The Influence of Musculoskeletal Pain On Exercise-Induced Hypoalgesia in Humans

an Experimental and Clinical Exploratory Approach

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BY
SIMON HANSEN

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CV

Simon Hansen qualified as a B.Sc. in Sports Science in 1998 from University of Copenhagen, where after he worked 1½ year as a scholarship student at University of Copenhagen within human neurophysiological movement research. This research, resulting in a number of international peer-reviewed articles, inspired him to work with patients. In 2003, Simon graduated as a physiotherapist from the Physiotherapist School of Copenhagen.

After full-time clinical practice and extensive post-graduate education focusing on musculoskeletal pain disorders, Simon in 2016 started at the Master of Pain Science and Multi-disciplinary Pain Management education at Aalborg University. After graduation in 2018, he continued in clinical practice and concurrently started as a part-time Research Assistant at Center for Sensory-Motor Interaction (SMI®), Aalborg University. He was affiliated with the Translational Pain Biomarkers research interest group and supervised by Associate Professor Kristian Kjær Petersen with a focus on understanding underlying mechanisms of responders and non-responders to exercise therapy. Throughout this work, Simon has been awarded a number of travel grants and presented several posters at international conferences; the IASP 17th World Congress on Pain (Boston, USA, 2018), the EFIC 11th Congress of the European Pain Federation (Valencia, Spain, 2019), the IASP Virtual Series on Pain & Expo (online, 2020-2021) and the OARSI Connect Virtual World Congress (online, 2021).

Simon is a Specialist in Musculoskeletal Physiotherapy approved by The Danish Society of Physiotherapy.

PREFACE

This PhD thesis and the following PhD defence are the true highlights of my time as a Research Assistant at SMI®, Department of Health Science and Technology, Aalborg University, Aalborg, Denmark. The research projects for this PhD thesis were conducted between October 2017 and June 2020. Associate Professor Kristian Kjær Petersen and The Aalborg University Talent Management Programme (j.no. 771126) are sincerely acknowledged for project support.

The longitudinal studies for this PhD project were conducted in collaboration with Viby Physiotherapy Clinic, Aarhus, Denmark, and the Danish Army Logistics, Aalborg, Denmark. The experimental study was conducted as an external research collaboration at the University of Southern Denmark in a laboratory at the Pain Center, University Hospital Odense, Odense, Denmark.

The thesis is a synthesis of three articles; two have been published in international peer-reviewed journals and one is under review. Throughout the thesis, these articles are referred to as:

Study I: Hansen S, Dalgaard RC, Mikkelsen PS, Sørensen MB, Petersen KK. Modulation of Exercise-Induced Hypoalgesia Following an Exercise Intervention in Healthy Subjects. Pain Med 2020 Sep 27;21(11):3556–3566.

Study II: Hansen S, Petersen KK, Sloth E, Manum LA, McDonald AK, Andersen PG, Vaegter HB. Hypoalgesia after painful versus non-painful muscle exercise – a randomized cross-over study in healthy individuals (under review: Pain Med).

Study III: 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 Jan;36(1):16–24.

The thesis will give an overview of the most important findings and additionally unpublished findings from Study I-III, and relate the findings to the existing literature within the field of exercise-induced hypoalgesia (EIH) in healthy individuals and individuals with chronic musculoskeletal pain. To enhance between-study comparability, all findings troughout the thesis are presented using the same metrics although other metrics may have been appilied in the articles related to Study I-III.

This thesis contributes to the current understanding of the relationship between the perception of pain and EIH, and explores the possibility of modulating EIH by exercise interventions.

ENGLISH SUMMARY

One out of five people suffer from chronic pain. Regular physical exercise may prevent more than 30 chronic disorders and is recommended as first choice of rehabilitating treatment for several chronic musculoskeletal (MSK) pain disorders to promote health, reduce co-morbidities and for pain relief.

Current evidence suggest that endogenous central pain inhibitory mechanisms are important for pain perception. It is also known that these central pain mechanisms may be impaired in chronic pain disorders across a wide variety of different etiologies. In addition, regular physical exercise may prevent and reduce chronic MSK pain by modulation of endogenous pain inhibitory mechanisms. However, some individuals do not experience this pain relieving effect. Therefore, understanding pain mechanisms related to pain perception and physical exercise may have the potential to optimise future pain relieving physical exercise interventions.

Exercise-induced hypoalgesia (EIH) is a well-established phenomenon defined as decreased pain sensitivity after acute exercise. EIH is considered a proxy of the balance between endogenous pain inhibitory and pain facilitatory mechanisms. EIH responses are highly variable or even hyperalgesic in chronic MSK pain populations, while moderate-large EIH responses robustly have been demonstrated in healthy individuals, suggesting that pain influence EIH. In addition, higher EIH is observed in physically active individuals compared to physically inactive individuals, indicating that regular physical activity (i.e. exercise interventions of week-month duration) may modulate EIH. Therefore, it was hypothesised that 1) pain is related to EIH, and 2) EIH is modifiable by exercise interventions.

This thesis is based on two longitudinal studies on healthy individuals (Study I) and individuals with chronic MSK pain (Study III), respectively, and one randomised controlled cross-over study on healthy individuals (Study II). The applied interventions were standardised approx. 7-week exercise interventions for Study I and Study III. Intramuscular injection of hypertonic saline was utilised as

an experimental model to investigate the isolated effect of pain on EIH (Study II). In all studies, EIH was assessed as change in pressure pain thresholds at exercising (local EIH) and non-exercising (remote EIH) muscles after acute exercise conditions, and EIH modulation was assessed as EIH change following the 7-week exercise interventions (Study I and Study III). Pain perception was assessed using well-accepted self-reported outcome measures in all studies.

No linear associations between pain intensity in relation to acute exercise and EIH responses in all investigated cohorts were found (Study I-III), and experimental pain did not influence EIH (Study II). In healthy individuals (Study I), remote EIH was modulated after the 7-week exercise intervention, whereas this effect was absent in chronic MSK pain individuals (Study III). An exploratory association between increased local EIH and decreased pain intensity after the exercise intervention was described in Study III.

In conclusion, this PhD project has provided novel evidence suggesting that the presence of pain does not influence or associate with EIH responses. EIH may be modulated in healthy individuals, albeit no EIH modulation occurred after the exercise intervention in chronic MSK pain individuals. However, increased EIH may be associated with pain relief following standardised exercise treatment in individuals with chronic MSK pain, which may indicate a link between endogenous pain inhibitory mechanism effectivity and the perception of pain. These novel findings add new knowledge into the translational interplay between EIH, the perception of pain and exercise interventions as treatment. The findings may have clinical implications in the guidance of individuals with chronic MSK pain using exercise for pain treatment, and the findings raise new important research questions on which factors influence EIH and pain.

DANSK RESUME

Én ud af fem voksne mennesker lider af kroniske smerter. Vedvarende fysisk træning kan forebygge mere end 30 kroniske sygdomme og er anbefalet som førstevalg ved rehabiliterende behandling af kroniske muskuloskeletale (MSK) smerter for at fremme sundhed, reducere følgesygdomme og som smertelindring.

Evidens foreslår at kroppens egne centrale smertehæmmende mekanismer er vigtige for reduktion af smerteopfattelsen. Det er ligeledes kendt at disse centrale mekanismer kan være forringede hos individer med kroniske smerter på tværs af et bredt spektrum af ætiologier. Endvidere kan vedvarende fysisk træning forebygge og reducere kroniske MSK smerter ved modulation af disse centrale smertehæmmende mekanismer. Dog er der nogle individer, der ikke oplever denne smertelindrende effekt af fysiske træning. En dybere forståelse af de underliggende smertemekanismer relateret til smerteopfattelse og fysisk træning har derfor potentialet til at optimere fremtidige smertelindrende fysiske træningsinterventioner.

Trænings-induceret nedsat smertefølsomhed (engelsk: exercise-induced hypoalgesi, EIH) er et velbeskrevet fænomen defineret som nedsat smertefølsomhed efter akut træning. EIH responset anses som indirekte mål for balancen mellem kroppens egne smertehæmmende og smertefremmende mekanismer. EIH responset er meget variabelt og kan endda vise øget smertefølsomhed hos individer med kroniske MSK smerter, mens det konsistent er moderat-stort hos raske, hvilket indikerer, at smerte påvirker EIH. Yderligere er EIH større hos fysisk aktive individer sammenlignet med inaktive individer, hvilket indikerer, at fysisk træning (f.eks. træningsinterventioner af uger-måneders varighed) kan modulere EIH. Det blev derfor hypotetiseret, at 1) smerte er relateret til EIH, og 2) EIH kan moduleres af træningsinterventioner.

Denne afhandling er baseret på to longitudinelle studier på henholdsvis raske (Studie I) og personer med kroniske MSK smerter (Studie III), samt et kontrolleret lodtrækningsstudie med overkrydsning på raske (Studie II). De benyttende interventioner var standardiserede ca. 7-

ugers træningsinterventioner på raske (Studie I) og individer med kroniske MSK smerter (Studie III). Intramuskulær smertegivende saltvandsindsprøjtninger blev anvendt som eksperimentel smertemodel for at undersøge den isolerede effekt af smerter på EIH (Studie II). I alle studierne blev EIH beregnet som ændringen i tryksmertetræsklen på aktive (lokal-EIH) og inaktive (fjern-EIH) muskler efter akut træning, og EIH modulationen blev udregnet som EIH ændringen efter de 7-ugers træningsinterventioner (Studie I og Studie III). Smerteopfattelsen blev i alle studierne vurderet med anerkendte selvrapporterede effektmål.

Der blev ikke fundet lineære sammenhænge mellem smerteintensitet i forbindelse med akut træning og EIH i alle de undersøgte populationer (Studie I-III), og eksperimentel smerte påvirkede ikke EIH (Studie II). Hos raske (Studie I) blev fjern-EIH moduleret efter den 7-ugers træningsintervention, mens denne effekt ikke blev observeret efter træningsinterventionen hos individerne med kroniske MSK smerter (Studie III). En mulig sammenhæng mellem øget lokal-EIH og nedsat smerteintensitet efter den 7-ugers træningsintervention blev fundet i Studie III.

Det kan konkluderes, at dette PhD projekt har frembragt ny evidens, der foreslår, at tilstedeværelsen af smerte ikke påvirker eller er sammenhængende med EIH. EIH kan muligvis moduleres i raske, mens der ingen EIH modulation blev fundet efter træningsinterventionen hos individer med kroniske MSK smerter. En mulig sammenhæng mellem øget EIH og smertelindring efter et træningsforløb kan forekomme hos individer med kroniske MSK smerter, hvilket antyder en sammenhæng mellem kroppens smertehæmmende mekanismer og opfattelsen af smerte. Disse nye fund tilføjer ny viden til det translatoriske sammenspil mellem EIH, opfattelsen af smerte og træningsinterventioner. Disse fund kan have kliniske implikationer i vejledningen af individer med kroniske MSK smerter ved anvendelse af træningsinterventioner som smertebehandling, og fundene rejser nye vigtige forskningsspørgsmål om hvilke faktorer, der influerer på EIH og smerter.

ACKNOWLEDGEMENTS

I would like to acknowledge and express my deepest gratitude to a number of persons, whose help and support have been essential for me and this project.

This thesis and the work leading up to it would not have been possible without the ongoing support from the most valuable in my life; my wife Dorthe and our lovely daughters Selma and Karla. Thanks for all your love, smiles and hugs even when my mind has been elsewhere ♥ ♥ ♥

Thanks to all the subjects who voluntarily accepted to participate in the experiments, and to the staff and officers at the 2nd Battalion of the Danish Army Logistics, Aalborg, Denmark who accepted to join forces to make Study I possible. Also, many sincere warm thoughts are send to my former colleagues at Viby Physiotherapy Clinic, Aarhus, Denmark, who recruited the participants for Study III, and to my other workplace at FysioDanmark Aarhus, Aarhus, Denmark, for giving me the flexibility to combine clinical practice and academia.

Sincere thanks to Dennis Boye Larsen for all your help and good energy and to all the other nice and friendly colleagues I have meet at Alborg University but not mentioned here. To all the secretaries at SMI®; homage to you for all the important administrative work in the background. Your work are very much appreciated and recognised.

Last, but not least, my two supervisors have been pivotal in all parts of this project. Thanks to Associate Professor Kristian Kjær Petersen, my main supervisor. You opened the door for me into academia, and you have been a guiding star throughout the entire project in a very competent, educational and constructive manner. I will always be grateful to you for giving me this opportunity! Thanks to Associate Professor Henrik Bjarke Vaegter, my co-supervisor. You have always been available for constructive and detailed discussions when needed, sharing your detailed knowledge within exercise and pain research.

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TERMS AND DEFINITIONS

Exercise-induced hypoalgesia (EIH) is used throughout the thesis as a term of decreased pain sensitivity (e.g. pressure pain thresholds) during or after one acute exercise condition.

Exercise condition is used throughout the thesis as term of the acute exercise used to test EIH.

Exercise intervention is used throughout the thesis as term of physical exercise programs with a typical duration of 6-12 weeks (but not limited to this duration), aiming at modulating experimental pain or clinical variables e.g. pain, disability, physical performance etc.

Throughout the thesis, the following terms are used in accordance with the definitions from the International Association for the Study of Pain (IASP)[1]:

Pain: "An unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage."

Pain threshold: "The minimum intensity of a stimulus that is perceived as painful."

CHAPTER 1. INTRODUCTION

The 2020-updated definition of pain by the International Association for the Study of Pain (IASP) with its concomitant notes highlights that pain is always a personal experience that cannot be questioned, and that pain may be influenced to varying degrees by biological and psychosocial factors[2].

1.1. THE BURDEN OF MUSCULOSKELETAL PAIN

Chronic pain is a global leading cause of disability affecting the society, the individual suffering from pain and also their relatives[3]. Around one in five individuals suffer from chronic pain[4,5]. Worldwide, the burden of musculoskeletal (MSK) pain disorders are increasing[6] with MSK pain being the most prevalent disorder with around 1.7 billion individuals that would benefit from rehabilitating treatment[7]. This makes MSK pain the main cause of years lived with disability[8]. On an individual level, this means that a 60-year old woman with chronic MSK pain such as osteoarthritis (OA) or low back pain may have lived more than a quarter of her life with pain and disability[3]. Several different treatment paradigms are now available[9–11] with varying success, and the focus on physical exercise as treatment for pain relief is increasing[12–16]. Therefore, this thesis will focus on physical exercise as treatment for chronic MSK pain.

1.2. PHYSICAL EXERCISE AS TREATMENT

Regular physical exercise or physical activity may prevent more than 30 chronic disorders[17] and is a multimodal treatment option recommended as first choice of rehabilitating treatment for several chronic pain disorders including MSK pain to promote health[12,13] reduce co-morbidities[13,14] and for pain relief[14–16]. Despite these recommendations, a large proportion of chronic pain individuals, irrespective of the pain disorder, do not gain beneficial and/or clinically relevant pain relief after exercise interventions[16], presumably because of the complexity of pain[2,18]. In example, pain relief following exercise interventions may be due to getting attention rather than the exercise

itself[19]. Furthermore, the pain relieving effect of exercise interventions in individuals with chronic MSK pain is independent of radiographic findings and there is discrepancy between pain intensity perception and tissue damage (e.g. on radiographic findings) in chronic MSK pain[18,20,21]. This implies that chronic MSK pain cannot be explained alone by local tissue damage[22–25]. Therefore, research has focused on sensitivity of the nervous system[26–29], which may be enhanced in chronic MSK pain individuals[23,25].

1.3. HYPERSENSITIVITY AND MECHANIMS

Hypersensitivity of the sensory nervous system may be caused by peripheral and/or central sensitisation[25,26,28,30] and acute exercise may decrease (hyper)-sensitivity. This well-established phenomenon is labelled exercise-induced hypoalgesia (EIH)[31–34]. The underlying neural substrates influencing sensitivity in relation to acute exercise has been reviewed extensively[31–33,35–43] and include, but are not limited to, opioids[44–48], nitric oxide[49,50] and endocannabinoids[48,51–53] as well as cardiovascular[47,54,55], noradrenergic[56–59] and serotonergic[60–62] mechanims. For instance, a recent human study[62] found that greater EIH after an isometric exercise, normally known to be painful[63], was associated with weaker serotonergic level in combination with a gene for stronger opioid signalling, which may suggest an antagonistic interaction between serotonergic and opioid EIH mechanisms possibly to avoid analgesia. In this respect, opiodergic, serotonergic and noradrenergic pathways are known to be involved in the descending pain inhibitory control pathways[64,65]. In 1979, Le Bars and colleagues in their pioneering research showed that continuous tonic nociceptive inputs inhibit other nociceptive inputs from convergent wide-dynamic range spinal dorsal horn neurons[66]. This “pain-inhibits-pain” phenomenon was labelled diffuse noxious inhibitory control (DNIC)[66,67]. The term DNIC is still used in animal studies, while the human proxy is known as conditioned pain modulation (CPM) and is assessed using psychophysical testing-modalities[68]. The underlying neuroanatomical structures involved in DNIC include the periaqueductal grey matter (PAG), locus coeruleus (LC) rostro-ventral medulla (RVM) and subnucleus reticularis dorsalis[65,69] (Figure 1). Also, noradrenergic pathways from various cortical areas are crucial for LC

and subnucleus reticularis dorsalis activity[64,65], collectively showing that DNIC, and possibly CPM, do not derive from one specific nucleus, but several brain areas and nuclei interact intrinsically in this descending pain modulatory phenomenon. Furthermore, pain-inhibits-pain phenomenons may also influence pain sensitivity during or after painful exercise in humans[70,71].

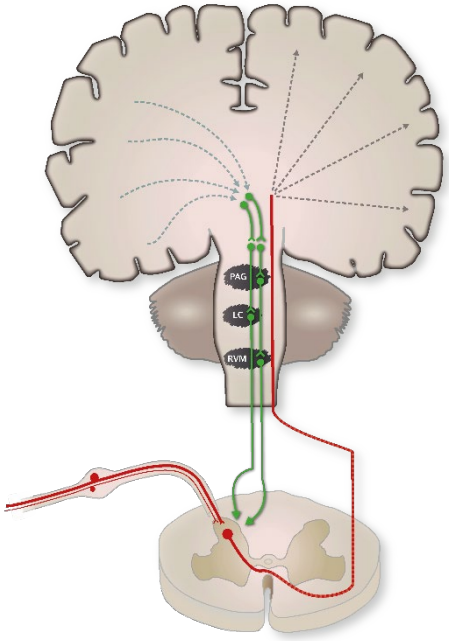

Figure 1. Descending pain modulation.
Descending pain modulation are the net effect of inhibitory (anti-nociceptive) and facilitatory (pro-nociceptive) supraspinal mechanisms acting on the spinal dorsal horns. PAG: periaqueductal grey matter. LC: Locus coeruleus. RVM: rostro-ventral medulla.

The immune system, triggered by exercise, may also influence hypersensitivity. For instance, pre-clinical evidence shows that physically uninjured inactive rodents have a larger pro-inflammatory response compared to active rodents[72]. Also, in humans, the pro-inflammatory cytokine IL6 plasma levels increase consistently during acute exercise[73] and this increase is exponentially related to exercise duration[74]. However, the IL-6 increase is short-lasting (<1 hour after

exercise) and also counteracted by increases in anti-inflammatory cytokines and cortisol during and after acute exercise[74].

Taken together, EIH is the net result a wide array of local, segmental, extra-segmental and supraspinal descending mechanisms. These endogenous mechanisms, and their possible interplay with exercise, may act to decrease pain sensitivity and/or pain perception for an exercising individual.

1.4. SENSORY TESTING AND EXERCISE-INDUCED HYPOALGESIA

Hypersensitivity may be assessed using quantitative sensory testing (QST) modalities to attain information on the central integration of sensory inputs as assessed by proxies of peripheral and central sensitisation in acute and chronic pain[25,26,28,30]. This mechanistic approach may offer translational information having the potential to understand the essential driving mechanisms behind the perception of pain and pain relieving treatments such as exercise interventions[25,75].

Numerous EIH reviews has been published within the last 10 years[31,33,35–37,39–43,76–78]. The EIH response is considered a proxy of the balance between endogenous pain facilitatory and pain inhibitory mechanisms during and after one exercise session[37,39]. In general, EIH responses are highly variable in chronic pain populations, while moderate-large hypoalgesic responses have been consistently demonstrated in healthy individuals[37,39], which may suggest that the presence of pain impairs EIH. However, pain may also inhibits pain in relation to exercise[37,39] suggesting that pain can facilitate EIH. Currently, the literature on the relationship between pain intensity and EIH in healthy individuals[47,55,63,71,79–87] and chronic MSK pain individuals[88–94] show conflicting results; see Appendix A and B for schematic overviews of cross-sectional EIH studies investigating associations between EIH and pain ratings and studies comparing exercise with and without experimental pain in healthy individuals and individuals with chronic MSK pain, respectively.

Pre-clinical studies show that exercise interventions may prevent chronic pain by modulation of central pain inhibitory and pain

facilitatory mechanisms[95,96], and that pain relief after exercise interventions may be linked to modulation of central pain inhibitory and pain facilitatory mechanisms[31,32]. In humans, higher EIH is observed in physically active individuals compared to physically inactive individuals in cross-sectional studies[97,98] indicating EIH may be increased by regular exercise, although other studies report conflicting results in healthy individuals[99–102]. Exercise interventions lasting 4-16 weeks increase pressure pain thresholds (PPTs) in chronic MSK pain disorders locally at the painful regions and to a lesser degree in remote regions[103], while temporal summation of pain (TSP) may be decreased[104] and CPM increased[105]. However, EIH modulation following exercise interventions in healthy individuals and chronic MSK pain was only investigated to a limited degree at the time of planning this PhD project; see Appendix C for a schematic overview of longitudinal human studies investigating EIH modulation by exercise interventions. Collectively, these studies show equivocal results regarding if EIH is modifiable by exercise interventions in healthy individuals[106–108] and individuals with chronic MSK pain[109,110].

In summary, pain may influence EIH. In spite of this, the direct influence of pain (i.e. experimental pain) on EIH has only been investigated to a limited degree. Also, the current sparse amount of literature question if EIH is modifiable by exercise interventions. Therefore, further knowledge on how pain intensity influences EIH, and if EIH may be modified by exercise interventions of longer duration may have important implications for the planning and recommendations regarding exercise interventions for the individual person suffering from pain[34,36] which again may have a positive impact on disability[12] and societal costs[6,7].

CHAPTER 2. AIMS AND OVERVIEWS

Based on the considerations in the introduction, the overarching aims of this PhD project were to explore:

- Relations between EIH and pain intensity (Study I-III), and
- if EIH is modifiable by exercise interventions (Study I and Study III).

It was hypothesised that:

- EIH was related to pain intensity, and
- EIH was modifiable by exercise interventions.

A schematic overview of the study designs and of the thesis and are presented in Figure 2 and Figure 3, respectively.

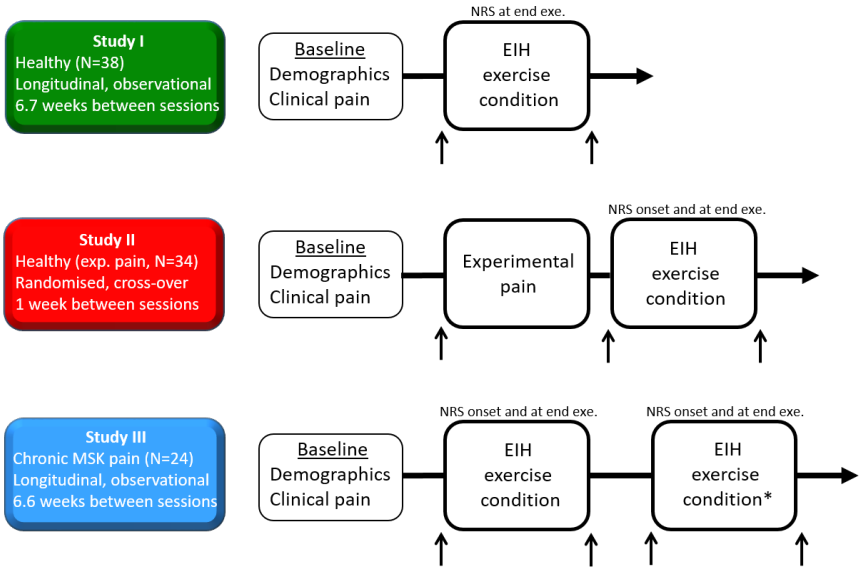

Figure 2. Schematic overview of study designs for study I-III.
*Exp. pain: Experimental pain by hypertonic saline injection. MSK: Musculoskeletal. EIH: Exercise-induced hypoalgesia; NRS: Numeric rating scale, for pain intensity assessment; *denotes exercise condition with unpublished data. Vertical arrows denote pressure pain threshold assessment.*

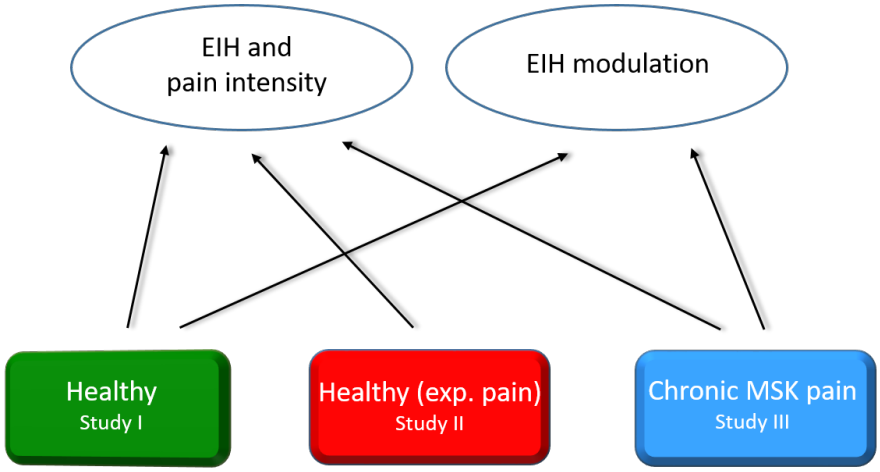

Figure 3. Overview of the current PhD thesis.
This thesis explores relations between pain intensity and EIH (Study I-III) and if EIH is modifiable by exercise interventions (Study I and Study III). Exp. pain: Experimental pain by hypertonic saline injection. MSK: Musculoskeletal.

Individuals with chronic painful knee OA was used in Study III to investigate chronic MSK pain. The three journal articles (Study I-III) are presented at a glance in Appendix D.

The following chapters provide a more in-detail description and discussion of the rationales for the methods (EIH assessment, pain ratings and the experimental pain model) utilised in this PhD project.

CHAPTER 3. EIH ASSESSMENT

EIH is assessed as decreased pain sensitivity during or after an acute exercise condition[37,39] (Figure 4).

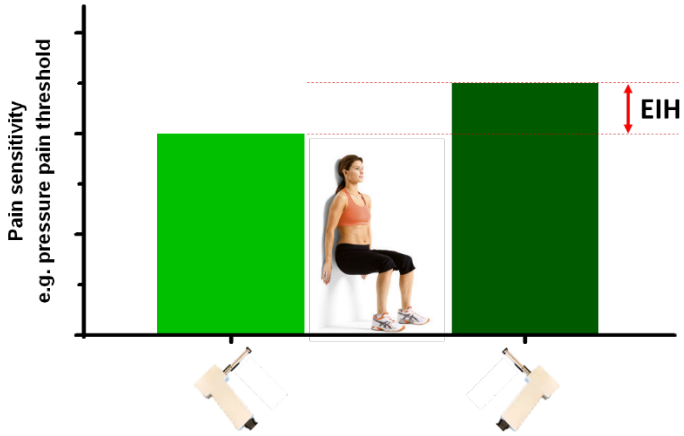

Figure 4. The concept of exercise-induced hypoalgesia assessment.
Exercise-induced hypoalgesia (EIH) is the increase in e.g. pressure pain thresholds after an acute exercise condition assessed with manual pressure algometry or other pain sensitivity modalities.

3.1. PRESSURE PAIN ALGOMETRY

EIH is most often assessed using PPT[33,37,39,76]. Both superficial and deep-tissue nociceptors are activated when assessing PPT[25] probably with inputs mainly from deep-tissue nociceptors (group III and IV)[111]. As physical exercise involves muscle effort, this makes PPT assessments more relevant in relation to EIH and exercise compared to thermal and tactile testing modalities which mainly assess gain or loss of function in skin sensory neurons[26,112].

In this PhD project, PPTs for EIH calculation were assessed using manual pressure algometry (Somedic AB, type II, Sweden; Figure 4) with a standardised pressure rate around 30 kPa/s and a 1-cm^2 probe. Computer-controlled algometry has also been utilised to assess pressure

pain sensitivity and EIH[90,113,114]. However, manual algometry and computer-controlled algometry are comparable for PPT assessment in healthy individuals[115], and manual algometry was the feasible option for Study I and Study III given the experimental settings and apparatus-availability constraints. Hence, manual pressure algometry was a consistent method throughout Study I-III.

In healthy individuals, PPT assessed with manual pressure algometry shows excellent within-session (intra-class correlation coefficients [ICC] ≥ 0.93) and good-excellent 1-week between-session (ICC 0.74 – 0.87) test-retest relative reliability at thigh and shoulder muscles[70,101,116], which were the primary assessment sites in Study I-III. Similarly, in knee OA individuals[117,118] and other MSK pain disorders[90], 1-3-week between-session test-retest reliability is good-excellent with ICCs ranging from 0.77 - 0.91. Further, two (Study I and Study II) and three (Study III) PPT assessments at each assessment site were made and averaged for each assessment time, as this enhances reliability compared to single assessments[119].

3.2. EIH RESPONSES AND EXERCISE CONDITIONS

In general, EIH responses can be categorized into three main categories. EIH may occur in exercising body regions (local EIH), within a few segments away from the primary exercising body regions (extra-segmental EIH) and at remote non-exercising regions (remote EIH)[37,39,76]. Generally, EIH responses are more pronounced at local sites compared to remote sites[37,39,76] which may suggest differential combined effects of local, segmental, extra-segmental and supraspinal descending mechanisms at exercising regions compared to non-exercising regions.

EIH may be assessed during or after different types of exercise. Isometric[70,120–123], resistance[92,124–130], aerobic[83,85,99,116,131–133], anaerobic[81,134,135], balance[136] and stretching[137] exercises have all been utilised in the assessment of EIH responses. Hence, no golden standard for EIH testing is currently available. The exercise conditions utilised to elicit EIH responses are presented and discussed below.

In Study I and Study II, the 3-minute isometric wall squat exercise was chosen as exercise condition, as this exercise condition has been shown to elicit EIH responses both at local exercising and remote non-exercising sites in healthy individuals[70,138]. Further, this exercise condition has shown no 1-week between-session systematic errors, although poor between-session relative reliability (ICCs: 0.03 – 0.43) and non-significant between-session agreement (Cohen's kappa < 0.13, $P > 0.43$) between EIH responders and non-responders has been observed in healthy individuals[70]. Lastly, it requires only simple equipment (a wall and a stopwatch) and is short-lasting, making it clinically feasible. Therefore, this exercise condition was considered optimal at the time of planning these two projects.

In Study III, The 2-minute lateral raise resistance exercise (shoulder condition) was chosen because the intensity is high and it involves remote muscles in relation to the painful knee. This increases the probability of inducing EIH[37], and significant EIH after upper-body resistance exercises in knee OA individuals has been documented[124]. Also, this exercise has been shown to decrease pain perception to standardised pressure pain methods in individuals with chronic MSK pain when performed two minutes each day for 10 weeks[139], indicating global effects on pain sensitivity by this exercise. Lastly, due to the simple nature of the exercise, it was feasible in the clinical setting. The 6-minute walk test (walk condition) was chosen as part of Study III for several reasons: First, it is part of the minimal core set of physical function tests for knee OA individuals endorsed by the Osteoarthritis Research Society International (OARSI)[140]. Second, pain flare during this exercise has been reported positively associated to TSP in knee OA individuals[141] which may indicate a relationship between pain flare during this exercise and other dynamic QST measures, such as EIH, as well. Third, as with the 3-minute isometric wall squat exercise, its simple nature made it feasible in the clinical experimental setting. Note that all pain intensity and EIH findings in relation to this walk condition are unpublished.

In study I, local EIH responses were assessed at the dominant quadriceps muscle, while remote responses were assessed at the contralateral middle deltoideus muscle. In Study II, local EIH responses were assessed at both quadriceps muscles (with experimental pain

injection in the right quadriceps muscle, see Chapter 5) and remote EIH was assessed at the left upper trapezius muscle. In study III, local EIH responses were assessed at the quadriceps muscle on the side with the most painful knee, while remote EIH was assessed at the contralateral middle deltoideus muscle (walk condition). For the shoulder condition, the same assessment sites were used with local responses being from the shoulder site and remote responses being from the thigh site.

In this thesis, all local and remote EIH responses will be presented as absolute change in PPTs immediately after exercise conditions compared to before the exercise condition[37]. Further, Cohen's d effect sizes[142] will be presented for all EIH responses to enhance between-site and between-study comparability.

3.3. PAIN MAY DECREASE EIH

EIH responses are often decreased or absent in chronic MSK pain individuals when compared to healthy individuals[37,39,76] which may indicate, that pain may decrease EIH. This is supported by studies on chronic MSK individuals showing decreased EIH after exercise with painful body regions compared to EIH after exercising non-painful body regions[122,124].

However, the existing literature is conflicting as no local EIH[136,143–146] or even hyperalgesia[126,147,148] also has been demonstrated in healthy individuals. These no-EIH responses may be due to different factors: 1) Time duration after exercise condition (PPT assessment ~1 hour after the exercise condition[136] due to using the entire German Research Network on Neuropathic Pain QST protocol)[28,29] since EIH is often abolished 15-30 minutes after exercise[37,39]; 2) the exercise condition may be of too low intensity to elicit EIH[145] as EIH is intensity-dependent in aerobic exercise[149–151]; 3) 40-minute resistance exercise[144] and eccentric resistance exercise[126,148] as exercise conditions. Further, uncontrolled factors such as disturbed sleep[152], systemic pro-inflammatory up-regulation[153], psychosocial influences[154] are all factors known to increase pain sensitivity and central pain facilitation may counteract EIH. Although speculative, these factors may also explain the general EIH difference between healthy individuals and

chronic pain individuals, as chronic pain individuals more often report decreased sleep quality[155], poorer psychosocial status[156,157] and have increased low-grade systemic inflammation[158] compared to healthy individuals. Additionally, subgroup differences have been reported in chronic MSK pain populations[94,159] with a subgroup of chronic knee OA individuals that presents a pain-inhibiting CPM response also presents with EIH compared to healthy contols[159]. Collectively, this suggests EIH heterogeneity within specific diagnosis of MSK pain disorders and within healthy individuals.

3.4. PAIN MAY INCREASE EIH

Exercise may be painful during or after exercise[160], which may increase EIH compared to non-painful exercise[83]. This is supported by findings showing that isometric exercise, as utilised in Study I and Study II, often is perceived as painful and elicit significant EIH responses[70,71]. Also, a systematic review and meta-analysis conclude that isometric exercise may induce EIH with a large effect size in healthy individuals[37]. Further, several studies report a positive relationship between EIH and CPM in healthy individuals[80,83,86,99,161,162] and individuals with chronic MSK pain[90,159], albeit controversial evidence exists[71,80,81].

CHAPTER 4. PAIN RATINGS

This chapter presents the pain ratings used in the current PhD project.

4.1. NUMERICAL RATING SCALE

Self-reported evaluation of pain intensity is considered a gold standard of pain assessment in experimental and clinical research[163,164]. In Study I-III, pain intensity ratings were assessed in relation to the exercise conditions and also as peak pain intensity within the last 24 hours (Study III) using numerical rating scales (NRS) from 0-10 with 0 indicating “no pain” and 10 indicating “worst imaginable pain”.

NRS is a reliable, valid and well-established unidimensional measure recommended for pain intensity assessment in experimental and clinical settings[163–165]. NRS may be less sensitive than visual analogue scale (VAS) ratings, but generally NRS and VAS are comparable[165] and chronic pain individuals prefer NRS rather than VAS[163,164]. Further, NRS was more applicable in relation to the exercise conditions due to its verbal execution. Additionally, a pain flare index (NRS at exercise condition end minus NRS at exercise condition onset) was calculated as a measure of pain flare during the individual exercise conditions (Study II and Study III) i.e. higher index indicate larger pain flare during exercise as used previosly[141] and in a recent EIH study[94]. Note that all pain flare findings are unpublished. Peak pain intensity within the last 24 hours was used in Study II as an indicator of the average pain intensity[166], as this rating has been used in similar studies in knee OA individuals[167–170].

4.2. KNEE INJURY AND OSTEOARTHRITIS OUTCOME SCORE

The Knee Injury and Osteoarthritis Outcome Score (KOOS) questionnaire was applied in Study I and Study III. KOOS is a composite self-administered questionnaire to individuals with knee injury or knee OA to evaluate temporal aspects of the pain disorder and for treatment evaluation[171]. KOOS contains five subscales: Pain, other

Symptoms (Symptoms), Function in Activities of Daily Living (ADL), Function in sport and recreation (Sport), and knee-related Quality of Life (QoL) with scores ranging from 0 indicating "worst" to 100 indicating "best" for each subscale. The questionnaire is reliable, valid and responsive in knee OA individuals[172–175]. Further, KOOS has been used previously to characterise healthy individuals from individuals with painful knee disorders[176,177]. The questionnaire also contains the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) and is freely available online at http://www.koos.nu making it more applicable and accessible compared to WOMAC.

In Study I, KOOS was used to identify any knee pain and functional impairments, as these factors have been related to a hyperalgesic response after acute exercise in knee pain individuals[114,124,159]. In Study III, all KOOS subscales and the average score of the four subscales for Pain, Symptoms, ADL, and QoL ($KOOS_4$) was applied to characterise the knee OA individuals and as the primary outcome measure. $KOOS_4$ has been applied previously in clinical longitudinal studies on painful knee OA individuals[178,179]. Moreover, a responder in clinical studies on knee OA individuals is specified by OMERACT-OARSI as having 1) more than 50% improvement in pain and function or 2) more than 20% improvement in two of the categories of Physical function, Pain and Global assessment[180], making KOOS ideal for this study.

CHAPTER 5. EXPERIMENTAL PAIN - SALINE INJECTION

The transition from acute to chronic MSK pain is still not well understood[23,25] which highlights the importance of translational research. Experimental pain models provide opportunities to investigate probable underlying mechanisms in the transition from pain-free/acute-chronic pain[25,181]. The models mimic certain aspects of painful conditions to investigate short-lasting muscle pain and sensitivity changes. In this respect, intramuscular injections of hypertonic saline is the most widely used exogenous model to induce pain and mechanical pressure hypersensitivity to imitate MSK pain in otherwise pain-free individuals[182]. It has been shown to produce a local deep muscle pain by activation of group III and IV muscle nociceptors[111] and the pain may refer to more distal body regions especially in chronic pain disorders[25,183]. However, this model and other experimental pain models such as nerve-growth factor injection[184] and delayed-onset muscle soreness (DOMS)[160] are only short-lasting and they can therefore only investigate the development of acute pain[25,181].

Hypertonic saline (5.8%) injection has not previously been utilised to investigate how pain influence EIH. Therefore, this model with injection into the quadriceps muscle 1-2 minutes before exercise, and isotonic saline (0.9%) injection as a control[185–187], was employed in Study II.

CHAPTER 6. EIH AND PAIN INTENSITY

This chapter presents the results from Study I-III in relation to the first aim of this thesis; to explore relations between EIH and pain intensity. In addition, the results will be compared and discussed in relation to previous human EIH studies on healthy individuals (with and without experimental pain) and individuals with chronic MSK pain.

6.1. EIH RESPONSES

Local EIH responses were found before exercise interventions in Study I and Study III (shoulder condition) with small-moderate effect sizes (Figure 5A). Similarly, local EIH responses with small-moderate effect sizes were evident after inducing experimental pain in Study II. In addition, significant remote EIH responses with small-moderate effect sizes were found in Study II (both conditions), while no remote EIH was observed in Study I and Study III before exercise interventions (Figure 5B).

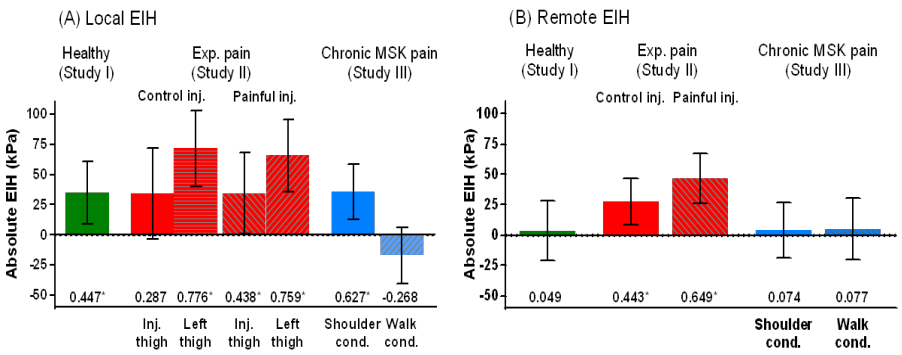

Figure 5. EIH responses at baseline for Study I-III.
*Local (A) and remote (B) EIH responses for Study I-III, as reflected by absolute change in PPT after acute exercise conditions. Shoulder cond.: 2-minute lateral raises; Exp.: Experimental; Inj.: Injection; MSK: Musculoskeletal. Error bars denote 95% confidence intervals. Values denote Cohen's d effect sizes for EIH responses *denotes significant EIH (within-study Bonferroni adjusted P<0.05). Walk condition (6-minute walk test) results are unpublished.*

In addition, no influence of pain on local and remote EIH were observed in Study II, as no between-session differences (control injection vs painful injection) were found (all $P < 0.135$, Cohen's d: 0.00 – 0.30).

At follow-up, significant local and remote EIH responses with moderate effect sizes were found in healthy individuals, while only local EIH (shoulder condition, moderate effect size) was observed in chronic MSK individuals (Figure 6).

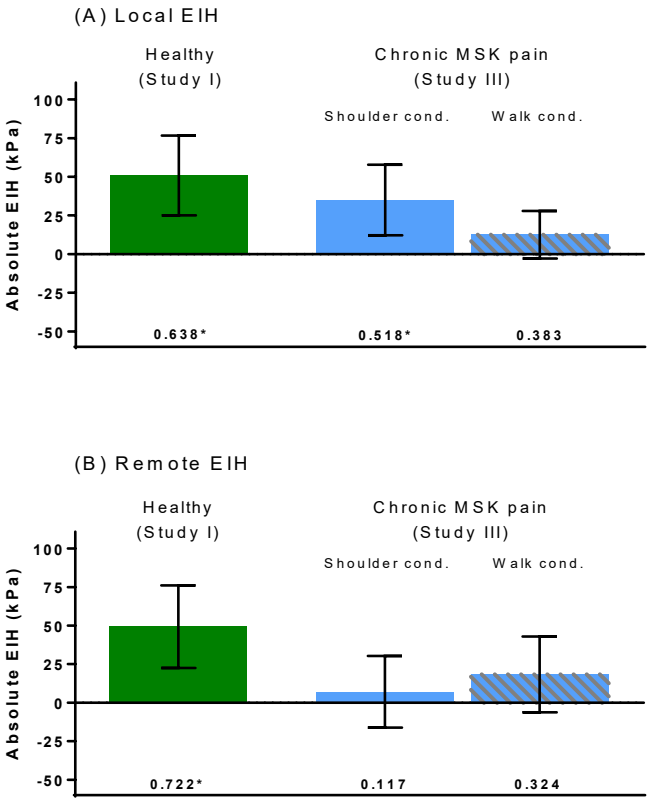

Figure 6. EIH responses following exercise interventions for Study I and Study III. *Local (A) and remote (B) EIH responses for Study I and Study III at follow-up, Shoulder cond.: 2-minute lateral raises; Exp.: Experimental; Inj.: Injection; MSK: Musculoskeletal. Error bars denote 95% confidence intervals. Values denote Cohen's d effect sizes for EIH responses. *denotes significant EIH (within-study Bonferroni adjusted P≤0.016). Walk condition (6-minute walk test) results are unpublished.*

6.2. ASSOCIATIONS BETWEEN PAIN INTENSITY AND EIH

Pain intensity ratings in relation to all exercise conditions and bivariate correlation analyses between pain intensity ratings and EIH responses for Study I-III (unpublished) are summarised in Appendix E and Appendix F, respectively.

Pain intensity was not significantly associated with EIH responses irrespective of pain intensity assessment time-point. This was observed in Study II (painful condition), where pain intensity in the injected leg at *onset* of the exercise condition was not significantly associated with subsequent local and remote EIH responses (all $P > 0.05$). Similarly, in Study III, pain intensity at onset of either exercise condition was not significantly associated with subsequent local and remote EIH responses at baseline (all $P > 0.05$) and follow-up (all $P > 0.05$). Furthermore, no significant associations between pain intensity at *the end* of the exercise condition and EIH responses were found in Study II (painful condition, all $P > 0.05$), or Study I and Study III at baseline (all $P > 0.05$) or follow-up (all $P > 0.05$). No significant associations between the *pain flare* index and local and remote EIH responses were found in Study II (painful condition) or Study III at baseline (all $P > 0.05$) and follow-up (all $P > 0.05$).

6.3. DISCUSSION

EIH responses were small-moderate (effect sizes: 0.287 – 0.776) in Study I and the control condition in Study II (Figure 5) after painful isometric exercise. These numerical values were larger than estimates from a recent systematic review with meta-analysis (including only three controlled studies using isometric exercise conditions), showing no consistent EIH after this type of exercise in healthy individuals[35]. This may suggest differential responses to exercise on EIH in healthy individuals or may be ascribed to methodological differences.

6.3.1. PRE-EXERCISE PAIN AND EIH

The influence of pain intensity at exercise onset has been investigated using other experimental pain models in healthy individuals. DOMS, as induced by eccentric contraction of the target muscle[188], in a remote body region may inhibit EIH after aerobic cycling[130] as EIH normally is induced after this type of exercise[91,116]. Also, when DOMS was present in the exercising quadriceps muscle, isometric exercise did not induce EIH when compared to a no DOMS group[63] conflicting the results from Study II where experimental pain did not decrease EIH. Further, using a CPM paradigm where pain was abolished shortly before exercise onset, decreased EIH compared to a control exercise condition without previous pain was found, indicating previous pain may decrease EIH[80].

In agreement with the findings from Study II and Study III, earlier studies in painful knee OA individuals described no significant associations between pre-exercise pain intensity[124] or other pain ratings (WOMAC)[159] and EIH. However, the study by Burrows and colleagues[124] suggests that pre-exercise (chronic) pain in the exercising body region may decrease EIH compared to healthy individuals. Similarly, no association between baseline pain intensity (peak pain intensity within the last week) and EIH was found in patellar tendinopathy individuals[92]. However, following EIH-inducing exercise, pain intensity may decrease in a subsequent exercise, which may indicate a link between EIH and the subsequent pain response during physical activity[189].

6.3.2. PAIN DURING OR AFTER EXERCISE AND EIH

The Study I null-findings on association between pain intensity and EIH response at the end of exercise, are in agreement with earlier studies in healthy individuals[71,79,81,82,84,85], yet contradicting evidence exists[47,63,87] (appendix A). Furthermore, studies have reported EIH and no change in pain intensity[79,87,129,190,191], decreased pain intensity[48,82,129,190–193] and increased pain intensity[148], while one study has reported hyperalgesia and increased pain intensity in response to acute exercise[126], collectively suggesting discrepant findings regarding the relationship

between EIH and pain intensity during and after exercise in healthy individuals.

In Study II (painful condition) and Study III, pain intensity at the end of acute exercise and EIH responses were not associated, which is in agreement with other studies in chronic MSK pain such as chronic neck-shoulder pain[194], temporomandibular disorder[195] and whiplash-associated disorder[89,93]. Additionally, earlier studies did not find a difference in EIH responses between individuals with fibromyalgia and healthy individuals, yet the fibromyalgia cohorts reported more intense pain after acute exercise[120,121], which support the no-relationship between EIH and pain intensity.

6.3.3. PAIN FLARE AND EIH

No significant linear relationships between pain flare during exercise and EIH responses were found in Study II (painful condition) or Study III. Interestingly, in a recent study, Vaegter and colleagues[94] reported that EIH was induced in chronic low back pain individuals with low pain flare index (NRS pain flare <2) after walking, while hyperalgesia was provoked in individuals with higher pain flare index (NRS pain flare ≥2). Furthermore, EIH and baseline pain intensity were associated with the pain flare[94]. Additionally, chronic MSK pain individuals with high pain sensitivity show lower EIH compared to low pain sensitivity individuals[90], and EIH was similar between low and high kinesiophobia individuals with chronic MSK pain, although high kinesiophobia individuals showed higher baseline pain intensity[91]. This may indicate that EIH can be elicited in individuals with chronic MSK pain who report high pain intensity, which may suggests within-diagnosis heterogeneity i.e. within-diagnosis subgroups.

6.3.4. PAINFUL VS NON-PAINFUL EXERCISES

A limited number of studies have compared EIH between painful and non-painful exercises in healthy individuals[47,55,83]. Greater EIH was found during painful cycling compared to non-painful cycling[83], and greater EIH was induced with painful high-pressure occlusion compared to low- or no-occlusion resistance training[47]. These findings

may indicate that pain induced by the exercise may increase EIH in healthy individuals. These findings contrast with the findings of Study II, where experimental pain did not affect EIH responses compared to the control session (Figure 5). Conversely, an earlier study reported lower EIH responses in the cuff-occluded arm (painful) compared to the non-occluded arm (non-painful) during cycling[55], which may suggest that remote pain during exercise can decrease EIH[55]. Methodological differences or simply that individuals may react differently to painful stimuli in relation to exercise may explain these contrasting findings.

No studies have investigated the influence of pain on EIH responses using exogenous experimental pain models in individuals with MSK pain. Earlier studies have examined EIH responses between painful vs non-painful exercises[122–124] and using different exercise intensities or durations[88,123,196]. For example, Lannersten & Kosek[122] observed EIH in individuals with shoulder myalgia only after a lower extremity exercise away from their clinical pain, while no EIH was elicited during shoulder exercise. When fibromyalgia individuals performed the two exercises, no EIH responses were found[122]. This may indicate that different EIH responses occur comparing individuals with generalised and local pain, and that exercising a pain-free region may be more optimal for EIH than exercising a painful region[122]. Study III supports the latter speculation, and is further supported by findings in knee OA individuals[124]. Additionally, lateral epicondylalgia individuals did not produce a local EIH response after painful forearm exercise[123]. This result corresponds to the walk condition no-EIH results from Study III, but conflicts with the results from Study II, which may indicate that pain duration influence EIH. Similarly, in fibromyalgia individuals, isometric exercise to fatigue, using shorter duration or maximal contractions, did not elicit EIH or changed pain intensity[88]. However, in fibromyalgia individuals, cycling at a self-selected intensity provoked similar EIH compared with a higher submaximal intensity, while pain perception decreased after exercises[196]. This could indicate that self-selected intensity may be preferred and recommended over higher intensities in treatment settings.

CHAPTER 7. EIH MODULATION AND PAIN INTENSITY

This chapter presents the results in relation to this thesis' second aim; to explore if EIH is modifiable by exercise interventions. The results will be compared and discussed in relation to previous literature on EIH modulation and modulation of other pain sensitivity measures and pain ratings. In addition, possible mechanisms influencing EIH modulation will be discussed.

7.1. EIH MODULATION

Seven-week exercise interventions did not modulate local EIH responses, as reflected in no changes in absolute EIH responses from baseline to follow-up, in healthy individuals or chronic MSK pain individuals, while remote EIH was significantly increased in healthy individuals (Figure 7).

An additional exploratory analysis revealed a significant association between absolute change in local EIH and absolute change in pain intensity (i.e. decreased pain intensity indicated by a negative numerical value) following the exercise intervention (Study III shoulder condition, r = -0.407; P = 0.048, unpublished) which may indicate, that increased EIH and pain relief following exercise interventions may be related. All other correlation analyses between change in EIH responses following exercise intervention and change in pain ratings were non-significant (all $P > 0.05$) (unpublished, see Appendix G).

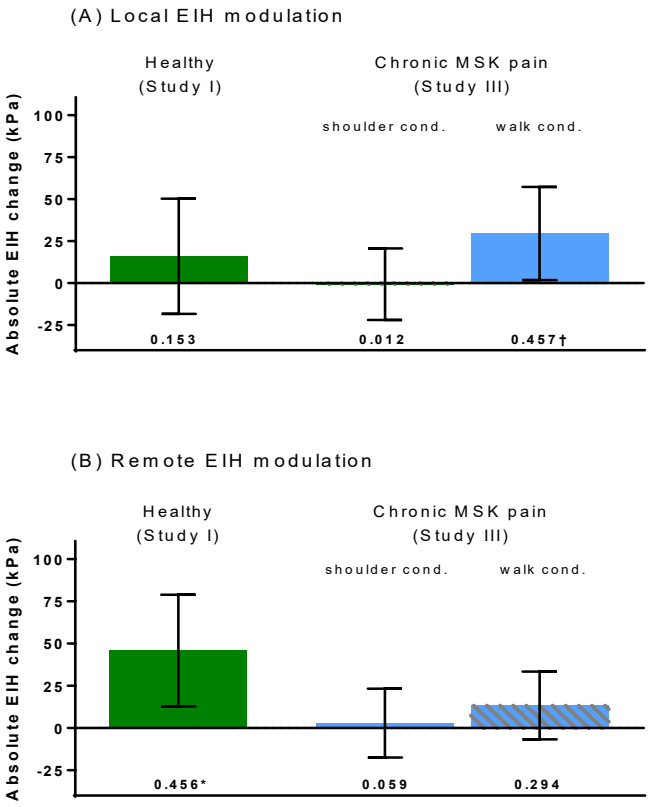

Figure 7. EIH modulation after exercise interventions in Study I and Study III. *Local (A) and remote (B) absolute EIH change after 7-week exercise interventions in Study I and Study III. Shoulder cond.: 2-minute lateral raises; MSK: Musculoskeletal. Error bars denote 95% confidence intervals. Values denote Cohen's d effect sizes for EIH modulation from baseline to follow-up. *denotes significant EIH modulation (within-study Bonferroni adjusted P=0.016). †denotes non-significant EIH modulation (within-study Bonferroni adjusted P=0.152) although error bars are not crossing zero. Walk condition (6-minute walk test) results are unpublished.*

7.2. DISCUSSION

Evidence on EIH modulation using threshold testing is scarce. Exercise interventions lasting at least six weeks in healthy individuals has only been investigated in Study I and one recent study[107] in pain-free overweight men. While the recent study[107] demonstrated that local and remote EIH could not be elicited at baseline using either high intensity

interval cycling or moderate intensity continuous cycling, Study I observed local EIH but not remote EIH at baseline. Furthermore, and in contrast to the recent study[107], the exercise intervention did increase remote EIH responses in Study I (Figure 7). However, EIH modulation, using pressure pain tolerances for EIH assessment, have been reported following 6-week moderate-vigorous aerobic cycling[108] and high-intensity interval cycling in healthy individuals[106], which could indicate that tolerance EIH responses may be more modifiable than EIH threshold responses.

Although a moderate effect size for local EIH modulation after the walk condition was observed in Study III no significant local or remote EIH modulation were found (Figure 7). This is in agreement with a previous study[109] reporting that neither neuromuscular exercise therapy (similar to the exercise therapy used in study III) for three months or total knee replacement in knee OA individuals, did change EIH responses despite an improvement in clinical pain intensity following both treatments[109]. Similar findings have been reported in knee OA individuals with moderate-severe pain intensity before total knee replacement at 6-month follow-up after surgery[114], and in rheumatoid arthritis individuals after two years of health-enhancing physical activity[110]. Hence, the existing evidence suggests, that there may be no significant EIH modulation in chronic MSK individuals regardless of the ability to induce pre-treatment EIH responses or not.

The type of exercise may be of importance for EIH modulation as eccentric exercise may affect PPT responses after acute exercise differently comparing two exercise sesssions[128,147,197,198]. For instance, Lau and colleagues[147] observed hyperalgesia immediately after both sessions, separated by four weeks, of eccentric exercise in healthy individuals, with hyperalgesia present up to three days only after the first session. In addition, the pain intensity ratings were elevated in the first session compared to the second session for up to three days[147]. Also, two sessions of eccentric exercise, separated by seven days, did not elicit local or remote EIH immediately after exercise in both sessions in healthy individuals[128,197,198]. However, PPTs were reduced (hyperalgesia) 24 hours only after the first session, showing a time-dependent modulation on deep-tissue pain sensitivity and pain intensity after this type of exercise[128,197,198]. Further studies are warranted to

investigate if longer exercise interventions using eccentric exercises may modulate EIH.

EIH and EIH modulation may not be the main driver of pain perception. This may be argued as no knee pain was reported in Study I before EIH testing (indicated by KOOS Pain subscale scores) at baseline and follow-up and no difference in pain intensity after acute exercise was found comparing baseline to follow-up albeit remote EIH was improved. This is further supported by the Study I-III findings on no associations between pain intensity and EIH responses. Pre-clinical evidence show that changes in PAG, RVM and spinal mechanisms (e.g. serotonergic, endocannabinoid, opiodergic and NMDA mechanisms) after exercise interventions may decrease (hyper)-sensitivity and be linked to EIH modulation[31,36]. Moreover, also from preclinical evidence, immune system mechanisms such as reduced glial cell activation and a balance-shift in pro- and anti-nociceptive inflammatory cytokines[36,96,199] may explain EIH modulation. Further, decreased low-grade inflammation after exercise interventions may be caused by fat loss, as fat loss is common after exercise interventions in humans[153,200,201] and a large proportion of individuals in the general population and in the military (Study I) are overweight or obese[202]. Also, improved psychosocial aspects negatively related to EIH in healthy individuals[154] may explain the EIH modulation in Study I, although this interaction is still inconclusive in healthy individuals[43].

A ceiling effect for EIH may exist, as significant baseline EIH responses did not increase following exercise interventions. A ceiling effect of psychophysical measures (i.e. PPT and CPM) is in agreement with recent studies showing that non-invasive brain stimulation did not modulate psychophysical measures in healthy individuals[203] and chronic low back pain individuals[204] although non-invasive brain stimulation may provide hypoalgesia in chronic pain individuals[205]. Similarly, recent findings in chronic low back pain individuals showed no modulation of CPM and TSP although clinical pain improved following guideline-recommended primary care treatment including exercise[206]. However, this is in contrast to studies showing modulation of CPM[105] and TSP[104] by exercise interventions in individuals with chronic MSK pain. The latter notion was not supported by Study III-results (not addressed in this thesis) as no TSP modulation was found

after exercise intervention. Furthermore, modulation of other central pain mechanisms (spatial summation of pain and widespread hyperesthesia) have been reported in knee OA individuals becoming pain-free after total knee replacement[207] suggesting that these mechanisms may be modulated by treatment. Lastly, and in agreement with the PPT findings in Study III, a recent systematic review with meta-analysis showed that PPTs are increased after exercise interventions in chronic MSK pain disorders locally at the painful region(s) and to a lesser degree in remote regions[103].

CHAPTER 8. METHODOLOGICAL CONSIDERATIONS AND LIMITATIONS

This chapter address considerations and limitations related to the utilised EIH exercise conditions, the sample size calculations and general considerations in relations to this PhD project.

8.1. EXERCISE CONDITIONS

EIH responses were elicited using a 3-minute isometric wall squat (Study I and Study II), a 2-minute lateral raises resistance exercise and the 6-minute walk test (Study III).

8.1.1. 3-MINUTE ISOMETRIC WALL SQUAT

In Study I, only local EIH was elicited after the wall squat test at baseline. This is in contrast to previous studies showing both local and remote EIH using this exercise condition[70,138] as also found in Study II. This difference may be explained by psychosocial factors such as cognitive stress during military training[202] which again may decrease EIH[154]. Additionally, natural variation or other unknown factors may explain this difference.

8.1.2. 2-MINUTE LATERAL RAISES

No EIH was induced at the knee (remote EIH) by the 2-minute lateral raises resistance exercise in Study IIII. Exercises with non-painful muscles have previously been able to induce EIH at the painful body region in individuals with painful knee OA[124], shoulder myalgia[122], chronic low back pain[208] and chronic neck pain following trauma[138]. The lack of remote EIH after this shoulder condition may be due to the shorter-lasting nature of this exercise condition compared to e.g. the study by Burrows and colleagues[124] who used three sets of three upper-body resistance exercises to induce EIH. Also, non-controlled shoulder pain at exercise onset was reported by five individuals (21%) at baseline (NRS: 2.6 ± 1.1, range: 1 - 4) and follow-up (NRS: 2.8 ± 0.8, range: 2

- 4) (unpublished). This may have affected the EIH responses, and may partly explain the lack of remote EIH after this exercise condition.

8.1.3. 6-MINUTE WALK TEST

The 6-minute walk test did not induce local EIH at the thigh or remote EIH at the shoulder in Study III. Other exercise conditions such as 15-minute cycling at 70% aerobic capacity[114,159] and 5-minute isometric knee extension at 50% maximal voluntary contraction[109,159] have previously been able to induce EIH in knee OA individuals, suggesting that the intensity of the 6-minute walk test may be too low to induce EIH. This is supported by findings showing that EIH after aerobic exercise is positively intensity-dependent[149–151]. Additionally, recent findings have shown no EIH after this exercise condition in healthy individuals[145] and individuals with chronic low back pain[94]. This highlights a possible limitation of this exercise condition for EIH testing using PPTs, while significant EIH may be obtained using pressure tolerance testing[145].

8.2. STATISTICAL CONSIDERATIONS

Small sample sizes are a limitation in neuroscience in general which may question the majority of study conclusions[209]. This limitation is probably also evident in the field of EIH research. The Study I-III sample sizes are therefore relevant to reflect upon.

8.2.1. STUDY I

The pre-determined sample size (36 individuals) for this observational study was based on an approximated moderate EIH modulation effect size of 0.6 after military training, an estimated standard deviation of 0.9, a statistical power of 80% and α=0.05. The actual effect size for the remote EIH modulation was 0.456 indicating that this study was slightly under-powered.

8.2.2. STUDY II

The pre-determined sample size (34 individuals) for this randomised cross-over study was based on an approximated medium effect size of 0.5 on the EIH response at the injected thigh after exercise with hypertonic saline (experimental pain session) compared to exercise with isotonic saline (control session), a statistical power of 80% and α=0.05.

The actual between-session EIH-difference effect size of 0.00 on the main outcome in this study shows, that the estimated effect size was clearly overestimated. However, a significant between-session EIH difference could not have been achieved by increasing the sample size.

8.2.3. STUDY III

The sample size calculation for this observational study was based on a statistical power of 80%, α=0.05 and a 20% improvement in $KOOS_4$ after exercise intervention, as this is a responder criteria in clinical studies on knee OA individuals stated by OMERACT-OARSI[180].

The aims of Study III were to predict pain-related outcome (change in $KOOS_4$) using baseline EIH and several other QST and clinical variables before exercise intervention, and to investigate EIH modulation following the exercise intervention. Hence, it could be argues that the sample size calculation was not optimal. However, as this was an observational proof-of-concept study, which for the first time utilised EIH as an outcome predictor following exercise therapy in a clinical setting, the inclusion of 24 individuals with painful knee OA was deemed acceptable at the time of planning this study. The sample size calculation for a larger-scale study of this kind should be based on a multiple regression analysis with 11 relevant predictors (the actual variables included in Study III) or more e.g. psychosocial factors[43] and age[210] as these variables may also influence EIH. This gives an estimated samples size of 110-150 individual also accounting for dropouts.

8.3. GENERAL CONSIDERATIONS

Several studies report EIH responses solely as the change in pain sensitivity in relation to acute exercise. This method may, however, be prone to methodological biases e.g. learning effects, participants expectations etc.[35,133]. In example, the first systematic review with meta-analysis found average moderate-large significant EIH effect sizes in healthy individuals after different exercise types[37]. However, most of the included studies in this meta-analysis[37] are observational studies without a control group or a resting control condition which reduces the likelihood of detecting a true effect[211]. In agreement with this, the most recent systematic review with meta-analysis, including only controlled EIH studies, conclude that EIH effect sizes range from no effect (isometric exercise) to large (aerobic exercise) in healthy individuals[35]. Therefore, a resting control condition of equal length as the exercise condition is recommended in EIH studies[35], albeit EIH is generally larger than the PPT change due to repeated testing in itself[70,101,116,212]. A resting control condition was not part of Study I and Study III due to time constraints at the experimental settings. In Study II, a control condition using non-painful isotonic saline was included in this more rigorous cross-over design. Not including a resting control condition is a limitation to all three studies.

Methodological artefacts related to the control injection itself may have occurred in Study II. This was observed as significant negative associations between EIH (local and remote) and pain intensity at the end of exercise in both the injected and non-injected leg (all $r_s \leq -0.523$, all $P \leq 0.031$). Furthermore, negative associations between the pain flare index and EIH (local and remote) in the non-injected leg (all $r_s \leq -0.502$, all $P \leq 0.024$) were found (Appendix F). These artefacts should be properly controlled in future studies.

Not including a non-exercising matched control group is also a limitation to Study I and Study III. This decreases the results' generalisability, and the results should be replicated in more controlled studies.

CHAPTER 9. CONCLUSIONS

This thesis explored two specific aims: 1) Relations between EIH and pain intensity and 2) if EIH may be modifiable by exercise interventions.

The main findings are summarised in Figure 8.

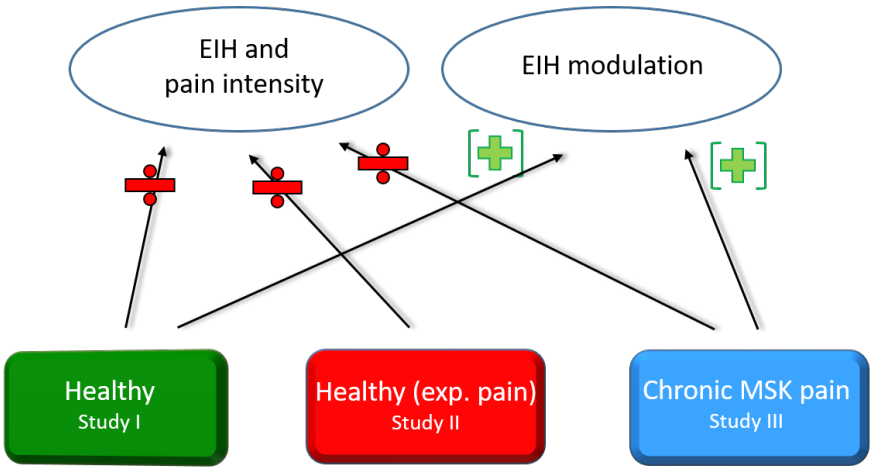

Figure 8. Overview of main findings from Study I-III.
No significant associations between exercise-induced hypoalgesia (EIH) and pain intensity were found in Study I-III, and pain did not influence EIH (Study II). Only remote EIH was modulated in healthy individuals (Study I), while no EIH modulation occurred in individuals with chronic musculoskeletal (MSK) pain (Study III). An association between EIH modulation and pain relief following exercise treatment was observed in Study III. Red minus denotes no significant findings. Green plus in brackets denotes some statistical significant findings.

No linear associations between pain intensity in relation to acute exercise and EIH responses in all investigated cohorts were found (Study I-III), and experimental pain did not influence EIH (Study II). In healthy individuals (Study I), remote EIH was modulated after the 7-week exercise intervention, whereas this effect was absent in chronic MSK pain individuals (Study III). An association between increased local EIH and pain relief after the 7-week exercise intervention was described in Study III.

Overall, all three included studies in this thesis have presented novel designs and findings within the scientific area of EIH. Utilizing longitudinal and experimentally controlled approaches, this thesis has attempted to add new knowledge into the translational interplay between EIH, the perception of pain and exercise interventions. The findings suggest that the perception of pain intensity is unrelated to EIH, while the linear relationship between EIH modulation and pain relief following exercise treatment warrants further research in individuals with chronic MSK pain.

CHAPTER 10. CLINICAL IMPLICATIONS AND FUTURE DIRECTIONS

10.1. CLINICAL IMPLICATIONS

Looking beyond methodological considerations and limitations, the presented results may have clinical implications in the guidance of individuals with chronic MSK pain using exercise interventions.

As no associations between EIH and pain intensity were found, this knowledge may be used by the clinician to inform the exercising individual, that any change in palpable pressure sensitivity after acute exercise may be unrelated to the perception of pain intensity before or during exercise.

Pain relief following exercise treatment may be due to modulation of endogenous pain inhibitory mechanisms[32,36,213] as also indicated by Study III-findings. Hence, this information may be used by the clinician to inform the individual with chronic MSK pain about possible mechanisms of pain relief following exercise interventions.

Lastly, low baseline EIH was related to poorer outcome following exercise intervention (Study III). However, the predictive value of QST[214,215] including EIH[216] is limited, and it is currently unclear if EIH could be advantageous in the shared-decision-making between the clinician and the paining individual regarding exercise selection for pain treatment.

10.2. FUTURE DIRECTIONS

No clear definition of EIH exist, as EIH is referred to as change in pain sensitivity, but also clinical pain, during and after acute exercise[39]. Further, EIH has been used as the change in clinical pain after exercise interventions in pain populations[15], collectively mixing pain sensitivity, clinical pain and measurement time frame. Additionally, EIH responses may be reported in absolute, relative or normalised values[37] complicating between-study comparisons if standardised effect sizes

(e.g. Cohen's d or z-scores) are not reported regardless of responses being statistically significant or not. Based on this, a consensus statement on EIH terminology and standardisation, similar to CPM recommendations[68], is warranted.

Most EIH studies have not used a resting control condition as mentioned previously, or the studies have used a fixed-order design with the rest condition always being performed before the exercise condition. Randomising order of conditions, as done in only a small number of studies[55,129,217], and Study III, and including a proper control group (e.g. healthy individuals compared to symptomatic individuals or a non-exercising control group) is warranted to further strengthen the evidence level of future EIH studies. As an example, and in direct continuation of Study III, a larger-scale controlled study (with or without a non-treated control group) would be relevant to investigate if EIH assessment may be relevant in clinical examinations as treatment outcome predictor or as treatment target. In support of such a study, a previous study[218] reported that pre-treatment CPM efficiency was associated with pain outcome following treatment with duloxetine (a serotonin and noradrenalin re-uptake inhibitor[219]) in neuropathic pain individuals in such a way that individuals with lower pre-treatment CPM gained better pain outcome following treatment[218]. However, if pre-treatment EIH may be used as treatment target and outcome predictor in larger scaled studies using exercise interventions or pharmacological treatments remains to be investigated.

A normative EIH database is non-existent and warranted. Such a database on healthy individuals and clinically relevant cohorts may also include optimal threshold limits for subgrouping individuals into two or more categories of EIH responders, no-responders and hyperalgesic-responders as examples. QST measures have previously been demonstrated superior over traditional diagnosis criteria to identify treatment responders and non-responders in individuals with e.g. painful chronic OA[114,167,169,170,220–223]. However, applied methods[70,101,212] to classify EIH responders and non-responders are rather arbitrary as no consensus exits on a clinical meaningful EIH. Future research should work towards a common understanding of a threshold between EIH responder and non-responders. Along that line, due to the complexity of the individual person's pain perception[2] and

the large EIH variability[37,39], it is not likely that EIH-testing will emerge as a single clinical diagnostic or predictive test, but it may be more promising in a more comprehensive pain phenotyping assessment for each individual person. This is supported by a study showing that a multimodal QST-score may be positively associated to pain intensity in chronic MSK pain[224]. Furthermore, multimodal QST-testing may be more reliable compared to single modality-testing[225].

For EIH to emerge as a relevant clinical tool, its reliability in well-controlled studies must be good-excellent. Despite the large amount of EIH studies, only few studies have examined the test-retest EIH reliability in healthy individuals[70,101,116,145,212]: Across these studies, between-subject reliability (e.g. ICC) was poor-moderate, while within-subject reliability (e.g. Cohen's kappa) was absent-moderate. Additionally, no studies have examined EIH reliability in MSK pain populations. Therefore, more EIH reliability studies are needed to find the most reliable EIH assessment methods and to establish if EIH may have a future role in the clinical management of healthy individuals and individuals with MSK pain.

EIH has primarily been assessed using exercise conditions of short duration (seconds-minutes), with only a limited number of studies having investigated exercise conditions of longer duration[136], using eccentric resistance exercise[128,147,197,198] or several resistance exercises[124,129,191]. The latter resembles common exercise modalities for individuals with MSK pain. From a clinical perspective, more studies assessing EIH after exercise conditions resembling an exercise session from clinically recommended treatment programs for MSK pain e.g. neuromuscular exercises for knee OA individuals[109,178,226] are needed to guide the individual person in relation to the expected immediate pain sensitivity response to exercise. Also, a 5-week eccentric exercise intervention in female neck pain individuals modulated CPM compared to controls[105] and eccentric exercise treatment may be beneficial for pain relief and improved function in tendinopathies[227–229]. If eccentric exercise interventions of at least five weeks in healthy individuals or MSK pain individuals may modulate EIH remains to be investigated.

Verbal information may influence EIH positively or negatively in healthy individuals[230,231] and communication about pain may induce

long-lasting hypoalgesia[232]. Similarly, conditioning visual information about painful movement may affect pain in relation to movement in healthy subjects[233]. However, the influence of verbal or visual information on EIH has not been investigated in individuals with MSK pain. Such new knowledge is highly relevant as beliefs and expectations are of utmost importance in the treatment of MSK pain disorders[234].

Lastly, the underlying neurophysiological mechanisms eliciting EIH responses are not fully understood as described in the introduction. While DNIC has been thoroughly investigated using electrophysiological methods in pioneering pre-clinical studies more than four decades ago[66,67], similar studies investigating EIH mechanisms during and after painful and pain-free exercise have not been conducted. Additionally, duloxetine treatment may increase an impaired CPM in painful rats[235], and ketamine (a NMDA-receptor inhibitor[236]) has been shown to decrease TSP in humans[237,238]. However, the effect of duloxetine and ketamine on EIH has not been investigated; such studies may be conducted using a controlled cross-over design as utilised in Study II to gain further insight into EIH mechanisms.

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APPENDICES

Appendix A. Cross-sectional studies investigating relationships between EIH and pain ratings in healthy individuals

Cross-sectional studies on healthy individuals investigating relationships between EIH and pain ratings using correlation analyses or comparing EIH responses in relation to exercise conditions with and without experimental pain

This non-systematic overview is organised by publication year, exercise condition (aerobic, anaerobic, isometric, resistance) population with control condition, pain sensitivity and pain ratings, and main findings.

STUDY	EXERCISE CONDITION Intensity (I) Duration (D)	POPULATION Total N (females) Age (years): mean	CONTROL CONDITION	PAIN SENSITIVITY AND PAIN RATINGS	MAIN FINDINGS
Umeda et al., 2010[82]	Isometric, hand dynamometer I: 25 % MVC D: 1: 1 min 2: 3 min 3: 5 min	N=50 (25) Age: 18-40 (range)	Within-subject (different durations as control)	PPT: forefinger dominant hand Pain ratings: verbal descriptors	↑ PPT and ↓ pain ratings after all three exercises **No correlation between pain ratings and EIH**
Ellingson et al., 2014[83]	Aerobic, cycling I: 1: 60 watt, painful cuffs on thighs 2: 60 watt, no cuffs D: 10 min	N=21 (21) Age: 30.6	Within-subject rest	Heat pain ratings (0-20): palm of right hand Pain ratings: MPQ, VAS	↓ Heat pain ratings during both exercises **Greater EIH after painful exercise compared to non-painful exercise.** **VAS during painful exercise negatively correlated to EIH for pain intensity and EIH for pain unpleasantness i.e. the higher pain during exercise, the greater EIH**
Bement et al., 2014[84]	Isometric, elbow flexors I: 25 % MVC D: To fatigue	N=26 (13) Age: 20.3	Within-subject rest	PPT: right index finger. Pressure pain ratings: NRS	↑ PPT after exercise **No correlation between pain ratings and EIH**
Stolzman et al., 2015[85]	Aerobic, treadmill I: VO_{2max} test D: To exhaustion	Normal weight N=33 (16) Age: 15.5 Overweight/obese N=29 (17) Age: 14.6	Within- and between-subject, rest as within control	Pressure pain perception: left fourth finger, left deltoid, right quadriceps Pain ratings: MPQ	↑ Pressure pain perception at all sites after exercise. No group difference. **EIH not correlated with clinical pain**

STUDY	EXERCISE CONDITION Intensity (I) Duration (D)	POPULATION Total N (females) Age (years): mean	CONTROL CONDITION	PAIN SENSITIVITY AND PAIN RATINGS	MAIN FINDINGS
Stolzman et al., 2016[86]	Aerobic, treadmill walking I: VO_{2max} test D: until fatigue	N=55 (29) Age: 15.2	-	PPTs at left nail bed, left deltoid and right quadriceps CPM Pain ratings: NRS	↑ PPT at deltoid and quadriceps after exercise PPT unchanged at nail bed after exercise CPM and EIH correlated **Peak pain intensity during exercise did not influence EIH**
Black et al., 2016[63]	Isometric, right knee extensors I: 25 % MVC D: To task failure	N=25 (0) Age: 22.8	Within-subject (without DOMS compared to with DOMS)	PPT: both quadriceps Blood pressure Pain ratings: VAS	↑ PPT during exercise and up to 15 min after exercise in exercising leg, but no change after exercise in resting leg, similar between conditions **Significant positive correlation between EIH and muscle pain intensity during exercise in both legs**
Lemley et al., 2016[87]	Resistance,, concentric isotonic, elbow flexors or knee extensors I: MVCC D: 3 x 30 reps, reps every 3 sec; plus 1 MVIC after each set; to "fatigue" (4.5 min)	Young N=34 (19) Age: 21.7 Older N=30 (12) Age: 71.3	Within-subject (between-sessions)	Pain thresholds (time): left index finger Pain ratings: NRS	↑ pain threshold in young and old women after elbow exercise, but not after knee exercise **Baseline pain rating negatively associated with EIH in elbow session, but not knee extensor session**
Jones et al., 2017[55]	Aerobic, cycling (recumbent, arms relaxed) with upper limp blood occlusion I: RPE ≥ 17 D: 5 min	N=36 (18) Age: 22.6	Within-subject rest (with blood occlusion)	PPT: right rectus femoris, both first dorsal interosseous Pain ratings (NRS)	↑ PPTs at all sites after exercise No effect of rest **EIH non-occluded arm > EIH occluded arm, suggesting pain med decrease EIH** (and peripheral factors contribute to EIH)
Foxen-Craft et al., 2017[79]	Isometric, dominant handgrip (N=68) I: 25% MVC D: 2 min	N=134 (82) Age: 21.98	Between-subject sham exercise (holding dynamometer without contraction, N=66)	Cold pressor pain tolerance. Pain ratings: VAS	↑ Pain tolerance after exercise compared to sham **Changes in pain tolerance not correlated with changes in pain intensity**

STUDY	EXERCISE CONDTION Intensity (I) Duration (D)	POPULATION Total N (females) Age (years): mean	CONTROL CONDITION	PAIN SENSITIVITY AND PAIN RATINGS	MAIN FINDINGS
Gajsar et al., 2018[80]	Aerobic, cycling with preceding cold pressor task I: 75% ATHR D: 15 min	N=31 (15) Age: 27.7	Within-subject rest, and cycling with preceding rest	PPT: non-dominant hand, biceps femoris, non-dominant side L3. CPM	↑ PPTs at all sites after exercise and rest ↓ PPT at hand when CPM before exercise No EIH at L3 and biceps when CPM before exercise **↓ EIH after the CPM condition in comparison to control condition**
Samuelly-Leichtag et al., 2018[81]	Anaerobic, cycling (Wingate Test) I: "All-out" D: 30 sec (plus 5 min warm-up)	N=30 (15) Age: 25.1	Between-subject control group (5 min warm-up + 30 sec rest) N=20 (10) Age: 23.6	PPT: dominant quadriceps and abductor pollicis CPM and TSP heat Pain ratings (NPS, 0-100)	↑ PPTs quadriceps after exercise compared to controls ↑ PPTs at quadriceps at hand in Wingate group, but not in controls ↓ CPM after Wingate, but not in control **No correlations pain intensity - EIH**
Alsouhibani et al., 2019[71]	Isometric, right knee extension I: 30% MVC D: 3 min	N=30 (15) Age: 19.3	Within-subject rest	PPT: quadriceps and upper trapezius CPM Pain ratings (NRS)	↑ PPTs at quadriceps after exercise No change in trapezius PPT after exercise No changes in PPT at both sites after rest Decreased CPM in systemic EIH responders compared to systemic EIH non-responders **No correlations between pain during/after exercise and EIH**
Hughes & Patterson 2020[47]	Resistance, unilateral leg press with dominant leg, 1. Low load resistance 2. High load resistance I: 70% 1RM D: 4 x 10 reps 3. Low pressure blood flow restriction (BFR40) 4. High pressure blood flow restriction (BFR80) I: (1,3,4): 30% 1RM D: 4 set (30, 15, 15, 15 reps)	N=12 (2) Age: 29	-	PPT: dominant and non-dominant quadriceps, dominant biceps brachii, non-dominant upper trapezius muscle Pain ratings (Borg)	↑ PPTs at all sites after all exercises; greatest increase after BFR80 in the exercising limb, less increase systemically comparable after exercise 1-3. Increase lasted 24h at dominant quadriceps in both restriction exercises. **EIH mediated by muscle discomfort (~58%) and β-endorphins (~42%)**

Appendix B. Cross-sectional studies investigating relationships between EIH and pain ratings in individuals with chronic musculoskeletal pain

Summary of cross-sectional studies on individuals with muskuloskeletal pain disorders investigating relationships between EIH and pain ratings using correlation analyses

This non-systematic overview is organised by publication year, population and exercise condition (aerobic, isometric, resistance) with control condition, pain sensitivity and pain ratings, and main findings.

STUDY	POPULATION Total N (females) Age (years): mean	EXERCISE CONDITION Intensity (I) Duration (D)	CONTROL CONDITION	PAIN SENSITIVITY AND PAIN RATINGS	MAIN FINDINGS
Bement et al., 2011[88]	Fibromyalgia N=15 (15) Age: 52	Isometric, elbow flexors 1: 25 % MVC, to exhaustion (max 5 min) 2: 25 % MVC, 2 min 3: 100 % MVC, 3 reps	Within-subject rest	PPT: right index finger. Pain ratings: VAS	Fibromyalgia: No change in PPT or pain ratings after any of the exercises Subgroup analysis: ↑PPT after exercise in younger patients and subjects with high pain sensitivity (lower baseline PPT). **EIH predicted decrease in clinical pain**, so that largest experimental pain relief also had the decrease in clinical pain intensity
van Oosterwijck et al., 2012[89]	Chronic whiplash associated disorder (WAD) N=22 (22) Age: 38.4	Aerobic, cycling I: 1: 75 % age-predicted heart rate (APHR) 2: Self-paced D: N/A	Healthy controls N=22 (22) Age: 37.1 (14.6)	PPT: hand, lower back and calf muscle Pain ratings: SF-36 / VAS	WAD: ↓ PPTs at back and calf after 75% APHR condition ↓ PPTs at calf and hand after self-paced exercise ↑ PPTs at back after self-paced exercise ↑ pain rating (VAS) after submaximal exercise, but not self-paced exercise Controls: ↑ PPTs after both exercises ↓ pain rating (VAS) after submaximal exercise **No significant associations between increased pain (pain flare) and hyperalgesia after exercise**

STUDY	POPULATION Total N (females) Age (years): mean	EXERCISE CONDITION Intensity (I) Duration (D)	CONTROL CONDITION	PAIN SENSITIVITY AND PAIN RATINGS	MAIN FINDINGS
Vaegter et al., 2016[90]	Chronic musculoskeletal (MSK) pain, Low pain sensitivity (LPS) N=30 (21) Age: 47.3 High pain sensitivity (HPS) N=31 (21) Age: 43.5	Aerobic, cycling I: 75% ATHR D: 15 min Isometric, dominant knee extensors I: 30% MVC D: 90 sec	Within-subject rest	Handheld PPT: both quadriceps, dominant biceps brachii and non-dominant trapezius Cuff PPT, PTT and TSP: calf Pain ratings: VAS at cuff tolerance level Clinical pain: peak NRS last 24h	↑ PPTs widespread after all exercises in both groups. Lower EIH in HPS group compared to LPS group **Clinical pain did not predict EIH**
Vaegter et al., 2018[91]	Chronic MSK pain N=54 (39) Age: 45.7 Low (N=23) and high (N=31) kinesiophobia	Aerobic, cycling I: 75% ATHR D: 15 min Isometric, dominant knee extensors I: 30 MVC D: 90 sec	-	Handheld PPT: both quadriceps, dominant biceps brachii and non-dominant trapezius. Pain ratings: NRS	↑ PPTs after both exercises in total group Kinesiophobia correlated with pain intensity No correlations EIH and kinesiophobia No EIH difference comparing high vs low kinesiophobia, but higher NRS in high kinesiophobia group
Straszek et al., 2019[92]	Patellofemoral pain N=29 (29) Age: 23	Resistance, painful leg 1: hip abduction 2: knee extension I: 12 RM D: 3 x 12 reps	-	Handheld PPT: patella and tibialis anterior at most painful leg, contralateral elbow Cuff PDT, PTT, TSP and CPM Pain ratings: NRS	No change in PPT at patella and elbow after both exercises ↑ PPT at tibialis anterior equally after both exercises ↑ cPDT equally after both exercises ↑ cPTT more after knee vs hip exercise **No association between baseline clinical pain and EIH after both exercises**

STUDY	POPULATION Total N (females) Age (years): mean	EXERCISE CONDITION Intensity (I) Duration (D)	CONTROL CONDITION	PAIN SENSITIVITY AND PAIN RATINGS	MAIN FINDINGS
Smith et al., 2020[93]	Chronic WAD N=40 (28) Age: 37.3	Aerobic, treadmill walking I: 75% ATHR D: 30 min Isometric, knee extension I: 20-25% MVC D: max 3 min (or until exhaustion)	Asymptomatic controls N=30 (23) Age: 40.4	PPT: right hand, left tibialis ant, right C5-6 CPM Pain ratings: VAS	↑ PPTs at hand during exercises in WAD No change at spine and tibialis ant. during exercises in WAD ↑ PPTs at all sites during and after both exercises in controls. Unchanged PPTs after exercise in WAD. **Baseline VAS did not predict EIH**
Vaegter et al., 2021[94]	Chronic low back pain Total N=96 (36) Age: 47.0	Aerobic, walking I: self-paced D: 6-min	-	PPT: back and calf Pain ratings: NRS	No change in PPT at both sites after exercise in total group ↑ PPT after walking in subjects with pain flare < 2 ↓ PPT after walking in subjects with pain flare ≥ 2 **Baseline EIH, NRS and pain thresholds associated with likelihood of pain flare during exercise**

Appendix C. Longitudinal EIH studies in healthy individuals and individuals with chronic musculoskeletal pain

Longitudinal physical exercise interventions with EIH testing before and after treatment must have been conducted for studies to be included in the non-systematic overview.

This non-systematic overview is organised by publication year and duration of exercise intervention with EIH condition, control condition, pain sensitivity and pain measures, and main findings

STUDY	TREATMENT	POPULATION N (females) Age (years): mean	EIH CONDITION Intensity (I) Duration (D)	CONTROL CONDITION	PAIN SENSITIVITY AND PAIN RATINGS	MAIN FINDINGS
Kosek et al., 2013[109]	Neuromuscular exercises, 3 month, 1-hour sessions twice a week Total joint replacement	Knee osteoarthritis (OA) N=66 (39) Age: 68.0 Hip OA N=47 (26) Age: 67.1 Healthy controls N=43 (23) Age: 68.9	Isometric contraction, knee extensors I: 50 % MVC D: Until exhaustion (max 5 min)	-	PPT: quadriceps, contralateral deltoid Pain ratings (VAS)	Knee OA: ↑ PPT during exercise Hip OA: ↑ PPT during exercise Healthy controls: ↑ PPT during exercise **No change in EIH following ET or surgery in knee OA and hip OA**
Jones et al., 2014[108]	Cycling, 6 weeks, 3 sessions per week at 75% heart rate reserve	Healthy Exercise group N=12 (11) Age: 24 Control group N=12 (10) Age: 22	Isometric handgrip, dominant hand I: 30%MVC D: 4 sec contraction, 4 sec rest as long as tolerable (max 10 min)	Between-subject	Ischemic pressure pain tolerance Pain ratings (NRS)	**↑ Pressure pain tolerance during EIH condition after treatment (EIH modulation) in exercise group but not in control.** No relationship between the change in duration of ischemic contractions and change in VO_2max for either group

STUDY	TREATMENT	POPULATION N (females) Age (years): mean	EIH CONDITION Intensity (I) Duration (D)	CONTROL CONDITION	PAIN SENSITIVITY AND PAIN RATINGS	MAIN FINDINGS
O'Leary et al., 2017[106]	Cycling, 6 weeks, 3 sessions a week. High intensity interval training (HIIT): 6-8 bouts of 5-min cycling Continuous training (CONT): similar energy expenditure as HIIT	Healthy HIIT group N=10 (2) Age: 27 CONT group N=10 (2) Age27	Isometric handgrip, right hand I: 30%MVC D: 4 sec contraction, 4 sec rest as long as tolerable (max 10 min)	Between-subject	Ischemic pressure pain tolerance Pain ratings (NRS)	**EIH modulation (↑ pressure pain tolerance during EIH condition) after treatment in HIIT but not CONT**
Hakansson et al., 2018[107]	Cycling, 6 weeks, 3 sessions per week with EIH conditions	Pain-free (over-weight) high intensity intervals N=16 (0) Age: 30 Moderate intensity continuous N=12 (0) Age: 26	Aerobic, cycling High intensity intervals I: 90–100% $Watt_{peak}$ (RPE ~15) D: 10 x 1-min intervals, total 24 min. Moderate intensity continuous I: 65– 75% HR_{peak} (RPE ~11) D: 30 min	Between-subject	PPT: right rectus femoris, tibialis anterior and trapezius, at first, middle and last session No pain ratings	No consistent hypoalgesic effect was observed after acute exercise; the effects varied widely, ranging from moderate hypoalgesia to moderate hyperalgesia. **No EIH modulation after 6-weeks intervention**
Löfgren et al., 2018[110]	Moderate-intensity physical activity at least 150 min per week and strength training twice weekly, 2 years	Rheumatoid arthritis N=46 (43) Age: 61	Isometric, right knee extensors I: 30% MVC D: until fatigue (max 5 min)	Healthy controls N=20 (16) Age: 60 (6)	PPT: right quadriceps, left deltoideus Supra-threshold pressure pain Pain ratings (VAS)	↑ PPTs at both sites after exercise; no between-group difference. **No EIH modulation after two years intervention**

STUDY	TREATMENT	POPULATION N (females) Age (years): mean	EIH CONDITION Intensity (I) Duration (D)	CONTROL CONDITION	PAIN SENSITIVITY AND PAIN RATINGS	MAIN FINDINGS
Hansen et al., 2020 (Study III)	Neuromuscular exercises, 6.6 weeks, 1-hour sessions twice a week	Knee OA N=24 (16) Age: 64.3	Resistance, shoulder abductions I: ~30 reps D: 2 min (to "exhaustion")	-	PPT: quadriceps on side of painful knee, tibialis anterior, contralateral deltoideus Pain ratings (KOOS, NRS, PDQ)	↑ PPTs at deltoideus, but not quadriceps, after exercise **No modulation of EIH following exercise therapy** Baseline EIH and PDQ predicted pain relief following exercise therapy
Hansen et al., 2020 (Study I)	Basic military training (strength, endurance, and agility/mobility), 6.7 weeks, Weekly: one to three sessions (45–60-minute each) plus 10–15 micro-sessions (10–15 minutes each)	Healthy N=38 Age: 20.5	Isometric, 3-minute wall squat	-	PPT: dominant quadriceps, contralateral deltoideus muscle Pain ratings (NRS)	↑ PPT at quadriceps, but not at deltoideus, at baseline ↑ PPT both sites at follow-up **↑ EIH at deltoideus following military training** Baseline hyperalgesic subgroup (26% of subjects) increased EIH following military training

Appendix D. The articles at a glance

The three journal articles of which the current thesis is based are presented at a glance in the following table.

	Study I	Study II	Study III
Main objectives	To explore if military training may modulate mechanistic pain-profiling measures including EIH. To explore if EIH subgroups may exist.	To compare EIH in the thigh muscle with experimental pain versus no experimental pain. To explore if pre-exercise experimental pain intensity was associated with EIH.	To explore associations between mechanistic pain-profiling measures including EIH before exercise therapy and self-reported pain relief after exercise therapy. To explore if EIH may be modulated by exercise therapy.
Design	Observational, longitudinal (6.7 weeks)	Randomised experimental crossover	Observational, longitudinal (6.6 weeks)
Intervention	Basic military training	Hypertonic/isotonic saline injection (right quadriceps muscle)	Standardised neuromuscular exercise therapy
Sample	38 healthy individuals Age: 20.5 (18-24)	34 healthy individuals Age: 25.5 (20-46)	24 painful knee OA individuals Age: 64.3 (51-78)
PPT measures before/after EIH condition	Dominant quadriceps muscle (local) Contralateral deltoideus muscle (remote)	Right quadriceps muscle (local, injected) Left quadriceps muscle (local, non-injected) Left upper trapezius muscle (remote)	Quadriceps muscle painful leg (local). Contralateral deltoideus muscle (remote)
EIH condition	3-minute isometric wall squat	3-minute isometric wall squat	2-minute shoulder lateral raises resistance exercise
Pain intensity measures	NRS at end of EIH condition	NRS before, at end and after EIH condition	Maximal NRS within last 24 hours
Main findings	Remote EIH improved after military training suggesting exercise may improve endogenous pain inhibitory mechanisms. Baseline hyperalgesic subgroup (26% of subjects) increased EIH following military training.	No difference between EIH responses between sessions, suggesting that acute pain itself in the exercising muscle does not reduce EIH. Pre-exercise pain intensity unrelated to EIH.	Pre-treatment EIH (and PainDETECT questionnaire) results associated with relative change in $KOOS_4$ after exercise therapy, indicating EIH as a novel predictor for treatment response after exercise therapy. EIH unchanged after exercise therapy.

Appendix E. Pain intensity ratings

Pain intensity ratings from exercising muscles in relation to EIH exercise conditions in Study I-III. Pain flare index calculated as pain intensity (numeric rating scale, NRS) difference from onset-end of the specific exercise condition as indicator of pain flare during the exercise condition. Values denote mean ± SD (95% confidence intervals) [range]. All Study III and pain flare index results are unpublished.

	Baseline mean ± SD (95% CI) [range]			**Follow-up** mean ± SD (95% CI) [range]		
NRS related to exercise cond.	**Onset**	**End**	**Pain flare index**	**Onset**	**End**	**Pain flare index**
Study I (healthy)						
thigh	N/A	6.5 ± 1.9 (5.8 – 7.1) [2 – 9]	N/A	N/A	6.1 ± 1.6 (5.6 – 6.6) [3 – 9]	N/A
Study II (experimental pain)						
Painful inj.						
Injected thigh	4.6 ± 2.1 (3.8 – 5.3) [1 – 9]	6.7 ± 2.5 (5.8 – 7.6) [0 – 10]	2.0 ± 2.3 (1.2 – 2.9) [-2 – 6]	N/A	N/A	N/A
Non-injected thigh	0.0 ± 0.2 (0.0 – 0.1) [0 – 1]	6.3 ± 2.6 (5.4 – 7.3) [0 – 10]	6.2 ± 2.5 (5.2 – 7.1) [0 - 10]			
Control inj.						
Injected thigh	0.3 ± 0.4 (0.1 – 0.4) [0 – 1]	6.5 ± 2.4 (5.6 – 7.4) [0 – 10]	6.1 ± 2.4 (5.2 – 7.0) [0 - 10]	N/A	N/A	N/A
Non-injected thigh	0.1 ± 0.3 (0.0 – 0.2) [0 – 1]	6.6 ± 2.4 (5.7 – 7.5) [0 – 10]	6.4 ± 2.4 (5.5 – 7.3) [0 - 10]			
Study III (chronic pain)						
Shoulder condition	0.4 ± 1.2 (0.0 - 1.0) [0 - 4]	2.2 ± 2.5 (1.1 – 3.2) [0 - 7]	1.6 ± 2.5 (0.6 – 2.7) [0 - 6]	0.6 ±1.2 (0.1 – 1.1) [0 -4]	1.5 ± 2.1 (0.6 – 2.4) [0 - 6]	0.9 ± 1.6 (0.3 – 1.6) [0 - 6]
Walk condition	1.5 ± 1.6 (0.8 – 2.2) [0 – 5]	3.8 ± 2.7 (2.7 – 5.0) [0 - 9]	2.2 ± 2.1 (1.4 – 3.2) [-1 – 6]	1.1 ± 1.2 (0.6 – 1.6) [0 - 3]	1.9 ± 1.6 (1.2 – 2.5) [0 - 5]	0.9 ± 1.6 (0.2 – 1.6) [-2 – 4]

Appendix F. Correlation analyses between pain intensity ratings in relation to exercise conditions and EIH responses

Bivariate correlation analyses between pain intensity ratings in relation to EIH exercise conditions and subsequent EIH responses for Study I-III.

All analyses are made using non-parametric methods (Spearman's r_s) to enhance between-study comparability. All findings in this appendix are unpublished results. NRS: Numeric rating scale; Pain flare index: NRS end of exercise condition minus NRS onset of exercise condition. Bold values denote significant findings ($P < 0.05$).

Study I (healthy)		**Baseline**		**Follow-up**	
NRS in relation to EIH condition (time) and EIH site	**Correlation**	**Local EIH**	**Remote EIH**	**Local EIH**	**Remote EIH**
Onset	R_s	N/A	N/A	N/A	N/A
	P				
End	R_s	0.134	-0.099	-0.032	0.079
	P	0.423	0.553	0.848	0.639
Pain flare index	R_s	N/A	N/A	N/A	N/A
	P				

Study II (exp. pain)		**EIH site**		
NRS in relation to EIH condition (time) and thigh	**Correlation**	**Local, injected thigh**	**Local, non-injected thigh**	**Remote**
Painful condition				
Onset – injected	R_s	0.040	-0.330	-0.150
	P	0.821	0.057	0.398
Onset – non-injected	R_s	-0.257	-0.275	0.204
	P	0.142	0.115	0.247
End – injected	R_s	0.084	0.200	0.046
	P	0.653	0.282	0.804
End – non-injected	R_s	0.220	0.262	0.042
	P	0.235	0.155	0.824
Pain flare index - injected	R_s	0.039	0.332	0.223
	P	0.332	0.068	0.228
Pain flare index – non-injected	R_s	0.225	0.272	0.032
	P	0.224	0.139	0.862
Control condition				
Onset – injected	R_s	-0.146	-0.241	-0.221
	P	0.410	0.169	0.209
Onset – non-injected	R_s	-0.090	0.048	-0.153
	P	0.613	0.789	0.387
End – injected	R_s	-0.373	-0.523	-0.411
	P	**0.035**	**0.002**	**0.019**
End – non-injected	R_s	-0.383	-0.509	-0.438
	P	**0.031**	**0.003**	**0.012**
Pain flare index - injected	R_s	-0.335	-0.468	-0.361
	P	0.061	**0.007**	**0.043**
Pain flare index – non-injected	R_s	-0.340	-0.502	-0.399
	P	0.057	**0.003**	**0.024**

Study III (chronic pain)		**Baseline**		**Follow-up**	
NRS in relation to EIH condition (time) and EIH site	**Correlation**	**Local EIH**	**Remote EIH**	**Local EIH**	**Remote EIH**
Shoulder condition					
Onset	R_s	0.088	0.165	-0.222	-0.287
	P	0.682	0.442	0.302	0.174
End	R_s	-0.211	0.112	-0.282	-0.327
	P	0.323	0.603	0.183	0.119
Pain flare index	R_s	-0.137	0.056	-0.331	-0.290
	P	0.522	0.796	0.114	0.170
Walk condition					
Onset	R_s	-0.214	-0.175	0.115	0.075
	P	0.316	0.414	0.593	0.728
End	R_s	0.080	-0.160	-0.314	0.124
	P	0.710	0.456	0.135	0.563
Pain flare index	R_s	0.312	-0.077	-0.279	0.203
	P	0.138	0.720	0.187	0.342

Appendix G. Correlation analyses between change in EIH and change in pain variables following treatment

Bivariate correlation analyses (Pearson's *r*, as all variables are normal-distributed) between absolute change in EIH following treatment and absolute change in clinical pain variables following treatment for Study III (i.e. improvement in NRS is a negative value, while KOOS improvement is positive values). All findings in this appendix are unpublished.

NRS: Numeric rating scale; KOOS: Knee Injury and Osteoarthritis Outcome Score; $KOOS_{pain}$: KOOS Pain subscale score; $KOOS_4$: average score of the KOOS subscale scores for Pain, Symptoms, Activities of Daily living and Quality of life. Bold value denotes significant finding ($P < 0.05$).

EIH site absolute change	**Pain variable following exercise treatment, absolute change**	***R***	***P***
Shoulder condition			
Local EIH	NRS	-0.407	**0.048**
	$KOOS_{pain}$	0.381	0.066
	$KOOS_4$	0.330	0.115
Remote EIH	NRS	-0.273	0.197
	$KOOS_{pain}$	-0.133	0.534
	$KOOS_4$	-0.403	0.051
Walk condition			
Local EIH	NRS	0.092	0.670
	$KOOS_{pain}$	0.031	0.887
	$KOOS_4$	0.078	0.717
Remote EIH	NRS	0.382	0.066
	$KOOS_{pain}$	0.102	0.637
	$KOOS_4$	0.148	0.491

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