15, 66]	.006*
WUR (Cervical) ²	10/1	1.9 [1.5, 2.5]	2.0 [1.4, 3.3]	.49

Legend: HC = Healthy Control; WAD = Whiplash-Associated Disorder; WUR = Wind-up ratio. Results presented as Median [Interquartile Range]. * $p \leq .05$. †P-value associated with Mann-Whitney U test.

¹Due to division by zero for some participants, WUR (Hand) used N = 25 for HC and N = 38 for WAD

²Due to division by zero for some participants, WUR (Cervical) used N = 26 for HC and N = 38 for WAD

Table 4: Group Exercise Results for Isometric (Knee Extension) and Aerobic (Treadmill) Exercise

	HC (n = 30)	WAD (n = 40)	<i>p</i> -value†
Aerobic (Treadmill)			
APMHR	137 (133, 141)	135 (131, 138)	.37
Peak power output (W)	597 (497, 697)	306 (229, 384)	< .001*
Maximum exercise HR (bpm)	146 ± 10	142 ± 15	.24
Maximal exercise BP (mmHg)	143/97 ± 6/5	143/94 ± 10/7	.63/.06
Peak RPE (/10)	5 [3, 6]	4 [3, 5]	.78
Change in neck pain (/10)	.03 (-.03, .10)	.14 (-.41, .69)	.70
Isometric (Knee Extension)			
Peak MVC (Nm)	158 (135, 180)	119 (105, 134)	.003*
Maximum exercise HR (bpm)	84 ± 12	82 ± 14	.55
Maximal exercise BP (mmHg)	138/93 ± 19/11	124/83 ± 12/11	.001*/.001*
Peak RPE (/10)	5 [3, 7]	5 [3, 7]	.43
Change in neck pain (/10)	0 (0, 0)	.04 (-.29, .37)	.81

Legend: HC = healthy controls; WAD = whiplash associated disorder; CI = confidence interval; IQR = interquartile range; APMHR = age-predicted maximum heart rate; HR = heart rate; BP = blood pressure; W = watts; RPE = rated perceived exertion; MVC = maximum voluntary contraction;

Results presented as Mean (95%CI or ± SD) except Peak RPE which is expressed as Median [Interquartile Range]. †P-value associated with Independent Samples T-Test (Mann-Whitney U test for Peak RPE). *p ≤ .05

Table 5: Estimated marginal mean PPT (kPa) for significant predictors of EIH in the hand and cervical spine regions for WAD participants

Baseline Predictor	Estimated mean hand PPT ¹		<i>p</i>	Estimated mean cervical spine PPT ²		<i>p</i>
Exercise Type - Aerobic	Pre-exercise	During-exercise		Pre-exercise	Post-exercise	
IPAQ Category						
LOW	253.4	326.1	.002	250.8	264.0	.42
MODERATE	306.3	345.0	.006	286.0	275.0	.28
HIGH	242.6	302.6	<.001	233.2	221.8	.32
Relative CPM (%) [†]						
ΔPPT = 0.20	269.5	343.8	<.001	264.1	254.4	.30
ΔPPT = 0.90	265.9	310.8	<.001	251.8	253.0	.88
Exercise Type - Isometric						
IPAQ Category						
LOW	278.8	294.7	.46	240.2	241.1	.96
MODERATE	304.4	350.9	.001	254.9	274.5	.06
HIGH	273.4	296.2	.14	244.9	211.0	.004
Relative CPM (%) [†]						
ΔPPT = 0.20	301.3	306.9	.65	253.2	224.7	.003
ΔPPT = 0.90	274.2	318.9	<.001	242.4	253.6	.16

Legend: PPT = Pressure Pain Threshold, CPM = Conditioned Pain Modulation; IPAQ = International Physical Activity Questionnaire

¹ Age ($F_{1,39} = 4.76$, $p=.036$) and sex ($F_{1,39} = 6.41$, $p=.015$) were controlled for in the hand model

² Age ($F_{1,39} = 8.83$, $p=.005$) was controlled for in the cervical spine model; Sex was non-significant and therefore not included in the model.

[†]Estimated mean PPT was assessed at the 25th ($\Delta\text{PPT} = 0.20$) and 75th ($\Delta\text{PPT} = 0.90$) percentile. ΔPPT refers to the relative change from average baseline PPT pre-immersion to peak PPT during immersion in the cold pressor test, and is calculated as $[(\text{PPT}_{\text{Peak}} - \text{PPT}_{\text{Baseline}}) / \text{PPT}_{\text{Baseline}}] * 100$.

Fig. 1: Timeline of research protocol. Black arrows indicate that pain sensitivity (PPTs) was measured over the cervical spine and tibialis anterior immediately prior to and following rest/exercise. Dashed arrows indicate that PPTs were measured in the hand region prior to and following rest, and prior to and during exercise (i.e. not following exercise).

Figure 2: Estimated marginal mean and 95% confidence intervals for hand PPTs prior to and during exercise in healthy control (HC) and WAD participants

Figure 3: Estimated marginal mean and 95% confidence intervals for cervical spine PPTs prior to and following exercise in healthy control (HC) and WAD participants

Figure 4: Estimated marginal mean and 95% confidence intervals for tibialis anterior PPTs prior to and following exercise in healthy control (HC) and WAD participants