Original Experimental

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Hypoalgesia after exercises with painful vs. non-painful muscles in healthy subjects – a randomized cross-over study

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Abstract

Objectives: Exercise-induced hypoalgesia (EIH) is a decrease in the pain sensitivity after exercise. Individuals with chronic pain show less EIH after one exercise session compared with pain-free individuals possibly due to pain in exercising muscles. The primary aim of this randomized controlled cross-over study was to compare the EIH response at the exercising thigh muscle following exercises performed with painful vs. non-painful muscles. Secondary aims were to explore if a reduced EIH response was confined to the painful muscle, and whether the muscle pain intensity and the EIH responses were negatively associated.

Methods: In two sessions, 34 pain-free participants received a painful (hypertonic saline, 5.8%) injection and a control (isotonic saline, 0.9%) injection in the right thigh muscle before performing a 3 min isometric wall squat exercise. Pressure pain thresholds (PPTs) were assessed at both thighs and the left neck/shoulder at baseline, after injections and after exercise. Pain intensities in the thighs were rated on numerical rating scales (NRS: 0–10).

Results: Hypertonic saline induced moderate thigh pain at rest (NRS: 4.6 ± 2.1) compared to the control injection (NRS: 0.3 ± 0.4; p<0.001). EIH at the thighs and neck/shoulder were not different between sessions (Injected thigh: 0 kPa; 95% CI: −51 to 52; Contralateral thigh: −6 kPa; 95% CI: −42 to 30; neck/shoulder: 19 kPa; 95% CI: −6 to 44). No significant associations between pain intensity ratings immediately after the Painful injection and EIH responses at any assessment sites were found (right thigh: β=0.08, 95% CI: −12.95 to 20.64, p=0.64, left thigh: β=−0.33, 95% CI: −27.86 to 0.44, p=0.06; neck/shoulder: β=−0.18, 95% CI: −15.11 to 4.96, p=0.31).

Conclusions: Pain in the area of an exercising muscle did not reduce local or systemic EIH responses.

Trial registration number: NCT04354948.

Keywords: exercise; exercise-induced hypoalgesia; experimental pain; isometric exercise; pain modulation; saline injection.

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Introduction

Exercise is considered first-line treatment for many chronic pain conditions [1], and hypoalgesia after a single exercise session, also known as exercise-induced hypoalgesia (EIH), is a well described phenomenon [2, 3]. In pain-free individuals, EIH is observed as temporary reductions in pain sensitivity (e.g., an increase in pressure pain threshold [PPT] or pain tolerance) [3], often with a more pronounced effect in exercising muscles compared to non-exercising muscles [4]. Exercises perceived as painful often result in larger EIH responses compared with non-painful exercises [5] suggesting a link between EIH and conditioned pain modulation (CPM) [6, 7] in pain-free individuals. Therefore, EIH has been considered a measure of endogenous pain inhibitory

control with activation of both peripheral and central pain inhibitory mechanisms [2]. Similar to CPM [8] more variance in the EIH response has been observed in individuals with chronic pain [2]. The reason for such variation may relate to whether exercises are performed in painful or non-painful body areas. In support of this hypothesis, Lannersten and Kosek [9] observed reduced EIH in patients with shoulder myalgia when isometric exercise was performed with the painful shoulder compared to exercise performed with the non-painful leg. Similar, Burrows et al. [10] found reduced EIH after lower-body resistance exercise compared with upper-body resistance exercise in patients with knee osteoarthritis. In addition to an increase in pain sensitivity, a recent study by Grimby-Ekman et al. [11] observed a small increase in clinical pain after a light aerobic exercise involving the painful regions in individuals with chronic neck-shoulder pain compared to pain-free individuals, suggesting that the hypoalgesic response after acute exercise is attenuated by exercising painful muscles. Based on these observations, it has been suggested that the reduced EIH responses is due to further input from an already painful (hypersensitive) body area [12] resulting in a net balance of pain facilitation and increased pain sensitivity after exercise. However, more studies investigating the influence of pain in the area of the exercising muscles on the EIH responses are needed to better understand the interaction between pain and the exercise effects. This knowledge will add to the current discussion whether exercising painful or pain-free body regions are optimal for a hypoalgesic response, which may have implications for exercise prescription in clinical settings and be more clinically relevant than previous studies inducing pre-exercise pain with the pain-inhibits-pain approach where pain is abolished before exercise [6, 7]. Using a human experimental pain model with injection of hypertonic saline to induce pain and local hyperalgesia [13], the primary aim of this randomized experimental cross-over study was to compare the EIH response at the exercising thigh muscle following an isometric squat exercise performed with experimental muscle pain vs. no experimental muscle pain. Secondary aims were to explore if EIH responses were reduced at the non-painful (non-injected) exercising thigh and at a remote non-exercising neck/shoulder muscle, and whether experimental thigh muscle pain intensity due to the injection and the EIH responses were negatively associated. We hypothesized that pain induced by the hypertonic saline injection would decrease the subsequent EIH responses compared with a non-painful control injection, and that experimental thigh muscle pain would be negatively associated to the EIH responses.

Materials and methods

This experimental cross-over study was pre-registered at clinicaltrials.gov (NCT04354948), approved by the ethical committee of the Region of Southern Denmark (S-20190081), and the Danish Data Protection Agency (20/30833). The experiments were conducted in accordance with the Helsinki Declaration between June 19th and September 9th 2020 in the laboratory at the Pain Center, University Hospital Odense. Oral and written informed consent were provided by all participants prior to enrolment. Participants received 500 DKK for participation in the study. The Consolidated Standards of Reporting Trials of Non-pharmacological Treatments (CONSORT NPT) were used as a guideline for reporting of this trial.

Participants

Pain-free individuals aged 18–50 years, adept in Danish and naive to hypertonic saline injections, were invited to participate in the study. Individuals were recruited by notifications at the University College Lillebaelt of Southern Denmark and through social media platforms. Exclusion criteria were any pain for more than two weeks within the last three months, any pain on the testing days, known mental illnesses, neurological diseases, inflammatory rheumatoid diseases or circulatory diseases in the form of heart or lung disease, any surgery within the last three month, pregnancy, addictive behavior to any kind of euphoric substances or opioids and consumption of alcohol on the day of participation. In the beginning of the first session, before the experiments, participants were familiarized with the PPT assessments and were shown how to perform the wall squat exercise without actually performing the exercise. In addition, demographic information (age, sex, weight and height) was obtained.

Randomization

A researcher (HVB, not involved in recruitment, the practical experiments and the data analyses) randomized and counterbalanced the session-order (Painful and Control), which were sealed in opaque envelopes. The researchers conducting the experiments (ES, LAM, AKM) were blinded to the sequence allocation until after participants were randomized, and thus not blinded to injection type during the PPT assessments. Participants and the researcher (SH) conducting the data analyses were blinded to the order of sessions.

Interventions

Participants attended two sessions, lasting approximately 30 min each, separated by one week. This between-sessions timeframe was chosen to avoid possible carry-over effects such as delayed-onset muscle soreness normally affecting pain variables for less than a week following acute exercise [14]. The experimental procedures are illustrated in Figure 1. In both sessions, manual PPTs at both thighs and the left neck/shoulder were assessed at baseline. Following these assessments in the first session, participants were randomized into one of two order of sessions: (A) injection with hypertonic saline (Painful) in session 1 and injection with isotonic saline (Control) in session 2, or (B) injection with isotonic saline (Control) in session 1 and injection

with hypertonic saline (Painful) in session 2. Then, an intra-muscular bolus injection of 1 mL sterile hypertonic saline (5.8%) or isotonic saline (0.9%) was given into the midline of the right quadriceps femoris (QF) muscle 20 cm proximal to the base of the patella. Injections were made manually using a 1 mL plastic syringe (Becton Dickinson, Madrid, Spain) with a disposable needle (27G, 0.40 × 38 mm, Misawa Medical Industry, Ibaraki-ken, Japan). The injections were administered by the same researcher (ES, LAM, AKM) across participants.

Next, participants performed an isometric wall squat exercise for a maximum of 3 min or until fatigue: Participants were instructed to stand upright with their back against the wall, feet parallel and shoulder-width apart and hands by their sides, lowering their back down the wall until a knee flexion angle of approximately 100° was reached and to maintain this position for 3 min. This exercise condition has previously been shown to induce a robust hypoalgesic EIH response in groups of pain-free individuals [4].

Outcomes

The primary outcome was the between-session difference in EIH response at the injected thigh muscle assessed as the absolute change in PPT (i.e., PPT immediately after exercise minus PPT at baseline). Secondary outcomes were the between-session differences in EIH responses at the contralateral non-injected thigh muscle and the contralateral neck/shoulder muscle as well as pain intensity in the thighs.

PPTs were assessed before the injection, after the injection and after the wall squat exercise using a handheld pressure algometer (Somedic Type II, Sweden, Horby) with a stimulation probe of 1 cm^2 placed perpendicularly to the skin and with the participant sitting on a plinth with foot support and arms resting on the thighs. Three sites were located and marked for the PPT assessments: Site 1; the right QF muscle (injected thigh), 15 cm proximal to the base of patella, so the assessment was in closeness, proximity to the injection, but not directly over the injection. Site 2; the left QF muscle (control thigh), 15 cm proximal to the base of patella. Site 3; the left upper trapezius muscle, 10 cm from the acromion in direct line with the 7th cervical vertebra. The pressure was increased at ~30 kPa/s until the participant defined the pressure as the first sensation of pain and pressed a button. Two PPT assessments were performed at each site, and the average for each site was used for statistical analyses. Twenty-second intervals between assessments were kept.

Pain intensity in both thighs were rated by the participants using two separate 0–10 numerical rating scales (NRS), with 0 defined as "no pain" and 10 "as worst imaginable pain". Pain intensities were rated at baseline, after the injections as well as at 1, 2, and 3 min into the wall squat exercise and 1 min after the wall squat exercise.

As reported in the pre-registration protocol, cuff-induced pain sensitivity and self-reported physical activity were collected at baseline in both sessions (prior to assessments of PPTs, injections and exercises). These measures will be reported in a later paper on the associations between self-reported physical activity and pain sensitivity.

Statistical analysis

The estimated number of participants were based on an *a priori* two-tailed sample size estimation. An average EIH effect size of 0.7 following short-term isometric exercise in healthy pain-free individuals has been reported in a previous meta-analysis [2]. As the effect of muscle pain on the EIH response has not been investigated previously, we decided on a medium between-intervention effect size of 0.5 (difference in EIH response at the thigh after exercise with experimental muscle pain compared to exercise without experimental muscle pain), a power of 0.80, and an alpha of <0.05. Using G*power version 3.1.9.2 (Düsseldorf, Germany), 34 participants were required in this within-subject repeated-measures study.

Results, in text and figures are presented as mean ± standard deviation (SD) with 95% confidence intervals (95% CI) unless stated otherwise. To test for normality, all data variables were inspected using QQ-plots and histograms, and analyzed using Shapiro-Wilks test. If QQ-plots, histograms (extreme values) and Shapiro-Wilks tests ($p \leq 0.05$) indicated non-normal distributed data non-parametric statistical analyses were applied.

Analysis of primary outcome: The EIH responses at the injected thigh were compared between sessions with Painful and Control injections using a student's paired samples *t*-test.

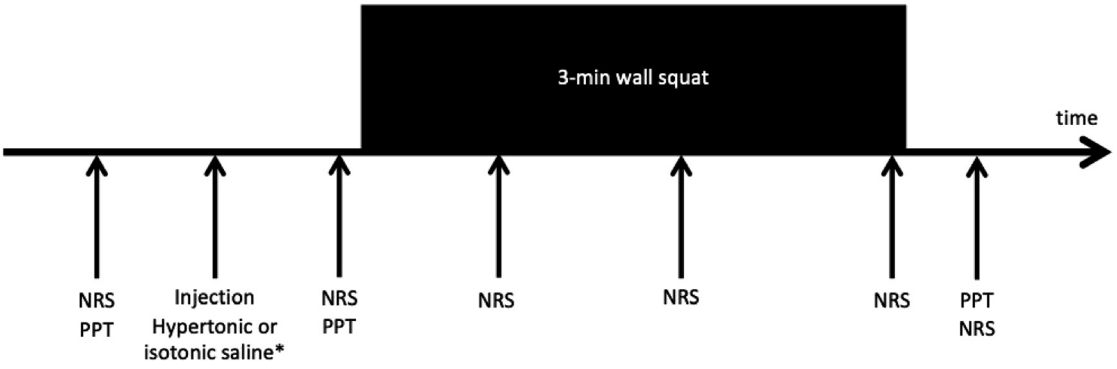

Figure 1: Experimental procedures during both sessions. Manual pressure pain thresholds (PPTs) were assessed at both quadriceps femoris muscles and the left upper trapezius muscle. Pain intensity was assessed by numerical rating scales (NRS: 0–10) immediately before and after the randomized and counterbalanced saline injections, after 1, 2, and 3 min of wall squat, and 1 min after the wall squat. Sessions were separated by one week.

Analyses of secondary outcomes: To explore the effect of muscle pain in the exercising thigh muscle on the EIH responses at the contralateral thigh (non-injected and exercising muscle) and remotely at the left trapezius (non-injected and non-exercising muscle), the EIH responses in sessions with Painful and Control injections were compared using two separate student's paired samples *t*-tests. Cohen's d effect sizes were calculated for the primary and secondary outcomes. Further, possible associations between pain intensity ratings immediately after the Painful injection (independent variable) and the EIH responses (dependent variable) were analyzed using individual univariate regression analyses with standardized coefficients β, 95% CI and p-values.

Additional not pre-specified analyses: Pain intensity induced by the Painful injection compared to the Control injection in the injected thigh was analyzed using a student's paired samples *t*-test. Possible effects on PPTs (e.g., local hypersensitivity and a remote pain-inhibits-pain phenomenon) of the Painful injection was also explored: Absolute changes in PPTs immediately after the Painful injection (i.e., PPTs immediately after injections minus PPTs at baseline) were compared with changes after the Control injection using 3 separate student's paired samples *t*-tests. Finally, possible between-session order effects on baseline PPTs were analyzed using a repeated-measures analysis of variance (RM-ANOVA) with the within-subject factors *site* (right thigh, left thigh, neck/shoulder) and *session* (session 1, session 2). p-Values<0.05 were considered significant for all analyses. SPSS version 25 (IBM Corporation, Armonk, New York, USA) was used for all statistical analyses.

As reported in the pre-registration protocol, cuff-induced pain sensitivity and self-reported physical activity were collected at baseline in both session (prior to assessments of PPTs, injections and exercises). These measures will be reported in a later paper on the associations between self-reported physical activity and pain sensitivity.

Results

Participant characteristics

Thirty-five participants were recruited and tested in this study as one participant became unwell (dizziness and had to lie down for approx. 5–6 min) after one of the injections and was unable to initiate the wall squat test immediately after the injection. Thirty-four participants (age: 25.5 ± 4.4 years [range: 20–46]; BMI: 24.4 ± 4.3 [range: 18.9–40.9]; 12 women) completed both sessions and were included in the analyses.

Baseline PPTs in the two sessions are presented in Table 1. No significant main effect of sessions ($F(1,66) = 2.62$, $p=0.12$) or interaction between sessions and sites ($F(2,66)=0.53$, $p=0.59$) were found indicating no between-sessions order effect on baseline PPTs.

EIH outcomes

Increases in PPTs (i.e., EIH) at all assessment sites were observed after the wall squat exercise in both sessions except in the injected thigh after the Control injection as indicated by the confidence interval overlapping zero (Table 1).

Primary outcome

No significant between-session EIH difference (EIH injected thigh in the Painful and Control session) was found (Table 1).

Secondary outcomes

No significant between-session EIH differences (EIH responses non-injected thigh and trapezius, respectively, in Painful and Control session) were found (Table 1).

Collectively, this indicates, that pre-exercise induced muscle pain in an exercising thigh muscle did not reduce the local or remote EIH responses.

Associations between experimental muscle pain intensity and the EIH responses after exercise

Pain intensity ratings increased during the wall squat exercise and decreased immediately after the exercise in both sessions (Table 2). No significant correlations between pain intensity ratings immediately after the Painful injection and EIH responses at any assessment sites were found (right thigh: $\beta=0.08$, 95% CI: −12.95 to 20.64, $p=0.64$, left thigh: $\beta=-0.33$, 95% CI: −27.86 to 0.44, $p=0.06$; neck/shoulder: $\beta=-0.18$, 95% CI: −15.11 to 4.96, $p=0.31$).

Exploratory outcomes

Wall squat performance and pain intensity

Thirty (88.2%) participants performed the wall squat exercise for the maximum 3 min in both sessions. One participant stopped the wall squat exercise due to fatigue in both sessions (session 1: after 1.35 min; session 2: after 1.32 min); two participants stopped the wall squat exercise due to fatigue in session one (after 2.03 and 2.39 min, respectively), while one participant stopped the wall squat exercise due to fatigue in the second session (after 2.03 min).

Table 1: Pressure pain thresholds at baseline and after the wall squat exercise in sessions with isotonic (Control) and hypertonic (Painful) injections.

	Session with Control injection (n=34)			Session with Painful injection (n=34)			Between-sessions EIH difference	p-Value	Effect size
	Baseline	After exercise	Within-session EIH response	Baseline	After exercise	Within-session EIH response			
PPT, kPa Right thigh (injected)	485 ± 218 (409–561)	518 ± 251 (431–606)	34 ± 108 (–4 to 72)	472 ± 237 (389–554)	506 ± 235 (424–588)	34 ± 97 (1–68)	0 ± 147 (–51 to 52)	0.988	0.00
PPT, kPa Left thigh (non-injected)	441 ± 203 (371–512)	513 ± 243 (428–598)	72 ± 89 (40–103)	455 ± 228 (376–535)	521 ± 256 (432–611)	66 ± 86 (36–96)	–6 ± 103 (–42–30)	0.749	0.06
PPT, kPa Shoulder	288 ± 126 (243–332)	315 ± 134 (268–362)	28 ± 54 (9–46)	282 ± 148 (230–334)	328 ± 168 (270–387)	47 ± 58 (26–67)	19 ± 73 (–6–44)	0.135	0.30

KPa, kilopascal. Data presented as mean ± SD (95% CI). p-Values based on paired *t*-tests for the absolute difference in PPTs from baseline to after exercise between the Painful and Control injection sessions. Effect sizes presented as Cohen's *d*.

Table 2: Pain intensity ratings in the thighs at baseline, after injections, during (1, 2, and 3 min) and 1 min after the wall squat exercise in sessions with Painful and Control injections.

	Time before and during wall squat					
	Baseline	After injection	1 min	2 min	3 min/fatigue	1 min after
Session with painful injection (n=34)						
Pain intensity – Injected thigh (NRS: 0–10)	0.0 ± 0.0 (0.0–0.0) [0–0]	4.6 ± 2.1[a] (3.8–5.3) [1–9]	4.4 ± 1.9 (3.9–5.2) [0–9]	5.7 ± 2.1 (5.0–6.5) [1–10]	6.7 ± 2.5 (5.8–7.6) [0–10]	2.1 ± 1.7 (1.6–2.7) [0–5]
Pain intensity – Contralateral thigh (NRS: 0–10)	0.0 ± 0.0 (0.0–0.0) [0–0]	0.0 ± 0.2 (0.0–0.1) [0–1]	2.7 ± 2.0 (2.0–3.4) [0–7]	4.9 ± 2.3 (4.1–5.7) [0–10]	6.3 ± 2.6 (5.4–7.3) [0–10]	1.3 ± 1.6 (0.8–1.9) [0–6]
Session with Control injection (n=34)						
Pain intensity – Injected thigh (NRS: 0–10)	0.0 ± 0.0 (0.0–0.0) [0–0]	0.3 ± 0.4[a] (0.1–0.4) [0–1]	3.1 ± 2.4 (2.2–3.9) [0–8]	5.4 ± 2.4 (4.6–5.3) [0–10]	6.5 ± 2.4 (5.6–7.4) [0–10]	2.0 ± 1.8 (1.4–2.7) [0–7]
Pain intensity – Contralateral thigh (NRS: 0–10)	0.0 ± 0.0 (0.0–0.0) [0–0]	0.1 ± 0.3 (0.0–0.2) [0–1]	3.1 ± 2.3 (2.3–3.9) [0–8]	5.5 ± 2.2 (4.7–6.3) [1–10]	6.6 ± 2.4 (5.7–7.5) [0–10]	2.1 ± 1.8 (1.4–2.7) [0–7]

Data presented as mean ± SD (95% CI) and [range]. [a]indicate significant difference between Painful and Control injection (p<0.001)

Effect of the injections on pain intensity and PPTs

The hypertonic saline injection induced moderate thigh pain at rest (Table 2; NRS: 4.6 ± 2.1, CI: 3.8–5.3, range: 1–9) compared to the Control injection (NRS: 0.3 ± 0.4, CI: 0.1–0.4, range: 0–1; p<0.001).

The absolute increase in trapezius PPT was significantly larger after the Painful injection (51.1 ± 43.1 kPa; 95% CI: 36.1–66.1) compared with the Control injection (16.4 ± 37.4 kPa; 95% CI: 3.4–29.5; p<0.001), indicating a remote pain-inhibits-pain effect of the Painful injection. The non-injected thigh PPT also increased after the Painful injection (43.3 ± 72.0 kPa; 95% CI: 18.1–68.4) but not after the Control injection (17.0 ± 66.9 kPa; 95% CI: –6.3 to 40.4), however there was no significant difference in PPT increase between sessions (p=0.09). The injected thigh PPT was significantly decreased after the Painful injection (–50.8 ± 134.5 kPa; 95% CI: –97.7 to –3.8) indicating that the Painful injection did induce local hypersensitivity. The injected thigh PPT was not significantly decreased after the Control injection (–16.8 ± 76.6 kPa; 95% CI: –43.5 to 9.9), however there was no significant difference in PPT decrease between sessions (p=0.24).

Discussion

Summary of results

This randomized controlled cross-over study is the first study to investigate the influence of experimental muscle pain in the exercising thigh muscle during exercise on the subsequent EIH responses following acute exercise. No between-session difference in EIH responses after exercise

with a painful muscle compared to exercise with non-painful muscles were found. Moreover, ratings of muscle pain intensity after the painful injection were not associated with the subsequent EIH responses following exercise. These findings suggest that pain per se in the exercising muscle does not reduce the hypoalgesic effects of exercise in pain-free individuals. The strengths of this study include pre-registration of the protocol, *a priori* sample size estimation, randomization and allocation concealment, and statistical analysis performed by a blinded investigator.

General discussion of findings

PPTs increased after the wall squat exercise in the non-painful session, although EIH was not robust in the injected thigh, which is in agreement with previous studies in pain-free individuals [4]. Studies of EIH in individuals with different pain conditions suggest that the hypoalgesic EIH response after acute exercise is attenuated when exercising painful muscles [9, 10] and therefore the current findings were unexpected. There are several possible explanations for these conflicting results: The painful hypertonic saline injection in the thigh muscle induced moderate pain and local hypersensitivity compared with baseline, which is in agreement with a previous study using the same needle size, concentration of hypertonic saline, and volume injected in the pelvic area [13]. However, the range in pain intensity in the current study spans from almost no pain (NRS: 1) to very intense pain (NRS: 9) which indicate large inter-individual pain responses to the same noxious stimuli. This may have affected the results and warrant further investigation. The pain intensity induced by the hypertonic saline injection was similar to pain intensity after the control injection 2 min into the exercise (Table 2). Increased pain intensity throughout the whole exercise might have had influenced the EIH response. Also, in the current study, the hypersensitivity was not significantly larger after the painful injection than after the control injection. The equivocal results may be related to the site of injection (muscle vs. ligament), which is supported by a previous study by Drew and colleagues who observed no differences in hypersensitivity after hypotonic saline injections in thigh muscles compared with isotonic control injections [15].

Reduced EIH in individuals with chronic pain has been associated with hypersensitivity, a common finding in painful areas in several clinically painful conditions [16]. The individuals included in this study showed no signs of a reduced pain-inhibits-pain response; in contrast they showed increased PPT in the neck/shoulder muscle and the non-injected thigh after the painful injection. A reduced pain-inhibits-pain response has previously been related to reduced EIH [17, 18]. Fingleton et al. observed reduced EIH after isometric and aerobic exercise in knee osteoarthritis patients who also showed reduced CPM while patients with an inhibitory CPM response also showed a positive EIH response [17]. Further, several studies have suggested shared systemic mechanisms involved in EIH and CPM responses [6, 7]. Collectively, this indicates that the effectiveness of central pain inhibitory mechanisms may be more important for the hypoalgesic response to exercise than the presence of pain itself.

In the current study, non-painful muscles were also exercised (the contralateral thigh) during the wall squat exercise after painful injection possibly activating more segmental or systemic effects counteracting a potential reduced local EIH response from exercising painful muscles, as previous studies have indicated that the EIH response is the net result of an array of locally and systemically acting pain inhibitory mechanisms involving both opioidergic and non-opioidergic mechanisms [2, 19].

The injection itself may have reduced the EIH response as the magnitude of the EIH response in the injected thigh was generally smaller compared to the non-injected contralateral thigh independently of injection type. This may suggest that the bolus injection itself or local intrinsic factors such as different weight bearing strategies or different muscle activation patterns after the injections might have influenced the results. In support of the latter, hypertonic saline injection has previously been shown to redistribute muscle activation patterns during isometric exercise [20].

The results may also be influenced by the duration of pain; injection with hypertonic saline only induced short-lasting pain that was almost normalized 4 min after the injection in contrast to chronic pain states that are consistent. Other pain models e.g., delayed-onset muscle soreness [21] or nerve-growth-factor injections [22] that induce longer lasting pain should be investigated in the future in relation to EIH.

The results may also be influenced by the distribution of pain. As highlighted in a recent EIH review [12], individuals with more widespread pain often show attenuated EIH both locally and systemically, while individuals with more localized pain show attenuated local EIH only when exercising painful muscles. In the current study, pain distribution after the painful injection was not assessed. However, a previous study injecting hypertonic saline into the thigh muscle observed a rather localized pain distribution [15], whereas studies demonstrating reduced EIH often have included individuals with more widespread pain [18, 23, 24].

The current results are in line with Kadetoff & Kosek [25] who found similar EIH responses after a one-legged isometric knee extension exercise to exhaustion in individuals with fibromyalgia compared with pain-free individuals, suggesting that the hypoalgesic response to acute exercise may be unrelated to the presence of clinical pain itself during exercise. Additionally, longitudinal studies reporting no change in EIH in spite of reduced clinical pain following long-term exercise programs [26, 27] or surgery [28] in knee pain populations, suggest that clinical pain and EIH are not directly related. Only a few studies have examined EIH calculated as change in clinical pain after acute exercise as highlighted in a recent review [12]. Future research on e.g., the change in clinical pain (instead of change in pain sensitivity measures) after acute exercise and its relation to improved clinical pain after long-term exercise programs are warranted.

The current findings indicate that the EIH responses are comparable after exercise performed with painful and non-painful muscle in pain-free individuals. Several studies that have investigated EIH, report that the utilized exercise is perceived as moderately painful [4, 29], and larger hypoalgesic responses are found after painful compared to non-painful exercises in pain-free individuals [5], suggesting that pain during exercise may be important for EIH. Further, previous studies have reported a positive correlation between CPM responses and EIH responses in healthy individuals [7, 30] suggesting shared mechanisms of CPM and EIH and that the individual's ability to pain inhibition is a trait rather than only related to exercise. This may be different in some chronic pain populations; Coombes and colleagues [31] showed that painful isometric exercises above the individual's pain threshold caused increased clinical pain responses during and after exercise in people with lateral epicondylalgia, while no increase in pain was observed with non-painful exercises performed below the pain threshold. This indicates an interaction between pain and EIH; painful exercises of higher exercise intensity may result in more input to central pain facilitatory mechanisms resulting in a net balance of pain facilitation and subsequently post-exercise increased pain sensitivity and clinical pain. Although acute hyperalgesia to exercise is common in musculoskeletal pain populations [12] studies on change in clinical pain after acute exercise are limited [12], and a recent systematic review with meta-analysis concluded that painful long-term exercise programs may be more beneficial than non-painful exercise for short-term, but not medium- and long-term, pain management in chronic musculoskeletal pain populations [32] highlighting the complexity between the acute exercise pain response and long-term outcome.

Limitations

This study is strengthened by its randomized cross-over design. However, in addition to the limitations discussed in the previous sections, this study did not include a no-exercise control condition as recommended in a recent review [33]. Also, the study was powered to detect a medium between-intervention effect and thus not adequately powered to detect smaller differences.

Conclusions

In conclusion, this study demonstrated that local and remote EIH responses were comparable after exercises performed with painful and pain-free muscles suggesting that the pain relieving effects of exercise is not attenuated by exercising painful body areas. In addition, pre-exercise pain intensity was not associated with the subsequent post-exercise hypoalgesic responses. This knowledge adds to the current discussion whether exercising painful or pain-free body regions are optimal for a hypoalgesic response, which may have implications for exercise prescription in clinical settings. Further research investigating factors and mechanisms associated with the reduced EIH response observed in some individuals with chronic pain conditions are warranted.

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Ethical approval: This study was approved by the local ethics committee of Region of Southern Denmark (S-20190081) and the Danish Data Protection Agency (20/30833).

References

1. Pedersen BK, Saltin B. Exercise as medicine - evidence for prescribing exercise as therapy in 26 different chronic diseases. Scand J Med Sci Sports 2015;25:1–72.
2. Naugle KM, Fillingim RB, Riley JL 3rd. A meta-analytic review of the hypoalgesic effects of exercise. J Pain 2012;13:1139–50.
3. Rice D, Nijs J, Kosek E, Wideman T, Hasenbring MI, Koltyn K, et al. Exercise-induced hypoalgesia in pain-free and chronic pain populations: state of the art and future directions. J Pain 2019;20:1249–66.

4. Vaegter HB, Lyng KD, Yttereng FW, Christensen MH, Sørensen MB, Graven-Nielsen T, et al. Exercise-induced hypoalgesia after isometric wall squat exercise: a test-retest reliability study. Pain Med 2019;20:129–37.
5. Ellingson LD, Koltyn KF, Kim J-S, Cook DB. Does exercise induce hypoalgesia through conditioned pain modulation? Psychophysiology 2014;51:267–76.
6. Alsouhibani A, Vaegter HB, Hoeger Bement M. Systemic exercise-induced hypoalgesia following isometric exercise reduces conditioned pain modulation. Pain Med 2019;20:180–90.
7. Gajsar H, Nahrwold K, Titze C, Hasenbring MI, Vaegter HB. Exercise does not produce hypoalgesia when performed immediately after a painful stimulus. Scand J Pain 2018;18:311–20.
8. Lewis GN, Rice DA, McNair PJ. Conditioned pain modulation in populations with chronic pain: a systematic review and meta-analysis. J Pain 2012;13:936–44.
9. Lannersten L, Kosek E. Dysfunction of endogenous pain inhibition during exercise with painful muscles in patients with shoulder myalgia and fibromyalgia. Pain 2010;151:77–86.
10. Burrows NJ, Booth J, Sturnieks DL, Barry BK. Acute resistance exercise and pressure pain sensitivity in knee osteoarthritis: a randomised crossover trial. Osteoarthritis Cartilage 2014;22: 407–14.
11. Grimby-Ekman A, Ahlstrand C, Gerdle B, Larsson B, Sandén H. Pain intensity and pressure pain thresholds after a light dynamic physical load in patients with chronic neck-shoulder pain. BMC Musculoskelet Disord 2020;21:266.
12. 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:e823.
13. Palsson TS, Graven-Nielsen T. Experimental pelvic pain facilitates pain provocation tests and causes regional hyperalgesia. Pain 2012;153:2233–40.
14. Lau WY, Blazevich AJ, Newton MJ, Wu SSX, Nosaka K. Changes in electrical pain threshold of fascia and muscle after initial and secondary bouts of elbow flexor eccentric exercise. Eur J Appl Physiol 2015;115:959–68.
15. Drew MK, Palsson TS, Hirata RP, Izumi M, Lovell G, Welvaert M, et al. Experimental pain in the groin may refer into the lower abdomen: implications to clinical assessments. J Sci Med Sport 2017;20:904–9.
16. Curatolo M, Müller M, Ashraf A, Neziri AY, Streitberger K, Andersen OK, et al. Pain hypersensitivity and spinal nociceptive hypersensitivity in chronic pain. Pain 2015;156:2373–82.
17. 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;33:395–404.
18. Vaegter HB, Graven-Nielsen T. Pain modulatory phenotypes differentiate subgroups with different clinical and experimental pain sensitivity. Pain 2016;157:1480–8.
19. Da Silva Santos R, Galdino G. Endogenous systems involved in exercise-induced analgesia. J Physiol Pharmacol 2018;69:3–13.
20. Hirata RP, Salomoni SE, Christensen SW, Graven-Nielsen T. Reorganised motor control strategies of trunk muscles due to acute low back pain. Hum Mov Sci 2015;41:282–94.
21. Dannecker EA, Koltyn KF. Pain during and within hours after exercise in healthy adults. Sports Med 2014;44:921–42.
22. Sørensen LB, Gazerani P, Sluka KA, Graven-Nielsen T. Repeated injections of low-dose nerve growth factor (NGF) in healthy humans maintain muscle pain and facilitate Ischemic contraction–Evoked pain. Pain Med 2020;00:1–11.
23. Hoeger Bement MK, Weyer A, Hartley S, Drewek B, Harkins AL, Hunter SK. Pain perception after isometric exercise in women with fibromyalgia. Arch Phys Med Rehabil 2011;92:89–95.
24. Staud R, Robinson ME, Price DD. Isometric exercise has opposite effects on central pain mechanisms in fibromyalgia patients compared to normal controls. Pain 2005;118:176–84.
25. Kadetoff D, Kosek E. The effects of static muscular contraction on blood pressure, heart rate, pain ratings and pressure pain thresholds in healthy individuals and patients with fibromyalgia. Eur J Pain 2007;11:39–47.
26. Hansen S, Vaegter HB, Petersen KK. Pretreatment exercise-induced hypoalgesia is associated with change in pain and function after standardized exercise therapy in painful knee osteoarthritis. Clin J Pain 2020;36:16–24.
27. Löfgren M, Opava CH, Demmelmaier I, Fridén C, Lundberg IE, Nordgren B, et al. Long-term, health-enhancing physical activity is associated with reduction of pain but not pain sensitivity or improved exercise-induced hypoalgesia in persons with rheumatoid arthritis. Arthritis Res Ther 2018;20:262.
28. Vaegter HB, Handberg G, Emmeluth C, Graven-Nielsen T. Preoperative hypoalgesia after cold pressor test and aerobic exercise is associated with pain relief 6 months after total knee replacement. Clin J Pain 2017;33:475–84.
29. Vaegter HB, Dørge DB, Schmidt KS, Jensen AH, Graven-Nielsen T. Test-retest reliability of exercise-induced hypoalgesia after aerobic exercise. Pain Med 2018;19:2212–22.
30. Lemley KJ, Hunter SK, Bement MKH. Conditioned pain modulation predicts exercise-induced hypoalgesia in healthy adults. Med Sci Sports Exerc 2015;47:176–84.
31. Coombes BK, Wiebusch M, Heales L, Stephenson A, Vicenzino B. Isometric exercise above but not below an individual's pain threshold influences pain perception in people with lateral epicondylalgia. Clin J Pain 2016;32:1069–75.
32. Smith BE, Hendrick P, Smith TO, Bateman M, Moffatt F, Rathleff MS, et al. Should exercises be painful in the management of chronic musculoskeletal pain? A systematic review and meta-analysis. Br J Sports Med 2017;51:1679–87.
33. Wewege MA, Jones MD. Exercise-induced hypoalgesia in healthy individuals and people with chronic musculoskeletal pain: a systematic review and meta-analysis. J Pain 2021;22:21–31.