Effect of prolonged experimental neck pain on exercise-induced hypoalgesia

Christensen, Steffan Wittrup McPhee; Cancino, Edith Elgueta; Simonsen, Morten Bilde; de Brito Silva, Priscila; Sørensen, Line Bay; Graven-Nielsen, Thomas; Hirata, Rogerio Pessoto

Published in:
Pain

DOI (link to publication from Publisher):
10.1097/j.pain.0000000000002641

Publication date:
2022

Document Version
Accepted author manuscript, peer reviewed version

PAIN Publish Ahead of Print
DOI: 10.1097/j.pain.0000000000002641

The effect of prolonged experimental neck pain on exercise-induced hypoalgesia

Steffan Wittrup McPhee Christensen[1,2]; Edith Elgueta Cancino[3,4]; Morten Bilde Simonsen[1,5]; Priscila de Brito Silva[2]; Line Bay Sørensen[3]; Thomas Graven-Nielsen[3]; Rogerio Pessoto Hirata[1,6]

[1]*Department of Health Science and Technology, Aalborg University, Aalborg, Denmark*

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

[3]*Center for Neuroplasticity and Pain (CNAP), Department of Health Science and Technology, Aalborg University, Aalborg, Denmark*

[4]*Center of Precision Rehabilitation for Spinal Pain, School of Sport, Exercise and Rehabilitation Sciences, University of Birmingham, Birmingham, United Kingdom*

[5]*Centre for Clinical Research, North Denmark Regional Hospital, Hjoerring, Denmark*

[6]*Sport Sciences – Performance and Technology, Faculty of Medicine, Department of Health Science and Technology, Aalborg University, Aalborg, Denmark*

Corresponding author: Associate Professor Steffan Wittrup McPhee Christensen Department of Health Science and Technology, Aalborg University, Fredrik Bajers Vej 7D-3, Aalborg, Denmark, 9220 Aalborg E, Denmark Phone: +45 99408889, E-mail: stc@hst.aau.dk URL: https://www.hst.aau.dk/

ABSTRACT

Neck pain is a common musculoskeletal problem often accompanied by reduced exercise-induced hypoalgesia (EIH) or hyperalgesia compared to an asymptomatic population. This study investigated EIH in a healthy population during experimental neck pain. Forty participants were randomized into this double-blinded parallel-group study. On four separate test days (Day0, Day2, Day4, Day15), participants completed the Neck Disability Index (NDI) and scored neck pain intensity during head movements on a numerical rating scale (NRS). At the end of Day0 and Day2, Nerve Growth Factor (NGF) or isotonic saline (control) was injected into the right splenius capitis muscle. Pressure pain thresholds (PPTs) were recorded bilaterally over splenius capitis (neck), temporalis (head) and tibialis anterior (leg) muscles on all days. On Day0, Day4 and Day15, PPTs were recorded before and after a hand-bike exercise. EIH was defined as the PPT increase caused by the exercise. Compared with the control-group, the NGF-group demonstrated higher NDI scores at Day2 and Day4 ($P<0.001,\eta^2>0.557$) and higher NRS scores ($P<0.03,\eta^2>0.09$) along with reduced neck PPTs ($P<0.01,d>0.44$) at Day2(Right:95%CI[26.0,54.0];Left:95%CI[6.8,26.9]), Day4(Right:95%CI[40.5, 67.9];Left:95%CI[6.9,28.2]) and Day15(Right:95%CI[5.6,37.2];Left:95%CI[6.9,34.8]). Across days, the EIH-effect was reduced at the neck site in the NGF-group compared to the control-group ($P<0.001,\eta^2_P=0.367$,95%CI[–34.5,-13.7]). At the head and leg sites, the NGF-group showed reduced EIH-effect compared to the control-group ($P<0.05,d>0.43$) on Day4(Head:95%CI[-61.4,-22.9];Leg:95%CI[-154.7,-72.4]) and Day15(Head:95%CI[-54.3,-7.6];Leg:95%CI[-122.7,-34.4]). These results indicate that a few days of clinically comparable neck pain and hyperalgesia might have a negative impact on EIH-responses and may help explain why some neck pain patients do not experience immediate positive effects of exercise.

Keywords: Neck pain; Exercise-induced hypoalgesia; Nerve growth factor; pressure pain thresholds

INTRODUCTION

Neck pain is one of the most considerable musculoskeletal problems [16;40], with many developing pain lasting for >3months [8;9]. Despite a multitude of possible treatment strategies [23;24], even comprehensive interventions seem to be no more effective than advice [22;35]. Although exercise training (multiple sessions), including strength and endurance exercises, has shown promise as treatment modalities [23], the influence on central pain mechanisms remains unclear [22;35].

Increased local and widespread pain sensitivity is often observed in neck pain patients compared to asymptomatic individuals [12;49;50;64]. Hyperalgesia, especially in distant areas away from the painful site, is suggested to be a sign of altered central pain processing [4;64]. This difference in central nociceptive processing between those with pain and asymptomatic subjects can also be observed in responses to exercise [39]. In healthy participants, exercise (single session) including resistance and aerobic exercises, lasting approximately 3 to 40minutes, have shown to decreased pain sensitivity, also known as exercise-induced hypoalgesia (EIH) [30;39]. The underlying mechanism of EIH remains unclear, but it has been suggested that exercise engages the endogenous pain modulatory system to decrease pain sensitivity, although this mechanism may not be working efficiently in patients with persistent pain [39;57]. Interestingly, other studies have found increased pain sensitivity following exercises consisting of 3-6 series of repeated arm-movements or submaximal cycling for approximately 2-5minutes in populations with persistent neck pain [12;59], which is contrasted by studies showing positive effects on pain sensitivity following exercise training 3-5times per week consisting of resistance-exercise with/without 30minutes of aerobic-

exercises over 2-6months [3;28]. Understanding this relationship between exercise and altered pain sensitivity is crucial to optimize exercise-rehabilitation of neck pain [12;22;59]. While studies have examined EIH in persistent neck pain [12;26;45;46;59] there is a paucity of studies investigating this in the early stages following the onset of neck pain. This lack of research may be explained by the difficulties of recruiting participants immediately before or after the onset of neck pain. Therefore, experimental pain models lasting several days provide an opportunity for investigating sensory changes during the early stage of neck pain [1;11]. Previous experimental neck pain studies have been limited by pain models lasting only minutes [11;13]. This short-lasting pain may not be sufficient to illuminate how pain may impact EIH over time. One study has used eccentric exercise to induce neck muscle pain lasting for a few days [1], while intramuscular injections of Nerve Growth Factor (NGF) have shown promise as a model inducing muscle pain lasting several days [42;47]. The advantage of an NGF-model compared to eccentric exercises is the ability to extend the duration of pain with repeated injections [37;52].

This study aimed to investigate the effect of NGF-induced experimental neck pain over sixteen days in healthy participants and assess pain sensitivity and EIH, compared to control injections of isotonic saline. It was hypothesized that: (i) the NGF-group would display increased pain sensitivity and decreased EIH following neck pain onset compared to both baseline and the control-group; and (ii) these changes will normalize once the pain had subsided.

METHODS

Participants

One hundred and forty-three participants reported interest in the study following advertisements on social media and at local educational facilities (Fig. 1). Forty-one did not report back after receiving further information regarding the study, and four were no longer interested. Of those remaining, 47 fulfilled the inclusion criteria: healthy pain-free participants between 18-50 years of age and able to speak and read Danish or English. Exclusion criteria were any neck or shoulder pain during the past six months, any previous neck or shoulder surgery, any current or previous recurring painful conditions or any neurologic, musculoskeletal or mental illnesses that could influence the results. Furthermore, regular use of analgesics, drug addiction, heavy exercise during the days leading up to the test sessions, left-hand dominance or pregnancy was also cause of exclusion. Participants were asked to refrain from starting new exercise regimes, whereas any ongoing regular exercise training (e.g., running, walking, cycling, fitness) was allowed since there is no consensus that physical activity levels influence EIH in a young population [7;36]. Participants were given written and verbal information about the study prior to providing their written informed consent. The study was approved by the local ethics committee (N-20180063). This study focusing on the effect of exercise on pain sensitivity is part of a larger project (Clinicaltrial.gov: NCT03848247) investigating potential effects of prolonged experimental neck pain on pain sensitivity, cortical excitability, and alterations in human movement.

As no previous study has used a similar experimental NGF neck pain model, the sample size calculations were based on Pressure Pain Threshold (PPT) data from the neck area in clinical neck pain [12]. Using G*power v3.1.9.4 (Heinrich-Heine-Universität, Düsseldorf, Germany), a sample size calculation was performed for a repeated measure analysis of variance (RM-ANOVA) with two groups, four days, 90% power, α 0.05 and a Cohen´s f for a medium effect size of f=0.27. The correlation between repeated measures was set at 0.3 to account for interindividual differences in

response to experimental neck pain, giving an estimated sample size of 36 needed. Therefore 40 participants (20 per group) were recruited for this study to be conservative.

Protocol

This study used a parallel-group design and was conducted in a laboratory setting at Aalborg University in 2019 with pilot testing starting in January and the final session was completed in May. Participants were randomized (balanced) to either a neck pain condition (NGF-group) or a control condition (control-group; Fig. 2). Participants had to participate on four separate days; Day0, Day2, Day4 and Day15. Each session lasted approximately 2-hours except for Day2 lasting approximately 30 min. All sessions started with participants reporting the sleep duration in the previous night, filling out the neck disability index (NDI), and describing any painful experience (see experimental pain section below). Day0, Day4 and Day15 followed the same protocol (Fig. 2). From a seated position on a chair without a backrest, pressure pain thresholds (PPTs) were assessed with participants leaning forward with their upper body and forehead resting on a table in front of them. PPTs were assessed before and immediately after an upper limb exercise session, followed by reassessing any potential painful experience. Participants were informed that the objective of the study was to investigate any potential relationship between neck pain, pain sensitivity and exercise but remained naïve to both the study hypothesis and how exercise may influence EIH-responses.

The protocol for Day2 included questionnaires and describing any potential painful experience before assessing PPTs. At the end of Day0 and Day2, participants received an injection of either Nerve Growth Factor (NGF) or Isotonic saline as a control injection depending on group allocation. Participants picked an opaque envelope containing information on group allocation, which was only reviled to the person giving the injection.

Experimental neck pain

A solution of sterile recombinant human NGF (0.5 ml, 5 μg, Skanderborg pharmacy, Denmark) was injected into the right (side of hand dominance) splenius capitis muscle at C3 level between the borders of the upper trapezius and the sternocleidomastoid muscles following a previously described protocol [11;13]. An intramuscular NGF injection causes muscle pain/soreness during muscle contractions and increased sensitivity to pressure stimulation lasting a few days [6;25;42;53]. Therefore, a protocol including two repeated NGF injections separated by 2-days was implemented to prolong the painful sensation [15;42;51]. For the control-group injections of 0.5 ml isotonic saline (0.9%) were used, which should not cause any discomfort during muscle contraction or increased mechanical sensitivity [6;11;13;20]. Both participants and assessors were blinded to group allocation.

Neck pain intensity was scored on an 11-point numerical rating scale (NRS; 0 = no pain, 10 = worst imaginable pain) [31] during non-standardized head movements where participants were asked to rotate their heads to look over their shoulders. The area of perceived pain was marked on a body chart [11-13]. The marked areas on body charts were extracted using Adobe Acrobat Pro (San Jose, CA, USA; v.2019.012.20034) in arbitrary units (a.u.) and the combined value (the area from anterior, posterior and side view) was used for further analysis. The NDI questionnaire [61], expressed as a percentage of scored items, was used to assess changes regarding disability associated with neck pain. Quality of pain was described by choosing words from the McGill pain questionnaire (MPQ) [17;33]. On Day4, all participants were asked which injection type, NGF or saline, they believed they received.

Pressure pain sensitivity

Pressure pain sensitivity was assessed by PPTs using a handheld pressure algometer (Somedic, Hörby, Sweden) mounted with a 1 cm^2 probe protected by a disposable cover. The pressure was steadily increased by 30 kPa/s. The threshold was defined as the instant that the pressure went from a pressure to first becoming painful, at which time point the participant would push a button wired to the algometer which recorded the pressure. PPTs were recorded at three bilaterally locations (six in total): 1) A neck site over the splenius capitis muscle (neck) just superior to the injection site (C3 level) [11-13]. 2) A segmental head site over the temporal muscle (head) above the base of the ear [11-13;29]. 3) A distal leg site over the upper one-third of the tibialis anterior muscle (leg) [10;32;42;44]. PPTs were always assessed first at the neck, then the head, followed by the leg site on one side of the body, followed by the contralateral side and this was repeated three times. The start side (right/left) for PPT was randomized in a balanced way for both groups (NGF/control) and was the same between sessions for each participant. The average of the three measurements for each site was used for further analysis.

Exercise-induced hypoalgesia

Upper limb exercise was performed using an adjustable hand-bike (SCI FIT Pro II; Tulsa, Oklahoma, USA) without a backrest. A progressive exercise protocol was adapted from previous studies [41;43]. Participants were asked to maintain 70 rounds per minute (RPM) during the entire hand-bike exercise, which was ensured by visually observing the display showing RPMs. Following 3 minutes with a bike resistance of 3.0 (arbitrary units), the resistance was increased by 0.5 a.u. every minute. The exercise session was stopped when the participants could no longer maintain 70 RPM or at a 16-minute time limit. The time of the last completed minute was noted and used as a target for the following sessions. Every minute during the hand-bike exercise, participants were

asked to rate any neck pain on the NRS scale. EIH was calculated by subtracting pre-exercise PPT values from post-exercise values.

Statistics

The statistical analyses were conducted using SPSS v.25 (IBM, Chicago, IL, USA). Demographic and sleep data were compared between groups using a Mann-Whitney U test. Completed minutes of exercise, NRS scores of neck pain, area of perceived pain, and NDI were assessed over days for each group (NGF, control) using a Friedman's ANOVA. A Bonferroni corrected Wilcoxon's test was used as a post-hoc test when indicated. Group comparisons at each time point were conducted using a Mann-Whitney U test and likewise, a Bonferroni correction was implemented to correct for multiple comparisons. For the Friedman's ANOVA the Kendall's W was reported, while Eta Squared (η^2) was reported for the Wilcoxon's and the Mann-Whitney U test.

PPT data were analysed for normality using the Shapiro-Wilk test, after which the appropriate statistical approach was selected accordingly. First, PPTs at Day0 were analysed for potential group differences as well as to confirm an EIH-response using a RM-ANOVA with Group (NGF, control) as between-subject factor and Side (right, left) and Time (Pre-, Post-exercise), after which pre-exercise PPTs at Day2, Day4 and Day15 were normalized (percentages) to baseline at Day0, thereby showing the change from baseline. Normalised PPT data were analysed with a RM-ANOVA with Group (NGF, control) as between-subject factor and Side (right, left) and Days (Day2, Day4, Day15) as within-subject factors. This was done separately for each site (neck, head, leg) and Bonferroni corrected pairwise comparisons were used for post-hoc analysis.

Changes in EIH were analysed using a RM-ANOVA with Group (NGF, control) as between-subject factor and Side (Right, Left) and Days (Day0, Day4, Day15) as within-subject factors. This was

done separately for each site (neck, head, leg), and Bonferroni corrected pairwise comparisons were used for post-hoc analysis and reported along with 95% CI and Cohen's *d*. For the RM-ANOVAs, the partial Eta Squared (η^2_P) and 95% CI was reported. A significance level of 0.05 was accepted. All data in text and figures/tables are presented as the mean and standard error of the mean (SEM) or median and interquartile range (25th and 75th percentile).

RESULTS

Of the 47 recruited participants, a total of 40 (20 in each group) completed the study (Fig. 1). Of those completing the study, three participants were unable to guess the type of injection they received when asked on Day4. No significant difference between groups was seen for age, height, weight or hours of sleep (Table 1).

Perceived pain intensity, area, quality and disability

For pre-exercise neck pain, the Friedman's ANOVA indicated a difference between days in the NRS scores during head movements (Table 2) for the NGF-group ($\chi^2(3)=51.503$, $P<0.001$, $W=0.858$). On Day2 and Day4, the NGF-group had higher pain NRS scores compared to Day0 and Day15 (Wilcoxon: $P<0.002$, $\eta^2>0.700$). Similarly, for the NGF-group, a difference between days was also found for neck pain during head movement post-exercise ($\chi^2(2)=36.273$, $P<0.001$, $W=0.906$) with significant higher NRS scores at Day4 compared to Day0 and Day15 (Wilcoxon: $P<0.001$, $\eta^2>0.742$) and at Day 15 compared to Day0 (Wilcoxon: $P=0.034$, $\eta^2=0.32$). No within-group difference was observed when comparing pre- and post-exercise pain NRS scores for the two groups. The NGF-group displayed higher pain NRS scores compared to the control-group on Day2, Day4 and Day15 at both pre- and post-exercise (Mann-Whitney: $P<0.03$, $\eta^2>0.09$).

For the perceived area of pre-exercise pain (Table 2; Fig. 3) the Friedman's ANOVA indicated a difference over days for the NGF-group ($\chi^2(3) = 49.281$, $P<0.001$, $W=0.821$) with the post-hoc test revealing larger areas of pain on Day2 and Day4 compared to Day0 and Day15 (Wilcoxon: $P<0.001$, $\eta^2>0.730$). Similarly, for the post-exercise pain assessment ($\chi^2(2) = 31.433$, $P<0.001$, $W=0.785$) larger pain areas were seen for the NGF-group on Day4 compared to Day0 and Day15 (Wilcoxon: $P<0.006$, $\eta^2>0.480$) while no differences were found when comparing areas of perceived pain pre- and post-exercise. Between-group differences were observed with the NGF-group displaying larger areas of pre-exercise pain on Day2, Day4 and Day15, as well as post-exercise on Day4 and Day15 (Mann-Whitney: $P<0.03$, $\eta^2>0.066$).

A difference over days was indicated for the NDI in the NGF-group by the Friedman's ANOVA ($\chi^2(3)=44.103$, $P<0.001$, $W=0.735$) with the post-hoc test showing higher scores at Day2 and Day4 compared to Day0 and Day15 (Wilcoxon: $P<0.002$, $\eta^2>0.658$) and the Mann-Whitney U test confirmed that these days were also higher compared to the control-group (Table 1; $P<0.001$, $\eta^2>0.557$). On Day2, the words chosen from the MPQ by >25% of the NGF-group were "Annoying" (65%), "Sore" (45%), "tiring" (35%) and "tight" (35%). On Day4, the words chosen by >25% of the NGF-group were "Annoying" (70%), "Sore" (55%), "pressing" (40%), "hot" (30%), and "tight" (30%). For the control-group no words were chosen by >25% on any day.

Pressure pain sensitivity at baseline

No significant baseline (Day0) group difference in PPTs was found for any site (Table 3). For all sites a main effect of time was observed (Neck: $F(1,38)=52.7$, $P<0.001$, $\eta^2_P=0.581$, 95%CI [-26.7, -15.1]; Head: $F(1,38)=21.0$, $P<0.001$, $\eta^2_P=0.357$, 95%CI [-36.6, -14.2]; Leg: $F(1,38)=8.1$, $P<0.007$, $\eta^2_P=0.177$, 95%CI [-51.2, -8.8]) with post-exercise PPT being significantly higher than pre-values. In addition, the analysis revealed a side difference for the neck and leg sites (Neck: $F(1,38)=7.0$,

P<0.012, η^2_P=0.157, 95%CI [-18.7, -2.5]; Leg: F(1,38)=4.6, P<0.039, η^2_P=0.108, 95%CI [1.3, 45.2]) with the right side compared to the left having lower PPTs at the neck while the opposite was true for the leg. No side-by-time interactions were observed for any site.

Pre-exercise pressure pain sensitivity

For normalised PPTs at the neck site, the RM-ANOVA showed a Group*Time*Side interaction (Fig. 4; F(2,76)=14.0, P<0.001, η^2_P=0.270). Post-hoc pairwise comparisons revealed reduced PPTs for the NGF-group compared to the control-group at Day2 on both sides (Right: 95%CI [26.0, 54.0], P<0.001, *d*=0.914; Left: 95%CI [6.8, 26.9], P=0.002, *d*=0.538), Day4 (Right: 95%CI [40.5, 67.9], P<0.001, *d*=1.268; Left: 95%CI [6.9, 28.2], P=0.002, *d*=0.527) and Day15 (Right: 95%CI [5.6, 37.2], P=0.009, *d*=0.433; Left: 95%CI [6.9, 34.8], P=0.005, *d*=0.477). Furthermore, the NGF-group displayed reduced PPTs on the right side on Day4 compared to Day2 (95%CI [-28.5, -4.6], P=0.004, d=0.776) and Day15 (95%CI [-52.0, -26.5], P<0.001, *d*=1.722) as well as on Day2 compared to Day15 (95%CI [-35.1, -10.4], P<0.001, *d*=1.029). Moreover, the NGF-group displayed reduced PPTs on the right side compared to the left side on Day2 (95%CI [-32.6, -15.4], P<0.001, *d*=1.260) and Day4 (95%CI [-46.9, -27.5], P<0.001, *d*=1.737).

For normalised PPTs at the head site, a significant Group*Time interaction was found (F(2,76)=4.58, P=0.013, η^2_P=0.108). The post-hoc comparison showed that the NGF-group displayed reduced PPT values compared to the control-group on Day2 (95%CI [-21.0, -3.3], P=0.009, *d*=0.440) and Day4 (95%CI [-27.4, -8.5], P<0.001, *d*=0.608). In addition, the NGF-group had lower PPTs on Day4 compared to Day2 (95%CI [-16.1, -0.2], P=0.042, *d*=0.577) and Day15 (95%CI [-17.9, -2.5], P=0.006, *d*=0.738).

For the leg site, a main group effect was seen with the NGF-group overall displaying reduced PPTs compared to the control-group (F(1,38)=5.49, P=0.024, η^2_P=0.126, 95%CI [-21.2, -1.5]).

Additionally, a Side*Time (F(2,76)=5.71, P=.005, η^2_P=.131) interaction was found. On Day2, lower values were found for the PPTs on the right compared to the left side (P=0.015, 95%CI [-11.8, -1.3], *d*=0.401). Furthermore, right sided PPTs were higher on Day15 compared to Day2 (P=0.027, 95%CI [0.8, 17.0], *d*=0.435) and Day4 (P=0.009, 95%CI [1.9, 16.1], *d*=0.503), while left sided PPTs were lower on Day4 compared to Day2 (P=0.003, 95%CI [-16.3, -2.8], *d*=0.562) and Day15 (P=0.001, 95%CI [-21.6, -5.3], *d*=0.652).

Exercise-induced hypoalgesia

No significant within or between groups difference was observed for the number of completed minutes during hand-bike exercise, with the overall number of minutes being 7.0 [5.0-10.0] for the NGF group and 6.5 [5.0-8.0] for the control group.

For the neck sites (Fig. 5), the EIH analysis revealed a group effect showing a smaller EIH-effect for the NGF-group compared to the control-group (F(1,38)=22.1, P<0.001, η^2_P=0.367, 95%CI [–34.5, -13.7]).

For the head site, a Group*Time interaction was found (F(2,76)=5.9, P=0.004, η^2_P=0.135). The post hoc comparison showed that the NGF-group displayed a lower EIH-effect compared to the control-group at Day4 (95%CI [-61.4, -22.9], P<0.001, *d*=0.728) and Day15 (95%CI [-54.3, -7.6], P=0.011, *d*=0.425). In addition, the control-group displayed an increased EIH-effect at Day4 compared to Day0 (95%CI [9.6, 49.2], P=0.002, *d*=0.831).

For the leg site, a Group*Time interaction was seen (F(2,76)=7.1, P=0.001, η^2_P=0.158). The NGF group had a lower EIH-effect compared to the control group at Day4 (P<0.001, 95%CI [-154.7, -72.4], *d*=0.883) and Day15 (P<0.001, 95%CI [-122.7, -34.4], *d*=0.569). In addition, at Day4

(P=0.014, 95%CI [11.6, 126.4], *d*=0.673) and Day15 (P=0.045, 95%CI [0.8, 103.8], *d*=0.569), an increased EIH was observed for the control-group compared to Day0.

DISCUSSION

This study demonstrated that intramuscular NGF-injections into healthy pain-free participants induced neck pain lasting for days and diminished EIH-response compared to a control-group. The NGF-group reported larger areas of pain, higher pain intensity and displayed local and widespread hypersensitivity to pressure compared to the control-group without neck pain.

Prolonged pain and disability

Neck pain intensity during head movements and area of perceived pain in the current study was comparable to previous findings for clinical neck pain [12;44]. Surprisingly, unlike previous observations based on clinical neck pain, this study did not find increased pain intensity or area of perceived pain following exercise. This discrepancy between clinical populations [12], where pain may arise from several cervical structures [8;14] and the current NGF-model, could be due to the model itself. NGF-Injections increase mechanical sensitivity which in turn can cause pain during muscle-contraction [6]. However, the injected muscle, splenius capitis, in the current study, was not directly involved in the exercise, which could explain why perceived pain did not change. An alternative explanation for the lack of increased intensity or area of pain following exercise could be that the NGF-model did not sufficiently sensitize central mechanisms. Another factor to consider is how pain develops over time [21] where the current model resembles acute-subacute pain, which may not be comparable to persistent neck pain. However, when considering some of the words most chosen by the participants to describe their experienced pain in the current study, such as

"annoying", "tiring" or "nagging", these represent evaluative and affective aspects of pain [34] which are believed to be increasingly present in persistent pain compared to more acute conditions [38]. This is supported by previous studies where words describing affective aspects were commonly reported by those with persistent neck pain [12;27] compared with healthy populations experiencing a short-lasting experimental neck pain [11;19].

Taken together, the pain intensity, area of perceived pain and the words chosen to describe pain, the current NGF-model may be suitable for mimicking the early phases of neck pain. This is supported by the NDI ratings, where the NGF-group had a median score of 10% on Day2 and Day4, which would be interpreted as mild disability [48;60].

Local and widespread hyperalgesia

This study found decreased PPTs locally over the neck site, ipsilateral to the injection, which was most pronounced on days with the highest pain intensity and could be considered a clinically meaningful reduction [62;63]. Furthermore, PPTs approximated baseline values at Day15. However, this was expected as NGF-injections increase mechanical sensitivity for days before normalising again, as observed in previous NGF-studies [6;42;53]. This reduced PPT at the ipsilateral neck site for the NGF-group compared to the control-group is in line with findings in both idiopathic and traumatic neck pain when compared to healthy controls [12;44]. Such findings could indicate that the current NGF-model may mimic early phases of neck pain. In contrast, a previous short-lasting experimental neck pain model, using intramuscular injection of hypertonic saline, showed the opposite effect, namely a decreased mechanical sensitivity immediately after injection, which is considered a sign of a healthy inhibitory response [11;13]. Compared to neck pain following eccentric exercise, which caused approximately 15% reduction in PPTs after two

days [1], the current NGF-model caused a 34% reduction in PPTs on the right neck site after two days and a 50% reduction on Day4, indicating it may be a more potent model.

In this study NGF was only injected in the right splenius capitis muscle, but still hyperalgesia was observed on the left side, indicating the potential presence of facilitated central pain mechanisms. This is also supported by the findings of decreased PPTs on the side of the head on Day2 and Day4 as well as the general reduced PPT on the leg site for the NGF-group when compared to the control-group. These novel findings, of local and widespread hypersensitivity, are not commonly found in studies using intramuscular NGF-injections [6;15;42;53]. The difference between previous findings of locally decreased PPTs and the current study showing widespread changes could be due to the injection site. Previous NGF-studies have targeted muscles in either arm-, leg- or jaw-muscles [2;6;15;42;53] where it may have been easier to reduce or avoid the use of the affected muscle. In comparison, the splenius capitis muscle is highly involved in rotating and extending the neck [5;54], which may be difficult to avoid during normal daily life and thereby increasing the frequency of nociceptive input to the nervous system. This in turn could lead to facilitated central pain mechanisms and widespread pressure hypersensitivity as observed for the NGF-group compared to the control-group. Despite the current study used healthy young participants, which could limit the clinical translatability of the results, the findings are in line with findings from clinical populations with persistent neck pain [12;49;50;64] and may reflect the changes in pain sensitivity potentially involved in the initial phases of persistent neck pain.

Side differences in PPT data, were observed for the neck and leg site at Day0 and the normalized data for the leg site on Day2. These side differences were unrelated to group allocation and while they could be related to the side of dominance, the cause remains unclear.

Exercise-induced hypoalgesia

The hand-bike exercise induced a significant EIH-response for all sites at Day0 and the magnitude of this changed within and between groups over days. Although still debated, the underlying mechanism behind EIH has been suggested to be closely related to endogenous pain modulation, which is typically known to be impaired in persistent painful conditions [39;57]. An impaired pain modulation also seems to be the case for neck pain of both idiopathic and traumatic origin compared to a healthy and pain-free population [12;59;64]. This study generally found lower EIH at all sites when comparing the NGF-group to the control-group, which is in line with studies showing decreased EIH for those in pain compared to healthy participants [12;39;46;57]. Although the NGF-group was expected to display lower EIH-response, the direction of these changes over time in the current study is contrasting. It was hypothesized that the NGF-group would display decreased EIH after the onset of pain compared to the control-group, which is in line with the literature [39;57]. Although there may be a tendency for a minute EIH-reduction over time for the NGF-group, this does not resemble the increase seen for the control-group following Day0. One explanation for such EIH-differences could be that the control-group had a more efficient EIH-response and thereby different from those in pain, which has previously been observed in clinical populations [18;56]. Although unlikely due to the randomization of groups, this theory could be supported by EIH results from the neck site where a main effect of group was observed (Fig. 5).

Nevertheless, this seems unlikely when considering the results from the head and leg sites where comparable results between groups were seen at Day0. One study has suggested that increased EIH at a follow-up session could be due to the initial EIH experience [55], which may explain the increase in EIH seen for the control-group. In contrast, a similar EIH-response as seen in the control-group, may have been inhibited for group receiving the NGF-injections at the end of Day0 and Day2. Interestingly, Vaegter et al. [58] suggested that neutral or positive pre-exercise expectations regarding the responses may facilitate EIH, whereas negative expectations may cause

reduced EIH or even a hyperalgesic response. In this study, both groups knew that they would receive either saline- or NGF-injections. If the control-group had a positive EIH experience on Day0 and then realized their group allocation following the injections, they would not expect any further pain during the study, which could potentially facilitate positive expectations and EIH-responses. In the NGF-group, the experience of pain could have reduced or reverted the EIH-responses [55;58]. Based on this, clinicians working with pain patients may play a vital role in regards to facilitating positive pre-exercise expectations [58], to optimize the initial experience of EIH. Especially when considering, that an initial positive experience, may influence EIH-responses in later sessions [55].

Limitations

When considering the EIH-response in the current study, it is difficult to interpret the clinical relevance as no changes over time were found for the group experiencing pain. Similarly, any potential influence of expectation on the results is speculative in the current study, as this was not assessed. However, expectations in combination with facilitated central pain processing could explain some of the mechanisms at play in the early phase of clinical neck pain.

Conclusion

This study is the first to show that repeated NGF-injections into a neck muscle can cause neck pain lasting for days and widespread hyperalgesia to pressure similar to what is seen in clinical neck pain. Additionally, this study indicates that neck pain lasting for days in a group of otherwise healthy participants can have a negative impact on the magnitude of a hypoalgesic response to exercise, as seen in those without pain. Different expectations could possibly explain the differentiated EIH-responses in those with and without pain as a result of pain.

ACKNOWLEDGMENTS

This study and Steffan Wittrup McPhee Christensen were funded by the Fund for research, quality and education in physiotherapy practice (Fysioterapipraksisfonden) and the Lundbeck Foundation for Health Care Research. Center for Neuroplasticity and Pain (CNAP) is supported by the Danish National Research Foundation (DNRF121). The authors have no other conflicts of interest to declare

REFERENCES

[1] Alsultan F, Cescon C, Heneghan NR, Rushton A, Barbero M, Falla D. Eccentric exercise and delayed onset muscle soreness reduce the variability of active cervical movements. J Biomech 2020;111:109962.

[2] Andersen H, Arendt-Nielsen L, Svensson P, Danneskiold-Samsoe B, Graven-Nielsen T. Spatial and temporal aspects of muscle hyperalgesia induced by nerve growth factor in humans. Exp Brain Res 2008;191(3):371-382.

[3] Andersen LL, Andersen CH, Sundstrup E, Jakobsen MD, Mortensen OS, Zebis MK. Central adaptation of pain perception in response to rehabilitation of musculoskeletal pain: randomized controlled trial. Pain Physician 2012;15(5):385-394.

[4] Arendt-Nielsen L, Morlion B, Perrot S, Dahan A, Dickenson A, Kress HG, Wells C, Bouhassira D, Mohr Drewes A. Assessment and manifestation of central sensitisation across different chronic pain conditions. Eur J Pain 2018;22(2):216-241.

[5] Basmajian JV, De Luca CJ. Muscles alive : their functions revealed by electromyography. Baltimore: Williams & Wilkins, 1985.

[6] Bergin MJ, Hirata R, Mista C, Christensen SW, Tucker K, Vicenzino B, Hodges P, Graven-Nielsen T. Movement Evoked Pain and Mechanical Hyperalgesia after Intramuscular Injection of Nerve Growth Factor: A Model of Sustained Elbow Pain. Pain Med 2015;16(11):2180-2191.

[7] Black CD, Huber JK, Ellingson LD, Ade CJ, Taylor EL, Griffeth EM, Janzen NR, Sutterfield SL. Exercise-Induced Hypoalgesia Is Not Influenced by Physical Activity Type and Amount. Med Sci Sports Exerc 2017;49(5):975-982.

[8] Bogduk N. The anatomy and pathophysiology of neck pain. Phys Med Rehabil Clin N Am 2011;22(3):367-382, vii.

[9] Borghouts JA, Koes BW, Bouter LM. The clinical course and prognostic factors of non-specific neck pain: a systematic review. Pain 1998;77(1):1-13.

[10] Chien A, Sterling M. Sensory hypoaesthesia is a feature of chronic whiplash but not chronic idiopathic neck pain. Man Ther 2010;15(1):48-53.

[11] Christensen SW, Hirata RP, Graven-Nielsen T. The effect of experimental neck pain on pressure pain sensitivity and axioscapular motor control. J Pain 2015;16(4):367-379.

[12] Christensen SW, Hirata RP, Graven-Nielsen T. Altered pain sensitivity and axioscapular muscle activity in neck pain patients compared with healthy controls. Eur J Pain 2017;21(10):1763-1771.

[13] Christensen SW, Hirata RP, Graven-Nielsen T. Bilateral experimental neck pain reorganize axioscapular muscle coordination and pain sensitivity. Eur J Pain 2017;21(4):681-691.

[14] Curatolo M, Bogduk N, Ivancic PC, McLean SA, Siegmund GP, Winkelstein BA. The role of tissue damage in whiplash-associated disorders: discussion paper 1. Spine (Phila Pa 1976) 2011;36(25 Suppl):S309-315.

[15] De Martino E, Zandalasini M, Schabrun S, Petrini L, Graven-Nielsen T. Experimental muscle hyperalgesia modulates sensorimotor cortical excitability, which is partially altered by unaccustomed exercise. Pain 2018;159(12):2493-2502.

[16] Diseases GBD, Injuries C. Global burden of 369 diseases and injuries in 204 countries and territories, 1990-2019: a systematic analysis for the Global Burden of Disease Study 2019. Lancet 2020;396(10258):1204-1222.

[17] Drewes AM, Helweg-Larsen S, Petersen P, Brennum J, Andreasen A, Poulsen LH, Jensen TS. McGill Pain Questionnaire translated into Danish: experimental and clinical findings. Clin J Pain 1993;9(2):80-87.

[18] Fingleton C, Smart KM, Doody CM. Exercise-induced Hypoalgesia in People With Knee Osteoarthritis With Normal and Abnormal Conditioned Pain Modulation. Clin J Pain 2017;33(5):395-404.

[19] Ge HY, Madeleine P, Cairns BE, Arendt-Nielsen L. Hypoalgesia in the referred pain areas after bilateral injections of hypertonic saline into the trapezius muscles of men and women: a potential experimental model of gender-specific differences. Clin J Pain 2006;22(1):37-44.

[20] Graven-Nielsen T. Fundamentals of muscle pain, referred pain, and deep tissue hyperalgesia. Scand J Rheumatol Suppl 2006;122(Journal Article):1-43.

[21] Graven-Nielsen T, Arendt-Nielsen L. Assessment of mechanisms in localized and widespread musculoskeletal pain. Nat Rev Rheumatol 2010;6(10):599-606.

[22] Griffin A, Leaver A, Moloney N. General Exercise Does Not Improve Long-Term Pain and Disability in Individuals With Whiplash-Associated Disorders: A Systematic Review. J Orthop Sports Phys Ther 2017;47(7):472-480.

[23] Gross A, Kay TM, Paquin J-P, Blanchette S, Lalonde P, Christie T, Dupont G, Graham N, Burnie SJ, Gelley G, Goldsmith CH, Forget M, Hoving JL, Brønfort G, Santaguida PL. Exercises for mechanical neck disorders. Cochrane Database Syst Rev 2015;1:CD004250.

[24] Gross A, Langevin P, Burnie SJ, Bédard-Brochu M-S, Empey B, Dugas E, Faber-Dobrescu M, Andres C, Graham N, Goldsmith CH, Brønfort G, Hoving JL, LeBlanc F. Manipulation and mobilisation for neck pain contrasted against an inactive control or another active treatment. Cochrane Database Syst Rev 2015;9(9):CD004249.

[25] Hayashi K, Shiozawa S, Ozaki N, Mizumura K, Graven-Nielsen T. Repeated intramuscular injections of nerve growth factor induced progressive muscle hyperalgesia, facilitated temporal summation, and expanded pain areas. Pain 2013;154(11):2344-2352.

[26] Ickmans K, Malfliet A, De Kooning M, Goudman L, Hubloue I, Schmitz T, Goubert D, Aguilar-Ferrandiz ME. Lack of Gender and Age Differences in Pain Measurements Following Exercise in People with Chronic Whiplash-Associated Disorders. Pain Physician 2017;20(6):E829-E840.

[27] Jesus-Moraleida FRd, Pereira LSM, Vasconcelos CdM, Ferreira PH. Multidimensional features of pain in patients with chronic neck pain. Fisioterapia em Movimento 2017;30:569-577.

[28] Karlsson L, Gerdle B, Ghafouri B, Backryd E, Olausson P, Ghafouri N, Larsson B. Intramuscular pain modulatory substances before and after exercise in women with chronic neck pain. Eur J Pain 2015;19(8):1075-1085.

[29] Kasch H, Stengaard-Pedersen K, Arendt-Nielsen L, Staehelin Jensen T. Pain thresholds and tenderness in neck and head following acute whiplash injury: a prospective study. Cephalalgia 2001;21(3):189-197.

[30] Koltyn KF. Exercise-induced hypoalgesia and intensity of exercise. Sports Med 2002;32(8):477-487.

[31] Kovacs FM, Abraira V, Royuela A, Corcoll J, Alegre L, Tomas M, Mir MA, Cano A, Muriel A, Zamora J, Del Real MT, Gestoso M, Mufraggi N, Spanish Back Pain Research N. Minimum detectable and minimal clinically important changes for pain in patients with nonspecific neck pain. BMC musculoskeletal disorders 2008;9:43.

[32] La Touche R, Fernandez-de-Las-Penas C, Fernandez-Carnero J, Diaz-Parreno S, Paris-Alemany A, Arendt-Nielsen L. Bilateral mechanical-pain sensitivity over the trigeminal region in patients with chronic mechanical neck pain. J Pain 2010;11(3):256-263.

[33] Melzack R. The McGill Pain Questionnaire: major properties and scoring methods. Pain 1975;1(3):277-299.

[34] Melzack R, Torgerson WS. On the language of pain. Anesthesiology 1971;34(1):50-59.

[35] Michaleff ZA, Maher CG, Lin CW, Rebbeck T, Jull G, Latimer J, Connelly L, Sterling M. Comprehensive physiotherapy exercise programme or advice for chronic whiplash (PROMISE): a pragmatic randomised controlled trial. Lancet 2014;384(9938):133-141.

[36] Ohlman T, Miller L, Naugle KE, Naugle KM. Physical Activity Levels Predict Exercise-induced Hypoalgesia in Older Adults. Med Sci Sports Exerc 2018;50(10):2101-2109.

[37] Proske U, Morgan DL. Muscle damage from eccentric exercise: mechanism, mechanical signs, adaptation and clinical applications. J Physiol 2001;537(Pt 2):333-345.

[38] Reading AE. A comparison of the McGill Pain Questionnaire in chronic and acute pain. Pain 1982;13(2):185-192.

[39] Rice D, Nijs J, Kosek E, Wideman T, Hasenbring MI, Koltyn K, Graven-Nielsen T, Polli A. Exercise-Induced Hypoalgesia in Pain-Free and Chronic Pain Populations: State of the Art and Future Directions. J Pain 2019;20(11):1249-1266.

[40] Safiri S, Kolahi AA, Hoy D, Buchbinder R, Mansournia MA, Bettampadi D, Ashrafi-Asgarabad A, Almasi-Hashiani A, Smith E, Sepidarkish M, Cross M, Qorbani M, Moradi-Lakeh M, Woolf AD, March L, Collins G, Ferreira ML. Global, regional, and national burden of neck pain in the general population, 1990-2017: systematic analysis of the Global Burden of Disease Study 2017. BMJ 2020;368:m791.

[41] Sawka MN, Foley ME, Pimental NA, Toner MM, Pandolf KB. Determination of maximal aerobic power during upper-body exercise. J Appl Physiol Respir Environ Exerc Physiol 1983;54(1):113-117.

[42] Schabrun SM, Christensen SW, Mrachacz-Kersting N, Graven-Nielsen T. Motor Cortex Reorganization and Impaired Function in the Transition to Sustained Muscle Pain. Cereb Cortex 2016;26(5):1878-1890.

[43] Schoenmakers P, Reed K, Van Der Woude L, Hettinga FJ. High Intensity Interval Training in Handcycling: The Effects of a 7 Week Training Intervention in Able-bodied Men. Front Physiol 2016;7:638.

[44] Scott D, Jull G, Sterling M. Widespread sensory hypersensitivity is a feature of chronic whiplash-associated disorder but not chronic idiopathic neck pain. Clin J Pain 2005;21(2):175-181.

[45] Smith A, Ritchie C, Pedler A, McCamley K, Roberts K, Sterling M. Exercise induced hypoalgesia is elicited by isometric, but not aerobic exercise in individuals with chronic whiplash associated disorders. Scand J Pain 2017;15:14-21.

[46] Smith A, Ritchie C, Warren J, Sterling M. Exercise-induced Hypoalgesia Is Impaired in Chronic Whiplash-associated Disorders (WAD) With Both Aerobic and Isometric Exercise. Clin J Pain 2020;36(8):601-611.

[47] Sorensen LB, Boudreau SA, Gazerani P, Graven-Nielsen T. Enlarged Areas of Pain and Pressure Hypersensitivityby Spatially Distributed Intramuscular Injections ofLow-Dose Nerve Growth Factor. J Pain 2019;20(5):566-576.

[48] Sterling M, Jull G, Kenardy J. Physical and psychological factors maintain long-term predictive capacity post-whiplash injury. Pain 2006;122(1-2):102-108.

[49] Sterling M, Jull G, Vicenzino B, Kenardy J. Characterization of acute whiplash-associated disorders. Spine (Phila Pa 1976) 2004;29(2):182-188.

[50] Sterling M, Treleaven J, Edwards S, Jull G. Pressure Pain Thresholds in Chronic Whiplash Associated Disorder: Further Evidence of Altered Central Pain Processing. Journal of Musculoskeletal Pain 2010;10(3):69-81.

[51] Summers SJ, Chipchase LS, Hirata R, Graven-Nielsen T, Cavaleri R, Schabrun SM. Motor adaptation varies between individuals in the transition to sustained pain. Pain 2019;160(9):2115-2125.

[52] Summers SJ, Schabrun SM, Hirata RP, Graven-Nielsen T, Cavaleri R, Chipchase LS. Effect of sustained experimental muscle pain on joint position sense. Pain Rep 2019;4(3):e737.

[53] Svensson P, Cairns BE, Wang K, Arendt-Nielsen L. Injection of nerve growth factor into human masseter muscle evokes long-lasting mechanical allodynia and hyperalgesia. Pain 2003;104(1-2):241-247.

[54] Takebe K, Vitti M, Basmajian JV. The functions of semispinalis capitis and splenius capitis muscles: an electromyographic study. Anat Rec 1974;179(4):477-480.

[55] Vaegter HB, Dorge DB, Schmidt KS, Jensen AH, Graven-Nielsen T. Test-Retest Reliabilty of Exercise-Induced Hypoalgesia After Aerobic Exercise. Pain Med 2018;19(11):2212-2222.

[56] Vaegter HB, Handberg G, Graven-Nielsen T. Hypoalgesia after exercise and the cold pressor test is reduced in chronic musculoskeletal pain patients with high pain sensitivity. Clin J Pain 2016;32(1):58-69.

[57] Vaegter HB, Jones MD. Exercise-induced hypoalgesia after acute and regular exercise: experimental and clinical manifestations and possible mechanisms in individuals with and without pain. Pain Rep 2020;5(5):e823.

[58] Vaegter HB, Thinggaard P, Madsen CH, Hasenbring M, Thorlund JB. Power of Words: Influence of Preexercise Information on Hypoalgesia after Exercise-Randomized Controlled Trial. Med Sci Sports Exerc 2020;52(11):2373-2379.

[59] Van Oosterwijck J, Nijs J, Meeus M, Van Loo M, Paul L. Lack of endogenous pain inhibition during exercise in people with chronic whiplash associated disorders: an experimental study. J Pain 2012;13(3):242-254.

[60] Vernon H. The Neck Disability Index: state-of-the-art, 1991-2008. J Manipulative Physiol Ther 2008;31(7):491-502.

[61] Vernon H, Mior S. The Neck Disability Index: a study of reliability and validity. J Manipulative Physiol Ther 1991;14(7):409-415.

[62] Walton DM, Levesque L, Payne M, Schick J. Clinical pressure pain threshold testing in neck pain: comparing protocols, responsiveness, and association with psychological variables. Phys Ther 2014;94(6):827-837.

[63] Walton DM, Macdermid JC, Nielson W, Teasell RW, Chiasson M, Brown L. Reliability, standard error, and minimum detectable change of clinical pressure pain threshold testing in people with and without acute neck pain. J Orthop Sports Phys Ther 2011;41(9):644-650.

[64] Xie Y, Jun D, Thomas L, Coombes B, Johnston V. Comparing central pain processing in individuals with non-traumatic neck pain and healthy individuals: a systematic review and meta-analysis. J Pain 2020.

FIGURE LEGENDS

Figure 1: Flow-chart showing the inclusion and exclusion of participants for the study

Figure 2: Study overview. Each session started with participants answering the NDI and recording any potential pain (NRS, body chart & MPQ) before PPTs were assessed. On Day0, Day4 and Day15, before participants completed a hand-bike exercise, PPT and pain experience were recorded once more. On Day2, the hand-bike exercise was not performed. On Day0 and Day2, participants received injections of NGF or isotonic saline.

Figure 3: Superimposed pre-exercise body chart drawings (posterior and right/injection side) for all test days (Day0, Day2, Day4 and Day15) and groups (NGF, Control). Darker markings indicate that these were more frequently chosen areas.

Figure 4: Mean (+SEM, N=40) normalised pressure pain thresholds (PPTs) for the NGF (n=20, solid bars) and the control (n=20, grey bars) groups at the neck, head and leg sites bilaterally (right, left) on Day2, Day4 and Day15. Significantly different compared to the control-group (*, $P<0.03$).

Significantly different compared to other days ($^{\#}$, P<0.05). Significantly different between sides ($^{¤}$,P<0.02).

Figure 5: Mean (+SEM, N=40) exercise-induced hypoalgesia (EIH) effects (pre-exercise PPT subtracted from post-exercise PPT) for the NGF (n=20, solid bars) and the control (n=20, grey bars) groups at the neck, head and leg on Day0, Day4 and Day15. Significant difference compared to the NGF-group (*, P<0.02). Significant difference compared to Day0 (§, P<0.05).

Table 1: Median and interquartile range (25th and 75th percentile) for Age, Height, Weight, Hours of sleep (all days) and NDI (all days). The NDI was expressed as a percentage of scored items. Significantly different from the control-group (*, P<0.001).

	NGF-group	**Control-group**
Age (years)	26.5 [23.0-28.0]	26.0 [23.8-28.0]
Height (cm)	175.0 [168.0-183.3]	172.5 [167.3-179.5]
Weight (kg)	72.0 [60.3-85.0]	69.5 [64.0-78.3]
Sleep (Hr):		
Day0	7.5 [7.0-8.0]	7.3 [6.8-8.0]
Day2	7.0 [6.9-8.0]	7.3 [6.9-8.0]
Day4	7.0 [6.0-7.6]	7.0 [6.4-7.1]
Day15	7.0 [6.5-8.0]	7.0 [6.4-7.6]
NDI:		
Day0	2.0 [0.0-2.5]	0.0 [0.0-2.1]
Day2	10.0 [5.8-14.5]*	0.0 [0.0-2.0]
Day4	10.0 [5.5-14.9]*	0.0 [0.0-0.5]
Day15	1.0 [0.0-2.9]	0.0 [0.0-2.0]

Table 2: Median (interquartile range: 25th and 75th percentile) pain scores using a numerical rating scale (NRS; 0 = no pain, 10 = worst imaginable pain) and area of perceived pain (a.u.) for both groups (NGF, Control) pre- and post-exercise on all days. Significant within-group difference compared to Day0 and Day15 (§, P<0.02) and to the control-group (*, P<0.005)

	NGF-group	**Control-group**
NRS score:		
Day0 (pre-exercise)	0.0 [0.0-0.0]	0.0 [0.0-0.0]
Day0 (post-exercise)	0.0 [0.0-0.0]	0.0 [0.0-0.0]
Day2	3.0 [2.0-4.0] §*	0.0 [0.0-0.0]
Day4 (pre-exercise)	3.0 [2.0-4.0] §*	0.0 [0.0-0.0]
Day4 (post-exercise)	3.0 [2.0-4.0] §*	0.0 [0.0-0.0]
Day15 (pre-exercise)	0.0 [0.0-1.0] *	0.0 [0.0-0.0]
Day15 (post-exercise)	0.0 [0.0-1.0] §*	0.0 [0.0-0.0]
Area of perceived pain:		
Day0 (pre-exercise)	0.0 [0.0-0.0]	0.0 [0.0-0.0]
Day0 (post-exercise)	0.0 [0.0-0.0]	0.0 [0.0-0.0]
Day2	26.0 [12.5-52.0] §*	0.0 [0.0-0.0]
Day4 (pre-exercise)	28.5 [9.0-50.5] §*	0.0 [0.0-0.0]
Day4 (post-exercise)	16.0 [8.25-38.0] §*	0.0 [0.0-0.0]
Day15 (pre-exercise)	0.0 [0.0-7.75]*	0.0 [0.0-0.0]
Day15 (post-exercise)	0.0 [0.0-6.75]*	0.0 [0.0-0.0]

Table 3: Mean (kPa ±SEM, N=40) pressure pain thresholds (PPT) values for the NGF (n=20) and the control (n=20) groups at the neck, head and leg sites bilaterally (right, left) pre- and post-exercise. Significant main effect of time (‡, P<0.007). Significant main effect of side ($^{¤}$, P<0.039).

	NGF-group		Control-group	
	Pre	**Post**	**Pre**	**Post**
Neck (Right)	166.0 ± 12.7$^{‡¤}$	184.9 ± 15.0	217.0 ± 23.0$^{‡¤}$	237.1 ± 20.4
Neck (Left)	182.7 ± 14.7‡	194.0 ± 15.8	218.7 ± 19.1‡	251.9 ± 22.2
Head (Right)	251.0 ± 15.4‡	280.9 ± 17.2	310.0 ± 24.9‡	331.2 ± 26.5
Head (Left)	262.4 ± 19.7‡	283.8 ± 18.8	316.8 ± 30.0‡	345.8 ± 28.9
Leg (Right)	533.7 ± 61.5$^{‡¤}$	561.5 ± 65.9	611.0 ± 46.0$^{‡¤}$	639.3 ± 52.3
Leg (Left)	495.0 ± 52.1‡	526.7 ± 58.9	599.3 ± 42.7‡	631.5 ± 52.2