NERVOUS SYSTEM SENSITISATION IN MUSCULOSKELETAL PAIN SYNDROMES

by

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Abstract

There is evidence that nervous system sensitisation plays a role in the chronicity of persistent musculoskeletal pain syndromes. The component mechanisms of sensitisation involved, however, and the extent to which they contribute to overall pain states, are not well understood. It is expected that a better understanding of the role these mechanisms play in chronic musculoskeletal pain would inform more effective intervention strategies. This thesis aimed to contribute to this understanding by investigating nervous system sensitisation processes involved in chronic musculoskeletal pain syndromes in general, and also specifically in two common persistent tendon pain problems - midportion Achilles tendinopathy and subacromial pain syndrome.

The first study in this thesis is a randomised controlled trial that investigates whether Gunn Intramuscular Stimulation (IMS), a form of intramuscular dry needling (IMDN) which aims to reduce nervous system sensitisation, adds therapeutic benefit beyond the use of rehabilitative exercise for midportion Achilles tendinopathy. The second study is an observational study that uses quantitative sensory testing (QST) and pain mapping to investigate nervous system sensitisation processes in subacromial pain syndrome. The third study is a systematic review and meta-analysis that investigates whether impaired condition pain modulation, a type of nervous system sensitisation, is present in chronic musculoskeletal pain syndromes.

The general conclusions were: (1) that no additional therapeutic benefit was gained by the addition of IMDN to rehabilitative exercise for midportion Achilles tendinopathy, (2) that there

is nervous system sensitisation to pressure pain present locally, segmentally and remotely/extrasegmentally in SAPS, as well as spreading pain, i.e., pain that is experienced outside the receptive fields of nociceptors innervating the affected tissue, and (3) that conditioned pain modulation is impaired in chronic musculoskeletal pain syndromes, but that only a low level of confidence can be placed in this conclusion as the quality of the body of research in this area is only moderate and there is evidence of publication bias.

Lay Summary

The goal of this research was to better understand chronic pain. Clinicians and scientists think that some people develop chronic pain, at least in part, because their nervous system becomes "sensitised" and exaggerates painful sensations. This research found that: (1) deep dry needling (acupuncture), a treatment that aims to treat sensitised nervous systems and reduce pain, does not help chronic Achilles tendon pain get better any faster than exercise does, (2) people with painful shoulder tendons have nervous systems that are sensitised to pressure pain in their shoulders, but also in other parts of their bodies as well, and (3) people with different chronic pain problems in many different parts of the body may have nervous systems that are sensitised to pain in a particular way, called "impaired conditioned pain modulation". It is hoped that this new knowledge about nervous system sensitisation in chronic pain will help create better treatments.

Preface

A version of chapter 2 has been published. Solomons L, Lee JJY, Bruce M, White LD, Scott A. Intramuscular stimulation vs sham needling for the treatment of chronic midportion Achilles tendinopathy: A randomized controlled clinical trial. PLoS One [Internet]. 2020 Sep 1 [cited 2022 Jun 8];15(9). I conceptualised the project with Alexander Scott. I devised the methodology, conducted history-taking and physical examination screening, and provided the interventions and follow-up sessions. I recruited participants with assistance from Paul Drexler, Evan Finnamore and Alex Scott. I performed the ultrasound scans and collected all outcome data, except for the ankle range measures which were taken by Margaret Bruce and Alexander Scott. Input on ultrasound scan analysis was contributed by Jenny J. Y. Lee and Alex Scott. I administered the project with assistance from Paul Drexler, Katrina Kwan, Heather Denton, Louisa Purcell, Lynita D. White and Alex Scott. I wrote the report with Alex Scott. Contributions to reviewing and editing of the report were made by Jenny J. Y. Lee and Lynita D. White. The statistical analysis was performed by Julian Ho. Ethics approval for the research was obtained from the UBC Clinical Research Ethics Board (H12-02008).

Chapter 3 is a report on work conducted at John L. K. Kramer's lab at ICORD (the Blusson Spinal Cord Centre at Vancouver General Hospital). I conceptualised the project, recruited participants and conducted history-taking and physical examination screening. I devised the methodology and administered the project. I conducted the investigation with Nicole Bailey, Hannah Goodings, Jessica McDougall and Cassandra Choles. I wrote the COVID protocol required to resume limited research when it was suspended completely for six months due to the

pandemic and communicated this protocol to participants. The statistical analysis with performed by Biljana J. Stojkova. Ethics approval for the research was obtained from the UBC Clinical Research Ethics Board (H18-01559).

Chapter 4 is a report on a systematic review and meta-analysis (SRMA). I conceptualised the project and devised the protocol (search strategy, selection criteria, data extraction criteria, risk of bias assessment strategy, meta-analysis strategy); screened the search results; extracted the data; and performed the risk of bias assessment. Charlotte Beck assisted in the development of the search strategy. Alexander Scott adjudicated any unresolved inconsistencies in search results screening and performed the meta-analysis. Kipling Squier duplicated the screening of the search results, duplicated the risk of bias assessment and verified the data extraction. John L. K. Kramer provided expertise on CPM. Karim Khan provided statistical expertise re metanalysis.

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List of Abbreviations

5-HT	serotonin
AMSMC	Allan McGavin Sports Medicine Centre
AS	Alex Scott
CI	confidence interval
CPM	conditioned pain modulation
DNIC	diffuse noxious inhibitory control
DFNS	Research Network on Neuropathic Pain
FTT	full thickness tear
HC	healthy controls
HP40	heat pain 40
ID	identification
IMDN	intramuscular dry needling
IMS	Intramuscular Stimulation
IPAQ	International Physical Activity Questionnaire
IQR	interquartile range
KS	Kipling Squier
LS	Lyndal Solomons
NA	noradrenaline
NPRS	numerical pain rating score
PAG	periaqueductal gray
PP40	pressure pain 40

PTT	partial thickness tear
QST	quantitative sensory testing
RVM	rostral ventromedial medulla
SAPS	subacromial pain syndrome
SP	Substance P
SRMA	systematic review and meta-analysis
VAS	visual analogue scale
VISA-A	Victorian Institute of Sports Assessment–Achilles

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Dedication

To Dr. C. Chan Gunn

Chapter 1: Introduction

1.1 Theme

The main theme of this thesis is nervous system sensitisation in chronic musculoskeletal pain syndromes. Accompanying this theme is the problem of management of a subset of chronic musculoskeletal pain syndromes thought to result, at least in part, from nervous system sensitisation - chronic tendon pain syndromes. Together, these concepts drive the three investigations that comprise this thesis: (1) the effect of intramuscular dry needling (IMDN), a commonly used but understudied conservative intervention for tendon pain, (2) the characterisation of nervous system sensitivity in chronic rotator cuff tendon pain; and (3) an evidence synthesis of conditioned pain modulation (CPM), a type of nervous system sensitisation, in chronic musculoskeletal pain syndromes at large.

Nervous system sensitisation is a process by which it is thought musculoskeletal pain conditions may become persistent or "chronic".(1–3) The term "nervous system sensitisation" is an umbrella term for many mechanisms by which the experience of pain is modified away from what is considered to be "normal" and becomes amplified.(4) It encompasses the concepts of both peripheral and central nervous system sensitisation.(4) Peripheral sensitisation is specific to the sensitisation of nociceptors in the peripheral nervous system, whereas central sensitisation encompasses functional changes in both neurons and circuits in nociceptive pathways throughout the central nervous system.(4) These changes include temporal, spatial and threshold alterations in pain sensibility resulting from increased membrane excitability, increased synaptic efficacy and reduced inhibition.(4) Nociception certainly plays a role in nervous system sensitisation as it

may stimulate both peripheral and central sensitisation mechanisms,(2) such that all three processes are interconnected and are considered to occur to varying degrees in painful musculoskeletal conditions.(5) Importantly, it is recognised that more effective pain interventions will result from a more precise understanding of which of these processes contribute to the pain experience in any particular pain syndrome.(4) This is a key concept motivating the research contained in this thesis.

Nervous system sensitisation is considered to be involved in the development of chronicity in musculoskeletal pain conditions at large, as mentioned above, including (with specific relevance to this thesis) chronic tendon pain states.(6–9) Tendon pain is a common musculoskeletal pain condition that often becomes chronic.(8) Current understanding of the mechanisms of pain in persistent tendon pain remains relatively limited, however, nervous system sensitisation is thought to be involved.(10) Recovery is often prolonged for tendon problems and therapeutic management is challenging.(8) This is the case for the two tendon problems investigated in detail in this thesis - midportion Achilles tendinopathy and rotator cuff tendinopathy,(8) and provides motivation for the two related studies.

The cornerstone of conservative management for both midportion Achilles tendinopathy and rotator cuff tendinopathy is rehabilitative exercise.(11–13) Even with appropriate rehabilitative efforts, however, recovery remains prolonged and is not always successful in both conditions.(14,15) The first study in this thesis looks at the effects on treatment outcome of the addition of IMDN, a commonly used treatment modality in physiotherapy, to a rehabilitative exercise program for midportion Achilles tendinopathy. Of particular relevance to the theme of

this thesis, other than the general difficulty of managing chronic tendon problems and the desire to find more effective interventions, is that the particular style of IMDN investigated in this study uses a treatment paradigm that aims to reduce nervous system sensitisation.(16) In support of this paradigm, there is a growing body of evidence that IMDN can reduce nervous system sensitivity, demonstrated by increases in reduced pressure pain thresholds (a sign of nervous system sensitisation), as discussed in detail in Section 4.2.4.

The second study in this thesis also relates to tendon pain, specifically the role that nervous system sensitisation plays in its pathophysiology. It is an observational study looking at nervous system function in painful rotator cuff tendinopathy. It uses quantitative sensory testing (QST) and pain mapping to investigate for the presence of nervous system sensitisation in cutaneous and deep tissues that relate to the rotator cuff by local nerve supply, segmental nerve supply and extrasegmental/remote nerve supply. The study was designed to investigate for patterns of nervous system sensitisation that incorporated current knowledge of the wide range of ways in which nervous system sensitisation might develop and present. As such, the design incorporated static tests, dynamic tests, and tests for different nociceptive modalities (thermal and mechanical); at local, segmental, and extrasegmental/remote sites. The goal was to create a testing framework that would allow for discernment of any patterns of nervous system sensitivity that might be characteristic of rotator cuff tendinopathy. As discussed earlier, in the overall theme for this thesis, the motivation for this goal was to provide a more precise understanding of the pain mechanisms in rotator cuff tendinopathy to inform more effective interventions.

The third study in this thesis, a systematic review and meta-analysis, relates to the theme of this thesis by investigating nervous system sensitisation in chronic musculoskeletal pain syndromes more broadly, but in this case a specific type of nervous system sensitisation - CPM (the "pain inhibits pain" phenomenon). Again, the goal was to better understand the pain mechanisms involved and thereby inform more effective interventions.

1.2 Achilles tendinopathy

1.2.1 Pathology

Healthy tendon tissue consists of bundles of collagen fibrils, consisting of predominantly Type I collagen, linked together into larger longitudinal structures called fibres, residing within a proteoglycan-rich extracellular matrix, maintained by specialised resident fibroblastic cells called tenocytes.(17) The fibres are organised into larger structures again, called fascicles, that are enclosed within a synovial structural envelope called the endotendon.(17) The endotendon is continuous with a fine connective sheath, called the epitendon, which in turn surrounds the whole tendon.(18) This whole structure is enclosed by a loose, fatty, areolar connective tissue called the paratendon.(18)

The predominant form of tendon injury observed in mid-portion Achilles tendinopathy is tendinosis,(19) presenting as pain, thickening and impaired function in the body of the tendon.(20,21) The term "tendinosis" refers to what is understood to be chronic degenerative change, characterised by cellular level damage that includes disorganisation of collagen fibres within the tendon core and also disorganisation of the endotendon, epitendon and paratendon

tissue.(19,22) Increased presence of Type III collagen, blood vessels, sensory nerves, tenocyte nuclei, leukocytes, vascular cells, macrophages, glycosaminoglycans, ground substance and inflammatory mediators (such as Substance P (SP) and prostaglandin E2) are also characteristic of tendinosis.(19,22) Areas of degenerative and/or reactive change are frequently observed (as evidenced by cell death and fibroblast reaction), as are areas of metaplasia (as evidenced by bony, cartilaginous, or adipocyte transformation).(22)

1.2.2 Tendon pain

The clinical presentation of persistent "overuse" tendon pain was historically labelled "tendinitis", as it was thought that the pain and "swelling" resulted from a classic cell-mediated inflammatory response to injury,(21) similar to the large-scale infiltration of inflammatory cells involved in inflammatory conditions such as rheumatoid arthritis.(23) A widely accepted suggestion was made to abandon the term "tendinitis",(24) however, when histopathological studies of painful tendinopathic tissue failed to demonstrate the presence of inflammatory cells in affected tendon tissue.(25,26) Other more recent studies, however, using more advanced laboratory techniques, *have* demonstrated the presence of inflammatory cells in tendinopathic tissue, including macrophages, mast cells, T-cells and leucocytes.(23) Although such findings have suggested that inflammation does play a role in the pathophysiology of tendinopathy,(23,27,28) it is not clear to what degree such inflammatory processes may play a role in pain-provocation in tendinopathy.(23) The use of corticosteroid injections and non-steroidal anti-inflammatories in tendinopathy suggests generally positive effects on pain in the short term, however, such anti-inflammatory treatments are not effective in all cases of tendinopathy or at all anatomic locations.(29,30) It also is not clear as to whether the positive

effects of these substances are due to their anti-inflammatory actions, or to other independent actions,(27) in the case of corticosteroids – indirect vasoconstriction or direct analgesia,(27) and in the case of NSAIDs – direct analgesia.(31) With this in mind, other sources of pain-provocation other than inflammation, that might be targeted for treatment in painful tendinopathy must be considered. In considering the potential causes of tendon pain, it is helpful to conceptualise that the experience of pain may result from four main processes – nociception, inflammation, nervous system injury (neuropathic pain) or nervous system "dysfunction".(1) Each of these pain-generating processes is discussed below.

Nociception is the simplest pain pathway, whereby primary sensory receptors, functionally specialised to detect high-threshold mechanical, thermal or chemical stimuli (that are potentially tissue-damaging), i.e., nociceptors, excite Aδ or C/Type III or IV nerve fibres, which carry electrical signals to the central nervous system that elicit the perception of pain.(32) This is a relatively simple system, with a clear tissue-protective function.

Inflammation is a process that occurs following tissue injury, and is seen to be an essential part of healing.(33) An array of local non-neuronal and neuronal cellular responses occur as part of the inflammatory process,(34) that includes the local release of a wide array of biochemicals.(1,35) Dramatic changes in the responsiveness of the nervous system take place, as one consequence of these events,(1) producing allodynia ("pain due to a stimulus that does not normally provoke pain")(36) and hyperalgesia ("increased pain from a stimulus that normally provokes pain")(36), such that previously innocuous stimuli produce pain and such that pain from noxious stimuli is amplified in intensity and duration.(37,38) Inflammatory pain, similarly

to nociception, has also been interpreted as serving a protective function – eliciting behavioural protection of healing tissue.(2) Inflammatory pain typically diminishes and resolves as healing progresses and completes,(1) but can become pathological – persisting well beyond typical healing timeframes (typically in the scenario of a failed healing response).(33) A single inflammatory episode can also cause prolonged nociceptor hyperexcitability, that persists beyond the resolution of the initial inflammatory episode, and is evident with exposure to subsequent episodes of local inflammation - a form of peripheral sensitisation,(39) as discussed below.

Neuropathic pain results from injury to the nervous system itself, to either its peripheral or central components.(40) In the peripheral nervous system, injury may cause both pain hypersensitivity to stimuli (hyperalgesia or allodynia) or spontaneous pain (pain that occurs without a stimulus).(40) Spontaneous pain may result from axon or cell body hyperexcitability foci and ectopic discharges, sympathetically-maintained pain (pain due to the development of sensitivity to circulating catecholamines and noradrenaline or sympathetic axon sprouting into the dorsal root ganglion), or disinhibition effects in the dorsal horn of the spinal cord.(40) It is thought that neuropathic pain may play a role in common pain syndromes such as myofascial and low back pain,(40) as well as in some forms of tendinopathy, such as rotator cuff tendinopathy (specifically, suprascapular neuropathy).(41)

Nervous system dysfunction as a source of pain, or “dysfunctional pain”, refers to those pain states that exist despite the lack of any evidence of noxious stimulus, inflammation or nervous system injury, such as fibromyalgia, irritable bowel syndrome and paroxysmal pain disorder.(40)

Inflammatory, neuropathic and dysfunctional pain states are all susceptible to both peripheral and central sensitisation processes, which occur due to the extreme plasticity of the nervous system.(1,2) Peripheral sensitisation, defined as “increased responsiveness and reduced threshold of nociceptive neurons in the periphery to the stimulation of their receptive fields”,(36) can occur in the presence or absence of inflammatory mediators, but is best understood when such mediators are present.(2) Inflammatory mediators enhance the excitability of nociceptors by their direct action on peripheral nerve terminal receptors, but also by altering nociceptor gene expression (which also acts to amplify local neurogenic inflammation).(2) Central sensitisation, defined as “increased responsiveness of nociceptive neurons in the central nervous system to their normal or subthreshold afferent input”),(36) is a use-dependent phenomenon.(1) The process incorporates activity-dependent sensitisation of post-synaptic receptors, disinhibition of inhibitory interneurons and activation of microglial cells in the central nervous system.(2) It may manifest as pain hypersensitivity, aftersensations, or enhanced temporal summation due to the resultant increases in synaptic efficacy and reductions in inhibition.(42) Central sensitisation has been demonstrated to occur in a wide range of pain states, including many musculoskeletal conditions such as myofascial pain, osteoarthritis and temporomandibular joint pain,(42) and also tendinopathy.(8)

As painful tendinopathy is accompanied by local structural change (in tendinosis: characteristic changes in tenocytes, extracellular matrix, collagen, and vasculature, and in tears: more extensive macroscopic disruption of tendon tissue)(22) it seems reasonable, however, that the initial instigation of pain perception would be signalled locally via nociceptors, whether as an instantaneous first line signal of impending or actual tissue damage, or later in the presence of an

inflammatory response to tissue damage. SP (a nociceptive stimulator), specifically, has been implicated as its concentrations have found to be increased in the early stages of tendon pain (by 12 weeks),(43) and SP-containing nerves have been found in tendinopathic tissues, as discussed below.(44) It has also been speculated that nociceptors may be activated in tendinopathy by other non-inflammatory neuromodulatory effects resulting from local tissue changes, such as altered tenocyte function, altered tissue pH, and neovascularisation.(45) That local nociceptors are the primary key to the pain of tendinopathy, regardless of how they are activated, however, seems to be accepted by most commentators.

Studies of the innervation of healthy rat Achilles tendons have shown that most nerve endings present in tendons terminate in the connective tissue sheaths that surround the primary, secondary and tertiary tendon fibre bundles (the endotendon), and the tendon itself (the epitenon and paratenon).(46) Nerve endings have also been shown to penetrate the fibre bundles, but very minimally.(46) The nerve endings that signal nociception in particular (i.e., nociceptors) in tendons, as in other deep somatic tissues, take the form of free nerve endings and are served by Group III and Group IV nerve fibres, (corresponding to cutaneous Aδ & C fibres respectively).(47) Notably, however, not all Group III and IV fibres convey nociception - they may alternatively convey non-nociceptive thermal or mechanical signals.(47) Electron microscopy of the Achilles tendon of the cat has revealed the presence of free nerve endings served by Group III and IV fibres.(48) Free nerve endings served by Group III fibres were found in venous and lymphatic vessels, in the connective tissues around blood vessels and small nerve fibre bundles, and also contacting collagen fibre bundles. Free nerve endings served by Group IV fibres were found in the connective tissue of blood vessels. Importantly, even at the level of

electron microscopy, free nerve ending morphology is not able to signify its function, i.e., whether it is noci-, mechano- or thermoreceptive,(47) and so it is not clear to what degree these tendon nerve endings may or may not serve nociception.

In disease states, the innervation pattern of tendons is altered. Following Achilles tendon rupture in rats, it has been shown that a progressive healing response ensues.(49) As part of this response, new nerves grow into the healing tissue, then retreat again on successful healing - a process that occurs similarly in other types of healing tissues.(49,50) In human Achilles tendons, it has also been shown that nerve ingrowth, notably including SP-positive fibres, into the tendon proper occurs both following rupture and in painful tendinosis.(44) Interestingly, the ingrowth of SP-positive nerves occurs to a greater degree in painful tendinosis than it does following rupture.(44) While it cannot be assumed that these SP-containing nerves are nociceptors – SP is also found in non-nociceptive neurons – it does seem that the majority of SP-containing nerves are indeed nociceptive.(51) SP-containing nerves, when stimulated, release SP from their primary afferent terminals.(52) This action forms part of the process of neurogenic inflammation, whereby vasodilatation and plasma extravasation occur in local tissues.(53) SP also seems to have a direct stimulatory effect on nociceptors.(54) In rats, SP has also been shown to potentiate the effect of glutamate on nociception in peripheral tissues.(55) It is possible that SP has similar effects in tendon tissue – increased free glutamate concentrations have been found in tendinopathic human Achilles and patellar tendons.(56,57) Regardless, such work helps to characterise the nerves that invade tendinopathic tissue, and given the biological properties of SP, SP-positive nerves arguably may be responsible, at least in part, for pain induction in tendinopathy.(58)

Nociception is a complex, plastic process, subject to the influence of many intra- and interneuronal processes.(32) Nociception also stimulates peripheral and central sensitisation mechanisms,(2) such that all three processes are interconnected and are considered to occur to varying degrees in painful musculoskeletal conditions.(5) Preliminary findings of local nociceptive processes, (43,44) peripheral(6) and central(7) nervous system sensitisation(8,9) provide early evidence that this is the case in painful tendinopathy.

1.2.3 Exercise for Achilles tendinopathy

A body of evidence has been built that demonstrates that eccentric exercise has the strongest evidence of efficacy of the conservative interventions, when compared with, for example, extracorporeal shockwave therapy, splinting/bracing, active rest, low-level laser therapy, concentric exercises (i.e., inferior to eccentric), foot orthoses, therapeutic ultrasound and topical glycerin trinitrate).(11,12) Despite this, eccentric exercise has been found to be ineffective in up to 44% of sufferers.(14) When eccentric exercise alone is compared with combined concentric-eccentric/plyometric programmes (e.g., (59)), however, evidence for effectiveness is equivalent.(60) Twenty percent of sufferers have been shown to have residual limitations, however, five years after initiation of this type of rehabilitative exercise programme.(61) Of note, pain relief has been demonstrated in patellar tendinopathy with heavy isometric loading (70% maximum voluntary contraction load, 5 sets of 45 second holds, at 60° knee flexion).(62) More recently, although eccentric exercise continues to be recommended, the recommendations for exercise modalities have been expanded to include heavy-load, low-speed (concentric and

eccentric) exercise(63) based on evidence of similar efficacy of this type of loading in the management of Achilles tendinopathy.(64)

Exercise management remains the primary intervention used in the management of Achilles tendinopathy,(11,63) and though this has been demonstrated to be the most effective conservative approach, there are also those who do not recover with this type of management, as already discussed. This ongoing shortfall in the current management of Achilles tendinopathy continues to prompt research to better understand tendon pain and better inform management strategies.

1.2.4 Dry needling treatment for musculoskeletal pain syndromes

Dry needling involves the insertion of fine solid (monofilament) needles into tissues for therapeutic effect. Various clinical effects have been credited to dry needling, but rigorous evidence about its effects and potential physiological mechanisms of actions is still lacking.(65) IMDN is a type of dry needling where dry needles are inserted into muscle tissue specifically. There is a growing body of evidence for its efficacy in treating various musculoskeletal pain syndromes, including several systematic reviews, which are described below.

A systematic review of IMDN for musculoskeletal conditions by Gattie, Cleland and Snodgrass(66) assessed six randomised controlled trials (336 patients, 325 controls) that investigated pain reduction following IMDN compared with controls/sham, in the immediate to 12-week timeframe after IMDN intervention. They found an overall “serious” risk of both bias and inconsistency, and “low” quality in the included studies (using the GRADE approach;

considering study design and risk of bias, heterogeneity, generalisability, the number of participants being less than 400, and "other", e.g., publication bias).(67) They found greater pain reduction following IMDN, with meta-analysis calculating a "large" standardised mean difference between groups of -0.70 (95% confidence interval (CI): -1.06, -0.34). Their review also assessed five randomised controlled trials (334 patients, 327 controls) that investigated change in pressure pain threshold following IMDN compared with controls/sham in the immediate to 12-week timeframe after IMDN intervention. They found an overall "serious" risk of both bias and inconsistency, a "strong" suspicion of publication bias, and "very low" quality in the included studies. They found a greater increase in pressure pain threshold following IMDN, with meta-analysis calculating a "large" standardised mean difference between groups of -0.80 (95% CI: -1.27, -0.32). The authors noted limitations that included the "low" to "very low" quality of evidence and the high heterogeneity in the body of evidence (I^2 = 78% for pain reduction; I^2 = 87% for pressure pain threshold increase). They concluded that there was "low-quality evidence suggesting a moderate effect [on pain] favouring dry needling over control/sham" (66 p142) and "very low–quality evidence suggesting a moderate effect [on pressure pain threshold] favouring dry needling over control/sham".(69 p143)

Another systematic review of IMDN by Boyles et al.,(68) investigating the effect of IMDN on myofascial pain in "all body regions", assessed 19 randomised controlled trials the authors deemed to be of "high quality" (scores of 6-10/10) by the PEDro (Physiotherapy Evidence Database) Scale).(66) A meta-analysis was not performed, with the authors' conclusions instead being based on the results of individual study group differences. They reported significant differences in pain reduction following IMDN compared with controls/other treatment in nine

trials, increased range of motion compared with controls in five trials, increased pressure pain threshold compared with controls in seven trials, and improved function compared with controls in five trials. The authors noted that limitations of their review included the wide variety of IMDN protocols used (that complicated the comparison of study outcomes and possibly jeopardised external validity), heterogeneity of study protocols (which led them to conclude that meta-analysis was not appropriate), the inclusion of English-speaking articles only and the potential of publication bias. Their conclusion was that the "majority of high-quality studies included in [their] review show measured benefit from [IMDN]… in multiple body areas, suggesting broad applicability … for multiple muscle groups".(69 p276)

A systematic review by Liu et al.(71) of IMDN for low back pain assessed 10 randomised controlled trials (324 patients; 349 comparisons) that investigated pain reduction post-intervention following IMDN compared with other treatments. They found greater pain reduction following IMDN, with meta-analysis calculating a "large" standardised mean difference between groups of -1.06 (95% CI: -1.77, -0.36; $p < .003$). The authors noted limitations that included risk of bias due to concerns with random assignment, blinding and publication bias; low-to-moderate quality evidence; large heterogeneity ($I^2 = 94\%$); large clinical heterogeneity (e.g., sample sizes, total intervention time); and generally relatively small sample sizes of the included studies. They concluded that "moderate evidence showed that dry needling … could be recommended to relieve the intensity of low back pain".(71 p1)

Another systematic review by Kietrys et al.(72) examined the use of IMDN for "upper-quarter" myofascial pain. They assessed pain reduction following IMDN compared with sham or placebo

immediately after treatment from four trials (86 patients, 83 controls) and calculated a "large" standardised mean difference favouring IMDN of 1.06 (95% CI: 0.05, 2.06). They also assessed pain reduction at four weeks from three trials (50 patients, 44 controls), again favouring IMDN, with a "large" standardised mean difference of 1.07 (95% CI: –0.21, 2.35). They noted limitations that included a limited search strategy, the small number of included studies, risk of bias related to blinding, and high heterogeneity (I^2 = 86% for immediately after Rx; I^2 = 84% for four weeks post-treatment). The authors assessment was to "recommend dry needling, compared to sham or placebo, for decreasing pain immediately after treatment and at four weeks in patients with upper-quarter myofascial pain".(72 p620)

A systematic review by He and Hua(73) of IMDN for plantar heel pain assessed seven randomised controlled trials (210 participants, 207 controls).(73) The authors found greater pain reduction following IMDN compared with placebo or other treatment with a raw mean difference of -15.50 on a 0-100 scale (95% CI: -19.48, -11.53; $p < .001$). Limitations noted by the authors were that some studies had a relatively small sample size ($n < 50$); there was high clinical and methodological heterogeneity, e.g., variation in needling technique, diagnostic criteria, study design; and that two of the randomised controlled trials were at high risk of bias due to lack of blinding. Heterogeneity was also considerable (I^2 = 57%). They concluded that "[dry] needling effectively reduced the heel pain due to plantar fasciitis".(73 p1933)

Since this research proposal was finalised, other systematic reviews have been published that, for the most part, show beneficial treatment effects on pain from dry needling interventions. The results of these reviews are summarised below in Table 1.1.

Study	Condition	Study Type	n	MA	Favours DN	Conclusion
Blanco-Díaz 2022(74)	Subacromial shoulder pain	RCTs	9	no	yes	"DN has been demonstrated to be effective at reducing the pain and disability produced by SAPS"(74 p1)
Hu 2018(75)	LBP	RCTs	16	yes	yes	"Compared with acupuncture and sham needling, DN is more effective for alleviating pain and disability at postintervention in LBP, while its effectiveness on pain and disability at follow-up was equal to acupuncture."(75 p1)
Llurda-Almuzara 2021(76)	Plantar heel pain/fasciitis	RCTs	6	yes	yes	"Moderate- to low-quality evidence suggests a positive effect of trigger point dry needling for improving pain intensity and pain-related disability in the short term and long term, respectively, in patients with plantar heel pain of musculoskeletal origin."(76 p1630)

Navarro-Santana 2020(77)	Neck pain	RCTs	28	yes	yes	"Moderate-to-low evidence suggesting that dry needling can be effective for improving neck pain intensity and related disability when compared with a comparative group immediately after and at short-, but not at mid-, term follow-ups in people with myofascial TrPs associated with neck pain symptoms. The effects were mostly observed when dry needling was compared with sham/placebo/waiting list/other forms of dry needling but not against other physical therapy interventions."(77 p31)
Navarro-Santana 2020(78)	Lateral epicondylalgia	RCTs	7	yes	yes	"Low to moderate evidence suggests a positive effect of dry needling for pain, pain-related disability, pressure pain sensitivity and strength at short-term in patients with lateral epicondylalgia of musculoskeletal origin."(78 p1328)

Navarro-Santana 2021(79)	Shoulder pain	RCTs	6	yes	yes	"Moderate- to low-quality evidence suggests positive effects of TrP dry needling for pain intensity (small effect) and pain-related disability (large effect) in nontraumatic shoulder pain of musculoskeletal origin, mostly at short term."(79 p1)
Pourahmadi 2021(80)	Tension-type headache, cervicogenic headache, and migraine	RCTs	9	yes	yes/equivocal (other therapies)	"Dry needling produces similar effects to other interventions for short-term headache pain relief, whereas dry needling seems to be better than other therapies for improvement in related disability in the short term."(80 p1)
Rahou-El-Bachiri 2020(81)	Knee pain	RCTs	10	yes	yes (for short term)	"Low to moderate evidence suggests a positive effect of trigger point dry needling on pain and related disability… at short-term"(81 p1)
Rodríguez-Huguet 2022(82)	Chronic neck pain	RCTs	8	no	yes	"DN can be an effective treatment option for [chronic neck pain], positive outcomes were achieved in the short-term and in the follow-up performed between

						three and six months, and this technique may offer better outcomes than a placebo intervention based on the application of simulated DN."(82 p1)
Sánchez-Infante 2021(83)	Myofascial trigger points	RCTs	42	yes	yes	"Low-quality evidence that the immediate to 72-hour (large) effect, 4- to 12-week (large) effect, 13- to 24-week (large) effect, and moderate-quality 1- to 3-week (moderate) effect suggested that DN performed by physical therapists was more effective than no treatment, sham DN, and other therapies for reducing pain."(83 p1)
Vier 2019(84)	Temporomandibular joint dysfunction	RCTs	7	yes	yes	"Meta-analysis showed that dry needling is better than other interventions for pain intensity…".(84 p3) Authors noted very low quality evidence.

Table 1.1: Summary of systematic reviews for dry needling therapies for musculoskeletal conditions published since this thesis proposal was finalised. MA – meta-analysis, DN – dry needling, RCT – randomised controlled trial, SAPS – subacromial pain syndrome, LBP – low back pain, TrP – trigger point, n – number of included studies

In general, the authors of the systematic reviews that were published both before the plan for this research thesis was finalised and since have found results almost entirely in favour of dry needling interventions. They have also generally assessed the body of evidence as being of low quality. The reviews, in general, have assessed a relatively small number of studies. Many of the meta-analyses have been undertaken with low numbers of studies and with high clinical, methodological and statistical heterogeneity. Despite this, the use of dry needling is used widely in clinical practice in musculoskeletal pain syndromes.(83) With respect to the study of dry needling for Achilles tendinopathy in particular, there is only one related published study of which I am aware, that of Zhang et al.(85). This study describes inserting dry needles using an acupuncture-type approach into "the painful area" (presumably the tendon), so does not provide useful information with respect the use of intramuscular dry needles for this condition. These factors together, prompted the undertaking of the randomised controlled trial presented in Chapter 2: "Intramuscular stimulation vs sham needling for the treatment of chronic midportion Achilles tendinopathy: a randomised controlled clinical trial". Importantly, all groups in the trial were provided with exercise rehabilitation as this was, and remains, the gold standard for Achilles tendinopathy conservative management, as discussed above.

1.3 Subacromial pain syndrome

1.3.1 Pathology and Pain

Subacromial pain syndrome (SAPS) was defined in 2014 by the Dutch Orthopaedic Association(86) as "all non-traumatic, usually unilateral, shoulder problems that cause pain, localised around the acromion, often worsening during or subsequent to lifting of the arm".(86

p314) The group suggests that this definition should include the clinical syndromes of bursitis, calcific tendinitis, tendinopathy, tears and degeneration of the rotator cuff, as well as biceps tendinitis. For the purposes of this thesis, it is considered that bicipital tendinitis is a separate clinical syndrome and is not included in the operational definition of SAPS. The subacromial-subdeltoid bursa, however, commonly exhibits pathology when the rotator cuff exhibits pathology.(87,88) It is a pain-sensitive structure(89) (innervated by the suprascapular nerve and the lateral pectoral nerve(90)) whose pathology may present very similarly on history-taking and physical examination as a symptomatic rotator cuff,(87,91) i.e., it may be a source of pain in people with pain localised around the acromion and have similar history and physical examination findings to rotator cuff pathology. The multiple syndromes of tendinitis, tendinopathy, tears and degeneration referred to in the Dutch Orthopaedic Association definition(86) are included, in this thesis, under the umbrella term of "tendinopathy" referring to "a spectrum of changes that occur in damaged and/or diseased tendons"(86 p833). Therefore, for the purposes of this thesis, SAPS is defined as "rotator cuff tendinopathy with or without subacromial-subdeltoid bursitis".

The pathological changes that occur in tendinopathy have been discussed above, as have pain considerations related to tendinopathy. For SAPS specifically, research has suggested that nervous system dysfunction is involved, including impaired CPM,(92) facilitated temporal summation to mechanical pain(92) and heat pain,(93) and reduced mechanical pain thresholds,(94) (95) (96) (97) (93) (98) both locally and remotely (which has been interpreted to suggests the presence of central sensitisation), however, conflicting observations have also being made.(99) These findings have prompted calls for more in-depth studies of nervous system

dysfunction in SAPS that include both static and dynamic testing, as well as local and remote testing.(92,97,98,100,101)

The mainstay of conservative management for SAPS, as for Achilles tendinopathy, is rehabilitative exercise,(13) which achieves a 65-80% success rate after one year.(15) The prolonged timeframe needed for recovery, however, as well as the 20-35% of people who do not recover by one year, clearly demonstrates room for improvement in management of this problem. These shortfalls support the value of investigating pain mechanisms in SAPS to inform its more effective management, as was undertaken and is presented in Chapter 3 of this thesis: "Quantitative sensory testing of nervous system dysfunction and sensitisation in chronic subacromial shoulder pain".

1.4 Referred pain and enlarged pain areas - pain mapping

A common feature of musculoskeletal pain syndromes is pain felt outside the area of the local tissue implicated as the source of pain,(102) i.e., outside the receptive fields (the spatial area within which a single neuron may be stimulated)(103) of the nociceptors innervating the implicated local tissue. This feature has been reported in, for example, chronic shoulder pain(94) and whiplash associated disorder.(104) Its presence suggests that nervous system components that are not neuroanatomically part of the relatively direct ascending pathway from nociceptors in the implicated tissue to the somatosensory cortical regions involved in pain perception can become active and modify the perceptual localisation of pain.(105)

There are two main processes by which this is suggested to occur: (1) enlarged pain areas and (2) referred pain.(106–108) "Enlarged pain areas" refers to pain felt in an area that extends beyond the receptive fields of the peripheral nociceptors involved and is thought to reflect the development of increased receptive fields in spinal cord dorsal horn neurons that have become "unmasked" by a reduced magnitude of descending inhibition in the central nervous system.(106,108) "Referred pain" refers to pain that is felt in areas remote and distinct from the implicated tissue (and the receptive fields of its peripheral nociceptors) and is also thought to result from the unmasking of dorsal horn neurons in the spinal cord.(106,107) Both, therefore, are seen to be the result of central sensitisation processes. (4,109,110) As previously discussed, there is evidence that central sensitisation occurs in SAPS. Pain hypersensitivity related to central sensitisation may result from various temporal, spatial and threshold changes in the central nervous system, with enlarged pain areas and the presence of referred pain resulting from reduced descending inhibition being just two manifestations of central sensitisation.(4) For this reason, pain mapping, a simple way to measure the presence of pain outside the area of the local tissue implicated as the primary source of pain, was included as part of the investigation framework of nervous system dysfunction in SAPS in this thesis. Related findings would be expected to inform more effective management of SAPS by providing a better understanding of the pain mechanisms involved, as discussed, relating to the comprehensive QST planned and described below.

1.5 "Spreading" sensitisation – local, segmental and remote testing

As discussed previously, central sensitisation processes can result in pain being felt in areas that extend beyond the immediate area (i.e., receptive fields) of implicated nociceptors and/or pain

being felt in areas remote and distinct from the implicated tissue (and the nociceptors that supply that tissue).(106–108) Graven-Nielsen and Arendt-Nielsen have used the term “spreading sensitisation” to describe pain “spreading” in this way.(3) They suggest that pain may “spread” first into the segmental ipsilateral and contralateral central nervous system at the level of the spinal cord, then extrasegmentally in the spinal cord and also to higher centres.(3) Evidence to support this model has been demonstrated both in animals (e.g., by the development of remote ipsilateral hyperalgesia, contralateral hyperalgesia, and the expansion of dorsal horn neuron receptive fields in response to noxious stimulation) (107,112,113) and in humans (e.g., by clinical observations of initially localised pain spreading beyond the immediate area of the implicated tissue and becoming generalised, the development of segmental and extrasegmental pain referral patterns in response to hypertonic saline injections, and by the development of increased activity in pain-related brain areas in chronic pain states).(106,114,115)

Investigation aligned with this model was incorporated into the design of the SAPS QST study in this thesis by testing somatosensory function in: (1) local areas, (2) segmental areas, and (3) extrasegmental/remote areas. Where possible, somatosensory function was tested in both the cutaneous and deep tissue nociceptors in each of these representative areas as relates to SAPS, (whereby the posterior rotator cuff tendons and the SASD bursa are both innervated by the suprascapular nerve, which has a C5, C6 segmental supply)(90,116). With this in mind, test areas were selected as follows: (1) local testing (although outside the region of the receptive fields of the nociceptors that directly innervate the rotator cuff tendon tissues, and so representing an “enlarged” pain area in relationship to the rotator cuff tendon tissues) was performed in the infraspinatus muscle (supplied, in common with the posterior rotator cuff tissues, by the

suprascapular nerve, which has a C5, C6 segmental supply); (2) segmental testing was performed in the deltoid muscle and in the skin over the deltoid muscle (both supplied by the axillary nerve which, in common with the suprascapular nerve, has a C5, C6 segmental supply); and extrasegmental testing was performed in the tibialis anterior muscle (supplied by the common peroneal nerve, which has an L4, L5 segmental nerve supply, i.e., remote to the C5, C6 suprascapular nerve supplying the rotator cuff) and in the skin over the lateral aspect of the shin below the knee (supplied by the lateral cutaneous nerve of the calf, which also has an L4, L5 segmental supply, i.e., again, remote to the C5, C6 suprascapular nerve). Any observable pattern of nervous system sensitivity that aligned with this model of spreading sensitivity, again, had the potential to provide insight into the pain mechanisms of SAPS, and so was incorporated into the study design. In particular, any observed patterns of sensitivity relating to local nerve supply (the suprascapular nerve), segmental nerve supply (the C5, C6 levels) and/or extrasegmental/remote nerve supply (L4, L5 levels) were considered to have the potential to be informative with respect to this model of spreading sensitisation. Of note, there is typically no cutaneous distribution of the suprascapular nerve (there being an exception in about 15% of people)(117), and so there was no “local” cutaneous testing that could be performed to complement the infraspinatus deep tissue (suprascapular nerve/C5, C6) testing as was possible for the deltoid/skin over deltoid (axillary nerve/C5, C6) and tibialis anterior/skin over the lateral aspect of the shin below the knee (common peroneal nerve/lateral cutaneous nerve of the calf/L4, L5) testing combinations.

1.6 Quantitative sensory testing

QST is a method of assessing somatosensory nervous system function, and in particular, to ascertain which particular nervous system pathways may be dysfunctioning in any particular pain

syndrome(118). It encompasses many tests and protocols that assess the various components of the somatosensory system(119) that respond to thermal, mechanical, electrical, or chemical stimuli, and may be used to test different body tissues, i.e., skin, muscles, viscera etc.(118) Tests may be "static", where testing focuses on simple singular sensory modality inputs aiming to stimulate just one nervous system pathway to a specified perception level, e.g., heat pain threshold, pressure pain tolerance; or "dynamic", where testing deliberately stimulates more complex nervous system function by using repetitive or multiple stimuli, e.g., temporal summation and CPM.(118)

QST is described as being "psychophysical", as it requires cognitive engagement of the person being tested in producing test outcomes.(120) The person being tested needs to perceive the sensation being tested and respond in some way to indicate that the specified threshold of sensation has been reached, e.g., by pressing a button or verbally reporting that this has occurred. Although QST test results are vulnerable to variability caused by testing protocols/procedures and test subject performance, this can be controlled for by using standardised protocols.(121)

QST has been used in this thesis to attempt to gain insight regarding the nervous system mechanisms involved in chronic SAPS. Details of the testing involved is discussed in the relevant chapter (Chapter 3). Current understanding of the mechanisms of tendon pain is limited, as discussed above.(10) This thesis looks to use QST to contribute to increasing the understanding of the pain mechanisms involved in SAPS.

1.7 Conditioned pain modulation

CPM, the "pain inhibits pain" phenomenon(122) is considered to be a normal function of the nervous system whereby the intensity of pain sensation felt in one area of the body is reduced in the presence of another pain stimulus elsewhere in the body (i.e., a "conditioning stimulus").(123) Impaired CPM (i.e., a relative lack of pain inhibition in response to a conditioning stimulus) is understood to be a form of central sensitisation that results from relatively reduced descending inhibition of pain in the central nervous system.(123)

A systematic review of chronic pain states in general found an overall effect of impaired CPM in people suffering from chronic pain compared to healthy controls.(124) Although there are reports of reduced CPM in various chronic musculoskeletal pain states,(124–127), there is conflicting evidence, even within singular pain conditions, e.g., whiplash associated disorder.(128,129)

It is not known whether there may be an overall effect of impaired CPM in chronic musculoskeletal pain states as opposed to chronic pain states in general. It may be that the mechanisms of chronicity are different in different body systems but are perhaps similar in the musculoskeletal system. Seeking clarity on this issue was the motivation for undertaking a systematic review and meta-analysis of CPM in chronic musculoskeletal pain conditions and the results of this review are reported in Chapter 4 of this thesis: "Chapter 4: Conditioned pain modulation in chronic musculoskeletal pain: a systematic review and meta-analysis".

1.8 Hypotheses and/or goals of the thesis

The goals and hypotheses for the three studies in this thesis are described below.

1.8.1 Intramuscular stimulation vs sham needling for the treatment of chronic midportion Achilles tendinopathy: a randomised controlled trial (Chapter 2)

The goal of this randomised controlled trial was to answer the question, "Is intramuscular stimulation (IMS) more effective than sham intramuscular stimulation/dry needling for the treatment of mid-portion Achilles tendinopathy?".

The main hypothesis for this study was that people with chronic midportion Achilles tendinopathy would have a greater improvement on the VISA-A questionnaire (Victorian Institute of Sports Assessment–Achilles; a valid and reliable disease-specific outcome measure)(130) if they received Gunn IMS (a type of IMDN) in addition to a rehabilitative exercise program compared to receiving sham IMDN in addition to the same exercise program or receiving the same exercise program alone. Secondary hypotheses were that the IMDN group would also have a greater improvement on a Global Rating of Change Likert scale (from 1 (very much improved) to 7 (very much worse)), a greater increase in ankle dorsiflexion range, and a greater reduction in tendon thickness, with measurements being taken at baseline, 6 weeks, 12 weeks, 26 weeks and 52 weeks.

1.8.2 Quantitative sensory testing of nervous system dysfunction and sensitisation in chronic subacromial shoulder pain (Chapter 3)

The goal of this cross-sectional study was to conduct QST and pain mapping of a SAPS population compared with healthy controls.

The primary hypothesis was that pressure pain threshold values for the deltoid muscle (C5, C6 segmental nerve supply) are lower for people with SAPS compared to healthy controls. The secondary hypotheses were that pressure pain threshold values for: (1) the infraspinatus muscle (C5, C6 segmental nerve supply), and (2) the tibialis anterior muscle (L4, L5 segmental nerve supply) are lower for people with SAPS compared to healthy controls. Exploratory hypotheses were that: (1) heat pain threshold values for (a) the skin over the deltoid muscle (C5, C6 segmental nerve supply), and (b) the skin over the lateral aspect of the shin below the knee (L4, L5 segmental nerve supply) are lower for people with SAPS compared to healthy controls; (2) pinprick mechanical pain threshold values for (a) the skin over the deltoid muscle (C5, C6 segmental nerve supply), and (b) the skin over the lateral aspect of the shin below the knee (L4, L5 segmental nerve supply) are lower for people with SAPS compared to healthy controls; (3) CPM response to pressure (a) the infraspinatus muscle (C5, C6 segmental nerve supply), (b) the deltoid muscle (C5, C6 segmental nerve supply), and (c) the tibialis anterior muscle (L4, L5 segmental nerve supply) is impaired for people with SAPS compared to healthy controls; (4) CPM response to heat for (a) the skin over the deltoid muscle (C5, C6 segmental nerve supply), and (b) the skin over the lateral aspect of the shin below the knee (L4, L5 segmental nerve supply) is impaired for people with SAPS compared to healthy controls; (5) temporal summation to punctate (sharp) pressure values for (a) the skin over the deltoid muscle (C5, C6 segmental

nerve supply), and (b) the skin over the lateral aspect of the shin below the knee (L4, L5 segmental nerve supply) is augmented for people with SAPS compared to healthy controls; and (6) the size of pain area for people with (chronic) SAPS is larger than localised acromial pain.

1.8.3 Conditioned pain modulation in chronic musculoskeletal pain: a systematic review and meta-analysis (Chapter 4)

The goal of this SRMA was to examine whether adults (persons 18 years of age or older) with chronic musculoskeletal pain have reduced CPM compared to healthy adults.

The hypothesis for this systematic review and meta-analysis is that adults with chronic musculoskeletal pain have impaired CPM compared to healthy adults.

Chapter 2: Intramuscular Stimulation vs Sham Needling for the Treatment of Chronic Midportion Achilles Tendinopathy: A Randomised Controlled Trial

2.1 Synopsis

Background: The insertion of filiform needles intramuscularly (a.k.a. intramuscular stimulation (IMS)/dry needling) has been suggested as a possible treatment for various painful musculoskeletal conditions. Our aim was to answer the question, is IMS more effective than sham IMS/dry needling for the treatment of Achilles tendinopathy?

Methods: 52 participants with persistent midportion Achilles tendinopathy began and 46 completed one of three treatment protocols which were randomly assigned: (Group 3) a 12-week rehabilitation program of progressive tendon loading plus IMS (n = 25), (Group 2) the same rehabilitation program but with sham IMS (n = 19), or (Group 1) a reference group of rehabilitation program alone (as an additional control) (n = 8). The *a priori* primary outcome measure was change in VISA-A questionnaire (Victorian Institute of Sports Assessment–Achilles) score at 12 weeks – VISA-A was also measured at 6 weeks, and at 6 and 12 months. Secondary outcome measures include the proportion of patients who rated themselves as much or very much improved (%), dorsiflexion range of motion (degrees), and tendon thickness (mm).

Results: The study retention was 94% at 12 weeks and 88% at 1 year. VISA-A score improved in all three groups over time ($p < .0001$), with no significant difference among the three groups in VISA-A score at the start of the study (mean + standard deviation: Group 3, 59 + 13; Group 2,

57 + 17; Group 1, 56 + 22), at 12 weeks (Group 3, 76 + 14; Group 2, 76 + 15; Group 1, 82 + 11) or at any other timepoint. The percentage of patients who rated themselves as much or very much improved (i.e., treatment success) was not different after 12 weeks (Group 3, 70%; Group 2, 89%; Group 1, 86%; $p = .94$), or at 26 weeks ($p = .62$) or 52 weeks ($p = .71$). No clinically significant effects of intervention group were observed in any of the secondary outcome measures.

Conclusion: The addition of IMS to standard rehabilitation for Achilles tendinopathy did not result in any improvement over the expected clinical benefit achieved with exercise-based rehabilitation alone.

2.2 Introduction

Mid-portion Achilles tendinopathy is accompanied by pain and impaired function in the Achilles tendon.(131) It is typically accompanied by structural changes visible on ultrasound such as thickening of the tendon, a feature which is present early in the development of pathology.(43) The pain of Achilles tendinopathy may be accompanied by altered central nervous system processing, such as reduced conditioned pain modulation and mechanical secondary hyperalgesia (7,9) although others have argued that Achilles tendinopathy pain is primarily driven by peripheral nociception(132) perhaps as a result of local nociceptive substances.(43,44)

Achilles tendinopathy is common in runners, with a recent cohort study reporting a 24% incidence among runners;(133) in that study, years of activity and weekly mileage were identified as risk factors, although not all studies have found such associations.(134) In another

study, the cumulative incidence of Achilles tendinopathy was 24% in former elite athletes from various sports (running, athletics, soccer, hockey, etc.) compared to 5.9% in matched controls.(135) The prognosis of Achilles tendinopathy is variable; in club-level adult and youth athletes, the median time to return to play from Achilles tendinopathy was 1.7-12.5 days, with an upper range of 30 days.(136) Others have reported that ongoing symptoms are present 5 years later in over a third of recreationally active adults who enrolled in a clinical trial.(61)

Current recommended treatment for those with persistent Achilles tendinopathy emphasises tendon-loading exercises and activity modification/education(63,131) with eventual referral to surgery for severe long-standing cases.(137,138) Placebo-controlled studies of proposed adjunct treatments such as shockwave therapy(139–141) or topical glyceryl trinitrate(142,143) have not clearly demonstrated a benefit for Achilles tendinopathy, showing small improvements,(140) improvements reported by a single study,(143) or no apparent benefit.(139,141,142) There are very few placebo-controlled studies of injection therapies(144–150) or analgesics (either topical(151) or oral(152)) to inform clinical decision-making.

The insertion of filiform needles intramuscularly, a.k.a. intramuscular stimulation (IMS), has been suggested as a possible treatment for Achilles tendinopathy.(153) Gunn et al.(154) suggested that, in people with Achilles tendinopathy, the gastrocnemius and soleus muscles may become “shortened” (i.e., demonstrate reduced extensibility) and deliver excessive mechanical stress to the Achilles tendon. They suggested that this muscle shortening may result from the formation of taut bands (sustained contraction in localised sections of the muscles), and that the insertion of filiform needles into these taut bands can assist in relaxing them, restoring their

extensibility and thereby normal mechanical loading of the tendon. They also suggested that local gastrocnemius and soleus muscle banding/shortening may often be accompanied by muscle banding/shortening in other muscles that share the same segmental nerve supply as the gastrocnemius, soleus and Achilles tendon, i.e., L5-S2. They hypothesised that the reason for this may be neuropathic dysfunction in the affected segments.(155)

There is great variability in the way filiform needles are used to treat musculoskeletal pain both in practice and in research settings, and there is no research on the use of filiform needles intramuscularly to treat Achilles tendinopathy. For other conditions, such as chronic low back pain, it has been suggested that dry needling may be a useful adjunct treatment, however further high quality studies are needed.(156) There is only one published study that uses a needle insertion approach to treat Achilles tendinopathy, conducted by Zhang et al.(85), but it is unclear where the filiform needles were inserted (skin, muscle, tendon etc.) and the conclusion of the study (that the effect of acupuncture-style needling on pain and function was superior to exercise) may be questioned. In their 2016 systematic review Cox et al.(157) rated the study by Zhang et al. as having a low risk of bias, however the acupuncture group: (1) was not blinded, (2) was not compared to a sham group, and (3) received 24 visits over eight weeks compared to apparently a single visit in the exercise group, which achieved lower-than-expected outcomes for exercise-based treatment.(85) Thus, there is very minimal evidence on which to base a recommendation for the use of dry needling of any kind in people with chronic Achilles tendinopathy.

The primary purpose of this study was to compare the clinical status of people with Achilles tendinopathy who receive IMS (Group 3) to those who receive sham needling (Group 2); a reference group (Group 1) received no needling. To our knowledge, this is the first placebo/sham controlled randomised controlled trial of an intramuscular dry needling (IMDN) technique for Achilles tendinopathy.

2.3 Methods

2.3.1 Study design

This was a parallel, randomised, single-blind, controlled trial at a single site: the University of British Columbia (Vancouver, Canada). A single investigator, Lyndal Solomons (LS), provided all treatments. Three treatment and data collection locations were used: the Centre for Hip and Health Mobility at Vancouver Coastal Health Research Institute (Vancouver), Kinetic Rehabilitation Centre (North Vancouver), and Canopy Integrated Health (North Vancouver). Patients were recruited by placing advertisements in public locations, local newspapers, and a Facebook page, and an entry in the Vancouver Coastal Health Research Institute trial database and newsletter. All patients provided written informed consent. The study was approved by the UBC Clinical Research Ethics Board (H12-02008). The trial was registered and kept up to date on the ISRCTN registry (70177540). As described below in the section on enrolment, the study took place in two phases but with identical outcome measures such that the data set could be combined and analysed as a whole.

2.3.2 Participants

Inclusion criteria were; 19 to 60 years of age, fluent in English, minimum symptom duration of three months, evidence of midportion Achilles tendinopathy on physical examination, i.e., pain location isolated to the mid-portion of the Achilles tendon and progressive loading causing increasing pain (double leg toe-raise, single leg toe-raise, jump, hop, hop for height, hop for distance), and indications by Gunn IMS assessment of neuropathic change in the L5-S2 segmental levels including the presence of taut muscle bands amenable to IMS. Exclusion criteria were; IMS contraindications (infection in the area, pregnancy, bleeding disorders, history of bacterial endocarditis, post-surgical implant in the past six months, major surgery in the last three months), previous treatment with IMDN (for blinding purposes), true leg length difference of greater than 0.5 inches, systemic inflammatory disease, previous corticosteroid injections, recent fluoroquinolone use, and the presence of other syndromes that cause pain in and around the Achilles tendon (determined by history-taking and physical examination). Age, sex, symptom duration and physical activity level (International Physical Activity Questionnaire) were recorded at the first visit.

2.3.3 Enrolment and Randomisation

After informed consent was obtained, and before any clinical assessment or baseline data collection, participants were randomised by a study coordinator who had no role in the initial assessment or treatment. The study coordinator was provided with a simple (unblocked, unbalanced) random allocation sequence with 42 allocations into three groups, generated by Alex Scott (AS), using a random sequence generator (Microsoft Excel). This list was used to allocate the first 21 participants (recruited from April 2013 to April 2014) into Group 3, Group 2 or

Group 1 conditions. In summer of 2014, an amendment to the protocol was submitted to the funder, ethics board and trial registry such that further allocations would be only to Groups 2 and 3 to focus on the primary question - comparison between IMS and sham needling. Thereafter a revised (unblocked, unbalanced) random allocation sequence with a further 42 allocations was generated. The remaining participants were recruited and allocated to Group 3 or Group 2 from October 2014 – December 2018. The same research assistant assigned participants to groups sequentially as they enrolled using the random allocation sequence and notified the researcher administering the interventions (LS) of participants group allocation just prior to their first treatment session. Only AS and a research assistant not involved in participant intervention or assessments had access to the random allocation sequence.

2.3.4 Blinding

Participants assigned to a needling group were blinded to their group allocation (IMS or sham IMS) until after having received their nine treatments and completed their 12-week outcome measures. It was not possible to blind the treating therapist (LS) to participant group allocation. Those assessing ankle range measures were blinded to group allocation. Ultrasound scans were taken by LS and interpretation of ultrasounds was performed by a research assistant blinded to group allocation. After 12 weeks, participants who received needling were asked which group they thought they were assigned to, and their answer was categorized as correct or incorrect.

2.3.5 Important changes to methods after trial commencement (such as eligibility criteria), with reasons

Initially, prior experience with Traditional Chinese Medicine Acupuncture was an exclusion criterion. This was removed prior to the start of recruitment (in April 2013) as it was seen as potentially an undue hindrance to recruitment. After enrolment of the first 21 participants, we amended the allocation ratio (as described above) to focus on the primary question (difference in outcome between IMS (Group 3) and sham IMS (Group 2) groups by making no further allocations to the exercise-only reference group (Group 1).

2.3.6 Interventions

All participants (Group 3, Group 2, and Group 1) received a standardised 12-week physiotherapy program provided by one of the investigators (LS), including a progressive isometric, concentric and eccentric training and kinetic chain strengthening program. All groups were prescribed the same standardised exercise programme designed to initially maintain (as pain allowed) and then, as tolerated, increase the ability of the muscle-tendon unit and kinetic chain to absorb load.(131) The programme was progressive, and eccentric loading of gastrocnemius-soleus-Achilles tendon complex loading was incorporated throughout. The first phase focused on isometric loading, the second on concentric and then load, range and speed were gradually increased.(132) Participants were progressed based on a "twenty-four-hour response" to exercise, specifically, there was not to be increased pain or stiffness the morning after undertaking the exercise, however pain during exercise was allowed. Participants were advised to keep the discomfort during activities of daily living to a level of no more than 5/10 on a scale of zero to 10, where zero is no pain at all and 10

is the worst possible imaginable pain. Participants filled out a training diary – the number of sessions completed was calculated as a percentage of prescribed sessions.

Group 3, the IMS treatment group, received IMDN treatment (APS Dry Needling Needles by Agupunt; Barcelona, Spain; 0.25-0.30 x 25-75 mm) once a week for the first six weeks of the trial and once every two weeks for the remainder, resulting in nine treatments overall. The choice of needle insertion points was individualised for each participant based on their assessment findings and using the neuropathic model approach described by Dr. C. Chan Gunn.(155) This approach considers the potential contribution of a dysfunctional nervous system to myofascial pain syndromes, and in the case of Achilles tendinopathy with a particular focus on treating dysfunction in the L5-S2 segments. Treatment involved the insertion of filiform needles into taut muscle bands (as palpated by the treating therapist) in both the spinal region and the lower limbs.

Group 2, the sham IMS group, received eight filiform needles (APS Dry Needling Needles by Agupunt; Barcelona, Spain; 0.25 x 25 mm) that were inserted superficially (1-2 mm maximum depth) in the buttock, posterior thigh and calf regions and were left in situ for ten minutes. The schedule and number of visits was the same as for Group 3. To avoid mimicking either IMS or purported Traditional Chinese Medicine Acupuncture effects: needles were inserted away from any taut muscle bands that were palpated by LS and were inserted superficially so that no muscle penetration occurred; purported Traditional Chinese Medicine Acupuncture meridians in the treatment area and Traditional Chinese Medicine Acupuncture points used to treat heel pain specifically were avoided; and it was ensured that no “deqi” sensation was elicited (i.e., aching, soreness, pressure).(158)

Group 1, the reference group (actively allocated only during the first portion of the study period, as described above), only received the exercise program. This intervention group was included as an additional, internal reference primarily to ensure that the exercise program was performing as expected.

2.3.7 Outcome measures

The outcome measures were designed and registered prior to the publication of a recent consensus on outcome measures.(131) There were no changes to outcome measures after registration of the protocol at ISRCTN, although we did alter the ultrasound analysis from what was described in our ethics protocol by focusing on tendon thickness rather than echo texture. The primary outcome for which the study was powered was the change from baseline in 12-week VISA-A (Victorian Institute of Sports Assessment – Achilles) questionnaire score, a valid and reliable disease-specific outcome measure(159) which also includes an activity-related pain scale. The VISA-A score was also measured at 6, 26 and 52 weeks.

The following secondary outcome measures were included *a priori*:

(1) A written, seven-point, patient-rated Likert scale of Global Rating of Change was filled in by patients, from 1 (very much improved) to 7 (very much worse). Ratings of "very much improved" or "much improved" were considered as treatment success, and were measured at 6, 12, 26, 52 weeks.

(2) Dorsiflexion range of motion was measured in two weightbearing positions (knee bent or straight) using an inclinometer placed midway along the anterior surface of the tibia (measured at weeks 0, 12 and 52).(160) Prior to measurement, participants performed stretches in a standing lunge with the middle of the heel and the second toe aligned in a straight line, with both the back knee straight and the back knee bent (15 second stretch, five times each). Range of motion was measured by one of two physiotherapy assessors who were both blind to treatment allocation.

(3) Tendon thickness in the anteroposterior plane at the point of maximal tendon thickening was measured using grey-scale B-mode ultrasound images (at weeks 0, 6, 12, 26 and 52). Participants lay prone on an examination bed with their foot positioned and stabilised by the person taking the scan such that the tendon was "flat" (i.e., lay passively straight) to achieve a perpendicular alignment between the Achilles tendon and the UTC transducer (Smartprobe 10L5; Terason 2000, Teratech, USA; UTC Technologies, Oldemarkt, Netherlands), i.e., in an individual-specific degree of dorsiflexion. The transducer (in its tracker) was positioned and stabilised by the person taking the scan so that the centre of the ultrasound head was placed over the centre of the posteromedial aspect of the tendon (the region most commonly affected by Achilles tendinopathy) to allow the ultrasound beam to interface with this region at a perpendicular angle. Once the foot and transducer were fixed in position, adjacent transverse images (2D) of the Achilles tendon were automatically captured at every 0.2 mm for 12 cm along the tendon axis and compiled to create a 3D scan for tomographic visualisation in the transverse, sagittal, and coronal planes. Each subsequent scan was obtained using the same method. The ultrasound probe was a Smartprobe 10L5 (Terason 2000, Teratech, USA) attached to an Ultrasound Tissue Characterisation tracking system (UTC Technologies, Oldemarkt, Netherlands). Transverse

images (2D) of the Achilles tendon were automatically captured every 0.2 mm for 12 cm along the tendon axis and compiled to create a 3D scan for tomographic visualisation in the transverse, sagittal, and coronal planes. Scans were de-identified and analysed with blinding of the time point and participant identification. To analyse the scans, the site of maximal thickening was identified at week 0. The same location was used for all subsequent measures by measuring from the calcaneal insertion.

2.3.8 Sample size calculation

We assumed that the change in VISA-A from 0 to 12 weeks with exercise alone would be 20 points,(159) with a normal distribution and a standard deviation of 12(147). We assumed the minimum clinically important difference in VISA-A score to be 12 points.(159) Given these assumptions, to answer the primary question, the trial was powered (at 0.80, with alpha of 0.05) to detect a difference of 12 points between the Group 3 (IMS) and Group 2 (sham needling) groups, which for an independent t-test would require a sample size of 16 in each group.

2.3.9 Statistical analysis and treatment of missing data

Statistical analysis was conducted in R 3.6.0. Data are presented to two significant figures with the standard deviation in parentheses. For the primary and secondary outcome measures, an independent blinded statistical analysis was conducted by a professional statistician. For the main outcome measure (VISA-A), linear modeling was employed to incorporate the repeated nature of the measurements. For the primary endpoint of VISA-A, we used a model of VISA-time = αSUBJECT + VISAbaseline$*$group + log(time) + ϵtime. The covariance structure was Correlation(ϵt1,ϵt2) = $\phi|t1-t2|$, to account for the fact that the longer the time interval between

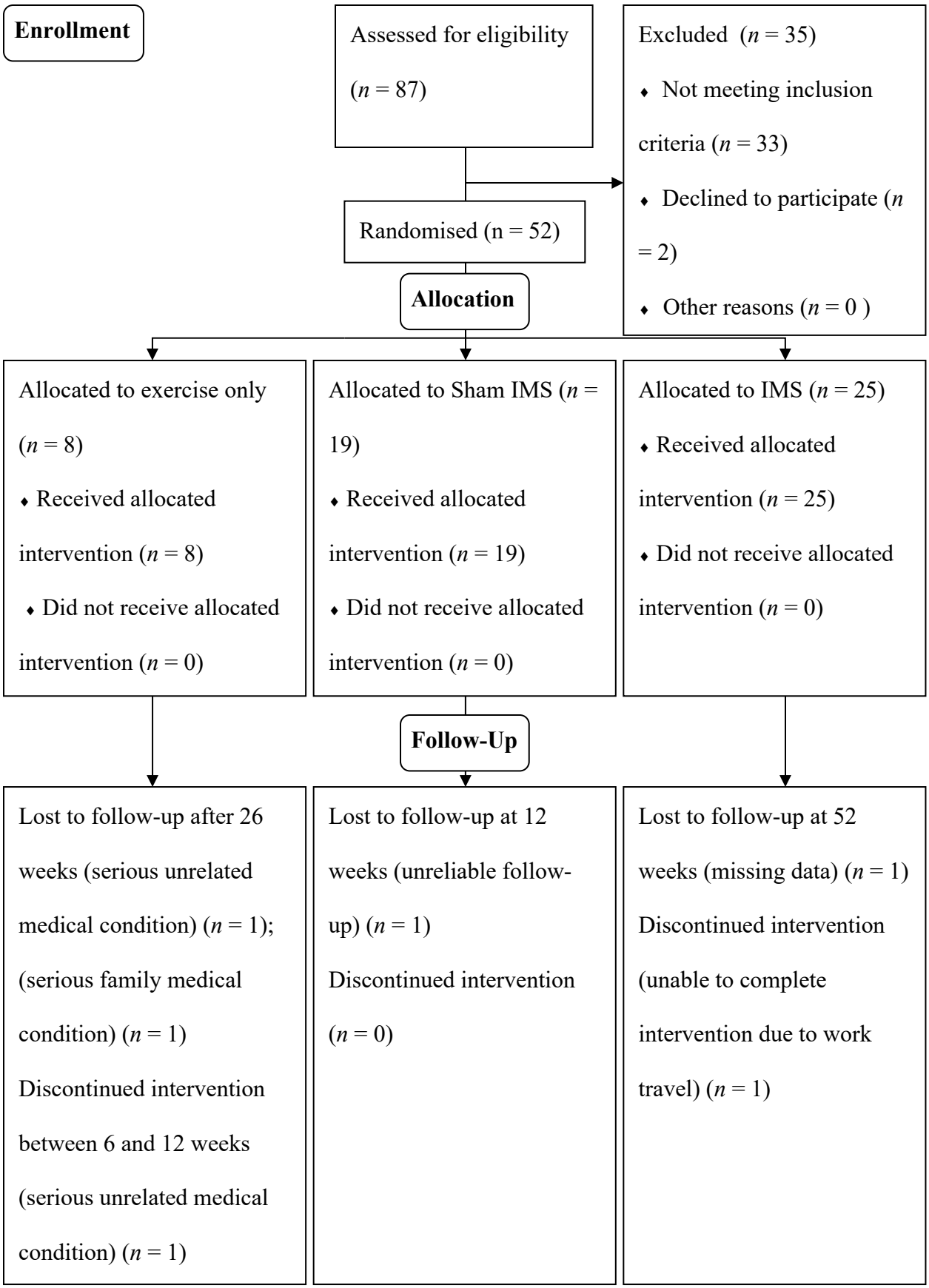
two observations, the weaker their correlation is. We also tested for interactions between group and time, and between baseline VISA and group. The same modelling process and covariance structure was used for dorsiflexion range of motion (both bent and straight knee) and tendon thickness. Global Rating of Change was treated as a binary variable (success or failure) and tested using Pearson's chi-squared test. All available data for all patients who received their allocated treatment were included in the analysis, regardless of whether their data set was complete. All patients were analysed according to their allocated treatment (i.e., intention to treat). We did not impute or replace any missing values but rather fitted all the available data to the model. Actual, not modelled, data are presented. An interim analysis was planned at the midpoint of recruitment, and this was conducted slightly ahead of schedule after the first 18 subjects were enrolled, in order to support release of further grant funds.

2.4 Results

2.4.1 Participants

Recruitment began in April 2013 and was completed in December 2018. The trial was stopped because the target sample size for Group 2 and Group 3 were achieved. Fifty-two participants were allocated into three groups (Figure 2.1) which were demographically and clinically similar (Table 2.1). Thirty-one (60%) participants identified running as the likely mechanism of injury to their Achilles tendon. Other reported mechanisms of injury were walking (n = 7, 13% of participants), jumping (n = 3, 6%), soccer (n = 3, 6%), hiking (n = 2, 4%), basketball (n = 1, 2%), bus driving (n = 1, 2%), swing dancing (n = 1, 2%), squash (n = 1, 2%) and tennis (n = 1, 2%). Twenty participants (64% of runners) had stopped running due to their Achilles pain. On entry to

the study, 24 participants were walking for exercise, two were hiking, 13 were running, one per activity were still participating in soccer, basketball, bus driving and swing dancing.


Enrollment
Assessed for eligibility (n = 87)
Excluded (n = 35)
Not meeting inclusion criteria (n = 33)
Declined to participate (n = 2)
Other reasons (n = 0)
Randomised (n = 52)
Allocation
Allocated to exercise only (n = 8)
Received allocated intervention (n = 8)
Did not receive allocated intervention (n = 0)
Allocated to Sham IMS (n = 19)
Received allocated intervention (n = 19)
Did not receive allocated intervention (n = 0)
Allocated to IMS (n = 25)
Received allocated intervention (n = 25)
Did not receive allocated intervention (n = 0)
Follow-Up
Lost to follow-up after 26 weeks (serious unrelated medical condition) (n = 1); (serious family medical condition) (n = 1)
Discontinued intervention between 6 and 12 weeks (serious unrelated medical condition) (n = 1)
Lost to follow-up at 12 weeks (unreliable follow-up) (n = 1)
Discontinued intervention (n = 0)
Lost to follow-up at 52 weeks (missing data) (n = 1)
Discontinued intervention (unable to complete intervention due to work travel) (n = 1)

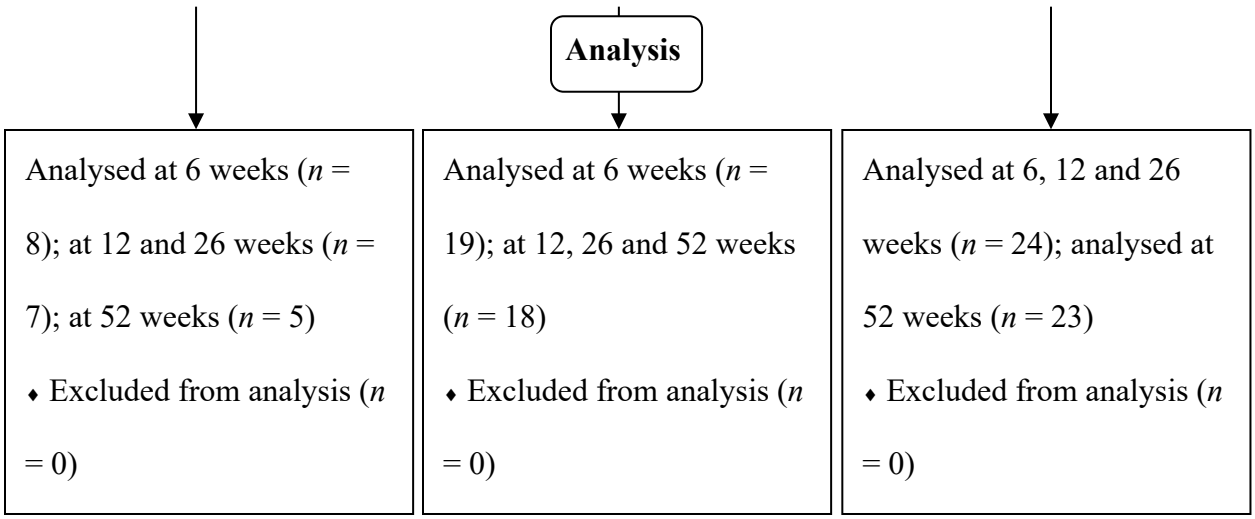


Figure 2.1: CONSORT flow diagram

Baseline Data	Exercise only (Group 1)	Sham IMS (Group 2)	IMS (Group 3)	Overall
Female, male	5 (63%), 3 (38%)	8 (42%), 11 (58%)	15 (60%), 10 (40%)	28 (54%), 24 (46%)
VISA-A: mean (SD)	56.1 (24.1)	58.5 (17.0)	57.7 (12.7)	57.8 (15.9)
Age: mean (SD)	47 (7.2)	46 (7.6)	51 (5.8)	48 (7.0)
Physical Activity level (IPAQ)				
1 = inactive,	0 (0%)	0 (0%)	1 (42%)	1 (2.0%)
2 = minimally active,	0 (0%)	6 (67%)	9 (38%)	15 (30%)
3 = highly active	8 (100%)	3 (33%)	14* (58%)	35 (67%)
Symptom Duration: mean (SD) in months	7.6 (7.5)	18 (16)	21 (15)	18 (15)

Table 2.1: Baseline characteristics of study subjects. *One missing data point. IMS – intramuscular stimulation, SD – standard deviation, IPAQ – International Physical Activity Questionnaire: a self-reported measure of physical activity

2.4.2 Success of blinding

At 12 weeks, when asked to state what group they thought they were in, most participants had difficulty selecting one or the other group. There were three missing data points; two from Group 3 and one from Group 2. In Group 3, 81% of participants correctly guessed they received IMS. This is perhaps understandable because IMS typically elicits noticeable sensations. In Group 2, 67% of participants correctly guessed they were in the sham needling group. Thus, the blinding may be said to be only partially successful.

2.4.3 Missing data

Most missing data were a result of participants' leaving the study (i.e., all subsequent measurements after a certain time point were missing), yielding a retention rate of 94% at 12 weeks and 88% at one year. After one year, the number of participants who had left the study were two (Group 3), one (Group 2), and three (Group 1). Missing tendon thickness values also occurred at a particular time point for 3 participants due to unusable or missing ultrasound scans: one at 6 weeks and two at 26 weeks. Global Rating of Change values were missing for a Group 3 participant at 26 weeks, but not at the other timepoints. The linear modeling did not impute missing values but fitted all available data to the model. All analysis was completed by the original assigned group.

2.4.4 Exercise compliance

Based on the information recorded by patients in the exercise diaries, the percentage of participants who completed at least 75% of their prescribed exercise sessions was 83% (Group 3), 92% (Group 2) and 100% (Group 1).

2.4.5 Primary outcome: VISA-A at 12 weeks

Across all groups, the majority (71%) of patients experienced an improvement in VISA-A score of ≥ 12 points from 0 to 12 weeks; the improvement in VISA-A in all groups was statistically significant ($p < .001$). There was no significant difference in the magnitude of improvement between treatments ($p = .13$), and no significant interaction between group and time ($p = .51$) or group and baseline VISA-A ($p = .32$) (Figure 2.2). The mean (and standard deviation) improvements in VISA-A score were: Group 3, 18 (13); Group 2, 18 (11); Group 1, 26 (21).

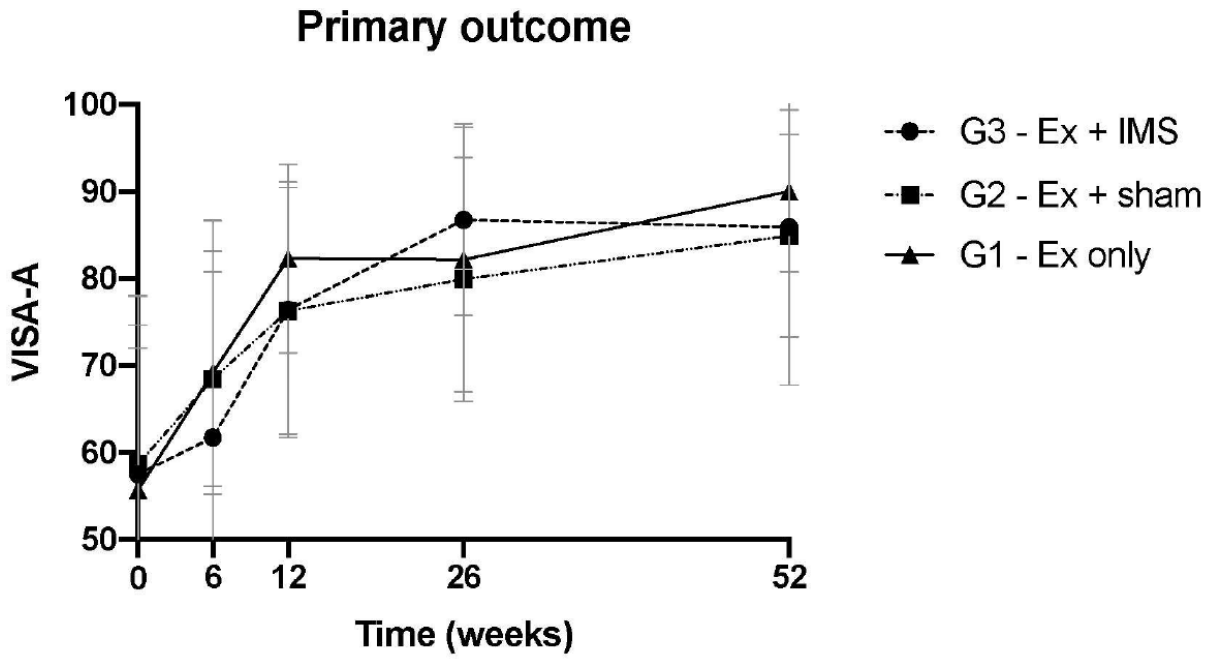


Figure 2.2: Change in symptom severity over time. Mean and standard deviation are shown. VISA-A - Victorian Institute of Sport Assessment-Achilles questionnaire score. Ex - exercise. IMS - intramuscular stimulation (i.e., dry needling)

2.4.6 Secondary outcomes

The percentage of patients who rated themselves as much or very much improved (i.e., "treatment success") was not significantly different at any timepoint (Table 2.2: 12 weeks, p = .94; 26 weeks, p = .62; 52 weeks, p = .71). Pearson's Chi-squared testing showed there was no significant difference in the distribution of Global Rating of Change rating between Groups 1, 2 and 3 at any time point (Figure 2.3).

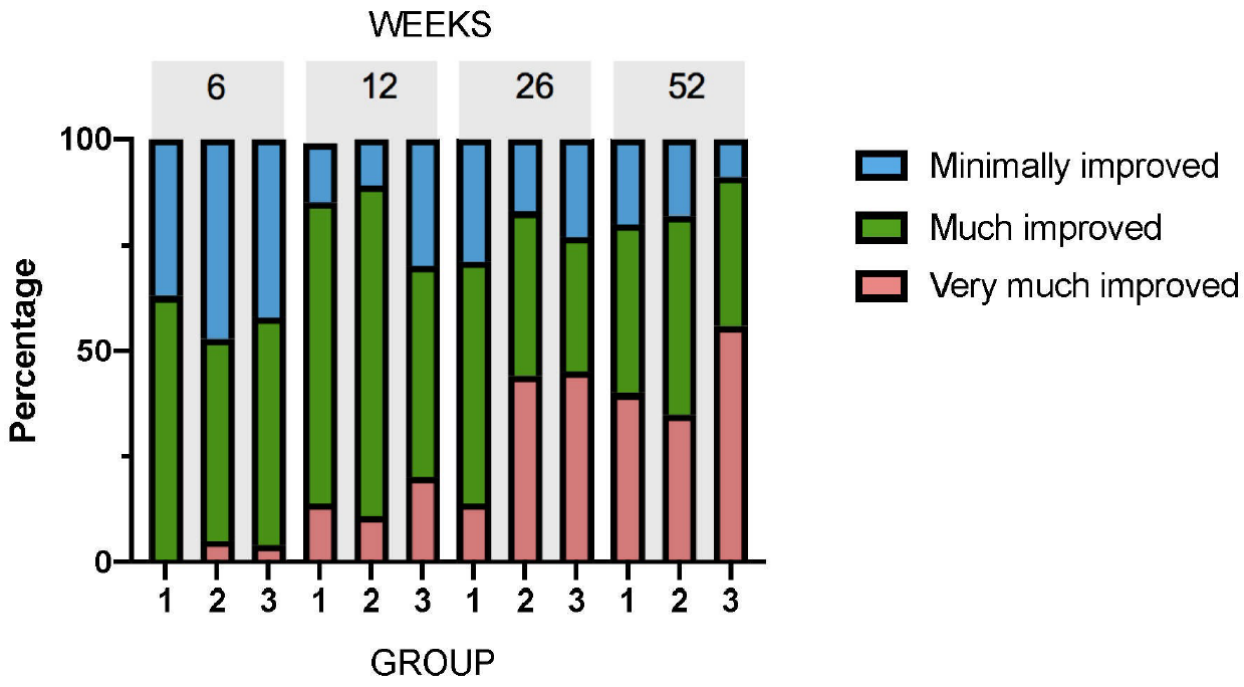


Figure 2.3: Comparison of Global Rating of Change over time. 1 = very much improved, 2 = much improved, 3 = minimally improved. Group 1 - exercise only, Group 2 - sham IMS + exercise, Group 3 – IMS + exercise. Not shown on graph: one participant in Group 3 was unchanged (Global Rating of Change = 4) at 26 weeks, and one participant in Group 4 was minimally worse (Global Rating of Change = 5) at 52 weeks

On the whole, the tendon thickness significantly decreased over 12 months regardless of group allocation (p < .01) (Table 2.2), despite that fact that there was a significant interaction between

baseline value and group (p = .007) indicating a slight allocation bias for this outcome measure. Dorsiflexion range of motion did not differ between groups when measured either with the knee straight (p = .124) or bent (p = .474) and did not significantly improve over time (p = .277 and p = .328 and respectively); baseline range of motion was the only significant predictor (p < .001 for both straight and bent knee).

	Baseline	6 weeks	12 weeks	26 weeks	52 weeks
Tendon thickness (mm)					
Group 3	9.2 (2.3)	9.3 (2.1)	8.9 (2.1)	9.0 (2.3)	8.6 (2.4)
Group 2	8.2 (1.6)	8.2 (1.7)	8.1 (1.6)	7.9 (1.6)	7.5 (1.3)
Group 1	8.7 (1.7)	8.1 (2.0)	8.4 (2.6)	8.2 (2.6)	6.3 (0.3)
Range of motion, SK (degrees)					
Group 3	41 (11)	—	45 (11)	—	47 (11)
Group 2	44 (8.2)		43 (7.6)		43 (9.5)
Group 1	34 (4.8)		37 (1.8)		36 (9.3)
Range of motion, BK (degrees)					
Group 3	45 (8.6)	—	48 (9.0)	—	50 (7.8)
Group 2	47 (7.9)	—	47 (7.3)	—	46 (10)
Group 1	42 (4.6)	—	43 (2.4)	—	45 (4.7)
Treatment success (%)					
Group 3	—	58	71	78	92
Group 2	—	52	89	83	82

Group 1	—	63	85	71	80

Table 2.2: Secondary outcome measures. Group 3 - IMS + exercise; Group 2 - sham IMS + exercise; Group 1 - exercise only. Values are presented as mean (standard deviation). SK - straight knee. BK - bent knee.

2.4.7 Side effects

Most individuals in the IMS group reported acute localised sensation when the needles were inserted to the depth of the muscle, reporting a deep ache of variable intensity and/or a muscle twitch/contraction. Many in the IMS group also reported post-treatment soreness of variable intensity and duration. Both of these were expected treatment effects, and likely contributed to the difficulty in blinding to group allocation, as in contrast there was almost no sensation reported from needle insertion to the depth of the skin only in the sham IMS group – only the very occasional reporting of mild sharpness - and no reporting of post-treatment soreness. When a sensation of sharpness on needle insertion occurred in either needling group, the needle was immediately removed, and replaced at an adjacent site where no sharpness was elicited and left *in situ* in that location. As expected, occasional minor bruising resulted from some of the needle insertions in the IMS group. There were no severe adverse events reported (including pneumothorax, organ injury, nervous system injury or infection). No patient withdrew due to needling side effects.

2.5 Discussion

This study failed to demonstrate a greater improvement in symptoms of Achilles tendinopathy in patients who received IMS and exercise, compared either to sham IMS and exercise, or to exercise alone. The magnitude of improvement observed in all three groups in the primary outcome measure (VISA-A) was as expected, based on previous studies with similar exercise programs.(161)

In some jurisdictions, IMDN, including IMS, is widely used by physiotherapists and other health professionals as an adjunct treatment for tendinopathies and other conditions, with some support for conditions such as low back pain.(156) There is minimal evidence on whether this treatment technique (or other needle-based techniques such as acupuncture) is effective for Achilles tendinopathy, or other tendinopathies.

A variety of theories have been advanced about the potential mechanisms whereby IMDN may influence musculoskeletal pain; however, since there did not appear to be an important clinical effect on Achilles tendon pain in this trial, we will not speculate about potential treatment mechanisms here. Regarding the possible effect in this study of IMS on muscle length, however, the main mechanism that has been proposed for this effect is the induction of a local twitch response. The local twitch response has been described as a reflex contraction of the muscle fibres that form a taut band that can be elicited by inserting a needle into the band.(162) local twitch responses induced by IMDN have been associated with the following changes within muscles: a reduction in excess electrical activity,(163) a reduction in local excess levels of the

neurotransmitters Substance P and calcitonin gene related peptide,(164) and a reduction in mechanical hypersensitivity.(165) We did not document whether a local twitch response occurred with intramuscular needle insertions in the IMS group in this study.

Another limitation is that we were not able to objectively measure one of the prescription parameters for the type of needling used here (presence of taut bands within muscles of the L5-S2 segments). We recognise that a technology has not been developed that is able to identify or measure differences in hardness of different regions of muscle with the robustness required for a study of this kind, although shear wave elastography shows promise if the technology can be further refined. Nevertheless, manual therapists attest to the ability of the human sense of touch to palpate differences in hardness of different regions of the same muscle. This was the method used to determine the location for intramuscular needle insertions in this study, and we acknowledge this method as a limitation.

This study has some other limitations. Our sample size was relatively small, particularly for the exercise-only group which was mainly included as a reference group to ensure that the exercise program was performing similar to the reports. Our power calculation assumed that the standard deviation of the VISA-A score would be 12, and in fact it was 14. This indicates that for the primary outcome, the required sample size to detect difference between sham and IMS needling (the main study question) was 22 per group, which was nearly met for Group 2 and Group 3. Thus, although we cannot exclude the possibility that the trial was under-powered, nevertheless it seems unlikely that including a few more participants in the sham IMS group would alter the observed mean improvement in VISA-A (Group 3, 18 (13); Group 2, 18(11)). An interesting

finding is that blinding was only partially successful, and yet despite this a placebo effect was not observed for either needling group. The results of this study cannot be generalised to other patient populations such as elite athletes or those with insertional Achilles tendinopathy, or to patients who may be treated with needling alone rather than in combination with exercise.

Chapter 3: Quantitative Sensory Testing of Nervous System Dysfunction and Sensitisation in Chronic Subacromial Shoulder Pain

3.1 Synopsis

Background: Subacromial pain syndrome (SAPS) is a common musculoskeletal disorder, accounting for 85% of all shoulder complaints.(50) The mechanisms of pain production in this disorder are only partially understood, which hampers intervention strategies.

Objective: To conduct quantitative sensory testing (QST) and pain mapping of a SAPS population compared with healthy controls

Design: Cross-sectional study

Setting: People referred to specialist sports medicine physician care for SAPS pain in a sports medicine clinic in Vancouver, Canada

Patients: 21 SAPS patients and 21 age- and sex-matched healthy controls

Measurements: QST - static (pressure pain threshold, mechanical pain threshold, heat pain threshold) and dynamic (conditioned pain modulation (CPM) and temporal summation) - and body charts shaded for areas of pain related to shoulder pain

Results: The estimated mean difference of pain pressure threshold at deltoid in SAPS compared to healthy controls was -92.4 kPa (95% confidence interval (CI): -260.1, 75.3), adjusted for covariates. Reduced pressure pain thresholds in SAPS at infraspinatus (locally), with an unadjusted estimated mean difference of -203 kPa (95% CI: -383, -22.7), and tibialis anterior (remotely), with an unadjusted estimated mean difference of -196 kPa (95% CI: -376, -15.4), were observed. None of the exploratory hypotheses regarding heat pain threshold, mechanical pain threshold, CPM or temporal summation hypotheses could be confirmed with either statistical significance, or with estimated mean differences that suggested practical significance. Of the 21 SAPS participants, 19 demonstrated pain beyond the subacromial area in body chart reporting of areas of pain that the participants associated with their shoulder pain.

Limitations: There was insufficient power to detect the effect size of interest for the primary hypothesis related to the primary outcome, i.e., comparing the mean pressure pain threshold at deltoid between the healthy control and SAPS groups. The participants in the SAPS group were enrolled using convenience sampling. which is susceptible to self-selection bias.

Conclusions: People with SAPS demonstrate reduced pressure pain thresholds in local tissues, tissues that have the same segmental nerve supply and in remote tissues. They also demonstrate spreading sensitisation. They do not demonstrate reduced mechanical pain thresholds, heat pain thresholds, augmented temporal summation or impaired CPM. Further research is needed to elucidate the relative contributions of central sensitisation and peripheral sensitisation to this sensory profile.

3.2 Introduction

Chronic musculoskeletal pain is common, difficult to manage, is a major burden to those who suffer from it, and to society as a whole.(166) Individuals suffering from chronic pain are endlessly subjected to “an unpleasant sensory and emotional experience”(167), in itself a burden, but also find their personal relationships, social interactions, quality of life, mental health, finances and ability to work negatively impacted.(168) On a societal level, chronic pain is a great cost financially to the medical system and in terms of lost productivity.(169) Efforts to better manage chronic pain and reduce the tremendous burden it places on the individual and society are being made in the physical, pharmaceutical and psychological fields of medicine. It is widely accepted that the results of these efforts will be improved by devising interventions that target the specific underlying pain mechanisms involved.(4,170,171) This concept provides the rationale for the proposed study, which looks to investigate potential pain mechanisms in chronic SAPS.

The experience of pain does not necessarily have a linear relationship with stimulus input parameters, particularly in chronic pain states.(4,172) The pain system is subject to modulation due to the extremely plastic nature of the nervous system.(4,172) This plasticity is believed to be responsible for the development of chronic pain states in which net gain or an overall state of increased excitation in the components of the nervous system that convey the perception of pain is induced.(172) One of these plasticity mechanisms thought to cause musculoskeletal pain to become chronic is nervous system sensitisation.(1–3) Nervous system sensitisation includes both peripheral nervous system sensitisation - “increased responsiveness and reduced threshold of

nociceptive neurons in the periphery to the stimulation of their receptive fields",(36) and central nervous system sensitisation - "increased responsiveness of nociceptive neurons in the central nervous system to their normal or subthreshold afferent input",(36) and these processes are understood to interact.(172)

Peripheral sensitisation can occur in the presence or absence of inflammatory mediators, but is best understood when such mediators are present(2) - inflammatory mediators enhance the excitability of nociceptors by their direct action on peripheral nerve terminal receptors, but also by altering nociceptor gene expression (which also acts to amplify local neurogenic inflammation).(2) It manifests as hyperalgesia – "increased pain from a stimulus that normally provokes pain",(173) allodynia – "pain due to a stimulus that does not normally provoke pain",(173) and/or spontaneous pain.(174) Peripheral sensitisation is a process that occurs within the nociceptive system via altered function of nociceptors, and generally requires ongoing local pathology to occur.(4)

Central sensitisation is a use-dependent phenomenon(1,4) that involves changes in the properties of central nervous system neurons.(4) The process involves activity-dependent sensitisation of post-synaptic receptors, disinhibition of inhibitory interneurons and/or activation of microglial cells in the central nervous system.(2) It manifests as pain hypersensitivity, after-sensations, and/or enhanced temporal summation due to the resultant increases in synaptic efficacy and reductions in inhibition.(42) In contrast to peripheral sensitisation, central sensitisation can produce the perception of pain in the absence of peripheral pathology or noxious stimuli.(4) It involves altered function of the somatosensory system more broadly, in contrast to peripheral

sensitisation which occurs solely within the nociceptive system.(4) Central sensitisation has been shown to occur in a wide range of pain states, including many musculoskeletal conditions such as myofascial pain, osteoarthritis, temporomandibular joint pain(42) and tendinopathy.(8)

Peripheral noxious input can lead to both peripheral and central nervous system sensitisation.(172,175) Graven-Nielsen and Arendt-Nielsen (2010) suggest that this sensitisation can then "spread" to the segmental ipsilateral and contralateral central nervous system at the level of the spinal cord, then extrasegmentally in the spinal cord and also to higher centres.(5) Evidence to support the model of contralateral spread includes findings induced by intramuscular injection of acidic saline in rodents, i.e., the development of mechanical hyperalgesia (to von Frey filaments and noxious pinch) remotely unilaterally, and also contralaterally(112,113); and receptive field expansion to include the contralateral side (shown in extracellular recordings of spinal wide dynamic range neurons).(112) Evidence to support the model of segmental and extrasegmental spread includes the appearance of new receptive fields remote from an injection site of (noxious) bradykinin in animals(107); segmental and extrasegmental patterns of pain referral in humans in response to hypertonic saline injections(106); and clinical observations of initially localised pain spreading and becoming generalised.(114) Evidence to support the model of spread to higher centres includes functional MRI findings of increased activation in pain-related brain areas in response to nociceptive pressure stimulation in people with chronic low back pain and fibromyalgia.(115) This concept of spreading sensitisation forms the rationale for investigating local, segmental and extrasegmental pain modulation in this proposed thesis.

Another mechanism by which pain is proposed to become chronic is by dysfunction in the descending central nervous system pathways that facilitate or inhibit the transmission of nociceptive information.(176) For example, disruption of descending inhibitory pathways could upset the "balance" of endogenous pain modulatory systems to favour descending facilitatory pathways, which may promote and sustain chronic pain.(176) A component of the descending inhibitory system is the diffuse noxious inhibitory control (DNIC) system.(177) DNIC was first described by Le Bars et al. in 1979.(178) Their experiments in rats showed that the activation of convergent wide dynamic range dorsal horn neurons by noxious input could be inhibited by concurrent noxious stimulation of body regions outside the receptive field of the dorsal horn convergent neurons. This occurred in response to an array of concurrent noxious stimuli types – pinch, (bradykinin) injection, heat, and electrical stimulation. The effect was shown to occur by means of a "spino-bulbo-spinal loop",(179) with the subnucleus reticularis dorsalis (in the caudal medulla) shown to play a key role in the mechanism.(180) In humans, DNIC effects have been examined through a paradigm termed "conditioned pain modulation" (CPM)(181) - whereby remote stimulation with one noxious stimulus ("counter-irritation"(182)) reduces the pain intensity of a separate local noxious stimulus.(124,181) CPM can be assessed experimentally by comparing the pain response to a noxious test stimulus under two different conditions – alone, and during/after exposure to another painful stimulus applied to a remote body area. CPM is a normal function of a healthy nervous system and is considered to reflect the function of endogenous pain inhibition mechanisms, primarily of midbrain-centred descending inhibitory nervous system pathways,(183) but also of other higher brain-centred mechanisms involving cognition and emotion,(184,185) e.g., expectancy-induced analgesia.(182)

Impaired CPM may reflect impaired function of descending inhibitory pathways(177) and is a common feature of chronic pain, having been observed in osteoarthritis,(124) fibromyalgia,(124,186) chronic tension-type headache,(125) musculoskeletal shoulder pain,(92) irritable bowel syndrome,(187–190) temporomandibular disorder,(127) posttraumatic trigeminal neuropathy,(191) chronic pancreatitis,(192) and postherpetic neuralgia.(193) A systematic review conducted by Lewis et al. (2012)(124) concluded that CPM was impaired in people with chronic pain conditions. Meta-analysis found an overall large(194) effect size of 0.78 (95% CI: 0.48–1.08). The review included a myriad of chronic pain conditions for consideration, including neurogenic/neuropathic pain states, fibromyalgia, complex regional pain syndrome, irritable bowel syndrome, temporomandibular disorder, chronic fatigue, arthritis, stroke, whiplash, headache, migraine, vestibulodynia and pancreatitis.(124)

This study conducted pain mechanism-related research on the musculoskeletal condition of SAPS. The research included conducting quantitative sensory testing (QST) of people with SAPS to assess nervous system function in this disorder. Shoulder pain is the third most common musculoskeletal complaint,(195) and rotator cuff disorders are accepted to be the most common cause of shoulder pain, (195,196) with 85% of all shoulder complaints having been attributed to this diagnosis.(197) Tendinopathy – structural tendon abnormality causing pain, tendon thickening and impaired function - is a common source of chronic pain.(21) Rotator cuff tendinopathy occurs most often in the supraspinatus tendon, followed by the infraspinatus tendon, and, to a much lesser degree, the teres minor tendon.(198) Discriminating between these individual tendons of the posterior cuff as sources of pain is particularly difficult as anatomically they blend with each other and with the shoulder joint capsule.(199) This means that they

function together, not in isolation, and distribute forces between each other on loading. As such, they "test" together on diagnostic loading tests, even when such tests are biomechanically biased towards preferentially loading one component over another.(13,200) For this reason, the proposed study will examine pain sensitivity patterns in the posterior cuff tendons as a group, rather than attempting to discriminate pain as being generated by a specific tendon. Anatomical dissection studies demonstrate innervation of the posterior cuff tendons by the suprascapular nerve.(201) The subacromial-subdeltoid bursa commonly exhibits pathology when the posterior rotator cuff exhibits pathology.(87,88) It is a pain-sensitive structure(89) (innervated by the suprascapular nerve and the lateral pectoral nerve(90)) whose pathology may present very similarly on history-taking and physical examination as a symptomatic posterior cuff,(87,91) i.e., it may be a source of pain in people with superolateral shoulder pain and have similar history and physical examination findings to posterior cuff pathology. For this reason, the proposed studies will investigate SAPS (rotator cuff tendinopathy with or without subacromial-subdeltoid bursitis).

Tendon pain is a less well understood pathology, which adds to the value of investigating pain mechanisms in SAPS. That local nociceptors are the primary key to the pain of tendinopathy seems to be accepted by most commentators, regardless of whether they are activated by primary nociception, inflammation, by other non-inflammatory neuromodulatory effects resulting from local tissue changes, such as altered tenocyte function, altered tissue pH, and neovascularisation.(45) Nociception is a complex, plastic process, however, subject to the influence of many intra- and interneuronal processes.(32) Nociception also stimulates peripheral and central sensitisation mechanisms,(2) such that all three processes are interconnected and are

thought to occur to varying degrees in painful musculoskeletal conditions.(5) Evidence is emerging that this is the case in painful tendinopathy.(8) The attempt to develop treatments to address any one of these pain mechanisms seems worthwhile, in the management of tendon pain.

Nervous system dysfunction has been found to occur in chronic shoulder pain.(100,101) Details of key studies in this area are briefly summarised below.

1. Impaired CPM has been demonstrated by Valencia et al. (2012) in a chronic shoulder pain population awaiting surgical management.(92) The group was heterogenous, including complaints of anterior, lateral or posterior shoulder pain; and diagnoses of rotator cuff tendinopathy; adhesive capsulitis; and labral lesions. The test stimulus used was a suprathreshold heat pain stimulus (that provoked a 50/100 level of pain) applied to the thenar eminence of the nonsurgical side and the conditioning stimulus used was the cold pressor test (surgical side hand). Although the absolute difference in pain ratings was the same for those with shoulder pain as their age- and sex-matched controls, the percentage change was significantly less in the group with chronic shoulder pain (mean = 17) compared to the control group (mean = 54) (CIs not provided). Of interest in both clinical and research settings is whether or not reduced CPM is present in painful SAPS specifically, in part because it is the most common cause of shoulder pain,(195,196) and also because the pathogenesis of tendon pain generally is not well understood.
2. Valencia et al. (2012)(92) also found significantly facilitated temporal summation of heat pain in this group of chronic shoulder pain patients. Temporal summation of pain is defined as an increasing perception of pain intensity in response to repetitive noxious stimuli of equal

magnitude.(202) Its manifestation in humans is seen to be equivalent to the initial phases of "wind-up" demonstrated in animals, whereby quickly-repetitive noxious stimuli carried by peripheral C-fibre afferents causes a prolongation of fibre discharge of dorsal horn neurons in the spinal cord such that continuous discharge at an increased rate result.(203) Enhanced temporal summation is one of the manifestations of central sensitisation – the "increase in the excitability and synaptic efficacy of neurons in central nociceptive pathways".(42 pS2). Measures of temporal summation/wind-up have been used as proxies for neural plasticity and central sensitisation in the spinal cord.(204)

3. Gwilym et al. (2011)(94) found significantly reduced mechanical pain thresholds to punctate stimuli and increased pain levels to a 256 mN punctate stimulus (tested over the deltoid insertion), suggestive of central sensitisation,(205) in people with chronic shoulder pain attributed to subacromial impingement syndrome, compared with their unaffected side. They also found reduced mechanical pain thresholds in the shoulder pain group compared with a control group.
4. Hidalgo-Lozano et al. (2010)(95) found significantly reduced pressure pain thresholds, suggestive of peripheral and/or central sensitisation,(205) in six local shoulder muscles and also the tibialis anterior in people with chronic shoulder pain diagnosed as subacromial impingement syndrome compared with controls.
5. Paul et al. (2012)(96) found significantly reduced pressure pain thresholds in the local deltoid, but also the contralateral deltoid and tibialis anterior in people with chronic shoulder pain (diagnosed as subacromial impingement syndrome) compared with controls.
6. Coronado et al. (2011)(97) found significantly reduced pressure pain thresholds in local shoulder (supraspinatus, infraspinatus and the acromion combined) and distal (brachioradialis

and masseter combined) tissues on the affected side compared to the contralateral side in a mixed group of chronic shoulder pain patients.

7. Coronado et al. (2014)(93) found significantly reduced pressure pain thresholds in the ipsilateral acromion on the affected side compared to the contralateral side in a mixed group of chronic shoulder pain patients, but not at the ipsilateral masseter. They also found significantly reduced pressure pain thresholds bilaterally at the acromion in the shoulder pain group compared to healthy controls. Pressure pain threshold at the masseter was not significantly different between the shoulder pain group and controls. Thermal pain measures found significantly increased pain intensity perception with temporal summation of heat pain in the shoulder pain group compared to healthy controls. There was no significant difference in heat pain threshold or tolerance between groups. In a summary of their findings, the group used peripheral and central sensitisation indices based on 25^{th} and 75^{th} percentile categories to conclude that both peripheral and central sensitisation can occur in chronic shoulder pain, but that the pattern between individuals was not consistent.
8. Yan et al. (2017)(98) found significantly reduced pressure pain thresholds at four acupuncture point sites in the shoulder region in the affected side compared to the contralateral (unaffected) side in people with shoulder pain of longer than 6 weeks. They also found significantly reduced pressure pain thresholds at the same points in the unaffected side of the shoulder pain group compared to the same side in healthy controls. They used percentile-based sensitisation indices to conclude that both peripheral (37-77%) and central (43-63%) sensitisation occur in chronic shoulder pain, again in variable patterns between individuals, notably with 30-50% of people demonstrating both.

9. Kuppens et al. (2017) found no significant difference in pressure pain threshold, vibration detection threshold, mechanical detection threshold, temporal summation (110% pressure pain threshold), or CPM (test stimulus – pressure pain threshold, conditioning stimulus – cold pain to the thenar eminence) between a group with non-specific shoulder pain and healthy controls. Notably, the measures were all taken at sites remote from the shoulder – the upper trapezius, calf muscle and the dorsal middle finger.

These findings have prompted calls for more in-depth, well-conducted investigations of these nervous system functions in shoulder pain, particularly by expanding the focus of testing beyond what has been predominantly (static) pressure pain threshold testing(206) and including dynamic testing,(92,97,98,100,101) such as temporal summation and CPM that reflect the state of ascending facilitation and descending inhibition of pain signaling, respectively.(207) These calls add to the rationale for conducting comprehensive QST in people with SAPS.

The goal of QST is to ascertain which particular nervous system pathways may be dysfunctioning in the pain state under investigation.(118) The QST protocol proposed for this thesis was derived largely from a comprehensive standardised protocol devised by the German Research Network on Neuropathic Pain (DFNS)(119) including: heat pain threshold tests for Aδ and C fiber function; mechanical pain threshold tests for Aδ and C fiber function using weighted pinprick stimulators; and temporal summation to mechanical (pinprick) pain. Pressure pain threshold for deep pain sensitivity to blunt pressure (muscle Aδ/III and C/IV fiber function) was also tested. CPM was also tested, using a protocol derived from recommendations made by Yarnitsky et al. (2015).(208) Two test stimuli were used – pressure pain and heat pain, with a

cold water bath used as the conditioning stimulus for both. The combination of these tests assessed for small fibre dysfunction, central sensitisation and altered endogenous pain modulation in SAPS.

Another aspect of the descending pain inhibitory system, of which CPM is a part, is its function to localise pain perception to the relevant body tissue. Descending inhibition dampens excessive sensitivity to noxious stimuli under normal resting conditions.(109) This tonic activity is proposed to optimise sensory discrimination by restricting the extent of receptive fields in the dorsal horn.(109) Enlargements of receptive fields have been shown to occur following prolonged peripheral nociceptive input(4) and are considered to reflect the activation ("unmasking") of previously inactive ("silent") synaptic connections in the dorsal horn.(209) These changes are considered to be a feature of central sensitisation as they have been shown to occur as a result of primarily central (not peripheral) nervous system changes, e.g., increased receptive field size is not reduced by administration of local anaesthetic to an injury site.(4) The unmasking of receptive fields by reduced descending inhibition as is also considered to be a mechanism involved in the development of referred pain.(72)

Central sensitisation by the expansion and development of new receptive fields(209) is thought to manifest as enlarged pain areas and referred pain areas. Enlarged pain areas (i.e., "spreading pain" - pain that "spreads" outside the primary nociceptive area) and referred pain (pain in an area isolated and distinct from the primary nociceptive area, apparent spontaneously without stimulation of the referred pain area)(106–108) are accepted to be a common occurrence in musculoskeletal conditions(102) having been reported in, for example, chronic shoulder pain(94)

and whiplash associated disorder.(104) They are seen to represent the manifestation of central nervous system sensitisation.(106–108) In the case of enlarged pain areas, sensitisation results from the enlargement of the receptive field size of dorsal horn neurons.(106) Enlarged receptive field sizes have been demonstrated in both chronic neck pain and chronic low back pain.(210) In the case of referred pain, sensitisation results from dorsal horn neurons receiving input from new receptive fields.(107) Measurements of enlarged pain areas and referred pain areas are, therefore, useful proxies for assessing the presence of central sensitisation related to the expansion of dorsal horn neuron receptive field size or input to dorsal horn neurons from new receptive fields.(204) These findings provide the rationale for including pain mapping to assess for the extent of the pain area experienced in SAPS.

Endogenous modulation of pain includes both descending inhibition and descending facilitation mechanisms.(109) The endogenous modulation system is exceedingly complex, involving modulation by structures in the cortex, diencephalon, brainstem and spinal cord.(109) Exerting particular influence are the brainstem structures: the periaqueductal gray (PAG) in the midbrain and the rostral ventromedial medulla (RVM).(109,211) A large number of neurotransmitters and other modulators, including glial cells and immune cells, are involved in the modulation process.(109) Important and much-studied among these modulators are the neurotransmitters noradrenaline (NA) and serotonin (5-HT); and endogenous opioid peptides.(109) Multiple receptor classes exist for these transmitters and their function may be pro- or anti-nociceptive in the dorsal horn and throughout the neuroaxis, as is the case for many modulators involved in endogenous modulation.(109)

It is considered that under normal circumstances, there is a "balance" between descending inhibition and descending facilitation, but that in pathological pain states, this balance may be disrupted.(211) The CPM psychophysical paradigm has been used in pain research settings to assess for impaired CPM, interpreted as representing a loss of descending inhibition influence.(212)

In summary, this study assessed for nervous system dysfunction in chronic SAPS, the most common cause of chronic shoulder pain,(91,195,197) using static and dynamic QST. In light of the frequent treatment failure of many chronically painful musculoskeletal conditions, where treatment paradigms focus on abnormalities of local tissue structure, function or mechanics, it has been argued that knowledge of any coexisting nervous system dysfunction, increasingly considered to be part of the pathophysiology of chronic musculoskeletal pain syndromes, may inform more successful management and treatment strategies for these types of problems.(213,214)

3.2.1 Background/rationale

The overarching hypothesis for this study was that people with chronic SAPS would exhibit QST findings suggestive of nervous system sensitisation locally, segmentally and extrasegmentally (remotely) compared to healthy controls. Specifically, it was hypothesised that people with chronic SAPS would exhibit hyperalgesia (decreased heat pain threshold, reduced mechanical pain threshold, reduced pressure pain threshold, facilitated temporal summation, and impaired CPM), and that the magnitude of sensitisation would be greatest locally, then segmentally, then

extrasegmentally. It was also hypothesised that people with chronic SAPS would exhibit enlarged pain areas.

3.2.2 Objectives

3.2.2.1 Primary outcome – pressure pain threshold

The primary outcome of interest was pressure pain threshold. This was the primary outcome of interest as this test assesses sensitivity of deep tissues, which are implicated in SAPS pain.

3.2.2.1.1 Primary hypothesis

The primary hypothesis was that pressure pain threshold values for the deltoid muscle (C5, C6 segmental nerve supply) are lower for people with SAPS compared to healthy controls.

The deltoid location was the primary hypothesis because it is segmentally associated with SAPS neuroanatomically, but is not anatomically related as it is not, by definition, one of the rotator cuff tissues. The priority of the research objective was to seek information regarding the state of the nervous system more globally, rather than simply locally, and so the deltoid location was prioritised over the infraspinatus and tibialis anterior locations.

3.2.2.1.2 Secondary hypotheses

The secondary hypotheses were that:

1. Pressure pain threshold values for the infraspinatus muscle (C5, C6 segmental nerve supply) are lower for people with SAPS compared to healthy controls

2. Pressure pain threshold values for the tibialis anterior muscle (L4, L5 segmental nerve supply) are lower for people with SAPS compared to healthy controls

3.2.2.2 Exploratory outcomes

The exploratory outcomes were:

1. Heat pain threshold
2. Mechanical pain threshold
3. Conditioned pain modulation-pressure pain 40 (PP40) (CPM effect)
4. Conditioned pain modulation-heat pain 40 (HP40) (CPM effect)
5. Temporal summation score

3.2.2.2.1 Exploratory hypotheses on the exploratory outcomes

The hypotheses related to the exploratory outcomes were:

1. Outcome: heat pain threshold
    a. Heat pain threshold values for the skin over the deltoid muscle (C5, C6 segmental nerve supply) are lower for people with SAPS compared to healthy controls
    b. Heat pain threshold values for the skin over the lateral aspect of the shin below the knee (L4, L5 segmental nerve supply) are lower for people with SAPS compared to healthy controls
2. Outcome: mechanical pain threshold
    a. Pinprick mechanical pain threshold values for the skin over the deltoid muscle (C5, C6 segmental nerve supply) are lower for people with SAPS compared to healthy controls

b. Pinprick mechanical pain threshold values for the skin over the lateral aspect of the shin below the knee (L4, L5 segmental nerve supply) are lower for people with SAPS compared to healthy controls

3. Outcome: conditioned pain modulation-PP40 (CPM effect)
    a. Conditioned pain modulation-PP40 (absolute and percentage changes) for the infraspinatus muscle (C5, C6 segmental nerve supply) is impaired for people with SAPS compared to healthy controls
    b. Conditioned pain modulation-PP40 (absolute and percentage changes) for the deltoid muscle (C5, C6 segmental nerve supply) is impaired for people with SAPS compared to healthy controls
    c. Conditioned pain modulation-PP40 effect for the tibialis anterior muscle (L4, L5 segmental nerve supply) is impaired for people with SAPS compared to healthy controls
4. Outcome: conditioned pain modulation-HP40 (CPM effect)
    a. Conditioned pain modulation-HP40 for the skin over the deltoid muscle (C5, C6 segmental nerve supply) is impaired for people with SAPS compared to healthy controls
    b. Conditioned pain modulation-HP40 for the skin over the lateral aspect of the shin below the knee (L4, L5 segmental nerve supply) is impaired for people with SAPS compared to healthy controls
5. Outcome: temporal summation score

a. Temporal summation to punctate (sharp) pressure values for the skin over the deltoid muscle (C5, C6 segmental nerve supply) is augmented for people with SAPS compared to healthy controls
b. Temporal summation to punctate (sharp) pressure values for the skin over the lateral aspect of the shin below the knee (L4, L5 segmental nerve supply) is augmented for people with SAPS compared to healthy controls

6. Pain area

   The size of the pain area (in pixels) for people with (chronic) SAPS is larger than localised acromial pain.

3.3 Methods

3.3.1 Study design

The study design was cross-sectional. There were two groups – SAPS and healthy controls (age- and sex-balanced). Healthy control participants were purposively sampled based on the sample of SAPS participants using stratified random sampling, balanced for sex and age range in decades, i.e., 18-29, 30-39, 40-49 etc. This was done as previous research has shown significant differences in certain QST measures on this basis, e.g., increased pain sensitivity in women, and decreasing thermal and mechanical sensitivity with age.(215,216)

3.3.2 Setting

History-taking and physical examination screening were undertaken by Lyndal Solomons (LS) at Allan McGavin Sports Medicine Centre (AMSMC) (2553 Westbrook Mall, Vancouver). Both

groups had baseline demographic data collected by LS (AMSMC) and underwent QST by John Kramer's lab members at ICORD (the Blusson Spinal Cord Centre at Vancouver General Hospital). The period of recruitment was August 16, 2019, to November 12, 2021. The period of data collection was September 19, 2019, to December 15, 2021.

3.3.3 Participants

3.3.3.1 Population - sources and methods of selection

The target population was adults (18 years of age and older) with SAPS. The source population was adults with SAPS in the Greater Vancouver (BC, Canada) area. The sampling method for SAPS participants was convenience sampling in response to recruitment efforts made through AMSMC. The sampling method for healthy controls was purposive, based on SAPS participants' sex and age (as described above). Healthy control participants were recruited by word of mouth (team study members verbally asked friends and relatives if they would be willing to participate in the study) and by placing posters in the same neighbourhood as AMSMC (i.e., around UBC campus).

3.3.3.2 Eligibility criteria

3.3.3.2.1 Both SAPS and healthy control groups

Participants were excluded from either group if they: were under 18 years of age; were unable to communicate in English; had undergone previous surgery of any kind in the shoulder area (rotator cuff, bursa or otherwise); had sustained previous acromioclavicular joint disruption; had

undergone previous cervical spine surgery, e.g., fusion; had sustained previous glenohumeral joint dislocation or chronic instability (subjective reporting); or had a medical history of fibromyalgia or diabetes mellitus (Type 1 or Type 2).

3.3.3.2.2 SAPS group

Participants were excluded from the SAPS group if they had received injection therapy of any kind to the shoulder area (corticosteroid, platelet rich plasma, prolotherapy etc.) in the 12 weeks prior to study participation; used quinolone antibiotics during the 12 months preceding the onset of symptoms or since; or had historical (within 6 months of visit to AMSMC physicians) X-ray report findings of glenohumeral joint or acromioclavicular joint arthritis, or ultrasound imaging report findings that *did not* include findings of tendinosis, calcific tendinopathy, partial thickness tears (PTTs) (including rim rent tears), or full thickness tears (FTTs) of the supraspinatus, infraspinatus or teres minor tendons. Eligibility for inclusion in the SAPS group required strong clinical suspicion of SAPS as well as specific diagnostic criteria outlined in Appendix B.

3.3.3.2.3 Healthy control group

Eligibility for inclusion in the healthy control group required: no history of superolateral shoulder pain, or shoulder pain generally in the past 5 years; and no current musculoskeletal pain.

3.3.4 Variables

1. Pressure pain threshold (primary outcome variable)
2. Heat pain threshold (exploratory outcome variable)

3. Mechanical pain threshold (exploratory outcome variable)
4. Conditioned pain modulation-PP40 (exploratory outcome variable)
5. Conditioned Pain Modulation-HP40 (exploratory outcome variable)
6. Temporal summation score (exploratory outcome variable)
7. Pain area (exploratory outcome variable)

3.3.4.1 Potential confounders

1. Age – controlled for by age-balancing of groups in decades on recruitment; adjusted for statistically as covariate for primary hypothesis on the primary outcome
2. Sex – controlled for by balancing groups on recruitment; adjusted for statistically as covariate for primary hypothesis on the primary outcome
3. Physical activity level – adjusted for statistically as covariate for primary hypothesis on the primary outcome

3.3.5 Data sources/measurement

QST was undertaken by blinded assessors (JK lab members) and included tests for heat pain thresholds, mechanical pain (punctate/sharp) thresholds and pressure pain (mechanical – blunt) thresholds; temporal summation of pain (sharp) and CPM. QST encompasses a wide array of tests and protocols that generally assesses function of the somatosensory system in chronic pain conditions.(119) The goal of QST is to ascertain which particular nervous system pathways may be dysfunctioning in the pain state under investigation.(118) QST test results are vulnerable to variability caused by testing protocols/procedures and test subject performance, which can be controlled for by using standardised protocols.(121) An overview of the tests that were

performed is presented below in Table 3.1 and Table 3.2, with details of the methodologies in the following sections.

STATIC							
Test site			Local nerve supply	Segmental nerve supply	HPT	MPT	PPT
"Local"*	mm (deep)	infraspinatus muscle	suprascapular n.	C5, C6	-	-	✓
Segmental	mm (deep)	deltoid muscle	axillary n.	C5, C6	-	-	✓
	skin	skin over deltoid			✓	✓	-
Remote	mm (deep)	tibialis anterior muscle	common peroneal n.	L4, L5	-	-	✓
	skin	skin over lateral aspect of shin below the knee	lateral cutaneous n. of calf	L4, L5 (based on dermatome charts)	✓	✓	-

Table 3.1: An overview of the static quantitative sensory tests that were performed; *local to the posterior rotator cuff tissues and subacromial-subdeltoid bursa, i.e., the suprascapular nerve. Note that there were no cutaneous (skin) tests for "local" tissue (HPT or MPT) as the suprascapular nerve does not have a cutaneous supply, except for in 15% of people, where the cutaneous branch of the suprascapular nerve is presumed to be contained in the axillary nerve and supplies the skin on the proximal third of the lateral aspect of the arm within the territory of the axillary nerve(117)**. mm – muscle, n. – nerve, HPT – heat pain threshold, MPT – mechanical pain threshold, PPT – pressure pain threshold, ✓ – test performed, - – no test.**

DYNAMIC						
Test Site			CPM			Temporal summation (modality)
			CS modality	CS location	TeS modality	
"Local"*	mm (deep)	infraspinatus muscle	cold water bath pain at cold pain40	contralateral foot	blunt pressure pain at PP40	-
Segmental	mm (deep)	deltoid muscle			blunt pressure pain at PP40	-
	skin	skin over deltoid			contact heat pain at HP40	sharp mechanical pain

Remote	mm (deep)	tibialis anterior muscle		contralateral hand	blunt pressure pain at PP40	-
	skin	skin over lateral aspect of shin below the knee			contact heat pain at HP40	sharp mechanical pain

Table 3.2: An overview of the dynamic quantitative sensory tests that were performed; *local to the posterior rotator cuff tissues and subacromial-subdeltoid bursa, i.e., the suprascapular nerve. Note that there were no cutaneous (skin) tests for "local" tissue as the suprascapular nerve does not have a cutaneous supply, except for in 15% of people, where the cutaneous branch of the suprascapular nerve is presumed to be contained in the axillary nerve and supplies the skin on the proximal third of the lateral aspect of the arm within the territory of the axillary nerve(117)**. CPM – conditioned pain modulation, CS – conditioning stimulus, TeS – test stimulus, mm – muscle, PP40 – pressure pain40, HP40 – heat pain40**

Test sites were marked with a skin pen for use throughout the QST studies, as below:

1. Infraspinatus - point ¾ of the way along a line drawn from the inferior angle of the scapula to a point ¾ along the scapular spine from the lateral edge of acromial shelf to the base of the root of the spine of the scapula
2. Deltoid - midway between the lateral edge of the acromial shelf and the deltoid insertion
3. Tibialis anterior - 2 cm below the tibial tuberosity, 1 cm lateral to the anterior border of the tibia

4. Skin over the deltoid muscle - size of thermode contact area, i.e., 7.84 cm^2
5. Skin over the lateral aspect of the shin below the knee - size of thermode contact area, i.e., 7.84 cm^2

For the static QST test sites for the upper limb (shoulder), the side of testing for SAPS participants was the side of their shoulder pain complaint (or the worst shoulder if both shoulders were affected). The side of testing for SAPS participants was determined by the handedness of their matched SAPS participant, i.e., for the SAPS participant with whom the healthy control participant was matched, handedness was noted. If the SAPS participant's dominant hand was their affected shoulder side (or most affected shoulder side), the healthy control participant would also be tested on their dominant hand side. If the SAPS participant's affected shoulder side (or most affected shoulder side) was on their non-dominant hand side, the healthy control participant with whom they were matched would be tested on their non-dominant side.

For the static QST test sites for the lower leg, the leg ipsilateral to the upper limb static QST testing arm was used for testing. The ipsilateral side was used as the contralateral foot was "needed" as the conditioning stimulus site for the dynamic CPM testing. This avoided overstimulating one lower limb, by using it as the test site for both static and dynamic testing, as a consideration for participant comfort. Also laterality was considered unimportant regarding testing outcomes as the leg sites were used only to represent remote sites, which could be achieved by using either the ipsilateral or contralateral lower limb. This approach also allowed for consistency between participants testing exposures and the static and dynamic testing protocols, and avoided unnecessary skin marking for the participant.

For the dynamic QST testing, the same test sites were used as for the static tests, with the addition of contralateral limb test sites, that were only needed for CPM testing as conditioning stimulus sites. These sites were the foot contralateral to the shoulder that had been tested in the static tests, and the hand contralateral to the shoulder that had been tested in the static tests.

Static testing was performed in a single session. Dynamic testing was performed in a separate session 24 hours after, or as soon as otherwise possible after, the static testing session.

3.3.5.1 Pressure pain threshold (primary outcome variable)

Pressure pain threshold was measured using a manual computerised algometer, (JTECH Medical, Salt Lake City, UT, USA). Test sites were the infraspinatus, deltoid and tibialis anterior as described previously. An algometer tip size of 0.503 cm^2 (diameter 0.80 cm) (placed perpendicular to the skin) was used – a larger tip would require greater forces to be exerted by the tester (even though the pressure pain threshold magnitude is lower under these conditions), and hence greater strength required, such that the forces necessary would become too large to be practical; a smaller tip would have greater potential to cause tissue trauma due to higher resultant pressure pain threshold magnitudes(217) – especially in control groups (who presumably are not suffering from hyperalgesia and so will only experience pain once the stimulus reaches an intensity that is potentially tissue-damaging. A constant force application rate was used(217) - this is more easily achieved with a mechanical system, but is trainable with a manual system.(218) For a 0.503 cm^2 tip and a pressure application rate of 30 kPa/s, a force rate of 59.6 N/cm^2 will result (pressure = force/area). An application rate of 30 kPa/s was considered slow

enough to allow sufficient reaction time for participants to provide a more accurate pressure pain threshold without being so slow as to make it physically too demanding with respect to control and fatigue for the tester applying the algometer.(219)

Participants were taught to press the button at the onset of pain. Participants were given the opportunity to practice the protocol on an unrelated part of their body –muscles in the ventral aspect of the contralateral ulnar forearm – as many times as was needed for them to feel comfortable with the procedure and for the testers to have the opinion that the participant understood the procedure.(220) Once this had been achieved, all participants had one trial run on the test site before the actual measure was taken.

Reliability studies have shown various results with repeated testing in the same session. Typical outcomes of these studies were either that the test was reliable on repeated testing(221,222) or that the first measure was significantly different from those taken afterwards.(218,219,223) The approach for this study recognised that the first pressure pain threshold measure may not be accurate, and so this first measure was used as a practice run, but only one measure(218) (rather than an average of three) at the test-site was performed to reduce sensitisation effects that may have occurred in hyperalgesic participants. An interval of 45 seconds was observed between the practice run in the test location and the actual pressure pain threshold measure (in kPa) to prevent sensitisation effects from repeated stimulation.(224) Outcome measures were reported in kPa as this is the standard unit of measure for pressure pain threshold presented in most research settings.

3.3.5.2 Heat pain threshold (exploratory outcome variable)

The DFNS protocol for heat pain threshold was used, whereby heat pain threshold was determined by exposing the skin to alternating heating and cooling ramps, 3 times each, such that 3 measures each of heat pain threshold were recorded. The representative measure, the "actual" heat pain threshold, was obtained by calculating the mean of the three absolute heat pain threshold temperature values (in °C). This method of assessing heat pain threshold has a test-retest reliability of 0.881 (Pearson's correlation *r*) when tested on consecutive days, in people with sensory disturbances of varying aetiologies.(225)

The test was performed using a TSA 2001-II (MEDOC, Israel) thermal sensory testing device. A thermode with a contact area of 7.84 cm^2 was used. The baseline temperature was 32°C (middle of neutral range). Stimuli were ramped at 1°C/s. Once the stimulus reached the heat pain threshold – "when the impression of "warmth" or "heat" changes its quality towards an additional impression of a "burning", "stinging", "drilling" or "aching" sensation", participants immediately pushed a button which reversed the stimulus temperature back to 32°C. This procedure was repeated 3 times, with an interstimulus interval of 10 seconds between each of the 3 tests. The upper cut-off temperature was 50°C to avoid any injury to the skin. Participants were not able to see any information regarding the temperature they were experiencing. The test sites were the skin over the deltoid muscle and the skin over the lateral aspect of the shin below the knee. Participants underwent a training session where they experienced the test protocol at a site other than those tested (the skin on the ventral aspect of the ulnar side of the forearm).

3.3.5.3 Mechanical pain threshold (exploratory outcome variable)

The DFNS protocol for mechanical pain threshold was used, whereby mechanical pain threshold was determined by exposing the skin to pinprick stimuli using a set of seven weighted pinprick simulators that exert forces due to their weight (i.e., 8, 16, 32, 64, 128, 256, 512 mN; MRC Systems Pin Prick Stimulator Set, 0.25 mm flat circular contact area).(205) Each trial began with the 8 mN simulator and was followed by the next heaviest simulator until the participant felt the stimulation as painful, i.e., "sharp", "pricking" or "stinging" sensation (suprathreshold mechanical pain threshold). When this occurred, the next lightest stimulator was applied again sequentially until the participant said the sensation was no longer painful, i.e., felt as "blunt" and no longer "sharp", "pricking" or "stinging" (subthreshold mechanical pain threshold). At this point the nest heaviest stimulator was applied again sequentially until the stimulus was painful again, then reversed until it was no longer painful, and so on until 5 ascending and descending ramps had been completed to determine five suprathreshold and five subthreshold mechanical pain threshold values. The value of the mechanical pain threshold was determined to be the geometric mean of all of the five suprathreshold and five subthreshold mechanical pain threshold values (in mN). This method of assessing mechanical pain threshold has a test-retest reliability of 0.802 (Pearson's correlation *r*) when tested on consecutive days, in people with sensory disturbances of varying aetiologies.(225) Care was taken to ensure that the stimulator tip touched the skin at a 90° angle to the skin surface, that only the needle tip contacted the skin and that a "flowing" movement was used to touch the skin with the stimulator and remove it again that resulted in a stimulator/skin contact time of 1 second.(226) The test sites were the skin over the deltoid muscle and the skin over the lateral aspect of the shin below the knee. Participants

underwent a training session where they experienced the test protocol at a site other than those tested (the skin on the ventral aspect of the ulnar side of the forearm).

3.3.5.4 Conditioned pain modulation-PP40 and conditioned pain modulation-HP40 (exploratory outcome variables)

CPM was tested using protocols derived from recommendations made by Yarnitsky et al. (2015)(208). Noxious cold water immersion was used as the conditioning stimulus for all tests – 1-minute immersion at pain40, i.e., 40/100 visual analogue scale (VAS) (with > 20/100 VAS pain, i.e., > pain20 - mild to moderate pain levels being suggested to suffice).(208) A cold-water bath with a circulating fan was used. For upper limb test areas (test stimuli), the contralateral foot was immersed to the ankle level (conditioning stimulus). For lower limb test areas (test stimuli), the contralateral hand was immersed to the wrist level (conditioning stimulus).

For the deep tissue CPM tests (infraspinatus, deltoid and tibialis anterior muscles), i.e., "conditioned pain modulation-PP40", the test stimulus was blunt pressure applied using a manual computerised algometer (as described above for pressure pain threshold) at the participant's individualised PP40 (pain level of 40/100 VAS). The test stimulus magnitude was recorded in kPa. For cutaneous CPM tests (skin over the deltoid, and skin over the lateral aspect of the shin below the knee), i.e., "conditioned pain modulation-HP40", the test stimulus used was contact heat pain applied using a thermode (as described above for heat pain threshold), at the participant's individualised HP40 (pain level of 40/100 VAS). The test stimulus magnitude was recorded in °C.

A sequential (rather than parallel) protocol was used whereby the test stimulus was presented immediately after the conditioning stimulus, to avoid biases such as distraction.(208) Yarnitsky et al.(208) recommend performing each test twice, however, each test was only performed once in this protocol to avoid too heavy a burden on participants.

Upper limb tests (test stimuli) were followed by lower limb tests (test stimuli) for every participant, separated by a 10-minute break.(208) Tests were conducted in the same order for every participant – for upper limb tests, the order was: (1) HP40 at the skin over deltoid, (2) PP40 at infraspinatus, and (3) PP40 at deltoid. For lower limb tests the order was: (1) HP40 at shin skin, and (2) PPT40 at tibialis anterior.

Pre-conditioning PP40 and HP40 measures were determined on the day of testing, immediately prior to exposure to the conditioning stimulus. Familiarisation testing runs were carried out first on the ulnar aspect of the forearm contralateral to the test shoulder for both PP40 and HP40. To determine the participants' pre-conditioning HP40 temperatures, the area of skin to be tested was exposed to an increasing temperature ramp using a thermode (using the same system as for heat pain threshold), starting at a baseline temperature of 32°C and ramped at 1°C/s. Participants were asked to immediately push a button once the stimulus temperature reached a pain intensity of 40/100 VAS. This procedure was completed twice, and the average taken as representative - this temperature was recorded as that participant's pre-conditioning HP40 temperature (test stimulus) for each region of skin being tested. The pre-conditioning PP40 pressure was determined similarly, using a manual computerised algometer (using the same system as for pressure pain threshold), at a pressure application rate of 30 kPa/s (i.e., a force rate of 59.6 $N/cm^{2)}$, applied to

the muscle to be tested. The average of two PP40 measures was taken as representative and was recorded as that participant's pre-conditioning PP40 pressure (test stimulus) for each muscle being tested.

Each participant's pain40 cold temperature (conditioning stimulus) was determined while the CPM test was being conducted, by immersing the relevant extremity in a cold-water bath containing cold water, ice and a recirculating fan.(208) Once the temperature reached 40/100 VAS for each individual patient, a timer was started to ensure a 1-minute exposure at this level of pain. Ice or warm water was added by the tester during this timeframe to maintain the 40/100 VAS level of pain (the tester checked regularly with the participant verbally re level of pain). After the 1-minute exposure to 40/100 VAS pain, the test stimuli were applied.

On the testing day, the pre-conditioning HP40 temperature for the skin over the deltoid (the thermode was left in situ for post-conditioning stimulus retesting) was determined, and the pre-conditioning PP40 pressure was determined for the infraspinatus and deltoid muscles. CPM testing began with cold water immersion of the foot (contralateral to the affected, or most affected, shoulder) for 1 minute at pain40, followed immediately (and in a consistent order) by the application of: (1) the post-conditioning HP40 test stimulus to the skin over the deltoid (immediately), (2) the post-conditioning PP40 test stimulus to the infraspinatus (at t = 30s), and (3) the post-conditioning PP40 test stimulus to the deltoid (at t = 60s). Only one test of each of the post-conditioning test stimuli was performed to allow for rapid post-conditioning testing as CPM effects are short-lived(208).

A rest period of 10 minutes was then observed between the upper limb and lower limb CPM tests, i.e., 10 minutes from the time the foot was removed from the cold-water bath, until the pre-conditioning pain40 levels were determined for the lower limb test stimuli tests.

CPM testing continued with the pre-conditioning HP40 measure for the skin over the lateral shin (the thermode was then left in situ for post-conditioning stimulus retesting) being determined, and the pre-conditioning PP40 measure being determined for the tibialis anterior muscle. Again, the average of two measures was taken as representative and was recorded as each participant's pre-conditioning PP40 and HP40 test stimuli measures. Testing then continued with immersion of the hand contralateral to the shoulder that was tested in the static QST tests for 1 minute at cold pain40, followed immediately by (in consistent order) the application of: (1) the post-conditioning HP40 test stimulus to the skin over the lateral shin (immediately), and (2) the post-conditioning PP40 test stimulus to the tibialis anterior (at t = 30s). Again, only one test of each the post-conditioning test stimuli was performed to allow for rapid post-conditioning testing.

The "CPM effect" was determined as the difference in: a) HP40 measures (in °C), where the pre-conditioning HP40 measure was subtracted from the post-conditioning HP40 measure (after exposure to the conditioning stimulus, i.e., after 1 minute spent with the contralateral extremity immersed in 40/100 cold water), and b) PP40 measures (in kPa), where the pre-conditioning PP40 measure was subtracted from the post-conditioning PP40 measures (after exposure to the conditioning stimulus, i.e., after 1 minute spent with the contralateral extremity immersed in 40/100 cold water). Where the test results have been presented, post-conditioning pain inhibition is therefore indicated by a negative (< 0) value, post-conditioning pain facilitation is therefore

indicated by by a positive (> 0) value, i.e., CPM effect = post-conditioning HP40/PP40 minus pre-conditioning HP40/PP40. Data has been presented as both: (1) absolute values (absolute difference in HP40/PP40 scores) – "CPM effect-absolute change", and (2) percentage changes in HP40/PP40 scores – "CPM effect - percentage change".

3.3.5.5 Temporal summation – mechanical pain (exploratory outcome variable)

The experimental assessment of temporal summation in humans has been assessed using different modalities, including pressure (both sharp and blunt).(5,205) It requires a stimulus train, with each stimulus separated by a time interval of adequately short duration.(5)

The DFNS punctate pressure temporal summation testing protocol was used in this study (which has a test-retest reliability of 0.671 (Pearson's correlation *r*) when tested on consecutive days, in people with sensory disturbances of varying aetiologies,(225) with minor modifications, as described below.

1. A 256 mN weighted pinprick stimulator was applied to the skin(227) using a similar technique as outlined above for punctate mechanical pain threshold testing. If the 256 mN pinprick VAS score was rated as "0" three times, the same protocol was used with the 512 mN pinprick. If the 512 mN pinprick VAS score is rated as "0" three times, temporal summation was seen as being unable to be assessed for that individual,(226) and was handled as missing data.(225) (Inclusion of the 512 mN pinprick stimulator was a modification from the original DFNS protocol, which only used a 256 mN stimulator, and considered temporal summation as being unable to be assessed for any individual who rated the 256 MN pinprick NRPS score as "0" three times).

2. A single pinprick stimulus was applied, and an electronic VAS was used by the participant to immediately rate the level of pain on a 0-100 VAS scale, whereby any “sharp”, “pricking”, “stinging” or “burning” sensation was defined as being painful.(226) (The DFNS protocol recorded pain ratings as singular 0-100 NRPS scores recorded immediately after the application of the stimulus).
3. Then, after a 10-second interval, a train of 10 pinprick stimuli, at an interstimulus interval (ISI) of 1 second (timed by a metronome) were applied within a 1 cm^2 area in close proximity to the initial single stimulus.(226) Continuous pain measures were recorded by the participant using the electronic VAS.(225,227) (The DFNS protocol recorded singular NRPS scores only, after the 1^{st} and 10^{th} stimuli only).
4. This procedure was repeated 5 more times, at sites within a few centimetres of each other, such that 6 single VAS pinprick scores and 6 electronic VAS traces of pain scores (temporal summation pinprick scores) were recorded(226)
5. The first of the single pinpricks, followed by a train of pinpricks stimuli was collected as a familiarisation trace. The DFNS protocol did not include a familiarisation trace (i.e., only collected 5 total temporal summation measures).
6. The difference between each of the final VAS scores and the corresponding initial VAS score from the 10 pinprick stimulus trains was calculated and the average of these 5 different measures – the temporal summation to punctate (sharp) pressure value - was used for analysis(228)
7. If only 3 full traces were recorded, the differences between the 1st and 10th pinprick VAS scores were calculated for those 3 traces; the average of these 3 differences was taken to

represent the 4th and 5th trace differences; and the overall mean calculated as per 5 full traces

8. If only 4 full traces were recorded, the differences between the 1st and 10th pinprick VAS scores were calculated for those 4 traces; the average of these 4 differences was taken to represent the 5th trace difference; and the overall mean calculated as per 5 full traces

The test sites were as for mechanical pain threshold testing, i.e., the skin over the deltoid muscle and the skin over the lateral aspect of the shin below the knee. Participants underwent a training session where they experienced the test protocol at a site other than those tested (the skin on the ventral aspect of the ulnar side of the forearm).

3.3.5.6 Pain area

Participants in the SAPS group were asked to shade in the areas where they had pain related to their shoulder pain on body chart diagrams using Navigate Pain Software (Version: 2.8.2, Aglance Solutions ApS, Aalborg, Denmark). This software is able to calculate the area shaded in number of pixels.

3.3.6 Bias

SAPS participants were recruited using convenience sampling, which is susceptible to self-selection bias.

3.3.7 Study size

The study size was arrived at by power calculations made for the primary hypothesis – pressure pain threshold at deltoid for SAPS compared with healthy controls. A one-sided independent t-test was used to determine that a minimum sample size of $n = 8$ per group would be required to detect an effect size of d = 1.4 in the primary outcome measure pressure pain threshold at deltoid (using standard deviations of 80 and 50 in the SAPS and healthy control groups, respectively) with power of 85%, at alpha of 0.05. The data used for the power calculations was taken from a study on fibromyalgia patients (Maquet et al. 2004).(229) This data was selected because it was measured in a population recognised to have tenderness on muscle palpation; it provided data on a muscle in a similar body region to the deltoid such that tissue behavior would be expected to be similar, i.e., supraspinatus in the case of the fibromyalgia study, and deltoid in the context of the SAPS study; and because the study provided the requisite data to calculate an effect size from which a sample size number which would be expected to answer the research question could be identified, i.e., do individuals with SAPS have a lower pressure pain threshold in the deltoid muscle than healthy controls. The calculations are provided Table 3.3 below.

Pressure pain threshold in kPa (supraspinatus):		Ẍ (s)						d (ES) =			
Group 2 fibromyalgia ($\ddot{X}_2$, s_2) - for both lines a. and b. below (due to no males with fibromyalgia included in study)		120 (50)	s_1	s_2	$\ddot{X}_1$	$\ddot{X}_2$	$\ddot{X}_1 - \ddot{X}_2$	$\ddot{X}_1 - \ddot{X}_2/s'$	for:	power (%)	n =
a. Group 1 ($\ddot{X}_1$, s_1) HC (female)	a.	320 (80)	80	50	320	120	200	2.998127	d =1.4	85	8
b. Group 1 ($\ddot{X}_1$, s_1) HC (male)	b.	360 (60)	60	50	360	120	240	4.345716	d =1.4	85	8

Table 3.3 Power calculations made for the primary hypothesis. A one-sided independent t-test was used to determine that a minimum sample size of *n* = 8 per group would be required to detect an effect size (ES) of d = 1.4 on PPT at deltoid (using standard deviations of 80 kPa and 50 kPa in the SAPS and HC groups, respectively) with power of 85%, at alpha of 0.05. Data from Maquet 2004.(229) **Ẍ - mean, s – standard deviation, ES - effect size, PPT – pressure pain threshold, HC – healthy controls.**

3.3.8 Statistical methods

3.3.8.1 Primary outcome variable - pressure pain threshold

A linear mixed effect model (230) was used to estimate the effect of treatment group (SAPS or healthy control) on pressure pain threshold at different muscles (deltoid, infraspinatus and tibialis anterior). Based on the study design, a linear mixed effect model was developed by a professional academic statistician in order to obtain the most efficient estimates for the comparisons of interest. This approach was able to incorporate the finding that the pressure pain threshold observations within patients between locations were correlated. The statistical model selected was useful as it was able to incorporate these within-patient correlation structures into the analysis and, in so doing, was able to obtain more precise estimates.

For the primary outcome, the model was specified with a response variable of pressure pain threshold, main effects of Treatment Group and Location, and an interaction effect between Treatment Group and Location. A random intercept for Patient identification (ID) was specified to account for variability within patients. One model was fitted for the pressure pain threshold and then used to perform post-hoc comparisons between the treatment groups at different locations, in order of importance of the hypotheses. By this, the first test compared mean pressure pain threshold between the healthy controls and SAPS at deltoid, the second two tests compared mean pressure pain threshold between the healthy controls and SAPS at infraspinatus and tibialis anterior. These second two tests were considered equally important tests for the secondary analysis on the primary outcome (pressure pain threshold). Prioritising the hypotheses in this way avoided creating a multiple comparisons problem, such that the statistical testing of the

pressure pain threshold at deltoid data did not need to be adjusted for multiple testing. The secondary analysis on the primary outcome (pressure pain threshold) consisted of two tests (infraspinatus and tibialis anterior), therefore, a multiple comparisons adjustment (adjusted by False Discovery Rate(231) method) was applied to both tests.

The interaction of Treatment group and Location was included because this allowed for testing of the outcome differences between the treatment groups (SAPS and healthy controls) at different locations (deltoid, infraspinatus and tibialis anterior). The overall interaction effect and main effects were tested with an ANOVA Type III table, to determine which post-hoc pairwise tests would allow testing of the hypotheses of interest using the model estimates. As per Wei et al. (2012), post-hoc pairwise comparisons can be run when the interaction effect (Treatment Group x Location) on the outcome is significant, or when the interaction effect is insignificant but the main effects (Treatment Group and Location) are statistically significant.(232)

3.3.8.2 Exploratory outcome variables - heat pain threshold, mechanical pain threshold, conditioned pain modulation-PP40 and -HP40, and temporal summation score

The statistical methodology used for the exploratory analyses was the same as that used for the primary analysis. Linear mixed effect models were fitted for each of the exploratory outcomes. Each outcome served as a response variable, Treatment Group and Location as main effects, and an interaction effect between Treatment Group and Location. Random intercepts for Patient ID were specified to account for variability within patients. Post-hoc comparisons between the treatment groups at different locations were performed for each of the outcomes. The tests were considered equally important tests. Multiple comparisons adjustment (adjusted by False

Discovery Rate(231)) was applied for the number of tests within each outcome. The interaction of Treatment Group and Location was included because this allowed for testing of the outcome mean differences between the treatment groups at different locations. The overall interaction effect and main effects were tested with an ANOVA Type III table, to determine which post-hoc pairwise tests would allow testing of the hypotheses of interest using the model estimates.

3.3.8.3 Missing data

The linear mixed effects models that were used in this analysis rely on the Missing at Random (MAR) assumption to produce unbiased estimates of the effects of interest. Notably, for the primary hypothesis on the primary outcome, as there was no missing data for either group, there are no limitations or potential biases related to missingness.

3.4 Results

3.4.1 Participants

3.4.1.1 Numbers potentially eligible

155 people were identified as being potentially eligible for the SAPS group on intake to AMSMC. 27 people were identified as being potentially eligible for the healthy control group.

3.4.1.2 Numbers examined for eligibility

25 people who were identified as being potentially eligible consented to being examined for eligibility. All 27 people who were identified as being potentially eligible for the healthy control group consented to being assessed for eligibility.

3.4.1.3 Numbers confirmed eligible

23 people who were examined for eligibility were eligible. Both people who were found to be ineligible at this stage were excluded as the pain provoked on physical examination did not equal 5/10 or greater numerical pain rating score (NPRS) on at least one orthopaedic special test, as described in the inclusion criteria in section 3.3.3.2. 23 people who were identified as being potentially eligible for the healthy control group were eligible. Two of the people who were found ineligible at this stage were excluded because there was no one in the SAPS group who matched their age and/or sex. The other two were excluded because they had current musculoskeletal pain.

3.4.1.4 Numbers included in the study

21 people who were eligible for the SAPS group participated in the study. One person was not able to participate because there were travel restrictions in place at the time due to COVID. The other person withdrew from participating because of life stress related to COVID. 21 people who were eligible for the healthy control group participated in the study. One person withdrew from participating because they were concerned that the testing may be too painful. The other person withdrew from participating because of life stress related to COVID.

3.4.1.5 Numbers analysed

For pressure pain threshold, numbers were analysed for all participants for deltoid, infraspinatus, and tibialis anterior, i.e., 21 SAPS participants and 21 healthy controls. For heat pain threshold for the skin over the deltoid muscle, numbers were analysed for 20 SAPS participants and 20 healthy controls. For heat pain threshold for the skin over the lateral aspect of the shin below the knee, numbers were analysed for 20 SAPS participants and 21 healthy controls. For the one participant in the SAPS group, the measuring device was not working. For mechanical pain threshold, numbers were analysed for all participants for both the skin over the deltoid muscle and the skin over the lateral aspect of the shin below the the knee, i.e., 21 SAPS participants and 21 healthy controls. For conditioned pain modulation-PP40 for deltoid, numbers were analysed for 21 SAPS participants and 20 healthy controls. For conditioned pain modulation-PP40 for tibialis anterior, numbers were analysed for all participants, i.e., 21 SAPS participants and 21 healthy controls. For conditioned pain modulation-PP40 for infraspinatus, numbers were analysed for 21 SAPS participants and 19 healthy controls. For conditioned pain modulation-HP40 for the skin over the deltoid muscle and the skin over the lateral aspect of the shin below the knee, numbers were analysed for 20 SAPS participants and 21 healthy controls. For temporal summation score for the skin over the deltoid muscle, numbers were analysed for 16 SAPS participants and 16 healthy controls. For temporal summation score for the skin over the lateral aspect of the shin below the knee, numbers were analysed for 16 SAPS participants and 17 healthy controls. For pain area, numbers were analysed for all relevant participants, i.e., 21 SAPS participants.

3.4.2 Descriptive data

The characteristics of the participants are described below in Table 3.4.

Baseline data	HC n = 21	SAPS n = 21
Female, male: n (%)	11 (52%), 10 (48%)	11 (52%), 10 (48%)
Age in decades: n (%)		
18-29 years of age	1 (4.8%)	1 (4.8%)
30-39 years of age	2 (9.5%)	2 (9.5%)
40-49 years of age	4 (19%)	4 (19%)
50-59 years of age	7 (33.3%)	7 (33.3%)
60-69 years of age	4 (19.1%)	4 (19.1%)
70-79 years of age	3 (14.3%)	3 (14.3%)
Age in years: mean (SD)	52.6 (14.1)	53.9 (12.9)
Physical activity level (IPAQ)		
low: n (%)	1 (4.8%)	1 (4.8%)
medium: n (%)	2 (9.5%)	9 (42.9%)
high: n (%)	18 (85.7%)	11 (52.4%)
Total score in MET min/week: median (IQR)	3972 (3303)	2373 (3387)
SF-36 score %: mean (SD)		
Physical functioning	93.8 (19.4)	78.1 (16.6)
Role limitations due to physical health	97.6 (10.9)	54.8 (40.8)
Role limitations due to emotional problems	85.7 (27.0)	66.7 (42.2)

Energy/fatigue	66.4 (16.1)	52.6 (23.8)
Emotional well-being	80.8 (14.6)	69.9 (20.8)
Social functioning	93.5 (14.0)	78.0 (27.6)
Pain	94.3 (6.8)	53.7 (21.8)
General health	78.3 (14.4)	70.0 (18.1)
Health change	63.1 (20.3)	53.6 (22.8)
Arm dominance **- right, left, ambidextrous: n (%)**	17 (81.0), 3 (14.3), 1 (4.8)	17 (81.0), 4 (19.0), 0 (0)
Affected side **- dominant, non-dominant, neither (ambidextrous) n (%)**	-	14 (66.7), 6 (28.6), 1 (4.8)
SPADI score %: median (IQR)		
pain scale	-	34 (27.5)
disability scale	-	36 (39)
total score	-	30 (30)
WORC score %: median (IQR)	-	
Physical Symptoms	-	39.0 (26.9)
Sports/Recreation	-	54.8 (27.0)
Work	-	43.3 (30.2)
Lifestyle	-	33.1 (38.7)
Emotions	-	39.9 (33.9)
Total	-	43.2 (26.0)

Table 3.4: Description of characteristics of the participants. IPAQ – International Physical Activity Questionnaire: a self-reported measure of physical activity; SF-36 - 36-Item Short Form Health Survey: a self-reported quality of life measure; SPADI - Shoulder Pain and Disability Index: a self-reported measure of pain and disability related to shoulder problems; WORC - Western Ontario Rotator Cuff Index: a self-reported quality of life measure for patients with rotator cuff disease, SD – standard deviation, IQR – interquartile range, HC – heatlhy control, SAPS – subacromial pain syndrome

3.4.3 Outcome data

3.4.3.1 Pressure pain threshold (primary outcome variable)

3.4.3.1.1 Deltoid – primary hypothesis

Table 3.5 (below) shows the distribution of the outcome pressure pain threshold in HC and SAPS at deltoid. Missing values for all variables were denoted as "Missing".

Summary measure	Treatment Group	
	HC	SAPS
n	21	21
mean (kPa)	488.51	382.68
SD	260.61	268.15
min	87.20	87.20
max	1159.30	861.40
median	466.50	307.70

IQR	281.20	346.90
Q1	329.30	181.30
Q3	610.50	528.20
Missing	0	0

Table 3.5: Descriptive table for the primary outcome pressure pain threshold by treatment group at deltoid. min - minimum, max - maximum, Q1 - quartile 1, Q3 - quartile 3

The boxplots in Figure 3.1 (below) provide visual representations of the distributions of the outcome pressure pain threshold by treatment group at deltoid shown in Table 3.5. The median, spread, interquartile range (IQR) and range of each of the boxplots correspond to the corresponding number of observations in each cell in Table 3.5.

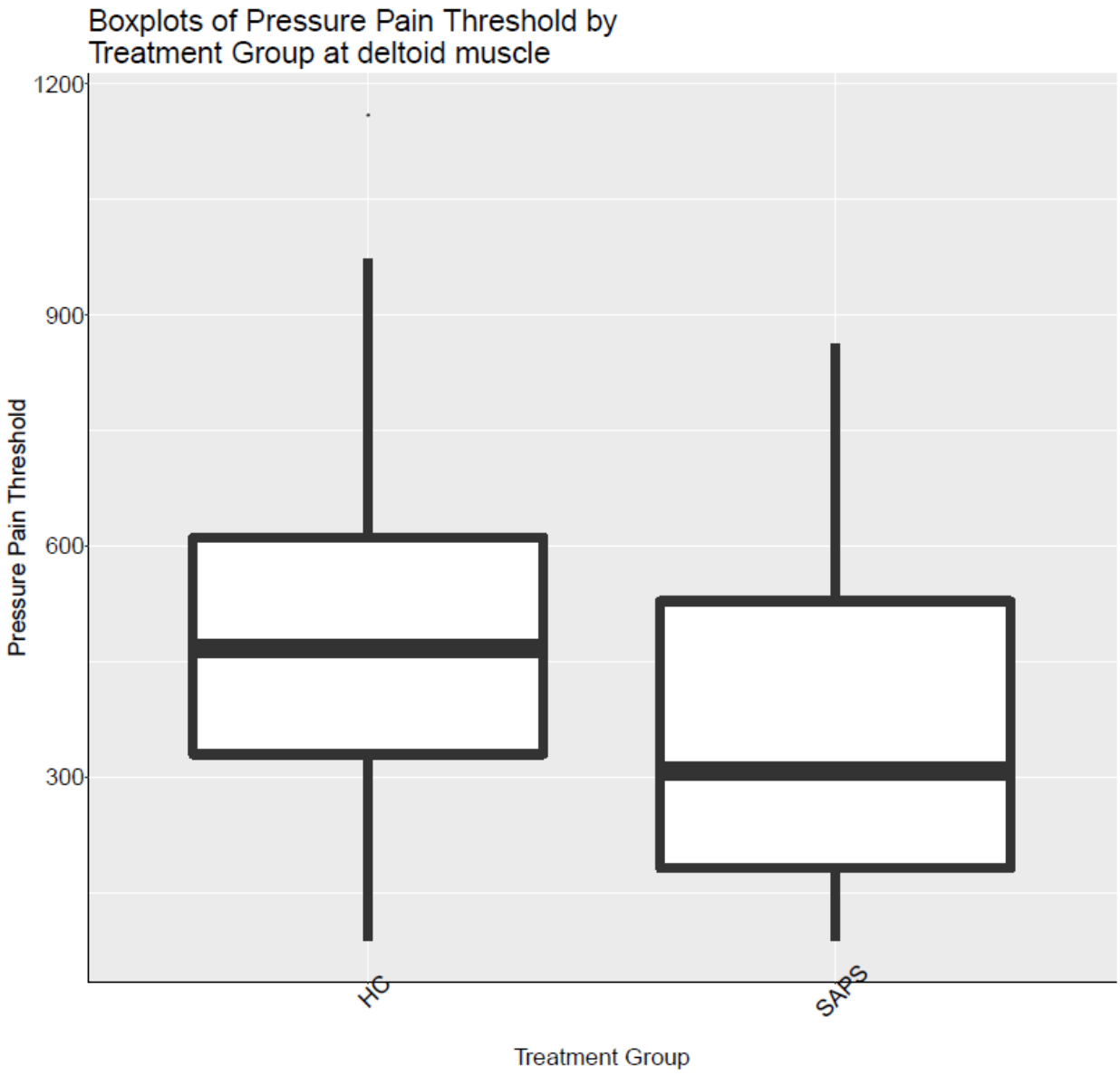


Figure 3.1: Side by side boxplots of pressure pain threshold measured at deltoid by treatment group

3.4.3.1.2 Infraspinatus and tibialis anterior – secondary hypotheses on the primary outcome

Table 3.6 (below) shows the distributions of the outcome pressure pain threshold in healthy controls and SAPS at infraspinatus and tibialis anterior. Missing values for all variables were denoted as "Missing".

Summary measure	Infraspinatus		Tibialis anterior	
	Treatment Group		Treatment Group	
	HC	SAPS	HC	SAPS
n	21	21	21	21
mean (kPa)	571.55	368.58	745.87	550.24
SD	248.35	233.28	386.22	324.31
min	151.90	81.30	151.90	199.90
max	1229.90	998.60	1489.60	1482.70
median	508.20	293.00	728.10	478.20
IQR	291.00	263.60	475.30	479.20
Q1	447.90	217.60	446.90	293.00
Q3	738.90	481.20	922.20	772.20
Missing	0	0	0	0

Table 3.6: Descriptive table for the primary outcome pressure pain threshold by Treatment Group at infraspinatus (left) and at tibialis anterior (right)

The boxplots in Figure 3.2 (below) provide visual representations of the distributions of the outcome pressure pain threshold by treatment group at infraspinatus (top) and tibialis anterior (bottom) shown in Table 3.6. The median, spread, IQR and range of each of the boxplots corresponds to the corresponding number of observations in each cell in Table 3.6.

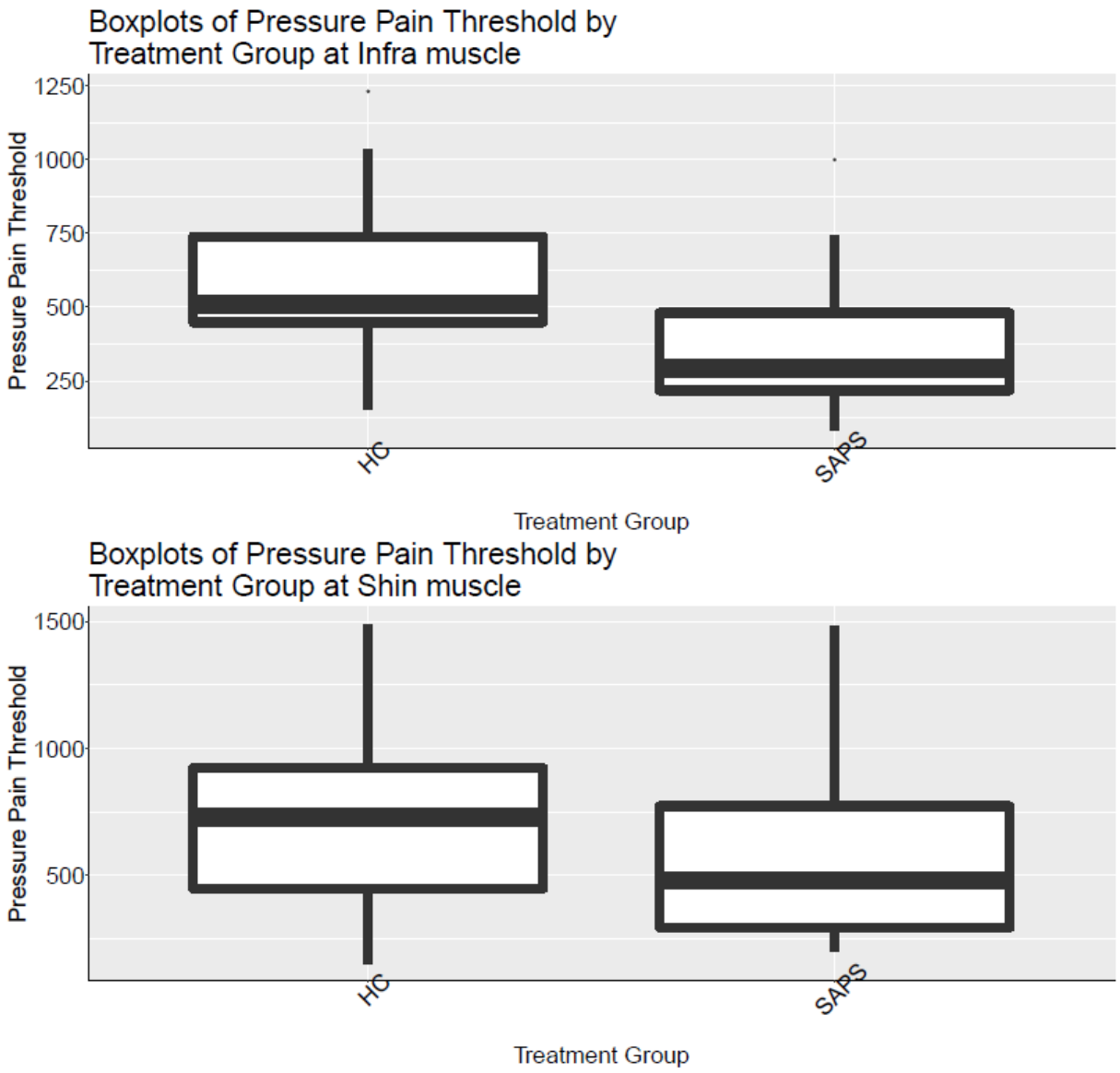


Figure 3.2: Side by side boxplots of pressure pain threshold measured at infraspinatus (top) and tibialis anterior (bottom) by treatment group

3.4.3.2 Heat pain threshold (exploratory outcome variable)

1. Skin over the deltoid muscle
2. Skin over the lateral aspect of the shin below the knee

Table 3.7 (below) shows the distributions of the outcome heat pain threshold at the skin over the deltoid muscle and the skin over the lateral aspect of the shin below the knee. Missing values for all variables were denoted as "Missing". Regarding missing data for the skin over the deltoid muscle - for the participant in the healthy control group, the data was saved, but the file was corrupted somehow (would not open properly). For the participant in the SAPS group, the measuring device was not working. Regarding missing data for the skin over the lateral aspect of the shin below the knee - for the one participant in the SAPS group, the measuring device was not working.

Summary measure	Deltoid		Shin	
	Treatment Group		Treatment Group	
	HC	SAPS	HC	SAPS
n	21	21	21	21
mean (°C)	46.73	46.38	47.18	46.94
SD	3.06	3.38	3.20	4.04
min	37.33	36.83	38.83	35.77
max	51.77	49.70	52.53	50.97
median	47.10	47.57	47.77	48.42
IQR	2.39	3.58	3.17	4.04
Q1	45.94	45.17	45.87	45.50
Q3	48.33	48.75	49.03	49.54
Missing	1	1	0	1

Table 3.7: Descriptive table for the exploratory outcome heat pain threshold by treatment group at the skin over the deltoid muscle (left) and at the skin over the lateral aspect of the shin below the knee (right)

The boxplots in Figure 3.3 provide visual representations of the distributions of the outcome heat pain threshold by treatment group at the skin over the deltoid muscle and at the skin over the lateral aspect of the shin below the knee shown in Table 3.7. The median, spread, IQR and range of each of the boxplots corresponds to the corresponding number of observations in each cell in Table 3.7.

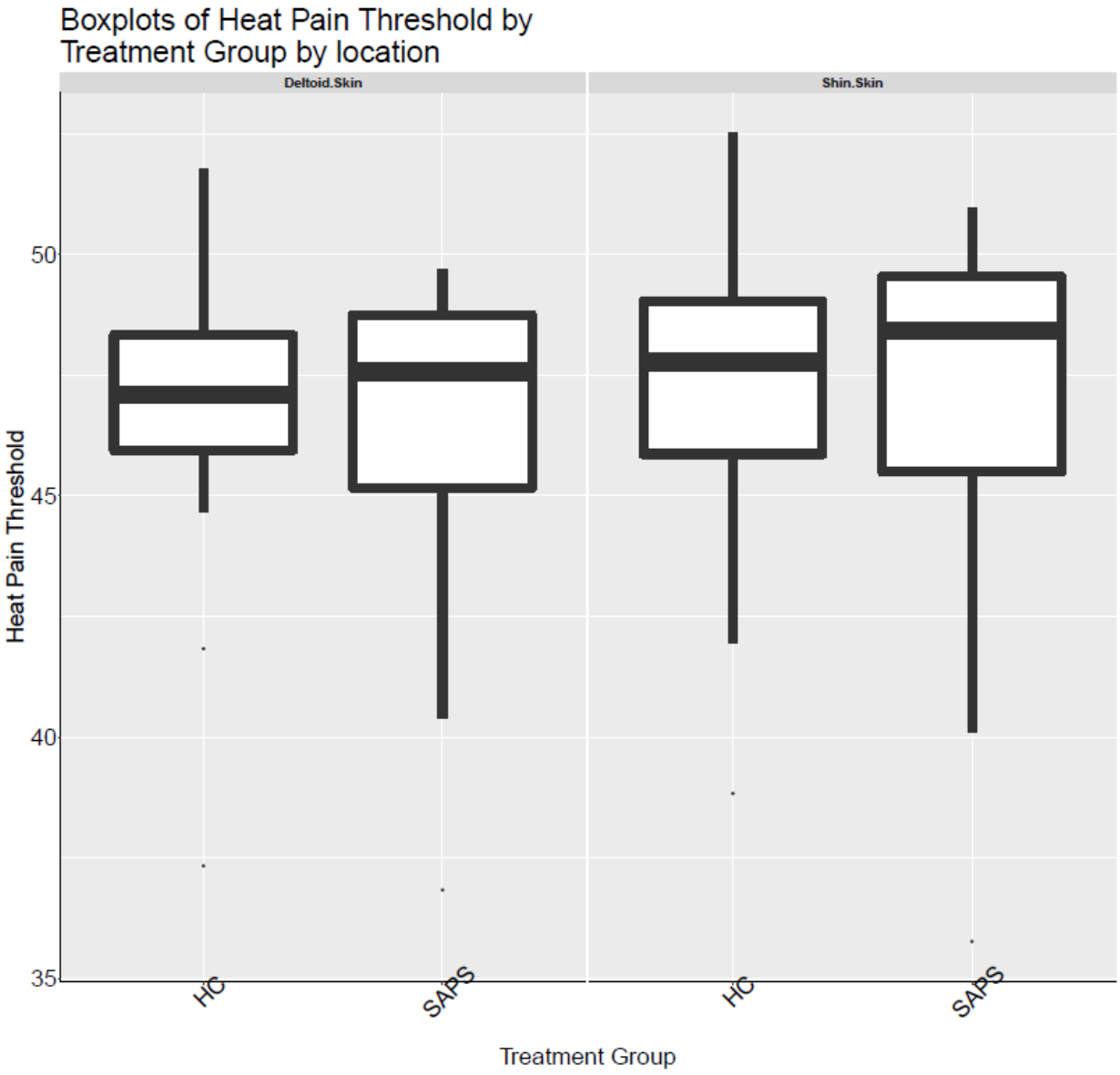


Figure 3.3 Side by side boxplots of heat pain threshold measured at the skin over the deltoid muscle (left) and at the skin over the lateral aspect of the shin below the knee (right) by treatment group

3.4.3.3 Mechanical pain threshold (exploratory outcome variable)

1. Skin over the deltoid muscle
2. Skin over the lateral aspect of the shin below the knee

Table 3.8 (below) shows the distributions of the outcome mechanical pain threshold at the skin over the deltoid muscle and the skin over the lateral aspect of the shin below the knee. Missing values for all the variables were denoted as "Missing".

Summary measure	Deltoid		Shin	
	Treatment Group		Treatment Group	
	HC	SAPS	HC	SAPS
n	21	21	21	21
mean (mN)	155.98	165.83	111.72	140.46
SD	151.99	138.89	128.89	124.33
min	8.00	8.00	8.00	8.57
max	512.00	512.00	445.72	362.04
median	90.51	97.01	51.98	103.97
IQR	193.60	158.86	81.57	226.80
Q1	45.25	64.00	29.86	25.99
Q3	238.86	222.86	111.43	252.79
Missing	0	0	0	0

Table 3.8: Descriptive table for the exploratory outcome mechanical pain threshold by treatment group at the skin over the deltoid muscle (left) and at the skin over the lateral aspect of the shin below the knee (right)

The boxplots in Figure 3.4 (below) provide visual representations of the distributions of the outcome mechanical pain threshold by treatment group at the skin over the deltoid muscle and at the skin over the lateral aspect of the shin below the knee shown in Table 3.8. The median, spread, IQR and range of each of the boxplots corresponds to the corresponding number of observations in each cell in Table 3.8.

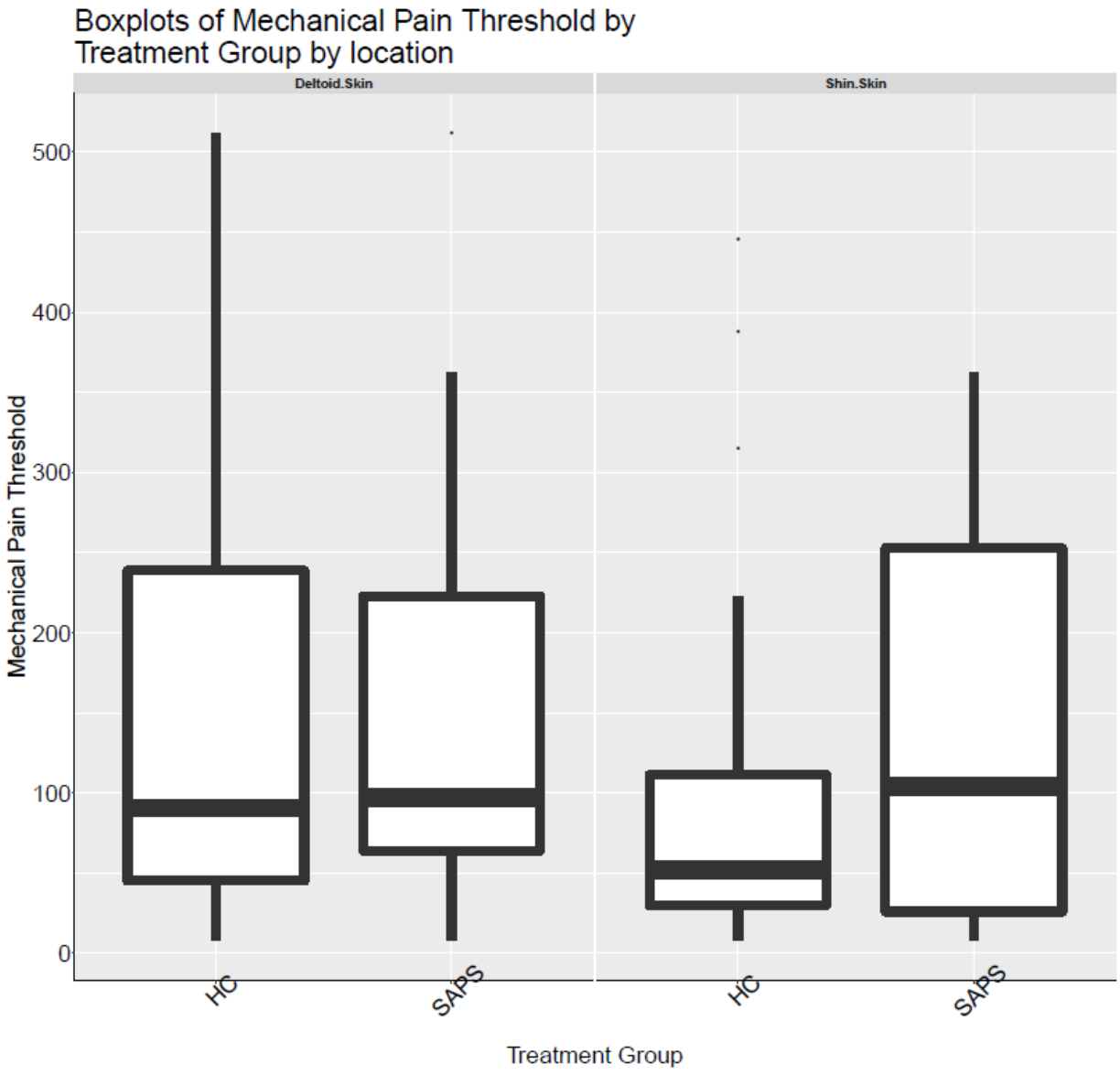


Figure 3.4: Side by side boxplots of mechanical pain threshold measured at the skin over the deltoid muscle (left) and at the skin over the lateral aspect of the shin below the knee (right) by treatment group

3.4.3.4 Conditioned pain modulation-PP40 (exploratory outcome variable)

1. CPM effect - absolute change
    a. Deltoid

b. Infraspinatus

c. Tibialis anterior

Table 3.9 (below) shows the distributions of the outcome CPM effect (absolute change) at deltoid, infraspinatus, and tibialis anterior. Missing values for all variables were denoted as "Missing".

Summary measure	Deltoid		Infraspinatus		Tibialis anterior	
	Treatment Group		Treatment Group		Treatment Group	
	HC	SAPS	HC	SAPS	HC	SAPS
n	21	21	21	21	21	21
mean (change in kPa)	-2.62	-33.48	5.24	-25.25	58.50	1.36
SD	117.50	108.32	117.80	59.75	69.10	130.16
min	-306.75	-289.60	-274.45	-151.40	-77.35	-257.30
max	218.55	229.85	247.50	92.20	165.15	394.40
median	3.68	-20.60	22.50	-10.25	66.10	-11.25
IQR	123.03	78.40	101.40	63.25	121.10	87.75
Q1	-54.07	-69.10	-29.60	-57.35	-2.00	-62.70
Q3	68.95	9.30	71.80	5.90	119.10	25.05
Missing	1	0	2	0	0	0

Table 3.9: Descriptive table for the exploratory outcome conditioned pain modulation-PP40 (CPM effect - absolute change) by treatment group at deltoid (left), infraspinatus (centre) and tibialis anterior (right)

Regarding missing data for deltoid - for the participant in the healthy control group, the measure was not taken as there was a bruise in the area.

Regarding missing data for infraspinatus - for both participants in the healthy control group the skin marking was not clear and so the tests were not performed.

The boxplots in Figure 3.5 (below) provide visual representations of the distributions of the outcome conditioned pain modulation-PP40 (CPM effect - absolute change) by treatment group at deltoid, infraspinatus and tibialis anterior as shown in Table 3.9. The median, spread, IQR and range of each of the boxplots corresponds to the corresponding number of observations in each cell in Table 3.9.

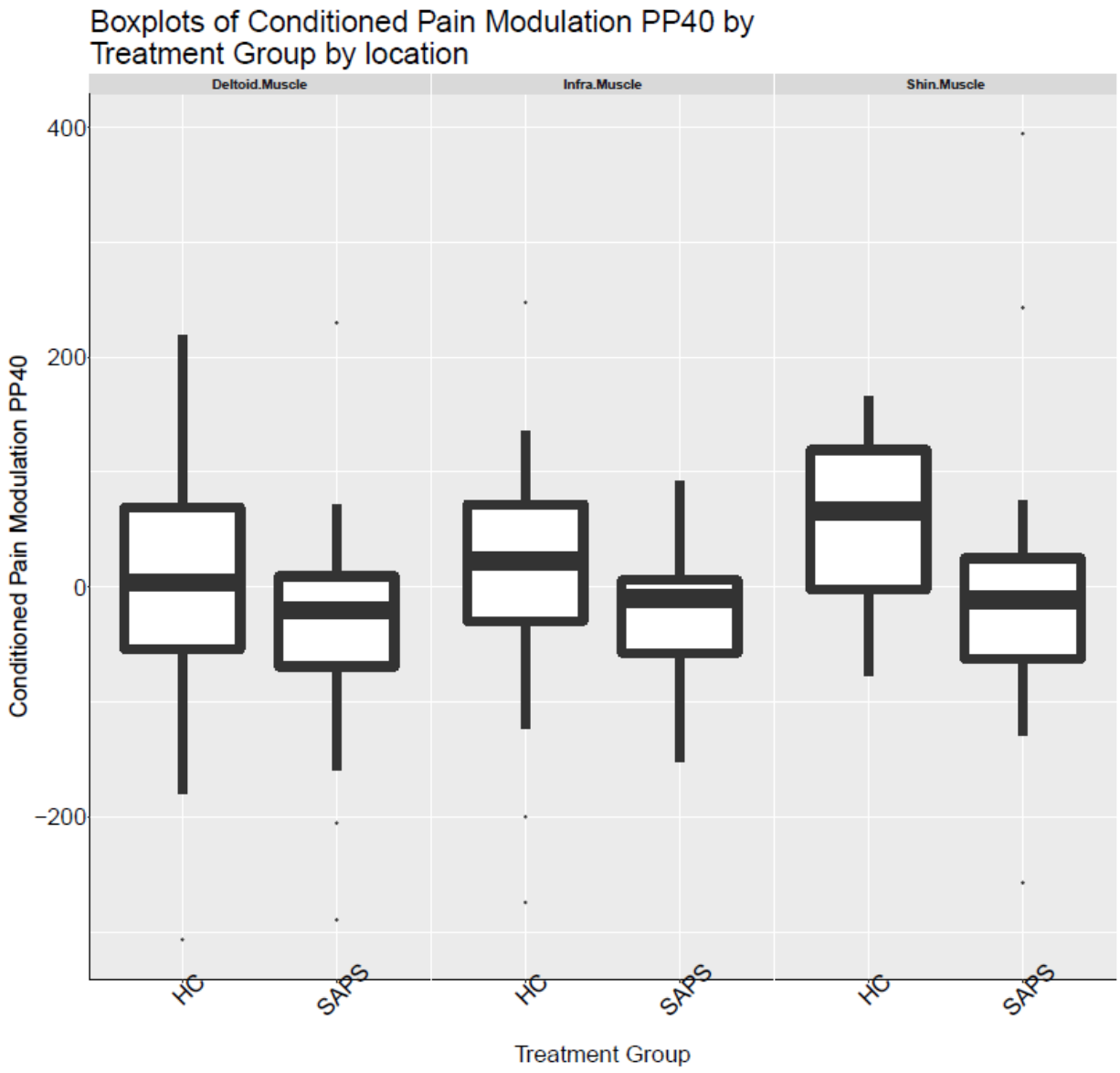


Figure 3.5: Side by side boxplots of conditioned pain modulation-PP40 (CPM effect - absolute change) measured at deltoid (left), infraspinatus (centre) and tibialis anterior (right) by treatment group

1. CPM effect - percentage change
    a. Deltoid
    b. Infraspinatus

c. Tibialis anterior

Table 3.10 (below) shows the distributions of the outcome CPM effect (percentage change) at deltoid, infraspinatus, and tibialis anterior. Missing values for all the variables were denoted as "Missing".

Summary measure	Deltoid		Infraspinatus		Tibialis anterior	
	Treatment Group		Treatment Group		Treatment Group	
	HC	SAPS	HC	SAPS	HC	SAPS
n	21	21	21	21	21	21
mean (% change)	-10.04	-17.28	-3.59	-10.23	10.01	-1.98
SD	37.05	28.95	29.72	21.99	12.28	18.80
min	-114.22	-63.72	-89.32	-68.16	-13.05	-31.05
max	29.28	27.04	26.85	32.22	38.45	36.20
median	0.86	-21.34	2.77	-3.91	10.55	-3.19
IQR	38.35	47.09	24.90	23.89	18.80	23.26
Q1	-25.84	-41.35	-8.22	-22.42	-0.37	-15.60
Q3	12.51	5.73	16.68	1.47	18.43	7.66
Missing	1	0	2	0	0	0

Table 3.10: Descriptive table for the exploratory outcome conditioned pain modulation-PP40 (CPM effect - percentage change) by treatment group at deltoid (left), infraspinatus (centre) and tibialis anterior (right)

Regarding missing data, please see details above in the description regarding absolute CPM change.

The boxplots in Figure 3.6 (below) provide visual representations of the distributions of the outcome conditioned pain modulation-PP40 (CPM effect - absolute change) by treatment group at deltoid, infraspinatus and tibialis anterior as shown in Table 3.10. The median, spread, IQR and range of each of the boxplots corresponds to the corresponding number of observations in each cell in Table 3.10.

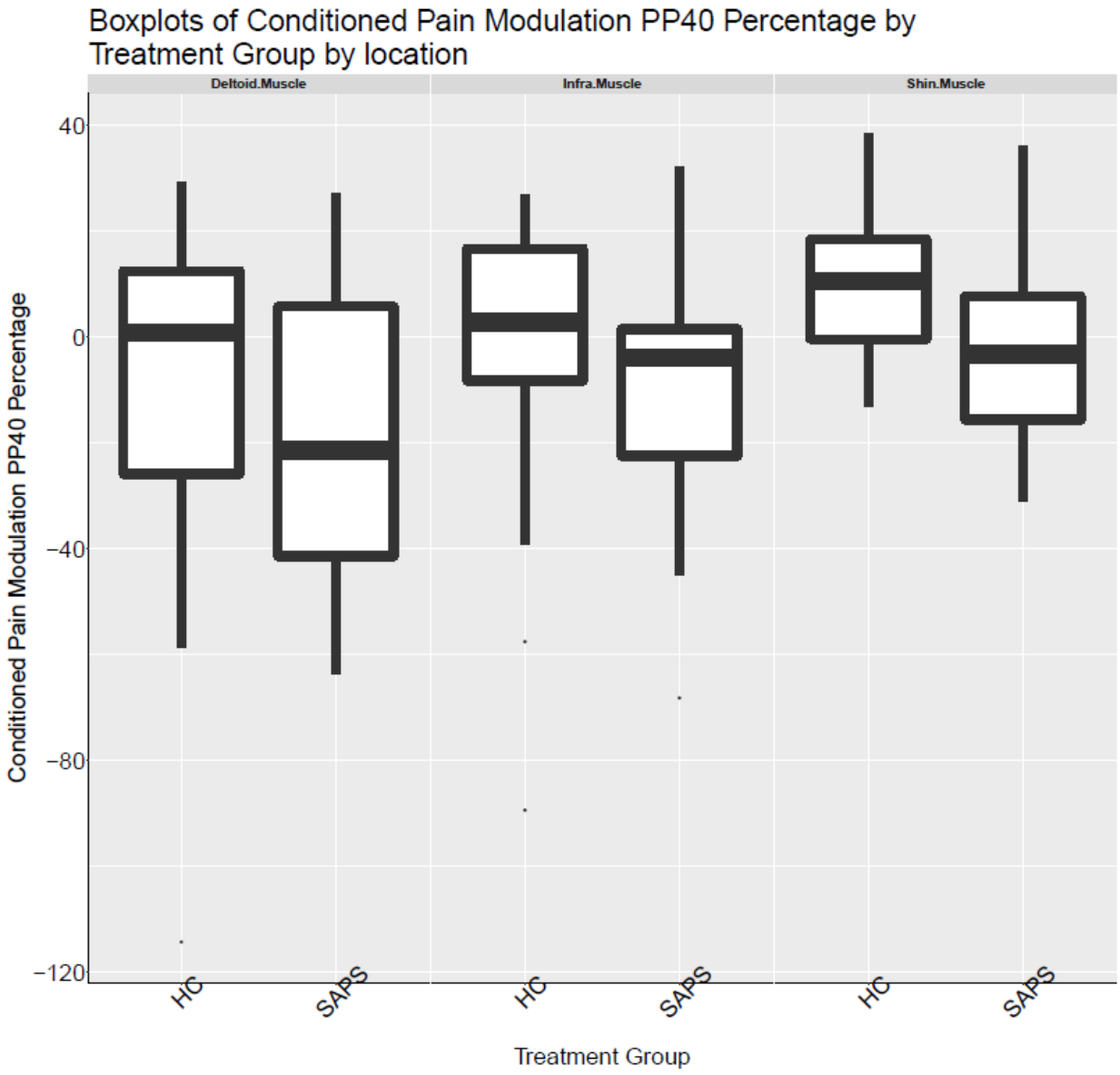


Figure 3.6: Side by side boxplots of conditioned pain modulation-PP40 (CPM effect – percentage change) measured at deltoid (left), infraspinatus (centre) and tibialis anterior (right) by treatment group

3.4.3.5 Conditioned pain modulation-HP40 (exploratory outcome variable)

1. CPM effect - absolute change
    a. Skin over the deltoid muscle

b. Skin over the lateral aspect of the shin below the knee

Table 3.11 (below) shows the distributions of the outcome CPM effect (absolute change) at the skin over the deltoid muscle and the skin over the lateral aspect of the shin below the knee. Missing values for all variables were denoted as "Missing".

Summary measure	Deltoid		Shin	
	Treatment Group		Treatment Group	
	HC	SAPS	HC	SAPS
n	21	21	21	21
mean (change in °C)	1.02	-0.09	0.61	0.27
SD	1.78	2.02	1.68	2.16
min	-0.95	-5.50	-1.85	-2.55
max	7.75	3.80	5.95	8.15
median	0.60	0.00	0.35	0.28
IQR	1.30	2.11	0.70	1.09
Q1	0.35	-0.94	0.10	-0.50
Q3	1.65	1.18	0.80	0.59
Missing	0	1	0	1

Table 3.11: Descriptive table for the exploratory outcome conditioned pain modulation-HP40 (CPM effect – absolute change) by treatment group at the skin over the deltoid muscle (left) and at the skin over the lateral aspect of the shin below the knee (right)

Regarding missing data for both the skin over the deltoid muscle and the skin over the lateral aspect of the shin below the knee - for the participant in the SAPS group, the heat measuring device was not working on the day of testing.

The boxplots in Figure 3.7 (below) provide visual representations of the distributions of the outcome conditioned pain modulation-HP40 (CPM effect - absolute change) by treatment group at the skin over the deltoid muscle and at the skin over the lateral aspect of the shin below the knee as shown in Table 3.11. The median, spread, IQR and range of each of the boxplots corresponds to the corresponding number of observations in each cell in Table 3.11.

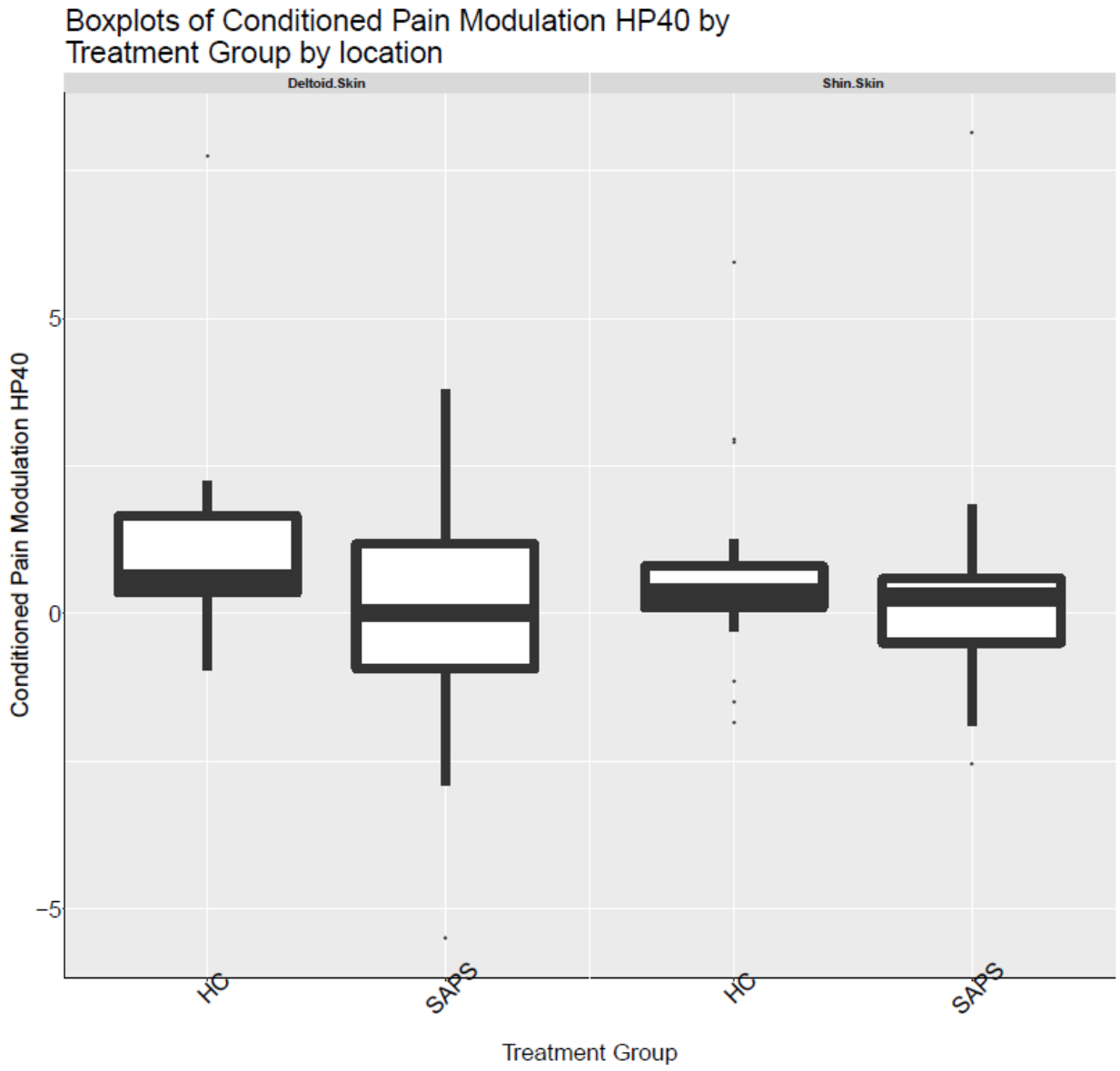


Figure 3.7: Side by side boxplots of conditioned pain modulation-HP40 (CPM effect - absolute change) measured at the skin over the deltoid muscle (left) and at the skin over the lateral aspect of the shin below the knee (right) by treatment group

1. CPM effect - percentage change
    a. Skin over the deltoid muscle
    b. Skin over the lateral aspect of the shin below the knee

Table 3.12 (below) shows the distributions of the outcome CPM effect (percentage change) at the skin over the deltoid muscle and the skin over the lateral aspect of the shin below the knee. Missing values for all variables were denoted as "Missing".

Summary measure	Deltoid		Shin	
	Treatment Group		Treatment Group	
	HC	SAPS	HC	SAPS
n	21	21	21	21
mean (% change)	2.26	-0.25	1.30	0.40
SD	4.02	4.54	3.68	4.41
min	-2.13	-11.51	-4.22	-5.71
max	17.40	9.50	13.36	15.84
median	1.25	-0.01	0.67	0.55
IQR	3.44	4.40	1.48	2.35
Q1	0.69	-1.95	0.20	-1.12
Q3	4.13	2.45	1.68	1.23
Missing	0	1	0	1

Table 3.12: Descriptive table for the exploratory outcome conditioned pain modulation-HP40 (CPM effect – percentage change) by treatment group at the skin over the deltoid muscle (left) and at the skin over the lateral aspect of the shin below the knee (right)

Regarding missing data, please see details above in the description regarding absolute CPM change.

The boxplots in Figure 3.8 (below) provide visual representations of the distributions of the outcome conditioned pain modulation-HP40 (CPM effect - percentage change) by treatment group at the skin over the deltoid muscle and at the skin over the lateral aspect of the shin below the knee as shown in Table 3.12. The median, spread, IQR and range of each of the boxplots corresponds to the corresponding number of observations in each cell in Table 3.12.

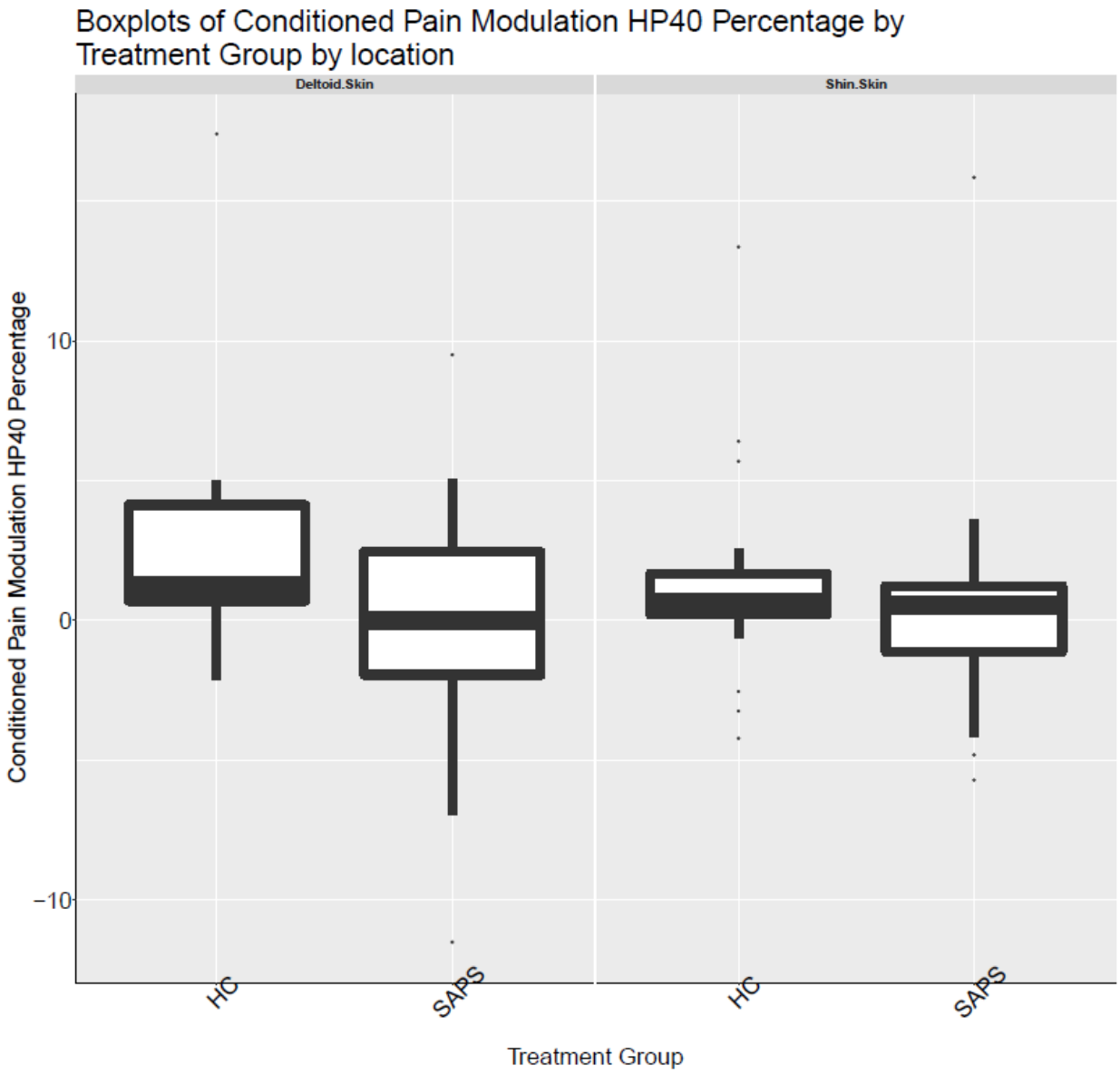


Figure 3.8: Side by side boxplots of conditioned pain modulation-HP40 (CPM effect - percentage change) measured at the skin over the deltoid muscle (left) and at the skin over the lateral aspect of the shin below the knee (right) by treatment group

3.4.3.6 Temporal summation score (exploratory outcome variable)

1. Skin over the deltoid muscle
2. Skin over the lateral aspect of the shin below the knee

Table 3.13 (below) shows the distributions of the outcome temporal summation score at the skin over the deltoid muscle and the skin over the lateral aspect of the shin below the knee. Missing values for all variables were denoted as "Missing".

Summary measure	Deltoid		Shin	
	Treatment Group		Treatment Group	
	HC	SAPS	HC	SAPS
n	21	21	21	21
mean	9.43	17.07	10.92	17.93
SD	9.38	19.74	9.48	17.35
min	-2.60	2.00	0.00	4.70
max	37.00	66.40	29.80	62.60
median	8.20	8.12	8.20	11.43
IQR	7.85	18.05	11.80	8.50
Q1	3.95	4.20	4.00	7.75
Q3	11.80	22.25	15.80	16.25
Missing	5	5	4	5

Table 3.13: Descriptive table for the exploratory outcome temporal summation score by treatment group at the skin over the deltoid muscle (left) and at the skin over the lateral aspect of the shin below the knee (right)

Regarding missing data, some participants (for deltoid: SAPS n = 3, healthy controls n = 3; for the shin: SAPS n = 2, healthy controls n = 1), who registered pain with the 256 mN stimulator had less than three traces (of five) that registered pain over the full stimulus train of five stimulus series, so that it was not possible to subtract the pain value at pinprick 1 from pinprick 10 in three or more traces, as required for analysis. The trace may have contained, for example, only one pain peak or, for example, only 3 pain sensations felt within a stimulus train of 10 pinpricks, where there should be a "continuous" sensation of pain stimulated, such that you would see summation of pain, if it was occurring, and to what degree.

Additionally, some participants (for deltoid: SAPS n = 2, healthy controls n = 2; for the shin: SAPS n = 2, healthy controls n = 3) did not register any pain with either the 256 mN pinprick stimulator or the 512 mN pinprick stimulator, so they had a completely blank summation trace (no pain, so no "summation" - or lack of summation - of pain), or had less than three traces (of five) that registered pain over the full stimulus train of five stimulus series, so that it was not possible to subtract the pain value at pinprick 1 from pinprick 10 in three or more traces, as required for analysis.

For one final participant (for the shin; SAPS n = 1), the testing equipment was not working on the day.

The boxplots in Figure 3.9 (below) provide visual representations of the distributions of the outcome temporal summation score by treatment group at the skin over the deltoid muscle and at the skin over the lateral aspect of the shin below the knee as shown in Table 3.13. The median,

spread, IQR and range of each of the boxplots corresponds to the corresponding number of observations in each cell in Table 3.13.

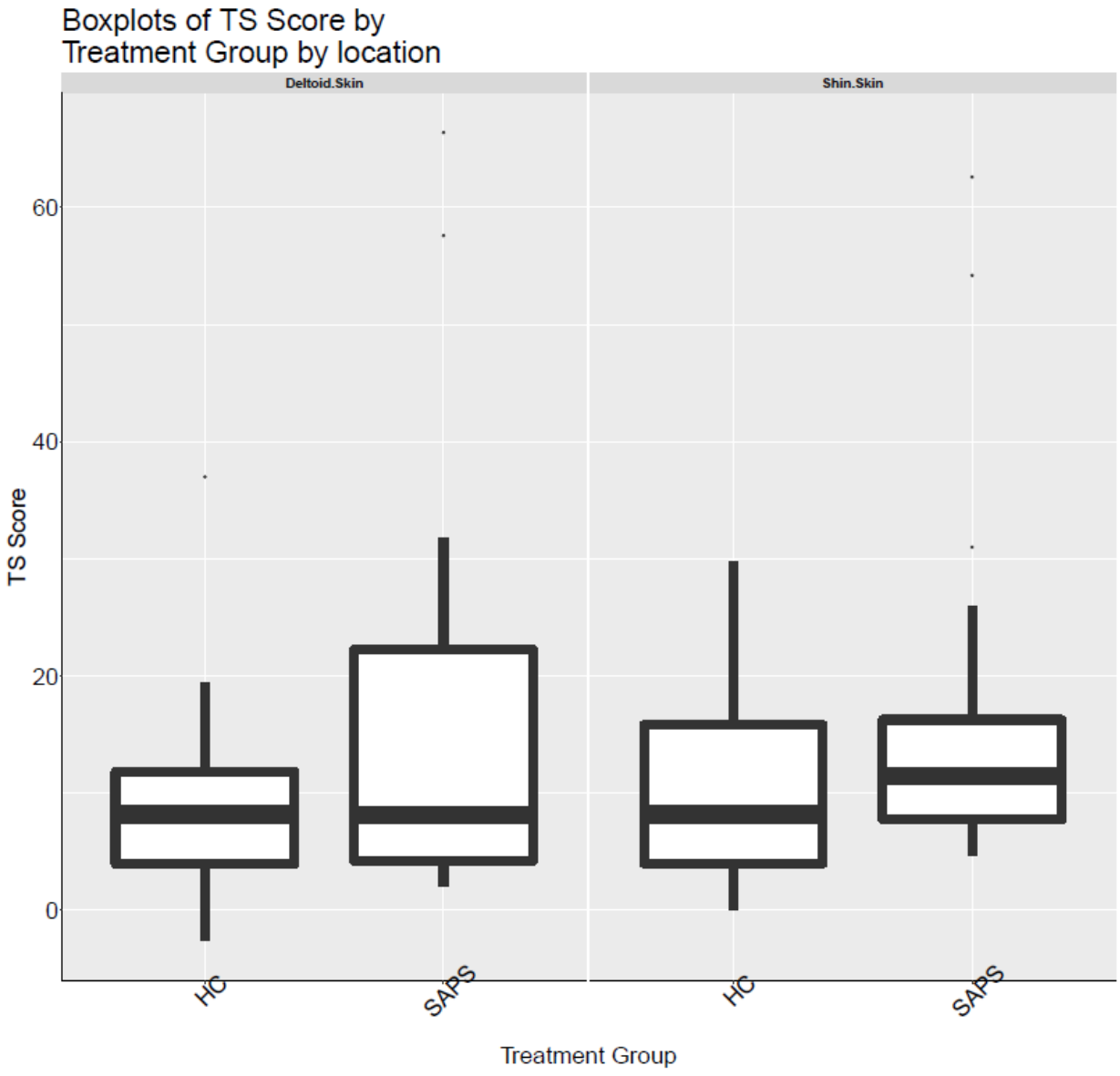


Figure 3.9: Side by side boxplots of temporal summation score (TS Score) measured at the skin over the deltoid muscle (left) and at the skin over the lateral aspect of shin below the knee (right) by treatment group

3.4.3.7 Pain area

Using the same body charting software that was used for participants shading in the areas where they had pain related to their shoulder pain, the localised subacromial area was found to have an area of approximately 92 pixels. The mean and standard deviation of the areas that SAPS participants shaded on their body chart is presented in Table 3.14 below.

Treatment Group	Mean (number of pixels)	SD	Min	Max
SAPS	5185.29	6154.51	72	21318

Table 3.14 Outcome: pain area. Mean, standard deviation (SD), minimum (Min) and maximum (Max) values

3.4.4 Main results

3.4.4.1 Pressure pain threshold (primary outcome variable)

3.4.4.1.1 Deltoid – primary hypothesis

Table 3.15 (below) indicates that the interaction effect on pressure pain threshold was insignificant, but the main effects for Treatment and Location were statistically significant; and so the comparisons of interest were performed using this model.(232)

	Chisq	Df	Pr(>Chisq)
(Intercept)	33.55	1	0.000
TreatmentGroup	5.09	1	0.024
Location	19.81	2	0.000
TreatmentGroup:Location	2.84	2	0.242

Table 3.15: Outcome: pressure pain threshold. Type III ANOVA table from the mixed effects model to test overall significance of the interaction and main effects (Treatment Group and Location)

Table 3.16 (below) shows the estimated marginal means for each of the treatment groups healthy controls and SAPS at deltoid along with their standard errors and 95% CIs. This table indicates that the hypothesis was supported by the data, i.e., the mean pressure pain threshold was lower in the SAPS group than in the healthy control group.

Treatment Group	Location	Marginal mean estimates (kPa)	SE	df	Lower CI	Upper CI
SAPS	Deltoid	383	63.6	56.8	255	510
HC	Deltoid	489	63.6	56.8	361	616

Table 3.16: Outcome: pressure pain threshold. Marginal mean estimates from the mixed effect model and their 95% CIs (lower CI and upper CI) at deltoid

Table 3.17 (below) shows the estimated mean pressure pain threshold difference between the SAPS and healthy control groups at deltoid. The estimated difference was in the hypothesised direction, i.e., mean pressure pain threshold was lower in the SAPS group than in the healthy control group, with an estimate of -105.838 (95% CI: -286.064, 74.388). Although the estimated effect supports the hypothesis, this difference is statistically insignificant based on the p-value = .244.

contrast	Location	Estimated effect	SE	df	t-ratio	p-value	Lower CI	Upper CI
SAPS - HC	Deltoid	-106	90	56.8	-1.18	0.244	-286	74.4

Table 3.17: Outcome: pressure pain threshold. Estimated effect of SAPS, i.e., estimated mean pressure pain threshold difference between the SAPS and HC at deltoid from the mixed effect model and their standard errors, p-values and 95% CIs

3.4.4.1.2 Deltoid – primary hypothesis adjusted for covariates

The following three covariates were included to adjust the effects of SAPS on pressure pain threshold at deltoid:

1. Age
2. Sex
3. IPAQ (International Physical Activity Questionnaire: a self-reported measure of physical activity) total score

These three covariates were selected based on subject matter knowledge that these covariates have an effect on pressure pain threshold. Use of a linear mixed effects model not only helps to estimate the magnitudes of the effects of SAPS on pressure pain threshold and to test for statistical significance, but also allows for the inclusion of covariates that may adjust the magnitudes of the effect size estimates, i.e., make them more accurate. Of note, even though the SAPS and healthy control groups are matched by age and sex, there may still be a leftover effect of these covariates on the pressure pain threshold that may be captured by including them in the model.

Table 3.18 (below) shows the distribution of pressure pain threshold by each level of the baseline covariate sex. The relationship between the outcome pressure pain threshold and the other two covariates, age and IPAQ score, are only explored with the scatterplots (see Figure 3.10), as both these baseline covariates and the outcome of interest are continuous variables.

Summary measure	Sex	
	Female	Male
n	22	20
mean	313.45	569.96
SD	209.19	262.16
min	87.20	199.90
max	737.90	1159.30
median	246.95	538.50

IQR	293.05	442.05
Q1	137.43	348.43
Q3	430.48	790.47
Missing	0	0

Table 3.18: Descriptive table for the primary outcome pressure pain threshold by sex at deltoid

Table 3.19 shows the distributions of the baseline covariates sex, age and IPAQ total score in healthy control and SAPS. p-values were obtained using the Fisher Exact test for the categorical covariate sex, the two-sample t-test for the continuous covariate age, and the Mann-Whitney-Wilcoxon Test for the covariate IPAQ total score. Missing values for all variables were denoted as "Missing". As expected, due to balancing of groups by age and sex in the study design, there were no significant differences in age and sex between healthy controls and SAPS. However, there was a statistically significant difference in IPAQ total score distributions between healthy controls and SAPS.

Outcome - sex			
Summary measure	Treatment Group		
	HC	SAPS	p-value
Female: n (%)	11 (52.38)	11 (52.38)	1.000
Male: n (%)	10 (47.62)	10 (47.62)	
Missing: n	0	0	

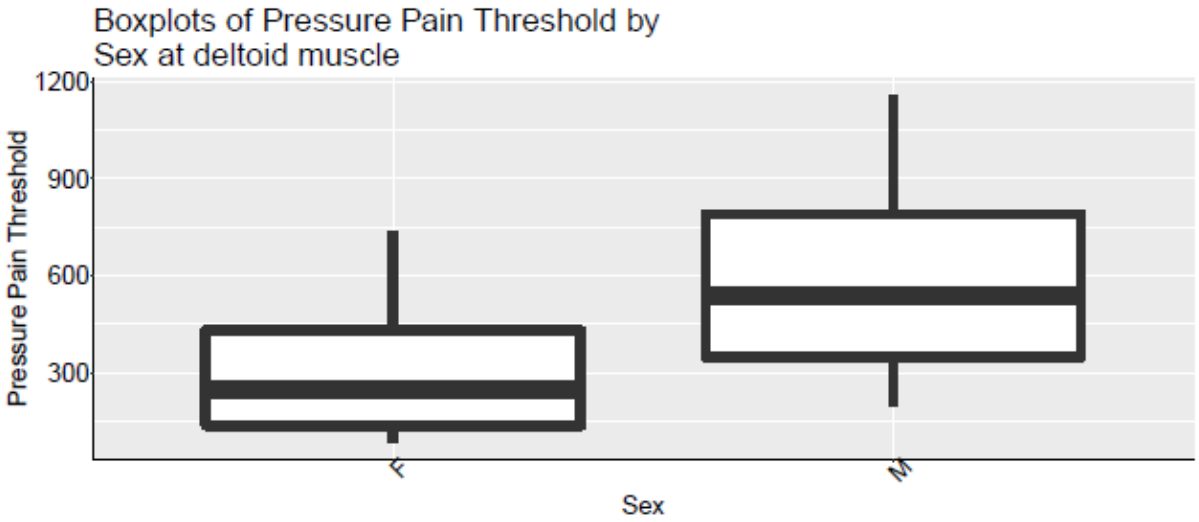
Outcome - Age			
Summary measure	Treatment Group		
	HC	SAPS	p-value
n	21	21	
mean	52.62	53.90	0.759
SD	14.11	12.86	
min	29	28	
max	77	76	
median	54	54	
IQR	19	13	
Q1	41	48	
Q3	60	61	
Missing	0	0	

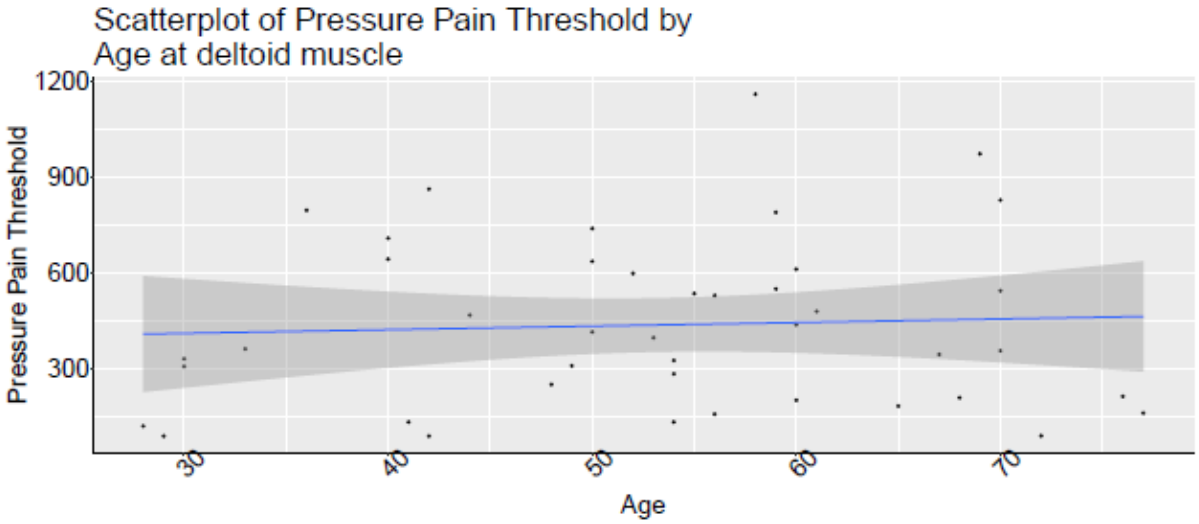
Outcome – IPAQ total score			
Summary measure	Treatment Group		
	HC	SAPS	p-value
n	21	21	
mean	5390.31	4052.50	0.038
SD	3591.07	3928.22	
min	594	628.5	
max	15918	13437	
median	3972	2373	

IQR	3303	3387	
Q1	3309	1386	
Q3	6612	4773	
Missing	0	0	

Table 3.19: Descriptive table for the baseline covariates sex, age, IPAQ total score by treatment group

Figure 3.10 (below) explores the relationship between the outcome pressure pain threshold and sex with boxplots and between the other two covariates - age and IPAQ total score - with scatterplots (as both these baseline covariates and the outcome of interest are continuous variables).


Boxplots of Pressure Pain Threshold by Sex at deltoid muscle
Pressure Pain Threshold
1200
900
600
300
F
M
Sex


Scatterplot of Pressure Pain Threshold by Age at deltoid muscle
Pressure Pain Threshold
1200
900
600
300
30
40
50
60
70
Age

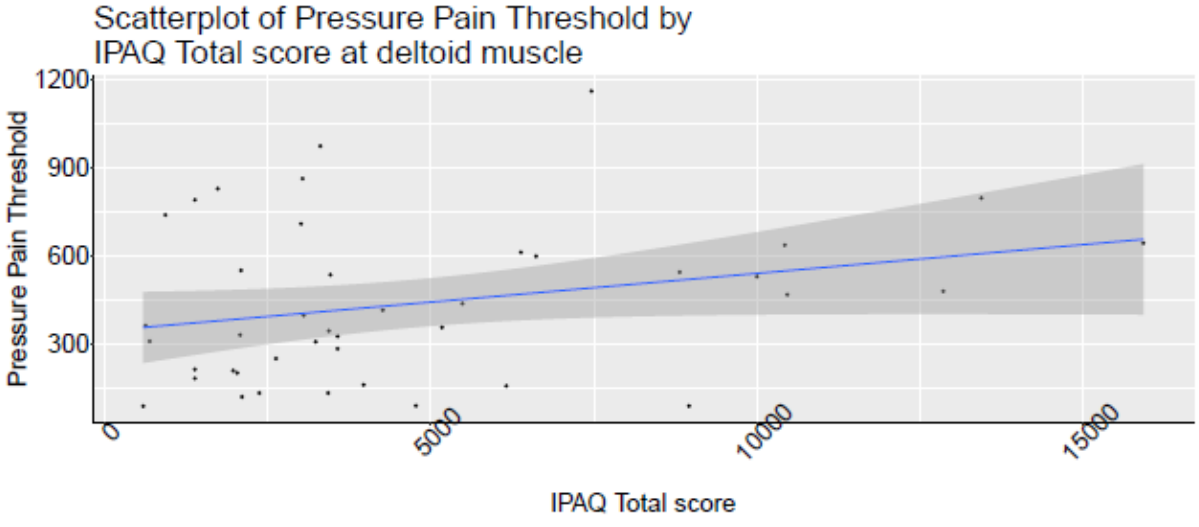

Scatterplot of Pressure Pain Threshold by IPAQ Total score at deltoid muscle
Pressure Pain Threshold
1200
900
600
300
0
5000
10000
15000
IPAQ Total score

Figure 3.10: Exploration of the associations between the covariates sex, age and IPAQ total score and pressure pain threshold at deltoid

Table 3.20 (below) indicates that the interaction effect Treatment Group x Location on pressure pain threshold was insignificant, but both main effects of interest (Treatment Group and Location) were statistically significant after adjusting for the baseline covariates.(232) Since the covariates were included in the model only to adjust for the effect of interest, interpretations of the effects of the baseline covariates on the pressure pain threshold have not been provided. The unadjusted and adjusted estimates for the mean pressure pain threshold differences between the healthy control group and the SAPS group at deltoid have been described below.

	Chisq	Df	Pr(>Chisq)
(Intercept)	0.241	1	0.623
Treatment Group	5.128	1	0.024
Location	19.813	2	0.000
Age	0.698	1	0.404
Sex	8.468	1	0.004
‘IPAQ Total Score‘	1.405	1	0.236
TreatmentGroup:Location	2.838	2	0.242

Table 3.20: Outcome: pressure pain threshold. Type III ANOVA table from the mixed effects model to test overall significance of the interaction and main effects (Treatment Group and Location) adjusted for the covariates: age, sex, IPAQ total score

Table 3.21 (below) shows the estimated marginal means for each of the treatment groups healthy controls and SAPS at deltoid along with their standard errors and 95% CIs adjusted for the covariates. This table indicates that the hypothesis was supported by the data, i.e., mean pressure pain threshold was lower in the SAPS group than in the healthy control group.

Treatment Group	Location	Marginal mean estimates (kPa)	SE	df	Lower CI	Upper CI
SAPS	Deltoid	395	70.2	56.1	254	535
HC	Deltoid	487	68.2	56.0	351	624

Table 3.21: Outcome: pressure pain threshold. Marginal mean estimates from the mixed effect model and their 95% confidence intervals (CL) at deltoid adjusted for the covariates: age, sex, 'IPAQ total score'

Table 3.22 (below) shows the estimated mean pressure pain threshold difference between the treatment groups SAPS and healthy controls at deltoid, adjusted for the covariates. The estimated difference was in the hypothesised direction, i.e., mean pressure pain threshold was lower in the SAPS group than in the healthy control group, with an estimate of -92.414 (95% CI: -260.116, 75.287). Although the estimated effect supports the hypothesis, this difference is statistically insignificant based on the p-value = .274.

contrast	Location	Estimated effect	SE	df	t-ratio	p-value	Lower CI	Upper CI
SAPS - HC	Deltoid	-92.4	83.7	55.7	-1.1	0.274	-260	75.3

Table 3.22: Outcome: pressure pain threshold. Estimated effect of SAPS, i.e., estimated mean pressure pain threshold difference between the SAPS and HC at deltoid from the mixed effect model and their standard errors, p-values and 95% CIs, adjusted for the covariates: age, sex, 'IPAQ total score'

Table 3.23 (below) shows the estimated differences in pressure pain threshold means for the SAPS and healthy control groups, unadjusted and adjusted for the covariates.

	contrast	Location	Estimated effect	SE	df	t-ratio	p-value	Lower CI	Upper CI
unadjusted	SAPS - HC	Deltoid	-105.8	90.0	56.8	-1.18	0.244	-286	74.4
adjusted	SAPS - HC	Deltoid	-92.4	83.7	55.7	-1.10	0.274	-260	75.3

Table 3.23: Outcome: pressure pain threshold. Estimated effect of SAPS, i.e., estimated mean pressure pain threshold difference between the SAPS and HC at deltoid from the mixed effect model and their standard errors, p-values and 95% CIs, unadjusted and adjusted for the covariates: age, sex, and 'IPAQ total score'

3.4.4.1.3 Infraspinatus and tibialis anterior – secondary hypotheses on the primary outcome

The Type III ANOVA table previously discussed above for the analysis of the primary hypothesis (deltoid pressure pain threshold) also corresponds to the infraspinatus and tibialis anterior locations. As discussed previously, Table 3.24 (below) indicates that the interaction effect on pressure pain threshold was insignificant, but the main effects for Treatment and Location were statistically significant; and so the comparisons of interest relating to the secondary hypothesis were also performed using this model.(232)

	Chisq	Df	Pr(>Chisq)
(Intercept)	33.55	1	0.000
TreatmentGroup	5.09	1	0.024
Location	19.81	2	0.000
TreatmentGroup:Location	2.84	2	0.242

Table 3.24: Outcome: pressure pain threshold. Type III ANOVA table from the mixed effects model to test overall significance of the interaction and main effects (Treatment Group and Location)

Table 3.25 (below) shows the estimated marginal means for each of the treatment groups healthy controls and SAPS at infraspinatus and tibialis anterior along with their standard errors and 95% CIs. This table indicates that the hypothesis was supported by the data, i.e., mean pressure pain

threshold was lower in the SAPS group than in the healthy control group in both locations: infraspinatus and tibialis anterior.

Treatment Group	Location	Marginal mean estimates (kPa)	SE	df	Lower CI	Upper CI
SAPS	Infraspinatus	369	63.6	56.8	241	496
HC	Infraspinatus	572	63.6	56.8	444	699
SAPS	Tibialis anterior	550	63.6	56.8	423	678
HC	Tibialis anterior	746	63.6	56.8	618	873

Table 3.25: Outcome: pressure pain threshold. Marginal mean estimates from the mixed effect model and their 95% CIs (CL) at infraspinatus and tibialis anterior

Table 3.26 (below) shows the estimated mean pressure pain threshold difference between the treatment groups SAPS and healthy controls at infraspinatus and tibialis anterior. The estimated difference was in the hypothesised direction for both locations. At infraspinatus, the mean pressure pain threshold was lower in the SAPS group than in the healthy control group with an estimate of -202.971 (95% CI: -383.197, -22.745). At tibialis anterior, the mean pressure pain threshold was lower in the SAPS group than in the healthy control group with an estimate of -

195.633 (95% CI:-375.859, -15.407). The estimated effect supports the hypothesis, and is statistically significant for both muscles based on the adjusted p-values for FDR (False Discovery Rate(231)), p-values = (.028, .034).

contrast	Location	Estimated effect	SE	df	t-ratio	p-value	Lower CI	Upper CI
SAPS - HC	Infraspinatus	-203	90	56.8	-2.25	0.028	-383	-22.7
SAPS - HC	Tibialis anterior	-196	90	56.8	-2.17	0.034	-376	-15.4

Table 3.26: Outcome: pressure pain threshold. Estimated effect of SAPS, i.e., estimated mean pressure pain threshold difference between the SAPS and HC at infraspinatus and tibialis anterior from the mixed effect model and their standard errors, p-values and 95% CIs

3.4.4.2 Heat pain threshold (exploratory outcome variable)

1. Skin over the deltoid muscle
2. Skin over the lateral aspect of the shin below the knee

Table 3.27 (below) indicates that the interaction effect of Treatment Group and Location on heat pain threshold was statistically insignificant, based on the ANOVA Type III table. The main effects of Treatment Group and Location were also insignificant. Regardless, post-hoc pairwise tests were performed to test the hypotheses of interest and obtain estimates of the outcome

differences between the healthy control group and the SAPS group at the skin over the deltoid muscle and at the skin over the lateral aspect of the shin below the knee.

	Chisq	Df	Pr(>Chisq)
(Intercept)	3750.409	1	0.000
TreatmentGroup	0.051	1	0.822
Location	2.291	1	0.130
TreatmentGroup:Location	0.000	1	0.994

Table 3.27 Outcome: heat pain threshold. Type III ANOVA table from the mixed effects model to test overall significance of the interaction and main effects (Treatment Group and Location)

Table 3.28 (below) shows the estimated marginal means for each of the treatment groups healthy controls and SAPS at the skin over the deltoid muscle (lower) and at the skin over the lateral aspect of the shin below the knee (upper) along with their standard errors and 95% CIs.

Treatment Group	Location - skin	Marginal mean estimates (˚C)	SE	df	Lower CI	Upper CI
SAPS	Shin	46.9	0.767	43.7	45.4	48.5
HC	Shin	47.2	0.748	43.7	45.7	48.7
SAPS	Deltoid	46.4	0.767	43.7	44.8	47.9

HC	Deltoid	46.6	0.752	44.5	45.1	48.1

Table 3.28: Outcome: heat pain threshold. Marginal mean estimates from the mixed effect model and their 95% CIs (CL) at the skin over the deltoid muscle (lower) and at the skin over the lateral aspect of the shin below the knee (upper)

Table 3.29 (below) indicates that the estimated effect was in the hypothesised direction (i.e., the mean outcome was lower in the SAPS group than in the healthy control group). At the skin over the lateral aspect of the shin below the knee, the estimated mean heat pain threshold in the SAPS group was insignificantly smaller (p-value = .823) than in the healthy control group by 0.241, with an estimated effect of -0.241 (95% CI: -2.4, 1.918). At the skin over the deltoid muscle, the estimated mean heat pain threshold in the SAPS group was insignificantly smaller (p-value = .827) than in the healthy control group by 0.237, with an estimated effect of -0.237 (95% CI: -2.401, 1.928).

contrast	Location - skin	Estimated effect	SE	df	t-ratio	p-value	Shin	Upper CI
SAPS - HC	Shin	-0.241	1.07	43.7	-0.225	0.823	-2.4	1.92
SAPS - HC	Deltoid	-0.237	1.07	44.1	-0.220	0.827	-2.4	1.93

Table 3.29: Outcome: heat pain threshold. Estimated effect of SAPS, i.e., estimated mean heat pain threshold difference between the SAPS and HC at the skin over the deltoid muscle and at the skin over the lateral

aspect of the shin below the knee from the mixed effect model and their standard errors, p-values and 95% CIs

3.4.4.3 Mechanical pain threshold (exploratory outcome variable)

1. Skin over the deltoid muscle
2. Skin over the lateral aspect of the shin below the knee

Table 3.30 (below) indicates that the interaction effect of Treatment Group and Location on mechanical pain threshold was statistically insignificant, based on the ANOVA Type III table. The main effects of Treatment Group and Location were also insignificant. Regardless, post-hoc pairwise tests were performed to test the hypotheses of interest and obtain estimates of the outcome differences between the healthy control group and the SAPS group at the skin over the deltoid muscle and at the skin over the lateral aspect of the shin below the knee.

	Chisq	Df	Pr(>Chisq)
(Intercept)	22.258	1	0.000
TreatmentGroup	0.466	1	0.495
Location	0.971	1	0.324
TreatmentGroup:Location	0.269	1	0.604

Table 3.30: Outcome: mechanical pain threshold. Type III ANOVA table from the mixed effects model to test overall significance of the interaction and main effects (Treatment Group and Location)

Table 3.31 (below) shows the estimated marginal means for each of the treatment groups healthy controls and SAPS at the skin over the deltoid muscle (lower) and at the skin over the lateral aspect of the shin below the knee (upper) along with their standard errors and 95% CIs.

Treatment Group	Location - skin	Marginal mean estimates ()	SE	df	Lower CI	Upper CI
SAPS	Shin	140	29.8	57.5	80.9	200
HC	Shin	112	29.8	57.5	52.1	171
SAPS	Deltoid	166	29.8	57.5	106.2	225
HC	Deltoid	156	29.8	57.5	96.4	216

Table 3.31: Outcome: mechanical pain threshold. Marginal mean estimates from the mixed effect model and their 95% CIs (CL) at the skin over the deltoid muscle and at the skin over the lateral aspect of the shin below the knee

Table 3.32 (below) indicates that the estimated effect was not in the hypothesised direction (i.e., the mean outcome was higher in the SAPS group than in the healthy control group). At the skin over the lateral aspect of the shin below the knee, the estimated mean mechanical pain threshold in SAPS group is insignificantly higher (p-value = .498) than in healthy control group by 28.744, with estimated effect of 28.744 (95% CI: -55.557, 113.045). At the skin over the deltoid muscle, the estimated mean mechanical pain threshold in SAPS group is insignificantly higher (p-value =

.816) than in the healthy control group by 9.852, with an estimated effect of 9.852 (95% CI: -74.449, 94.153).

contrast	Location - skin	Estimated effect	SE	df	t.ratio	p-value	Shin	Upper CI
SAPS - HC	Shin	28.74	42.1	57.5	0.683	0.498	-55.6	113.0
SAPS - HC	Deltoid	9.85	42.1	57.5	0.234	0.816	-74.4	94.2

Table 3.32: Outcome: mechanical pain threshold. Estimated effect of SAPS, i.e., estimated mean mechanical pain threshold difference between the SAPS and HC at the skin over the deltoid muscle and at the skin over the lateral aspect of the shin below the knee from the mixed effect model and their standard errors, p-values and 95% CIs

3.4.4.4 Conditioned pain modulation-PP40 (exploratory outcome variable)

1. CPM effect - absolute change
    a. Deltoid
    b. Infraspinatus
    c. Tibialis anterior

Table 3.33 (below) indicates that the interaction effect of Treatment Group and Location on Conditioned Pain Modulation-PP40 – absolute change was statistically insignificant, based on the ANOVA Type III table. The main effects of Treatment Group and Location were also

insignificant. Regardless, post-hoc pairwise tests were performed to test the hypotheses of interest and obtain estimates of the outcome differences between the healthy control group and the SAPS group at deltoid, infraspinatus and tibialis anterior.

	Chisq	Df	Pr(>Chisq)
(Intercept)	1.251	1	0.263
TreatmentGroup	0.853	1	0.356
Location	1.476	2	0.478
TreatmentGroup:Location	0.498	2	0.779

Table 3.33: Outcome: conditioned pain modulation-PP40 – absolute change. Type III ANOVA table from the mixed effects model to test overall significance of the interaction and main effects (Treatment Group and Location)

Table 3.34 (below) shows the estimated marginal means for each of the treatment groups healthy controls and SAPS deltoid (lower), infraspinatus (upper) and tibialis anterior (middle) along with their standard errors and 95% CIs.

Treatment Group	Location	Marginal mean estimates (kPa)	SE	df	Lower CI	Upper CI
SAPS	Infraspinatus	-25.25	22.6	114	-70.0	19.5
HC	Infraspinatus	4.99	23.7	115	-42.0	52.0
SAPS	Tibialis anterior	1.36	22.6	114	-43.4	46.1
HC	Tibialis anterior	58.50	22.6	114	13.8	103.2
SAPS	Deltoid	-33.48	22.6	114	-78.2	11.2
HC	Deltoid	-1.61	23.1	114	-47.4	44.2

Table 3.34: Outcome: conditioned pain modulation-PP40 – absolute change. Marginal mean estimates from the mixed effect model and their 95% CIs (CL) at infraspinatus and tibialis anterior

Table 3.35 (below) indicates that the estimated effect was not in the hypothesised direction (i.e., the mean outcome had a negative (less than zero) value at all three locations in the SAPS-HC comparisons (indicating that CPM was not impaired in the SAPS group compared to the healthy control group and that the SAPS group actually demonstrated more pain inhibition, or "more efficient" CPM than the healthy control group). At infraspinatus, the estimated mean conditioned pain modulation-PP40 - absolute change in the SAPS group was insignificantly more negative (p-value = .358) than in the healthy control group by 30.233, with an estimated effect of -30.233

(95% CI: -95.129, 34.663). At tibialis anterior, the estimated mean conditioned pain modulation-PP40 - absolute change in the SAPS group was insignificantly more negative (p-value = .076) than in the healthy control group by 57.133, with an estimated effect of -57.133 (95% CI: -120.382, 6.115). At deltoid, the estimated mean conditioned pain modulation-PP40 - absolute change in the SAPS group was insignificantly more negative (p-value = .326) than in the healthy control group by 31.867, with an estimated effect of -31.867 (95% CI: -95.903, 32.169).

contrast	Location	Estimated effect	SE	df	t-ratio	p-value	Lower CI	Upper CI
SAPS - HC	Infraspinatus	-30.2	32.8	114	-0.923	0.358	-95.1	34.66
SAPS - HC	Tibialis anterior	-57.1	31.9	114	-1.789	0.076	-120.4	6.12
SAPS - HC	Deltoid	-31.9	32.2	114	-0.986	0.326	-95.9	32.17

Table 3.35: Outcome: conditioned pain modulation-PP40 – absolute change. Estimated effect of SAPS, i.e., estimated mean conditioned pain modulation-PP40 – absolute change difference between the SAPS and HC at infraspinatus and tibialis anterior from the mixed effect model and their standard errors, p-values and 95% CIs

1. CPM effect - percentage change
    a. Deltoid
    b. Infraspinatus

c. Tibialis Anterior

Table 3.36 (below) indicates that the interaction effect of Treatment Group and Location on Conditioned Pain Modulation-PP40 – percentage change was statistically insignificant, based on the ANOVA Type III table. The main effects of Treatment Group and Location were also insignificant. Regardless, post-hoc pairwise tests were performed to test the hypotheses of interest and obtain estimates of the outcome differences between the healthy control group and the SAPS group at deltoid, infraspinatus and tibialis anterior.

	Chisq	Df	Pr(>Chisq)
(Intercept)	3.274	1	0.070
TreatmentGroup	0.671	1	0.413
Location	4.504	2	0.105
TreatmentGroup:Location	0.305	2	0.859

Table 3.36: Outcome: conditioned pain modulation-PP40 – percentage change. Type III ANOVA table from the mixed effects model to test overall significance of the interaction and main effects (Treatment Group and Location)

Table 3.37 (below) shows the estimated marginal means for each of the treatment groups healthy controls and SAPS deltoid (lower), infraspinatus (upper) and tibialis anterior (middle) along with their standard errors and 95% CIs.

Treatment Group	Location	Marginal mean estimates (kPa)	SE	df	Lower CI	Upper CI
SAPS	Infraspinatus	-10.23	5.65	110	-21.43	0.975
HC	Infraspinatus	-3.52	5.93	112	-15.28	8.236
SAPS	Tibialis anterior	-1.98	5.65	110	-13.18	9.226
HC	Tibialis anterior	10.01	5.65	110	-1.19	21.212
SAPS	Deltoid	-17.28	5.65	110	-28.48	-6.076
HC	Deltoid	-9.75	5.79	111	-21.22	1.715

Table 3.37: Outcome: conditioned pain modulation-PP40 – percentage change. Marginal mean estimates from the mixed effect model and their 95% CIs (CL) at infraspinatus, tibialis anterior and deltoid

Table 3.38 (below) indicates that the estimated effect was not in the hypothesised direction (i.e., the mean outcome had a negative (less than zero) value at all three locations in the SAPS-HC comparisons (indicating that CPM was not impaired in the SAPS group compared to the healthy control group and that the SAPS group actually demonstrated more pain inhibition, or "more efficient" CPM than the healthy control group). At infraspinatus, the estimated mean conditioned pain modulation-PP40 - percentage change in the SAPS group was insignificantly more negative (p-value = .415) than in healthy control group by 6.707, with estimated effect of -6.707 (95% CI:

-22.945, 9.532). At tibialis anterior, the estimated mean conditioned pain modulation-PP40 - percentage change in the SAPS group was insignificantly more negative (p-value = .137) than in the healthy control group by 11.986, with an estimated effect of -11.986 (95% CI: -27.828, 3.856). At deltoid, the estimated mean conditioned pain modulation-PP40 - percentage change in the SAPS group was insignificantly more negative (p-value = .354) than in the healthy control group by 7.525, with an estimated effect of -7.525 (95% CI: -23.556, 8.507).

contrast	Location	Estimated effect	SE	df	t-ratio	p-value	Lower CI	Upper CI
SAPS - HC	Infraspinatus	-6.71	8.20	111	-0.818	0.415	-22.9	9.53
SAPS - HC	Tibialis anterior	-11.99	7.99	110	-1.499	0.137	-27.8	3.86
SAPS - HC	Deltoid	-7.53	8.09	110	-0.930	0.354	-23.6	8.51

Table 3.38: Outcome: conditioned pain modulation-PP40 – percentage change. Estimated effect of SAPS, i.e., estimated mean conditioned pain modulation-PP40 – percentage change difference between the SAPS and HC at infraspinatus, tibialis anterior and deltoid from the mixed effect model and their standard errors, p-values and 95% CIs

3.4.4.5 Conditioned pain modulation-HP40 (exploratory outcome variable)

1. CPM effect - absolute change
    a. Skin over the deltoid muscle

b. Skin over the lateral aspect of the shin below the knee

Table 3.39 (below) indicates that the interaction effect of Treatment Group and Location on conditioned pain modulation-HP40 – absolute change. was statistically insignificant, based on the ANOVA Type III table. The main effects of Treatment Group and Location were also insignificant. Regardless, post-hoc pairwise tests were performed to test the hypotheses of interest and obtain estimates of the outcome differences between the healthy control group and the SAPS group at the skin over the deltoid muscle and at the skin over the lateral aspect of the shin below the knee.

	Chisq	Df	Pr(>Chisq)
(Intercept)	0.390	1	0.532
TreatmentGroup	0.336	1	0.562
Location	0.353	1	0.552
TreatmentGroup:Location	0.822	1	0.365

Table 3.39: Outcome: conditioned pain modulation-HP40 – absolute change. Type III ANOVA table from the mixed effects model to test overall significance of the interaction and main effects (Treatment Group and Location)

Table 3.40 (below) shows the estimated marginal means for each of the treatment groups healthy controls and SAPS at the skin over the deltoid muscle (lower) and at the skin over the lateral aspect of the shin below the knee (upper) along with their standard errors and 95% CIs.

Treatment Group	Location - skin	Marginal mean estimates (˚C)	SE	df	Lower CI	Upper CI
SAPS	Shin	0.267	0.428	78	-0.585	1.12
HC	Shin	0.614	0.418	78	-0.218	1.45
SAPS	Deltoid	-0.093	0.428	78	-0.945	0.76
HC	Deltoid	1.021	0.418	78	0.190	1.85

Table 3.40: Outcome: conditioned pain modulation-HP40 – absolute change. Marginal mean estimates from the mixed effect model and their 95% CIs (CL) at the skin over the deltoid muscle and at the skin over the lateral aspect of the shin below the knee

Table 3.41 (below) indicates that the estimated effect was not in the hypothesised direction (i.e., the mean outcome had a negative (less than zero) value at both locations in the SAPS-HC comparisons (indicating that CPM was not impaired in the SAPS group compared to the healthy control group and that the SAPS group actually demonstrated more pain inhibition, or "more efficient" CPM than the healthy control group). At the skin over the lateral aspect of the shin below the knee, the estimated mean conditioned pain modulation-HP40 – absolute change in the SAPS group was insignificantly more negative (p-value = .564) than in healthy control group by 0.347, with an estimated effect of -0.347 (95% CI: -1.538, 0.844). At the skin over the deltoid muscle, the estimated mean conditioned pain modulation-HP40 – absolute change in the SAPS

group was insignificantly more negative (p-value = .066) than in the healthy control group by 1.114, with an estimated effect of -1.114 (95% CI: -2.305, 0.077).

contrast	Location - skin	Estimated effect	SE	df	t-ratio	p-value	Lower CI	Upper CI
SAPS - HC	Shin	-0.347	0.598	78	-0.58	0.564	-1.54	0.844
SAPS - HC	Deltoid	-1.114	0.598	78	-1.86	0.066	-2.31	0.077

Table 3.41: Outcome: conditioned pain modulation-HP40 – absolute change. Estimated effect of SAPS, i.e., estimated mean conditioned pain modulation-HP40 – absolute change difference between the SAPS and HC at the skin over the deltoid muscle and at the skin over the lateral aspect of the shin below the knee from the mixed effect model and their standard errors, p-values and 95% CIs

2. CPM effect-percentage change
    a. Skin over the deltoid muscle
    b. Skin over the lateral aspect of the shin below the knee

Table 3.42 (below) indicates that the interaction effect of Treatment Group and Location on conditioned pain modulation-HP40 – percentage change was statistically insignificant, based on the ANOVA Type III table. The main effects of Treatment Group and Location were also insignificant. Regardless, post-hoc pairwise tests were performed to test the hypotheses of interest and obtain estimates of the outcome differences between the healthy control group and

the SAPS group at the skin over the deltoid muscle and at the skin over the lateral aspect of the shin below the knee.

	Chisq	Df	Pr(>Chisq)
(Intercept)	0.187	1	0.665
TreatmentGroup	0.478	1	0.489
Location	0.246	1	0.620
TreatmentGroup:Location	0.767	1	0.381

Table 3.42: Outcome: conditioned pain modulation-HP40 – percentage change. Type III ANOVA table from the mixed effects model to test overall significance of the interaction and main effects (Treatment Group and Location)

Table 3.43 (below) shows the estimated marginal means for each of the treatment groups healthy controls and SAPS at the skin over the deltoid muscle (lower) and at the skin over the lateral aspect of the shin below the knee (upper) along with their standard errors and 95% CIs.

Treatment Group	Location - skin	Marginal mean estimates (%)	SE	df	Lower CI	Upper CI
SAPS	Shin	0.403	0.932	78	-1.452	2.26
HC	Shin	1.304	0.910	78	-0.507	3.12
SAPS	Deltoid	-0.250	0.932	78	-2.106	1.61
HC	Deltoid	2.264	0.910	78	0.453	4.08

Table 3.43: Outcome: conditioned pain modulation-HP40 – percentage change. Marginal mean estimates from the mixed effect model and their 95% CIs (CL) at the skin over the deltoid muscle and at the skin over the lateral aspect of the shin below the knee

Table 3.44 (below) indicates that the estimated effect was not in the hypothesised direction (i.e., the mean outcome had a negative (less than zero) value at both locations in the SAPS-HC comparisons (indicating that CPM was not impaired in the SAPS group compared to the healthy control group and that the SAPS group actually demonstrated more pain inhibition, or "more efficient" CPM than the healthy control group). At the skin over the lateral aspect of the shin below the knee, the estimated mean conditioned pain modulation-HP40 – percentage change in the SAPS group was insignificantly more negative (p-value = .491) than in healthy control group by 0.9, with an estimated effect of -0.9 (95% CI: -3.493, 1.692). At the skin over the deltoid muscle, the estimated mean conditioned pain modulation-HP40 – percentage change in the SAPS group was insignificantly more negative (p-value = .057) than in the healthy control group by 2.514, with an estimated effect of -2.514 (95% CI: -5.107, 0.079).

contrast	Location - skin	Estimated effect	SE	df	t-ratio	p-value	Lower CI	Upper CI
SAPS - HC	Shin	-0.90	1.3	78	-0.691	0.491	-3.49	1.692
SAPS - HC	Deltoid	-2.51	1.3	78	-1.930	0.057	-5.11	0.079

Table 3.44: Outcome: conditioned pain modulation-HP40 – percentage change. Estimated effect of SAPS, i.e., estimated mean conditioned pain modulation-HP40 – percentage change difference between the SAPS and HC at the skin over the deltoid muscle and at the skin over the lateral aspect of the shin below the knee from the mixed effect model and their standard errors, p-values and 95% CIs

3.4.4.6 Temporal summation score (exploratory outcome variable)

1. Skin over the deltoid muscle
2. Skin over the lateral aspect of the shin below the knee

Table 3.45 (below) indicates that the interaction effect of Treatment Group and Location on temporal summation score was statistically insignificant, based on the ANOVA Type III table. The main effects of Treatment Group and Location were also insignificant. Regardless, post-hoc pairwise tests were performed to test the hypotheses of interest and obtain estimates of the outcome differences between the healthy control group and the SAPS group at the skin over the deltoid muscle and at the skin over the lateral aspect of the shin below the knee.

	Chisq	Df	Pr(>Chisq)
(Intercept)	24.622	1	0.000
TreatmentGroup	1.867	1	0.172
Location	0.122	1	0.727
TreatmentGroup:Location	0.022	1	0.883

Table 3.45: Outcome: temporal summation score. Type III ANOVA table from the mixed effects model to test overall significance of the interaction and main effects (Treatment Group and Location)

Table 3.46 (below) shows the estimated marginal means for each of the treatment groups healthy controls and SAPS at the skin over the deltoid muscle (lower) and at the skin over the lateral aspect of the shin below the knee (upper) along with their standard errors and 95% CIs.

Treatment Group	Location - skin	Marginal mean estimates (/100)	SE	df	Lower CI	Upper CI
SAPS	Shin	17.85	3.60	54.0	10.62	25.1
HC	Shin	10.98	3.52	52.4	3.92	18.0
SAPS	Deltoid	16.51	3.60	54.0	9.28	23.7
HC	Deltoid	8.85	3.60	54.0	1.62	16.1

Table 3.46: Outcome: temporal summation Score. Marginal mean estimates from the mixed effect model and their 95% CIs (CL) at the skin over the deltoid muscle and at the skin over the lateral aspect of the shin below the knee

Table 3.47 (below) indicates that the estimated effect was in the hypothesised direction (i.e., the mean outcome was higher in the SAPS group than in the healthy control group). At the skin over the lateral aspect of the shin below the knee, the estimated mean temporal summation score in the SAPS group was insignificantly higher (p-value = .178) than in the healthy control group by 6.868, with an estimated effect of 6.868 (95% CI: -3.231, 16.968). At the skin over the deltoid

muscle, the estimated mean temporal summation score in the SAPS group was insignificantly higher (p-value =.139) than in the healthy control group by 7.658, with an estimated effect of 7.658 (95% CI: -2.564, 17.88).

contrast	Location - skin	Estimated effect	SE	df	t.ratio	p-value	Lower CI	Upper CI
SAPS - HC	Shin	6.87	5.04	53.3	1.36	0.178	-3.23	17.0
SAPS - HC	Deltoid	7.66	5.10	54.0	1.50	0.139	-2.56	17.9

Table 3.47: Outcome: temporal summation score. Estimated effect of SAPS, i.e., estimated mean temporal summation Score between the SAPS and HC at the skin over the deltoid muscle and at the skin over the lateral aspect of the shin below the knee from the mixed effect model and their standard errors, p-values and 95% CIs

3.4.4.7 Pain area

Statistical testing was not carried out on the results of the pain area outcome. It was clear, however, from the data that enlarged pain areas were present in the vast majority of participants (19 of 21) and of a magnitude that indicates that these findings have practical significance (localised subacromial area approximately 92 pixels, compared with 5185 pixels (standard deviation 6155) in the SAPS group.

Further to the description provided above regarding the number of pixels shaded, further qualification of this data is provided in Table 3.48 below with respect to the areas of the body that were shaded in by the participants.

Pain area	n
"Subacromial"	21
Lateral shoulder (deltoid region)	17
Scapular region	12
Periscapular and/or adjacent thoracic spine region	3
Anterior arm (biceps region)	5
Posterior arm (triceps region)	6
Neck region (ipsilateral)	5
Upper trapezius region (ipsilateral)	10
Head region (ipsilateral)	1
Forearm – radial aspect	3
Forearm – ulnar aspect	0
Below wrist	0

Table 3.48: Outcome: pain area. Body regions shaded by participants where they had pain related to their shoulder pain

3.5 Discussion

3.5.1 Key results

It was hypothesised that SAPS would be associated with lower pressure pain thresholds (locally, segmentally and remotely); and that heat pain thresholds and mechanical pain thresholds would be similarly reduced. It was also hypothesised that tests devised as a measure of centrally-mediated pain modulation, i.e., CPM (to both heat and pressure pain) and temporal summation (to mechanical pain) would also show signs of nervous system sensitisation in SAPS (locally, segmentally and remotely).

The primary hypothesis for reduced pressure pain threshold at deltoid in SAPS was not confirmed with statistical significance, however, the estimated mean difference of -92.4 kPa (95% CI: -260.1, 75.3), adjusted for covariates, suggests practical significance. The secondary hypotheses for reduced pressure pain threshold in SAPS at infraspinatus (locally), with an estimated mean difference of -203 kPa (95% CI: -383, -22.7), and tibialis anterior (remotely), with an estimated mean difference of -196 kPa (95% CI: -376, -15.4), were both confirmed, both with statistical significance. Neither of these estimates were adjusted for covariates. The magnitude of both these estimated mean differences also suggests practical significance. None of the exploratory hypotheses regarding heat pain threshold, mechanical pain threshold, CPM or temporal summation hypotheses were confirmed with either statistical significance, or with estimated mean differences that suggested practical significance.

It was also hypothesised that people with SAPS would demonstrate spreading sensitisation beyond the subacromial area in body chart reports indicating areas of pain that the participants associated with their shoulder pain. In the vast majority (19 of 21) of SAPS participants, this was demonstrated. Observation of the body chart shading revealed patterns suggestive of deep tissue pain (muscular and/or myofascial and/or myotendinous) rather than patterns suggestive of dermatomes or localised cutaneous innervation. This correlates with the findings of practically significantly reduced pressure pain thresholds in this group of people with SAPS locally (infraspinatus), segmentally (deltoid) and remotely (tibialis anterior), rather than changes in the skin areas that relate to the corresponding segmental innervations.

In observing the outcomes for pressure pain threshold testing for those two participants who only shaded in the subacromial area on their body charts, there was not a corresponding relationship of having high pressure pain thresholds in the three areas tested - infraspinatus, deltoid and tibialis anterior. The individual pressure pain thresholds for these participants (raw data) and the SAPS group marginal mean estimates (for reference) are provided below in Table 3.49. Both these participants were male. Their age and IPAQ total scores (covariates) are also provided, along with the SAPS group median and interquartile range for IPAQ total score, for reference.

Participant	pressure pain threshold infraspinatus	pressure pain threshold deltoid	pressure pain threshold tibialis anterior	IPAQ total score
Participant (#4): male, age 70	361	827	906	1737
Participant (#9): male, age 48	249	272	278	2628
SAPS Group marginal mean estimates (CI 95%)	369 (241,496)	383 (255, 310)	550 (423,678)	
SAPS Group marginal mean estimates adjusted for covariates age, sex and IPAQ total score (CI 95%)	-	395 (254,535)	-	
SAPS Group IPAQ total score (median, IQR)				2373 (3387)

Table 3.49: Individual data for the two participants who shaded only the subacromial area in their body charts for pressure pain thresholds at infraspinatus, deltoid and tibialis anterior, along with their individual data relating to sex, age and IPAQ total scores (covariates)

3.5.2 Limitations

3.5.2.1 Limitations of study design

1. Insufficient power

A major limitation of the study design was that there was insufficient power to detect the effect size of interest for the primary hypothesis related to the primary outcome, i.e., comparing the mean pressure pain threshold at deltoid between the healthy control and SAPS groups. This limitation occurred because there was limited information regarding standard deviations in healthy control and SAPS patients in the previous literature. This study did, however, generate data descriptions for these groups, which provides a good basis for future studies.

2. Convenience Sampling

The participants in the SAPS group were enrolled using convenience sampling. Participants were those who consented to participate in the study so self-selection biases may have occurred. The same is true for the healthy control group, who also consented to participate.

3.5.2.2 Limitations of the analysis

The linear mixed effects models that were used in this analysis rely on the missing at random (MAR) assumption to produce unbiased estimates of the effects of interest. This is a more flexible model then the missing completely at random (MCAR) assumption. The MAR model assumes that, for the involved data set, the probability related to observations being missing is independent of the missing data's actual values; that the probability of data observations being missing is the same only within the groups defined by the observed data. It is not possible to verify the plausibility of this assumption, and if the missing data mechanism for the outcomes deviates from this assumption, this may allow for bias in the effect size estimates. The magnitude of such a bias which would relate to the extent to which the assumption was violated for the data set. Of note, for the data related to the primary hypothesis for the primary outcome, there was no

missing data for either group, and so there are no related limitations or potential biases related to missingness for this analysis.

3.5.3 Interpretation

The data from this study demonstrated a global sensitivity to mechanical pain (i.e., reduced pressure pain threshold) in the deep tissues of this group of people with SAPS (degenerative rotator cuff tendinopathy with or without subacromial-subdeltoid bursitis). This was demonstrated by statistically significant reductions in pressure pain threshold at infraspinatus, which is a local muscle directly involved in SAPS pathophysiology (supplied locally by the suprascapular nerve; and with a C5, C6 segmental nerve supply); and at tibialis anterior (which has an L4, L5 segmental nerve supply). Although not statistically significant, a practically significant estimated mean reduction in pressure pain threshold at deltoid of 92.4 kPa, (which along with infraspinatus also has a C5, C6 segmental nerve supply but is supplied locally by the axillary nerve) was also demonstrated at a magnitude of approximately half that of the reduction at infraspinatus and tibialis anterior. This estimated mean reduction in pressure pain threshold at deltoid was in keeping with the anticipated effect size derived by the initial power calculations, however, the wide variability in pressure pain threshold outcomes meant that this estimated mean difference was not statistically significant at deltoid.

Pressure pain threshold is a static sensory test, and tests for deep pain sensitivity to blunt pressure (muscle Aδ/III and C/IV fiber function). Static sensory tests were also conducted for heat pain threshold (Aδ and C fiber function) and mechanical pain threshold (Aδ and C fiber function). These cutaneous sensory tests did not demonstrate any increase in pain sensitivity in

people with SAPS at either the skin over the deltoid (also supplied by the axillary nerve locally, along with the deltoid muscle, with a C5, C6 segmental nerve supply) or the skin over the lateral aspect of the shin below the knee (supplied by the lateral cutaneous nerve of the calf, sharing an L4, L5 nerve supply with the tibialis anterior muscle) - the measurements were not different from those of the people in the healthy control group. This suggests that whatever process is causing sensitivity in the deep tissues is not affecting nerve supply related to the superficial tissues either in heat nociception or mechanical nociception. The nerve that directly supplies the posterior rotator cuff tissues, the suprascapular nerve, does not have a cutaneous nerve supply and so there was no equivalent cutaneous test locally to that of the infraspinatus muscle for deep tissue sensitivity.

Temporal summation did show some augmentation in the SAPS group, although the magnitude of this difference was not statistically significant and may be of only questionable practical significance. Regardless, there was some degree of temporal summation augmentation demonstrated in this group of people with SAPS which may suggest the presence of this aspect of central sensitisation in SAPS, whereby quickly-repetitive noxious stimuli carried by peripheral C-fibre afferents causes a prolongation of fibre discharge of dorsal horn neurons in the spinal cord such that continuous discharge at an increased rate results.(203) This augmentation was demonstrated in both the C5, C6 distribution (skin over the deltoid) and the L4, L5 distribution (skin over the lateral aspect of the shin below the knee), i.e., both segmentally and remotely with respect to the rotator cuff tissues (C5, C6 distribution).

CPM has been described in the literature as being a normal function of a healthy nervous system(183) and that impaired CPM may reflect impaired function of descending inhibitory pathways.(177) This study demonstrated that on average people with SAPS have more inhibition by this mechanism, rather than less. The CPM efficiency measured in all locations and modalities, both cutaneous nociception (heat pain) and deep tissue nociception (pressure pain) - i.e., the infraspinatus (local C5, C6), deltoid (segmental C5, C6) and tibialis anterior (remote) muscles; and the skin over the deltoid (segmental C5, C6) and the skin over with a lateral aspect of the shin below the knee (L4, L5) – on average, was in a direction that suggested more efficient CPM in people with SAPS rather than less. These findings were not statistically significant and may not be practically significant, but considered on average, do not suggest the presence of central sensitisation by this mechanism in SAPS.

Further to this discussion, it is remarkable that across both groups, i.e., SAPS and healthy controls, there were participants who demonstrated facilitation, there were participants who demonstrated inhibition, and there were participants who demonstrated neither. The individual data demonstrated a remarkable consistency for one of these three responses within patients, i.e., patients who facilitated pain at one location tended to facilitate pain at all locations and both modalities. The same was observed in individual patient data for inhibition of pain and for no alteration of pain perception in the presence of a conditioning stimulus. As this response was so variable, it seems baseless to suggest, even considering the discussion in the previous paragraph, that CPM plays a consistent role in pain modulation for people with SAPS.

There was a clear demonstration of spreading sensitisation in this group of people with SAPS, as demonstrated by pain mapping by participants. The patterns of pain that were indicated on the body charts followed patterns of deep tissue anatomy rather than cutaneous anatomy. This finding corresponds with the findings of reduced pressure pain threshold that were demonstrated in static sensory testing of deep tissues. The mechanism that accounts for this pattern of sensitisation in people with SAPS suggests either a system-wide peripheral sensitisation process of some sort occurring in the deep tissues, perhaps similar in some way to people who suffer from fibromyalgia (a condition typified by widespread tissue sensitivity); or some preferential central sensitisation process by which the neural pathways responsible for pain in the deep tissues are affected while sparing those parts of the neuroaxis that regulate pain sensitivity in the cutaneous tissues.

The mechanical hypersensitivity demonstrated in this group of people with SAPS may result from peripheral sensitisation, central sensitisation or a combination of both. As noted in the introduction, these processes are considered to interact,(172) with peripheral sensitisation being a process that occurs within the nociceptive system via altered function of nociceptors, generally requiring ongoing local pathology to occur.(4) Ongoing local pathology was one of the inclusion criteria for this study, and so this suggests peripheral sensitisation may, by definition, occur in SAPS. Central sensitisation, in contrast, can produce the perception of pain in the absence of peripheral pathology or even noxious stimuli.(4) The presence of central sensitisation is suggested by the widespread pain reported in body charting by the SAPS group in this study, and also by reductions in pressure pain threshold in tissues remote to the pain complaint. This relationship is not conclusive, however, as this pattern of pain sensitivity may also be explained

simply by some biological cause of peripheral sensitisation that is occurring throughout the deep tissues in SAPS, as previously discussed.

In comparison with previous research, this study demonstrates extensive pain profiles of people with SAPS - investigating nervous system dysfunction in both static and dynamic modes of nociceptive responses in both deep and cutaneous tissues, across multiple modalities (heat pain, mechanical pain, pressure pain), and across strategically-selected body locations (local, segmental, and remote locations) in a SAPS population well-defined by thorough physical examination and imaging procedures, and understood by contemporary definitions of the pathophysiology of the condition.

Previous research has conducted sensory testing in subsets of the comprehensive assessment undertaken in this study, which does not give a complete picture of the state of the nervous system in this condition. Notably, findings that were found to be statistically significant in many of these studies had effect sizes similar to those in this study but with much smaller variability, such that findings were found to be statistically significant, but were perhaps not practically significant. The relative functional differences in subsets of the nociceptive system are more easily seen with comprehensive sensory testing as has been performed in this study.

The question of spreading local pain (related to enlarged receptive fields) and referred pain (related to new receptive fields)(106) and whether it occurs in SAPS or not, and what the mechanism of such widespread spread pain may be is also well informed by this data set. The patterns drawn by the SAPS participants give an indication of the structures that are causing pain

- these patterns were more indicative of deep tissue pain rather than dermatomal pain or pain from isolated cutaneous nerves. This pain mapping, considered in the light of the other findings of this study, reinforces the impression that people with SAPS have a system-wide sensitivity of the deep tissues, and that even though temporal summation may be occurring to some degree, the practical significance of this finding is of a much smaller magnitude then the findings of reduced pressure pain threshold and spreading sensitivity in the deep tissues. As discussed previously, this spreading sensitivity may be occurring due to peripheral sensitisation processes, central sensitisation processes, or a combination of the two. This would be a helpful area of study for future research.

With tendon pain being a less well understood pathology with respect to its related pain mechanisms, demonstrating this particular sensory profile in people with SAPS should help to guide research and inform practice related to intervention strategies for this group of patients.

3.5.4 Generalisability

The findings of this research is generalisable to people suffering from SAPS of 12 weeks or more duration referred to specialist sports medicine physicians and defined by the inclusion and exclusion criteria for this study, which includes a minimum of 5/10 NRPS pain elicited by orthopedic special tests. The influence of selection bias inherent in convenience sampling should be considered, however, when generalising both to the sports medicine-referred population as well as to the wider SAPS population.

3.6 Other information

3.6.1 Funding

Physiotherapy Foundation of Canada.

Chapter 4: Conditioned Pain Modulation in Chronic Musculoskeletal Pain: A Systematic Review and Meta-Analysis

This is a report on a systematic review and meta-analysis (SRMA) conducted to investigate for the presence of conditioned pain modulation (CPM) in chronic musculoskeletal pain syndromes.

4.1 Introduction

4.1.1 Rationale

Chronic musculoskeletal pain is a major burden to those who suffer from it, and to society as a whole.(166) Individuals suffering from chronic pain are endlessly subjected to "an unpleasant sensory and emotional experience",(167) in itself a burden, but also find their personal relationships, social interactions, quality of life, mental health, finances and ability to work negatively impacted.(168) On a societal level, chronic pain is a great cost financially to the medical system and in terms of lost productivity.(169)

Reduced CPM is a common feature of chronic pain, having been observed in many types of chronic pain conditions, including osteoarthritis,(124) fibromyalgia,(124,186) chronic tension-type headache,(125) musculoskeletal shoulder pain,(100) irritable bowel syndrome,(187–190) temporomandibular disorder,(127) posttraumatic trigeminal neuropathy,(191) chronic pancreatitis,(192) and postherpetic neuralgia.(193) CPM is considered to be a normal function of a healthy nervous system. It refers to the function in the nervous system whereby remote

stimulation with one noxious stimulus (“counter-irritation”)(182) reduces (“inhibits”) the pain intensity of a separate local noxious stimulus.(124,181) CPM is understood to reflect the function of endogenous pain inhibition mechanisms, primarily of midbrain-centred descending inhibitory nervous system pathways,(183) but also of other higher brain-centred mechanisms involving cognition and emotion,(184,185), e.g., expectancy-induced analgesia.(182)

A systematic review conducted by Lewis et al.(124) concluded that CPM was impaired in people with chronic pain conditions. Meta-analysis found an overall large(194) effect size of 0.78 (95% confidence interval (CI) 0.48–1.08). The review included a myriad of chronic pain conditions for consideration, including neurogenic/neuropathic pain states, fibromyalgia, complex regional pain syndrome, irritable bowel syndrome, temporomandibular disorder, chronic fatigue, arthritis, stroke, whiplash, headache, migraine, peripheral vascular disease, vestibulodynia and pancreatitis.(124)

In light of the frequent failure of treatment for many chronically painful musculoskeletal conditions, where treatment paradigms focus on abnormalities of local tissue structure, function or mechanics, it has been argued that knowledge of any coexisting nervous system dysfunction, increasingly considered to be part of the pathophysiology of chronic musculoskeletal pain syndromes, may inform more successful management and treatment strategies for these types of problems.(213,214)

4.1.2 Objectives

This SRMA examined whether adults (persons 18 years of age or older) with chronic musculoskeletal pain have reduced CPM compared to healthy adults.

4.2 Methods

This systematic review was conducted and reported in accordance with PRISMA guidelines. Please see below for details about protocol registration.

4.2.1 Eligibility criteria

4.2.1.1 Study characteristics

Studies that met the criteria specified below were included in the review.

4.2.1.1.1 Study designs – cross-sectional

Studies needed to be either cross-sectional or of another study type that included a relevant component of investigation that was cross-sectional, but only that part of the report was analysed. In the case of experimental trials, only data gathered prior to exposure to any intervention was considered. They must have included a patient group (chronic musculoskeletal pain) and a healthy control group (healthy adults without chronic musculoskeletal pain) - as defined below.

4.2.1.1.2 Participants – chronic musculoskeletal pain

Studies needed to investigate CPM in adults with chronic musculoskeletal pain defined as "pain that persists or recurs for longer than 3 months … arising from bone(s), joint(s), muscle(s), vertebral column, tendon(s) or related soft tissue(s)"(233), that was attributable to structural or biomechanical changes in the relevant tissue(s).

The coexistence of neuropathic dysfunction, e.g., altered pain, mechanical or thermal thresholds, were not grounds for exclusion, however, a musculoskeletal origin of the pain needed to be highly probable. Reports must have specified the use of accepted confirmatory diagnostic criteria as inclusion criteria that confirmed the implicated musculoskeletal tissue as the primary location of pain.

Excluded were studies of patients with:

1. Chronic musculoskeletal pain due to infectious diseases, metabolic disorders, crystal deposition or auto-immune processes
2. A history of nervous system injury, e.g., a stroke or nerve trauma, or disease e.g., diabetic neuropathy
3. Radicular pain
4. Neuralgia ("pain in the distribution of a nerve or nerves"(173), e.g., trigeminal neuralgia, postherpetic neuralgia
5. "Chronic primary pain" defined as "chronic pain in one or more anatomical regions that is characterised by significant emotional distress (anxiety, anger/frustration or depressed mood)

or functional disability (interference in daily life activities and reduced participation in social roles)",(233) including, but not limited to fibromyalgia and non-specific low back pain

4.2.1.1.3 Interventions – CPM testing

Studies needed to undertake CPM testing, whereby they used a testing protocol of superimposition of a "test stimulus" (local noxious stimulus) on the background of a "conditioning stimulus" (remote noxious stimulus - "counter-irritation"); and recorded an outcome measure of the magnitude of the test stimulus both prior to and during and/or immediately after exposure to the conditioning stimulus; and that evoked significant CPM in the control group were included.

Any studies that used only a homotopic CPM paradigm (i.e., the test and conditioning stimuli were applied in the same ipsilateral area of the body) were excluded. If the study also included a heterotopic paradigm, it was included, but the homotopic data was not considered.

Any studies that used only neurophysiological test stimuli were excluded. If the study also included a psychophysical test stimuli, it was included, but the neurophysiological test stimuli data were not considered.

4.2.1.1.4 Comparators – healthy controls

Studies needed to include a healthy control group (as defined above) who were tested for CPM to compare with the chronic musculoskeletal pain group (as defined above).

4.2.1.1.5 Outcomes – CPM efficiency (for both groups)

Studies needed to record some measure of "CPM efficacy", defined as the difference in magnitude of the "test stimulus" (local noxious stimulus) measure, e.g., pressure pain threshold, prior to and during or immediately after exposure to the conditioning stimulus (remote noxious stimulus - "counter-irritation").

4.2.1.2 Report characteristics

Reports of studies that were available in English, up until and including Oct 20th, 2020, to Jan 19th, 2021 (depending on the information source searched), were included.

4.2.2 Information sources

Search strategies of the literature were developed using appropriate medical subject headings (MeSH) and key words. The following electronic databases were searched: MEDLINE (OVID interface, 1946 to October 20th, 2020), EMBASE (OVID interface, 1974 to October 20th, 2020), CINAHL (EBSCO*host*, 1982 to October 20th, 2020), and PsycINFO (EBSCO*host*, 1979 to October 21st, 2020).

The following clinical trial registries were searched for relevant trial results that may only have been published in these registries: ClinicalTrials.gov (http://clinicaltrials.gov) (October 20th, 2020), World Health Organisation International Clinical Trials Registry Platform (http://apps.who.int/trialsearch) (January 18th, 2021), Open Trials (http://explorer.opentrials.net) (January 18th, 2021), and Cochrane Central Register of Controlled Trials (CENTRAL)(January 18th, 2021). PROSPERO (October 13th, 2020) and the Cochrane Database of Systematic Reviews

(CDSR) were searched for relevant completed systematic reviews (January 18th, 2021). Reference lists of systematic reviews were hand searched for relevant studies. ProQuest Dissertations & Theses Global (https://search-proquest-com) for relevant dissertations and theses (January 18th, 2021). Web of Science Core Collection (Clarivate Analytics) (January 19th, 2021) and EMBASE (OVID interface) were searched for relevant conference abstracts. See Table 4.1 below for a summary of the details of the searches of these information sources below. The reference lists of included studies identified by the search were scanned for relevant studies. They were also citation searched in PubMed for articles that have cited these papers.

Database/Collection	Interface/source	Search date
MEDLINE	OVID	(From 1946 to) October 20th, 2020
EMBASE	OVID	(From 1974 to) October 20th, 2020
CINAHL	EBSCO*host*	(From 1982 to) October 20th, 2020
PsycINFO	EBSCO*host*	(From 1979 to) October 21st, 2020
ClinicalTrials.gov	http://clinicaltrials.gov)	October 20th, 2020
World Health Organisation International Clinical Trials Registry Platform	http://apps.who.int/trialsearch	January 18th, 2021

Open Trials	http://explorer.opentrials.net	January 18th, 2021
Cochrane Central Register of Controlled Trials (CENTRAL)	https://www.cochranelibrary.com/central	
PROSPERO	https://www.crd.york.ac.uk/prospero/#searchadvanced	October 13th, 2020
Cochrane Database of Systematic Reviews (CDSR)	https://www.cochranelibrary.com/cdsr/reviews	January 18th, 2021
ProQuest Dissertations & Theses Global	https://search-proquest-com	January 18th, 2021
Web of Science Core Collection	Clarivate Analytics	January 19th, 2021

Table 4.1: Display of information sources consulted in the search strategy - database/collection, interface/source, and search date

4.2.3 Search strategy

The search strategy was developed with the assistance of a Health Sciences Librarian with expertise in systematic review searching (Charlotte Beck). The MESH and keyword searches used in the MEDLINE strategy were then adapted as required to the other databases. The MEDLINE strategy in Appendix C.

4.2.4 Selection process

Search results were uploaded for handling to Covidence (a web-based software platform; Covidence systematic review software, Veritas Health Innovation, Melbourne, Australia.

Available at www.covidence.org), where all records and data were handled throughout the review.

Forms containing screening questions were created in Covidence for two separate and sequential stages of screening, as described subsequently, and used to screen search results by application of the inclusion/exclusion criteria. Stage 1 involved screening titles and abstracts (as necessary and when available). Screening was undertaken independently by LS and Kipling Squier (KS) against inclusion and exclusion criteria with the assistance of Covidence software. Stage 2 involved screening the residual search results that appeared to meet the inclusion criteria, and any titles where it was not possible to ascertain whether they met the inclusion criteria based on the title and/or abstract alone by assessing their full text. This screening was also undertaken independently by LS and KS against inclusion and exclusion criteria with the assistance of Covidence software. Any discrepancies were discussed and, where possible, resolved by consensus. If necessary, Alex Scott (AS), was consulted.

If there was inadequate information in a report to decide whether it met the inclusion criteria, the corresponding author was emailed twice within two weeks. If there was no response within three weeks, the report was not included, and was listed as a 'potentially relevant study'. Seventeen studies, mostly conference proceedings fell into this category. For a listing of these studies, please see Appendix A.

All reports on a study were considered to ensure all trial outcomes were considered. Author names and sample sizes were juxtaposed to attempt to ensure duplicate publications were not included, assisted by Covidence software.

A record of decisions made for each article was recorded in the Covidence system. A flowchart was created according to the PRISMA recommendations documenting the study selection process and is presented in the results section of this report.

4.2.5 Data collection process

Data extraction forms were created in the Covidence system and used to extract data independently by LS. Extraction forms were used to collect general information, study characteristics, participant characteristics, test protocol details and outcome data/results, as detailed below. The extracted data was verified by KS. Any inconsistencies were reviewed by discussion. There were no unresolved inconsistencies.

If the data sought for extraction was not available or was unclear in the reports, this information was sought from the corresponding author by email, twice within two weeks. If there was no response within three weeks, the data was noted as "unable to acquire data from author".

4.2.6 Data items

The following variables were sought and extracted:

1. General information

a. Identification features of the study: author, article title, citation, record number (to uniquely identify study)
b. Type of publication (e.g., journal article, conference abstract)
c. Country of origin
d. Source of funding

2. Study characteristics
    a. Study design, e.g., cross-sectional, cohort, case–control, randomised controlled trial
    b. Study inclusion and exclusion criteria
    c. Recruitment procedures used - blinding
    d. Number of participants per group, i.e., patient group(s), control group
3. Participant characteristics

    Characteristics of participants at the beginning of the study
    a. Sociodemographic characteristics: age, sex, marital status, level of education, and employment status
    b. Pain condition of patient population (control population are healthy adults as per inclusion criteria)
    c. Primary relevant psychosocial assessment scores as provided (e.g., Fear Avoidance Beliefs Questionnaire (FABQ), Tampa Scale for Kinesiophobia (TSK), Pain Coping Inventory (PCI), Multidimensional Pain Inventory (MPI), Minnesota Multiphasic Personality Inventory (MMPI), Beck Depression Inventory, Cognitive Self-Statements scale etc.)
4. Test protocol details
    a. Test stimulus

i. Stimulus type (e.g., heat, cold, electrical, mechanical pressure (blunt/sharp), "pain-6 temperature")
ii. Method of application (e.g., thermode, water bath, von Frey filaments, force dynamometer)
iii. Pain assessment type (e.g., subjective pain intensity rating, subjective pain tolerance, suprathreshold pain response)
iv. Units of measurement (e.g., visual analogue scale (VAS)/numerical pain rating score (NPRS) 0-10, 0-100; Newtons (e.g., pressure pain threshold, mA)
v. Test protocol
vi. Test stimulus site

b. Conditioning stimulus
i. Stimulus type (e.g., cold-water immersion (cold pressor test), hot-water immersion, ischemic pain)
ii. Method of application (e.g., thermode, bath, cuff)
iii. Units of measurement (e.g., °C, mmHg)
iv. Test protocol
v. Test stimulus site

c. Conditioned pain modulation paradigm/calculations, i.e., test interpretation
d. Retest interval
e. Test order - randomised or consistent sequence

The main outcomes for which data were sought were:

1. Pain intensity measures recorded in response to exposure to the test stimulus before (at "baseline") and after exposure to the conditioning pain stimulus OR change scores assessed using a standardised measure (e.g., subjective pain reports using a VAS or an NPRS), or
2. Physical intensity measures recorded in response to exposure to the test stimulus before (at "baseline") and after exposure to the conditioning pain stimulus OR change scores assessed using a standardised measure (e.g., temperature in degrees Celsius for heat pain threshold, force in Newtons for pain pressure threshold). When measures were taken at more than one time point during exposure to the conditioning stimulus, the arithmetic mean of all measures taken during exposure to the conditioning stimulus were calculated as representative (if not already calculated). When measures were taken both during and after exposure to the conditioning stimulus, only measures taken during exposure were used., i.e., 4.i. and 4.ii. below.

This data allowed for calculation of the magnitude of the inhibitory effects elicited by the conditioning stimuli on both groups and allowed for comparisons of the effect size between groups, which was the primary goal in conducting this systematic review.

All outcome data/results for which data were sought were:

1. Unit of assessment/analysis (e.g., pain score as VAS/NPRS 0-10, 0-100, temperature in °C (e.g., pain threshold), pressure in N (pressure pain threshold))
2. For patient group(s) and control group
    a. Number of participants enrolled
    b. Number of participants included in analysis

c. Number of withdrawals, exclusions, lost to follow-up

d. Summary outcome data

    i. Outcome measure at baseline (prior to conditioning) - means and standard deviations*

    ii. Outcome measure during and/or following conditioning - means and standard deviations*

    iii. Results of study analysis (e.g., CPM result - impaired/not impaired; difference or no difference, e.g., “decreased CPM response in OA vs. controls")

* If data was not presented as means and standard deviations, data were converted to this form. If the data provided in the report could not be converted to this form, the corresponding author was emailed by LS, twice within two weeks. If there was no response within three weeks, and the effect size and variance could not be otherwise back-computed, the data was not included for meta-analysis.

4.2.7 Risk of bias assessment in individual studies

Risk of bias of individual studies were assessed using the Appraisal Tool for Cross-Sectional Studies (AXIS) critical appraisal tool (included as Sub-Appendix D.2).(234) This tool assesses twenty study components in five categories: 1. Introduction, 2. Methods, 3. Results, 4. Discussion, and 5. Other. A judgement was made as to whether each criteria had been met, classified as “Yes”, “No” or “Don’t know/Comment”. An overall quality rating of “high”, “medium” or “low” was assigned and a summary figure of these assignments for all included studies was created in table form and presented in the report.

It was anticipated that all relevant studies would have a cross-sectional design, or that if not, the data that was extracted would be cross-sectional in nature, e.g., baseline measures before an intervention was applied. If the design was not cross-sectional, the critical appraisal methods were adapted as appropriate.

Critical appraisal (including risk of bias) forms based on the AXIS critical appraisal tool were created in a spreadsheet and used to classify assignment independently and in duplicate by LS and KS. Any inconsistencies were reviewed by discussion. There were no unresolved inconsistencies.

4.2.7.1 Use of the AXIS tool for Critical Appraisal

The AXIS tool(234), with specific adaptations made related to the subject matter of this SRMA, was used to conduct critical appraisal of the selected studies. The adaptations are described in Sub-Appendix D.1). The AXIS tool assesses the risk of bias for individual studies, as well as the quality of the study design, and the reporting completeness of the study.(235) Reporting was considered important to assess for this SRMA, as without complete reporting, an assessment of risk of bias or level of quality cannot be made.(235)

4.2.8 Effect measures

The standardised mean difference was the effect measure used in the quantitative synthesis for the outcome under analysis in this SRMA, i.e., the difference in CPM magnitude elicited in the chronic musculoskeletal pain population under investigation compared with the observed CPM magnitude of healthy controls.

4.2.9 Synthesis methods

4.2.9.1 Eligibility determination for synthesis

All studies selected by the reported search strategy and subsequent screening stages that were found to be eligible, based on the inclusion and exclusion criteria described, were considered eligible for quantitative synthesis. Where the required data was subsequently found to not be available either from the primary report, or by request from the corresponding author as described previously, the associated study was determined to be ineligible for quantitative synthesis. All studies, regardless of whether the required outcome for meta-analysis was available, were included in the narrative synthesis.

4.2.9.2 Data preparation

For those studies that did not present the outcome data required for meta-analysis in mean and standard deviation form in the primary report, the relevant data was converted mathematically to mean and standard deviation form. This mean and standard deviation data was then converted to standardised mean differences form for meta-analysis in Review Manager (RevMan) ([Computer program]. Version 5.3. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014).

4.2.9.3 Results tabulation and visual display(s)

Result tabulations and visual displays were created by hand from the extracted data collected in Covidence, or otherwise extracted or calculated from information in the selected studies, or otherwise calculated in RevMan.

4.2.9.4 Meta-analysis methodology

Effects sizes (standardised mean differences) and CIs (95%) for the difference in magnitude of CPM between the patient and control groups were calculated in RevMan for each report using the means and standard deviations of the outcome measure at baseline (prior to conditioning) and after conditioning. (Note: conversion to this form, as possible, was performed in the data extraction stage. Data that could not be converted to this form was not included in the meta-analysis).

Heterogeneity of sample effect sizes were assessed by consideration of the statistics I^2 , tau-squared (T^2), and tau (T; √tau^2), and interpreting the heterogeneity as low, medium, or high (236).

Data was combined, and a summary effect size estimate and CI (95%) was calculated, using a random effects model. Although all studies provided a measure of the difference in CPM magnitude between the chronic musculoskeletal pain group under investigation and a healthy population group, the underlying tissue location of pain across the collective patient population selected for this review varied considerably, as did the CPM testing protocols used. Therefore, it seemed reasonable to assume that there may have been differences between the individual study

outcomes that, at least in part, reflected true underlying study population differences (random variation), rather than simply sampling error (conditional variation) alone. For this reason, a random effects model was used over a fixed effects model.(237,238)

The computations were done in RevMan, using the DerSimonian and Laird method.(239) The majority of studies used one single test stimulus modality at one single test site location in one single patient group. For these studies, standardised mean differences were entered into the meta-analysis as described above. In studies that assessed CPM using more than one test stimulus (n = 3),(240–242) and/or at more than one location (n = 5) ,(129,242–245) and/or in more than one subgroup of the defined musculoskeletal pain population (n = 2),(243,245) the CPM values were combined and entered as a single value, as detailed below.

For studies that assessed one single test stimulus type at more than one location (n = 5) ,(129,242–245) or in more than one subgroup of the defined musculoskeletal pain population (n = 2),(243,245) the outcome data from all locations and/or subgroups was averaged and the standard deviation was averaged using the formula below. The decision to handle the relevant data in this way was supported by the finding that, for those studies that used the same test stimulus type at multiple different locations in the same individual, the CPM effect was found generally to be highly similar at the different test locations.

$$\text{Average S.D.} = \sqrt{((n_1-1)s_1^2 + (n_2-1)s_2^2 + \ldots + (n_k-1)s_k^2) / (n_1+n_2 + \ldots + n_k - k)}$$

where:

- n_k: Sample size for k^{th} group
- s_k: Standard deviation for k^{th} group
- k: Total number of groups

Regarding the use of multiple test stimulus types in a single study, all the studies included in the meta-analysis used blunt pressure as one of the test stimuli used, except for one study which only used heat as a test stimulus.(127) For those studies that used blunt pressure as one of the test stimuli, only the blunt pressure outcomes were entered into the meta-analysis.

The output data has been presented in table and forest plot forms.

4.2.9.5 Heterogeneity exploration among study results

4.2.9.5.1 Subgroup analyses

Subgroup analyses were pre-specified to explore possible sources of heterogeneity, based on the test stimulus type, the conditioning stimulus type and parallel/concurrent vs sequential protocols. We proposed these analyses as they may have been able to contribute to investigation of the heterogeneity of the effects.(237) However, the numbers of studies with data available for meta-analysis was low (n = 15), and the distribution of covariates was uneven between the subgroups.

This made undertaking subgroup analysis inappropriate, as it is was unlikely to provide useful information.(237)

4.2.9.6 Sensitivity analyses

A pre-specified sensitivity analysis was conducted based on the critical appraisal/risk of bias assessment (by omitting studies that were assessed as having low quality as assessed by the AXIS tool) to explore this as a possible source of heterogeneity. A sensitivity analysis was also performed based on the exclusion of one study from the meta-analysis that was designated as an outlier(127) during the synthesis stage of the review as its 95% CI lay outside the 95% CI of the summary effect.(265)

4.2.9.7 Narrative synthesis

A narrative synthesis was undertaken for all studies retrieved by the selection process, regardless of whether or not it was possible to extract the data from them that was required for meta-analysis. The synthesis provides a brief summary of the study characteristics and issues related to bias and quality for those elements expected to assist in the interpretation of the results of this SRMA.

4.2.9.8 Quantitative synthesis

There was not methodological or clinical heterogeneity among the included studies of a magnitude that would prevent meta-analysis, and the necessary outcome data could be obtained for 16 (i.e., more than the predetermined minimum number of five studies) of the 23 studies that

met the inclusion criteria. Therefore the study data that was able to be extracted was quantitatively synthesised, and a meta-analysis was performed.

4.2.10 Meta-biases - reporting bias assessment

The possibility of publication bias was assessed using a funnel plot, as more than the pre-specified number of 10 studies were included in the meta-analysis. The plot was inspected visually for the general appearance of a narrowing funnel shape and symmetry, and the plot has been provided in the report.

4.2.11 Certainty assessment

The strength of the body of evidence was assessed using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach(67), with some omissions as were appropriate (considering this SRMA did not assess the effects of an "intervention").

The GRADE approach considers limitations in study design or execution (risk of bias), inconsistency of results, indirectness of evidence, imprecision, and publication bias; as well as factors that may increase the quality of evidence, such as a large effect size. The approach is used to determine the extent to which there can be confidence that the summary effect estimate is correct according to the following categories: (1) "high" - we are very confident that the true effect lies close to that of the estimate of the effect; (2) "moderate" - we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different; (3) "low" - our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect;

and (4) “very low” - we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect (67).

Using this approach, a rating of the overall confidence in the estimate for this SRMA, i.e., the presence of impaired CPM in chronic musculoskeletal pain syndromes, was assigned. A GRADE Evidence Profile has been presented, without reference to risk, as this is not relevant to the subject matter of this SRMA.

4.3 Results

4.3.1 Study selection

Figure 4.1 describes the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review.

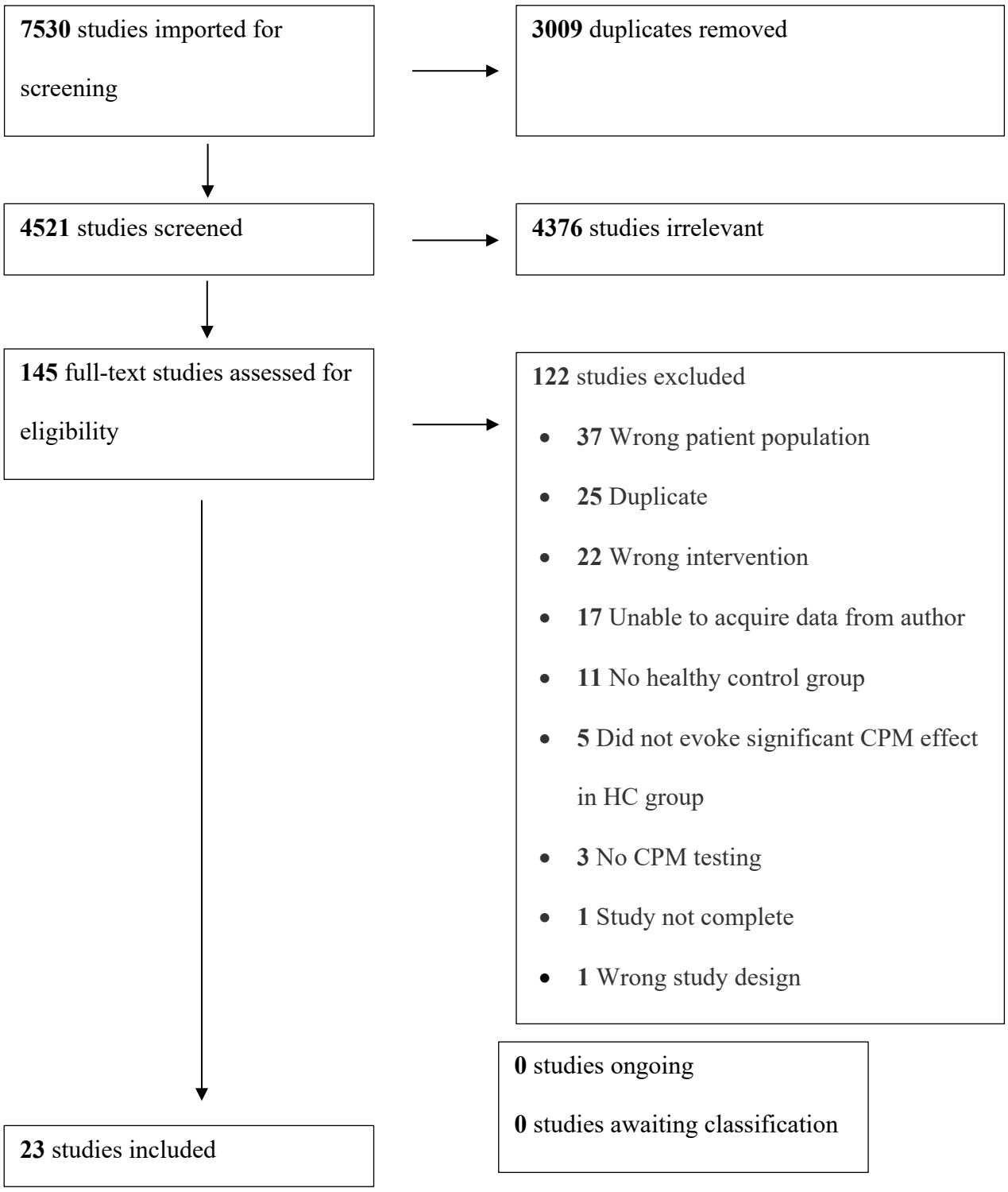


Figure 4.1: Results of the search and selection process, from the number of records identified in the search to the number of studies included in the review.

4.3.2 Study characteristics

Table 4.2 (below) cites each included study and present its characteristics of interest – the country in which the study was conducted, the overall design of the entire study on which the report was based, the pain condition studied, the sample size for each group of participants, the age of each group of participants and the biological sex of the participants.

Study ID	Country	Study design	Pain condition	Patient group n	Control group n	Patient group - Age	Control group - Age	Sex (male/female)
Arendt-Nielsen 2010(246)	Denmark	Cross-sectional	Knee OA	Grp 1 =24; Grp 2 = 24	24	Grp 1 = 63.6 (1.5); Grp 2 = 61.7 (1.7): *	61.6 (1.6): *	Both
Cathcart 2010(247)	Australia	Case-control	CTTH	Grp 1 =23; Grp 2 = 23	25	Grp 1 = 26.2 (6.6); Grp 2 = 29.3 (9.1): **	25.8 (6.4): **	Both
Christensen 2020(248)	Denmark	Cross-sectional	Chronic low back pain	22	22	40.5 (11.5): **	39.3 (11.1): **	Both

Coppieters 2018(128)	Belgium	Uncontrolled effectiveness	WAD	34	28	38 (25 - 47): ***	25 (22 - 44); ***	Both
Daenen 2013(129)	Belgium	Cross-sectional	WAD	35	31	43.8 (± 9.58): ¥	43.19 (± 16.11): ¥	Both
Edwards 2016(249)	United States	Uncontrolled effectiveness	Knee OA	35	39	57.9 ± 10.7: ~~¥¥~~	59.8 ± 8.9: ~~¥¥~~	Both
Graven-Nielsen 2012(240)	Denmark	Uncontrolled effectiveness	Knee OA	46	21	65 (40 - 86): ~~¥¥¥~~	60 (40 - 81): ~~¥¥¥~~	Both
Heredia-Rizo 2019(250)	Denmark	Cross-sectional	NSP	19	20	46.8 (1.3): *	41.7 (2.5): *	Female
Kashima 1999(251)	Japan	RCT	Masticatory myalgia	20	20	28.1 (8.0): **	25.2 (7.0): **	Female
King 2009(127)	United States	Cross-sectional	TMD	14	28	31.0 (10.2): **	28.6 (10.8): **	Female

Kosek 2000(252)	Sweden	Uncontrolled effectiveness	OA hip	15	15	52 (29 - 66): ¥¥¥	50 (30 - 67): ¥¥¥	Both
Kothari 2015(244)	Denmark	Cross-sectional	TMD	34	34	33.0 (1.8): *	30.1 (1.9): *	Both
Kothari 2016(253)	Denmark	Cross-sectional	TMD	Grp 1 = 43; Grp 2 = 15	41	37.2 (1.9) (for both): *	32.0 (1.9): *	Both
Leffler 2002(241)	Sweden	Cross-sectional	Trapezius myalgia	10	10	38 (24 - 55 years): ¥¥¥	38 (23 - 54): ¥¥¥	Both
Mkumbuzi 2021(254)	South Africa	Case-control	Achilles tendinopathy	123	100	46 (36 - 52): ***	37 (31 - 50): ***	Both
Moana-Filho 2019(255)	United States	Cross-sectional	TMD	22	17	36.0 (14.7): **	34.5 (13.7): **	Female
Oono 2014(242)	Denmark	Cross-sectional	TMD	16	16	43.0 (4.0): *	38.9 (3.4): *	Both

Plinsinga 2020(256)	Australia	Cross-sectional	GTPS	39	41	51 (9): **	53 (11): **	Both
Poluha 2020(257)	Brazil	Cross-sectional	TMD	30	30	33.4 ± 13.53: ¥	31.36 ± 10.64: ¥	Both
Sandrini 2006(258)	Italy	Cross-sectional	CTTH	17	20	32 ± 12: ¥	32 ± 7: ¥	Both
Serrano-Munoz 2019(259)	Spain	Cross-sectional	WAD	15	15	39.7 (3.1): *	40.5 (3.45): *	Both
Smith 2020(260)	Australia	Cross-sectional	WAD	40	30	37.3 (13.6): **	40.4 (14.3): **	Both
Tompra 2016(7)	Netherlands	Cross-sectional	Achilles tendinopathy	20	23	42.9 (13.5): **	362.2 (12.3): **	Both

Table 4.2: Citation for each included study and its characteristics of interest. RCT - randomised controlled trial, OA – osteoarthritis, CTTH - chronic tension-type headache, TMD - temporomandibular disorder, WAD - whiplash associated disorder, GTPS - greater trochanteric pain syndrome, NSP -

nonspecific neck/shoulder pain, * - mean (SEM); ** - mean (SD), * - median (IQR), ¥ - (mean ± ?), ¥¥ - (? ± ?), ¥¥¥ - ("average", range), § - (mean; age ± SEM), ID - identification**

Table 4.3 (below) summarises the main CPM testing details for each included study – the test stimulus modality, the conditioning stimulus modality and the results of the study analysis as described by the author(s). The majority of studies used blunt pressure as the test stimulus modality (n = 20). Of these, 18 used blunt pressure as a static test, and two used blunt pressure as a dynamic test (temporal summation).(129,247) Of all the studies that used blunt pressure as the stimulus modality, all but one used a dynamometer - one used a pressure cuff (static test).(250) Three studies used heat as a test stimulus.(127,241,259) One study used an electrical stimulus.(258) Three studies included a second test stimulus(240–242), all of which used static blunt pressure (dynamometer) at pressure pain threshold as the first test stimulus. One of these studies used a pressure cuff as the second test stimulus(240); one used static blunt pressure (dynamometer) again, but to an intensity of pressure pain tolerance,(242) and the third used heat.(241)

Study ID	Test stimulus modality	Conditioning stimulus modality	Results of study analysis
Arendt-Nielsen 2010(246)	Pressure - blunt (dynamometer)	Pressure - blunt (cuff)	CPM impaired in patient groups 1 & 2
Cathcart 2010(247)	Temporal summation: pressure - blunt (dynamometer)	Pressure - blunt (cuff)	CPM impaired in patient groups 1 & 2
Christensen 2020(248)	Pressure - blunt (dynamometer)	Cold water immersion	CPM impaired in patient group
Coppieters 2018(128)	Pressure - blunt (dynamometer)	Cold water immersion	CPM facilitated in patient group 2
Daenen 2013(129)	Temporal summation: pressure - blunt (dynamometer)	Pressure - blunt (cuff)	CPM impaired in patient group
Edwards 2016(249)	Pressure - blunt (dynamometer)	Cold water immersion	CPM impaired in patient group
Graven-Nielsen 2012(240)	1. Pressure - blunt (dynamometer); 2. Pressure - blunt (cuff)	Pressure - blunt (cuff)	CPM impaired in patient group

Heredia-Rizo 2019(250)	Pressure - blunt (cuff)	Pressure - blunt (cuff)	No difference in CPM between patient group and controls
Kashima 1999(251)	Pressure - blunt (dynamometer)	Pressure - blunt (cuff)	CPM impaired in patient group
King 2009(127)	Heat (thermode)	Cold water immersion	CPM impaired in patient group
Kosek 2000(252)	Pressure - blunt (dynamometer)	Pressure - blunt (cuff)	CPM impaired in patient group
Kothari 2015(244)	Pressure - blunt (dynamometer)	Cold water immersion	No difference in CPM between patient group and controls
Kothari 2016(253)	Pressure - blunt (dynamometer)	Cold water immersion	CPM impaired in patient group 1
Leffler 2002(241)	1. Pressure - blunt (dynamometer); 2. Heat (thermode)	Pressure - blunt (cuff)	No difference in CPM between patient group and controls
Mkumbuzi 2021(254)	Pressure - blunt (dynamometer)	Cold water immersion	No difference in CPM between patient group and controls
Moana-Filho 2019(255)	Pressure - blunt (dynamometer)	Heat (thermode)	No difference in CPM between patient group and controls

Oono 2014(242)	Pressure - blunt (dynamometer; PPT & PPTol))	Pressure - blunt (craniofacial compressive device with 4 probes around skull	CPM impaired in patient group
Plinsinga 2020(256)	Pressure - blunt (dynamometer)	Cold water immersion	CPM impaired in patient group
Poluha 2020(257)	Pressure - blunt (dynamometer)	Cold water immersion	CPM impaired in patient group
Sandrini 2006(258)	Electrical (surface electrodes)	Cold water immersion	CPM impaired in patient group
Serrano-Munoz 2019(259)	Heat (thermode)	Cold water immersion	CPM impaired in patient group
Smith 2020(260)	Pressure - blunt (dynamometer)	Cold water immersion	CPM impaired in patient group
Tompra 2016(7)	Pressure - blunt (dynamometer)	Cold water immersion	CPM impaired in patient group

Table 4.3: Summary of the main CPM testing details for each included study. PPT – pressure pain threshold, PPTol – pressure pain tolerance

Table 4.4 (below) summarises details of the test stimulus parameters used. The majority (n = 18) used a pain assessment type of subjective pain threshold. It is noteworthy that the most recent expert consensus recommendations on CPM testing in a research setting(208) are that the test stimulus be of $\geq$ 4/10 VAS intensity. Five of the studies used a subjective pain intensity rating for the

pain assessment type. Of those, only two met the recommendations of ≥ 4/10 VAS(127,241). For those two studies that incorporated a second test stimulus in the study design, only one met consensus recommendations – a heat stimulus of 7/10 VAS.(241)

	Test stimulus pain assessment type:			
Study ID	Subjective pain threshold	Subjective pain intensity rating	Specifics of subjective pain intensity rating	Meets consensus recommendations(208)
Arendt-Nielsen 2010(246)	✓			
Cathcart 2010(247)	✓			
Christensen 2020(248)	✓			
Coppieters 2018(128)	✓			
Daenen 2013(129)		✓	Pain levels not reported	
Edwards 2016(249)	✓			
Graven-Nielsen 2012(240)	✓ (1. Dynamometer) ✓ (2. Cuff)			
Heredia-Rizo 2019(250)	✓			

Kashima 1999(251)	✓			
King 2009(127)		✓	40-50% eVAS	✓
Kosek 2000(252)	✓			
Kothari 2015(244)	✓			
Kothari 2016(253)	✓			
Leffler 2002(241)	✓ (1. Dynamometer)	✓ (2. Heat)	7/10 VAS (2. Heat)	✓ (2. Heat)
Mkumbuzi 2021(254)	✓			
Moana-Filho 2019(255)	✓			
Oono 2014(242)	✓			
Plinsinga 2020(256)	✓			
Poluha 2020(257)	✓			
Sandrini 2006(258)		✓	Pain levels not reported	
Serrano-Munoz 2019(259)		✓	"Pain-3", i.e., 3/10 NPRS	
Smith 2020(260)	✓			

Tompra 2016(7)	✓			

Table 4.4: Summary of the test stimulus parameter details for each included study. VAS - visual analogue scale, eVAS - electronic visual analogue scale, NPRS - numerical pain rating scale

For the conditioning stimulus, 13 studies used noxious-cold water immersion. Nine used blunt pressure - eight of which used a pressure cuff, with one study using a craniofacial compressive device.(242) One study used a heat stimulus.(255) For details, please see table 4.4 above. Of those who used cold water immersion, only five used a subjective pain intensity rating as the pain assessment type, but all of those five met the expert consensus recommendations to use mild to moderate pain intensity (≥ 2/10 VAS).(208) The remainder (n = 8) used a fixed water temperature. Of the eight studies that used cuff pressure as a conditioning stimulus, seven used subjective pain intensity rating as the pain assessment type, using VAS ratings ranging from 3/10 to 7/10. One study used subjective pain threshold.(261) The single study that used the craniofacial compressive device induced a subjective pain intensity rating of 5/10 VAS.(242) The study that used a heat thermode also used a subjective pain rating - 30/100 NPRS plus 1°C.(255) For details, please see Table 4.5 below.

	Conditioning stimulus pain assessment type				
Study ID	Subjective pain threshold	Subjective pain intensity rating	Pain intensity not standardised	Specifics of subjective pain intensity rating	Meets consensus recommendations
Arendt-Nielsen 2010(246)		✓		4/10 VAS	✓
Cathcart 2010(247)		✓		3/10 VAS	✓
Christensen 2020(248)			2 min. immersion or max. time tolerated in 4°C water		
Coppieters 2018(128)			2 min. immersion or max. time tolerated in 12°C +/- 1°C water		
Daenen 2013(129)		✓		3/10 NPRS	✓
Edwards 2016(249)			30s immersion at 4°C		
Graven-Nielsen 2012(240)		✓		4/10 VAS	✓

Heredia-Rizo 2019(250)		✓		70% of pain tolerance threshold	✓
Kashima 1999(251)	✓				
King 2009(127)		✓		20-30% eVAS	✓
Kosek 2000(252)		✓		≥ 30/100 VAS	✓
Kothari 2015(244)			1.5 min. immersion at 2-4°C water		
Kothari 2016(253)			3 min. immersion in 2-4°C water		
Leffler 2002(241)		✓		≥ 7/10 VAS or max. 45 weight lifts if ≥ 7/10 VAS not induced in <45 lifts	✓
Mkumbuzi 2021(254)		✓		≥ 4/10 NPRS	✓

Moana-Filho 2019(255)		✓		30/100 NPRS + 1°C	✓
Oono 2014(242)		✓		5/10 VAS	✓
Plinsinga 2020(256)		✓		4-6/10 NPRS	✓
Poluha 2020(257)		✓		50-70/100 NPRS	✓
Sandrini 2006(258)			5 min. immersion in 5-6°C water		
Serrano-Munoz 2019(259)			30s immersion in 12°C water		
Smith 2020(260)			2 min. immersion or max. time tolerated in 5°C water		
Tompra 2016(7)		✓		5/10 NPRS	✓

Table 4.5: Summary of the conditioning stimulus parameter details for each included study. VAS - visual analogue scale, eVAS - electronic visual analogue scale, min. – minute(s), max. - maximum, s – second(s), NPRS - numerical pain rating scale. Consensus recommendations from (208)**.**

Table 4.6 (below) describes details regarding the test stimulus and conditioning stimulus sites, and relates them to the pain condition under study and the results of the study analysis. The test stimulus site is described in terms of whether or not the site was in the area of the pain condition, or at another site. For those studies where the test site was in an area other than the pain condition, the site location is described, as well as the laterality of that site related to the side of the pain condition, i.e., whether the site was on the same side as the pain complaint (ipsilateral) or the opposite side (contralateral). The conditioning stimulus site is also described regarding its laterality compared to the side of the pain complaint. The test stimulus site and the conditioning stimulus site are also juxtaposed. As well as describing the details outlined above, this table also serves to illustrate the degree of variability in the CPM test protocols of the selected studies. This observation is important as the most recent expert recommendations call for more consistency in CPM testing protocols to allow for better comparisons between data sets, including consistency regarding test locations in at least one of a recommended two rounds of CPM testing performed - they recommend an upper limb and a lower limb test site.(208)

		Test stimulus site			
Study ID	Pain condition	Test stimulus site in area of pain condition	Other site. (Laterality related to side of pain condition - painful or most painful side).	Conditioning stimulus site laterality - related to test stimulus site	Results of study analysis (CPM of patient group compared to controls)
Arendt-Nielsen 2010(246)	Knee OA	✓	✓ (2nd site - tib. ant.; 3rd site - ECRL). Laterality not reported.	Inconsistent (always left)	Impaired
Cathcart 2010(247)	CTTH	✓ (Right UFT)	✓ 2nd test site (right 3rd middle phalanx)	Contralateral (always left)	Impaired (patient groups 1 & 2)
Christensen 2020(248)	Chronic low back pain	✓		Inconsistent (always right)	Impaired

Coppieters 2018(128)	WAD		✓ (Ipsilateral quad.)	Contralateral	Facilitated (patient group 2)
Daenen 2013(129)	WAD	✓ (Right UFT)	✓ 2nd test site (right thigh)	Contralateral (always left)	Impaired
Edwards 2016(249)	Knee OA		✓ (Contralateral UFT)	Inconsistent (always right)	Impaired
Graven-Nielsen 2012(240)	Knee OA	✓		Inconsistent (always left)	Impaired
Heredia-Rizo 2019(250)	NSP		✓ (Calf ipsilateral to pain condition)	Contralateral	No difference
Kashima 1999(251)	Masticatory myalgia		✓ (Contralateral webspace)	Contralateral	Impaired
King 2009(127)	TMD		✓ (Left palm)	Contralateral (always right)	Impaired

Kosek 2000(252)	OA hip		✓ (Contralateral hip/calf)	Contralateral	Impaired
Kothari 2015(244)	TMD	✓	✓ 2nd test site (thenar muscles - patients most painful TMD side; controls -dominant hand)	Ipsilateral	No difference
Kothari 2016(253)	TMD	✓	✓ (2nd site - thenar muscles; dominant hand)	Inconsistent (always dominant side)	Impaired
Leffler 2002(241)	Trapezius myalgia		✓ (Right mid-thigh)	Contralateral (always left)	No difference
Mkumbuzi 2021(254)	Achilles tendinopathy	✓		Contralateral (patient group); not reported (controls)	No difference

Moana-Filho 2019(255)	TMD	✓		N/A - mean of both sides used for test stimulus	No difference
Oono 2014(242)	TMD	✓ 2 sites (TMJ & masseter)	✓ 3rd test site (left forearm)	N/A - bilateral conditioning site (skull compression)	Impaired
Plinsinga 2020(256)	GTPS	✓		Contralateral (patient group); not reported (controls)	Impaired
Poluha 2020(257)	TMD	✓	✓ 2nd test site (thenar muscles (dominant hand)	Inconsistent (always non- dominant side)	Impaired
Sandrini 2006(258)	CTTH		✓ (Retromalleolar site)	Contralateral	Impaired

Serrano-Munoz 2019(259)	WAD		✓ (Dominant thenar eminence)	Contralateral	Impaired
Smith 2020(260)	WAD	✓		N/A - central test site (mid-CSp)	Impaired
Tompra 2016(7)	Achilles tendinopathy	✓		Contralateral	Impaired

Table 4.6: Description of the test stimulus and conditioning stimulus sites, related to the pain condition under study and the results of the study analysis. OA - osteoarthritis, CTTH - chronic tension-type headache, TMD - temporomandibular disorder, WAD - whiplash associated disorder, GTPS - greater trochanteric pain syndrome, NSP - nonspecific neck/shoulder pain, tib. ant. - tibialis anterior; ECRL - extensor carpi radialis longus, quad. - quadriceps, CSp - cervical spine, UFT - upper fibres of trapezius, LB - low back, TMJ - temporomandibular joint

Regarding demographic and psychosocial measures, no studies reported the marital statement status of participants, only two reported level of education and only one reported employment status. Fourteen studies did not include a psychosocial assessment questionnaire. Of those that did, the primary questionnaires used were the Pain Catastrophising Scale (n = 6), the State-Trait Anxiety Inventory (n =

2) and the Patient Health Questionnaire for depression (n = 1). None of these outcomes lend further understanding to the results of this SRMA than is already discussed elsewhere in this report, and so they will not be discussed further.

4.3.3 Risk of bias in studies

4.3.3.1 Overall AXIS ratings

In terms of overall quality, none of the studies rated were appraised as being of high quality, based on the descriptions provided above. Twenty of the studies were appraised as being of medium quality and the remaining three as low.(246,258,259). A summary of the overall ratings is provided in Table 4.7 below. A detailed summary of the assessments for each of the AXIS items, and the overall quality rating (high, medium or low) is provided in Tables E.1.1a-d (Appendix E.1).

	Overall quality rating		
AXIS items	High	Medium	Low
Study	High quality = appraised as "Yes" on all six key items AND both secondary items	Medium quality = appraised as "Yes" on three or more key items with or without "Yes" on either or both secondary items	Low quality = appraised as "Yes" on two or less key items with or without "Yes" on either or both secondary items
Arendt-Nielsen 2010(246)			✓
Cathcart 2010(247)		✓	

Christensen 2020(248)		✓	
Daenen 2013(129)		✓	
Edwards 2016(249)		✓	
Graven-Nielsen 2012(240)		✓	
Heredia-Rizo 2019(250)		✓	
Kashima 1999(251)		✓	
King 2009(127)		✓	
Kosek 2000(252)		✓	
Kothari 2016(253)		✓	
Kothari 2015(244)		✓	
Leffler 2002(241)		✓	
Mkumbuzi 2021(254)		✓	
Moana-Filho 2019(255)		✓	
Oono 2014(228)		✓	
Plinsinga 2020(256)		✓	
Poluha 2020(257)		✓	
Sandrini 2006(258)			✓

Serrano-Munoz 2019(259)			✓
Tompra 2016(7)		✓	
Coppieters 2018(130)		✓	
Smith 2020(260)		✓	

Table 4.7: Results of risk of bias assessment and critical appraisal/quality assessment using a modified version of the AXIS tool – overall assessment of high, medium or low quality.

4.3.3.2 Risk of bias

The Cochrane group suggests that risk of bias in particular, as compared to critical appraisal (of which risk of bias is a component), should be considered in undertaking SRMAs, and so those issues related to risk of bias only are discussed separately here.(262) Cross-sectional studies are susceptible to the three common sources of bias - selection bias, information bias and confounding.(263) The major source of bias for cross-sectional studies, however, is selection bias, with information bias and confounding considered to be relatively minor sources.(263) Selection bias is considered in AXIS items 5, 6, 7 and 13 of the AXIS tool. Information bias is considered in items 8, 9, 11, 15 and 16. Confounding is considered in item 2. Items 1, 3, 4, 10, 12, 14, 17, 18, 19 and 20 relate more directly to critical appraisal/quality assessment. Full details of the component parts of the risk of bias assessment are provided in Table E.2.1-E.2.6 in Appendix E.2. All included studies were assessed as having an overall level of some concern regarding risk of bias. The results for risk of bias assessment in the three domains (selection,

information and confounding), and overall, is summarised in Table 4.8 below. Generally, the risk of bias assessment demonstrated remarkable similarity across all 23 included studies.

	Risk of bias			
Study	Selection	Confounding	Information	Overall
Arendt-Nielsen 2010(246)	SC	H	SC	SC
Cathcart 2010(247)	SC	H	SC	SC
Christensen 2020(248)	SC	H	SC	SC
Coppieters 2018(128)	SC	H	SC	SC
Daenen 2013(129)	SC	H	SC	SC
Edwards 2016(249)	SC	H	SC	SC
Graven-Nielsen 2012(240)	SC	H	SC	SC
Heredia-Rizo 2019(250)	SC	H	SC	SC

Kashima 1999(251)	SC	H	SC	SC
King 2009(127)	SC	H	SC	SC
Kosek 2000(252)	SC	H	SC	SC
Kothari 2016(253)	SC	H	SC	SC
Kothari 2015(244)	SC	H	SC	SC
Leffler 2002(241)	SC	H	SC	SC
Mkumbuzi 2021(254)	SC	H	SC	SC
Moana-Filho 2019(255)	SC	H	SC	SC
Oono 2014(242)	SC	H	SC	SC
Plinsinga 2020(256)	SC	H	H	SC
Poluha 2020(257)	SC	H	SC	SC
Sandrini 2006(258)	SC	H	H	SC

Serrano-Munoz 2019(259)	SC	H	SC	SC
Smith 2020(260)	SC	H	SC	SC
Tompra 2016(7)	SC	H	SC	SC

Table 4.8: Results of risk of bias assessment related to the three domains (selection, information and confounding), and overall, using a modified version of the AXIS tool. SC – some concerns, H - high

4.3.4 Results of individual studies

Table 4.9 (below) displays the key data (the test stimulus measure prior to conditioning and following conditioning) extracted for synthesis from all the included studies from which it was possible to obtain this data. For reference, accompanying information regarding the number of test types, test site number, patient group number, the test stimulus modalities and the units of measurement are also presented alongside this key data. Citations for the studies from which it was not possible to obtain the means and standard deviations for key data are Cathcart 2010(247), Coppieters 2018(128), Edwards 2016(249), Poluha 2020(257), Sandrini 2006(258), Serrano-Munoz 2019(259) and Smith 2020.(260)

				TeS measure prior to conditioning: mean (SD)		TeS measure following conditioning: mean (SD)	
Study ID	TeS no. - Site no./Patient group no.	TeS modality	Unit of measurement	Patient group	Control group	Patient group	Control group
Arendt-Nielsen 2010(246)	Site 1/ Grp 1	Pressure - blunt (dynamometer)	kPa	483.33 (244.95)	608.33 (244.95)	533.33 (29.39)	683.33 (34.29)

Arendt-Nielsen 2010(246)	Site 1/Grp 2	Pressure - blunt (dynamometer)	kPa	591.67 (244.95)	-	608.33 (34.29)	-
Arendt-Nielsen 2010(246)	Site 2/Grp 1	Pressure - blunt (dynamometer)	kPa	400 (150.73)	476.92 (150.74)	492.31 (24.5)	561.54 (29.39)
Arendt-Nielsen 2010(246)	Site 2/Grp 2	Pressure - blunt (dynamometer)	kPa	407.69 (188.42)	-	507.69 (29.39)	-
Arendt-Nielsen 2010(246)	Site 3/Grp 1	Pressure - blunt (dynamometer)	kPa	300 (113.05)	353.85 (150.74)	330.77 (14.7)	392.31 (19.6)
Arendt-Nielsen 2010(246)	Site 3/Grp 2	Pressure - blunt (dynamometer)	kPa	369.23 (150.74)	-	361.54 (19.6)	-
Christensen 2020(248)	Site 1	Pressure - blunt (dynamometer)	kPa	291.67 (116.67)	308.33 (116.67)	341.67 (133.33)	400 (141.67)
Daenen 2013(129)	Site 1	TS: pressure - blunt (dynamometer)	VAS/NPRS 0-10	2.54 (2.67)	2.1 (2.1)	2.49 (2.92)	0.7 (2.1)
Daenen 2013(129)	Site 2	TS: pressure - blunt (dynamometer)	VAS/NPRS 0-10	2.22 (3.55)	2.07 (2.16)	2.23 (3.36)	0.73 (1.84)

Graven-Nielsen 2012(240)	TeS1	1. Pressure - blunt (dynamometer)	kPa	239.8 (72.67)	215.82 (49.1)	292.86 (89.97)	389.8 (112.23)
Graven-Nielsen 2012(240)	TeS2	2. Pressure - blunt (cuff)	kPa	25.2 (10.6)	34.5 (12.1)	26.7 (17.4)	39.8 (17.13)
Heredia-Rizo 2019(250)	Site 1	Pressure - blunt (cuff)	kPa	18.11 (8.25)	20.7 (8.47)	20 (7.18)	28.15 (12.7)
Kashima 1999(251)	Site 1	Pressure - blunt (dynamometer)	N	2.48 (1.03)	4.17 (1.18)	2.8 (0.95)	5.25 (1.66)
King 2009(127)	Site 1	Heat (thermode)	VAS/NPRS 0-100	42.3 (2.1)	39.9 (1.6)	50.2 (2.3)	31.9 (1.7)
Kosek 2000(252)	Site 1	Pressure - blunt (dynamometer)	kPa	248.72 (111.22)	336.41 (71.5)	256.41 (119.17)	453.33 (117.18)
Kothari 2015(244)	Site 1	Pressure - blunt (dynamometer)	kPa	129.32 (54.33)	164.32 (35.78)	164.32 (78.19)	214.55 (50.36)
Kothari 2015(244)	Site 2	Pressure - blunt (dynamometer)	kPa	378.86 (103.29)	470.86 (106.62)	415.43 (99.96)	521.14 (109.96)

Kothari 2016(253)	Site 1/Grp 1	Pressure - blunt (dynamometer)	kPa	115.79 (39.53)	161.51 (35.81)	143.75 (49.93)	214.14 (50.55)
Kothari 2016(253)	Site 1/Grp 2	Pressure - blunt (dynamometer)	kPa	100 (38.16)	-	134.21 (54.16)	-
Kothari 2016(253)	Site 2/Grp 1	Pressure - blunt (dynamometer)	kPa	360.83 (89.6)	447.5 (106.72)	403.33 (84.33)	496.67 (117.39)
Kothari 2016(253)	Site 2/Grp 2	Pressure - blunt (dynamometer)	kPa	339.17 (81.07)	-	375 (43.65)	-
Leffler 2002(241)	TeS1	1. Pressure - blunt (dynamometer);	kPa	527.7 (233.3)	593.6 (309.7)	610.6 (234)	730 (168)
Leffler 2002(241)	TeS2	2. Heat (thermode)	°C	45 (2.2)	44.1 (1.6)	45.8 (3.6)	45.9 (0.9)
Mkumbuzi 2021(254)	Site 1	Pressure - blunt (dynamometer)	kPa	417 (146)	601 (196)	458 (142)	633 (183)
Moana-Filho 2019(255)	TeS1 - Site 1	Pressure - blunt (dynamometer)	kPa	123.1 (115.3)	141.5 (119.5)	141.5 (37.6)	158.2 (44.3)

Oono 2014(242)	TeS1 - Site 1	Pressure - blunt (dynamometer; PPT)	kPa	105.2 (50.8)	137.7 (44)	110.3 (56.4)	176 (46.4)
Oono 2014(242)	TeS1 - Site 2	Pressure - blunt (dynamometer; PPT)	kPa	102.8 (38)	115.2 (30.4)	100.5 (43.2)	159.5 (35.6)
Oono 2014(242)	TeS1- Site 3	Pressure - blunt (dynamometer; PPT)	kPa	187.6 (62)	215.9 (49.2)	213.3 (86)	329.4 (55.2)
Oono 2014(242)	TeS2 - Site 1	Pressure - blunt (dynamometer; PPTol))	kPa	166.4 (55.2)	215.9 (73.6)	165 (66.8)	268.8 (98.4)
Oono 2014(242)	TeS2 - Site 2	Pressure - blunt (dynamometer; PPTol))	kPa	345.4 (111.2)	377.4 (75.2)	335.9 (137.2)	522.3 (98)
Plinsinga 2020(256)	Site 1	Pressure - blunt (dynamometer)	kPa	203.25 (71.99)	343.23 (123.68)	247.82 (86.24)	463.81 (158.19)
Tompra 2016(7)	Site 1	Pressure - blunt (dynamometer)	kPa	253 (80.5)	671.4 (215.7)	289.4 (114.3)	831.9 (213.3)

Table 4.9: Key data (the test stimulus measure prior to conditioning and following conditioning) extracted for synthesis from all the included studies from which it was possible to obtain this data; and the number of test types, test site number, patient group number, the test stimulus modalities and the test stimulus units of measurement. no. - number, TeS - test stimulus, PPTol - pressure pain tolerance, TS - temporal summation, SD - standard deviation, VAS - visual analogue scale, NPRS - numerical pain rating scale

4.3.5 Syntheses

4.3.5.1 Narrative synthesis

The search and selection process retrieved studies that represent quite well the range of common musculoskeletal pain syndromes, including muscular pain, tendon pain, joint pain, and pain syndromes that likely receive pain contributions for more than one tissue type, e.g., chronic low back pain and nonspecific neck/shoulder pain. Specifically, the pain conditions represented were trapezius myalgia (n = 1), masticatory myalgia (n = 1), chronic tension-type headache (n = 2), Achilles tendinopathy (n = 2), knee osteoarthritis (n = 3), hip osteoarthritis (n = 1), temporomandibular disorder (n = 6), greater trochanteric pain syndrome (n = 1), nonspecific neck/shoulder pain (n = 1), whiplash associated disorder (n = 4), and chronic low back pain (n = 1).

Of these 23 studies, 17 concluded that CPM was impaired in the patient group compared to healthy controls, five concluded that there was no difference in CPM between patient group and controls and one concluded that CPM was facilitated (i.e., the test stimulus pain intensity was *greater* after exposure to the conditioning stimulus) in the patient group.

Most used pressure pain as a test stimulus modality. Most used noxious cold-water immersion as the conditioning stimulus modality. Of note, most used a test stimulus intensity of subjective pain threshold, e.g., pressure pain threshold. This is of note as the most recent expert consensus recommendations(208) are that the test stimulus should be of a minimum intensity of 40/100 VAS for CPM testing. Only one study met this recommendation. The consequences of only

using a test stimulus at pain threshold intensity is not known with respect to the existence or magnitude of any CPM effect. This issue is potentially important in this SRMA, where the goal is to ascertain the most precise estimate possible of a summary effect size. Regarding the conditioning stimulus, 15 studies met the expert consensus recommendation that the stimulus intensity should be at least 20/100 VAS. Five studies did not meet this recommendation and the remaining three studies did not provide enough information to be able to ascertain whether they met this recommendation. Regarding the recommendation that the test stimulus should be measured before and after at least one minute of exposure to the conditioning stimulus, only four studies met this standard. These recommendations were published in 2015. Of the studies selected, 12 were published in 2016 or later, however there was no consistent pattern regarding the standards of the test stimulus, conditioning stimulus or CPM protocol with respect to whether studies were published before or after the expert consensus recommendations were published.

All 23 studies were assessed as having a high risk of bias due to confounding. Age, sex and menstrual cycle were generally not well controlled, and all but one study did not blind assessors. Almost all studies were assessed as having some level of concern regarding risk of information bias, with two studies assessed as having high risk. All studies were assessed as having a level of "some concern" regarding selection bias and all studies were assessed as having an overall risk of bias of "some concern". These elements all contribute to lowering the confidence in the summary effect size calculated by meta-analysis, and in the strength of the body of evidence as a whole. This observation is seen as a limitation of the evidence, and as such, is discussed in the limitations section and implications of the results section of this report.

For further details on individual studies, please refer to the results sections of this report pertaining to study characteristics and risk of bias in studies.

4.3.5.2 Quantitative synthesis

The necessary outcome data could be obtained for 16 (i.e., ≥ 5 , as per the planned protocol) of the 23 studies that met the inclusion criteria for the review. The methodological and clinical heterogeneity among these studies was not of a degree that suggested meta-analysis was inappropriate,(264) therefore, the study data was quantitatively synthesised and a meta-analysis was performed. Visual inspection of the resulting forest plot revealed that one study(127) was a clear outlier. Examining the 95% CIs, it was clear that this study met the definition of an outlier whereby it's 95% CI (-24.79, -15.62) lay well outside the 95% CI of the pooled effects (-1.70, -0.69).(265) As a result, the decision was made to exclude this study from the meta-analysis, and the meta-analysis results were recalculated with the remaining 15 studies.

4.3.5.2.1 Meta-analysis

Characteristics and risk of bias among contributing studies

The musculoskeletal pain conditions of the patient groups for the 15 studies appropriate for meta-analysis were knee osteoarthritis (n = 2), chronic low back pain (n = 1), whiplash associated disorder (n = 1), nonspecific neck/shoulder pain (n = 1), masticatory myalgia (n = 1), temporomandibular disorder (n = 4), hip osteoarthritis (n = 1), trapezius myalgia (n = 1), Achilles tendinopathy (n = 2), and greater trochanteric pain syndrome (n = 1). (For the studies that met the systematic review inclusion criteria but were not able to be quantitatively

synthesised (n = 8), the conditions of the patient groups were knee osteoarthritis (n = 1), chronic tension-type headache (n = 2), whiplash associated disorder (n = 3) and temporomandibular disorder (n = 2)).

The risk of bias assessments for the studies that were included in the meta-analysis were remarkably similar. All were assessed as having a high risk of confounding. All were assessed as having some concern regarding risk of selection bias. All were assessed as having some concern regarding risk of information bias, except one(256) which was assessed as having a high risk. All 15 studies were assessed as having some concern regarding overall risk of bias; all but one to be of medium quality and one of low quality on critical appraisal. For further discussion of this assessment please see Section 4.3.3.

Summary estimate and its precision

Figure 4.2 below presents, in forest plot form, for each included study for which the relevant data could be obtained (15 of the 23 studies that met inclusion criteria): (1) the mean and standard deviations of the magnitude of CPM for each group (patient group and control group) and (2) the standardised mean differences in CPM between the patient and control groups and their 95% CIs. The weighting allocated to each study is also provided. A negative (less than zero) value for the standardised mean difference indicates a greater magnitude of CPM pain inhibition ("CPM efficiency") in the control group. There was evidence of a greater magnitude of CPM pain inhibition in healthy controls compared with patients with painful chronic musculoskeletal conditions, with 13 of 15 studies favouring a greater magnitude of CPM pain inhibition in

healthy controls (-0.98 [95% CI: -1.39, -0.58], Z = 4.74 (P < .00001)). The magnitude of this effect size would be categorised as “large” according to “Cohen’s effect sizes” that are often used as guiding rules for interpreting effect size magnitudes.(194) The summary confidence interval extends into a magnitude (-0.58) that would be considered “moderate” by this same system of interpretation.

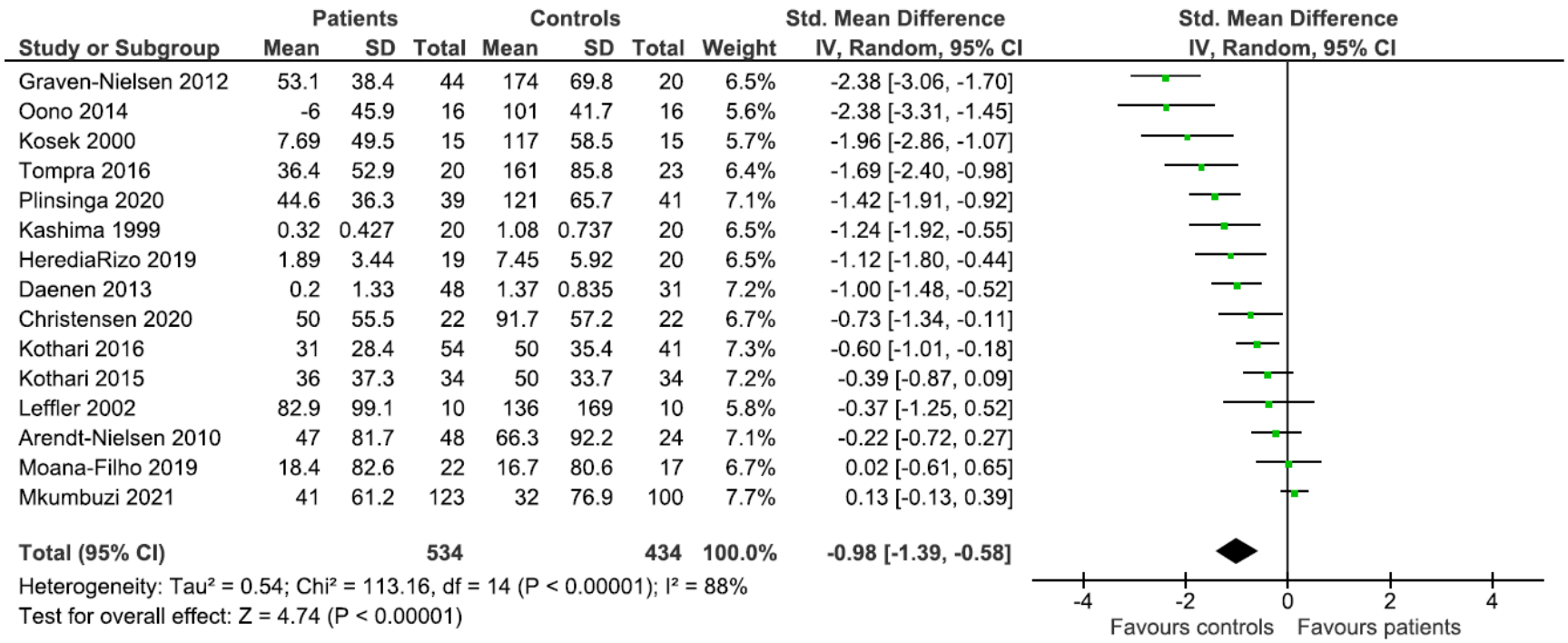


Figure 4.2: Results of individual studies - standardised mean differences and their 95% CIs, and overall effect. Std. Mean Difference - standardised mean difference, IV - independent variable, Cl - confidence interval

Measures of statistical heterogeneity

The value of the chi-squared ((χ^2, or Chi^2) statistic, a measure of heterogeneity (whereby heterogeneity, unless otherwise specified herein, refers to statistical heterogeneity, rather than methodological or clinical heterogeneity), was computed to be 113.16 (df = 14 (P < .00001). Further investigation of heterogeneity was then computed using the I^2 test. The I^2 test, which is used to indicate the *proportion* of observed dispersion (differences in values) in effect sizes from study to study that is real (i.e., true variation in effect sizes) rather than spurious (i.e., due to random error),(266) considers the value of the chi-squared ((χ^2, or Chi^2) statistic and its degrees of freedom whereby I^2 = (Q – df/Q) x 100%, and where Q is the Chi^2 statistic.(237) By this formula, I^2 was computed to be 88%, estimating that the percentage of the variability in the effect estimates (i.e., the difference in the CPM magnitudes between patients and healthy controls between the different studies) that was due to heterogeneity (i.e., true variation in effect sizes), rather that sampling error (i.e., chance) was 88%, which represents considerable heterogeneity.(237) To further illustrate the meaning of the value of this statistic, it is understood that I^2 reflects the extent of overlap of the confidence intervals and can be viewed as a measure of *inconsistency* across the underlying true effects.(266) In recognition that the I^2 value for this SRMA is large, (whereby I^2 values of 30-60% are considered to represent "moderate" heterogeneity; 50-90% "substantial" heterogeneity and 75-100% "considerable" heterogeneity),(237) this report later speculates about possible causes for this heterogeneity.(266)

The value of tau-squared (T^2), another measure of heterogeneity (the *variance* of the observed effect size estimates), was computed to be 0.54. The value of T^2 is used to assign weights in the

random effects model by the DerSimonian and Laird method. Tau (T; √tau^2) is the standard deviation of the effect size estimates. As T^2 was computed to be 0.54, T, therefore, had a value of 0.73. This value can then be used to describe the distribution of effect sizes around the mean, giving a sense for the range of true effect sizes, thereby allowing consideration of the implications of this range.(266) With the summary effect being -0.98, and T being 0.73, it is expected that 95% of the true effects will fall in the range of -0.98 plus or minus 1.96T (±1.44), or -2.42 to 0.46. Of note, this allows for true effect size values in the "no difference" (zero/close to zero) and positive (greater than zero) range of values. Notably, both T^2 and T reflect the amount of true heterogeneity (the variance and the standard deviation respectively) in the effect sizes. In contrast, I^2 reflects the proportion of observed dispersion that is due to this heterogeneity.(266)

Returning to importance of the I^2 value of 88%, the interpretation of its importance can be considered from the perspective of both the magnitude and direction of the effects. The magnitude of the effects (as represented by the values of the standardised mean differences), was quite wide-ranging (-2.38 - 0.13), however, there was a consistent trend in the direction of the effects being less than zero, i.e., favouring controls - 13 of the 15 studies included in the meta-analysis had a negative effect size value.

Direction of the effect

As discussed above, the more important element of the analysis for this SRMA is the consistent trend in the direction of the effects, i.e., less than zero in 13 of the 15 studies, with the positive

values for the two studies that had positive values having a relatively very small magnitude (0.13 and 0.02). Five studies, however, had confidence intervals that crossed zero(241,244,246,254,255). Overall, this distribution of effect sizes suggests that there is a greater magnitude *inhibitory* CPM effect (i.e., more efficient pain inhibition mechanism produced by the endogenous pain mechanisms of the nervous system) available in healthy populations (i.e., those free of chronic musculoskeletal pain) then in chronic musculoskeletal pain populations.

Possible causes of heterogeneity among study results

Possible causes of heterogeneity pre-specified for subgroup analysis prior to undertaking the systematic review were:

1. Test stimulus type (modality)
2. Conditioning stimulus type (modality)
3. Parallel/concurrent vs sequential protocols
4. Risk of bias/quality level

Unfortunately, as discussed subsequently, there were not enough studies, and subgroup distribution was of such an unequal extent, that it was not possible to undertake any of these pre-specified subgroup analyses with any expectation that the results would be meaningful.

Other possible causes of heterogeneity were the differences in CPM testing protocols and real differences in the CPM physiology of the musculoskeletal pain conditions being investigated.

There was such a degree of variability in the CPM testing protocols (for further details, please see Section 4.3.2), that it is not methodologically possible to discern within the context of this SRMA what, if any, elements of the overall protocols may have been responsible for any degree of heterogeneity in the effect size magnitudes between studies. It has been noted elsewhere in this report, however, that expert consensus guidelines on CPM testing have been published,(208) and yet these recommendations do not seem to have been consistently adopted in research settings, at least within the group of studies selected for this SRMA. The consequences of this for this SRMA and for future research are discussed later in this report.

For interest, Table 4.10 (below) displays the results of each study by pain condition type compared to the standardised mean differences (and 95% CIs) of the individual effect size estimates in descending order of effect size magnitude from the 15 included studies from which the requisite outcome data could be obtained. The weight statistically assigned to each study is also displayed and it is noteworthy that these are very similar between studies. While acknowledging that this data has not been meta-analysed and that subgroup analysis is not statistically appropriate for this dataset, as discussed subsequently given the low number of studies available for meta-analysis and the uneven numbers of studies within subgroups, it is nevertheless interesting to observe that there is no discernible pattern related to the overall tissue type (e.g., tendon pain, joint pain, muscle pain) implicated as the pain source and the magnitude of the effect size estimate.

Study	Pain condition	Weight	Std. Mean Difference, 95% Cl
Graven-Nielsen 2012(240)	Knee OA	6.50%	-2.38 [-3.06, -1.70]
Oono 2014(242)	TMD	5.60%	-2.38 [-3.31, -1.45]
Kosek 2000(252)	OA hip	5.70%	-1.96 [-2.86, -1.07]
Tompra 2016(7)	Achilles tendinopathy	6.40%	-1.69 [-2.40, -0.98]
Plinsinga 2020(256)	GTPS	7.10%	-1.42 [-1.91, -0.92]
Kashima 1999(251)	Masticatory myalgia	6.50%	-1.24 [-1.92, -0.55]
Heredia-Rizo 2019(250)	NSP	6.50%	-1.12 [-1.80, -0.44]
Daenen 2013(129)	WAD	7.20%	-1.00 [-1.48, -0.52]
Christensen 2020(248)	Chronic low back pain	6.70%	-0.73 [-1.34, -0.11]
Kothari 2016(253)	TMD	7.30%	-0.60 [-1.01, -0.18]
Kothari 2015(244)	TMD	7.20%	-0.39 [-0.87, 0.09]
Leffler 2002(241)	Trapezius myalgia	5.80%	-0.37 [-1.25, 0.52]
Arendt-Nielsen 2010(246)	Knee OA	7.10%	-0.22 [-0.72, 0.27]
Moana-Filho 2019(255)	TMD	6.70%	0.02 [-0.61, 0.65]
Mkumbuzi 2021(254)	Achilles tendinopathy	7.70%	0.13 [-0.13, 0.39]

Table 4.10: Pain condition type, standardised mean differences (and 95% CIs) and study weight of the 15 included studies from which the requisite outcome data could be obtained. OA – osteoarthritis, CTTH – chronic tension-type headache, TMD – temporomandibular disorder, WAD – whiplash associated disorder, GTPS – greater trochanteric pain syndrome, NSP – nonspecific neck/shoulder pain, Std. Mean Difference – standardised mean difference, SD – standard deviation, IV – independent variable, Cl – confidence interval

4.3.5.2.2 Subgroup Analyses

As described in the methods section, subgroup analyses were pre-specified to explore possible sources of heterogeneity.(237) The numbers of studies with data available for meta-analysis was low (n = 15) and the distribution of covariates was uneven between the subgroups, however, which made undertaking subgroup analysis inappropriate.(237)

4.3.5.2.3 Sensitivity Analyses

One study(127) that met inclusion criteria for the systematic review and for which the necessary outcome data could be obtained for quantitative synthesis was designated as an outlier during the synthesis stage as its 95% CI (-24.79, -15.62) lay outside the 95% CI of the pooled effects (-1.70, -0.69).(265) As a result, the decision was made to exclude this study from the primary metanalysis. Adding this study back into the meta-analysis computed an overall outcome estimate of -1.20 [-1.70, -0.69] (4.83 (P < .00001)), compared to the original primary meta-analysis overall outcome estimate of -0.98 [-1.39, -0.58] (Z = 4.74 (P < .00001)). Including the outlier study also resulted in the I^2 test for heterogeneity being computed to be 92%, compared to 88% originally. This sensitivity analysis – removal of an outlier - demonstrated a meaningful reduction in the effect size estimate as well as a reduction in the estimate of the proportion of the observed dispersion in effect sizes resulting from real variation in effect sizes, rather than from random error (the I^2 value). These findings support the decision to exclude this study from the primary analysis as an outlier.

The primary meta-analysis otherwise included all studies selected by the systematic review process for which the requisite outcome data could be obtained, regardless of quality or risk of bias assessment. A pre-specified sensitivity analysis was planned, however, based on the critical appraisal/risk of bias assessment. All studies were assessed as having some concern overall regarding risk of bias, i.e., they were all assessed as having similar risk. Only one study(246) was assessed as having low quality – the remainder were assessed as being of medium quality. A sensitivity analysis that omitted the single study that was assessed as being of low quality from the primary analysis was, therefore, conducted. Removing this study from the meta-analysis computed an overall outcome estimate of -1.04 [-1.47, -0.61] (Z = 4.71 (P < .00001)), compared to the primary meta-analysis overall outcome estimate of -0.98 [-1.39, -0.58] (Z = 4.74 (P < .00001)). The I^2 test for heterogeneity was unchanged at 88%. This sensitivity analysis demonstrated that the original primary results were robust as the overall outcome estimate was essentially unchanged.(264)

Table 4.11 (below) displays the details of the two sensitivity analyses and compares them to the primary meta-analysis.

	Patients	Controls	Std. Mean Difference				
Study	Total	Total	IV, Random, 95% Cl	Tau^2	Chi^2	I^2	Test for overall effect
Primary meta-analysis (*n* = 15)	534	434	-0.98 [-1.39, -0.58]	0.54	113.16	88%	Z = 4.74 (P < .00001)
Outlier included (*n* = 16)	548	462	-1.20 [-1.70, -0.69]	0.91	182.98	92%	Z = 4.83 (P < .00001)
Study of low quality removed (*n* = 14)	486	410	-1.04 [-1.47, -0.61]	0.58	109.98	88%	Z = 4.71 (P < .00001)

Table 4.11: Results of primary analysis and sensitivity analyses - standardised mean differences and their 95% CIs, Tau^2, Chi^2, I^2, and overall effect. Std. Mean Difference - standardised mean difference, IV - independent variable, Cl - confidence interval

4.3.6 Reporting biases

There was no risk of bias assessed due to missing results (arising from reporting biases) as there were no missing results in the studies included for meta-analysis.

4.3.7 Certainty of evidence

An assessment of the evidence included in the primary meta-analysis was undertaken, the goal being to determine the extent to which there can be confidence that the estimate of the effect is correct. The assessment was performed across all those studies, i.e., for the body of evidence. Of note here, as described earlier in this report, this SRMA assessed that both the quality and risk of bias were remarkably similar between all the included *individual* studies. For details on this assessment, please see Section 4.3.3.

Regarding certainty of the evidence, the GRADE approach was used to assess the quality of the *body* of evidence, whereby quality is graded as “high”, “moderate”, “low” or “very low”.(67) As the data considered in this meta-analysis was generated by observational studies, the starting (but modifiable) assessment of quality of evidence was “low”, as per the GRADE approach to determining the quality of evidence.(267)

Considering the five factors that GRADE describes may lead to rating down the quality of evidence, i.e., (1) risk of bias, (2) inconsistency, (3) indirectness, (4) imprecision, and (5) publication bias, there were enough concerns to rate the quality of evidence down one level to “very low”, as described below.

With respect to risk of bias, there was flawed measurement of CPM when considered against the most recent expert guidelines(208) and there was failure to adequately control confounding. For further discussion of these assessments, please see Section 4.3.3.2 and Appendix E.2.

Regarding inconsistency, i.e., "unexplained heterogeneity of results",(67) a thorough exploration of potential causes of the heterogeneity, within the limitations of what information was available from this body of evidence, did not reveal a sure explanation for the heterogeneity. (Please see Section 4.3.5.2.1 for a more detailed discussion of this investigation). Although the heterogeneity could not be explained with sureness, by which on one hand it is recommended to downgrade the quality of evidence, on the other hand it is also recommended that marked inconsistency may nevertheless not reduce confidence in results, depending on the decision to which it refers.(67) In the setting of this SRMA, it is acknowledged that the magnitude of the effect size cannot be described with confidence, however, the *direction* of the effect can be described with some level of confidence. This confidence comes from the range of the summary confidence interval being entirely less than zero (i.e., contains only negative values, indicating only an inhibitory CPM effect in healthy controls contrasted with the chronic musculoskeletal pain population) (-0.98 [95% CI: -1.39, -0.58], $Z = 4.74$ ($P < .00001$)). In the context of CPM, this observation alone potentially adds value to our understanding of pain mechanisms in chronic musculoskeletal pain and may have clinical meaning. The purpose of a systematic review, however, is not to provide recommendations for treatment, but simply to estimate the effect size.(67)

Regarding directness, i.e., the degree to which the research directly investigates the outcome of interest, i.e., the CPM effect, in the population of interest, i.e., the chronic musculoskeletal

population as a whole,(67) there were no major concerns. For a discussion of this, please see Section 4.3.5.2.1 relating to study population characteristics and Sections 4.3.3.1 and 4.3.3.2 relating to selection bias. Although the methodology for measuring the CPM effect differed between studies, all studies did demonstrate the elicitation of the CPM effect, at least in the healthy control group - this was, in fact, one of the inclusion criteria for the review - and the populations included covered a wide range of tissue locations of pain, including painful joint, tendon and muscle conditions as well as conditions that were likely to generate nociception from multiple tissue sources, e.g., chronic low back pain.

Regarding imprecision, the 95% CI around the effect size estimate was -1.39 -
-0.58 which, although wide, only encompassed negative values, suggesting a greater inhibitory CPM effect in healthy controls compared to chronic musculoskeletal pain populations. As discussed previously, this is likely the only outcome of this SRMA that can be discussed with even a low level of confidence, as opposed to the absolute magnitude of the true effect size.

Regarding publication bias, a funnel plot was produced in RevMan and inspected visually for asymmetry. The resultant funnel plot (please see Figure 4.3 below) is a scatter plot of the effect estimates from each of the individual studies included in the meta-analysis, plotted along the x-axis, against the standard error (a measure of each study's precision) on the y-axis.

In general, where there is no bias or between-study heterogeneity, the overall shape of such plotted estimates will take the shape of an inverted "funnel" (narrower at the top and wider at the bottom) that is symmetrical about a centre line representing the summary effect estimate

calculated by the meta-analysis. Visual inspection of the funnel plot generated for this meta-analysis reveals an asymmetrical shape whereby a disproportionate number of smaller studies (with relatively larger standard errors) show a greater magnitude effect in favour of healthy controls (a greater magnitude of an inhibitory CPM effect). In contrast, larger studies (with relatively smaller standard errors) show a disproportionate representation of a lesser magnitude effect size in favour of controls, and a trend towards the no difference line. This distribution suggests that smaller studies reporting no difference may not have been available for selection by the SRMA selection process, potentially due to publication bias whereby studies that do not show a significant effect are less likely to be selected for publication.(268)

There was a large degree of heterogeneity in the studies included for meta-analysis, as previously discussed. The asymmetry in the funnel plot reflects a correlation between study sizes and effect estimates.(268) Another possible source for this asymmetry other than publication bias is poor methodological quality in smaller studies.(268) An analysis of methodology of each of the included studies as described above, however, demonstrates that this is not the case for this group of studies. Asymmetry in the funnel plot may also simply have occurred due to chance as only a relatively small number of studies were included by the SRMA selection process in this meta-analysis. Despite these other considerations, for the reasons explained, it is considered likely that there was publication bias present in this body of work. Particularly in the realm of cross-sectional studies, as opposed to randomised controlled trials that are more likely to be registered and hence found during the selection process, it is very likely that cross-sectional studies on this subject have been conducted but not published or otherwise made available for consideration.(268) Statistical testing for funnel plot asymmetry was not undertaken, in part

because the asymmetry is clearly apparent on visual inspection and so statistical testing did not seem necessary, but also because statistical tests for funnel plot asymmetry have low power and it has been recommended that such testing need only be used in a minority of meta-analyses.(268) The obvious visual asymmetry of the final plot for this meta-analysis suggested that statistical testing did not need to be undertaken.

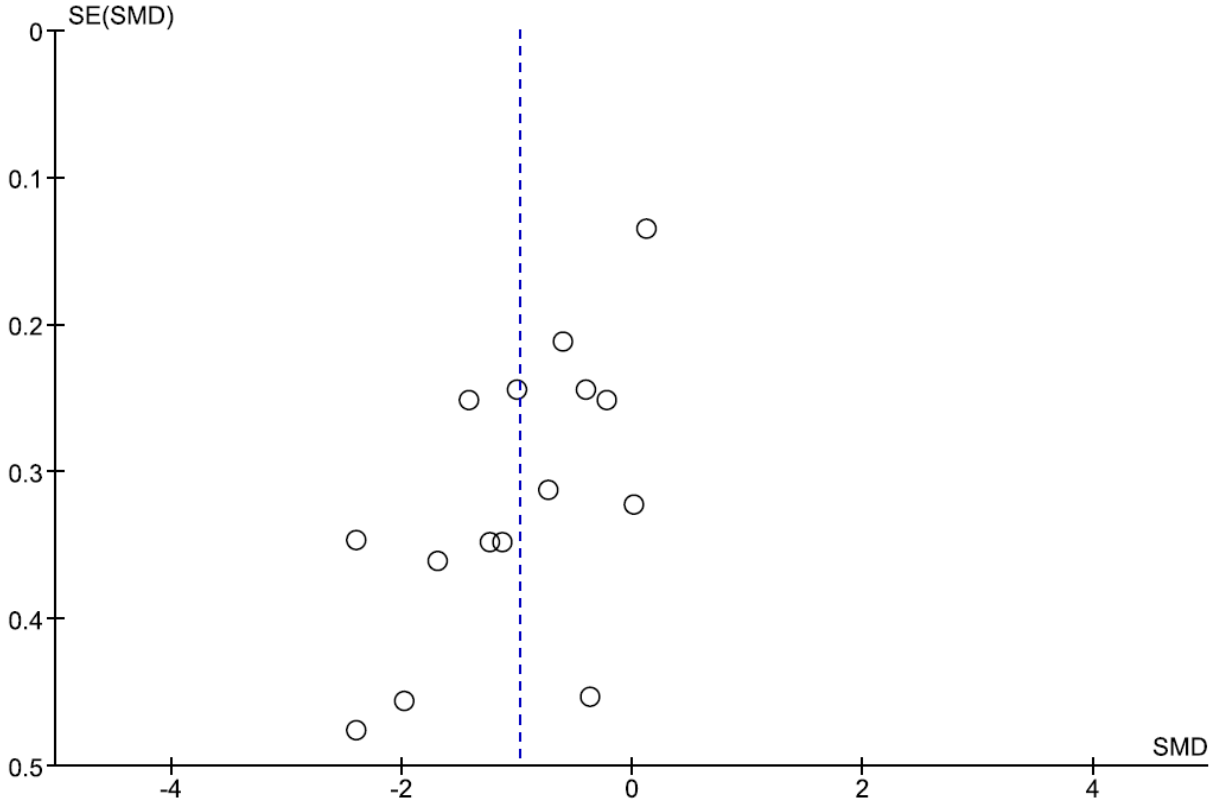


Figure 4.3: Funnel plot of the studies included in the meta-analysis plotting the standardised mean difference of each individual study against its standard error. SMD – standardised mean difference, SE – standard error.

Ultimately, the certainty of evidence rating for the effect size for this body of evidence was assessed as being very low. Please see Table 4.12 below for a summary of this assessment using the GRADE Evidence Profile framework.

	Factors that may decrease certainty of evidence					
No. of studies (No. of patients)	Risk of bias	Indirectness	Inconsistency	Imprecision	Other considerations	Certainty of Evidence
15 studies (534 patients)	Serious[a]	Not serious	Not serious	Not serious	Not serious	Very low

Explanations

a. Risk of bias assessment assigned high risk of bias for all studies related to confounding and some concerns for all studies re selection and information bias (except for two studies that were assessed as having high risk of information bias). By the GRADE categories, this translated to assigning risk of bias for both the "flawed measurement" and "failure to control confounding" categories. Therefore, the quality was downgraded by one level (one level total).

Table 4.12: Summary of this assessment of certainty of evidence rating using the GRADE Evidence Profile framework. No. - number

4.4 Discussion

4.4.1 General Interpretation

Caution should be exercised in the interpretation of the findings of this meta-analysis, in consideration of the high degree of unexplained heterogeneity in the effect sizes from the included studies, the assessment of a level of "some concern" regarding overall bias in the body of evidence, the finding of evidence of publication bias for this subject matter and the results of a GRADE analysis being that of having very low confidence in the summary effect estimate.

Having acknowledged this, the finding of this SRMA does align generally with Lewis et al.'s(124) review analysis that CPM efficiency was reduced in the general chronic pain population. Whereas Lewis et al.'s(124) review included a myriad of chronic pain conditions, as detailed previously, this SRMA refines its investigations to musculoskeletal system pain populations specifically. Interestingly, the effect size was of a similarly large magnitude and the confidence interval was similarly wide in both these studies. Lewis et al.(124) found a summary effect size estimate magnitude of 0.78 [95% CI: 0.48, 1.08), and this SRMA found a summary effect size estimate magnitude of 0.98 [95% CI: 0.58, 1.39]. The unexplained heterogeneity was also similarly large in both reviews, with Lewis et al.'s(124) review calculating an I^2 value of 87%, compared to an I^2 value of 88% for this SRMA.

In contrast to the methodology of this SRMA, however, Lewis et al's(124) review acknowledged having violated the assumption of independence in the data by entering data from the same patient/control groups twice where there were, for example, two different test stimuli used in the

same set of patients and controls. In contrast, in this SRMA the data was either entered singly for one test stimulus (preferentially blunt pressure was used) or combined and averaged and entered as a single data set into the meta-analysis (for details, please see Section 4.2.9.4).

In contrast to this SRMA, Lewis at al's(124) review was dominated by pain conditions that were not musculoskeletal - of the 30 studies that were included, seven were of patients with fibromyalgia and six were of patients with irritable bowel syndrome. Although some musculoskeletal pain syndromes were included - headache (n = 4), arthritis (n = 3) and temporomandibular disorder, the other conditions included were post-stroke pain, Parkinson's disease, neuralgia, vestibulodynia, and pancreatitis. In common with this SRMA, however, Lewis et al.(124) found the conditioning stimulus most often used in their included studies was immersion in noxious-cold water (17 of 30 studies), and the most common test stimulus used was pressure (15 of 30 studies). The greater number of studies included in Lewis et al's(124) review (n = 30 compared with n = 15 for this SRMA) allowed for subgroup analysis for both conditioning stimulus type and test stimulus type. This was a prespecified plan for this SRMA, however, the number of selected studies, together with the uneven distribution of characteristics, was not considered to permit these subgroup analyses to have the ability to produce calculations of any meaning, and so they were not undertaken. Of note, Lewis et al.(124) found that when these subgroup analyses were conducted across their dataset, however, they did not significantly influence the summary effect size.

In common, Lewis et al.(124) also reported similar limitations in individual studies regarding age- and sex-matching of the patient and control populations and lack of blinding of assessors.

Again, in contrast, however, Lewis et al.(124) determined that there was not clear evidence of publication bias, whereas this review did find evidence of publication bias.

4.4.2 Limitations

4.4.2.1 Limitations of the evidence

Unfortunately, only 23 studies that met the inclusion criteria for this SRMA were retrieved, and only 15 of those were able to provide data that could be meta-analysed. These 15 studies showed a large degree of heterogeneity, as discussed above. Had a larger number of studies been available to be retrieved, it may have been that subgroup analysis would have been able to explain some of this heterogeneity. The relatively small sample sizes typical in studies of this nature may also have contributed to imprecise estimates. Variable methodologies for CPM testing between studies may also have hindered the calculation of a more precise summary effect estimate. It may also be that the physiological nature of CPM is subject to influences yet to be discerned and accounted for in study designs as confounding factors.

Other limitations include an overall risk of bias that was assessed to be of a level of some concern, with confounding being a universal concern for all studies and both selection and information bias also being of some concern.

The GRADE assessment of certainty in the summary effect size was only very low. This is in part because observational studies begin at a level of “low” in the GRADE approach, with upgrading only seen to be appropriate under exceptional circumstances. However, even if this

body of evidence had started at a “high” level (as for randomised controlled trials) in this assessment scale, there were many characteristics of the body of evidence that would have resulted in downgrading by several levels, whereas only one level of downgrade was available in the GRADE assessment structure (i.e., to “very low”) as was assessed due to the weaknesses discussed above.

4.4.2.2 Limitations of the review processes

It was not possible to include seven studies that met selection criteria in the meta-analysis as it was not possible to either obtain or convert the requisite data to mean and standard deviation form. It is not known what effect this may have had on the computations of the meta-analysis.

Pre-specified subgroup analyses were planned; however, these were not undertaken due to a low number of studies with an uneven distribution of characteristics being retrieved to be entered into the meta-analysis. As discussed, a meta-analysis of CPM in the general chronic pain population (rather than musculoskeletal pain population specifically) conducted similar subgroup analyses to those planned for this SRMA, however, and found that theses analyses did not affect the summary estimate in their population. This suggests that there may have been a similar outcome for these subgroup analyses, had there been enough studies available for meta-analysis in this SRMA.

Due to feasibility issues, data extraction was performed by one reviewer (LS) and verified by another (KS), which may have introduced some errors. However, as the data was reviewed many

times by LS in the compilation of this report, and also by AS (who conducted the meta-analysis), it is unlikely that any errors have persisted to the final meta-analysis and general reporting.

Due to feasibility issues, reports being written in English was an inclusion criterion, rather than including reports in any language. It is unknown what effect this may have had on this SRMA, either qualitatively or quantitatively.

4.4.3 Implications of the results

The outcome of this SRMA is that impaired pain inhibition by CPM mechanisms is present in musculoskeletal pain syndromes generally. Considering the limitations of the evidence as described above, however, there should be hesitancy with regards to the certainty of this suggestion.

The goal of this systematic review with meta-analysis was to investigate whether inefficiency of pain inhibition by CPM mechanisms was a potential neuropathological contributor to pain chronicity in musculoskeletal pain populations. The setting of this goal was justified in light of the frequent treatment failure of many chronically painful musculoskeletal conditions, where treatment paradigms focus on abnormalities of local tissue structure, function or mechanics. It was hoped that by undertaking an SRMA on this topic that light may be shed on this issue to inform more successful management and treatment strategies of chronic musculoskeletal pain syndromes. For this question to be answered with confidence, however, it seems that more research is required, with more uniform and stringent approaches taken in conducting such future research. It may be that if future studies apply the recommendations for conducting CPM

research of the 2015 expert consensus panel(208) that the experimental induction of CPM would be more uniform across studies, and result in more precise estimates of the effect. This may result in less heterogeneity of effect sizes across studies which, combined with better control of confounding, may then be able to generate a summary effect size estimate in which the scientific community may have confidence. It may be that the biology of CPM depends on yet-to-be-determined factors, that currently create unknown confounding in research settings. Without data in which the scientific community may have confidence, this may remain an answered question.

4.5 Other information

4.5.1 Registration and protocol

In accordance with the guidelines, our systematic review protocol was registered with the International Prospective Register of Systematic Reviews (PROSPERO) on August 24th, 2020, and was last updated on August 24th, 2020 (registration number CRD42020205975). The protocol can be accessed at https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42020205975

No protocol amendments were made.

4.5.2 Support

No financial support for this SRMA was provided.

Chapter 5: Conclusion

5.1 Conclusions regarding goals and hypotheses

5.1.1 Intramuscular stimulation vs sham needling for the treatment of chronic midportion Achilles tendinopathy: a randomised controlled clinical trial (Chapter 2)

The randomised controlled trial demonstrated that Gunn Intramuscular Stimulation (IMS); a form of intramuscular dry needling (IMDN) for chronic midportion Achilles tendinopathy did not deliver additional benefits in outcome to rehabilitative exercise alone. Rehabilitative exercise is the current primary recommendation for conservative management of this condition. There was no additional improvement in the VISA-A questionnaire (Victorian Institute of Sports Assessment–Achilles) scores (a measure of pain and function in Achilles tendinopathy), Global Rating of Change scores (as measured by a Likert scale), dorsiflexion range of motion (with either a bent or straight knee), or anteroposterior diameter of the damaged part of the tendon. This was the case at six weeks, 12 weeks, six months and 12 months.

5.1.2 Quantitative sensory testing of nervous system dysfunction and sensitisation in chronic subacromial shoulder pain (Chapter 3)

The observational quantitative sensory testing (QST) study demonstrated that people with chronic SAPS (rotator cuff tendinopathy with or without subacromial-subdeltoid bursitis) exhibit deep tissue sensitivity to pressure pain, locally, segmentally and extrasegmentally/remotely. They also exhibit “spreading” pain beyond the local region/nociceptive receptive fields of the subacromial area. They do not demonstrate cutaneous sensitivity to heat pain or mechanical

(pinprick) pain locally, segmentally or extrasegmentally/remotely. They also do not demonstrate impaired CPM to pressure or heat stimuli; or facilitated temporal summation to painful mechanical pinprick stimuli locally, segmentally or extrasegmentally/remotely.

5.1.3 Conditioned pain modulation in chronic musculoskeletal pain: a systematic review and meta-analysis (Chapter 4)

The systematic review and meta-analysis demonstrated that people with chronic painful musculoskeletal conditions exhibit impaired CPM.

5.2 Overall analysis and integration in light of current research

5.2.1 Intramuscular stimulation vs sham needling for the treatment of chronic midportion Achilles tendinopathy: a randomised controlled clinical trial (Chapter 2)

The randomised controlled trial that was conducted and presented in Chapter 2 of this thesis did not find that Gunn IMS (a form of IMDN) improved pain or function in midportion Achilles tendinopathy beyond the benefits of rehabilitative exercise.

To explain this apparent conflict with previous literature that has shown that IMDN is helpful in treating pain and function in many musculoskeletal conditions (as discussed in the Introduction), it may be that this trial was underpowered, however, as discussed in the report of this study presented in Chapter 2, this seems unlikely. Another explanation may be that the therapeutic benefits of IMDN are specific to the nature of the musculoskeletal condition being treated, e.g., the tissue type involved, the degree of pain, the mechanism of injury or stage of recovery.

Another explanation for the apparent conflict may be that the therapeutic benefits of IMDN are specific to the nature of the intervention. IMDN's reported ability to reduce nervous system sensitisation may relate to the type of nervous system sensitisation occurring in any particular musculoskeletal condition, or individual. As demonstrated in the QST study of SAPS (presented in Chapter 3), a particular pattern of nervous system sensitisation is exhibited in the SAPS patient population, but it may be that other chronic musculoskeletal conditions demonstrate different patterns of nervous system sensitisation. Therefore, the specific mechanisms of IMDN may be helpful in treating some types of nervous system sensitisation but not others; this would explain why IMDN is helpful in treating some chronically painful musculoskeletal conditions and not others. With respect to midportion Achilles tendinopathy in particular, it is not known with the same degree of comprehensiveness as has been demonstrated by the SAPS QST study presented in Chapter 3 of this thesis, what pattern of nervous system sensitisation is present in this condition. One report has suggested that the predominant type of nervous system sensitisation in midportion Achilles tendinopathy may be central, however, the extent of QST in this study was limited to local CPM testing only – the test stimulus was applied to the painful part of the tendon.(269) If the underlying patterns of nervous system sensitisation were known for the conditions for which IMDN has been found to be helpful, this may provide insight into any relationship between patterns of nervous system sensitisation and the usefulness of IMDN. Should such relationships exist, the use of IMDN in clinical practice might be able to be applied in a more targeted way.

Further research relating these two concepts, i.e., which conditions are helped by IMDN and what is the underlying pattern of nervous system sensitisation for these conditions, would be helpful for clinicians to better discern which painful musculoskeletal problems would be expected to benefit from IMDN. In addition, further research elucidating in a more comprehensive way any pattern of nervous system sensitisation present in Achilles tendinopathy may be helpful to: (1) understand if such a pattern of sensitisation in Achilles tendinopathy is similar or different to the pattern of sensitisation demonstrated in SAPS, or indeed other tendon pain problems (pending further research in these areas as well), and (2) inform better treatment interventions.

5.2.2 Quantitative sensory testing of nervous system dysfunction and sensitisation in chronic subacromial shoulder pain (Chapter 3)

The QST study of SAPS in this thesis demonstrated a pattern of generalised deep tissue pressure sensitivity in this patient population. This could be interpreted as either peripheral or central nervous system sensitisation. The current state of understanding of nervous system sensitisation, however, would lean towards interpreting this pattern as one of predominantly central nervous system sensitisation, as further discussed. Firstly, pressure pain sensitivity is currently generally interpreted as such.(4) Secondly, even if deep tissue pressure sensitivity was instead predominantly a form of peripheral nervous system sensitisation, the pattern of deep tissue sensitivity that presents in SAPS in this QST study, is not localised to the tissues of the rotator cuff, i.e., it is not localised to the tissues supplied by the suprascapular nerve, and presumably, therefore, also not localised to the nociceptors directly innervating the affected rotator cuff tendons (that were not possible to test directly), based on the findings of generalised deep tissue

pressure sensitivity in this population. Tissues supplied by the axillary nerve (represented here by the deltoid muscle) also show sensitivity to deep pressure, as do tissues supplied by the common peroneal nerve (represented here by the tibialis anterior muscle). This suggests a sensitivity problem that is either system-wide in the peripheral tissues, perhaps as the result of some biochemical or peripheral neurophysiological mechanism, or otherwise a sensitivity problem that exists throughout the nervous system, thereby suggesting a problem of central nervous system sensitisation rather than peripheral.

This study also revealed a pattern of spreading pain in this patient population. This observation is also suggestive of central nervous system sensitisation. It is interesting to note that the pattern of spreading pain followed anatomical patterns of deep tissues (muscular and/or myofascial and/or myotendinous), as this suggests some commonality with the results of the QST testing discussed above that demonstrated pressure sensitivity of deep tissues rather than sensitivity of cutaneous tissues.

It is also interesting that this patient population did not demonstrate impaired CPM. This is particularly interesting considering the results of the SRMA that is presented in Chapter 4 of this thesis, which found an overall effect that CPM was impaired in chronic musculoskeletal pain conditions. Of particular note, it was found in the SAPS QST study that participants in both the SAPS and the healthy control groups demonstrated CPM that was impaired, facilitated, or neither/unmodulated (neither impaired nor facilitated); rather than all impaired, all facilitated or all unmodulated. This suggests that interpretation of CPM testing may be better interpreted as a categorical variable, i.e., impaired, facilitated, or unmodulated; rather than as a continuous

variable, whereby a mean value does not perhaps adequately represent the reality of the variability of CPM function in any population, healthy or otherwise, and may, therefore, be misleading.

In considering this apparent conflict in results, it may be that impaired CPM is a feature of certain types of chronically painful musculoskeletal conditions, but not others - similar to the discussion regarding the apparent conflict in the results of IMDN not being helpful for treating midportion Achilles tendinopathy but being helpful for treating other painful musculoskeletal conditions. Further research is needed to determine if some type of specificity also applies to this scenario, i.e., whether impaired CPM is present in certain types of chronically painful musculoskeletal conditions but not others, e.g., by further and more rigorous testing of CPM effects in subgroups of musculoskeletal populations, noting that there is currently limited good quality evidence in the existing literature, as revealed by the SRMA that is presented in Chapter 4 in this thesis. Of particular relevance to this discussion, subgroup analysis by patient population was not possible in the Chapter 4 SRMA due to the limited number of studies eligible for selection and meta-analysis.

5.2.3 Conditioned pain modulation in chronic musculoskeletal pain: a systematic review and meta-analysis (Chapter 4)

The results of this SRMA were there was an overall large effect size of CPM being impaired in chronic musculoskeletal pain conditions compared to healthy controls. Interestingly, the effect size was similar to that found in an SRMA investigating CPM in chronic pain conditions overall.(124)

Evidence of publication bias was demonstrated by analysis of a funnel plot of the studies that were included in this meta-analysis. Specifically, there was a disproportionate number of smaller studies (with relatively larger standard errors) showing a greater magnitude effect in favour of healthy controls (a greater magnitude of an inhibitory CPM effect), and larger studies (with relatively smaller standard errors) showing a disproportionate representation of a lesser magnitude effect size in favour of controls, and a trend towards the no difference line. These limitations are discussed in detail in chapter 4, but is it is important to consider these issues with respect to the overall analysis and integration of this research into the wider literature.

Another issue with this research is mentioned above in the discussion of the SAPS QST study, i.e., whether or not a mean CPM effect value accurately represents the endogenous pain modulation function in any group of participants, given that what is of interest is whether an individual's pain modulation system is inhibiting pain, facilitating pain or neither by this mechanism.

It is acknowledged in the literature that CPM is not always inhibitory, even in healthy populations.(270) That is, the pain experience is not always modulated in an inhibitory way under CPM experimental conditions, but can be modulated in a faciliatory way, i.e., an increased pain rating or equivalent indicator is observed in the presence of a conditioning stimulus.(270) It has been suggested, however, that inhibitory CPM is observed in approximately 80% of healthy people,(271)and that an inhibitory CPM effect of about 30% is an "average" response in healthy people.(272) Regardless, the results from the QST study in this thesis suggests that facilitatory or

unmodulated CPM may be more common in healthy populations than has been suggested in the existing literature. This may, again, represent publication bias in this body of evidence, but there is no report in the literature of a systematic investigation of this.

It is noted in the reports that were selected for this SRMA that individual point data was only presented for one study, and this data was presented graphically,(256) which made the details difficult to discern. The remainder of studies only reported mean or median scores, so it was not possible to discern the contribution to these scores of individual participants regarding whether they had inhibitory, facilitatory or unmodulated CPM effects. For the one study that did present their individual point CPM data graphically,(256) it appears from the graph that two of the 41 healthy controls had a facilitatory response to CPM testing (negative graphed gradient) and two had no change in pain (no gradient/flat line), rather than an inhibitory response (positive gradient).

5.3 Significance and contribution

5.3.1 Intramuscular stimulation vs sham needling for the treatment of chronic midportion Achilles tendinopathy: a randomised controlled clinical trial (Chapter 2)

The randomised controlled trial in this thesis investigating whether or not there are additional therapeutic benefits from adding IMDN to rehabilitative exercise for midportion Achilles tendinopathy suggests that this is not the case. The significance of this is: (1) that while other musculoskeletal conditions *do* seem to benefit from IMDN, midportion Achilles tendinopathy does not, and (2) that this suggests a specificity of action for the therapeutic benefits of IMDN,

and so it should not be assumed that IMDN is helpful in the treatment of all musculoskeletal conditions simply because it is helpful in some. The contribution of this finding is that it suggests that there is a specificity of action for IMDN that is important to consider in clinical practice.

This contribution is particularly meaningful to clinicians as it adds nuance to the broader findings of researchers that IMDN reduces nervous system sensitisation.(66,68,78). It does this by demonstrating that IMDN may not beneficially reduce all forms of nervous system sensitisation, as it did not add benefit to outcomes in midportion Achilles tendinopathy beyond that of rehabilitative exercise, the pain of which is considered to result at least in part from nervous system sensitisation(269). This is significant as it suggests that the ability of IMDN to reduce nervous system sensitisation is specific to the type occurring in the condition or individual being treated. It is also significant as it suggests that even if IMDN *does* reduce nervous system sensitisation in mid-portion Achilles tendinopathy, it does not do so in a way that is additive to the benefits already gained from rehabilitative exercise.

5.3.2 Quantitative sensory testing of nervous system dysfunction and sensitisation in chronic subacromial shoulder pain (Chapter 3)

The results of the SAPS QST undertaken as part of this thesis are significant in that: (1) they provide a clear picture of the pattern of nervous system sensitisation present in SAPS, considering both cutaneous and deep tissues; local, segmental, and extra segmental/remote nervous system relationships; multiple sensory nociceptive modalities (pressure, mechanical (pinprick), heat); static and dynamic tests; as well as the extent of “spreading” pain, and (2) this picture was obtained in the context of SAPS being defined by a contemporary understanding of

the condition, with participants having been thoroughly screened to ensure their symptoms were attributable to this condition. These results provide insight into the nature of pain in SAPS - a common chronic musculoskeletal condition that can be difficult to treat and of long duration, and therefore of significance to treating clinicians and to people who suffer from this condition. The contribution of these results is that they are expected to inform better intervention strategies.

5.3.3 Conditioned pain modulation in chronic musculoskeletal pain: a systematic review and meta-analysis (Chapter 4)

The contributions of this SRMA investigating whether or not CPM is impaired in chronic painful musculoskeletal conditions are that: (1) it provides a measure of the degree and magnitude to which this occurs, based on the current literature, and (2) it provides a critical appraisal of the contributing literature, which found that study quality is only "medium" (versus "high" or "low"); there are widespread deficiencies regarding control of confounders, blinding and risk of bias generally; as well as a general lack of adherence to the current expert recommendations for CPM testing protocols.(208) Although the meta-analysis produced an effect size direction that indicates there is CPM impairment in chronic musculoskeletal pain conditions, and also an estimate of the magnitude of this effect, evidence of publication bias casts doubt, at least on the magnitude of the effect, and possibly also the direction. The significance of this research is twofold: (1) it demonstrates that the overall impression created by the current literature that CPM is impaired in chronic musculoskeletal conditions is supported by meta-analysis, however, (2) the current body of evidence that creates this impression is at considerable risk of bias, lacks quality, and shows evidence of being subject to publication bias that overestimates the magnitude of impairment.

5.4 Strengths and limitations

5.4.1 Intramuscular stimulation vs sham needling for the treatment of chronic midportion Achilles tendinopathy: a randomised controlled clinical trial (Chapter 2)

This trial may have been underpowered, however, as discussed previously, this is unlikely to be the case. Also, use of the VISA-A questionnaire may not have given an adequately sensitive measure of participants' improvements as many of the participants were not engaged in sporting activities, and 40 of the 100 points on the VISA-A questionnaire are dedicated to participation in sporting activities. This means that improvements may not have been gauged sufficiently incrementally that real differences between groups could be revealed. A similar situation existed with the Global Rating of Change score - many participants commented that they would have liked an option of "moderately improved" as this more accurately reflected their situation, whereas they were forced to choose between "minimally improved" and "much improved". Again, this may have meant that improvements may not have been gauged sufficiently incrementally that real differences between groups could be revealed.

5.4.2 Quantitative sensory testing of nervous system dysfunction and sensitisation in chronic subacromial shoulder pain (Chapter 3)

The SAPS QST study would have benefited from a larger sample size, both from the perspective of the large standard deviations associated with the measures taken and also from the perspective of the statistical consequences of multiple testing. This would have allowed for more power to detect statistically significant differences between groups, had they existed. Despite this, it is

arguable that the feasibility of testing a sample size that was adequately large to cater to both these issues is highly questionable in the context of the resources available in the time and setting of this thesis, and many other research settings. It is also arguable that, despite these limitations, a clear pattern of nervous system sensitisation was nevertheless revealed, such that the practical significance of the results generated were meaningful.

5.4.3 Conditioned pain modulation in chronic musculoskeletal pain: a systematic review and meta-analysis (Chapter 4)

The main limitations of the SRMA were the relatively poor quality and the risk of bias of the body of evidence, as well as the evidence of publication bias. This meant that the conclusions drawn from the meta-analysis could only be made with hesitation. The review did, however, provide an assessment of the body of evidence at large, which allowed for recommendations to be made regarding improving the quality of future research on this topic.

5.5 Potential applications

5.5.1 Intramuscular stimulation vs sham needling for the treatment of chronic midportion Achilles tendinopathy: a randomised controlled clinical trial (Chapter 2)

The results of the randomised controlled trial looking at the effect of IMDN in midportion Achilles tendinopathy can be applied to clinical decision-making processes regarding the overall management of this condition. In particular, clinicians might consider that the evidence does not suggest that the use of IMDN will improve patient outcomes beyond a good rehabilitative programme with respect specifically to pain and function in midportion Achilles tendinopathy.

This suggestion is not meant to imply that patients may not benefit concurrently from other purported therapeutic benefits of IMDN.

5.5.2 Quantitative sensory testing of nervous system dysfunction and sensitisation in chronic subacromial shoulder pain (Chapter 3)

The QST study of SAPS contributes to a better understanding of the mechanisms of pain chronicity in this persistent and difficult-to-treat shoulder condition. For practitioners providing pain interventions generally, understanding the mechanism of pain production is essential to providing effective interventions. This study provides evidence of a particular pattern of nervous system sensitisation in SAPS, i.e., of generalised deep tissue sensitivity to pressure and of spreading pain. The applicability of this is: (1) it provides clinicians with useful information to integrate into their clinical decision-making framework when treating SAPS, and (2) it should stimulate further efforts to research and develop intervention strategies that directly address this particular aspect of the pain problem in SAPS.

5.5.3 Conditioned pain modulation in chronic musculoskeletal pain: a systematic review and meta-analysis (Chapter 4)

This SRMA draws attention to the lack of quality evidence in this body of research. It also draws attention to evidence of publication bias in this topic. It provides specific suggestions regarding how this quality could be improved, while also being able to hesitantly acknowledge some low-level evidence that CPM may be impaired in chronic musculoskeletal pain syndromes generally. The applicability of these findings for clinicians is to consider the presence of impaired CPM in their overall management strategies of chronically painful musculoskeletal conditions, and for

researchers is to consider aspects of design that improve the quality and reduce the risk of bias in future research on this topic.

5.6 Possible future research directions

5.6.1 Intramuscular stimulation vs sham needling for the treatment of chronic midportion Achilles tendinopathy: a randomised controlled clinical trial (Chapter 2)

The finding that there is no difference in outcome in midportion Achilles tendinopathy when IMDN is added to rehabilitative exercise draws attention to the possibility that, although certain chronic musculoskeletal pain syndromes may benefit from IMDN, this may not be the case for all such syndromes, as if often extrapolated in clinical practice. This should prompt further research to ascertain for which conditions the addition of IMDN to standard management is useful, and in what ways, and for which conditions it is not.

5.6.2 Quantitative sensory testing of nervous system dysfunction and sensitisation in chronic subacromial shoulder pain (Chapter 3)

Further research, following on from the findings of the SAPS QST study in this thesis, might be to investigate pressure pain sensitivity in SAPS in areas of the body other than the C5/C6 and L4/L5 segments that were tested in this study. It would be interesting to measure the extent of pressure pain sensitivity at other segmental levels across the neuroaxis.

Further research might also be stimulated that uses a similar framework of QST to this study to investigate nervous system function in other conditions. This may reveal patterns of nervous

system sensitivity in other tendinopathies or chronic musculoskeletal pain syndromes that are equally informative and useful.

5.6.3 Conditioned pain modulation in chronic musculoskeletal pain: a systematic review and meta-analysis (Chapter 4)

The findings of this SRMA may prompt further investigations of CPM in chronic musculoskeletal conditions that are of a higher quality and that adhere more closely to the recommendations of the expert consensus panel previously mentioned(208) regarding CPM testing protocols. Whether CPM is an important feature of chronic musculoskeletal pain syndromes is an important question to answer convincingly, again, because a better understanding of pain mechanisms guides more effective pain management strategies.

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Appendices

Appendix A Potentially relevant studies for which information could not be obtained

Potentially relevant studies – studies for which there was inadequate information in the report to decide whether it met the inclusion criteria. The corresponding author was emailed twice within two weeks, but there was no response within three weeks.

1. Valencia C, Vallandingham R, Demchak T. Effect of high and low frequency TENS on central pain processing in patients with knee osteoarthritis and healthy controls. In: Journal of Pain Conference: 34th Annual Scientific Meeting of the American Pain Society. Palm Springs, CA United State: Churchill Livingstone Inc. 2015; 2015. p. var.pagings.
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and behavioral measures. In: Journal of Pain 2017; Conference. United States: Churchill Livingstone Inc.; 2017. p. 18 (4 Suppl. 1) (p. S30).

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Appendix B Diagnostic criteria for SAPS

1. Superolateral shoulder pain of ≥ 12 weeks' duration, and
2. History findings, whereby there are aspects of the history sufficient to cause moderate to strong clinical suspicion of SAPS considering: pain location (superolateral shoulder with or without pain that radiates into the lateral shoulder and arm; and aggravating activities (pain with overhead activity, night pain with inability to sleep on the affected side, weakness during abduction and/or external rotation, pain with combing hair, holding a hair dryer, removing a wallet from a back pocket or similar) or biomechanically similar, and
3. (a) Combined physical examination findings, or (b) Combined history and physical examination findings, as described below
    a. Combined physical examination findings, whereby the following elements together were seen by the examiner together to elevate the probability of SAPS to strong or very strong: active and passive movement assessment (standard, all planes; in particular, screening for capsular pattern of global restriction indicative of adhesive capsulitis); cervical spine screening (all planes, plus overpressure); orthopaedic special tests (Neer, Hawkins-Kennedy, Painful Arc, Resisted External Rotation, Drop Arm test and Empty Can - where for all tests a positive test = the test reproduces the pain complaint; and differential diagnoses screening as appropriate (e.g., acromioclavicular joint, biceps tendon, superior labrum), or
    b. Combined history and physical examination findings
        i. Both positive of Neer and Hawkins-Kennedy tests;
        ii. Two of three positive of Hawkins-Kennedy, Painful Arc and Resisted External Rotation tests;

iii. Two of three positive of Painful Arc, Resisted External Rotation and Drop Arm tests;

iv. Three or more positive of Neer, Hawkins-Kennedy, Painful Arc, Resisted External Rotation and Empty Can tests;

v. All three positive of age ≥ 39 years, Painful Arc test and history of "popping" or "clicking"; or

vi. All three positive of age ≥ 65 years, night pain and Resisted External Rotation test (positive = weakness).

The reported diagnostic value of these combinations are

i. Both positive of Neer and Hawkins-Kennedy tests (see Table B.1 below); for rotator cuff tears (PTTs or FTTs) (reference standard – arthroscopy), where

    1. Neer: positive test = pain, and

    2. Hawkins-Kennedy: positive test = pain.(273)

Combination	SN (%)	SP (%)	PV+ (%)	PV- (%)	Study source
Both positive	83	56	43	56	MacDonald et al. 2000(273) (QUADAS 8/14(274))

Table B.1: Reported diagnostic combinations for both positive of Neer and Hawkins-Kennedy tests. Note: CIs not reported. SN – sensitivity, SP – specificity, PV+ - positive predictive value, PV- - negative predictive value.

ii. Two of three positive of Hawkins-Kennedy, Painful Arc and Resisted External Rotation tests (see Table B.2 below); for "subacromial impingement", defined as bursitis, "tendinitis" or tears (FTTs or PTTs) (reference standard – arthroscopy), where

1. Hawkins-Kennedy: positive test = pain,
2. Painful Arc: positive test = pain, and
3. Resisted External Rotation: positive test = giving way due to weakness or pain.(275)

Combination	SN	SP	LR+	LR-	Study source
2 of 3 positive	-	-	5.03	-	Park et al. 2005(275) (QUADAS 10/14(274))

Table B.2: Reported diagnostic combinations for two of three positive of Hawkins-Kennedy, Painful Arc and Resisted External Rotation tests. Note: Confidence intervals not reported. SN – sensitivity, SP – specificity, LR+ – positive likelihood ratio, LR- – negative likelihood ratio, - – not reported.

iii. Two of three positive of Painful Arc, Resisted External Rotation and Drop Arm tests (see Table B.3 below); for FTTs (reference standard – arthroscopy), where

1. Painful Arc test: positive test = pain,

2. Resisted External Rotation test: positive test = giving way due to weakness or pain, and
3. Drop Arm test: positive test = sudden drop or severe pain.(275)

Combination	SN	SP	LR+	LR-	Study source
2 of 3 positive	-	-	3.57	-	Park et al. 2005(275) (QUADAS 10/14(274))

Table B.3: Reported diagnostic combinations for two of three positive of Painful Arc, Resisted External Rotation and Drop Arm tests. Note: Confidence intervals not reported. SN – sensitivity, SP – specificity, LR+ – positive likelihood ratio, LR- – negative likelihood ratio, - - not reported.

iv. Three or more positive of Neer, Hawkins-Kennedy, Painful Arc, Resisted External Rotation and Empty Can tests (see Table B.4 below); for bursitis or "degeneration" of (superficial) supraspinatus tendon (reference standard – arthroscopy), where
    1. Neer: positive test = pain,
    2. Hawkins-Kennedy: positive test = pain,
    3. Painful Arc test: positive test = pain,
    4. Resisted External Rotation test: positive test = weakness (compared to unaffected shoulder), and

5. Empty Can: positive test = weakness (compared to unaffected shoulder).(276)

Combination	SN % (95% CI)	SP % (95% CI)	LR+ (95% CI)	LR- (95% CI)	Study source
3 or more positive	75 (54-96)	74 (61-88)	2.93 (1.60-5.36)	.34 (.14-.80)	Michener et al. (2009)(276) (QUADAS-2 – low risk of bias(277))

Table B.4: Reported diagnostic combinations for three or more positive of Neer, Hawkins-Kennedy, Painful Arc, Resisted External Rotation and Empty Can tests. SN – sensitivity, SP – specificity, CI – confidence interval, LR+ – positive likelihood ratio, LR- – negative likelihood ratio, - - not reported.

v. All three positive of age ≥ 39 years, Painful Arc test and history of "popping" or "clicking" (see Table B.5 below); for supraspinatus tendinopathy (including FTTs and PTTs), or bursitis (reference standard – ultrasound), where
    1. Age ≥ 39 years,
    2. Painful Arc test: positive test = pain, and
    3. History of "popping" or "clicking".(278)

Combination	SN % (95% CI)	SP % (95% CI)	LR+ (95% CI)	LR- (95% CI)	Study source
All 3 positive	38 (27-51)	99 (88-100)	32.2 (2.01-514.6)	0.63 (0.52-0.76)	Chew et al. (2010)(278) (QUADAS 2 – low risk of bias(277))

Table B.5: Reported diagnostic combinations for all three positive of age ≥ 39 years, Painful Arc test and history of "popping" or "clicking". SN – sensitivity, SP – specificity, CI – confidence interval, LR+ – positive likelihood ratio, LR- – negative likelihood ratio.

vi. All three positive of age ≥ 65 years, night pain and Resisted External Rotation test (positive = weakness) (see Table B.6 below); for rotator cuff tears (PTTs or FTTs) (reference standard - double contrast arthrography)(279)

1. Age ≥ 65 years,
2. Presence of night pain, and
3. Resisted External Rotation test: positive test = weak.(279)

Combination	SN (%)	SP (%)	PV+ (%)	LR+	LR-	Study source
All 3 positive	49*	95	93	9.84*	0.54*	Litaker et al. (2000)(279) (QUADAS > 10/14(280))

Table B.6: Reported diagnostic combinations for all three positive of age ≥ 65 years, night pain and Resisted External Rotation test (positive = weakness). Note: Confidence intervals not reported. *Values reported in Hegedus et al. (2012)(98), not original paper. SN – sensitivity, SP – specificity, PV+ - positive predictive value, LR+ – positive likelihood ratio, LR- – negative likelihood ratio.

Those who satisfied the above criteria were excluded if pain did not equal 5/10 or greater NPRS on at least one of the included orthopaedic special tests; or if there were findings on X-ray of glenohumeral joint or acromioclavicular joint arthritis.

Those who satisfied the above criteria were included if there were findings on ultrasound of tendinosis, calcific tendinopathy, PTTs (including rim rent tears), or FTTs of the supraspinatus, infraspinatus or teres minor tendons. Ultrasound findings were gathered from ultrasound reports filed by radiologists at the relevant referral site where the ultrasound imaging was performed.

Appendix C MEDLINE search strategy

1 diffuse noxious inhibitory control/

2 conditioned pain modulation.mp.

3 diffuse noxious inhibitory control*.mp.

4 DNIC.mp.

5 (heterotopic adj5 (conditioning or counter-stimulation or counterstimulation)).mp.

6 (endogenous pain adj5 (inhibit* or control* or modulat*)).mp.

7 (endogenous adj5 (analg* or descending analg*)).mp.

8 (descending pain adj5 (system* or pathway* or mechanism* or activit* or circuit* or modulat* or inhibit*)).mp.

9 (descending adj5 (modulat* pain or inhibit* pain or analg* system* or analg* pathway* or analg* mechanism* or analg* activit* or analg* circuit*)).mp.

10 (deficient adj5 pain modulat*).mp.

11 (inhibitory pain mechanism* or (inhibitory mechanism* adj5 pain)).mp.

12 (counter-irrita* or counter-stimul*).mp.

13 cold pressor.mp.

14 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 [all DNIC terms]

15 low back pain/ or chest pain/ or chronic pain/ or flank pain/ or headache/ or metatarsalgia/ or musculoskeletal pain/ or myalgia/ or pelvic girdle pain/ or neck pain/ or piriformis muscle syndrome/ or nociceptive pain/ or pain, referred/ or pelvic pain/ or Tension-Type Headache/

16 ((pain* adj5 (TMJ or temporomandibular or neck or chest or rib* or shoulder or scapula* or rotator cuff or arm or elbow or forearm or wrist or thumb or carpometacarpal* or metacarpal* or hand or finger or back or facet joint* or zygapophyseal or zygapophysial or zygapophyseal or

apophyseal or Z-joints or lumbosacral or pelvic or pelvis or sacroiliac or glute* or hip or piriformis or iliotibial or ITB or groin or adductor or leg or knee or patellofemoral or menisc* or shin or ankle or heel or plantar fasc* or tarsometatarsal* or metatarsal* or foot or feet or toe or referred or referral or bone* or joint* or muscle* or muscular or musculoskeletal or vertebral column or spinal or paravertebral or tendon* or soft tissue)) or headache).mp.

17 musculoskeletal diseases/ or cartilage diseases/ or chondromalacia patellae/ or fasciitis/ or fasciitis, plantar/ or foot deformities, acquired/ or bunion/ or hallux limitus/ or hallux rigidus/ or hallux valgus/ or hallux varus/ or metatarsal valgus/ or metatarsus varus/ or heel spur/ or posterior tibial tendon dysfunction/ or hand deformities, acquired/ or temporomandibular joint disorders/ or spondylitis, ankylosing/ or arthralgia/ or shoulder pain/ or exp osteoarthritis/ or sacroiliitis/ or spondylarthritis/ or osteoarthritis, spine/ or bursitis/ or femoracetabular impingement/ or patellofemoral pain syndrome/ or shoulder impingement syndrome/ or temporomandibular joint dysfunction syndrome/ or medial tibial stress syndrome/ or musculoskeletal pain/ or myalgia/ or myofascial pain syndromes/ or tendinopathy/ or elbow tendinopathy/ or tennis elbow/ or enthesopathy/ or de quervain disease/

18 (arthropath* or arthralg* or arthritis or osteoarthritis or spondylarthritis or chondropath* or enthesopath* or fasciitis or musculoskelet* pain* or tendinopath* or tendonopath* or tend?nitis or bursitis or bursal or capsulitis or femoracetabular impingement).mp.

19 iliotibial band syndrome/ or tibial meniscus injuries/ or whiplash injuries/ or rotator cuff injuries/

20 (syndrome* adj5 (temporomandibular or tmj or "rotator cuff" or impingement or piriformis or patellofemoral or iliotibial or "carpal tunnel" or "medial tibial stress" or de*quervain* or ITB or "tarsal tunnel" or "myofascial pain")).mp.

21 (tennis elbow or golfer* elbow or frozen shoulder or chondromalacia patella* or shin splint* or osteoarthritis or whiplash or heel spur* or bunion or heel spur).mp.

22 15 or 16 or 17 or 18 or 19 or 20 or 21

23 14 and 22

Appendix D Risk of bias assessment and critical appraisal - methods

D.1 Adaptations to the AXIS tool

The AXIS tool items are arranged to align with the standard sections of a study report, i.e., introduction, methods, results, discussion and "other". The AXIS item related to the introduction section considers clarity of the stated study objectives. The AXIS items related to the methods section considers study design appropriateness, justification of sample size, definition of the target population, appropriateness of the sampling process, representativeness of the selection process (including consideration of non-response), appropriateness and "correctness" of measurements, and reproducibility of methods. The AXIS items related to the results section considers the adequacy and completeness of data description, non-response bias, and internal consistency. The AXIS items related to the discussion section considers the appropriateness of the discussion and the consideration of limitations. The AXIS items related to the "other" section considers funding sources/conflicts of interest and ethics/consent.

Designation of items of key and secondary importance in the AXIS tool

For the purposes of this SRMA, the AXIS tool was adapted to designate certain items as having key importance (six items, as described below) or secondary importance (two items, as described below) to the specific subject matter. Additional questions were assigned to these items to appraise these important items thoroughly. More weight was also assigned to these items in the overall quality rating, as described below.

The key and secondary items were used to assign firm definitions of “high”, “medium”, or “low” quality, with the remainder of the items being used to potentially move studies to a lower level of critical appraisal, i.e., appraisals of “Yes” on other items could not move appraisal of a study “up” a level, but enough appraisals of “No” could move them down. By this appraisal protocol:

1. “High” quality was assigned to studies that were appraised as “Yes” on all six key items *and* both secondary items
2. “Medium” quality was assigned to studies that were appraised as “Yes” on three or more key items with or without “Yes” on either or both of the secondary items
3. “Low” quality was assigned to studies that were appraised as “Yes” on two or less key items with or without “Yes” on either or both of the secondary items

The items that were designated as having key importance were items 2, 3, 5, 6, 8 and 9. The original AXIS items were:

- Item 2. Was the study design appropriate for the stated aim(s)?
- Item 3. Was the sample size justified?
- Item 5. Was the sample frame taken from an appropriate population base so that it closely represented the target/reference population under investigation?
- Item 6. Was the selection process likely to select subjects/participants that were representative of the target/reference population under investigation?
- Item 8. Were the risk factor and outcome variables measured appropriate to the aims of the study?
- Item 9. Were the risk factor and outcome variables measured correctly using instruments/measurements that had been trialled, piloted or published previously?

The items that were designated as having secondary importance were items 10 and 11. The original AXIS items were:

- Item 10. Is it clear what was used to determine statistical significance and/or precision estimates?
- Item 11. Were the methods (including statistical methods) sufficiently described to enable them to be repeated?

Specific adaptations to the AXIS tool for this SRMA

The modifications made to these items specifically for the purposes of this SRMA are described below.

For Item 2 (Was the study design appropriate for the stated aim(s)?). This item was used specifically to incorporate the consideration of design regarding confounders/comparability of cases and controls and blinding. Specifically, the following four questions were considered:

1. Was age(281,282) ensured to be similar across groups by:
   i. Confirming lack of statistically significant difference between groups after enrolment through collection and analysis of baseline demographics
   ii. Stratifying enrolment to ensure equal distribution between groups, or
   iii. Adjusting for statistically if found to be different between groups
2. Was sex(283) ensured to be similar across groups? (assessed as in i, ii, and iii as above)
3. Was the potential confounding effect of menstrual cycle phase controlled for by testing:

i. All relevant participants outside their menstrual phase of the menstrual cycle (this is "preferred", as there is evidence that the CPM effect is reduced during the menstrual phase(283–285)), or
ii. All relevant participants during the menstrual phase of the menstrual cycle

4. Was the tester blinded to case versus control status(286)?

The answers to 1, 2, 3 and 4 above needed all to be "Yes" to be rated as an overall "Yes" for AXIS item 2.

The following suggested confounders were not considered in the critical appraisal as there is only ambiguous or preliminary evidence of a confounding effect:

i. Oral contraceptive use, as the evidence is "ambiguous"(283)
ii. Expectation, as there is evidence that both distraction and CPM reduce pain, but through separate mechanisms(271)
iii. Genes (high 5- HTT- expressing genotypes), as only one study(287) of medium quality provides preliminary evidence of a confounding effect, although this study does provide "moderate evidence for larger CPM effects in people with high 5- HTT- expressing genotypes"(283)
iv. Physical activity level, as only one study(288) of medium quality provides preliminary evidence of a confounding effect, whereby higher levels of physical activity seemed to correlate with more CPM(283)

Of note, there is evidence that there is no confounding effect for the following characteristics:

i. Ethnic background(283)

ii. Psychosocial factors (such as mood, self-efficacy, and reactivity),(281) and

iii. Demographic factors(270)

For Item 5 (Was the sample frame taken from an appropriate population base so that it closely represented the target/reference population under investigation?). This item was used to incorporate the consideration of sampling bias. Specifically, the following questions were considered:

1. Is the sampling frame (the list or source of the study population used to recruit participants into the study) "exactly the same" composition/structure as the target population, i.e., is it representative of the target population?
2. Was a convenience sample used (this typically results in non-representative or biased sample that can't be used to make assumptions about the characteristics of the target population)?(234)

If the answers to 1 and 2 above, together suggested that the group of enrolled participants were largely representative of the target population, the study was given an overall rating of overall "Yes" for AXIS item 5.

For Item 6 (Was the selection process likely to select subjects/participants that were representative of the target/reference population under investigation?). This item was used to incorporate the consideration of selection bias. Specifically, the following questions were considered:

1. Were widely accepted diagnostic criteria for the musculoskeletal condition under investigation used?

2. Were characteristics that could confound re the musculoskeletal condition under investigation excluded)?

If the answers to 1 and 2 above, together suggested that the group of enrolled participants were largely representative of the target population, the study was given an overall rating of "Yes" for AXIS item 6. Study design considerations regarding inclusion and exclusion criteria, in particular, were considered when assessing this item.

For Item 8 (Were the risk factor and outcome variables measured appropriate to the aims of the study?). This item was used to incorporate the consideration of measurement validity. Specifically, the following questions were considered regarding whether the CPM testing methodology used was appropriate for measuring CPM efficiency(286):

1. Was the test stimulus painful at a minimum of pain40 (pain level of 40/100 NPRS)?
2. Was the conditioning stimulus painful at a minimum of pain20 (pain level of 20/100 NPRS)?
3. Was the test stimulus applied and measured before and "after" (parallel or sequential) at least one minute of exposure to the conditioning stimulus?

The answers to all three of 1, 2 and 3 above, needed to be "Yes" to write an overall "Yes" for AXIS item 8.

For Item 9 (Were the risk factor and outcome variables measured correctly using instruments/measurements that had been trialled, piloted or published previously?). This item was used specifically to incorporate the consideration of measurement reliability. Specifically, the following questions were considered:

1. Could the test stimulus be reproduced and produce identical results if measured repeatedly, so that the measurements would be exactly the same if performed by another researcher?
2. Could the conditioning stimulus be reproduced and produce identical results if measured repeatedly, so that the measurements would be exactly the same if performed by another researcher?
3. Could the overall CPM paradigm be reproduced and produce identical results if measured repeatedly, so that the measurements would be exactly the same if produced by another researcher?

The answers to all three of 1, 2 and 3 above, needed to be "Yes" to rate an overall "Yes" for AXIS item 9.

D.2 AXIS tool: appraisal of cross-sectional studies

	Question	**Yes**	**No**	**Don't know/ Comment**
Introduction				
1	Were the aims/objectives of the study clear?			
Methods				
2	Was the study design appropriate for the stated aim(s)?			
3	Was the sample size justified?			
4	Was the target/reference population clearly defined? (Is it clear who theresearch was about?)			

5	Was the sample frame taken from an appropriate population base so that it closely represented the target/reference population under investigation?			
6	Was the selection process likely to select subjects/participants that were representative of the target/reference population under investigation?			
7	Were measures undertaken to address and categorise non-responders?			
8	Were the risk factor and outcome variables measured appropriate to the aims of the study?			
9	Were the risk factor and outcome variables measured correctly using instruments/measurements that had been trialled, piloted or published previously?			
10	Is it clear what was used to determined statistical significance and/or precision estimates? (e.g., p-values, confidence intervals)			
11	Were the methods (including statistical methods) sufficiently described to enable them to be repeated?			
Results				
12	Were the basic data adequately described?			

13	Does the response rate raise concerns about non-response bias?			
14	If appropriate, was information about non-responders described?			
15	Were the results internally consistent?			
16	Were the results presented for all the analyses described in the methods?			
Discussion				
17	Were the authors' discussions and conclusions justified by the results?			
18	Were the limitations of the study discussed?			
Other				
19	Were there any funding sources or conflicts of interest that may affect the authors' interpretation of the results?			
20	Was ethical approval or consent of participants attained?			

Appendix E Risk of bias assessment and critical appraisal – results

E.1 Detailed summary of the assessments for each of the AXIS items, and the overall quality rating (high, medium or low)

	Introduction	Methods				
AXIS items	1	2	3	4	5	6
Study	Were the aims/objectives of the study clear?	Was the study design appropriate for the stated aim(s)?	Was the sample size justified?	Was the target/reference population clearly defined? (Is it clear who the research was about?)	Was the sample frame taken from an appropriate population base so that it closely represented the target/reference population under investigation?	Was the selection process likely to select subjects/participants that were representative of the target/reference population under investigation?

Arendt-Nielsen 2010(246)	Y	N	N	Y	N	Y
Cathcart 2010(247)	Y	N	N	Y	Y	Y
Christensen 2020(248)	Y	N	Y	Y	Y	Y
Daenen 2013(129)	Y	N	Y	Y	Y	Y
Edwards 2016(249)	Y	N	N	Y	Y	Y
Graven-Nielsen 2012(240)	Y	N	N	Y	Y	Y
Heredia-Rizo 2019(250)	Y	N	Y	Y	Y	Y
Kashima 1999(251)	Y	N	N	Y	Y	Y

King 2009(127)	Y	N	N	Y	Y	Y
Kosek 2000(252)	Y	N	N	Y	Y	Y
Kothari 2016(253)	Y	N	N	Y	Y	Y
Kothari 2015(244)	Y	N	N	Y	Y	Y
Leffler 2002(241)	Y	N	N	Y	Y	Y
Mkumbuzi 2021(254)	Y	N	N	Y	Y	Y
Moana-Filho 2019(255)	Y	N	Y	Y	Y	Y
Oono 2014(242)	Y	N	N	Y	Y	Y

Plinsinga 2020(256)	Y	N	Y	Y	Y	Y
Poluha 2020(257)	Y	N	Y	Y	Y	Y
Sandrini 2006(258)	Y	N	N	Y	DK	Y
Serrano-Munoz 2019(259)	Y	N	N	Y	Y	DK
Tompra 2016(7)	Y	N	N	Y	Y	Y
Coppieters 2018(128)	Y	N	N	Y	Y	Y
Smith 2020(260)	Y	N	Y	Y	Y	Y

Table E.1.1a

	Methods continued…				Results	
AXIS items	7	8	9	10	11	12
Study	Were measures undertaken to address and categorise non-responders?	Were the risk factor and outcome variables measured appropriate to the aims of the study?	Were the risk factor and outcome variables measured correctly using instruments/measurements that had been trialled, piloted or published previously?	Is it clear what was used to determined statistical significance and/or precision estimates? (e.g., p-values, CIs)	Were the methods (including statistical methods) sufficiently described to enable them to be repeated?	Were the basic data adequately described?

Arendt-Nielsen 2010(246)	N	N	Y	Y	Y	Y
Cathcart 2010(247)	N	N	Y	Y	Y	N
Christensen 2020(248)	N	N	Y	Y	Y	Y
Daenen 2013(129)	N	N	Y	N	Y	Y
Edwards 2016(249)	N	N	Y	N	Y	Y
Graven-Nielsen 2012(240)	N	N	Y	Y	Y	Y
Heredia-Rizo 2019(250)	N	N	Y	Y	Y	Y
Kashima 1999(251)	N	N	Y	Y	Y	Y

King 2009(127)	N	N	Y	N	Y	Y
Kosek 2000(252)	N	N	Y	N	Y	Y
Kothari 2016(253)	N	N	Y	Y	Y	Y
Kothari 2015(244)	N	N	Y	Y	Y	Y
Leffler 2002(241)	N	N	Y	Y	Y	Y
Mkumbuzi 2021(254)	N	N	Y	Y	Y	Y
Moana-Filho 2019(255)	N	N	Y	Y	Y	Y
Oono 2014(242)	N	N	Y	Y	Y	Y
Plinsinga 2020(256)	N	N	N	Y	Y	Y

Poluha 2020(257)	N	N	Y	Y	Y	Y
Sandrini 2006(258)	N	N	N	Y	N	Y
Serrano-Munoz 2019(259)	N	N	Y	N	Y	Y
Tompra 2016(7)	N	N	Y	N	Y	Y
Coppieters 2018(128)	N	N	Y	Y	Y	Y
Smith 2020(260)	N	N	Y	Y	Y	N

Table E.1.1b

	Results				Discussion	
AXIS items	13	14	15	16	17	18

Study	Does the response rate raise concerns about non-response bias?	If appropriate, was information about non-responders described?	Were the results internally consistent?	Were the results presented for all the analyses described in the methods?	Were the authors' discussions and conclusions justified by the results?	Were the limitations of the study discussed?
Arendt-Nielsen 2010(246)	DK	N	Y	Y	Y	N
Cathcart 2010(247)	DK	N	Y	Y	Y	Y
Christensen 2020(248)	DK	N	Y	N	Y	Y
Daenen 2013(129)	DK	N	Y	Y	Y	Y
Edwards 2016(249)	DK	N	Y	Y	Y	Y

Graven-Nielsen 2012(240)	DK	N	Y	Y	Y	Y
Heredia-Rizo 2019(250)	DK	N	Y	Y	N	Y
Kashima 1999(251)	DK	N	Y	Y	Y	N
King 2009(127)	DK	N	Y	Y	Y	Y
Kosek 2000(252)	DK	N	Y	Y	Y	N
Kothari 2016(253)	DK	N	Y	Y	Y	N
Kothari 2015(244)	DK	N	Y	Y	Y	N
Leffler 2002(241)	DK	N	Y	Y	Y	N

Mkumbuzi 2021(254)	DK	N	Y	Y	Y	Y
Moana-Filho 2019(255)	DK	N	Y	Y	Y	Y
Oono 2014(242)	DK	N	Y	Y	Y	Y
Plinsinga 2020(256)	DK	N	Y	Y	Y	Y
Poluha 2020(257)	DK	N	Y	Y	Y	Y
Sandrini 2006(258)	DK	N	Y	Y	Y	N
Serrano-Munoz 2019(259)	DK	N	Y	Y	Y	N
Tompra 2016(7)	DK	N	Y	Y	Y	N
Coppieters 2018(128)	DK	N	N	Y	Y	Y

Smith 2020(260)	DK	N	Y	Y	Y	Y

Table E.1.1c

	Other		Overall quality rating		
AXIS items	19	20	High	Medium	Low
Study	Were there any funding sources or conflicts of interest that may affect the authors' interpretation of the results?	Was ethical approval or consent of participants attained?	High quality = appraised as "Yes" on all six key items AND both secondary items	Medium quality = appraised as "Yes" on three or more key items with or without "Yes" on either or both secondary items	Low quality = appraised as "Yes" on two or less key items with or without "Yes" on either or both secondary items

Arendt-Nielsen 2010(246)	N	Y			✓
Cathcart 2010(247)	N	Y		✓	
Christensen 2020(248)	N	Y		✓	
Daenen 2013(129)	N	Y		✓	
Edwards 2016(249)	N	Y		✓	
Graven-Nielsen 2012(240)	DK	Y		✓	
Heredia-Rizo 2019(250)	N	Y		✓	
Kashima 1999(251)	DK	N		✓	

King 2009(127)	N	Y		✓	
Kosek 2000(252)	DK	N		✓	
Kothari 2016(253)	N	Y		✓	
Kothari 2015(244)	N	Y		✓	
Leffler 2002(241)	DK	Y		✓	
Mkumbuzi 2021(254)	N	Y		✓	
Moana-Filho 2019(255)	N	Y		✓	
Oono 2014(242)	N	Y		✓	
Plinsinga 2020(256)	N	Y		✓	

Poluha 2020(257)	N	Y		✓	
Sandrini 2006(258)	DK	Y			✓
Serrano-Munoz 2019(259)	N	Y			✓
Tompra 2016(7)	N	Y		✓	
Coppieters 2018(128)	N	Y		✓	
Smith 2020(260)	N	Y		✓	

Table E.1.1d.

Tables E.1.1a, b, c and d: Results of risk of bias assessment and critical appraisal/quality assessment using a modified version of the AXIS tool – 20 items and overall assessment of high, medium or low quality. N – no, Y – yes, DK – don't know (information not reported)

E.2 Detailed summary of each of the risk of bias assessments, and the overall rating (high, medium or low)

Generally, the risk of bias assessment demonstrated remarkable similarity across all 23 included studies. The risk of confounding was assessed in AXIS item 2 and looked for some form of study design consideration around the known confounders of CPM effects - age, sex and menstrual cycle phase, as well as whether there was blinding of assessors. All 23 studies were assessed as having a high risk of bias due to confounding. All but one study(258) did not blind assessors. All but one study - the same study(258) - did not control for menstrual cycle phase. About half the studies (n = 12) did not control for age and about one third (n = 8) did not control for sex. One study(249) did not report details that allowed determination of whether age or sex were controlled in the study design. This information is summarised in Table E.2.1 below.

Study	1. Age	2. Sex	3. Menstrual cycle phase	4. Blinding	Overall	Risk of bias due to confounding
Arendt-Nielsen 2010(246)	Y	Y	N	N	N	H
Cathcart 2010(247)	N	N	N	N	N	H
Christensen 2020(248)	Y	Y	N	N	N	H

Coppieters 2018(128)	N	Y	N	N	N	H
Daenen 2013(129)	Y	Y	N	N	N	H
Edwards 2016(249)	DK	DK	N	N	N	H
Graven-Nielsen 2012(240)	Y	Y	N	N	N	H
Heredia-Rizo 2019(250)	N	Y	N	N	N	H
Kashima 1999(251)	N	Y	N	N	N	H
King 2009(127)	N	Y	N	N	N	H
Kosek 2000(252)	Y	Y	N	N	N	H
Kothari 2015(244)	Y	Y	N	N	N	H
Kothari 2016(253)	Y	Y	N	N	N	H

Leffler 2002(241)	Y	Y	N	N	N	H
Mkumbuzi 2021(254)	N	N	N	N	N	H
Moana-Filho 2019(255)	Y	Y	N	N	N	H
Oono 2014(242)	Y	Y	N	N	N	H
Plinsinga 2020(256)	N	N	N	N	N	H
Poluha 2020(257)	N	N	N	N	N	H
Sandrini 2006(258)	N	N	Y	Y	N	H
Serrano-Munoz 2019(259)	N	N	N	N	N	H
Smith 2020(260)	N	N	N	N	N	H
Tompra 2016(7)	N	N	N	N	N	H

Table E.2.1: Results of risk of bias assessment related to confounding using a modified version of the AXIS tool (item 2). N – no, Y – yes, DK – don't know (information not reported), H – high. The assessment of 1, 2, 3 and 4 above needed all to be "Yes" to be rated as an overall "Yes" for AXIS item 2.

Regarding the risk of selection bias, again there was remarkable similarity across all 23 studies. All were assessed as having some concerns regarding selection bias. All studies but two were assessed as having taken a sample frame from an appropriate population base so that it closely represented the target population under investigation. One study was assessed as not having done this(243); and one other study(258) did not report enough information to make an assessment in this regard. The selection process of all studies but one(259) was assessed as being likely to have selected participants who were representative of the target population under investigation. The one study(259) that was not, did not report enough information to make an assessment. It was assessed that none of the 23 studies had taken measures to address and categorise non-responders. This resulted in all studies being assigned "don't know" regarding whether the response rate raised concerns about non-response bias as there was no information provided regarding this potential source of bias. For details, see Table E.2.2 below.

Study	Sample frame representative	Participants representative	Non - response measured	Non-response bias concerns	Risk of selection bias
Arendt-Nielsen 2010(246)	N	Y	N	DK	SC
Cathcart 2010(247)	Y	Y	N	DK	SC
Christensen 2020(248)	Y	Y	N	DK	SC
Coppieters 2018(128)	Y	Y	N	DK	SC
Daenen 2013(129)	Y	Y	N	DK	SC
Edwards 2016(249)	Y	Y	N	DK	SC
Graven-Nielsen 2012(240)	Y	Y	N	DK	SC
Heredia-Rizo 2019(250)	Y	Y	N	DK	SC

Kashima 1999(251)	Y	Y	N	DK	SC
King 2009(127)	Y	Y	N	DK	SC
Kosek 2000(252)	Y	Y	N	DK	SC
Kothari 2015(244)	Y	Y	N	DK	SC
Kothari 2016(253)	Y	Y	N	DK	SC
Leffler 2002(241)	Y	Y	N	DK	SC
Mkumbuzi 2021(254)	Y	Y	N	DK	SC
Moana-Filho 2019(255)	Y	Y	N	DK	SC
Oono 2014(242)	Y	Y	N	DK	SC
Plinsinga 2020(256)	Y	Y	N	DK	SC

Poluha 2020(257)	Y	Y	N	DK	SC
Sandrini 2006(258)	DK	Y	N	DK	SC
Serrano-Munoz 2019(259)	Y	DK	N	DK	SC
Smith 2020(260)	Y	Y	N	DK	SC
Tompra 2016(7)	Y	Y	N	DK	SC

Table E.2.2: Results of risk of bias assessment related to selection bias using a modified version of the AXIS tool (items 5, 6, 7 and 13). N – no, Y – yes, DK – don't know (information not reported), SC – some concerns

AXIS items 5 and 6 were considered of key importance in the assessment of the included studies with consideration for the subject matter and, as such, additional specific questions were asked of each of the included studies relating to these two items. Details regarding the outcome of these assessments is provided in Table E.2.3 below. For AXIS item 5, two specific questions were asked: (1) Is the sampling frame "exactly the same" composition as the target population, i.e., is it representative of the target population?; and (2) Was a convenience sample used (as this typically results in non-representative or biased samples that can't be used to make assumptions about the characteristics of the target population)?(289) If the answers to questions (1) and (2)

together suggested that the sample frame was largely representative of the target population, the study was given an overall rating of "yes" on AXIS item 5 (Please note that when the answer to question (2) was "yes", this was not a favourable assessment). For AXIS item 6, two specific questions were asked: (1) Were widely accepted diagnostic criteria for the musculoskeletal condition under investigation used?; and (2) Were characteristics that could confound regarding the musculoskeletal condition under investigation excluded?. If the answers to questions (1) and (2) together suggested that the group of enrolled participants were largely representative of the target population, the study was given an overall rating of "yes" on AXIS item 6. Details regarding the assessments of these items for each of the included studies is provided in Table E.2.3 below.

Study	Sample frame representative	Convenience sample used*	Overall low risk of sampling bias	Accepted diagnostic criteria used	MSK-confounding factors excluded	Overall low risk of selection bias
Arendt-Nielsen 2010(246)	DK	DK	N	Y	N	Y
Cathcart 2010(247)	Y	Y	Y	Y	Y	Y
Christensen 2020(248)	Y	DK	Y	Y	Y	Y

Coppieters 2018(128)	Y	Y	Y	Y	Y	Y
Daenen 2013(129)	Y	DK	Y	Y	Y	Y
Edwards 2016(249)	Y	Y	Y	Y	Y	Y
Graven-Nielsen 2012(240)	Y	Y	Y	Y	Y	Y
Heredia-Rizo 2019(250)	Y	Y	Y	Y	Y	Y
Kashima 1999(251)	Y	Y	Y	Y	Y	Y
King 2009(127)	Y	Y	Y	Y	Y	Y
Kosek 2000(252)	Y	Y	Y	Y	Y	Y
Kothari 2015(244)	Y	Y	Y	Y	N	Y
Kothari 2016(253)	Y	Y	Y	Y	Y	Y

Leffler 2002(241)	Y	Y	Y	Y	Y	Y
Mkumbuzi 2021(254)	Y	Y	Y	Y	Y	Y
Moana-Filho 2019(255)	Y	Y	Y	Y	Y	Y
Oono 2014(242)	Y	Y	Y	Y	Y	Y
Plinsinga 2020(256)	Y	Y	Y	Y	Y	Y
Poluha 2020(257)	Y	Y	Y	Y	Y	Y
Sandrini 2006(258)	DK	DK	DK	Y	N	DK
Serrano-Munoz 2019(259)	Y	Y	Y	Y	Y	Y
Smith 2020(260)	Y	Y	Y	Y	Y	Y
Tompra 2016(7)	Y	Y	Y	Y	Y	Y

Table E.2.3: Results of risk of bias assessment related to selection bias detailed by the modified versions of items 5 and 6 of the AXIS tool. *Please note that an assessment of "Yes" for this question is not favourable regarding risk of bias. MSK – musculoskeletal, N – no, Y – yes, DK – don't know (information not reported), H – high

Regarding the risk of information bias, again there was remarkable similarity across all 23 studies. All but two were assessed as having some concerns regarding information bias. The remaining two(256,258) were assessed as having high risk of information bias. Regarding the component AXIS items that contributed to the overall risk of information bias assessment:

1. For AXIS item 8, none of the studies were assessed as having measured the outcome variables in a way that was appropriate to the aims of the study
2. For AXIS item 9, all but two(256,258) were found to have used measurement methods that had been trialled, piloted or previously published
3. For AXIS item 11, all but one(258) were found to have used methods that were described enough to enable them to be repeated
4. For AXIS item 15, all but one(128) were found to have presented results that were internally consistent
5. For AXIS item 16, all but one(248) were found to have presented all the analyses described in the methods

For details, see Table E.2.4 below.

Study	Measurements valid	Measurements reliable	Statical methods reliable	Results internally consistent	All results presented	Risk of information bias
Arendt-Nielsen 2010(246)	N	Y	Y	Y	Y	SC
Cathcart 2010(247)	N	Y	Y	Y	Y	SC
Christensen 2020(248)	N	Y	Y	Y	N	SC
Coppieters 2018(128)	N	Y	Y	N	Y	SC
Daenen 2013(129)	N	Y	Y	Y	Y	SC
Edwards 2016(249)	N	Y	Y	Y	Y	SC
Graven-Nielsen 2012(240)	N	Y	Y	Y	Y	SC
Heredia-Rizo 2019(250)	N	Y	Y	Y	Y	SC

Kashima 1999(251)	N	Y	Y	Y	Y	SC
King 2009(127)	N	Y	Y	Y	Y	SC
Kosek 2000(252)	N	Y	Y	Y	Y	SC
Kothari 2016(253)	N	Y	Y	Y	Y	SC
Kothari 2015(244)	N	Y	Y	Y	Y	SC
Leffler 2002(241)	N	Y	Y	Y	Y	SC
Mkumbuzi 2021(254)	N	Y	Y	Y	Y	SC
Moana-Filho 2019(255)	N	Y	Y	Y	Y	SC
Oono 2014(242)	N	Y	Y	Y	Y	H
Plinsinga 2020(256)	N	N	Y	Y	Y	SC

Poluha 2020(257)	N	Y	Y	Y	Y	H
Sandrini 2006(258)	N	N	N	Y	Y	SC
Serrano-Munoz 2019(259)	N	Y	Y	Y	Y	SC
Smith 2020(260)	N	Y	Y	Y	Y	SC
Tompra 2016(7)	N	Y	Y	Y	Y	SC

Table E.2.4: Results of risk of bias assessment related to information bias detailed by the modified versions of the AXIS tool (items 8 and 9) and items 11, 15 and 16. N – no, Y – yes, SC – some concerns, H - high

AXIS items 8 and 9 were considered of key importance in the assessment of the included studies with consideration for the subject matter and, as such, additional specific questions were asked of each of the included studies relating to these two items.

For AXIS item 8, three specific questions were asked to ascertain if the CPM testing methodology used was appropriate for measuring CPM efficiency as per the most recent expert recommendations(286). These questions were: (1) Was the test stimulus painful at a minimum of pain40 (i.e., a pain level of ≥ 40/100 NPRS)?; (2) Was the conditioning stimulus painful at a

minimum of pain20 (i.e., a pain level of ≥ 20/100 NPRS)?; and (3) Was the test stimulus applied and measured before and "after" (i.e., parallel, i.e., during, or sequential method) at least one minute of exposure to the conditioning stimulus? If the answers to all three questions were "Yes", the study was given an overall rating of "Yes" on AXIS item 8. Of note, all but one(127) study received a "No" assessment for question (1) regarding the test stimulus magnitude. Only about ¼ of the studies (*n* = 6) (242,244,253,255,258,260) received a "Yes" assessment for question (3) regarding the CPM paradigm used. Details regarding the outcome of these assessments are provided in Table E.2.5 below.

Study	TeS ≥ 40/100 NPRS	CS ≥ 20/100 NPRS	CPM before and "after" ≥ 1 min. CS exposure	Overall
Arendt-Nielsen 2010(246)	N	Y	N	N
Cathcart 2010(247)	N	Y	N	N
Christensen 2020(248)	N	DK	N	N
Coppieters 2018(128)	N	N	N	N
Daenen 2013(129)	N	Y	N	N

Edwards 2016(249)	N	N	N	N
Graven-Nielsen 2012(240)	N	Y	N	N
Heredia-Rizo 2019(250)	N	Y	N	N
Kashima 1999(251)	N	Y	N	N
King 2009(127)	Y	Y	N	N
Kosek 2000(252)	N	Y	N	N
Kothari 2016(253)	N	DK	Y	N
Kothari 2015(244)	N	DK	Y	N
Leffler 2002(241)	N	Y	N	N
Mkumbuzi 2021(254)	N	Y	N	N
Moana-Filho 2019(255)	N	Y	Y	N
Oono 2014(242)	N	Y	Y	N

Plinsinga 2020(256)	N	Y	N	N
Poluha 2020(257)	N	Y	N	N
Sandrini 2006(258)	N	N	Y	N
Serrano-Munoz 2019(259)	N	N	N	N
Smith 2020(260)	N	N	Y	N
Tompra 2016(7)	N	Y	N	N

Table E.2.5: Results of risk of bias assessment related to information bias detailed by the modified version of item 8 of the AXIS tool. N – no, Y – yes, DK – don't know (information not reported), TeS – test stimulus, min. – minute, CS – conditioning stimulus

For AXIS item 9, three specific questions were asked to ascertain if the CPM testing methodology used was adequately described to assess whether there may have been systematic error in the measurements. These questions were: Could the (1) test stimulus; (2) conditioning stimulus; and (3) overall CPM paradigm be reproduced and produce identical results if measured repeatedly, so that the measurements would be exactly the same if performed by another researcher? If the answers to all three questions were "Yes", the study was given an overall rating of "Yes" on AXIS item 9. Of note:

1. All but one study(258) received a “Yes” assessment for question (1) regarding the test stimulus description
2. All 23 studies received a “Yes” assessment for question (2) regarding the conditioning stimulus description
3. All but two studies(256,258) received a “Yes” assessment for question (3) regarding the CPM paradigm description

Even though these issues at face value relate more to reliability than validity, this assessment was made in this context primarily to inform AXIS item 8 which assesses the validity of the CPM-related measures. Without adequate descriptions, as queried in AXIS item 9, the validity of the measurements could not be determined, and so in the context of this SRMA, a thorough (reproducible) description of the methods was seen to be necessary to assess whether there may have been any systematic error(290) present in CPM assessment. Of note, generally this was well done across all 23 studies, which lends confidence in the assessments made in AXIS item 8. Details regarding the outcome of the AXIS item 9 assessments are provided in Table E.2.6 below.

Study	TeS reproducible	CS reproducible	CPM paradigm reproducible	Overall
Arendt-Nielsen 2010(246)	Y	Y	Y	Y
Cathcart 2010(247)	Y	Y	Y	Y
Christensen 2020(248)	Y	Y	Y	Y
Coppieters 2018(128)	Y	Y	Y	Y
Daenen 2013(129)	Y	Y	Y	Y
Edwards 2016(249)	Y	Y	Y	Y
Graven-Nielsen 2012(240)	Y	Y	Y	Y
Heredia-Rizo 2019(250)	Y	Y	Y	Y
Kashima 1999(251)	Y	Y	Y	Y
King 2009(127)	Y	Y	Y	Y

Kosek 2000(252)	Y	Y	Y	Y
Kothari 2016(253)	Y	Y	Y	Y
Kothari 2015(244)	Y	Y	Y	Y
Leffler 2002(241)	Y	Y	Y	Y
Mkumbuzi 2021(254)	Y	Y	Y	Y
Moana-Filho 2019(255)	Y	Y	Y	Y
Oono 2014(242)	Y	Y	Y	Y
Plinsinga 2020(256)	Y	Y	N	N
Poluha 2020(257)	Y	Y	Y	Y
Sandrini 2006(258)	N	Y	N	N
Serrano-Munoz 2019(259)	Y	Y	Y	Y
Smith 2020(260)	Y	Y	Y	Y

Tompra 2016(7)	Y	Y	Y	Y

Table E.2.6: Results of risk of bias assessment related to information bias detailed by the modified version of item 9 of the AXIS tool. N – no, Y – yes, TeS – test stimulus, CS – conditioning stimulus

Appendix F Sensory Testing Equipment

Figure F.1: Pressure pain threshold testing equipment. Image reproduced with permission from Medoc Advanced Medical Systems.

Figure F.2: Mechanical Pain Threshold testing equipment. Image reproduced with permission from Medoc Advanced Medical Systems.

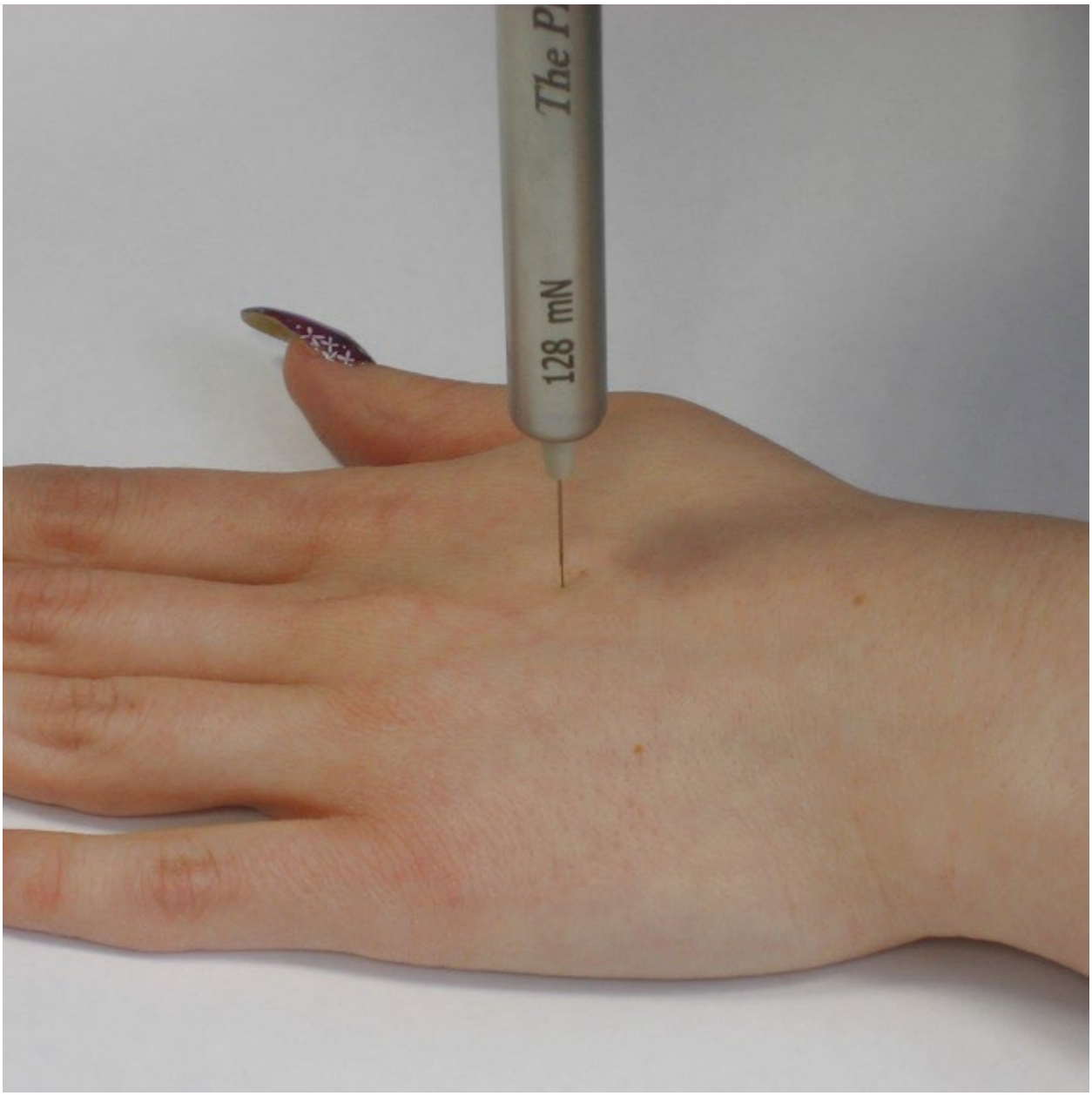

Figure F.3: PinPrick stimulator equipment in use. Image reproduced with permission from Medoc Advanced Medical Systems.

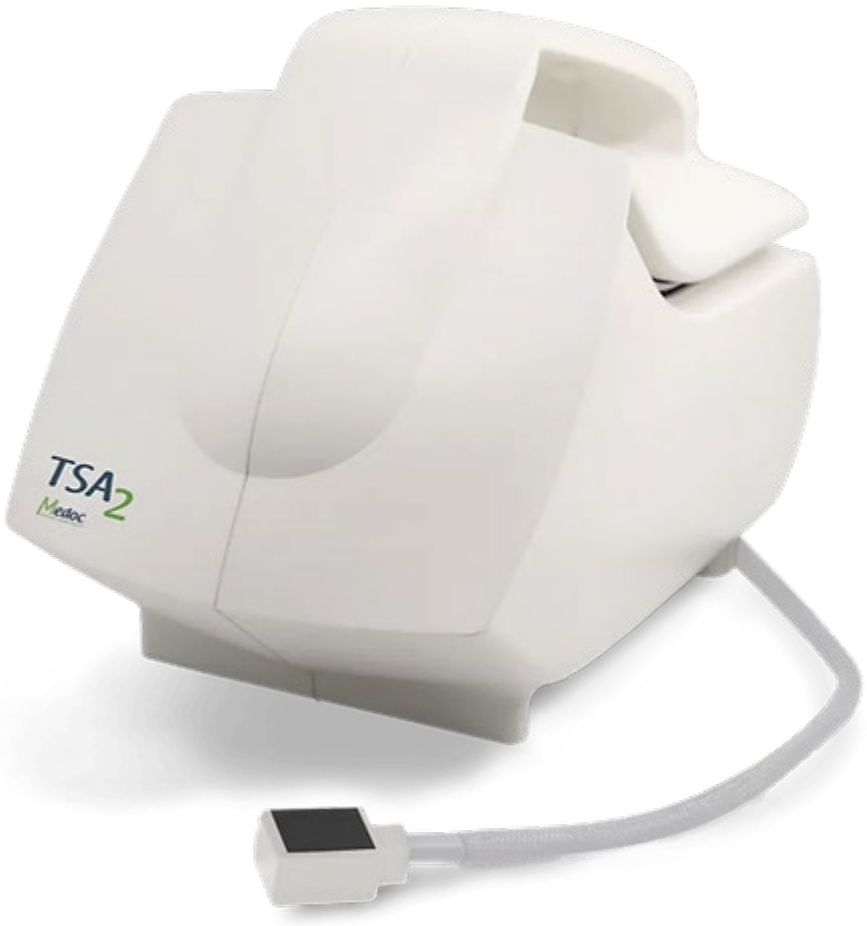

Figure F.4: Heat Pain Threshold testing equipment. Image reproduced with permission from Medoc Advanced Medical Systems.