Associations Between the Nociceptive and Autonomic Nervous Systems in People with Knee Osteoarthritis and Fibromyalgia

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Attestation of Authorship

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person (except where explicitly defined in the acknowledgements); nor material that to a substantial extent has been submitted for the award of any other degree or diploma of a university or other institution of higher learning.

Signed

Neil R Bossenger
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Abstract

Osteoarthritis is one of the most prevalent chronic pain conditions, with a lifetime risk of developing knee osteoarthritis (KOA) estimated to be up to 47%. Structural knee damage bears little relationship to pain; thus, evidence is emerging that alterations in nociceptive function contribute to pain. The nociceptive and autonomic nervous systems closely interact and influence each other to modulate pain. Other chronic pain conditions, including fibromyalgia (FM), exhibit autonomic nervous system (ANS) dysfunction. ANS dysfunction may be implicated in OA-related pain, but this has yet to be examined. Therefore, the aim of this thesis was to examine the function of the ANS in people with KOA.

Three studies were designed to investigate autonomic and nociceptive function in people with KOA, people with FM, and healthy, pain free controls at rest and in response to three types of stressors: (1) nociceptive stress, (2) mental stress, and (3) exercise stress. People with FM were included to demonstrate that the experimental procedures were able to detect ANS dysfunction. Quantitative sensory testing, including mechanical and thermal pain thresholds, was used to assess static and dynamic function of the nociceptive system. Heart rate variability was used to assess the parasympathetic nervous system, while impedance cardiography and electrodermal activity were used to assess the sympathetic nervous system. Assessments were made before, during, and after exposure to the stressors.

Static mechanical and thermal pain thresholds were not different in the KOA and FM groups in any of the studies. However, consistent evidence of resting ANS dysfunction was found in the KOA and FM groups compared to controls. People with KOA demonstrated reduced vagal tone at rest, but additionally showed reduced vagal withdrawal in response to nociceptive stress, acute mental stress, and acute exercise stress. There was some evidence of sympathetic hyperactivity at rest in the KOA and FM groups, as well as some evidence of a ceiling effect of blunted sympathetic reactivity when exposed to acute stress. Cardiac sympathetic reactivity was found to be dampened in people with KOA in response to both nociceptive and exercise stress. The findings of reduced tonic vagal activity, elevated resting sympathetic activity, and reduced autonomic modulation in response to stress raise the potential of a blunted ability to adapt to stress and modulate nociception. In support of this, in response to nociceptive stress, acute mental stress, and acute exercise stress, people with KOA and people with FM demonstrated an impairment of conditioned pain modulation, mental stress-induced hypoalgesia, and exercise-induced hypoalgesia, respectively.

These studies are the first to show evidence of ANS dysfunction in people with KOA at rest, and in response to three types of stressors. The results suggest that people with KOA may be less adept at responding to stress due to diminished autonomic flexibility, and at risk of impaired modulation of nociception when exposed to acute stress. These findings offer potential for future research to investigate ways to normalise ANS function and examine the impact on KOA pain.

Abbreviations

ACC	Anterior cingulate cortex
ACR	American College of Rheumatology
ACTH	Adrenocorticotropin hormone
ANOVA	Analysis of variance
ANS	Autonomic nervous system
BMI	Body mass index
BP	Blood pressure
BPI	Brief pain inventory
bpm	Beats per minute
BRS	Baroreflex sensitivity
CFS	Chronic fatigue syndrome
CNS	Central nervous system
COVAS	Computerised visual analogue scale
CPM	Conditioned pain modulation
CPT	Cold pain threshold
CRP	C-reactive protein
DASS-21	Depression, anxiety, and stress scales
DBP	Diastolic blood pressure
DNIC	Diffuse noxious inhibitory control
ECG	Electrocardiographic
EDA	Electrodermal activity
EIH	Exercise-induced hypoalgesia
EMG	Electromyographic
FM	Fibromyalgia
fMRI	Functional magnetic resonance imaging
HF	High frequency
HP60	Heat pain 60 (0 – 100)
HPT	Heat pain threshold
HPtol	Heat pain tolerance
HR	Heart rate
HRV	Heart rate variability
IBS	Irritable bowel syndrome
ICC	Intraclass correlation coefficient
ICG	Impedance cardiography
IL-6	Interleukin-6
IMP	Impedance pulsatile signal
ISI	Interstimulus interval
KOA	Knee osteoarthritis

LF	Low frequency
MRI	Magnetic resonance imaging
MVC	Maximal voluntary contraction
NFR	Nociceptive flexion reflex
NRS	Numerical rating scale
NTS	Nucleus tractus solitarius
nuHF	Normalised units of high frequency
OA	Osteoarthritis
PASAT	Paced Auditory Serial Addition Task
PBN	Parabrachial nucleus
PCS	Pain catastrophising scale
PEP	Pre-ejection period
PNS	Parasympathetic nervous system
PPT	Pressure pain threshold
PVSAT	Paced Visual Serial Addition Task
QST	Quantitative sensory testing
RA	Rheumatoid arthritis
RMSSD	Root mean square of successive differences between RR intervals
RSA	Respiratory sinus arrhythmia
RVM	Rostroventromedial medulla
SBP	Systolic blood pressure
SCL	Skin conductance level
SCR	Skin conductance response
SDNN	Standard deviation of the NN interval
SNS	Sympathetic nervous system
STD HR	Standard deviation of instantaneous heart rate
THA	Total hip arthroplasty
TKA	Total knee arthroplasty
VLF	Very low frequency
VLM	Ventrolateral medulla
WOMAC	Western Ontario and McMaster Universities Osteoarthritis Index

Chapter 1. Introduction

"To describe pain solely in terms of intensity is like specifying the visual world only in terms of light flux without regard to pattern, colour, texture and many other dimensions of the visual experience" [1] – Melzack and Wall: The authors who introduced the revolutionary gate control theory of pain [2].

1.1. Rationale of the research

In New Zealand, approximately 1 in 6 people aged 15 and over have at least one type of arthritis [3]. A report for Arthritis New Zealand estimates the financial cost of arthritis in 2010 to be $3.2 billion and health sector costs approximately $700 million [3]. Hospital costs represent a third of health sector costs [3]. Osteoarthritis (OA) is the most common form of arthritis, with a risk of developing painful knee osteoarthritis (KOA) estimated to be 47% over the course of a person's life [4]. OA is characterised by joint pain, stiffness, and decreased function [5]. The incidence of OA increases markedly over the age of 50 [4] and is associated with significant morbidity, physical disability, and raised health costs in middle-aged and elderly groups [6]. Apart from ageing, other risk factors for the development of OA include genetic predisposition, abnormal biomechanics, obesity, and previous joint trauma [7]. The end-stage treatment for OA is joint replacement, often due to severe, unremitting joint pain. OA of the hip and knee is reported to be responsible for over 90% of joint replacements in New Zealand [8], dominating public inpatient costs [3]. Data from the NZ Joint Registry show a 52.2% increase in the number of total knee arthroplasty (TKA) surgeries from 2001 to 2011, compared to an 8.2% increase for total hip arthroplasty (THA) [4]. Projections for 2026 predict a 183% increase in TKA [4], which will place an extraordinary burden on the NZ health care system, including hospital beds, rehabilitation, and demand for orthopaedic surgeons. More effective strategies for conservative management of OA-related pain need to be developed in order to delay or prevent TKA [4].

Interestingly, imaging studies indicate that approximately half of people with structural alterations consistent with KOA do not experience pain [9, 10]. Conversely, 10 – 15% of people with severe knee pain have no radiographic evidence of OA [11]. The challenge of finding new treatments in OA partly results from a lack of understanding around the pathogenesis of OA and the pathophysiology of OA-related pain. The progression of OA and manifestation of symptoms do not necessarily correlate, coupled with interindividual variability over time [7]. Evidence is emerging that suggests OA may be partly a systemic phenomenon, with the autonomic nervous system (ANS) hypothesised to play a multifactorial role in the pathogenesis of OA [12, 13]. Chronic pain is usually the reason people elect to undergo TKA [14]. Symptomatic KOA commonly show signs of altered central nociceptive processing, with pain spreading beyond the involved joint, and increased sensitivity to painful stimuli at distant sites in the leg and forearm [15, 16]. Normalisation of this sensitisation following successful TKA implies that central mechanisms of pain processing are altered, and at least partly maintained by, joint pathology [16]. However, approximately 20% of people undergoing TKA for the first time experience chronic post-operative pain [17, 18]. A suggested reason for this is persistent central sensitisation, including dysfunction of descending pain modulation [14].

The interaction between the nociceptive and autonomic nervous systems is a growing field of study. People with chronic pain conditions, such as fibromyalgia (FM) [19], irritable bowel syndrome (IBS) [20], complex regional pain syndrome (CRPS) [21], and rheumatoid arthritis (RA) [22], demonstrate alterations in both nociceptive processing and ANS function. It is hypothesised that ANS dysfunction can lead to changes in nociceptive processing. For example, reduced vagal tone at rest may place a person at risk of chronic pain due to a floor effect of diminished autonomic capacity to adapt to noxious stimuli and/or mental stress [23-25]. This is because the nociceptive and autonomic nervous systems are tightly intertwined through shared anatomy and brain structures in their role of adapting to threat and modulating pain [26-29]. Persistent nociceptive input may also lead to alterations in ANS function [30]. Indeed, people with chronic conditions, such as CRPS [21] and FM [31], exhibit ongoing pain maintained, at least in part, by ANS dysfunction. There have been no studies providing an in-depth examination of ANS function in people with OA. Historically, OA has been considered a peripheral pathology affecting joint nociception. However, evidence is emerging that arthritic pain may be strongly influenced by altered nociceptive function within the central nervous system (CNS) [32]; changes in ANS function [13, 33, 34]; and chronic, low-grade inflammation that is driven by the sympathetic nervous system (SNS) [7, 22, 35]. Collectively, these studies point toward the potential role of ANS function in OA-related pain, although direct evidence is lacking [13, 33].

1.2. Aims and hypotheses

Three studies were undertaken in this research with comparisons made between people with KOA; people with FM; and, healthy, pain free controls. People with FM were chosen because there is evidence that ANS dysfunction is common in this population [19, 20, 31, 36-41] and were expected to show differences to controls, however, the main focus of the studies was the KOA group. The research aimed to answer the following questions:

I. Is there evidence of resting ANS dysfunction and increased pain sensitivity in people with KOA?
II. How does cold water conditioning (nociceptive stressor) affect the nociceptive and autonomic nervous systems in people with KOA?
III. How does mental arithmetic (mental stressor) influence the nociceptive and autonomic nervous systems in people with KOA?
IV. How does an acute isometric muscle contraction (exercise stressor) influence the nociceptive and autonomic nervous systems in people with KOA?

It was hypothesised that people with KOA and FM would exhibit ANS dysfunction and altered pain sensitivity at rest compared to pain free controls (Figure 1A). In response to acute nociceptive, mental, and exercise stressors, people with KOA and FM would demonstrate a floor effect of reduced vagal withdrawal, and a ceiling effect of blunted SNS reactivity (Figure 1C). In addition, it was hypothesised people with KOA and FM would exhibit impairment of conditioned pain modulation (CPM), mental

stress-induced hypoalgesia, and exercise-induced hypoalgesia (EIH); and, increased clinical pain intensity immediately following the stressor(s), and for up to 15 mins after the stressor(s).

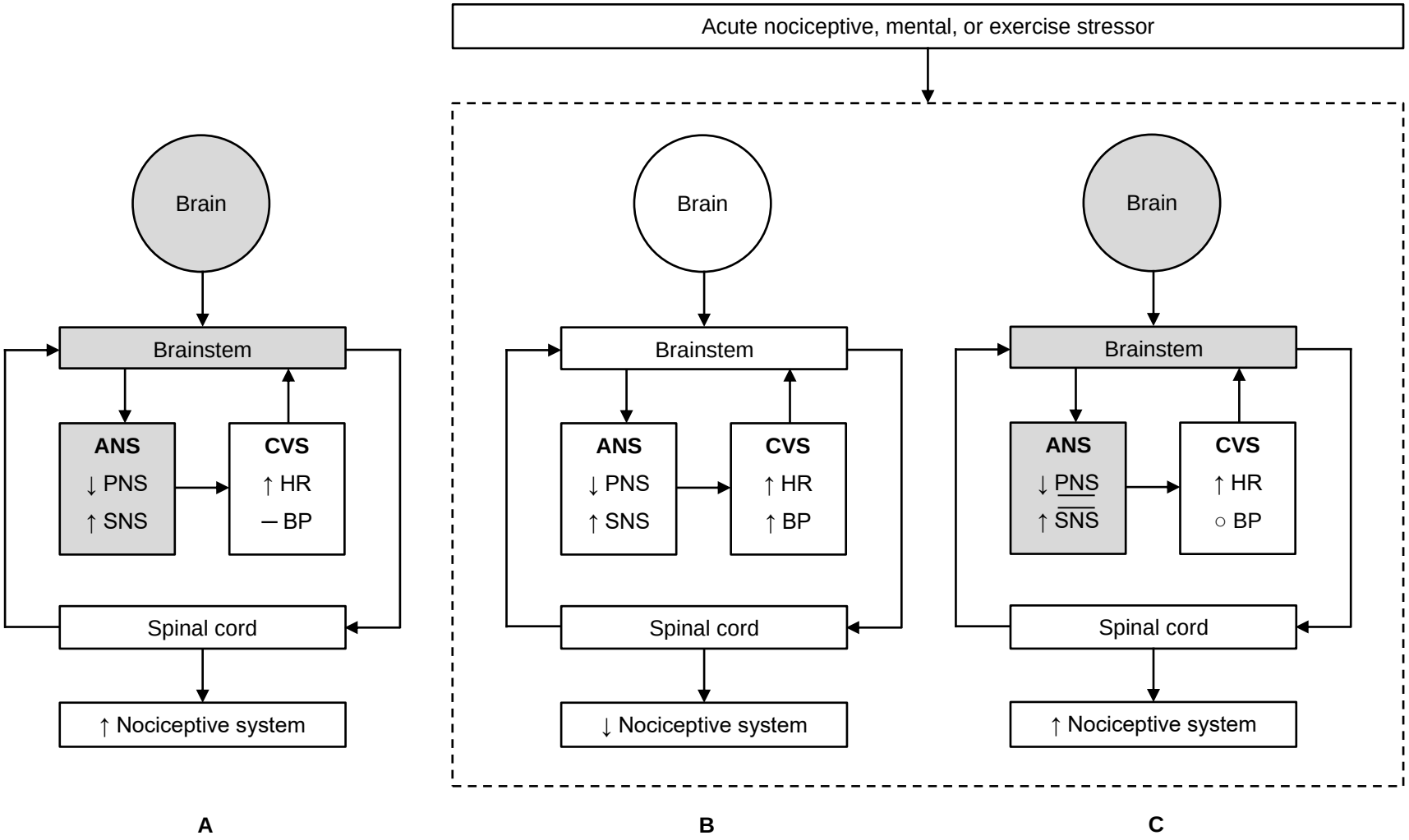
1.3. Structure of the thesis

The first chapter (Introduction) has introduced the challenge of OA-related pain and presented four specific questions the research addresses. The second chapter (Literature review) delves into the literature surrounding the nature of chronic and OA-related pain, what drives it, and how it is modulated. The review covers the relationships between the nociceptive and autonomic nervous systems, and how each is measured. Lastly in the literature review, the impact of both acute mental and exercise stress on these two systems is explored. The third (Study 1), fourth (Study 2), and fifth (Study 3) chapters aim to answer the four proposed questions by investigating the function of the nociceptive and autonomic nervous systems in people with KOA, people with FM, and pain free controls at rest; as well as when exposed to acute nociceptive, mental, and exercise stress. These three chapters and their respective studies present the results, ideas, strengths and limitations to each of the research questions individually. The sixth chapter (Summary) brings the findings of the studies together, including recommendations for future research.

1.4. Significance of the research

The research may have significance for people with OA-related pain; health professionals involved in managing OA and other chronic pain conditions; and, researchers with an interest in the mechanisms of ANS dysfunction and management of OA pain. The three studies will illustrate whether ANS dysfunction is present in KOA and how it may be related to measures of nociceptive processing. If ANS dysfunction exists in OA, then this study will provide a basis for future research to explore whether manipulation of the ANS positively impacts pain. Normalising ANS function in OA may not only improve pain – and the ability to cope with pain – but also support cardiovascular health and resilience to mental, emotional, and/or physical stress.

Figure 1. ***Altered function at rest in people with chronic pain (A); normal response to acute stress in pain free controls (B); and, altered response to acute stress in people with chronic pain (C).***



Note. ANS = autonomic nervous system; BP = blood pressure; CVS = cardiovascular system; grey = area of dysfunction; HR = heart rate; overscore = ceiling effect; PNS = parasympathetic nervous system; SNS = sympathetic nervous system; underscore = floor effect; ○ = blunted response

Chapter 2. Literature review

2.1. Literature search

Chapter 2 provides an extensive review of literature relating to OA and the nociceptive and autonomic nervous systems to provide background and context of the thesis. The review was undertaken using material published up to 2019 in peer-reviewed journals located using standard academic databases. The literature review begins with the epidemiology of OA and its impact on people. The review provides background of normal nociceptive function and pain, and what changes occur in the central and peripheral nervous systems of people with OA that may contribute to ongoing pain. Methods of measuring the nociceptive system using static and dynamic quantitative sensory testing are reviewed relative to chronic pain conditions, including OA, and how they compare to testing in healthy populations. Within each technique, the psychometric properties are discussed. The ANS is then introduced; how it controls the cardiovascular system and how, in turn, this is related to nociception and modulation of pain. Select methods for measuring the ANS relevant to the studies undertaken in this thesis are described in detail together with their psychometric properties. Differences in autonomic function are highlighted between pain free and chronic pain populations. Lastly, the impact of acute mental and exercise stress on both nociception and the ANS are outlined with literature findings relevant to healthy and chronic pain populations, including OA. Within each section, gaps within the existing literature are highlighted to provide rationale for the three experimental studies conducted within this thesis.

2.2. Epidemiology of osteoarthritis

In New Zealand, currently a quarter of people 65 and over have OA [42], and by 2030, this age group will comprise a quarter of the NZ population [4]. OA of the knee is one of the most common forms of arthritis [43] with a lifetime risk of developing symptomatic KOA estimated to be up to 47% [4]. Chronic knee pain, reduced joint motion, and reduced quadriceps strength are associated with KOA, with approximately 60% of people with KOA reporting the sensation of “knee instability, giving way, or buckling” [44]. Together, these factors reduce mobility and impair the ability to perform activities of daily living, leading to ongoing functional disability. KOA carries a great public health burden [3, 8], with the risk of reduced mobility attributable to KOA alone being greater than any other condition in people > 65 years old [9, 45]. Hunter et al. [9] describe OA as a heterogeneous disorder characterised by “progressive cartilage loss, subchondral bone remodelling, osteophyte formation, and synovial inflammation”, which results in joint pain, stiffness, and disability. Primary KOA, as defined by the American College of Rheumatology (ACR), can be classified three ways: knee pain plus clinical examination and laboratory findings; knee pain plus clinical examination and radiographic findings; and, knee pain plus clinical examination only [46]. Clinical diagnosis of KOA according to ACR criteria should include three of the following six findings: “age > 50 years, morning stiffness < 30 mins duration, crepitus on active motion, tenderness of the bony margins of the joint, bony enlargement noted on examination, and a lack of palpable warmth of the synovium” [46]. Pain is the main symptom of OA [47], and severe

OA-related pain of the knee has been associated with buckling [48], falling [49], hyperalgesia around the knee [43], and functional disability [45]. OA pain was once viewed as arising primarily through peripherally mediated joint nociception [50]. However, OA pain is now known to have complex underlying CNS mechanisms that modulate peripheral nociception and strongly contribute to OA symptomatology [51]. Moreover, the experience of pain does not always correlate with the level of joint degeneration [11]. While progressive OA may induce pain and joint dysfunction, approximately 50% of people with clear radiographic evidence of OA are asymptomatic, which is referred to as structure-symptom discordance [9, 11]. Therefore, structural alterations and joint degeneration alone are not able to account for the variation in pain levels [52].

2.3. Osteoarthritis and the nociceptive system

2.3.1. Nociception and pain

The pathophysiology of OA-related pain includes four distinct processes: activation of nociceptors in the affected joint; transmission of nociceptive signals from peripheral to CNS structures; perception of nociceptive signals in the cortex; and, finally, modulation of all such nociceptive signals peripherally in the dorsal horn of the spinal cord, and supraspinal areas, e.g. cortex, thalamus, and limbic structures [47, 53, 54]. There is a distinction between nociception and pain. In the presence of a noxious stimulus, nociception refers to the neural encoding induced by a noxious event, whereas pain is the abstract perception and interpretation of that event and may, in turn, be influenced by a range of different contextual factors, including thoughts and emotions [55-57]. Nociceptive signals are propagated by activation of specialised receptors, called nociceptors, which then transmit this information via the spinal cord to the cortex, where it is processed and interpreted to produce the pain experience [53]. Pain is both a sensory and emotional experience [58]. The sensory-discriminative component of the pain experience includes the localisation, physical quality, and intensity of the noxious stimulus, while the affective-motivational component creates emotional and contextual colouring, helping to shape a behavioural response to pain [55, 59, 60].

2.3.2. Drivers of osteoarthritic pain

There are numerous structural changes in KOA that can contribute to ongoing tissue damage, inflammation and, ultimately, pain [61]. Hunter et al. [9] state that knee pain is most commonly associated with structural changes evident on magnetic resonance imaging (MRI), such as subarticular bone attrition, subchondral bone marrow lesions, synovitis, and effusion. Acute pain may arise when nociceptors are exposed to mechanical stimulation by structural degradation and subsequent synovitis [9, 62]. Even though OA has long been considered a non-inflammatory condition, De Lange-Brokaar et al. [35] point to evidence that inflammation is present in the synovial tissue of people with OA [35]. The authors reviewed 100 articles investigating inflammation in OA synovial tissue and concluded: “inflammation is common in OA and characterised by immune cell infiltration and cytokine secretion” [35]. The composition of OA-related inflammation also seems to be different to that of other arthritic

conditions, such as RA, for example [35]. Systemic inflammation may also play a role in OA [33, 62]. C-reactive protein (CRP), a biomarker of inflammation, was measured in a population sample of 1025 women and found to be highly associated with both painful and radiographic KOA [63]. Lee et al. [64] found serum CRP levels to be elevated in people with OA compared to controls, and the OA group also showed heightened pain sensitivity. The authors suggested that people with high levels of CRP are more at risk of developing OA and that systemic, as well as local joint inflammation, may play a role in its pathogenesis [63, 64].

2.3.3. Peripheral and central sensitisation

Repetitive noxious afferent input from a joint due to tissue damage or inflammation can lead to enhanced excitation of peripheral nociceptors, causing peripheral sensitisation [52, 61, 65, 66]. When nociceptors are exposed to repetitive noxious stimulation their activation thresholds are reduced, giving rise to spontaneous activity [67, 68]. Peripheral sensitisation is also brought about by increased activity of secondary messenger systems releasing inflammatory mediators, such as bradykinin, prostaglandins, mast cells, substance P, and histamine [67, 69]. In turn, increased peripheral noxious input from a joint can lead to central sensitisation, a state of increased excitability in spinal dorsal horn neurons where amplification of afferent input within the CNS generates increased sensitivity to pain [61, 65, 66, 70]. Central sensitisation is further defined by enlarged receptive fields, increased synaptic efficacy of large and small diameter afferent fibres, and impairment of endogenous pain inhibitory and/or facilitatory systems [15, 71-73]. Peripheral and central sensitisation are considered two key underlying mechanisms of OA-related pain [15, 74]. Together, these processes lead to pain sensitisation characterised by increased responsiveness to noxious stimuli (hyperalgesia), painful responses to usually innocuous stimuli (allodynia), and wind-up [71]. Woolf [66] defines wind-up as: “repeated low frequency stimulation of a neuron, eliciting a progressive increase in action potential firing over the course of the stimulus.” Wind-up can lead to increased neuronal discharge within the spinal cord, even if the input remains constant [75-77]. Once initiated, central sensitisation is able to be maintained in the absence of significant ongoing peripheral nociceptive input from an OA-damaged knee joint [15, 66, 74, 78].

2.3.4. Endogenous pain inhibitory pathways

An important mechanism thought to be involved in the initiation and maintenance of central sensitisation in OA and other chronic pain conditions is dysfunction of endogenous pain modulation from brain to spinal cord [73, 79, 80]. Numerous factors, including nociceptive stimulation, can trigger descending pain inhibition, involving noradrenergic, serotonergic, and opioidergic inhibitory pathways [72, 73, 81]. The endogenous opioid system is central to pain modulation, with opioid receptors found throughout the CNS, including the anterior cingulate cortex (ACC), amygdala, hypothalamus, periaqueductal grey (PAG), rostroventromedial medulla (RVM), and spinal cord dorsal horn [82]. The PAG of the brainstem is an area known to be involved in antinociception [83, 84] and there is evidence that people with OA show abnormal activity in this region by way of hyperalgesia to noxious stimulation [78, 85]. Another supraspinal mechanism of pain inhibition is the diffuse noxious inhibitory control (DNIC) system. The

DNIC system is a spinal-medullary-spinal pathway that ascends through the spinoreticular tract and synapses in brainstem, which functions to inhibit pain in distant areas when a new or secondary pain is introduced [79, 86]. DNIC mechanisms were first observed in rats where spinal neurons were inhibited by noxious nociceptive input applied outside of their own inhibitory/excitatory segmental receptive fields [87, 88]. The DNIC system is considered to be mediated by neurons in the subnucleus reticularis of the caudal medulla, which receives nociceptive input and then projects to the spinal cord dorsal horn [79]. DNIC is the term used to describe lower brainstem mediated inhibitory mechanisms in animals, but the human counterpart, measured indirectly, is referred to as conditioned pain modulation (CPM) [80, 88]. A relationship exists between the intensity of the new noxious stimulus and the strength of resulting CPM hypoalgesia, such that with a strong noxious stimulus the inhibitory effects of CPM can last up to several minutes and sometimes even abolish the activity of nociceptive neurons [86, 89-91]. Impaired descending inhibition of nociceptive input reduces the excitation threshold of dorsal horn neurons, increasing discharge, leading to ongoing pain [15, 79, 87]. People with chronic OA-related pain may have impaired CPM, which can contribute to dysfunctional descending inhibition and/or facilitation of nociception, increased pain intensity, and spread pain to distant areas of the body [15, 16, 92, 93].

2.4. Quantitative sensory testing and the nociceptive system

2.4.1. Introduction

Quantitative sensory testing (QST) refers to the standardised psychophysical evaluation of the nociceptive system and pain perception pathways [94-97]. QST is one method for phenotyping the mechanisms driving OA-related pain; e.g. peripheral and central sensitisation, and/or dysfunction of descending pain modulation [97, 98]. Static QST assesses perceptual responses from non-painful to painful sensations using a number of controlled test modalities, including pressure, vibration, electrical, and thermal stimuli [96, 99, 100]. A stimulus is applied to a site on the body, e.g. joint or muscle, as the person is asked to push a button or stop the test when they first perceive changing sensation (perceptual threshold), first experience pain (pain threshold), or when they cannot tolerate any further stimuli (pain tolerance) [97, 99, 101]. Dynamic QST is used to assess pain processing and provides insight into underlying central pain mechanisms. CPM, a dynamic QST measure, is commonly used to assess the function of the DNIC system in humans [79, 80, 88]. Chalaye et al. [91] define CPM as: “an experimental procedure that compares the pain produced by a noxious test stimulus before and after (or sometimes during) application of a second noxious conditioning stimulus applied to a remote area of the body”. Studies have found fair to excellent test-retest reliability for thermal detection thresholds [100, 102], thermal pain thresholds [91, 95, 100, 103, 104], mechanical thresholds [95, 97, 105, 106], and CPM [89, 104, 107-109].

2.4.2. Static quantitative sensory testing

Mechanical pressure, cold, and heat pain is largely determined by both small myelinated A-delta and unmyelinated C fibres [96, 100, 101]. Noxious thermal and pressure sensations are conveyed centrally

via spinothalamic and other ascending nociceptive pathways [96, 101]. Pressure algometry quantifies a person's pressure pain threshold (PPT), the point at which pain is perceived following application of a mechanical pressure stimulus of increasing intensity [110]. Likewise, heat pain threshold (HPT) and cold pain threshold (CPT) produced by thermode, is the change in sensation from warmth to heat pain, and innocuous cold to cold pain respectively [91]. People with chronic pain typically present with somatosensory abnormalities, including pressure and thermal hyperalgesia [111-115]. Previous studies have shown people with KOA, and people with FM, to exhibit reduced PPTs [74, 97, 116, 117], reduced HPTs [51, 114, 115, 118], and increased CPTs [5, 115, 118] compared to controls. Although thermal thresholds are not commonly assessed in OA [51], Moss et al. [5] found significantly increased cold hyperalgesia in people with KOA compared to pain free controls. Reduced thresholds have also been found not only at the affected joint, but at distant sites not impacted by OA, including the lower back [119], trapezius [64], and forearm [112]. The measurement of PPTs in KOA has been shown to be reliable [105, 120]. In sum, people with KOA show increased sensitivity to several different types of painful input, both locally at the affected joint and distant, pain free areas [5, 112]. Local hyperalgesia at the affected joint can be due to peripheral and/or central sensitisation, whereas increased pain sensitivity at sites distant to the area of pathology is suggestive of central sensitisation in KOA [15, 97].

2.4.3. Dynamic quantitative sensory testing

Various conditioning and test stimuli have been used to evaluate CPM, including heat, cold, electrical, and mechanical pressure [109]. Immersion of the hand into cold water, serving as the conditioning stimulus, is a technique shown to have excellent within-session reliability [109]. Impaired CPM has been suggested as an underlying mechanism of chronic pain conditions, especially those with long term pain [72, 73, 79, 87, 114, 121]. In healthy people, conditioning pain usually reduces brief forms of experimental pain [89, 107, 122], whereas in chronic pain populations, including KOA [15, 92], hip OA [92], and FM [72, 114, 121], CPM has been reported to be absent or dysfunctional. Restoration of the CPM response has been demonstrated in people with hip OA following THA [92] and KOA following TKA [16, 123]. These data suggest that continuous nociceptive input over time can drive neuroplastic changes, increase facilitation of pain at a brainstem level, and impair CPM [16, 85, 117]. Decreased inhibitory CPM effectiveness has been well established in people with FM [20, 72, 91, 104, 114, 121], making this population appropriate to draw comparisons with other painful conditions, such as KOA.

2.5. Associations between the nociceptive and autonomic nervous systems

2.5.1. Introduction

The ANS serves as the interface between the internal and external environment to maintain homeostasis and modulate pain [26, 27]. The autonomic and neuroendocrine systems promote adaptation to the environment via a process called allostasis [124, 125]. Allostasis is defined as the ability of the body to physiologically adapt to the external environment [124-126]. Allostatic load is a measure of the cumulative physiological burden placed on allostatic systems, namely: the ANS,

hypothalamic-pituitary-adrenal axis, cardiovascular system, and various other metabolic process [29, 124, 125]. Functional diseases may develop when allostatic systems are either overworked, fail to turn off when no longer needed, or do not respond adequately to the initial threat [124, 127]. Adaptation is critically dependant on the CNS modulating nociception and autonomic output [26, 29]. The interactions between the nociceptive and autonomic nervous systems at peripheral and central nervous system levels are extensive [26-28] and Treister et al. [128] state that "the ANS is profoundly affected by the experience of pain". There are numerous areas within the neuraxis that receive convergent visceral and nociceptive input and these, in turn, stimulate specific sympathetic and parasympathetic responses [26, 129]. These regions include the ACC, insular cortex, amygdala, hypothalamus, PAG in the mesencephalon, parabrachial nucleus (PBN) in the pons, nucleus tractus solitarius (NTS) in the medulla, RVM, ventrolateral medulla (VLM) and raphe nuclei [26-28, 73, 81]. There is increasing evidence that ANS function can influence the nociceptive system. This is highlighted in chronic pain conditions such as CRPS – a condition with known sensory and autonomic abnormalities [21]. The modulation of nociception and the ability to dampen pain sensitivity is dependent on the functional interaction of these two systems [130]. Recent research points toward an association between ANS dysfunction and chronic pain, suggestive of sympathovagal imbalance playing a role in the maintenance of chronic pain [23, 24, 31, 37, 38, 131, 132].

2.5.2. Autonomic brainstem nuclei

There is increasing evidence that several autonomic brainstem nuclei generate stimulus-specific patterns of autonomic response in order to modulate nociceptive input [129, 133]. Nociceptive drive from spinal and trigeminal neurons are subject to descending inhibitory modulation by brainstem nuclei such as the PAG, RVM, locus coeruleus, and NTS [73, 81]. Noxious input causes these nuclei to activate hypoalgesic descending pathways in response to pain [27, 28, 133]. For example, one way the PAG alters the perception of pain is to release endogenous opioids, which bind to opioid receptors of the RVM, activating descending inhibitory pathways that act on the dorsal horn [82, 83, 134, 135]. The lateral and dorsolateral columns of the PAG also initiate sympathetic responses associated with tachycardia and hypertension, mediated by neurons of the VLM (Figure 2), that activate sympathetic preganglionic neurons controlling the cardiovascular system [28, 136, 137]. Conversely, neurons in the ventrolateral PAG excite sympathoinhibitory reactions associated with bradycardia and hypotension [27, 137]. It is postulated that dysfunction in these brainstem nuclei may facilitate pain in chronic conditions, such as migraine [133] and FM [104], raising the possibility that abnormal descending nociceptive inhibition may occur concurrently with abnormal autonomic [20] and, hence, cardiovascular responses [31].

2.5.3. Baroreflex system and baroreflex sensitivity

A change in state of the cardiovascular system can, in turn, modulate nociceptive processing and the perception of pain [138-140]. Blood pressure (BP) and heart rate (HR) are effects of autonomic control, with vascular resistance constantly being adjusted by the antagonistic influences of the sympathetic and parasympathetic nervous systems [141]. Painful stimuli, and subsequent autonomic reactions,

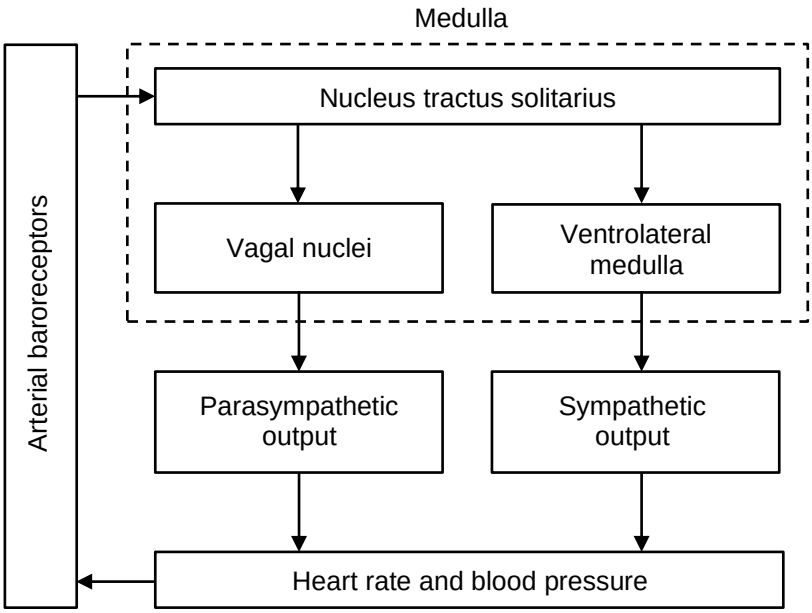
have been extensively studied to elucidate the relationship between the nociceptive and autonomic nervous systems [138, 142-144]. The baroreflex system (Figure 2) constitutes an important mechanism of autonomic cardiovascular control by assessing and maintaining beat-to-beat regulation of BP, and also moderates the relationship between pain and BP [140, 141, 143, 145]. Baroreflex sensitivity (BRS) is defined as “the change in interbeat interval in milliseconds per unit change in BP” [141] and chiefly falls under the control of the parasympathetic nervous system (PNS); i.e. cardiac vagal control [146, 147]. It has been proposed that an increase in BP is associated with a decrease in the perception of pain, a phenomenon known as hypertension-induced hypoalgesia [140, 148]. A noxious stimulus impacts the baroreflex system by initially causing vagal withdrawal, followed by an increase in sympathetic activity, raising HR and BP; a higher BP then activates arterial baroreceptors that, in turn, trigger descending pain inhibitory systems [140, 143, 148, 149]. In healthy, normotensive people, there is an inverse relationship between BRS and pain intensity [143]. Impairment of the baroreflex system, e.g. vagal dysfunction, may have an adverse effect on cardiovascular reactivity, reduce BRS, and, in turn, diminish the responsivity of descending pain inhibitory pathways [138, 150]. Bruehl et al. [140] highlight the function of baroreceptors in mediating the relationship between pain sensitivity and BP in multiple studies, including: electrical stimulation of baroreceptor/vagal afferents that induce antinociception [151]; surgical denervation of baroreceptor afferents that eliminates hypoalgesia in experimentally hypertensive rats [152] and hypertension induced by pharmacological pressor agents [153]; and, increases in baroreceptor stimulation from renal clip application, also resulting in hypoalgesia [154]. Research findings also show that acute mental stress can induce sharp increases in BP, which stimulate baroreceptors to produce an attenuation of pain [155]. In sum, experimental stimulation of baroreceptors impacts ANS function, BRS, and pain intensity [140, 143, 145].

2.5.4. Nucleus tractus solitarius

The NTS (Figure 2), located in the dorsomedial medulla, serves as the interface between the autonomic and sensory systems, receiving input from all organs of the body, spinal cord, and may also play a role in ANS dysfunction [30, 140, 156, 157]. Electrical stimulation of the NTS and vagus nerve has shown to yield reliable antinociceptive effects since it synapses directly and indirectly with other brainstem nuclei involved in analgesia, including the locus coeruleus, PAG, RVM, VLM, and raphe nuclei [26, 31, 81, 140, 158]. There is some evidence that tonic vagal afferent activity into the NTS is, at least partly, responsible for tonic descending inhibition of nociception at the dorsal horn through stimulation of these brainstem nuclei [30]. The NTS is the first synapse of the baroreflex system, receiving convergent afferent input from vagal afferents and spinal laminae neurons projecting nociceptive signals [27, 30, 144, 157]. Nociception is modulated by the NTS via the baroreflex system as changes in BP and HR influence the autonomic brainstem nuclei [140, 144, 159]. Figure 3 illustrates a simplified model of how nociceptive input in a healthy person activates the NTS to modulate ANS nuclei that alter BP and HR, which then, in turn, trigger descending inhibitory nociceptive pathways. In addition, the diagram shows how the NTS is related to descending inhibition of nociception via the PAG and RVM through monoaminergic pathways. These pathways are also under influence from descending

cortical/neuromatrix pathways and can be dysfunctional (facilitatory) in chronic pain conditions [73, 135].

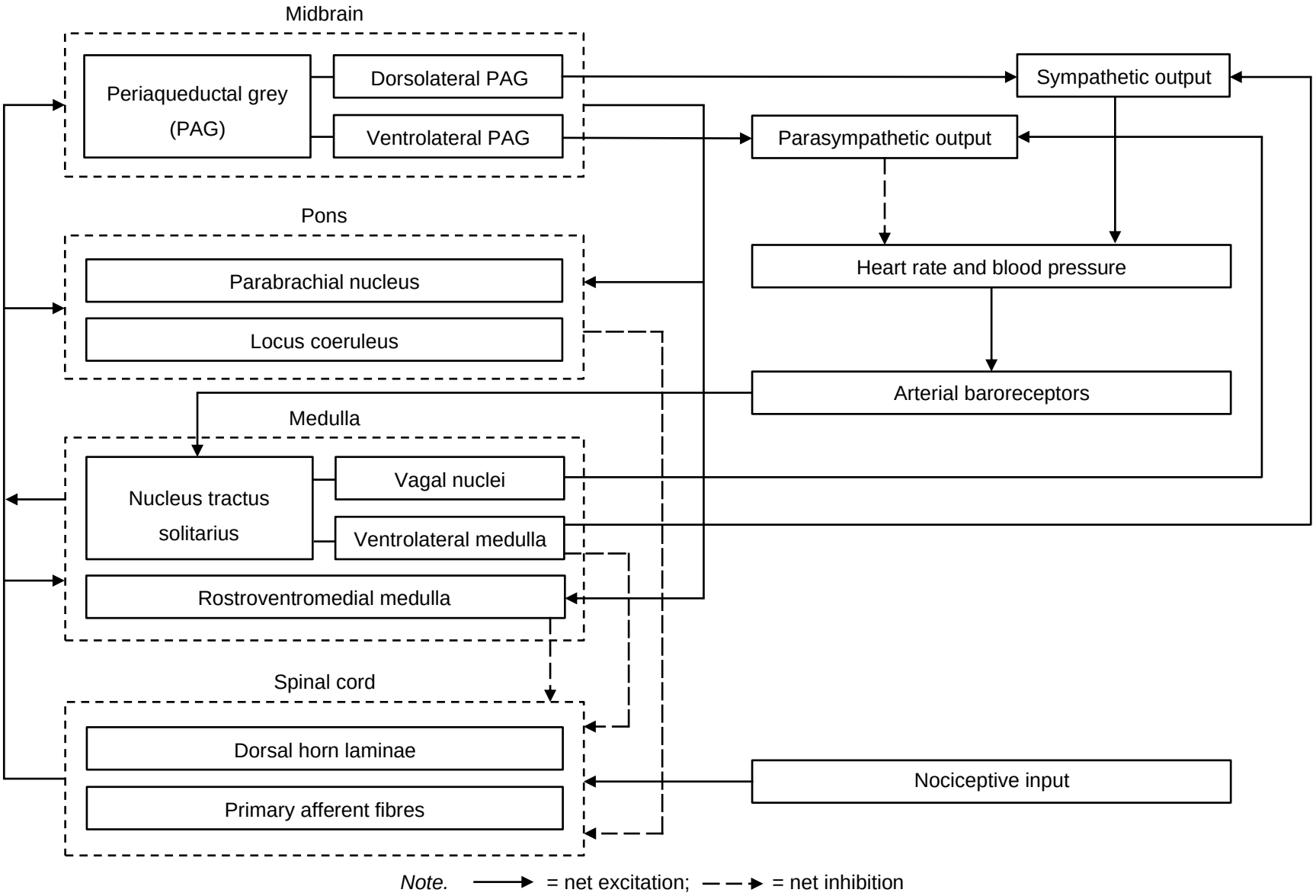
Figure 2. ***Simplified diagram of the baroreflex system.***



2.5.5. Nociceptive stress and cardiovascular reactivity

Chalaye et al. [91] examined the relationship between CPM and cardiovascular reactivity during cold water conditioning (nociceptive stressor) in healthy people and found a significant positive relationship between the increase in BP and magnitude of CPM. Cold water immersion increases muscle sympathetic nerve activity, HR, and BP [160], and the authors found that cold water conditioning significantly reduced the intensity of painful heat stimulation to the opposite arm, indicating effective CPM. Results also showed a positive correlation between heat pain tolerance and baseline systolic BP, indicating that people with higher BP are able to sustain greater nociceptive stimulation. Chalaye et al. [104] performed a second, similar study in people with FM and found that weaker BP responses during cold water conditioning were associated with decreased efficacy of inhibitory CPM. In addition, despite the same stimulus temperature, the FM group reported greater pain intensity during cold water conditioning compared to controls. Together, these findings point to an impairment of descending pain inhibitory and/or increased descending pain facilitatory mechanisms in FM. The results of these two studies show that the modulation of pain, and CPM specifically, is related to autonomic reactivity. Cardiovascular responses induced by experimental pain can potentially predict the endogenous capacity of a person to inhibit pain [91]. This may have important implications for chronic pain conditions, such as FM, where functional deficits are known to exist in the baroreflex system due to decreased autonomic control of BP and HR, especially under conditions of pain and stress [31, 72, 104, 138].

Figure 3. ***Simplified diagram showing normal interaction between the nociceptive and baroreflex systems to generate descending inhibition.***



2.6. Assessment of the autonomic nervous system

2.6.1. Introduction

Measuring autonomic indices has become important as emerging evidence is suggestive of an association between ANS dysfunction and chronic pain conditions, such as FM and CRPS, and the need for non-verbal, non-motor measures of pain [19, 21, 131, 142]. Autonomic responses to sensory events, such as pain, occur at involuntary cortical and subcortical levels to facilitate appropriate behavioural responses [27]. The cardiovascular and sudomotor systems are targets of ANS control and their output can be measured [31, 161, 162]. Several methods have been proposed to objectively tease apart pure sympathetic or parasympathetic activity, including heart rate variability (HRV) [23, 163], cardiac pre-ejection period (PEP) [164-166], skin conductance level (SCL) [167, 168], and skin conductance response (SCR) [133, 168]. Collectively, SCL and SCR refer to electrodermal activity (EDA) [169]. HRV represents both sympathetic and parasympathetic contributions to sympathovagal balance [29, 170], while sudomotor activity (i.e. sweating) and PEP are chiefly sympathetic [162, 164].

2.6.2. Heart rate variability

The baroreflex system is integral to the short term regulation of BP, modulating alterations in heart period due to phasic BP changes in response to various behaviours [31]. The baroreflex system is also a significant source of autonomic cardiac influence and the primary generator of HRV [31, 171]. HRV is defined as the amount of HR fluctuation around the mean HR [163, 172]. HRV is influenced by multiple hormonal, physical, and neural inputs, including response to experimental pain [173]. These factors generate specific, observable rhythms in a series that provides quantitative, non-invasive measure of ANS regulatory action [174]. The variance in HR can be evaluated by time or frequency domain measures [172]. For time domain measures, data for the calculation of HRV is the sequence of time intervals between heart beats (RR interval) determined by a continuous electrocardiographic (ECG) recording (Figure 4) [163]. Each QRS complex is detected and the normal-to-normal (NN) interval is determined, which is the instantaneous HR [163]. Several time domain variables can then be calculated, including: mean HR, standard deviation of the NN interval (SDNN), and the root mean square of successive differences between RR intervals (RMSSD) [172]. Frequency domain methods involve spectral analysis of HRV by analysing power, or variance, as a function of the changing HR frequency [163, 172]. The differential influence of the SNS and PNS on the sinoatrial node is due to the opposite effects of noradrenaline (SNS) and acetylcholine (PNS) [29]. The end result of sympathetic activation is an acceleration of slow diastolic depolarisation (seconds), resulting in lower frequencies, whereas parasympathetic influences on the sinoatrial node are brief (milliseconds), due to acetylcholine being rapidly hydrolysed, resulting in higher frequencies [163]. Consequently, the PNS is the only arm of the ANS capable of inducing quick, high frequency changes to the timing of the heart beat [29]. Spectral analysis attempts to disentangle these rhythms and separate sympathetic and parasympathetic contributions that underlie autonomic reactivity to nociceptive stimulation [130]. Appelhans et al. [173] describe HRV in the high frequency (HF) band (0.15 – 0.40 Hz) to reflect respiratory sinus arrhythmia (RSA), the RR oscillation associated with breathing, driven by the PNS; whereas low frequency (LF)

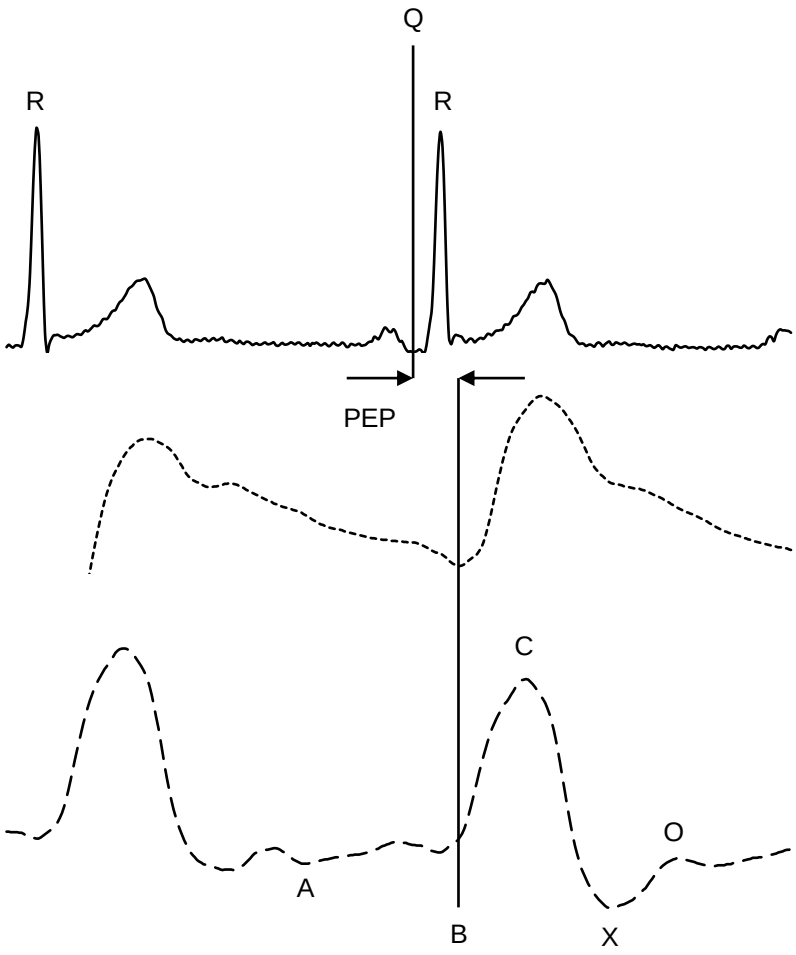
HRV (0.04 – 0.15 Hz) is influenced by both the SNS and PNS. Very low frequency (VLF) HRV is strictly greater than 0.00 Hz but less than 0.04 Hz [175]. The physiological explanation of the VLF component of HRV is not well defined and is usually attributed to non-harmonic properties that reflect slow, regulatory mechanisms such as thermoregulation [163, 176]. For this reason, VLF is typically avoided when interpreting HR and HRV recordings [163].

There is considerable evidence for moderate to excellent reliability of HRV measures during controlled resting conditions in adult participants [163, 177-183]. In well controlled studies, of sound methodology, with trained investigators, high intraclass correlation coefficient (ICC) values have been reported for time domain (ICC = 0.84 – 0.90) and frequency domain (ICC = 0.67 – 0.96) measures [177, 184]. Sinnreich et al. [182] conducted the largest reliability study on resting HRV recordings and concluded that HRV measures derived from 5-minute recordings, as per recommended guidelines [163], are stable and characteristic of an individual. The Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology [163] states: “frequency domain analysis is preferred to time domain analysis when investigating short-term recordings of HRV and that 5 mins is the advised recording time for a stationary system”. Schroeder et al. [185] also found HRV to show good repeatability and recommended a 5-minute recording length, in accordance with guidelines [163]. VLF and LF HRV measures are less reliable and repeatable [182]; hence, were not used as outcome measures in the current studies.

2.6.3. Pre-ejection period

PEP, a systolic time interval, is a non-invasive measure of cardiac contractility, a function which is primarily controlled by beta adrenergic mechanisms [165, 186, 187]. PEP is the best validated measure of cardiac sympathetic activity, derived from impedance cardiography (ICG) [164, 165, 188]. Each heart beat changes the volume and velocity of blood in the aorta, producing a change in electrical resistance (dZ) of the thorax to electrical alternating current. This is called the impedance pulsatile signal (IMP), generated and recorded from an external electrical signal passed across the midaxillary line. The ICG signal is the change in impedance over time (dZ/dt) and is the mathematical first derivative of the dZ (IMP) tracing [189] (Figure 4). PEP (Figure 4) is defined as the interval between left ventricular depolarisation, reflected by the Q wave on the ECG signal, to the opening of the aortic valve, corresponding to the B point on the ICG signal [164, 166]. The A, C (dZ/dt max), X, and O points correspond to atrial contraction, maximal ejection during systole, closure of the aortic valve, and diastole respectively [166, 190]. Pharmacological blockade studies show that PEP is a specific measure of cardiac sympathetic activity, with PEP shortening as sympathetic activity increases [164, 191]. ICG measurements, such as PEP, have been shown to be reliable [192, 193]. Furthermore, a systematic review by Parry et al. [193] found ICG to be a reliable and valid non-invasive application for measuring cardiovascular parameters.

Figure 4. ***Electrocardiographic, impedance pulsatile, and impedance cardiography signals.***



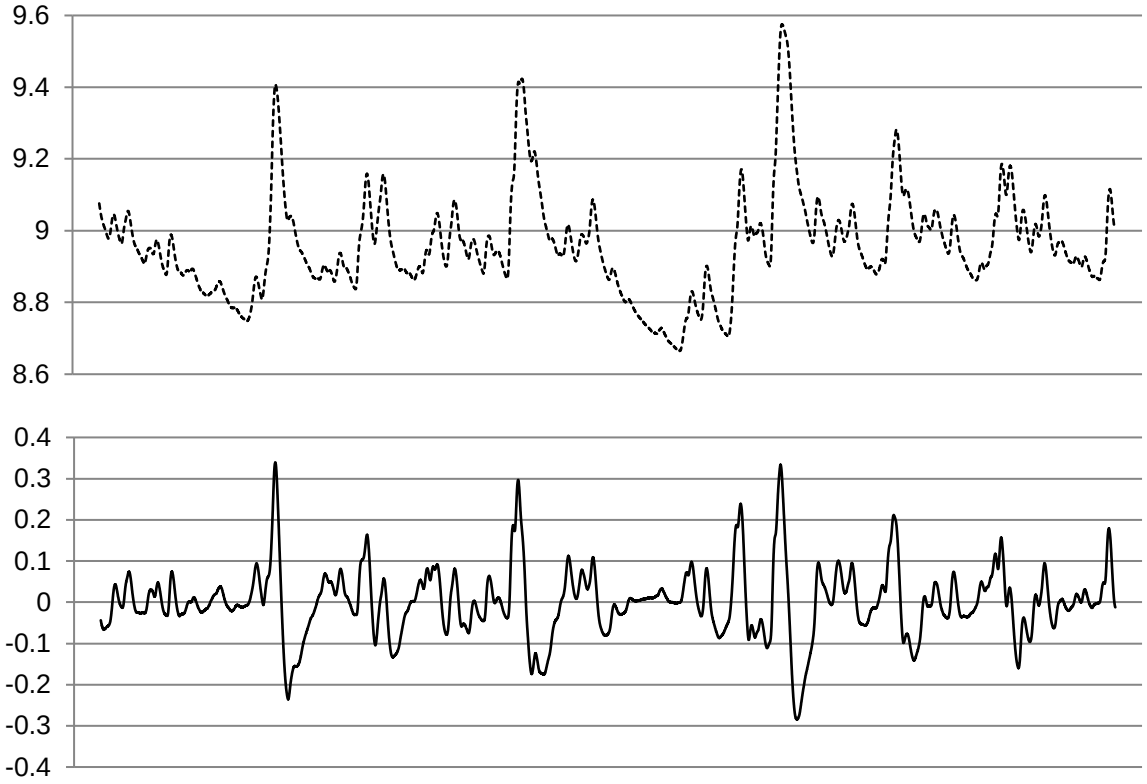
Note. ———— = ECG; ---------- = IMP (dZ); — — — — = ICG (dZ/dt)

2.6.4. Electrodermal activity

EDA is measured in two ways: tonic and phasic. Tonic EDA is referred to as the tonic level of electrical conductivity of the skin, or skin conductance level (SCL) [168]. SCL can also be described as gradual changes in the mean level of EDA over time [194]. Skin conductance response (SCR) is a brief increase in electrical conductivity of the skin relative to increased sweat gland activity, typically in response to a stimulus [195, 196]. Painful stimuli in healthy people induce activation of the SNS, resulting in an increase of SCL and greater number of SCRs [128, 133, 142, 197, 198]. The PNS has little influence on sudomotor function compared to the SNS, where preganglionic cholinergic neurons, derived from the hypothalamus and controlling thermoregulation, synapse with postganglionic sympathetic cholinergic sudomotor axons [162]. Electrical changes of the epidermis are due to activation of eccrine sweat glands via discharge of preganglionic sympathetic B fibres and postganglionic unmyelinated C fibres [196]. Electrodes placed on the fingertips measure conductance through the skin as small, direct electrical current passes through. As sweat gland activity rises, electrical conductivity of the skin momentarily increases resulting in a SCR, which ranges between 0.1 and 2.0 micro Siemen (μS) units

(Figure 5) [168, 195]. The reliability of EDA is less represented in the literature and, therefore, was not the primary ANS outcome measure for the current studies. However, several studies have found good test-retest reliability when measuring SCR [199-202]. Furthermore, a recent study by Breimhorst et al. [202] found sufficient reliability of SCR to differentiate between a range of painful stimuli, including mechanical and thermal pain.

Figure 5. ***Skin conductance response measured in micro Siemen (µS) units.***



Note. ---------- = unfiltered; ———— = filtered to 0.05 µS

2.7. Autonomic nervous system function at rest and in response to nociceptive stress

2.7.1. Autonomic nervous system function at rest in people with chronic pain

Autonomic function at rest has been explored in a number of studies examining different chronic pain populations, with a focus on HRV. A systematic review of 51 studies by Tracey et al. [23] found that people with chronic pain had significantly reduced HF HRV compared to healthy controls, indicating a loss of normal parasympathetic activity (vagal tone) at rest. Approximately 25% of the studies in the review included people with FM. The results of these studies consistently show reduced HF HRV in people with FM at rest compared to controls [40, 41, 138, 203]. Meeus et al. [204] also performed a systematic review and found, in 10 studies, HRV to be reduced in people with FM compared to controls. To date, no studies have examined HRV in OA [6, 33, 205]. However, ANS function has been studied in RA. Adlan et al. [206] performed a systematic review of 40 studies investigating ANS function in RA. Thirteen of these studies assessed HRV in people with RA in comparison to controls. Eight of the 13

studies demonstrated reduced HF HRV at rest in people with RA compared to controls, and 5 studies showed no difference between the groups. While almost exclusively dominated by studies in FM and RA, the existing literature suggests that chronic pain conditions are commonly associated with reduced HF HRV, indicating a loss of normal tonic vagal tone at rest.

Chronic pain may be the end result of maladaptation to the ongoing perception of threat, ultimately resulting in dysfunction of supraspinal pathways, increased allostatic load, endocrine dysfunction, and/or aberrant autonomic outflow [29, 126]. Sympathoexcitatory pathways in the brainstem, e.g. PAG, VLM, are under tonic inhibition of the prefrontal cortex, and during times of threat, the prefrontal cortex becomes hypoactive, leading to disinhibition of these circuits and sympathetic dominance [29, 207, 208]. Evidence tends to support elevated levels of sympathetic activity, including higher resting heart rates in people with chronic conditions such as arthritis [34], FM [167], chronic low back pain [209], and CRPS [21] compared to pain free controls. Previous research has also shown elevated SCL and a greater number of SCRs at rest in people with chronic pain compared to controls [36, 118, 167, 210-212]. Three studies investigated SCR in people with FM [19, 36, 213]. Of the three studies, two found impairment of SNS function in people with FM by way of increased latency and amplitude of SCR compared to controls at rest [19, 36]. No evidence of ANS dysfunction via SCR was found in the third study as there were no significant differences in latency and amplitude compared to controls [213]. The authors speculated that SCR may not be ideal for assessing ANS function as a whole since it only evaluates the sudomotor component of the SNS. Three studies investigated SCR in RA, all of which found similar results of significantly increased latency of SCR in RA compared to controls [214-216]. To date, SNS activity has not been examined in OA directly.

It is not known whether ANS dysfunction occurs in OA at rest. If it does, this may influence nociceptive processing and OA-related pain in at least two ways. Firstly, parasympathetic activation is associated with increased vagal facilitation of the NTS that, in turn, facilitates brainstem regions known to be involved in mediating descending pain inhibition, such as the PAG and RVM [30, 73, 84, 134, 151, 158]. Thus, decreased tonic parasympathetic activity at rest may, at least partly, explain diminished descending pain inhibition that is often observed in chronic pain conditions such as OA. Indeed, Cremeans-Smith et al. [217] recorded HR in people with KOA before TKA and found that those with elevated baseline HR before surgery were at greater risk of chronic post-operative pain. Secondly, the ANS is known to play a key role in modulating inflammation. For example, Drummond et al. [22] suggests that sympathetic activation “modulates proliferation of immune cells, their migration to sites of inflammation, and cytokine production”. Vagal activity regulates immune reactivity via cholinergic anti-inflammatory pathways by inhibiting release of proinflammatory cytokines [33, 218]. Disruption of anti-inflammatory vagal reflexes can facilitate nociceptive input and has been demonstrated in autoimmune and inflammatory disorders such as RA and IBS [13, 219, 220]. Thus, there may be a relationship between altered ANS function and inflammation in OA [33]. Yadav [221] studied 45 people with RA and found a significant correlation between disease activity score and reduced LF, HF, and total power HRV compared to healthy controls. The authors concluded that HRV was significantly reduced in people with

RA and associated with the disease process [221]. Seven studies reviewed by Adlan et al. [206] reported an association between inflammation and ANS function in RA. People with high CRP levels were more frequently observed to demonstrate ANS dysfunction, mainly by way of vagal withdrawal [206]. While unexplored to date, if ANS dysfunction does exist in OA at rest, this may have a pro-inflammatory effect that, in turn, may increase peripheral sensitisation and joint nociceptive output contributing to OA-related pain [33, 222].

2.7.2. Heart rate variability and acute experimental pain in healthy people

Autonomic responses to acute experimental pain, which focus on HRV, have been examined primarily in healthy populations [142-144, 223-230]. Three studies assessed ANS function in response to acute experimental pain in people with chronic pain, but either focused on cardiovascular parameters other than HRV, e.g. BP [104, 138], or used a time frame that was too short (i.e. 2 mins) [20] for adequate HRV recording as per recommended guidelines [163]. According to a systematic review by Koenig et al. [130], the typical ANS response to nociceptive stress in healthy people is reciprocal activation of decreased vagal activity and increased SNS activity. This has been demonstrated using various techniques, including mechanical pressure stimuli [225], cold water conditioning [228-230], hypertonic saline infusion [144], and noxious thermal pain [173]. However, to illustrate the complexity of autonomic responsivity in healthy people, Huang et al. [224] showed both LF and HF HRV to decrease in painful hot water immersion and increase in cold water. The authors suggest various mechanisms for the augmentation of both vagal and SNS activity during cold water pain, such as changes in peripheral vasoconstriction and vasomotion that alter BP and peripheral vascular resistance. Ye et al. [227] also found both LF and HF HRV to decrease from baseline to painful hot water immersion, stating that both vagal and SNS activity is suppressed when pain is produced. Therefore, there appears to be variance in how the ANS responds to noxious stimulation depending on the type of stimulus. Two studies involving healthy controls examined the relationship between pain ratings and HRV and found no significant correlations, despite there being changes in HRV in response to noxious stimulation [226, 231]. These data highlight that the response to pain is complex, and influenced by multiple neurophysiological factors aside from the ANS, including the motivational-affective and cognitive-evaluative components of pain perception [226, 232, 233]. To date, the ANS, and HRV specifically, has not been investigated in OA in response to a nociceptive stressor.

2.8. Impact of acute mental stress on the nociceptive and autonomic nervous systems

2.8.1. Introduction

Stress is a ubiquitous experience in life that is often said to cause or exacerbate illness. However, the concept of stress is subjective, and how each person copes with their environment is different [124]. A perception of threat, and the feeling of lack of safety, appears to be central to defining “stress” created by mental events [29]. Although many aspects of daily living may not seem to qualify as stressful to one

person, the effects on another may be negative, taking a cumulative, physiological toll on adaptive systems, ultimately resulting in detrimental physiological change [124].

Pain is recognised to intertwine sensory and emotional components, with psychological stress being a risk factor for chronic musculoskeletal pain [234, 235]. The unpleasantness of pain is an affective state with a strong level of arousal that initiates withdrawal from noxious stimuli [173]. This is part of a natural reaction to threat generated by excitation of the SNS, commonly referred to as the "fight or flight" response [236]. Ongoing pain following nociceptor activation is correlated with activation of the SNS, including cardiovascular, sudomotor, and sympathetic preganglionic neurons innervating the adrenal medulla [127]. The consequence of SNS activation from exposure to stress is increased HR, BP, EDA, and increased levels of plasma catecholamines, noradrenaline and adrenaline [127, 237]. However, stress exposure also modulates the PNS [237]. A meta-analysis by Brindle et al. [238] examined SNS and PNS contributions to cardiovascular reactivity and revealed that exposure to acute stress elicited both beta adrenergic sympathetic activation and vagal withdrawal to a similar extent. Research shows that autonomic responses to acute stressors, such as experimental pain [130] and mental arithmetic [31, 239], may be abnormal in chronic pain populations compared to healthy controls, which will be discussed in more detail in the following sections. Briefly, in healthy people, acute stress typically leads to vagal withdrawal and/or increased SNS activation, with a subsequent increase in HR and BP [228]. However, in chronic pain populations, functional deficits may exist in the ANS response such that autonomic and, in turn, cardiovascular regulation appears to be diminished or blunted in response to acute nociceptive and mental stress [31, 214, 238]. In turn, blunted cardiovascular reactivity leads to reduced BP-mediated baroreceptor activation, contributing to a decrease in descending pain inhibition and an impaired ability to modulate pain under conditions of stress [22, 31, 104, 140, 228]. Several studies have examined the nociceptive and autonomic effects of laboratory stress in people with FM [22, 31, 240-245], RA [22, 214, 240], and CRPS [21, 239]. The effects of acute mental stress tend to increase pain [22, 239], increase inflammation [246, 247], increase SCR [34, 211], and reduce HRV [21, 31] in chronic pain populations.

To date, the effects of mental stress on HRV and pain have not been examined in OA directly. However, Veldhuijzen et al. [247] recorded CRP (a marker of inflammation), BP, and HR in people with RA and OA during a brief mental arithmetic stress task in the standing position. This is the only study found analysing the effects of stress on two ANS parameters in an OA population. Results showed that combined mental and postural stress increased CRP in people with RA, but not OA. Furthermore, BP and HR increased significantly in both groups in response to acute mental stress [247]. No measures of pain or nociceptive processing were undertaken in either group. Stress may influence inflammation, at least in part, due to its autonomic effects. For example, Veldhuijzen et al. [247] found that acute stress elicited an acute inflammatory response in both RA and OA populations by way of raised white blood cell count. Hirano et al. [246] compared the influence of mental stress on neuroimmune function in RA and OA groups that underwent TKA and THA under general anaesthesia. Interleukin-6 (IL-6), cortisol, and adrenaline were recorded before anaesthesia was administered on the day of the

operation, and 30 mins later when the person was under general anaesthesia. The same blood markers measured the day before the operation, at the same time of day, served as the control levels. Results revealed that levels of IL-6, cortisol, and adrenaline were markedly increased in the RA group who lay on the operating table under significant mental stress due to the impending operation. However, in the OA group, blood levels remained within the normal range and no significant changes were observed [246]. No autonomic indices were recorded in this study.

2.8.2. The Paced Auditory Serial Addition Task

The Paced Auditory Serial Addition Task (PASAT) is a mental arithmetic task known to effectively evoke acute psychological stress [248]. The PASAT was devised by Gronwall et al. [249] and first used to examine the effects of traumatic brain injury on the rate and performance of information processing [249, 250]. Additionally, the PASAT has been used to examine cognitive performance in other neurological conditions such as chronic fatigue syndrome (CFS), whiplash, and depression [250]. The application of the PASAT involves presenting a series of numbers from 1 to 9 to a person, who is then instructed to add each number to the one that immediately preceded it; i.e. the second number is added to the first, the third number to the second, and so on. For example, if the digits 4, 7 and 3 were presented, the person would correctly answer with the sums of 11 and 10, respectively. The answer must be provided before the next number is presented in order for the response to be scored as correct. The requirement is that correct responses are sustained over multiple number presentations until the end of the trial. The classic Gronwall version used for this thesis comprises 61 items per trial [251]. Variants of the PASAT exist, such as a visual version called the Paced Visual Serial Addition Task (PVSAT) [250], or versions that contain fewer items per trial [252]. Gronwall et al. [249] altered the speed of information processing by presenting the same sequence of numbers at different rates. The time between presentations of each number is referred to as the interstimulus interval (ISI). The PASAT incrementally increases processing demands of sustained attention and working memory by decreasing the ISI of each trial [251]. Five ISI rates were originally used: 2.4 s, 2.0 s, 1.6 s, 1.2 s, and 0.8 s [250]. However, 0.8 s was dropped due to consistently poor results of correct responses [250]. Results of the PASAT are typically scored as either: number of correct responses for each trial (maximum = 60); or, the total number of correct responses summed across four blocks of trials (composite score). Mathias et al. [253] suggest a practice period in their use of the PASAT, in order to become familiar with the task before longer testing, but this may reduce the stress the novelty of the task is meant to induce. Regardless of pain condition or mental status, the PASAT is reported to be challenging, stressful, and frustrating [250]. Holdwick et al. [254] state that even people in a positive frame of mind can be negatively impacted by the PASAT, reporting increased levels of anxiety, sadness, and even hostility. The authors suggest that people undertaking the PASAT test should be warned that it will be an unpleasant emotional experience [254].

2.8.3. Effects of acute mental arithmetic on the nociceptive and autonomic nervous systems in healthy, pain free people

Numerous studies have examined the stressful effects of mental arithmetic on ANS function [21, 22, 31, 230, 248, 255-258]. Acute mental stress, such as that induced by mental arithmetic, is a stimulus that can generate circulatory reactions that resemble classical defence reactions resulting in major cardiovascular changes [127, 257, 259, 260]. The PASAT is a significant autonomic cardiac stressor [248, 253, 258, 260-265], acting through both vagal withdrawal [238, 266] and beta adrenergic sympathetic activation [238, 259, 267, 268]. Results consistently show the PASAT to significantly increase HR [253, 258, 261], decrease RMSSD [261], increase systolic BP (SBP) and diastolic BP (DBP) [258, 263], reduce HRV [248, 264], shorten PEP [265], and increase SCR [256]. Studies that have examined the time course of the effects of the PASAT recorded ANS variables for up to 33 mins following completion of the task [263]. Effects of the PASAT on ANS indices appear to be short lasting and may not persist beyond the period of the task, especially for healthy responders [269]. One study showed cardiovascular variables to return to baseline in as little as 2 mins following completion of a stressful task [270].

Only two studies have examined the between session reliability of the impact of the PASAT on cardiovascular variables, including HR, SBP, DBP, and PEP [263, 265]. Both studies are from the same research group and used the standard PASAT and the Pearson correlation coefficient for measures of reliability, which is not an optimal statistical analysis for reliability testing due to the inability to detect systematic error. In the first study, Ring et al. [263] found SBP, DBP, and HR to be elevated during the PASAT compared to the rest and recovery phases. Correlational analyses for 3-day temporal stability were performed on cardiovascular parameters during the baseline rest phase and during the PASAT. The absolute values of HR, SBP, and DBP during the PASAT demonstrated moderate to strong correlations when compared across sessions: HR (r = 0.44), SBP (r = 0.80), and DBP (r = 0.68) [263]. In the second study, Willemsen et al. [265] examined the effects of the PASAT on cardiovascular reactions in healthy males over two sessions separated by 28 days. During the PASAT, SBP, DBP, and HR increased at the beginning of the task and remained elevated for the duration of the task [265]. PEP shortened during the PASAT and remained reduced for the 9-minute duration of the stressor [265]. Pearson's correlation coefficients were calculated for baseline values, PASAT task levels, and change scores to determine test-retest reliability across sessions. All cardiovascular variables during the PASAT were considered reliable; as were the change scores from baseline to the PASAT: PEP (r = 0.80), HR (r = 0.73), SBP (r = 0.52), and DBP (r = 0.65).

The PASAT has also been shown to modulate the experience of pain. Acute mental stress can activate descending pain inhibitory control systems via supraspinal mechanisms [271]. This is known as mental stress-induced hypoalgesia [272, 273]. Mental stress-induced hypoalgesia is characterised by a reduction in nociception following exposure to mental stress through descending inhibitory opioid and non-opioid neural pathways [273]. The cardiovascular system is also recruited during mental stress and its responses are similar to that of exercise, involving areas of the brain responsible for cardiovascular

modulation; e.g. medial prefrontal and insular cortex [258]. Mental arithmetic elicits increases in BP and cardiac output, and decreases total peripheral resistance in healthy people [274]. In healthy, pain free people, the PASAT can inhibit experimentally induced pain [230, 257, 271, 273, 275, 276]. A range of painful stimuli have been used in these studies, including saline injection [230], mechanical pressure [273], noxious electrical [257, 271, 275], and heat [276]. Results consistently show that pain ratings were reduced during performance of the PASAT compared to when the same noxious stimuli were presented on their own [230, 257, 271, 275, 276]. Bendixen et al. [230] exposed participants to the PASAT during hypertonic saline-evoked jaw muscle pain and found an increase in HR and BP, as well as a reduction in pain. It was proposed that the hypertension-related hypoalgesia could be due to baroreceptor activation, observed by the inverse correlation between pain reduction from the PASAT and rise in BP [230]. However, changes in pain and ANS variables during mental stress may not always be related. Terkelsen et al. [271] demonstrated that while the PASAT reduced pain ratings, changes in pain and unpleasantness during the PASAT did not correlate with changes in HR. These findings suggest that the pathways responsible for the decrease of mean RR interval during mental arithmetic may not be common with pathways that relieve pain. In a separate study by the same team, Terkelsen et al. [257] found that the PASAT coupled with painful sural nerve stimulation, and the PASAT alone, both significantly decreased the mean RR interval. However, decreases in mean RR interval during the PASAT and pain did not correlate with the inhibition of pain induced by PASAT alone. The PASAT induced a significant reduction in mean RR interval during non-painful stimulation compared to rest plus painful stimulation; and, induced an even greater reduction during painful stimulation. However, total HRV was unchanged when the PASAT was combined with painful stimulation [257]. This could suggest that while pain induces sympathetic activation, the combination of mental stress and pain contributes to both cardiac sympathetic activation and vagal withdrawal such that there may be no significant change in total HRV [238, 257]. Subsequently, measuring the impact of mental stress on the ANS needs to be performed separately from acute pain assessments since pain influences the ANS.

Few studies have examined the impact of mental stress on pain sensitivity, assessed using QST. However, three studies [271, 277, 278] showed that mental arithmetic inhibited pain ratings associated with the nociceptive flexion reflex (NFR), but had different effects on the NFR itself. The NFR is defined as “a spinal polysynaptic defence response that can be elicited in humans by painful electrical stimulation of the foot causing its withdrawal from the noxious stimulus”; and, is considered an indirect measure of spinal nociceptive processing [271, 279]. McIntyre et al. [278] found that, during mental arithmetic, the NFR was larger and yet, pain ratings were attenuated [278]. The authors postulated that the reduction in pain perception was due to an increase in physiological arousal (i.e. faster HR and shorter RR interval) and supraspinal antinociceptive mechanisms, while the increase in NFR was due to the absence of systolic inhibition – and, therefore, reduced descending inhibition – caused by mental stress [278]. In contrast. Willer et al. [277] showed that mental stress, induced by a 30 sec serial subtraction task, not only reduced perception of pain, but also significantly reduced the size of the NFR response. Terkelsen et al. [271] examined how the NFR changed in healthy people by focusing on painful stimuli; or, distracting away from painful stimuli using the PASAT; and how, in turn, the

cardiovascular system responded. When focusing on painful stimulation without mental stress, there was no impact on pain perception, NFR, or HR [271]. Yet, distraction with the PASAT reduced pain and unpleasantness ratings, mean RR interval, and all HRV variables significantly – but not the NFR response [271]. Since HRV parameters only changed during mental stress, when pain perception was diminished, this may suggest that the ANS may play a role in pain modulation produced by mental arithmetic [271]. Therefore, to summarise these three studies, only Willer et al. [277] found that at least some of the pain inhibitory effects of mental arithmetic are mediated at a spinal level by way of increased descending inhibition, while the other two studies showed a facilitatory effect [278], and no effect at all on spinal nociception despite reduced pain, suggesting a supraspinal mechanism of action [271].

In sum, mental arithmetic has been shown to be a reliable mental stressor that, in healthy people, enhances SNS activity, reduces vagal activity; and, typically reduces experimental pain through altered supraspinal and, in some cases, spinal nociceptive processing [257, 271, 278].

2.8.4. Effects of acute mental arithmetic on the nociceptive and autonomic nervous systems in people with chronic pain

In contrast to the effects of mental arithmetic in healthy, pain free people, exposing chronic pain populations to mental arithmetic can result in increased levels of pain [22, 31, 167, 239], reduced BRS [31, 280], decreased HR reactivity [167, 209, 281], increased EDA relative to controls [34, 167], and longer recovery times [22, 167, 209]. However, people with chronic pain are not homogenous in their baseline ANS activity and reactivity to mental stress [212, 282]. Cluster analyses by Thieme et al. [212, 283] in two studies revealed four different ANS groups in people with FM when exposed to mental arithmetic. The largest group was characterised by high BP and HR, and stable SCL and electromyographic (EMG) responses, recorded from the left and right trapezius muscles. The second group showed reduced BP, HR, SCL, and EMG responses. The third group displayed increased BP, HR, and SCL, and reduced EMG responses. Lastly, the fourth group showed increased EMG responses, but unchanged BP, HR, and SCL.

It has been previously assumed that people who display exaggerated cardiovascular reactions to stress (i.e. increased BP and HR) are at increased risk of cardiovascular disease [260, 284]. However, evidence is emerging that smaller magnitude or blunted cardiovascular stress reactivity (i.e. reduced BP and HR response) may have a more negative prognostic value for health and behavioural outcomes [260, 269, 284]. Brindle et al. [285] define blunted cardiovascular reactivity as: “an objectively measured cardiovascular response to acute laboratory-based psychological stress that is comparatively lower than typically observed, and may reflect an inability to effectively mobilise the stress-response system to cope with stressful situations”. Blunted stress reactivity can therefore be considered a marker of dysfunction in the systems that control autonomic, cardiovascular, and neuroendocrine changes [260]. Indeed, people classified as “blunted reactors” showed reduced activity in the ACC and amygdala when exposed to stress – areas of the brain not only involved in emotional regulation, but also nociceptive and autonomic processing [26, 260, 284]. The PASAT is able to separate exaggerated from blunted

autonomic reactors [260, 284, 285]. Bibbey et al. [284] used the PASAT as a cardiovascular reactivity screening procedure to determine impulsivity in young adults with blunted or exaggerated HR reactions. Cardiovascular reactivity was calculated as the mean PASAT level minus the baseline level. Blunted reactors were defined as the bottom 15% of HR reactions, and exaggerated reactors the top 15%. It was found that participants with blunted HR reactivity were more impulsive [284]. Ginty et al. [260] also used the PASAT as a screening tool to separate exaggerated from blunted reactors before examining neural activity with functional MRI (fMRI). The authors found the two kinds of reactors displayed similar baseline heart rates, yet, differed significantly in their reactivity to mental stress [260]. Donadio et al. [269] referred to these two reactivity groups as responders and non-responders. In a study examining the effects of mental stress on muscle sympathetic nerve activity and BP, Donadio et al. [269] found that the PASAT was associated with a reduction of muscle sympathetic nerve activity and weaker BP increases during the task in non-responders compared to responders. This may impact people who are non-responders, or blunted reactors, in everyday life by virtue of the ANS not being able to respond appropriately to stressful situations [29, 269, 286].

Several studies have compared baseline autonomic activity, and autonomic reactivity to mental arithmetic, between healthy controls and chronic pain populations [21, 167, 209, 212, 283, 287]. As described previously, Thieme et al. [212, 283] characterised four groups for people with FM with varied ANS changes in response to mental arithmetic. Two other studies also examined the effects of the PASAT in chronic pain populations with known ANS dysfunction, including FM [167] and CRPS [21]. Terkelsen et al. [21] measured HR and HRV while Thieme et al. [167] recorded HR, SCL, and BP. Of particular interest in these studies are the differences in baseline levels and reactivity of ANS function between chronic pain groups and healthy controls. Participants with chronic pain demonstrated higher baseline HR in both studies compared to healthy controls [21, 167]. At rest, people with CRPS displayed similar autonomic values (RR interval, RMSSD, total HRV) to the control group during the PASAT, indicating ANS dysfunction in people with CRPS [21]. However, Terkelsen et al. [21] found that the response to mental stress by way of reduced HF HRV was similar in both groups. Thieme et al. [167] recorded not only a higher resting HR in people with FM compared to controls, but also a higher resting SCL. After exposure to mental arithmetic, the SCL increased significantly in the FM group and remained elevated in the proceeding 4 min recovery phase, while the SCL returned to baseline during recovery in controls [167]. The SCL being higher and prolonged in people with FM is suggestive of enhanced SNS response to stress [167]. Although FM participants showed a higher HR during mental arithmetic, HR reactivity in healthy controls was greater, with a greater reduction in HR during recovery [167]. Thieme et al. [167] concluded that blunted stress reactivity may be due to high baseline ANS levels, which create a ceiling effect when faced with acute stress, leading to reduced BRS that may, in turn, impair descending inhibition of nociceptive pathways.

Mental arithmetic has been shown to increase levels of pain across a number of chronic pain conditions, including FM [22, 31, 167], RA [22], chronic tension-type headache [288, 289], abdominal pain [280], and CRPS [239]. Drummond et al. [22] used a mental arithmetic stressor similar to the PASAT in a

study involving people with FM and RA. Experimentally induced pain ratings increased similarly in both FM and RA groups after 10 mins of mental arithmetic, and increased further after 15 and 20 mins of continuous mental arithmetic [22]. However, the differences between the groups became apparent when controlling for psychological distress since baseline levels of depression and anxiety can influence changes in pain during mental stress [22, 280]. Pain ratings were significantly greater than baseline in the FM group after 15 mins of mental arithmetic compared to the RA group [22]. In a different study, using a similar mental arithmetic task, Drummond et al. [239] showed that pain significantly decreased in healthy people, while increasing in people with CRPS. Some participants with CRPS even developed abnormal sensations in the affected limb. Abnormally high SNS activity in people with ANS dysfunction can produce peripheral vasoconstriction and, over time, lead to muscle ischemia that sensitises nociceptors, resulting in sensory abnormalities [113, 239]. In chronic pain populations, experimentally induced pain can increase current pain, and last longer than the stimulus. Cathcart et al. [288] examined pericranial muscle PPTs in people with chronic tension-type headache after exposing them to mental arithmetic and were able to induce headache in 91% of participants. The authors found reduced PPTs and reported an increase in intensity of headache throughout the course of the mental arithmetic task [288]. Sandrini et al. [289] demonstrated similar findings of reduced PPTs in people with chronic tension-type headache following stressful mental arithmetic. These findings show that mental stress not only triggers pain, but can also aggravate existing pain [290].

There are limited studies examining the effects of mental arithmetic on both pain and the ANS together in chronic pain populations. Two studies assessing the impact of mental arithmetic on these variables in FM consistently showed that mental arithmetic significantly increased pain ratings and altered HR, SCL, and BP [31, 167]. Thieme et al. [167] found several abnormalities in subjective pain ratings and stress reactivity levels in response to mental arithmetic. Results showed an increase in pain ratings, HR, and SCL from baseline to mental arithmetic in the FM group compared to healthy controls [167]. In the recovery phase following mental arithmetic, FM participants displayed elevated levels of HR and SCL, while these parameters returned to baseline during recovery in controls [167]. Consistent with the variance of ANS responses to mental arithmetic observed in FM populations [212, 283], BP rose to equal extent in both FM and control groups during mental arithmetic [167]. Reyes del Paso et al. [31] found that reduced BP and BRS were associated with increased pain ratings when exposing people with FM to acute mental arithmetic. ANS variables were recorded during the stressor using ICG/ECG and finger BP measurements. Results showed the RR interval was lower in the FM group at rest and during the mental arithmetic task compared to controls. For ICG parameters, stroke volume and left ventricular ejection time were lower in the FM group at rest and during the arithmetic task, indicating decreased sympathetic influences on the myocardium [31]. SBP changes across each epoch were similar in both groups, however, the DBP reaction was smaller in the FM group. BRS was lower in the FM group during the task, and since the baroreflex system influences the heart through vagal control [140], HRV was also reduced in all frequency bands during mental arithmetic [31].

In sum, people with chronic pain typically report increased levels of pain when exposed to mental arithmetic, yet yield mixed ANS results with normal, blunted, and exaggerated autonomic reactivity during the task. There is also some evidence of prolonged recovery time for ANS function to return to baseline after the stressor. To date, both nociceptive and ANS measures have not been studied together in OA after exposure to stressful mental arithmetic.

2.9. Impact of acute exercise on the nociceptive and autonomic nervous systems

2.9.1. Introduction

Acute exercise can change the perception of pain [291, 292]. The concept of pain sensitivity reduction in healthy people [291] and athletes [293, 294] through exercise is called exercise-induced hypoalgesia (EIH). Umeda et al. [291] define EIH as: “increases in pain thresholds and tolerances, as well as reductions in pain ratings during and following exercise”. EIH is typically measured by applying a noxious stimulus before and after exercise to assess for changes in pain sensitivity [292, 295-297]. Several laboratory techniques have been used to produce pain and assess EIH, including noxious electrical [298, 299], heat [300, 301], and mechanical pressure stimuli [116, 291, 302]. Various types of exercise have been employed to assess EIH, including aerobic exercise [293, 294, 303-307], dynamic resistance exercise [308, 309], and isometric exercise [93, 116, 117, 291, 299-302, 306, 310-322]. Typical aerobic exercises include running, stepping, or stationary cycling [295, 323]. Isometric exercise is a static contraction that does not involve a change in joint angle, whereas dynamic resistance exercise produces joint movement, usually through isotonic muscle contraction [295]. A meta-analysis by Naugle et al. [295] concluded that all three types of exercise can decrease levels of experimental pain in healthy people. However, isometric exercise was found to have the largest effect size. In healthy people, pain threshold and pain intensity differences were largest for isometric and dynamic resistance exercise compared to aerobic exercise [295]. EIH effectiveness is also dependent on exercise intensity and duration [319, 323]. Results from EIH studies point to hypoalgesia following aerobic exercise occurring most consistently after high intensity, e.g. ≈70% maximal oxygen uptake, and durations longer than 10 mins [295, 319]. Koltyn et al. [323] suggest that there may be an influence of interaction between duration and intensity, with high intensity exercise performed for a short duration, and moderate intensity exercise performed for a longer duration, both producing hypoalgesia. However, not all people are able to engage in high intensity cycling or running [292]. Therefore, it is important to determine whether other forms of exercise, such as isometric exercise, and relative intensities are associated with EIH. Specific hypoalgesic responses to intensity and duration of isometric exercise will be reviewed in Section 2.9.2.

Several potential mechanisms have been proposed for the hypoalgesic response following exercise. The first is the natural increase in BP that exercise stimulates due to the physiological demand of the activity [291]. Research has shown the functional interactions between the nociceptive and autonomic nervous systems, since the nuclei in the brainstem associated with pain modulation also control BP [140, 148, 291]. It is postulated that exercise may induce EIH by increasing BP, which activates arterial

baroreceptors, resulting in increased supraspinal inhibition of pain and stimulation of brain regions that modulate pain, including the endogenous opioid system [139, 140, 148, 153, 299]. The relationship between EIH and the increase in BP with exercise is discussed in more detail in Section 2.9.2. Multiple studies have demonstrated that BP increases with exercise and, in healthy people, attenuates pain perception [291, 299, 311, 320, 324]. Activation of the endogenous opioid system during exercise is the most commonly tested hypothesis for EIH [292]. Endogenous opioids are widely distributed in areas of the CNS involved in regulating the ANS, e.g. NTS, dorsal vagal nucleus, nucleus ambiguous; and, the nociceptive system, e.g. dorsal horn, peripheral nociceptors [82, 325]. Opioid receptors are also present in peripheral sympathetic and cardiovascular structures that exert inhibitory effects on sympathetic transmission [325]. Of special interest is the beta endorphin system, which contributes to the regulation of BP, pain perception, and thermoregulation [325]. Exercise, typically performed at high intensity, stimulates beta endorphin release, which is thought to be involved in EIH [292, 293, 325, 326]. Kemppainen et al. [303] showed that the release of adrenocorticotropin hormone (ACTH) from the anterior pituitary gland during exercise is also involved in EIH. ACTH is released in equal quantities and concomitantly with beta endorphin and, together, they create an effect of hypoalgesia following exercise [303, 327]. Two studies by Droste et al. [304, 328] measured the effects of exercise on pain thresholds, plasma beta endorphin, cortisol, and catecholamine levels. Both studies found pain thresholds and opioid levels to increase during exercise [304, 328]. Several other studies have also consistently shown increased plasma beta endorphin levels following exercise [329-331]. However, there is often a mismatch in timing between the EIH response and increased plasma endorphin levels [304, 332, 333] and several studies have shown that opioid antagonists may attenuate, but do not abolish EIH [292, 297, 334], suggesting that other non-opioid mechanisms are also involved. Hypoalgesic effects are exerted by catecholamines during exercise [326]. Descending pain inhibitory action on the dorsal horn is partly noradrenergic, and spinal laminae involved in nociceptive processing are known sites of alpha-2 adrenergic receptors and noradrenaline containing neurons [140, 335]. Noradrenaline, through the action of alpha-2 adrenoceptors, plays a pain suppressive role [334-336]. EIH may also be related to local segmental inhibitory effects. Exercise can stimulate skin or muscle afferents through gate control mechanisms that compete with nociceptive afferents in the dorsal horn, potentially contributing to EIH through gate control mechanisms [326].

2.9.2. Effects of acute isometric exercise on the nociceptive and autonomic nervous systems in healthy, pain free people

In healthy, pain free populations, isometric exercise can inhibit experimentally induced pain [291, 297-301, 306, 310, 312, 313, 315, 317, 319, 322, 337]. A range of painful stimuli have been used in these studies, including mechanical pressure [291, 297, 300, 301, 306, 312, 313, 315, 317, 319, 337], heat [297, 298, 300, 310, 317, 322], and electrical stimulation [298, 299]. In all but two of the studies [298, 315], results consistently showed that pain thresholds significantly increased, and pain ratings decreased, following isometric exercise. Isometric exercise can elicit pain inhibitory effects during and immediately following the contraction, with effects diminishing over time [117, 295]. Three studies

showed that that the effects of EIH can last up to 15 mins [306, 320, 337], while Kosek et al. [313] revealed that PPTs tend to return to baseline 30 mins following contraction.

Most exercise studies use a fatiguing protocol of either high intensity or lower intensity but long duration muscle contractions when assessing EIH. Gandevia [338] defines muscle fatigue as: “any exercise-induced reduction in force generating capacity”. Hoeger Bement et al. [319] claim that fatigue does not need to be reached in order for EIH to occur. In fact, EIH can be produced during and following short duration, e.g. 1.5 – 5 mins, and low intensity isometric exercise, e.g. 15 – 50% maximal voluntary contraction (MVC) [116, 291, 302, 311, 339]. There does, however, appear to be an intensity and duration threshold for EIH. Submaximal (≈25% MVC) isometric exercise of 3 min duration tends to produce the largest EIH effect [291, 300]. Umeda et al. [291] found PPTs to be higher in women immediately following 3 mins of isometric exercise; however, the change in pain threshold following 5 mins was small. Hoeger Bement et al. [319] state that a 25% MVC sustained for 2 mins does not induce EIH. Other studies have also shown EIH to occur following an intensity of as low as 21% MVC of the quadriceps [302], yet the duration must be performed for longer than 2 mins in order to produce hypoalgesia [319]. For durations shorter than 2 mins, Koltyn et al. [311] found that increasing exercise intensity to 40 – 50% MVC was sufficient to produce EIH. In sum, in order to induce EIH at 20 – 25% MVC, task duration of 3 mins is sufficient. However, for task durations < 3 mins, MVC intensity must be increased to 40 – 50%. Various isometric exercise techniques involving different muscle groups have been employed to induce EIH, the most common being a submaximal (≈25% MVC) isometric handgrip exercise, consisting of squeezing a hand dynamometer [291, 297, 300, 310, 312, 322]. Other studies have used exercises such as wall squat [301], shoulder rotation [117], and contraction of knee extensor [116, 302, 306, 315, 337] or elbow flexor muscles [306, 319, 337] using isokinetic dynamometers.

Several studies have assessed the effect of isometric exercise on pain ratings and thresholds in distant or contralateral body parts in addition to the one undertaking exercise [117, 312, 313, 317, 320, 337]. The hypoalgesic effect of exercise has been found to be multisegmental and not isolated to the contracting muscle, so called remote EIH [312, 313, 337]. This suggests that central mechanisms are, at least partly, responsible for EIH, such as increased systemic secretion of beta endorphins, activation of endogenous descending inhibitory pain systems, or increases in BP [295, 313]. However, the effect of remote EIH appears to be less consistent compared to the local EIH effect [340, 341]

The impact of short duration isometric exercise on the ANS can differ to other forms of exercise [342]. In healthy people, isometric exercise tends to increase BP [320, 342-347], increase HR [320, 343-348], decrease BRS [344], decrease PEP [345]; and, typically, reduce HF HRV [344, 345, 348, 349] and increase LF HRV [342, 344, 347-349]. However, isometric exercise is unique to others forms of exercise insofar that it can also induce both parasympathetic and sympathetic activation simultaneously [342, 346, 347]. Weippert et al. [342] highlighted the complexity of the ANS by comparing isometric exercise to dynamic exercise at similar heart rates. To achieve the same net effect on HR during both types of exercise, different mechanisms occur in the ANS during isometric contraction. Compared to dynamic

exercise, isometric exercise can exhibit higher increases in BP, RMSSD, and HF HRV [342]. It has been proposed that in order to achieve the same HR in both forms of exercise, dual autonomic activation occurs during isometric exercise such that there is an increase in both vagal and SNS activity [342, 346, 347]. Isometric exercise simultaneously accumulates metabolites within the muscle during static contraction, which triggers chemosensitive afferents that reflexively increase BP [346], and increases vagal cardiac efferent activity to maintain a constant HR [342]. In sum, there are three ways the ANS affects cardiovascular activity during exercise, all of which can yield the same net effect on HR: (1) reciprocal activation of the two autonomic branches; (2) dual activation of the two autonomic branches; or (3) sole vagal withdrawal or sole sympathetic activation [342, 346, 350].

Modulation of BP and HR during exercise is complex, being affected by multiple afferent pathways [351]. In healthy people at rest, an increase in BP usually induces a decrease in HR through the baroreflex system [351]. During isometric exercise, however, an increase in BP is accompanied by a concomitant increase in HR [351]. There are two hypotheses put forward to explain this. The first is that a reduction in BRS during exercise leads to an increase in both BP and HR [351, 352]. The second is that the CNS resets the baroreflex response to allow a higher operating BP during exercise without changing its sensitivity [343, 353]. Upon resetting the baroreflex system, the CNS perceives relative hypotension and acts to increase HR, initially through vagal withdrawal, and thereafter through sympathetic vasoconstriction [351, 352]. During isometric exercise, SNS activity is increased chiefly by muscle afferents, whereas the CNS raises HR and cardiac output via vagal withdrawal [352]. This is observed by a reciprocal increase in LF HRV and decrease in HF HRV [344, 354]. Following isometric exercise, HR returns to baseline, whereas BP remains elevated above rest. In turn, HF HRV returns to baseline while LF HRV remains elevated until the release of circulatory vasoconstriction [351]. Overall, this suggests that the maintenance of BP and HR during isometric exercise is controlled by both arms of the ANS: the vagally mediated baroreflex mechanism modulating HR; and, peripheral sympathetic activation maintaining BP [351, 352].

The effects of submaximal isometric exercise on ANS variables appear to be short lasting, especially in young, healthy people, with one study showing cardiovascular indices returning to baseline in as little as 2 mins following completion of a two-minute, 30% MVC isometric exercise [345]. Boutcher et al. [345] demonstrated that there may be differences in autonomic function between younger and older adults. The authors showed that, following isometric exercise, older people (≈60 years old) had smaller increases in PEP than younger people (≈21 years old) due to the beta adrenergic influence on the myocardium becoming blunted with age [345, 355]. Older people displayed significantly higher BP and lower HRV throughout rest, during isometric contraction, and recovery, indicating reduced vagal control [345]. Furthermore, Sarmento et al. [349] found that sedentary older adults displayed significantly higher cardiac sympathetic activity, and lower vagal activity, than age-matched active older adults at baseline and during isometric exercise. Active older adults exhibited greater HR variance in response to isometric exercise, implying that not only age, but also physical fitness impacts ANS function [349].

To date, reliability of HRV to submaximal isometric exercise has not been examined. However, three studies examined the between-session reliability of the impact of isometric exercise on ANS variables, including mean arterial pressure, HR, SBP, DBP, and SCR [356-358]. Turley et al. [358] assessed cardiovascular responses to 3 mins of submaximal isometric contraction in healthy men at 10, 20, and 30% MVC two to three weeks apart. Submaximal contractions were performed from lowest to highest intensity in order to eliminate the chance of previously fatiguing contractions affecting cardiovascular responses. Absolute delta values were calculated as the exercise value minus the pre-exercise value [358]. In the case of cardiovascular indices, intraclass correlation coefficients were moderate for all contraction intensities: SBP (ICC = 0.44 – 0.69), DBP (ICC = 0.46 – 0.61), and HR (ICC = 0.57 – 0.87). Pepin et al. [357] performed 3 trials of supine isometric handgrip exercise at 30% MVC to fatigue in people with multiple sclerosis, with a minimum of 24 hours between trials. Reliability estimates at the point of fatigue were higher than the findings of Turley et al. [358]: mean arterial pressure (ICC = 0.88), and HR (ICC = 0.97) [357]. Finally, Faulstich et al. [356] analysed temporal stability (≈14 days) of psychophysical reactivity to a broad range of mental and physical stressors, including 3 mins of 15% MVC handgrip isometric exercise, using Pearson's correlation coefficient as opposed to ICC. Pearson test-retest correlations for autonomic variables were high during isometric exercise: HR (r = 0.73), SBP (0.67), DBP (r = 0.74), SCR (r = 0.54).

Several studies have examined both ANS and nociceptive outcome measures in response to isometric exercise in healthy people to assess the relationship between the two systems. Results consistently showed that pain thresholds elevated during and following isometric exercise in conjunction with increases in BP [291, 299, 311, 314, 320] and HR [311, 320]. Ring et al. [299] found that isometric exercise increased BP proportionally to the intensity of the contractions (1%, 15%, 25% MVC) and that pain ratings from electrical stimulation were attenuated in a linear fashion. Mediation analysis showed that differences in pain ratings between the three isometric exercises were fully accounted for by variations in DBP [299]. Umeda et al. [291, 314] also examined the relationship between changes in BP and changes in pain perception but found no correlation. BP elevated in a dose-response manner, with longer durations of isometric exercise producing greater elevations in BP. However, in contrast to Ring et al. [299], pain perception was not attenuated in a linear fashion with BP. Other studies have also examined the relationship between BP and nociception and found no correlation [291, 311, 314, 320]. Possible reasons for the discrepancy between studies include duration of the exercise, methodology, and mechanisms contributing to EIH other than BP elevations. Ring et al. [299] used a phasic model of pain, delivering electrical stimuli during the isometric exercise, while Umeda et al. [291, 314] used a tonic model of pain, applying a continuous pressure stimulus before and after manipulating BP through isometric exercise.

In sum, low intensity submaximal isometric exercise (20 – 25% MVC) can inhibit experimental pain at local and distant sites in healthy people, provided the contraction is held for > 2 mins. The ANS response to isometric exercise is variable depending on the intensity and duration, reflected by either: (1) reciprocal activation of the two autonomic branches; (2) dual activation of the two autonomic branches;

or (3) sole vagal withdrawal or sole sympathetic activation. Acute increases in BP and HR are also reliably associated with isometric exercise, yet evidence is mixed when correlating changes in BP to EIH. While BP responses may be graded in a dose-dependent manner with duration of exercise, changes in BP are not reliably associated with the magnitude of EIH.

2.9.3. Effects of acute isometric exercise on the nociceptive and autonomic nervous systems in people with chronic pain

The hypoalgesic response to isometric exercise in people with chronic pain can be variable for physiological and methodological reasons. This may include the kind of exercise being performed; its relative intensity; type of chronic pain condition [295]; and, whether painful muscles are involved in the exercise or not [117, 316]. This is evident by some groups experiencing hypoalgesia, hyperalgesia, or no change in pain sensitivity following isometric exercise [316]. The varied pain responses to exercise in chronic pain populations may also, in part, be due to functional deficits in descending nociceptive inhibitory pathways, including aberrant ANS and cardiovascular responses that initiate EIH [109, 320, 326, 359]. Other possibilities may comprise: reduced sympathetic activation, required to promote blood flow and oxygenation of muscles during exercise, thereby leading to increased ischaemic muscle pain; or, adrenal insufficiency in response to isometric exercise [360, 361].

In chronic pain populations, EIH can be impaired in distant locations as well as the exercising muscle [116, 117, 317, 326]. EIH is also dependent on whether the contracting body part is painful or not [117]. Kosek et al. [116] showed PPTs to decrease following isometric exercise in FM compared to controls. However, PPTs were only assessed at the contracting muscle and the increase in pain could be due to peripheral sensitisation [317]. FM pain can arise from peripheral factors, such as altered muscle fibre structure; sensitisation of muscle nociceptors (evident by increased EMG post-exercise); and/or muscle ischemia during exercise [113]. Indeed, muscle ischemia can be a strong driver of peripheral nociception due to the mechanical pressure of isometric exercise inducing nociceptive stimulation, which may mask descending inhibition of nociception or activate descending facilitatory centres in the brainstem that counteract descending inhibition [362-365]. Staud et al. [317] found that people with FM exhibited reduced thermal and mechanical pain thresholds after isometric exercise, not only on ipsilateral and contralateral sides, but also in remote contralateral muscles compared to healthy controls, indicating a more widespread/centrally mediated dysfunction of EIH in FM.

It appears that people experiencing joint pain and/or muscle pain can display different EIH responses. Some exercise-based studies have demonstrated impairment of EIH in people with FM [116, 317], and people with CFS [366] – a condition where widespread musculoskeletal pain is a cardinal feature [367]. However, studies involving isometric exercise have shown that, at least at the group level, EIH is intact in KOA populations [93, 368, 369]. Vaegter et al. [368] not only found a normal EIH response via increased PPTs post-exercise in 15 people with resting KOA pain (≈3 out of 10), but that EIH results were similar following TKA. Kosek et al. [370] also found EIH to be intact in KOA, showing an increase in PPTs during isometric exercise that was comparable to controls, even though the KOA group

demonstrated lower PPTs at baseline. Similarly, Friden et al. [371] found normal EIH by way of increased PPTs in postmenopausal women with RA. The authors postulated that EIH during isometric exercise may be intact in people with joint pain, e.g. OA [93, 368, 369] and RA [371], yet impaired in people with muscular pain when exercising painful muscles because pain facilitatory mechanisms from the brainstem, e.g. the RVM, can potentially override exercise induced pain inhibition [117, 372]. However, evidence of the EIH response between joint and muscle pain groups is not clear-cut. Subgroups of joint pain have been identified based on the function of endogenous pain inhibitory systems and whether the exercise involves loading the painful joint [93, 308]. As discussed in Section 2.4.3, CPM can be impaired in people with OA [15, 16]. Fingleton et al. [93] subdivided people with KOA into those with normal and abnormal CPM function. The authors assessed PPT in these two groups before and after submaximal isometric exercise and found that the KOA group with abnormal CPM demonstrated impaired EIH via decreased PPTs following exercise [93]. In contrast, the KOA group with normal CPM, as well as healthy controls, showed increased PPTs in response to isometric exercise, suggesting normal EIH function [93]. Interestingly, at baseline there were no differences between groups in terms of age, severity of X-ray degeneration, or number of people with bilateral knee pain [93]. Similarly, Burrows et al. [308] showed a discrepancy in EIH function when a KOA group exercised upper limbs versus lower limbs. In contrast to healthy, pain free controls, the KOA group only demonstrated EIH, via increased PPTs at all sites, when exercising the upper limbs. When the KOA group exercised the lower limbs, neither the upper nor lower limb PPTs increased significantly. These findings highlight how exercising remote, non-painful limbs may generate systemic hypoalgesia, as well as the individualised function of EIH in people with KOA [308]. The findings of Burrows et al. [308] are supported by previous research in other chronic pain populations. Lannersten et al. [117] compared PPTs at the quadriceps and infraspinatus muscles in people with shoulder myalgia and FM to healthy controls during isometric exercise. PPTs were measured during exercise of the contracted muscle, the resting homologous contralateral muscle, and distant contralateral muscle. PPTs increased at all sites during contraction of both muscles in healthy controls, but not in those with FM, who demonstrated reduced PPTs at all sites during contraction of both the quadriceps and infraspinatus muscles. In contrast, those with shoulder myalgia showed increased PPTs at all sites during contraction of the non-painful quadriceps, but not during contraction of the painful infraspinatus, suggesting that in people with localised pain conditions, EIH may be impaired when exercising a painful body part but remain intact when exercising a non-painful one [117, 326].

Section 2.9.2 revealed isometric exercise to reliably perturb the cardiovascular system in healthy people. However, evidence is limited when examining the effect of acute isometric exercise on the ANS in chronic pain populations. ANS dysfunction is postulated as a contributing factor to muscle pain conditions, such as FM, due to a ceiling effect of autonomic function [37-39]. Elevated baseline levels of SNS activity, and reduced vagal activity, found in FM can lead to attenuated adaptability to physical stress [38, 41, 113]. Martinez-Lavin [37] suggested that, due to basal sympathetic hyperactivity, chronic overstimulation of beta adrenergic receptors leads to their downregulation and/or desensitisation and, in turn, blunted cardiovascular reactivity to the stress of exercise. Reduced peripheral sympathetic

reactivity to muscle contraction is one suggested mechanism for pain when exercising [37]. Indeed, Elam et al. [373] showed people with FM to have decreased muscle sympathetic discharge compared to controls when performing a static hand grip exercise.

It could be expected, based on the divergent effects of acute mental stress on HR and BP, between pain free and chronic pain groups, that acute isometric exercise would show similar differences in how the cardiovascular system responds. However, multiple studies have demonstrated that HR and BP rise and fall to a similar extent in both populations in response to short duration isometric exercise [317, 320, 360, 361, 374]. No studies to date have examined the effects of isometric exercise on the ANS in OA populations. Kadetoff et al. [320] showed HR in FM participants to increase more than controls during muscle contraction but put this down to the FM participants not being as conditioned to exercise. It is possible that evidence of ANS dysfunction was not elicited in the chronic pain groups in these studies because either the exercise was too short [317], or because HR did not exceed 100 beats per minute (bpm) [320, 360]. Rowell [353] explains that when exercise is mild, and HR is < 100 bpm, elevations in HR can be sustained by vagal withdrawal alone. However, when exercise is more intense, inducing a HR of > 100 bpm, maintaining sufficient cardiac output requires both vagal withdrawal and sympathetic activation [353]. It is then, in this instance of sympathetic recruitment, that dysfunction in chronic pain populations may become evident. Indeed, Hallman et al. [359] found that people with chronic neck-shoulder pain demonstrated blunted BP responses to longer submaximal isometric exercise (≈3 mins) compared to controls. Giske et al. [361] elicited heart rates of > 100 bpm with repetitive submaximal isometric contractions until exhaustion and still no differences in HR were found between FM and healthy control groups. However, levels of plasma adrenaline were measured during and following muscle contractions and found to be significantly diminished in the FM group compared to controls [361]. Therefore, evidence of ANS dysfunction can become apparent via reduced reactivity of the sympathoadrenal reflex if the exercise is of sufficient duration and intensity [360].

It is challenging to delineate specific SNS and PNS effects from HRV because sympathetic and parasympathetic activities have reciprocal effects on each other, and are also affected by mechanical events unrelated to cardiac autonomic activity, such as atrial stretching or changes in thoracic pressure during respiration [170, 375, 376]. Only two studies have examined HRV in response to isometric exercise in people with chronic pain, both involving chronic neck-shoulder pain [359, 377]. The results of these studies provide no clear characteristic HRV response to acute isometric exercise for people with chronic pain because the outcome measures limit drawing specific conclusions about SNS and vagal function. In addition, both studies only included one type of pain condition. Hallman et al. [359] provided results for both LF and HF HRV, and showed LF HRV to increase in the pain group in response to a static handgrip exercise compared to controls. While the pain group displayed lower HF HRV at baseline, the effect on HF HRV to isometric exercise was not different between groups [359]. Shiro et al. [377] only reported the LF/HF ratio of HRV in response to bilateral, isometric trapezius contraction. Results showed the LF/HF ratio to significantly increase in controls during the exercise but not in the chronic pain group, with the authors concluding that people with chronic neck-shoulder pain display

“reduced sympathetic nerve activity” [377]. However, LF HRV is a poor indicator of SNS activity because the lower frequencies of HRV comprise an aggregate of activity from both sympathetic and parasympathetic branches [170, 175]. Consequently, Billman [170] states that the LF/HF ratio cannot accurately quantify cardiac sympathovagal balance due to the complex, non-linear, non-reciprocal interactions between the SNS and PNS. The LF/HF ratio is also influenced by mechanical factors such as respiration [375, 376]. Increases or decreases in respiration frequency can alter the RR interval and, hence, HRV [170, 375, 376].

In sum, people with chronic pain display variable EIH, and mixed effects on the ANS, following acute isometric exercise with a number of factors that seem to influence results, including: the nature of the pain condition; whether the painful limb is exercised or not; the intensity of the exercise; and, the baseline integrity of descending pain inhibitory mechanisms. Except for increases in SNS activity, evidence is currently lacking to elucidate specific ANS response patterns to acute isometric exercise in chronic pain groups. Furthermore, no studies to date have examined the ANS response to acute isometric exercise in people with OA.

Chapter 3. Nociceptive and autonomic function in people with knee osteoarthritis and fibromyalgia

3.1. Introduction

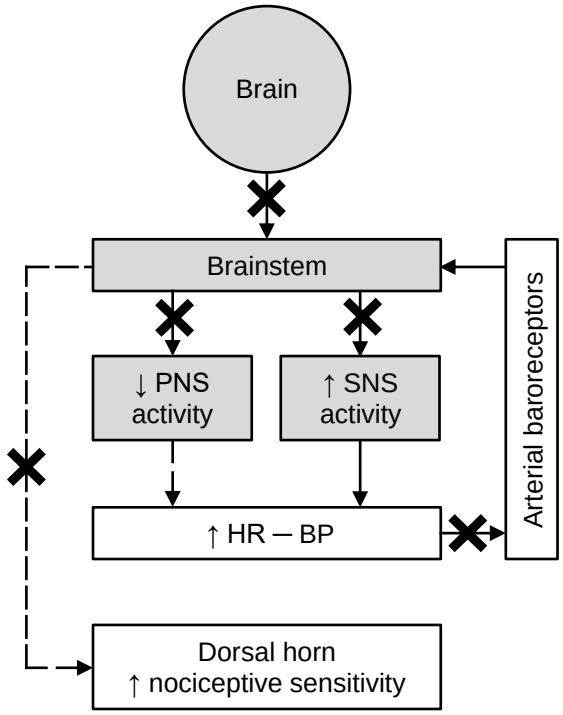
The following chapter outlines the methodology used by describing the study aim, design, participants, procedure, outcome measures, and statistical analyses.

The aim of the first study was to examine for evidence of ANS dysfunction and altered nociceptive processing in people with KOA and FM at rest; and, in addition, to examine the effects of nociceptive stress on the nociceptive and autonomic nervous systems. The following specific hypotheses were tested:

I. At rest, people with KOA and FM (Figure 6) would exhibit reduced HF HRV, reduced PEP, increased EDA; reduced PPT, HPT, and CPT; and, impaired CPM compared to pain free controls (Figure 7).

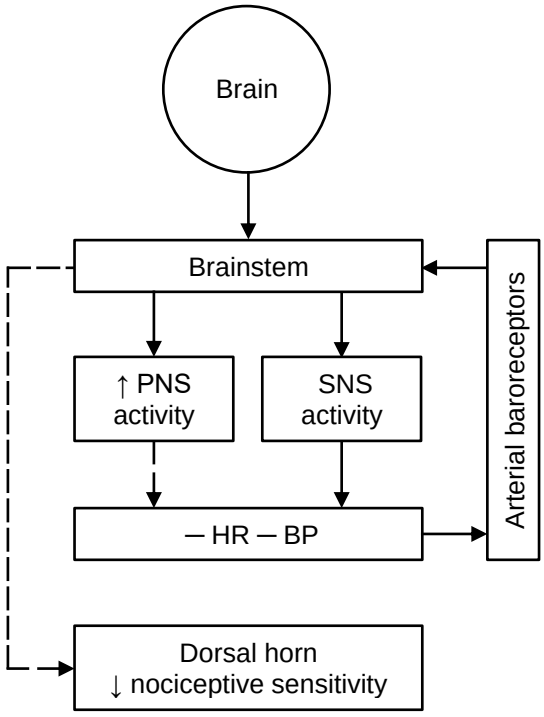
II. Immediately following cold water conditioning, pain free controls would demonstrate a reduction in HF HRV, reduced PEP, and increased EDA. In comparison to controls, these changes will be reduced in the KOA and FM groups.

Figure 6. ***Hypothesis of reduced high frequency heart rate variability, reduced pre-ejection period, increased electrodermal activity, and increased nociceptive sensitivity in people with knee osteoarthritis at rest.***



Note. BP = blood pressure; grey = area of dysfunction; HR = heart rate; PNS = parasympathetic nervous system; SNS = sympathetic nervous system; ⟶ = excitation; ⇢ = inhibition; ✕ = dysfunctional pathway

Figure 7. ***Hypothesis of increased high frequency heart rate variability and reduced nociceptive sensitivity in a pain free control at rest.***



Note. BP = blood pressure**;** HR = heart rate; PNS = parasympathetic nervous system; SNS = sympathetic nervous system**;** ⟶ = excitation; ⇢ = inhibition; ─ = within normal limits

3.2. Method

3.2.1. Study design

The study was a cross-sectional, single session design undertaken at the AUT Biomechanics Laboratory, North Shore Hospital, Auckland.

3.2.2. Sample size

A sample size calculation was undertaken using an alpha level of 0.05 and power of 0.8 with G*Power 3.1.9.2 [378]. HF HRV was chosen as the primary outcome measure because HRV has not previously been investigated in people with OA. An effect size of 0.8 was determined based on a previous study investigating HF HRV in people with RA compared to healthy controls at rest [379]. Due to limited literature to guide effect size to power the sample size calculation, RA is the most similar arthritide to OA. A two-group *t*-test with .05 one-sided significance level revealed that for an effect size of 0.8, 21 participants were required per group. For three groups, the total number of participants required was *N* = 63. The anticipated effect size for the difference in HF HRV between people with FM and controls is similar [31], therefore, the sample size will be adequate to detect this difference.

3.2.3. Participants

Participants for the study were sourced by advertising (Appendix A) in outpatient departments at the local hospital, web-based social media, and professional networks. Participants were assigned to one of three groups: people with KOA, people with FM, and healthy, pain free controls. Controls were matched for age and gender for the KOA group. The KOA and FM groups were not individually matched. Controls were selected so the overall age and gender group characteristics were equivalent to the KOA and FM groups. Thus, a larger sample size of controls was recruited to match the disparate age and gender characteristics of the KOA and FM groups.

Participants included in the KOA and FM groups were required to meet the following inclusion criteria:

- Aged 18 years and over.
- Diagnosed by a clinician with KOA or FM and fulfilled ACR criteria [46, 380, 381].
- Had experienced pain for at least 3 months on most days.
- Had experienced pain with a minimum level of 3 out of 10 in the previous 7 days.

Participants were included in the healthy control group if they satisfied the following inclusion criteria:

- Aged 18 years and over.
- Pain free and not on pain medication.
- No history of chronic pain.
- Did not suffer any major neurological, psychological or cardiovascular disorder.

Participants were excluded from the study if they had any of the following:

- Cardiac conditions; e.g. arrhythmia or pacemaker due to unwanted artefacts that affect beat-to-beat signals [382].
- Hypertension; i.e. systolic BP > 140 mmHg and/or diastolic BP > 90 mmHg [253].
- Medications that affect cardiovascular activity; e.g. calcium channel blockers [23] or beta blockers [104].
- Medications that may alter activity of the ANS; e.g. sympathomimetic drugs that stimulate the SNS, such as adrenaline; sympatholytic drugs that inhibit the SNS, such as beta blockers [383]; or anxiolytics, such as benzodiazepine [359].
- Inability to provide informed consent and/or comprehend fluent English.
- Current cigarette smoker.

Demographic information was collected from each participant and the nature of each participant's pain from the FM and KOA groups determined using the following:

- Brief Pain Inventory (BPI; Appendix B). The BPI is designed to measure pain severity (BPI severity), and the extent to which that pain interferes in the life of a person with chronic pain (BPI interference). The BPI assesses pain at its worst, least painful, average level, and current state ("right now") using a 0 to 10 numerical rating scale for each item. BPI severity is reported as the mean of these four pain items. The BPI measures how much pain interferes with seven daily activities, including general activity, walking, work, mood, enjoyment of life, relations with other people, and sleep. Also using a numerical rating scale, 0 implies no interference, while 10 is complete interference. BPI interference is reported as the mean of these seven interference items. The BPI is reported to be valid for people with non-cancer, chronic pain conditions [384-386].

- Pain Catastrophising Scale (PCS; Appendix C). Pain catastrophising can lead to worsening of chronic pain [387]. The PCS is a 13-item tool derived from definitions of catastrophising [56, 388]. A total PCS score of 30 out of 52 corresponds to the 75th percentile of PCS scores in samples of people with chronic pain and represents a clinically relevant level of catastrophising [388]. People who score between the 50th and 75th percentile on the PCS are considered at moderate risk for the development of chronicity [388]. The PCS has been used as a screening measure for risk of prolonged pain and disability, and has shown good reliability and validity in people with chronic pain [56, 389, 390].

- Depression, Anxiety, and Stress Scales (DASS-21; Appendix D). The DASS-21 is a self-report measure divided into three subscales of low positive affectivity (depression), physiological hyperarousal (anxiety), and stress [391]. Each subscale is divided into 7 four-point items, rated 0 to 3 [392]. Results for depression, anxiety, and stress are calculated by summing the scores of the relevant items for each subscale, ranging from normal to severe. The DASS-21 has been reported to show good reliability and validity in the assessment of common mental health problems [391-395].

- Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC; Appendix E). The WOMAC is a widely used, valid and reliable tool for assessing pain, stiffness, and physical function in people with OA of the hip and knee [396, 397]. The WOMAC pain subscale consists of 5 items; the stiffness subscale, 2 items; and, the physical function subscale consists of 17 items. All items are scored on a numerical rating scale from 0 to 4, with higher scores representing more pain or difficulty. Maximum possible scores for the pain, stiffness, and physical function subscales are 20, 8, and 68 respectively [398].

Participants were asked to refrain from taking analgesic medication, e.g. opioidergic [23, 93, 104] or anti-inflammatory medication [359], for 24 hours prior to data collection, and from taking caffeine and tobacco products 6 hours prior to data collection [104].

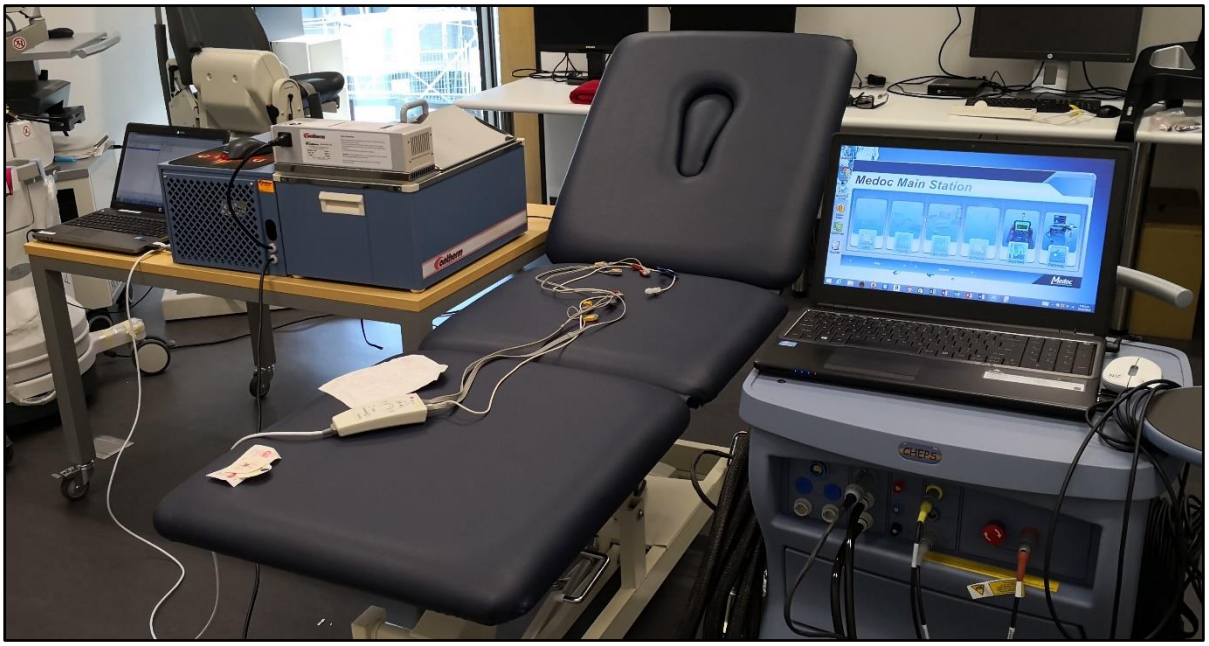
3.2.4. Ethical considerations

Ethical approval was obtained from the Health and Disability Ethics Committees (HDEC): approval number 18/CEN/45 (Appendix F). Consultation was also provided by the Matauranga Maori Committee. During the design and implementation of the study the principles of the Treaty of Waitangi, including partnership, participation and protection were applied, and the recruitment process ensured that all eligible participants had equal opportunity to take part in the study regardless of ethnicity. Each participant was fully informed with a participant information sheet (Appendix G) and consented to participation in the study by signing a consent form (Appendix H). The privacy of each participant was maintained at all times by assigning participant codes.

3.2.5. Study procedure

Figure 8 shows the laboratory set-up for assessing nociceptive and ANS outcome measures, which included, from left to right: a laptop computer to record ICG/ECG cardiovascular data using Cardio Vascular Lab (Medis, Germany); a circulating, temperature-controlled, cold water bath (Contherm, NZ) to assess CPM; an ICG device (CardioScreen 1000, Medis, Germany) with custom ICG sensors (Medis, Germany) to capture haemodynamic parameters; and, the Medoc PATHWAY Model ATS Pain and Sensory Evaluation System (Medoc, Israel) that incorporated an algometer, a thermode, and a computerised visual analogue scale (COVAS).

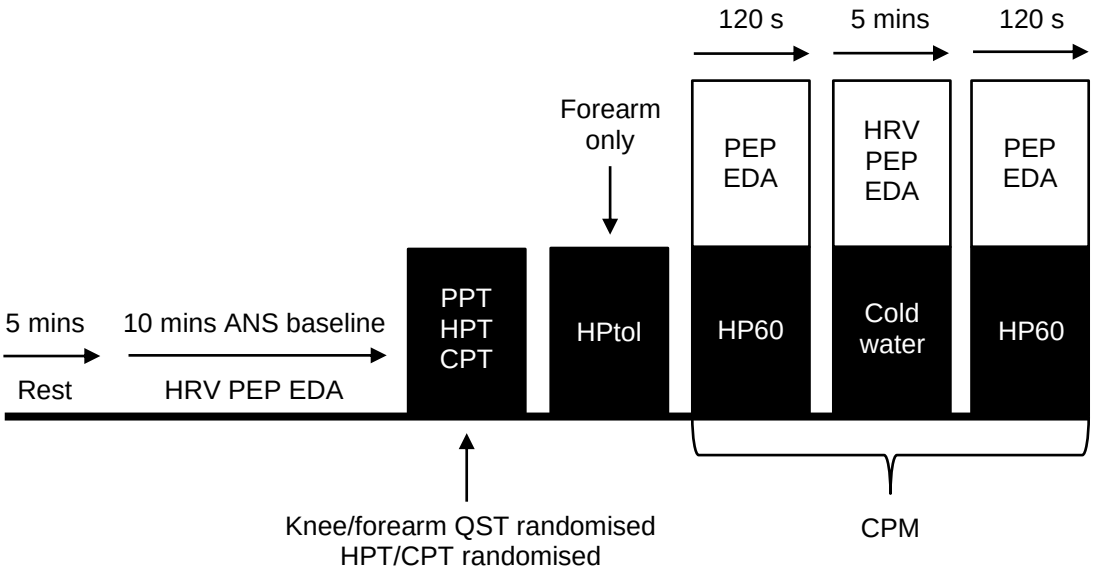
Figure 8. ***Laboratory set-up for assessing nociceptive and autonomic nervous system outcome measures.***



Following the collection of demographic information, participants were asked to recline in the supine position with the torso elevated at 30° for 5 mins before resting ICG/ECG and EDA data were recorded (Figure 9). HRV, PEP, and EDA were continuously recorded for 10 mins to establish an autonomic baseline prior to QST assessments (Figure 10). QST outcome measures were then recorded at the knee and forearm in random order. PPT was recorded first, followed by heat and cold pain thresholds in random order. Heat pain tolerance (HPtol) was measured last, before CPM (Figure 10). The measurement of thresholds and tolerance is outlined in Section 3.2.6. The heat test stimulus for CPM was applied on the volar forearm of all participants at an individualised temperature that elicited a mean heat pain intensity of 60 out of 100, known as heat pain 60 (HP60). The formula for determining HP60 is described in Section 3.2.6. After calculating the participant's HP60, CPM testing was performed. The HP60 stimulus was applied continuously for 120 s, before and after cold water conditioning. PEP and EDA were continuously recorded during this time. The length of time required for optimal analysis of HRV is at least 5 mins [163]. Therefore, cold water conditioning for CPM was 5 mins in length. All ANS variables were recorded during this time.

Figure 9. ***Participant resting at 30° before and during autonomic baseline recording.***

Figure 10. ***Procedure for cross sectional study assessing nociceptive and autonomic nervous system outcome measures.***



Note. ANS = autonomic nervous system; CPT = cold pain threshold; EDA = electrodermal activity; HP60 = heat pain 60 (0 – 100); HPT = heat pain threshold; HPtol = heat pain tolerance; HRV = heart rate variability; PEP = pre-ejection period; PPT = pressure pain threshold

3.2.6. Nociceptive outcome measures

Nociceptive processing was assessed using static and dynamic QST measures. Static QST outcome measures included: PPT, HPT, and CPT. Dynamic QST outcome measures were HP60 pain ratings (recorded using a COVAS), and CPM.

I. Pressure pain threshold

PPT was assessed using a computerised pressure algometer with a 1 cm^2 rounded tip (AlgoMed, Medoc, Israel). The algometer was placed perpendicular to the skin at two locations: one test site and one control site. For the KOA group, the test site was 2 cm distal to the inferior edge of the medial patella on the involved knee [15], while the control site was the volar forearm on the ipsilateral limb, 5 cm distal to the elbow along the radial border [15]. For the FM group, the test site was the left volar forearm only, 5 cm distal to the elbow along the radial border [15]. For the control group, the two sites were the same as the KOA group, except involving left limbs only. Algometer pressure increased at a constant rate of 30 kPa/s. Participants were instructed to report when the sensation produced by the probe changed from pressure to pain (PPT) and the value recorded. The procedure was performed three times at each location with a 30 s interval between stimuli and the average used for further analysis [368].

II. Heat pain threshold

HPT was evaluated with a 30 x 30 mm thermode (PATHWAY Model ATS, Medoc, Israel) applied to the skin at the two locations described above. Figure 11 shows the thermode placement at the knee and Figure 12A shows the thermode placement at the forearm. Thermode temperature was initially set at 32°C and slowly increased at a rate of 0.3°C/s [91]. Participants were instructed to report when the sensation produced by the thermode changed from heat sensation to pain (HPT). The procedure was performed three times at each location with a 30 s interval between stimuli and the average used for further analysis.

Figure 11. ***Thermode placement at the knee for assessment of heat/cold pain threshold.***

III. Cold pain threshold

CPT was evaluated with a 30 x 30 mm thermode (PATHWAY Model ATS, Medoc, Israel) applied to the skin at the two locations described above. Figure 11 shows the thermode placement at the knee and Figure 12A shows the thermode placement at the forearm. Thermode temperature was set at 32°C and slowly decreased at a rate of 1°C/s from baseline to a minimum temperature of 0°C [103]. Participants were instructed to report when the sensation produced by the thermode changed from cold sensation to pain (CPT). The procedure was performed three times at each location with a 30 s interval between stimuli and the average used for further analysis.

IV. Heat pain tolerance

HPtol was recorded identically to HPT, except the test site was always the volar forearm (Figure 12A). Participants were instructed to allow the heat pain stimulus to rise beyond their HPT, and report when the heat pain produced by the thermode became unbearable (HPtol). The procedure was performed three times and averaged to determine HPtol. HPtol was only recorded for the calculation of HP60 and not used as an outcome measure.

V. Heat pain 60 and computerised visual analogue scale scores

The HP60 test stimulus used for CPM in this study was applied to the volar forearm (Figure 12A) for 120 s at a constant temperature that elicited a mean pain intensity of 60 out of 100. HP60 is an individually adapted temperature based on HPT and HPtol values using the following formula: HPT + ([HPtol – HPT]/2) [91]. If a participant's pain rating was not 60 out of 100, the temperature was adjusted incrementally by 0.5°C to achieve HP60. Participants were told the temperature may increase, decrease, or remain constant during the 120 s heat test stimulus interval to reduce expectations [91]. Participants rated their pain continuously using a COVAS (Figure 12C), ranging from 0 (no pain) to 100

(worst pain imaginable). HP60 scores were recorded to quantify pain ratings at baseline and during the stressor, and to calculate CPM.

VI. Conditioned pain modulation

CPM is a dynamic QST measure. CPM effectiveness was determined by comparing two-minute HP60 scores (Figure 12C) before and after painful cold water conditioning (Figure 12B) based on methodology used by Chalaye et al. [91]. Heat pain was the test stimulus upon which the conditioning effect of immersing the arm in painfully cold water was tested [399]. The 30 x 30 mm thermode (PATHWAY Model ATS, Medoc, Israel) was applied to the volar forearm in all groups, and the HP60 test stimulus applied for 120 s. Participants were instructed to rate the heat pain continuously using the COVAS (Figure 12C) and told the temperature may increase, decrease, or remain constant during the 120 s heat test stimulus interval to reduce expectations [91]. For the conditioning stimulus of cold water, participants immersed their arm opposite to the heat pain stimulus (contralateral to the involved knee in the KOA group) up to the wrist in 12°C cold water for 5 mins and were told not to move or contract their arm during immersion [91]. If the person was not able to withstand the cold pain, they were permitted to move their hand in and out of the water for 10 s as required in order to achieve 5 mins. Participants were instructed to rate the cold pain intensity continuously using the COVAS. The HP60 test stimulus was applied a second time, at the original HP60 temperature, immediately following the 5-minute conditioning period. Participants again rated the heat pain continuously using the COVAS for 120 s and told the temperature may increase, decrease, or remain constant. CPM magnitude was calculated by subtracting the mean HP60 score of the second heat test stimulus from the first. CPM magnitude was reported as percentage change of the mean HP60 score [400]. Effective pain inhibitory mechanisms are reported as negative values (pain inhibited after conditioning) and pain facilitation reported as positive values (pain increased after conditioning) [400].

Figure 12. ***Thermode placement at the forearm for assessment of heat/cold pain threshold, heat pain tolerance, and heat pain 60 out of 100 (A); cold water conditioning at 12℃ with the arm immersed up to the wrist (B); and, the COVAS with finger-controlled sliding scale (C).***

A **B** **C**

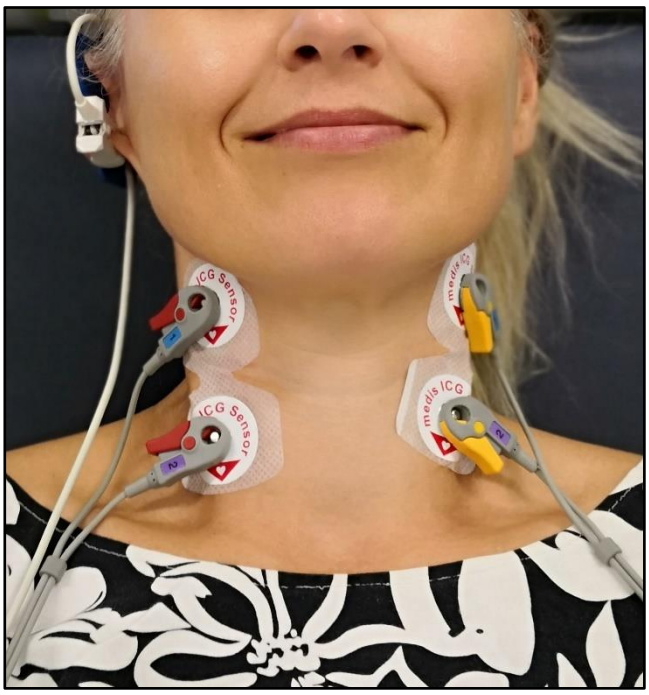
3.2.7. Autonomic nervous system outcome measures

Autonomic outcome measures assessed were: HF HRV, PEP, SCL, SCR, and HR.

I. Heart rate variability

Data for the calculation of HRV is the sequence of time intervals between RR intervals, determined from continuous ECG recordings using Cardio Vascular Lab (Medis, Germany). Pregelled 46 mm x 88 mm Ag/AgCl ICG/ECG electrodes were placed at each side of the participant's neck (Figure 13) and on each side of the thorax along the midaxillary line. The ECG signal was sampled at 200 Hz and time locked to the R wave. Kubios HRV Premium version 3.5.0 (Kubios Oy, Finland) was used to examine the variability of RR intervals, including time and frequency domains, and automatically reject artefacts in the ECG [401, 402]. The following time-domain variables were recorded for analysis: mean HR, standard deviation of instantaneous HR values (STD HR), and the square root of the mean squared differences between successive RR intervals (RMSSD), an HRV parameter that indicates parasympathetic function, which can be used as a measure of short term variability [401]. The generalised frequency bands for short term HRV recordings are VLF (0-0.04Hz) [175], LF (0.04-0.15Hz) [403] and HF (0.15-0.4Hz) [173]. In frequency-domain methods, a power spectrum density estimate is calculated for RR interval series, and frequency bands are extracted from this. The frequency band extracted for this study was the absolute and relative power of HF, and the HF band power in normalised units (nuHF), obtained over a 5-minute period. Normalised spectral HRV measures express quantities on a percentage scale basis and nuHF is the index of modulation of parasympathetic activity [175]. A higher score indicates greater parasympathetic activity; therefore, HF HRV (nu) was used as the main HRV outcome measure.

Figure 13. ***Electrode placement at the neck for impedance cardiography recording, including ground clip on the ear lobe.***



II. Pre-ejection period

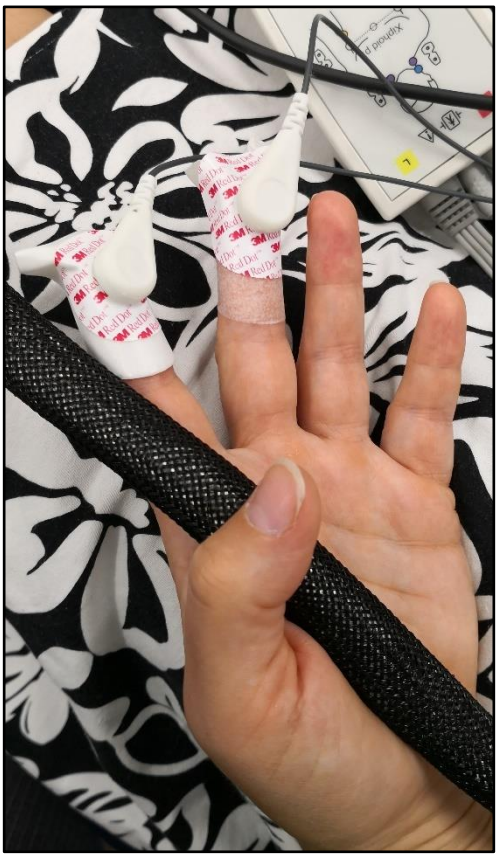
Using the same data from the ICG/ECG recording, ICG/ECG data was collected and analysed using Cardio Vascular Lab (Medis, Germany). Each heart beat changes the volume and velocity of blood in the aorta, producing a change in electrical resistance (dZ) of the thorax to electrical alternating current. This is called the impedance pulsatile signal (IMP), generated and recorded from an external electrical

signal passed across the midaxillary line (Figure 4). The ICG signal is the change in impedance over time (dZ/dt) and is the mathematical first derivative of the dZ (IMP) tracing [189]. The ECG signal was sampled at 200 Hz and time locked to the R wave to enable 30-second ensemble averaging of the ICG (dZ/dt) signal for data analysis. PEP was computed as the time period between the Q wave on the ECG signal, and the upstroke (B point) on the ICG (dZ/dt) signal [164]. PEP, indexing the interval between onset of electrical stimulation of the ventricles and aortic valve opening, is expressed in milliseconds. Increases in sympathetic activity results in shortening of PEP [164]. To describe changes in cardiac sympathetic reactivity, the mean PEP of the last 30 s of each minute for each stressor ($PEP_{stressor}$), e.g. cold water conditioning, was subtracted from the mean PEP of the last 30 s of each minute at baseline ($PEP_{baseline}$) [164, 404, 405]. $PEP_{baseline}$, $PEP_{stressor}$, and ΔPEP were reported.

III. Skin conductance level and response

EDA was recorded by placing a pair of 6 cm diameter pregelled Ag/AgCl electrodes (Red Dot, 3M) on the palmer tips of the index and middle fingers of the hand (Figure 14), after being pre-treated with ethanol wipes, at least 10 cm away from any region on the forearm receiving thermal stimulation [142]. Data were sampled at 32 Hz using a NeXus-10 MKII and BioTrace software (MindMedia, Netherlands). Two values of EDA were determined from the recorded data: tonic and phasic EDA, which were measured at baseline and during the stressor. Tonic EDA is referred to as the tonic level of electrical conductivity of the skin, or skin conductance level (SCL) [168]. Skin conductance response (SCR) is referred to as the phasic change in electrical conductivity of the skin, typically in response to a stimulus. SCL at baseline and during the stressor were calculated as the mean amplitude of the EDA signal during the last 10 s of each minute [128]. SCR was calculated by determining the number of events (spikes per minute) at baseline, and during the stressor, with a threshold level of 0.03 µS (Figure 5) [168, 194]. Changes in tonic EDA were calculated by subtracting the mean $SCL_{stressor}$ from the mean $SCL_{baseline}$. Changes in phasic EDA were calculated by subtracting the mean $SCR_{stressor}$ from the mean $SCR_{baseline}$. $SCL_{baseline}$, $SCR_{stressor}$, ΔSCL, and ΔSCR were reported.

Figure 14. ***Electrode placement at the fingertips for recording of electrodermal activity.***



3.2.8. Data analysis

Normality of distribution of data was analysed using the Kolmogorov-Smirnov test and variables that were not statistically significant were classed as having normal distribution. Normally distributed dependent variables were analysed by parametric analyses.

To compare characteristics of the three groups, continuous variables were compared between groups using one-way analysis of variance (ANOVA). Significant main effects were followed up with two-sided Dunnett's test to compare the KOA and FM groups to the control group. Ordinal data from questionnaires was compared between groups using Kruskal-Wallis tests, with follow-up Mann-Whitney U tests for significant results. Gender distribution across the groups was analysed using the chi-square test.

To compare the outcome measures at baseline among the three groups, one-way ANOVA was used. Significant main effects were followed up with one-sided Dunnett's test to compare the KOA and FM groups to the control group. Comparisons were not made between KOA and FM groups because this was not an outcome of interest and the studies were not powered to detect this. To determine the effect of cold water conditioning, paired *t*-tests were used to compare baseline and post cold water conditioning data within each group. Effect sizes and 95% confidence intervals were determined from the difference scores between the KOA-control and FM-control comparisons.

Difference values (i.e. change scores) in outcome measures from baseline to immediately following cold water conditioning were compared between groups using a one-way ANOVA. Significant main effects were followed up with one-sided Dunnett's test to compare the KOA and FM groups to the control group.

For equivalent non-parametric analyses, between group comparisons were made using Kruskal-Wallis tests, with follow-up Mann-Whitney U tests for significant results. Within group comparisons were made using Wilcoxon Signed Rank tests. The alpha level for all statistical procedures was set to .05, and all statistical analyses were performed using IBM SPSS Statistics for Windows version 28 (Armonk, NY: IBM Corp).

3.3. Results

3.3.1. Recruitment

During the period of recruitment, the worldwide COVID-19 pandemic led to several lockdown periods in the Auckland region, and a general hesitancy on the part of participants to attend hospital-based appointments for research involving face-to-face data collection. As such, only forty-two of the planned 63 participants were able to be recruited: 15 pain free people, 14 people with KOA, and 13 people with FM. All 42 participants met the inclusion criteria and completed the study. It was discovered during data processing that one control participant exhibited a previously undiagnosed ectopic beat, and another

control participant displayed an abnormal breathing pattern during baseline recording. These anomalies impacted measurements of HF HRV; therefore, HF HRV data was removed from data analyses for these two participants. Data collection took place from May 2019 to June 2021.

3.3.2. Participant characteristics

Participant characteristics are shown in Table 1. The age of participants ranged from 21 to 75 years, with 8 male and 34 female participants.

Table 1. ***Participant characteristics. Data are mean (SD) unless otherwise stated.***

Characteristic	Control *n* = 15	KOA *n* = 14	FM *n* = 13	Omnibus *p* value
Age, years	53 (10)	60 (9)	47 (14)	.17
Gender female, *n* (%)	11 (73)	10 (71)	13 (100)	.11
Height, cm	173 (9)	173 (9)	164 (6)*	.01#
Weight, kg	75 (12)	94 (25)*	84 (13)	.03#
BMI, kg/m^2	25 (3)	31 (7)*	31 (5)*	.003#
BPI severity	2 (3)	18 (10)*	20 (7)*	< .001#
BPI interference	1 (1)	30 (19)*	36 (19)*	< .001#
DASS-21:				
Depression	1 (1)	4 (3)*	6 (4)*	< .001#
Anxiety	1 (1)	3 (5)	6 (4)*	< .001#
Stress	2 (2)	5 (4)*	9 (5)*	< .001#
PCS	6 (7)	17 (13)*	22 (11)*	< .001#
WOMAC		44 (23)		

Note. BMI = Body Mass Index; BPI = Brief Pain Inventory; DASS-21 = Depression, Anxiety and Stress Scales; FM = fibromyalgia; KOA = knee osteoarthritis; PCS = Pain Catastrophising Scale; SD = standard deviation; WOMAC = Western Ontario and McMaster Universities Osteoarthritis Index; * = significant difference from control; # = significant difference between groups

There was a significant difference among groups for age ($F_{2,39}$ = 4.75, p = .01); however, follow-up two-sided Dunnett's test was not significant for the KOA-control comparison (p = .17) and the FM-control comparison (p = .27). There were no significant differences in gender between groups ($\chi^2(2)$ = 4.45, p = .11). There was a significant difference in height, weight, and body mass index (BMI) among groups. The KOA group were similar in height to controls (p = .99), but were significantly heavier (p = .02), and with a significantly greater BMI (p = .01). The FM group were significantly shorter than controls (p = .01), similar in weight (p = .33), but with a significantly greater BMI (p = .004). As expected, BPI severity,

BPI interference, DASS-21, and PCS values were all significantly higher in the KOA and FM groups compared to control (all $p < .001$). The mean WOMAC score for the KOA group was 44 ($SD = 23$).

3.3.3. Data distribution

The following ANS variables were not normally distributed and were analysed using non-parametric methods: SCR, ΔHF HRV, ΔSCR, and ΔPEP, where delta is the difference from baseline to immediately following cold water conditioning. The remaining ANS variables were normally distributed and were analysed using parametric methods.

The following QST variables were not normally distributed and were analysed using non-parametric methods: HPT and CPT for the arm, as well as PPT for both the knee and arm. The remaining QST variables were normally distributed and were analysed using parametric methods.

3.3.1. Pressure pain threshold

PPT results are shown in Table 2. There was no significant difference between the KOA and control groups in PPT at the knee ($H_1 = 1.60$, $p = .21$). There were also no significant differences between all three groups in PPT at the arm ($H_2 = 3.78$, $p = .15$).

3.3.2. Heat pain threshold

HPT results are shown in Table 2. There were significant differences among groups in HPT at the knee ($F_{2,39} = 3.36$, $p = .045$); however, follow-up one-sided Dunnett's test was not significant for the KOA-control comparison ($p = .95$) and the FM-control comparison ($p = .12$). There were no significant differences among groups in HPT at the arm ($H_2 = 5.80$, $p = .06$).

3.3.3. Cold pain threshold

CPT results are shown in Table 2. There were no significant differences among groups in CPT at the knee ($F_{2,39} = 0.86$, $p = .43$) or CPT at the arm ($H_2 = 2.28$, $p = .32$).

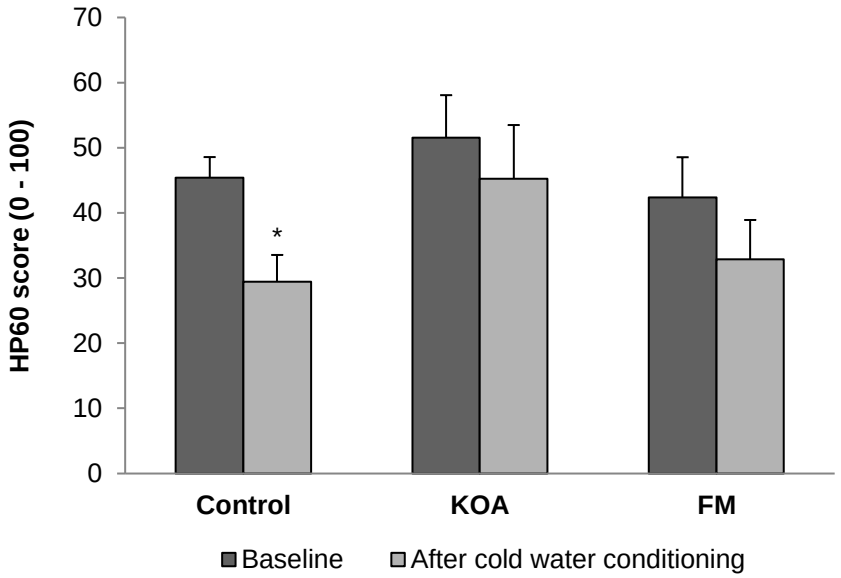
3.3.4. Heat pain 60 scores and conditioned pain modulation

Group results for HP60 scores are shown in Figure 15. While HP60 scores often began at 60 out of 100, pain ratings diminished over time to a similar extent in all three groups. Indeed, there were no significant differences among groups in HP60 scores at baseline ($F_{2,39} = 0.74$, $p = .48$). Therefore, baseline pain ratings were equivalent across all three groups (Table 2). There were no significant differences among groups in cold water pain ($F_{2,39} = 0.82$, $p = .45$). Therefore, all three groups experienced similar pain intensity during the conditioning stimulus (Table 2). Cold water conditioning led to a significant decrease in HP60 scores for the control group ($t_{14} = 4.33$, $p < .001$). In contrast, the KOA ($t_{13} = 1.23$, $p = .24$) and FM ($t_{12} = 1.66$, $p = .12$) groups did not show significant change in HP60 scores immediately following cold water conditioning. The responder rate for each group, demonstrating

a CPM effect (i.e. negative percentage change indicating a reduction of pain sensitivity after conditioning), is shown in Table 2.

HP60 scores and CPM results are shown in Table 2. No significant differences in percentage change values were found among groups in CPM from baseline to immediately following cold water conditioning ($F_{2,39}$ = 1.05, p = .36).

Figure 15. ***Heat pain 60 scores at baseline and immediately following cold water conditioning.***



Note. FM = fibromyalgia; HP60 = heat pain 60; KOA = knee osteoarthritis; * = significant difference from baseline; error bars are one standard error of the mean

Table 2. ***Raw QST and CPM values. Data are mean (SD).***

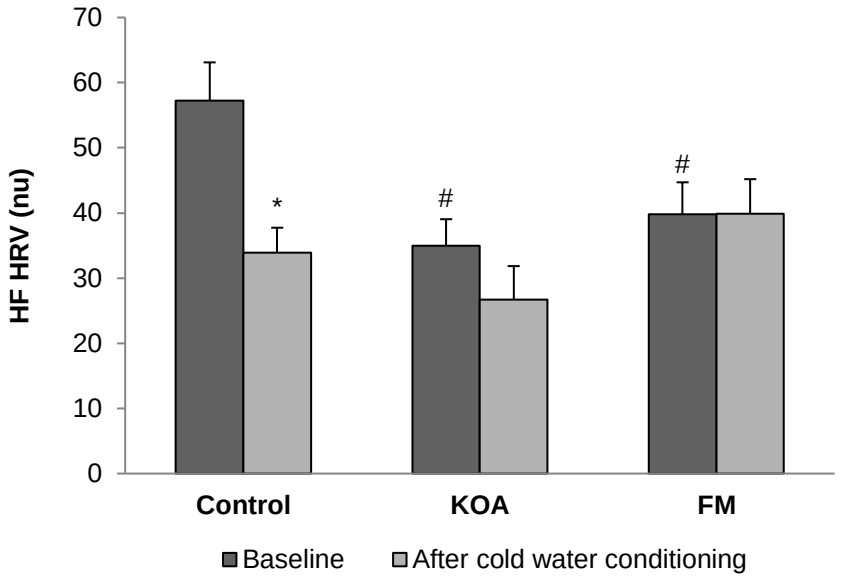
Variable	Control *n* = 15	KOA *n* = 14	FM *n* = 13	Omnibus *p* value	ES (95% CI) KOA-control	ES (95% CI) FM-control
PPT knee, kPa	321 (140)	240 (118)		.21	0.62 (-0.12 – 1.37)	
PPT arm, kPa	278 (127)	233 (91)	190 (94)	.15	0.41 (-0.33 – 1.14)	0.78 (0.01 – 1.55)
HPT knee, °C	39.2 (3.6)	40.5 (2.6)	37.5 (2.6)	.045[#]	-0.41 (-1.15 – 0.32)	0.54 (-0.22 – 1.29)
HPT arm, °C	39.2 (3.6)	39.2 (3.2)	36.6 (2.9)	.06	0.00 (-0.73 – 0.73)	0.79 (0.02 – 1.56)
CPT knee, °C	11.1 (12.3)	14.9 (13.6)	17.3 (11.8)	.43	0.29 (-0.44 – 1.03)	0.51 (-0.24 – 1.27)
CPT arm, °C	12.4 (12.5)	17.7 (11.8)	20.1 (8.6)	.32	0.44 (-0.30 – 1.17)	0.71 (-0.06 – 1.47)
HP60 score, 0 – 100:						
Before cold water conditioning	45.45 (12.11)	51.57 (24.36)	42.35 (6.20)	.48	0.32 (-0.41 – 1.06)	-0.32 (-1.06 – 0.43)
After cold water conditioning	29.45 (15.88)	45.24 (30.86)	32.90 (21.70)		0.65 (-0.10 – 1.40)	0.18 (-0.56 – 0.93)
CPM, % change	-35.83 (27.47)	-17.51 (36.86)	-15.10 (57.88)	.36	0.57 (-0.18 – 1.31)	0.47 (-0.28 – 1.22)
CPM responder rate, *n* (%)	14 (93)	10 (71)	8 (62)			
Cold water pain, 0 – 100	56.53 (21.19)	68.01 (25.23)	63.57 (26.80)	.45	0.49 (-0.25 – 1.23)	0.29 (-0.45 – 1.04)

Note. CI = confidence interval; CPM = conditioned pain modulation; CPT = cold pain threshold; ES = effect size; FM = fibromyalgia; HPT = heat pain threshold; HP60 = heat pain 60; KOA = knee osteoarthritis; PPT = pressure pain threshold; QST = quantitative sensory testing; SD = standard deviation; [#] = significant difference between groups

3.3.5. Heart rate variability

Group results for HF HRV are shown in Figure 16. There were significant differences among groups in HF HRV at baseline ($F_{2,37}$ = 5.49, p = .01). Follow-up one-sided Dunnett's test demonstrated both the KOA (p = .003) and FM (p = .02) groups to have significantly lower HF HRV at baseline compared to the control group. Cold water conditioning led to a significant decrease in HF HRV for the control group (t_{12} = 6.49, p < .001). In contrast, the KOA (t_{13} = 1.10, p = .07) and FM (t_{12} = -0.04, p = .97) groups did not show significant change.

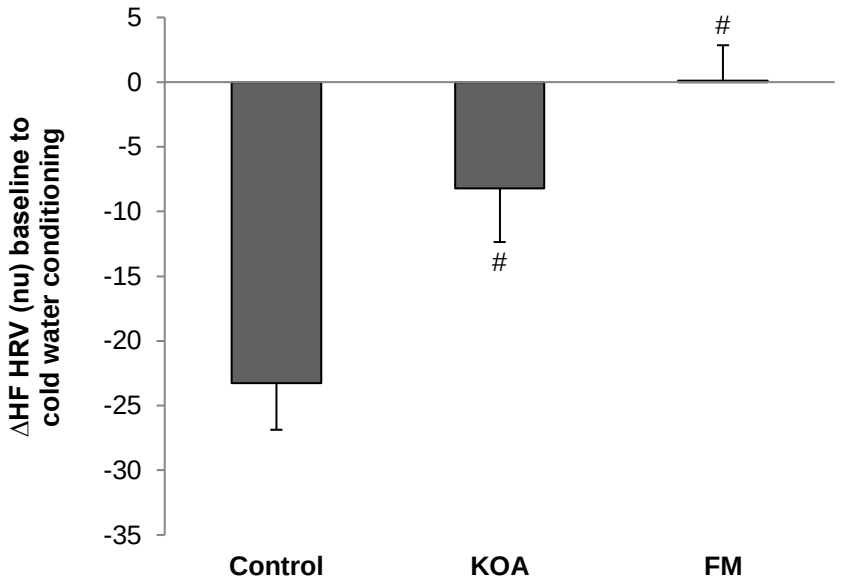
Figure 16. ***High frequency heart rate variability at baseline and immediately following cold water conditioning*****.**



Note. FM = fibromyalgia; HF HRV = high frequency heart rate variability; KOA = knee osteoarthritis; * = significant difference from baseline; # = significant difference from control; error bars are one standard error of the mean.

Change values for HF HRV are shown in Figure 17 and Table 3. Change values in HF HRV from baseline to immediately following cold water conditioning were significantly different among groups (H_2 = 14.35, p < .001). Follow-up Mann-Whitney U tests showed that the change in HF HRV was significantly smaller for both the KOA (p = .03) and FM (p < .001) groups compared to the control group.

Figure 17. ***Change values in high frequency heart rate variability from baseline to immediately following cold water conditioning.***



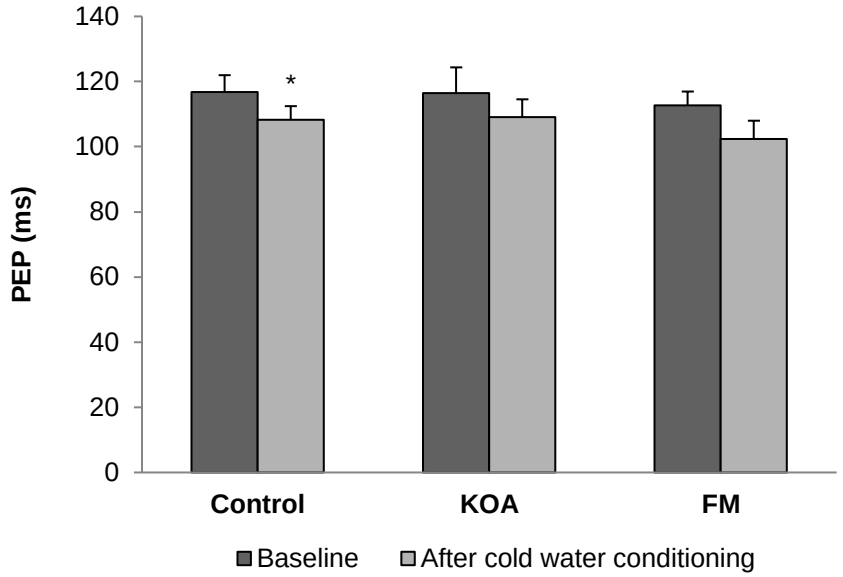
Note. FM = fibromyalgia; HF HRV = high frequency heart rate variability; KOA = knee osteoarthritis; # = significant difference from control; error bars are one standard error of the mean

3.3.6. Pre-ejection period

Group results for PEP are shown in Figure 18. There were no significant differences among groups in PEP at baseline ($F_{2,39}$ = 0.14, p = .87). Cold water conditioning led to a significant decrease in PEP in the control group (t_{14} = 3.04, p = .01). In contrast, the KOA (p = .24) and FM (p = .09) groups did not show significant change.

Change values for PEP are shown in Table 3. The change in PEP from baseline to cold water conditioning was not significantly different among groups (H_2 = 0.12, p = .94).

Figure 18. ***Pre-ejection period at baseline and immediately following cold water conditioning.***



Note. FM = fibromyalgia; KOA = knee osteoarthritis; PEP = pre-ejection period; * = significant difference from baseline; error bars are one standard error of the mean

3.3.7. Skin conductance level and response

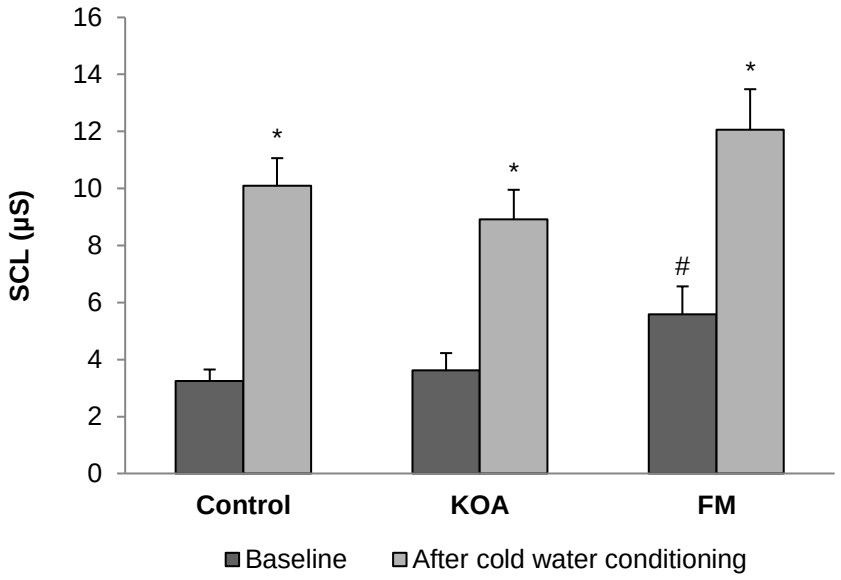
Group results for SCL are shown in Figure 19. There were significant differences among groups in SCL at baseline ($F_{2,39}$ = 3.35, p = .045). Follow-up one-sided Dunnett's test showed that SCL at baseline was significantly greater in the FM group than the control group (p = .02), while the KOA group were not significantly different to controls (p = .50). Cold water conditioning raised SCL significantly from baseline in all three groups (all p < .001).

Change values for SCL are shown in Table 3. The change in SCL from baseline to immediately following cold water conditioning was not significantly different among groups ($F_{2,39}$ = 0.67, p = .52).

Group results for SCR are shown in Figure 20. There were significant differences among groups in SCR at baseline (H_2 = 7.37, p = .03); however, follow-up Mann-Whitney U tests showed that the KOA group were not significantly different to the control group (p = .48), and while the FM group demonstrated a greater number of SCRs at baseline, there was no significant difference to controls (p = .053).

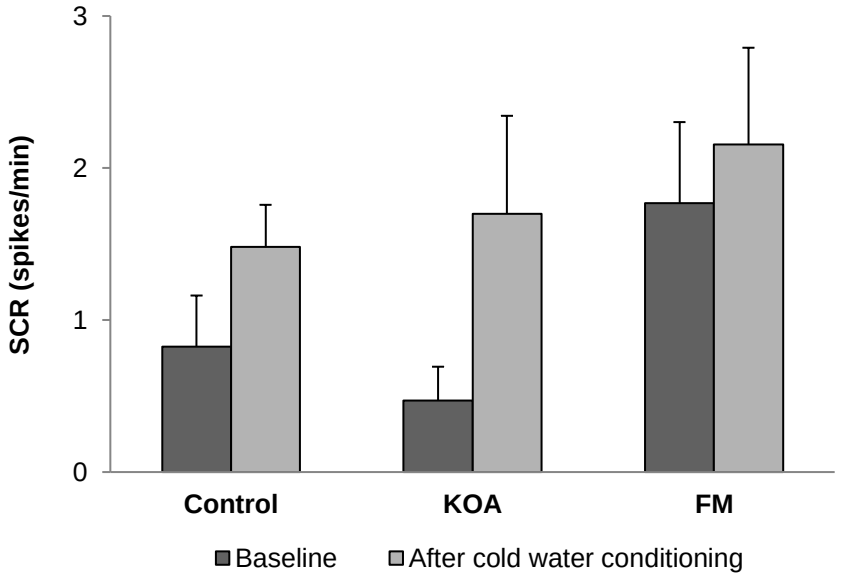
Change values for SCR are shown in Table 3. The change in SCR from baseline to immediately following cold water conditioning was not significantly different among groups (H_2 = 0.72, p = .70).

Figure 19. ***Skin conductance levels at baseline and immediately following cold water conditioning.***



Note. FM = fibromyalgia; KOA = knee osteoarthritis; SCL = skin conductance level; * = significant difference from baseline; # = significant difference from control; error bars are one standard error of the mean

Figure 20. ***Skin conductance responses at baseline and immediately following cold water conditioning.***



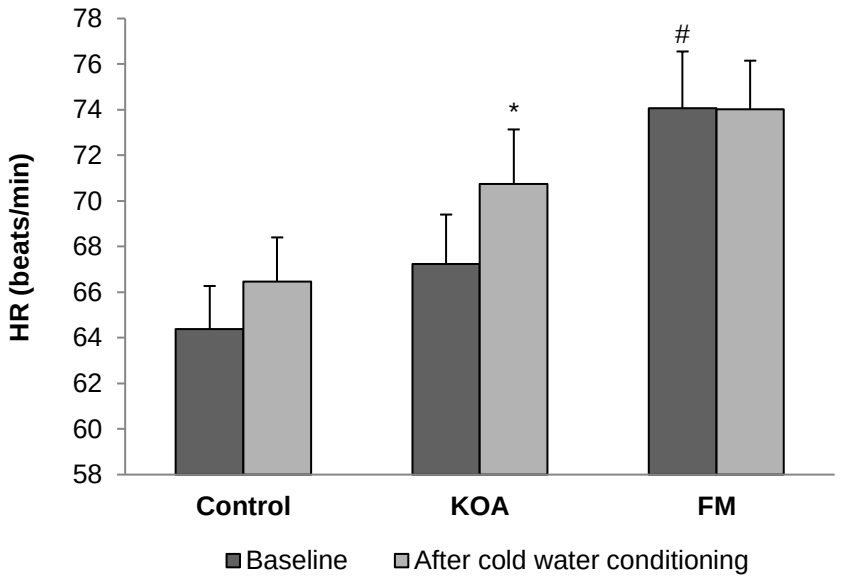
Note. FM = fibromyalgia; KOA = knee osteoarthritis; SCR = skin conductance response; error bars are one standard error of the mean

3.3.8. Heart rate

Group results for HR are shown in Figure 21. There were significant differences among groups in HR at baseline ($F_{2,39}$ = 5.14, p = .01). Follow-up one-sided Dunnett's test showed the FM group to have significantly higher HR at baseline compared to the control group (p = .003), while the KOA group were not significantly different to controls (p = .28). Only the KOA group showed a significant rise in HR from baseline to immediately following cold water conditioning (t_{13} = -2.76, p = .02). There were no significant differences from baseline to immediately following cold water conditioning in the control (t_{14} = -1.76, p = .10) and FM (t_{12} = 0.02, p = .98) groups.

Change values for HR are shown in Table 3. The change in HR from baseline to immediately following cold water conditioning was not significantly different among groups ($F_{2,39}$ = 1.74, p = .19).

Figure 21. ***Heart rate at baseline and immediately following cold water conditioning.***



Note. FM = fibromyalgia; HR = heart rate; KOA = knee osteoarthritis; * = significant difference from baseline; # = significant difference from control; error bars are one standard error of the mean

Table 3. ***Change values in ANS outcome measures from baseline to immediately following cold water conditioning. Data are mean (SD).***

Variable	Control *n* = 15	KOA *n* = 14	FM *n* = 13	Omnibus *p* value	ES (95% CI) KOA-control	ES (95% CI) FM-control
ΔHF HRV, nu	-23.28 (12.93)	-8.23 (15.44)*	0.11 (9.86)*	< .001[#]	1.06 (0.28 – 1.84)	2.01 (1.10 – 2.93)
ΔPEP, ms	-8.54 (2.81)	-7.43 (6.02)	-10.34 (5.69)	.94	0.24 (-0.49 – 0.97)	-0.41 (-1.16 – 0.34)
ΔSCL, µS	6.85 (0.84)	5.29 (0.90)	6.47 (1.29)	.52	1.79 (0.93 – 2.66)	0.36 (-0.39 – 1.10)
ΔSCR, spikes/min	0.65 (0.43)	1.23 (0.54)	0.39 (0.67)	.70	-1.19 (-1.98 – -0.40)	0.47 (-0.28 – 1.22)
ΔHR, beats/min	2.07 (1.18)	3.52 (1.28)	-0.04 (1.54)	.19	-1.18 (-1.97 – -0.39)	1.55 (0.71 – 2.40)

Note. ANS = autonomic nervous system; CI = confidence interval; ES = effect size; FM = fibromyalgia; HF HRV = high frequency heart rate variability; HR = heart rate; KOA = knee osteoarthritis; PEP = pre-ejection period; SCL = skin conductance level; SCR = skin conductance response; SD = standard deviation; * = significant difference from control; [#] = significant difference between groups

3.4. Discussion

3.4.1. Introduction

The first aim of this study was to determine whether people with KOA and FM would exhibit ANS dysfunction and altered nociceptive processing at rest. There was some evidence of ANS dysfunction, but limited evidence for differences in nociceptive processing in the participants with KOA and FM, except for impaired CPM. The second aim of this study was to evaluate the function of the ANS in people with KOA and FM in response to a painful conditioning stimulus. The results showed lower resting vagal tone and smaller changes in ANS function in response to a cold water conditioning stimulus in participants with KOA and FM compared to pain free controls. This section discusses these findings in more detail.

3.4.2. Nociceptive system function at baseline

The hypothesis was not supported for reduced knee PPT, HPT, and CPT in the KOA and FM groups compared to controls. It was expected that there would be significant differences among groups when measuring mechanical and thermal pain thresholds at rest, revealing nociceptive sensitisation in the KOA and FM groups. Indeed, previous research has found that people with KOA exhibit reduced PPTs [15, 16, 43, 51, 74, 97, 112], reduced HPTs [51], and increased CPTs [5] compared to healthy controls. Reduced thresholds at the knee may indicate altered peripheral nociceptor sensitivity and/or hyperexcitability of nociceptive pathways [74, 112, 406]. Nociceptor receptive fields can become enlarged through peripheral sensitisation, causing hyperalgesia in areas adjacent to and/or remote from the affected joint [51, 407]. Degenerative changes within the knee joint, as well as inflammatory processes, can also contribute to peripheral sensitisation, resulting in reduced mechanical pain thresholds [43, 51, 406, 407]. In the current study, no significant differences in knee PPT were found between the KOA and control groups. Knee PPT in the KOA group, while not significantly different, was lower than controls with an effect size of 0.62. Three previous studies assessing knee PPT, using a similar method and location, found significantly reduced thresholds in people with KOA compared to healthy controls [15, 16, 112]. Arendt-Nielsen et al. [15], Graven-Nielsen et al. [16], and Wylde et al. [112] used sample sizes of 24, 48, and 107, and revealed effect sizes of 0.55, 0.95, and 1.41 respectively. Fingleton et al. [93] used a sample size of 40 and reported results similar to the current study, with no significant difference found between controls and people with KOA at rest, yet showed a large effect size of 3.69. The effect size of the current study was comparable to Arendt-Nielsen et al. [15], but smaller than the others. This suggests that while sample size may have been a factor in the lack of significant findings for knee PPT, there may also be other differences between the studies leading to discrepancies in the findings [408]; e.g. testing sites, resting pain levels, or participant characteristics. Indeed, the mean WOMAC score of the participants in Wylde et al. [112] was 60 compared to 44 in the current study, suggesting higher levels of pain and disability may account for the variability and difference in significance of knee PPT results and effect sizes. Graven-Nielsen et al. [16] tested multiple sites around the knee whereas only a single test site was chosen for the current study

to minimise participant burden, facilitate comparison across groups, and because the medial compartment is most commonly affected by OA [112, 409].

Thermal thresholds are not commonly assessed in KOA [5, 51]. In the current study, no significant differences in knee HPT and CPT were found in the KOA and FM groups compared to controls. Lee at al. [64] and Moss et al. [5], with sample sizes of 26 and 23 respectively, examined HPT at the knee and also found no differences between KOA and control groups. Moss et al. [5] found their study to have a ≈50% power to detect a 2°C difference in HPT between groups. Therefore, with a 1 – 2°C difference in HPT between the KOA and control groups in the current study, the sample size would need to be much larger in order to detect a difference in HPT, if a difference exists.

The hypothesis was not supported for reduced arm PPT, HPT, and CPT in the KOA and FM groups compared to controls. The results for all three arm variables showed trends toward greater pain sensitivity in the KOA and FM groups compared to controls, however, the variability in groups likely meant that the sample size was insufficient to detect significant differences in these outcome measures between groups. It has been reported that people with KOA display increased sensitisation at sites distal to the knee [97, 112]. Indeed, previous studies testing PPTs at remote anatomical sites have consistently found lowered thresholds in people with OA, suggestive of widespread sensitisation in central nociceptive pathways and/or impaired descending inhibition of spinal nociceptive pathways [16, 74, 97, 112].

3.4.3. Parasympathetic nervous system function at baseline

The brain and heart have a bidirectional connection mediated by the vagal system, which can be measured using HRV [29]. The brain regulates the ANS through the central autonomic network, comprised of the following key structures in the context of this and proceeding discussions: the prefrontal cortex, for tonic inhibition of brainstem sympathoexcitatory circuits [29, 207, 218, 410]; amygdala, for threat and stress detection [411]; PAG, for nociceptive modulation [82, 83, 412, 413]; and, the NTS, for cardiovascular control [26]. The primary output of this network is mediated via preganglionic sympathetic and parasympathetic neurons that converge on the sinoatrial node of the heart [25, 208]. Therefore, the central autonomic network is directly linked to HRV – the changing time series of heart rate – based upon painful or stressful experiences and, in turn, neural regulation of the heart [208, 414]. Thus, HRV is a measure that is responsive to both nociception and stress [29, 84, 412, 413, 415].

Assessment of the PNS showed both the KOA and FM groups had reduced HF HRV at rest compared to the control group, supporting the hypothesis. This is in line with previous meta-analytic evidence that chronic pain populations exhibit reduced vagal tone [23]; and, specifically, people with FM display reduced HF HRV at rest [40, 41, 138, 203, 204]. The FM group was included in the current study to demonstrate that the experimental procedures were able to detect this dysfunction; however, the focus of the study was on the KOA group. To date, this is the first study to demonstrate reduced resting vagal

tone in people with OA. Elevated tonic vagal activity may be important to a person's ability to adapt to pain [173, 413], while diminished tonic vagal activity may adversely affect tonic descending inhibition of nociception [30]. Vagal withdrawal is the first autonomic response to threat and, as such, if vagal tone is already reduced at baseline, creating a floor effect, this may not be able to occur [25, 29, 413, 416]. Greater autonomic reactivity, including vagal withdrawal, may be involved in triggering descending pain modulation [412, 413]. Therefore, resting vagal dysfunction may contribute to increased pain sensitivity in people with chronic pain [23, 131]. Indeed, there is consistent evidence that resting vagal dysfunction is associated with chronic pain [23, 131, 132] in multiple conditions, including FM [19, 41], CRPS [21], chronic neck-shoulder pain [359, 417], IBS [20], and chronic low back pain [418].

The PAG and RVM are nuclei within the brainstem that rely on input from structures such as the cortex and NTS, mediated by vagal afferents, in order to process and modulate nociceptive information [73, 81, 84]. The magnitude of change in HF HRV (i.e. having "more in the tank" at baseline [25]) correlates with the amount of hypoalgesia experienced when the PAG is stimulated [84]. The PAG has few direct projections to the spinal cord, yet uses the RVM, which does project directly to the dorsal horn [73]. Modulation of nociception at the dorsal horn is then mediated by opioidergic or monoaminergic mechanisms via the RVM, e.g. serotonin, noradrenaline [73, 81, 419]. Diminished inhibition of nociception at the dorsal horn can lead to ongoing pain and occurs through attenuation of activity in these descending inhibitory pathways [73, 81].

Higher resting vagal tone is linked to better executive function, stress management, and emotional regulation [25, 29, 218, 286]. In terms of overall self-regulation, Laborde et al. [25] use the phrase, "the higher the better", when it comes to resting vagal tone. Low resting vagal tone is associated with prefrontal hypoactivity, disinhibition of the amygdala, sympathetic hyperactivity, and thus may predict the inefficient allocation of cognitive resources, such as attention and working memory [29, 218, 237, 410, 420]. Therefore, low resting vagal tone may be a measure for increased risk to stress exposure [410].

In sum, the efficacy of descending pain inhibitory mechanisms relies on high resting tonic vagal afferent activity into the NTS and, in turn, the PAG/RVM prior to noxious stimulation in order to modulate nociception and increase pain thresholds [26, 30, 84, 412, 416]. Thus, the reduced resting vagal tone shown by the KOA and FM groups in the current study may potentially contribute to the pain experienced at rest by these people and, subsequently, create a vagal floor effect upon exposure to acute stress.

3.4.4. Sympathetic nervous system function at baseline

The hypothesis of reduced PEP and increased EDA at baseline in the KOA group compared to controls was not supported, although the hypothesis of increased SCL at baseline in the FM group compared to controls was supported. Evidence is limited when it comes to the SNS and OA [33]. Indeed, this is the first study to investigate EDA in OA. These results may imply that sympathetic dysfunction is not a

feature of OA at rest, even though Courties et al. [33] suggest mechanisms for bone and joint degradation in OA that are potentially facilitated by SNS hyperactivity. As expected from previous studies [19, 37, 118, 212], the FM group showed signs of SNS hyperactivity, reflected in increased SCL at rest compared to the control group. This finding differs to Reyes del Paso et al. [421], however, who found resting SCL to be lower in people with FM compared to controls. PEP values of the KOA and FM groups were statistically equivalent to controls, consistent with the findings of Barakat et al. [131]. Another recent study also found PEP in FM to be equivalent to healthy controls at baseline [422]. The different findings in the outcome measures, both of which assess the SNS, may be explained by the fact that sympathetic outflow varies to different regions and is not maintained by a single unitary sympathetic tone [423, 424]. Sympathetic responses operate in a bifurcated manner, centrally and peripherally, including via different neurotransmitters [191, 423, 424]. For example, adrenaline can have a profound effect on myocardial contractility, influencing PEP measures, but little impact on sudomotor (EDA) activity [423].

3.4.5. Nociceptive system response to a painful conditioning stimulus

The results of this study supported the hypothesis that people with KOA exhibit impaired descending inhibition and/or increased descending facilitation of nociceptive pathways; i.e. impaired CPM. Baseline pain ratings (HP60 scores) of the test stimulus were equivalent among groups, as was the pain intensity reported during the cold water conditioning stimulus. Immediately following cold water conditioning, pain free controls showed a significant decrease in HP60 scores compared to baseline, indicating effective CPM. HP60 scores did not reduce significantly in the KOA and FM groups immediately following cold water conditioning, demonstrating impairment of descending inhibition of nociceptive pathways. These data support previous findings of CPM inefficiency in OA [15, 92] and FM [72, 114, 121]. In contrast to these findings, however, Fingleton et al. [93] identified subgroups within a sample of people with KOA where CPM was found to be intact. Kosek et al. [92] suggest that impairment of CPM in OA may be maintained by ongoing nociceptive input from the affected joint(s), such that higher levels of pain intensity may override the effects of efficient CPM, whereas low pain intensity may not impair CPM. It is also important to note that healthy, older adults have been shown to exhibit decreased CPM efficiency compared to younger adults [425]. Therefore, while reduced CPM effectiveness may be an indicator for increased pain sensitivity [426], variance does seem to exist within different samples [93, 425].

3.4.6. Parasympathetic nervous system response to a painful conditioning stimulus

Immediately following cold water conditioning, HF HRV was significantly reduced in the control group, but not in the KOA and FM groups. These findings supported the hypothesis of expected vagal withdrawal in response to a painful conditioning stimulus in the control group, and impaired vagal withdrawal in the KOA and FM groups. A systematic review of 20 studies by Koenig et al. [130] consistently showed vagal withdrawal, via decreased HF HRV, in healthy people in response to experimental pain. While previous studies have demonstrated vagal withdrawal in healthy controls in response to cold water conditioning [228, 229], this is the first study to show reduced vagal withdrawal in people with OA. In response to a noxious stimulus, nociceptive feedback via spinal laminae and vagal

afferents to the NTS induce autonomic changes [27, 30, 144, 157]. These changes initially result in vagal withdrawal, closely followed by increased sympathetic activation, which raises HR and BP, stimulating arterial baroreceptors [140, 146]. The baroreceptors then provide a feedback loop to the NTS, which engages brainstem nuclei, such as the PAG and RVM, to modulate descending nociceptive pathways [104, 140].

To date, no studies have directly assessed HF HRV in response to a nociceptive stressor in a chronic pain population for at least 5 mins. As such, a direct comparison of HRV results is not possible. However, two studies reported on HRV in people with FM in response to cold water conditioning compared to controls. In the first study, Chalaye et al. [20] found nonsignificant inverse effects of cold water conditioning on HF HRV between people with FM and controls, such that the FM group showed decreased HF HRV, and the control group showed increased HF HRV. However, HRV recordings were only 2 mins long, during reportedly "mild pain" of the fingers, since the authors stated "prolonged immersion times are not feasible" for people with chronic pain at 12°C. The current study achieved 5-minute long HRV recordings in all three groups during cold water conditioning at 12°C, which all participants were able to tolerate. In the second study, Reyes del Paso [138] only recorded HRV before and after cold water conditioning, thus, a direct comparison of results is not possible. However, the changes reported in other cardiovascular parameters generally support the findings of the current study by way of reduced vagal reactivity in people with FM.

Historically, the parasympathetic and sympathetic branches of the ANS were seen to work reciprocally on a linear spectrum [238, 427, 428]. However, a contemporary view is that the two branches function within what Bernston et al. [427, 428] refer to as the "autonomic space". The PNS and SNS operate independently of each other, co-activating and co-inhibiting, to allow for a variety of responses to stress [238, 258]. Yet, even so, in this autonomic space vagal activity holds predominant control of the heart; e.g. sympathetic HR effects are small when background vagal tone is high [429]. Therefore, reliable cardiac vagal withdrawal is required to regulate antinociceptive responses to noxious stimuli, and a floor effect of reduced resting vagal tone, as observed in the KOA and FM groups in the current study, may play a role in the attenuated cardiovascular responses to pain and nociception [25, 104, 430]. A person with adequate autonomic reaction to pain is more efficient in triggering pain modulating mechanisms [413] because: (1) resting vagal tone is high [25, 29, 375]; and (2) the baroreflex system is intact [31, 143, 431]. There is an inverse relationship between BRS and pain perception [432], such that blunted stress/noxious reactivity may lead to increased pain sensitivity in people with chronic pain due to aberrant autonomic and cardiovascular function [31, 140, 143, 144, 431].

3.4.7. Sympathetic nervous system response to a painful conditioning stimulus

The results for sympathetic cardiac reactivity to a painful conditioning stimulus supported the hypothesis of reduced PEP in the control group, but not the KOA and FM groups; however, the difference in changes values between groups did not reach significance. Hence, the hypothesis of smaller changes in PEP in the KOA and FM groups was not supported. PEP shortened significantly in the control group

immediately following cold water conditioning, but not in the KOA and FM groups. Experimentally induced pain increases sympathetic activity [198]. Therefore, it was expected that PEP would shorten immediately following cold water conditioning in healthy controls as beta adrenergic activity and cardiac contractility increases [186, 433]. PEP in chronic pain populations is not well represented in the literature and results appear mixed. Indeed, this is the first study to examine PEP in OA. A recent study by Reyes del Paso et al. [431] found PEP to increase during cold water conditioning in controls as well as people with FM. In accord with the findings of this study, the same authors previously found lowered myocardial contractility in FM in response to a stressor [138]. The results of the current study potentially demonstrate reduced sympathetic modulation in the KOA and FM groups with PEP values remaining equivalent to baseline following the painful conditioning stimulus. Blunted sympathetic reactivity can affect autonomic cardiovascular regulation, potentially reduce BRS, and, in turn, impact pain sensitivity [31, 143, 431].

The hypothesis of increased EDA immediately following cold water conditioning in the control group was supported, but the hypothesis of smaller changes in EDA in the KOA and FM groups was not supported. An attenuated sympathetic response was not detected immediately following cold water conditioning in KOA or FM as SCL significantly increased in all groups. An increase in SCRs were detected in all groups immediately following cold water conditioning, but these changes did not reach significance. Increased SCRs have been observed in response to a stressor in healthy controls [128, 133, 142, 197], people with inflammatory arthritis [215, 216], and FM [211, 283]. However, sudomotor activity in response to a painful stressor has not been examined previously in OA.

The complexity of SNS responses to stressors makes clear interpretation of these findings difficult, with different drivers from brainstem nuclei to end organs, such as heart and skin; e.g. adrenergic versus cholinergic receptor activation [133, 283, 434]. This so-called “autonomic response specificity” refers to different stimuli producing unique patterns of autonomic responses [129, 258, 283]. While one type of stimulus, e.g. cold, may induce a rise in SCRs, or shortening of PEP, another type of stimulus may have the opposite effect [129]. Vetrugno et al. [434] suggest that normal sudomotor activity cannot rule out sympathetic dysfunction in chronic conditions. While neither the KOA or FM group demonstrated dampened sympathetic outflow to the skin following cold water conditioning, this does not necessarily mean these populations are without sympathetic dysfunction in other regions; e.g. sympathetic discharge to the heart [191, 434].

3.5. Strengths and limitations

The strengths of this study include: the range of validated QST and ANS measures collected in a controlled laboratory environment; KOA and control groups that were broadly matched in age and sex distributions; participants who were medication free; participants whose anxiety and depression levels fell within normal limits, thus reducing potential confounding of the measurements [435]; and creating a nociceptive stressor (cold water conditioning at 12°C) that could be withstood by participants long enough to record HRV for the recommended time frame of 5 mins [163], yet be sufficiently

painful/stressful. This study, however, is not without its limitations. COVID-19 restrictions led to the recruitment of fewer participants than planned in the time available. The small sample size may have limited the opportunity to detect significant differences between groups that have been found in previous studies [15, 16, 112]. A single knee PPT test site at the medial joint line was chosen in this study to reduce participant burden, facilitate comparison across groups and because the medial compartment is most commonly affected by OA [112, 409]. However, testing the most painful site for each person or the use of multiple sites in the peripatellar region may have increased the likelihood of finding lower PPTs in the KOA group [16]. Finally, previously undiagnosed cardiac issues in some participants resulted in the loss of ECG data. A larger sample size would accommodate for such unforeseen circumstances in future research.

3.6. Conclusion

This is the first study to date to demonstrate vagal dysfunction in people with KOA. Both the KOA and FM groups displayed reduced vagal tone at rest, and reduced vagal withdrawal in response to a painful conditioning stimulus. Reduced tonic vagal activity may impact tonic descending inhibition of nociception in people with KOA and FM. Vagal withdrawal in response to a stressor is a key autonomic reaction that contributes to nociceptive modulation via the baroreflex system. The floor effect of reduced vagal tone at rest in people with KOA and FM may contribute to ongoing chronic pain in these groups. Despite finding no evidence for reduced mechanical pain thresholds, dynamic quantitative sensory testing found evidence of impaired CPM in people with KOA and FM, suggesting a loss of descending inhibition and/or increase in descending facilitation of nociceptive pathways in these populations. Assessment of the SNS demonstrated reduced sympathetic reactivity in people with KOA and FM, indicated by a smaller reduction in PEP when exposed to a painful conditioning stimulus compared to the control group. However, conflicting findings from sudomotor measures of sympathetic reactivity suggest the need for further investigation.

Chapter 4. Effects of acute mental stress on nociception and the autonomic nervous system in people with knee osteoarthritis and fibromyalgia

4.1. Introduction

The following chapter outlines the methodology used by describing the study aim, design, participants, procedure, outcome measures, and statistical analyses.

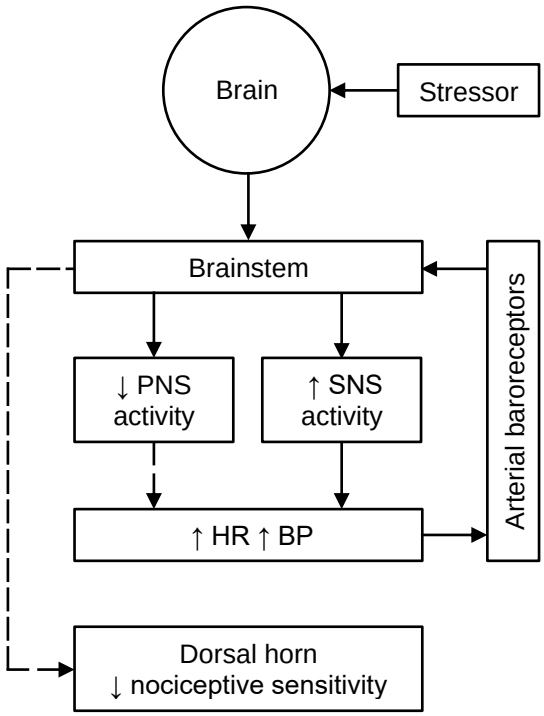
The aim of the second study was to examine the effects of acute mental stress on the nociceptive and autonomic nervous systems in people with KOA and FM. The following specific hypotheses were tested:

I. At rest, people with KOA and FM would exhibit reduced HF HRV, reduced PEP, and increased EDA compared to pain free controls.

II. Immediately following mental arithmetic, pain free controls (Figure 22) would demonstrate a reduction in HF HRV, reduced PEP, increased EDA; and, reduced HP60 scores. In comparison to controls, these changes in the ANS outcome measures would be reduced in the KOA and FM groups. In contrast to pain free controls, HP60 scores would be increased in KOA and FM groups (Figure 23).

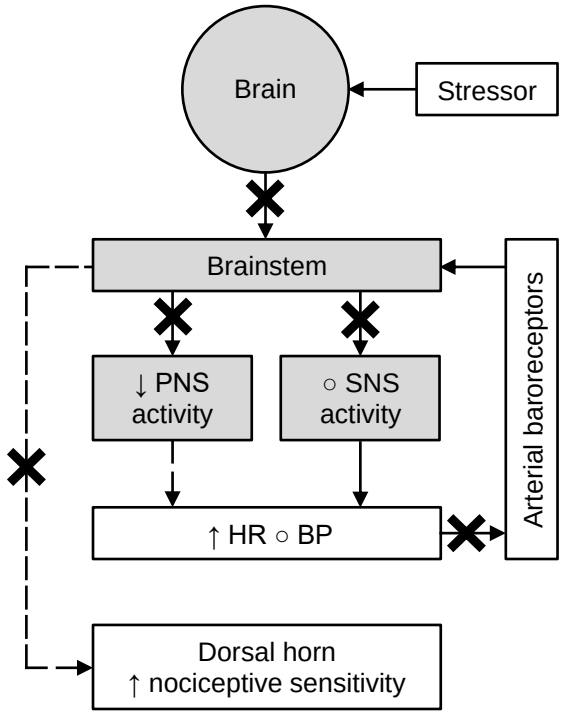
III. Fifteen minutes after mental arithmetic, HF HRV, PEP, EDA, and HP60 scores would be equivalent to baseline values in the control group. In comparison to baseline, any changes in ANS outcome measures and HP60 scores would be maintained in the KOA and FM groups at 15 mins.

Figure 22. ***Hypothesis of a pain free control exhibiting normal autonomic function resulting in reduced nociceptive sensitivity in response to acute mental stress.***



Note. BP = blood pressure**;** HR = heart rate; PNS = parasympathetic nervous system; SNS = sympathetic nervous system**;** ⟶ = excitation; ⇢ = inhibition

Figure 23. ***Hypothesis of a floor effect of reduced high frequency heart rate variability, blunted sympathetic reactivity, and increased nociceptive sensitivity in response to acute mental arithmetic in people with knee osteoarthritis.***



Note. BP = blood pressure; grey = area of dysfunction; HR = heart rate; PNS = parasympathetic nervous system; SNS = sympathetic nervous system; ○ = blunted response; ✕ = dysfunctional pathway; ⟶ = excitation; ⇢ = inhibition

4.2. Method

4.2.1. Study design

The study was a cross-sectional, experimental design undertaken at the AUT Biomechanics Laboratory, North Shore Hospital, Auckland.

4.2.2. Sample size

A sample size calculation was undertaken using an alpha level of .05 and power of 0.8 using G*Power 3.1.9.2 [378]. HF HRV was chosen as the primary outcome measure; however, no previous studies were available to estimate the effects of acute mental stress on HRV in people with OA. Therefore, an effect size of 0.72 was determined based on a previous study assessing HF HRV in people with CRPS in response to stressful mental arithmetic [21]. CRPS is a chronic pain condition that may resemble some similarities to OA in aberrant nociceptive processing and hypothesised ANS dysfunction. A two-group *t*-test with .05 one-sided significance level revealed that for an effect size of 0.72, 25 participants were required per group. For three groups, the total number of participants required was $N = 75$.

4.2.3. Participants

The same participant groups (KOA, FM, and pain free control) and inclusion/exclusion criteria from the first study were used. Demographic information was collected from each participant and the nature of each participant's pain was determined using the same questionnaires as the first study. Participants were asked to refrain from taking analgesic medication for 24 hours prior to data collection, and from taking caffeine and tobacco products 6 hours prior to data collection.

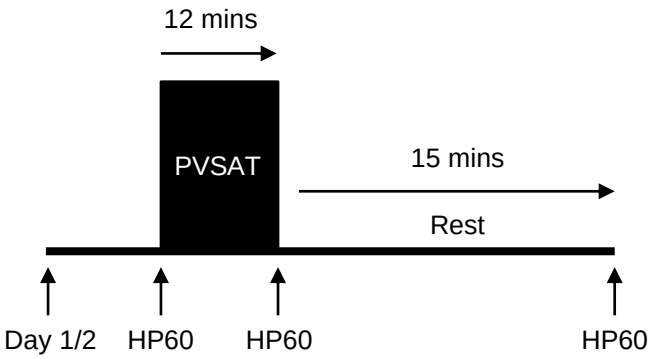
4.2.4. Ethical considerations

Ethical approval was obtained from the Health and Disability Ethics Committees (HDEC): approval number 18/CEN/45 (Appendix F). Consultation was also provided by the Matauranga Maori Committee. During the design and implementation of the study the principles of the Treaty of Waitangi, including partnership, participation and protection were applied, and the recruitment process ensured that all eligible participants had equal opportunity to take part in the study regardless of ethnicity. Each participant was fully informed with a participant information sheet (Appendix G) and consented to participation in the study by signing a consent form (Appendix H). The privacy of each participant was maintained at all times by assigning participant codes.

4.2.5. Study procedure

Participants were exposed to an acute mental arithmetic stressor in the form of a computerised, visual version of the PASAT called the Paced Visual Serial Addition Task (PVSAT) while nociceptive and ANS outcome measures were recorded. Data collection was conducted over two separate days, in random order, in order for the nociceptive measures not to confound the ANS recordings. One day assessed nociceptive outcome measures (Figure 24), while the other day examined ANS outcome measures (Figure 25).

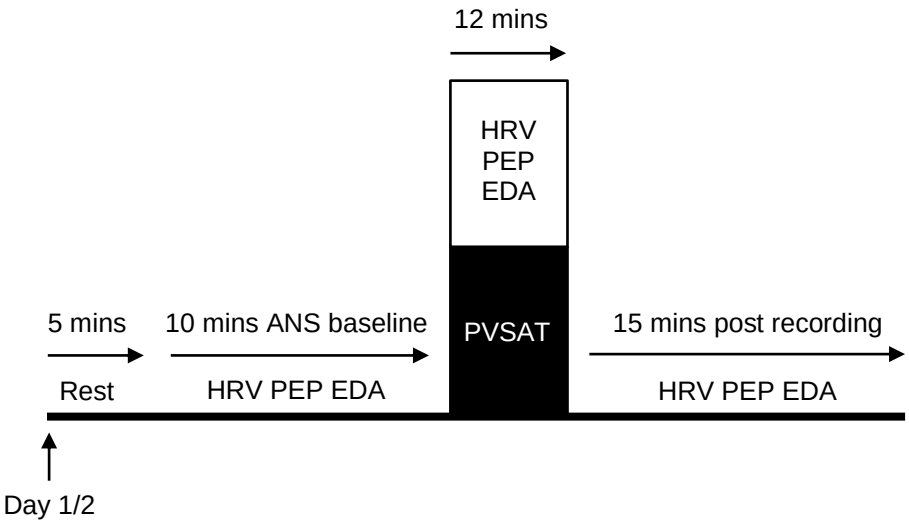
Figure 24. ***Procedure for assessing nociceptive outcome measures in response to the Paced Visual Serial Addition Task.***



Note. HP60 = heat pain 60 (0 – 100); PVSAT = Paced Visual Serial Addition Task

Nociceptive outcome measures were recorded before, immediately following, and 15 mins after the PVSAT. ANS outcome measures were recorded for 10 mins before (baseline), during (Figure 27), and continuously for 15 mins after the PVSAT. Participants were asked to recline in the supine position with the torso elevated at 30° during both sessions.

Figure 25. ***Procedure for assessing autonomic nervous system outcome measures in response to the Paced Visual Serial Addition Task.***

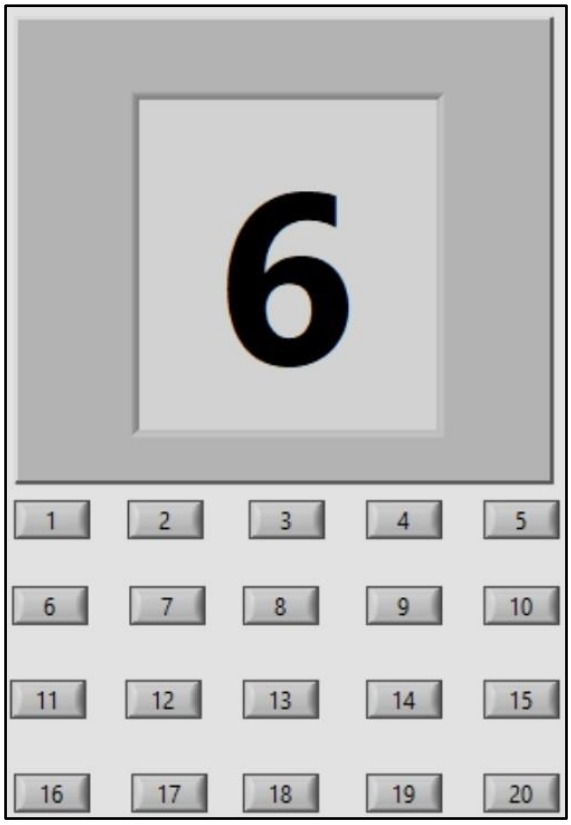


Note. ANS = autonomic nervous system; EDA = electrodermal activity; HRV = heart rate variability; PEP = pre-ejection period; PVSAT = Paced Visual Serial Addition Task

4.2.6. Acute mental stress intervention

The PVSAT (Figure 26) is a computerised, visual version of the PASAT programmed in LabVIEW (National Instruments, USA). The program presented a random series of numbers from 1 to 9 to the participant and they were instructed to consecutively sum pairs of numbers such that each number was added to the one immediately preceding it and mouse-click the correct answer onscreen [251]. For example, if the digits 4, 7 and 3 were presented, the participant would correctly click the buttons 11 and 10, respectively, into the application. This response requirement was sustained over 61 items per trial according to the standard Gronwall version [249-251]. The PVSAT consisted of 4 trials with an ISI rate of 2.4 s for each trial. The ISI rate remained constant, rather than incrementally decreasing across each trial, because the PVSAT is more difficult with a mouse-click response than the verbal response of the original PASAT. Pilot testing indicated that shorter ISIs were too quick to complete the task, leading to participant frustration and premature task termination. A 2.4 s ISI was sufficient to induce mental stress across the entire task and ensure participants did not give up. The examiner allowed a 60 s rest interval between trials [251]. The duration of each trial is calculated by multiplying the duration of the ISI by 60; e.g. 2.4 s x 60 = 144 s [250]. The total time of the intervention, including rest breaks, was 12 mins 36 s.

Figure 26. ***The computerised Paced Visual Serial Addition Task program.***



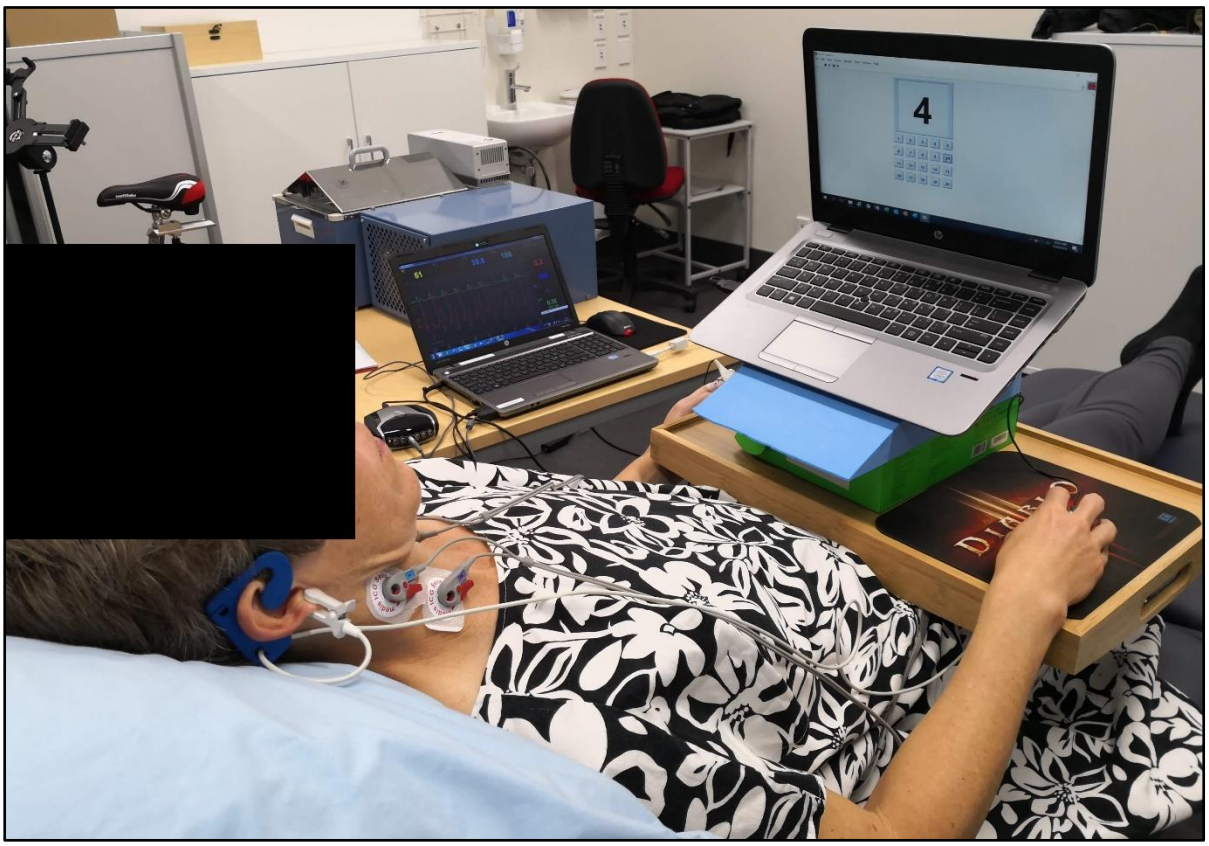
4.2.7. Nociceptive outcome measures

Using procedures described in the first study, HP60 scores were recorded for 120 s before, immediately following, and 15 mins after the PVSAT (Figure 24). Participants were told the temperature may increase, decrease, or remain constant during the 120 s heat test stimulus interval to reduce expectations. HP60 scores were recorded to quantify pain ratings at baseline, immediately following, and 15 mins after the PVSAT; as well as to calculate the modulatory effects of the PVSAT on pain ratings. Pain modulation of the PVSAT was determined by subtracting the mean HP60 score of the second heat test stimulus (during the PVSAT) from the first (baseline). PVSAT pain modulation was reported as percentage change of the mean HP60 score. Effective pain inhibitory mechanisms are reported as negative values (pain inhibited after acute mental stress) and pain facilitation reported as positive values (pain increased after acute mental stress). In addition, a verbal numerical rating scale (NRS) of 0 (no pain) to 10 (worst pain imaginable) was reported to evaluate the level of a participant's current KOA/FM pain (at rest) between each trial.

4.2.8. Autonomic nervous system outcome measures

HF HRV, PEP, SCL, SCR, and HR were recorded 10 mins before (baseline), during (Figure 27), and continuously for 15 mins after the PVSAT using procedures described in the first study.

Figure 27. ***Participant lying supine at 30° using a mouse to complete the onscreen Paced Visual Serial Addition Task during autonomic nervous system recording.***



4.2.9. Data analysis

Normality of distribution of data was analysed using the Kolmogorov-Smirnov test and variables that were not statistically significant were classed as having normal distribution. Normally distributed dependent variables were analysed by parametric analyses.

To compare characteristics of the three groups, continuous variables were compared between groups using one-way ANOVA. Significant main effects were followed up with two-sided Dunnett's test to compare the KOA and FM groups to the control group. Ordinal data from questionnaires was compared between groups using Kruskal-Wallis tests, with follow-up Mann-Whitney U tests for significant results. Gender was analysed using the chi-square test.

To compare the outcome measures at baseline among the three groups, one-way ANOVA was used. Significant main effects were followed up with one-sided Dunnett's test to compare the KOA and FM

groups to the control group. Comparisons were not made between KOA and FM groups because this was not an outcome of interest and the studies were not powered to detect this. To determine the effect of the PVSAT, repeated measures ANOVAs were used to compare the outcome measures across three time periods: baseline, immediately following, and 15 mins after PVSAT. The Huynh-Feldt correction factor was used when Epsilon < 1. Significant main effects were investigated with planned comparisons between baseline, immediately following, and 15 mins after PVSAT. PVSAT performance was calculated as the percentage of total correct answers across four blocks of trials and analysed using one-way ANOVA. Effect sizes and 95% confidence intervals were determined from the difference scores between the KOA-control and FM-control comparisons.

Difference values (i.e. change scores) in outcome measures from baseline to immediately following the PVSAT, and from baseline to 15 mins after the PVSAT, were compared between groups using one-way ANOVA. Significant main effects were followed up with one-sided Dunnett's test to compare the KOA and FM groups to the control group.

For equivalent non-parametric analyses, between group comparisons were made using Kruskal-Wallis tests, with follow-up Mann-Whitney U tests for significant results. Comparisons over the three time periods (baseline, immediately following, and 15 mins after PVSAT) were analysed within each group using Friedman tests, with significant results followed up with Wilcoxon Signed Rank tests. The alpha level for all statistical procedures was set to .05, and all statistical analyses were performed using IBM SPSS Statistics for Windows version 28 (Armonk, NY: IBM Corp).

4.3. Results

4.3.1. Recruitment

During the period of recruitment, the worldwide COVID-19 pandemic led to several lockdown periods in the Auckland region, and a general hesitancy on the part of participants to attend hospital-based appointments for research involving face-to-face data collection. As such, only forty-two of the planned 54 participants were able to be recruited: 15 pain free people, 14 people with KOA, and 13 people with FM. All 42 participants met the inclusion criteria and completed the study. It was discovered during data processing that one control participant exhibited a previously undiagnosed ectopic beat, and another control participant displayed heart beat errors. These anomalies impacted measurements of HF HRV; therefore, HF HRV data was removed from data analyses for these two participants. Data collection took place from May 2019 to June 2021.

4.3.2. Participant characteristics

Participant characteristics are shown in Table 1 and described in Section 3.3.2.

4.3.3. Data distribution

At baseline, only SCR was not normally distributed and was analysed using non-parametric methods. For comparisons over time, the following ANS variables were not normally distributed in at least one group and analysed using non-parametric methods: HF HRV, HR, and SCR. Difference values from baseline to immediately following the PVSAT (Δ_0), and from baseline to 15 mins after the PVSAT (Δ_{15}), that were not normally distributed included: Δ_0SCL, Δ_0HR, Δ_{15}PEP, Δ_{15}SCL, and Δ_{15}SCR. The remaining ANS variables were normally distributed and analysed using parametric methods.

Scores for self-reported pain (NRS values) were not normally distributed in the KOA group, but normally distributed in the FM group, and analysed using non-parametric and parametric methods respectively. NRS values were zero across all four PVSAT trials in the control group; therefore, no further analyses were performed on these data.

HP60 scores were normally distributed for all groups at baseline, immediately following, and 15 mins after the PVSAT, and were analysed by parametric analyses. Difference values in HP60 scores immediately following the PVSAT (Δ_0HP60), and 15 mins after the PVSAT (Δ_{15}HP60), were not normally distributed and analysed using non-parametric methods. The PVSAT performance scores (percentage correct answers) were normally distributed in both sessions and analysed using parametric methods.

4.3.4. Self-reported pain

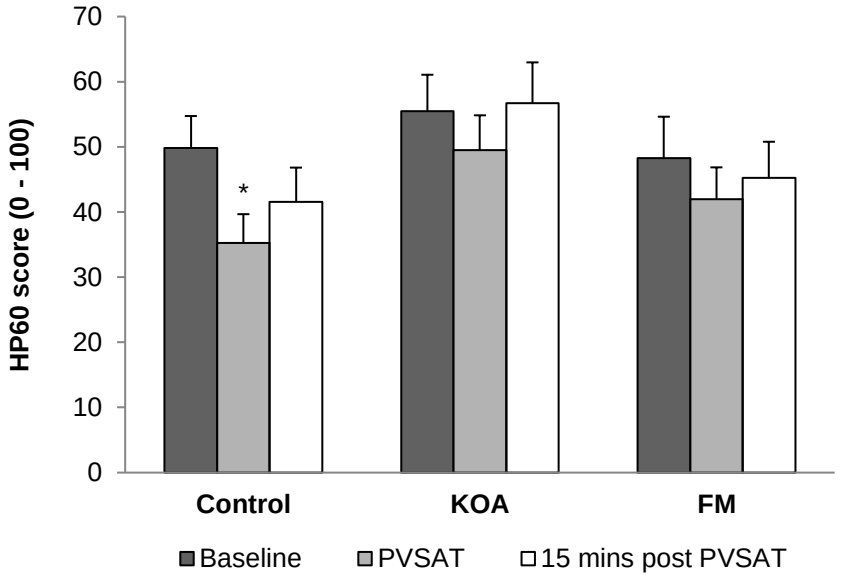
NRS results are shown in Table 4. As expected, there were significant differences in NRS at baseline in both the KOA-control and FM-control comparisons (both $p < .001$). No further analyses were performed on these data. There were no significant differences over time across the four blocks of PVSAT trials in either the KOA ($\chi^2(4) = 5.21$, $p = .27$) or FM ($F_{2.5,30.0} = 2.58$, $p = .08$) groups for self-reported pain.

4.3.5. Heat pain 60 scores

Group results for HP60 scores are shown in Figure 28. There were no significant differences among groups in HP60 scores at baseline ($F_{2,39}$ = 0.45, p = .64). Therefore, baseline pain ratings were equivalent across all three groups (Table 4). The main effect of time was significant for HP60 scores in the control group ($F_{2,28}$ = 4.69, p = .02). Compared to baseline, HP60 scores were significantly lower for the control group immediately following the PVSAT (p = .004), but not 15 mins after the PVSAT (p = .12). The main effect of time was not significant for HP60 scores in the KOA ($F_{2,26}$ = 1.33, p = .28) and FM ($F_{2,24}$ = 0.54, p = .59) groups.

Raw and change values for HP60 scores are shown in Table 4. The percentage change in HP60 scores from baseline to immediately following the PVSAT were not significantly different among groups (H_2 = 1.70, p = .43). Similarly, the percentage change in HP60 scores from baseline to 15 mins after the PVSAT were not significantly different among groups (H_2 = 1.53, p = .47).

Figure 28. ***Heat pain 60 scores at baseline, immediately following, and 15 mins after the Paced Visual Serial Addition Task.***



Note. FM = fibromyalgia; KOA = knee osteoarthritis; HP60 = heat pain 60; PVSAT = Paced Visual Serial Addition Task; * = significant difference from baseline; error bars are one standard error of the mean

Table 4. ***Self-reported pain, raw, and change QST values. Data are mean (SD).***

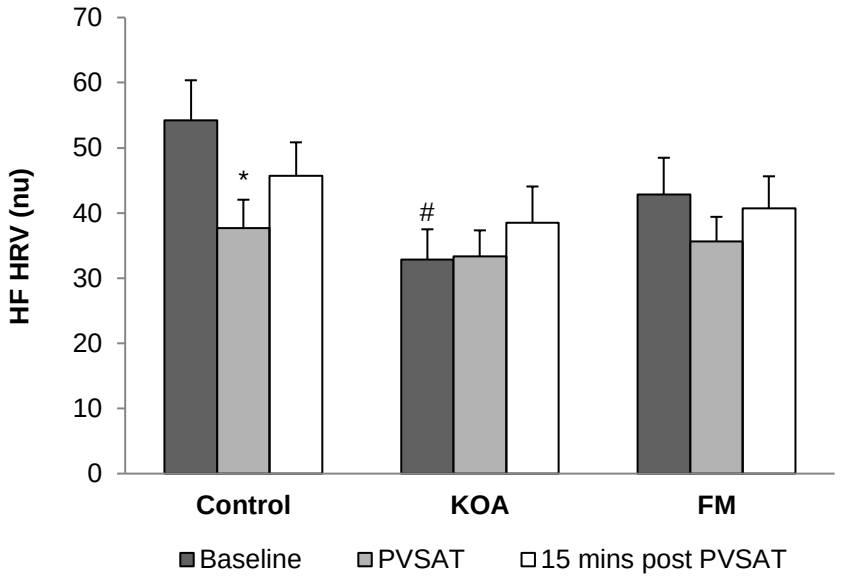
Variable	Control *n* = 15	KOA *n* = 14	FM *n* = 13	Omnibus *p* value	ES (95% CI) KOA-control	ES (95% CI) FM-control
NRS, baseline, 0 – 10	0 (0)	3 (3)	4 (2)	< .001[#]		
NRS, trial 1, 0 – 10	0 (0)	3 (3)	4 (2)			
NRS, trial 2, 0 – 10	0 (0)	3 (3)	4 (2)			
NRS, trial 3, 0 – 10	0 (0)	2 (3)	5 (2)			
NRS, trial 4, 0 – 10	0 (0)	3 (3)	5 (2)			
HP60 score, 0 – 100:						
Baseline	49.87 (18.85)	55.46 (20.98)	48.27 (22.92)	.64	0.28 (-0.45 – 1.01)	-0.08 (-0.82 – 0.67)
Immediately following PVSAT	35.23 (17.20)	49.47 (20.10)	41.93 (17.76)		0.76 (0.01 – 1.52)	0.38 (-0.37 – 1.13)
Fifteen mins after PVSAT	41.58 (20.27)	56.71 (23.42)	45.21 (20.11)		0.69 (-0.06 – 1.44)	0.18 (-0.56 – 0.92)
HP60 score, % change:						
Δ_0HP60	-24.32 (36.33)	-4.28 (51.35)	1.13 (56.72)	.43	0.45 (-0.28 – 1.19)	0.54 (-0.21 – 1.30)
Δ_{15}HP60	-12.18 (35.30)	11.09 (53.91)	9.52 (61.04)	.47	0.52 (-0.23 – 1.26)	0.44 (-0.31 – 1.20)

Note. CI = confidence interval; ES = effect size; FM = fibromyalgia; KOA = knee osteoarthritis; HP60 = heat pain 60; NRS = numerical rating scale; PVSAT = Paced Visual Serial Addition Task; QST = quantitative sensory testing; SD = standard deviation; [#] = significant difference between groups; Δ_0 = difference from baseline to immediately following the PVSAT; Δ_{15} = difference from baseline to 15 mins after the PVSAT

4.3.6. Heart rate variability

Group results for HF HRV are shown in Figure 29. There were significant differences among groups in HF HRV at baseline ($F_{2,37}$ = 3.87, p = .03). Follow-up one-sided Dunnett's test showed the KOA group to have significantly lower HF HRV at baseline compared to the control group (p = .01), while the FM group were not significantly different (p = .13). The main effect of time was significant for HF HRV in the control group ($F_{2,24}$ = 5.95, p = .01). Compared to baseline, HF HRV was significantly lower for the control group immediately following the PVSAT (p = .002), but not 15 mins after the PVSAT (p = .13). The main effect of time was not significant for HF HRV in the KOA ($F_{2,26}$ = 1.21, p = .31) and FM ($\chi^2(2)$ = 4.31, p = .12) groups.

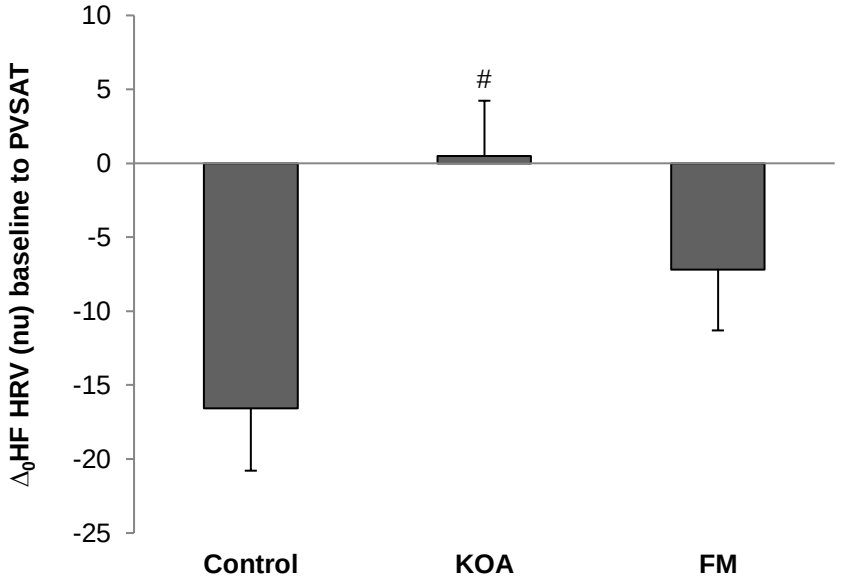
Figure 29. ***High frequency heart rate variability at baseline, immediately following, and 15 mins after the Paced Visual Serial Addition Task.***



Note. FM = fibromyalgia; HF HRV = high frequency heart rate variability; KOA = knee osteoarthritis; PVSAT = Paced Visual Serial Addition Task; * = significant difference from baseline; # = significant difference from control; error bars are one standard error of the mean

Change values for HF HRV are shown in Figure 30 and Table 5. Change values in HF HRV from baseline to immediately following the PVSAT were significant among groups ($F_{2,37}$ = 4.57, p = .02). Follow-up one-sided Dunnett's test showed change values for the KOA group were significantly smaller compared to the control group (p = .004), while the FM group were not significantly different (p = .10). Change values in HF HRV from baseline to 15 mins after the PVSAT were not significantly different among groups ($F_{2,37}$ = 2.80, p = .07).

Figure 30. ***Change values in high frequency heart rate variability from baseline to immediately following the Paced Visual Serial Addition Task.***



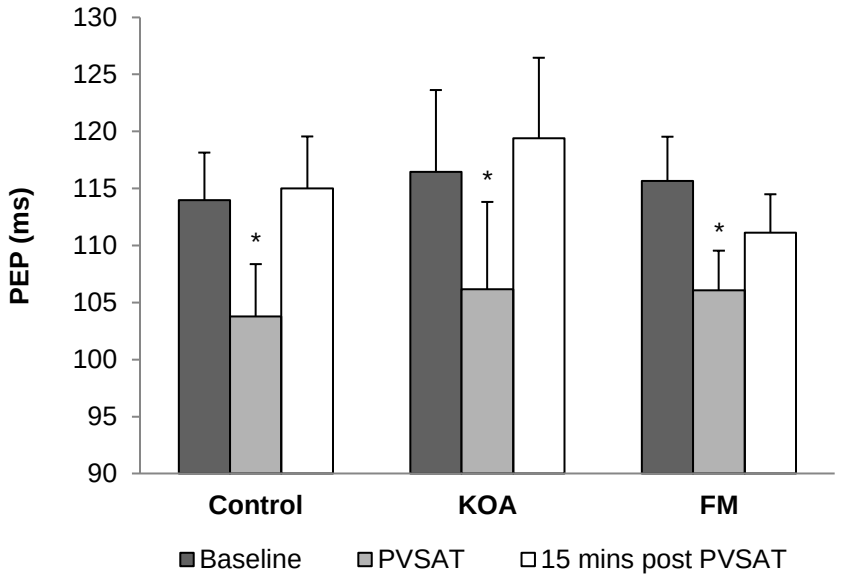
Note. FM = fibromyalgia; HF HRV = high frequency heart rate variability; KOA = knee osteoarthritis; PVSAT = Paced Visual Serial Addition Task; # = significant difference from control; Δ_0 = difference from baseline to immediately following the PVSAT; error bars are one standard error of the mean

4.3.7. Pre-ejection period

Group results for PEP are shown in Figure 31. There were no significant differences among groups in PEP at baseline ($F_{2,39}$ = 0.06, p = .94). The main effect of time was significant for PEP in the control ($F_{1.2,14.9}$ = 8.84, p = .01), KOA ($F_{1.7,22.4}$ = 10.45, p < .001), and FM ($F_{1.7,19.8}$ = 5.89, p = .013) groups. Compared to baseline, PEP was significantly lower in all three groups immediately following the PVSAT (all p < .03), but was not significantly different from baseline 15 mins after the PVSAT (all p > .10).

Change values for PEP are shown in Table 5. The change in PEP from baseline to immediately following the PVSAT was not significantly different among groups ($F_{2,39}$ = 0.01, p = .99). Similarly, the change in PEP from baseline to 15 mins after the PVSAT was not significantly different among groups (H_2 = 3.57, p = .17).

Figure 31. ***Pre-ejection period at baseline, immediately following, and 15 mins after the Paced Visual Serial Addition Task.***



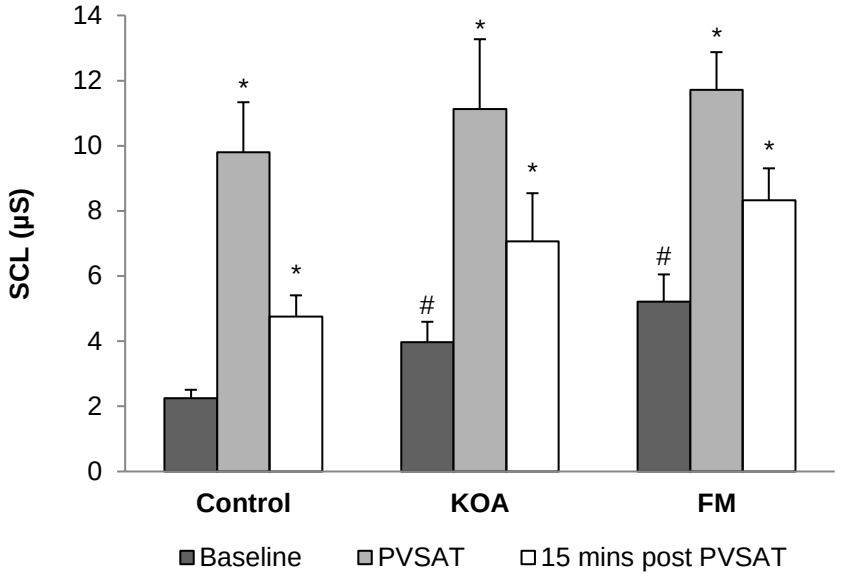
Note. FM = fibromyalgia; KOA = knee osteoarthritis; PEP = pre-ejection period; PVSAT = Paced Visual Serial Addition Task; * = significant difference from baseline; error bars are one standard error of the mean

4.3.8. Skin conductance level and response

Group results for SCL are shown in Figure 32. There were significant differences among groups in SCL at baseline ($F_{2,39}$ = 6.21, p = .01). Follow-up one-sided Dunnett's test showed that SCL at baseline was significantly greater in both the KOA (p = .04) and FM (p = .001) groups compared to the control group. The main effect of time was significant for SCL in the control ($F_{1.3,15}$ = 17.93, p < .001), KOA ($F_{1.2,15.5}$ = 16.55, p < .001), and FM ($F_{1.6,18.7}$ = 18.99, p < .001) groups. SCL was raised significantly from baseline in all three groups immediately following the PVSAT (all $p \leq .001$), and 15 mins after the PVSAT (all p < .01).

Change values for SCL are shown in Table 5. The change in SCL from baseline to immediately following the PVSAT was not significantly different among groups (H_2 = 0.23, p = .89). Similarly, the change in SCL from baseline to 15 mins after the PVSAT was not significantly different among groups (H_2 = 0.56, p = .76).

Figure 32. ***Skin conductance levels at baseline, immediately following, and 15 mins after the Paced Visual Serial Addition Task.***

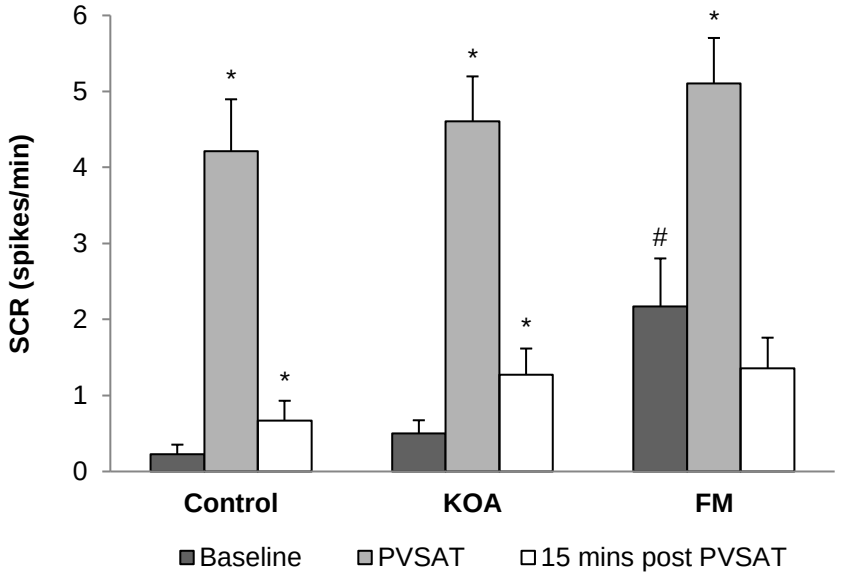


Note. FM = fibromyalgia; KOA = knee osteoarthritis; PVSAT = Paced Visual Serial Addition Task; SCL = skin conductance level; * = significant difference from baseline; # = significant difference from control; error bars are one standard error of the mean

Group results for SCR are shown in Figure 33. There were significant differences among groups in SCR at baseline (H_2 = 8.48, p = .01). Follow-up Mann-Whitney U tests showed that the KOA group were not significantly different to the control group (p = .11), while the FM group had a significantly greater number of SCRs (p = .01). The main effect of time was significant for SCR in the control ($\chi^2(2) = 25.08$, $p < .001$), KOA ($\chi^2(2) = 21.55$, $p < .001$), and FM ($\chi^2(2) = 9.53$, p = .01) groups. SCR was raised significantly from baseline in all three groups immediately following the PVSAT (all $p < .01$). Fifteen minutes after the PVSAT, SCR was raised significantly from baseline in the control (p = .02) and KOA (p = .01) groups, but not the FM group (p = .41).

Change values for SCR are shown in Table 5. The change in SCR from baseline to immediately following the PVSAT was not significantly different among groups ($F_{2,39}$ = 0.94, p = .40). Similarly, the change in SCR from baseline to 15 mins after the PVSAT was not significantly different among groups ($\chi^2(2) = 5.29$, p = .07).

Figure 33. ***Skin conductance responses at baseline, immediately following, and 15 mins after the Paced Visual Serial Addition Task.***



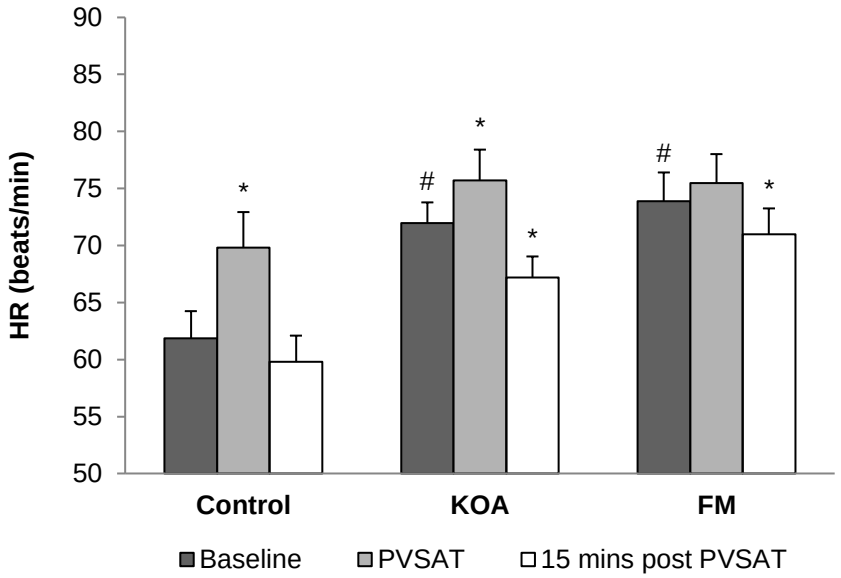
Note. FM = fibromyalgia; KOA = knee osteoarthritis; PVSAT = Paced Visual Serial Addition Task; SCR = skin conductance response; * = significant difference from baseline; # = significant difference from control; error bars are one standard error of the mean

4.3.9. Heart rate

Group results for HR are shown in Figure 34. There were significant differences among groups in HR at baseline ($F_{2,39}$ = 8.35, p < .001). Follow-up one-sided Dunnett's tests showed that HR was significantly higher at baseline in both the KOA (p = .003) and FM (p < .001) groups compared to the control group. The main effect of time was significant for HR in the control ($F_{1.7,20.9}$ = 33.03, p < .001), KOA ($\chi^2(2)$ = 17.71, p < .001), and FM ($F_{2,24}$ = 9.49, p < .001) groups. HR was raised significantly from baseline immediately following the PVSAT in the control (p < .001) and KOA (p = .04) groups, but not in the FM group (p = .20). Fifteen minutes after the PVSAT, HR was significantly lower than baseline in both the KOA (p = .004) and FM (p = .01) groups, but not in the control group (p = .06).

Change values for HR are shown in Table 5. Change values in HR from baseline to immediately following the PVSAT were significant among groups ($\chi^2(2)$ = 8.84, p = .01). Follow-up Mann-Whitney U tests showed the FM group to display significantly less change in HR than the control group (p = .003), while the KOA group were not significantly different to controls (p = .06). The change in HR from baseline to 15 mins after the PVSAT was not significantly different among groups ($F_{2,39}$ = 1.87, p = .17).

Figure 34. ***Heart rate at baseline, immediately following, and 15 mins after the Paced Visual Serial Addition Task.***

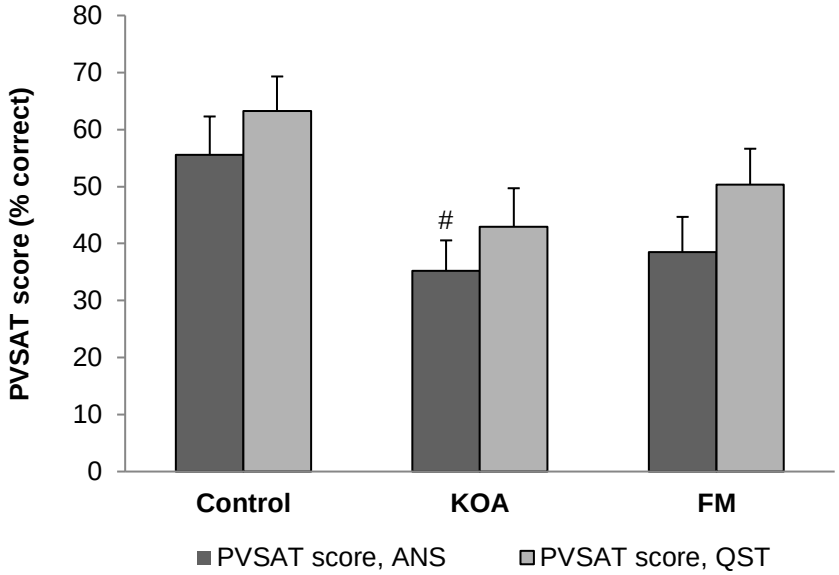


Note. FM = fibromyalgia; HR = heart rate; KOA = knee osteoarthritis; PVSAT = Paced Visual Serial Addition Task; * = significant difference from baseline; # = significant difference from control; error bars are one standard error of the mean

4.3.10. Paced Visual Serial Addition Task performance

Group results for PVSAT performance are shown in Figure 35. There were significant differences among groups in PVSAT performance during ANS testing ($F_{2,39}$ = 3.27, p = .049), but not during QST testing ($F_{2,39}$ = 2.69, p = .08). Follow-up one-sided Dunnett's test showed the KOA group scored significantly less than the control group during the ANS session (p = .02), and while the FM group also scored lower than controls, the difference was not significant (p = .052).

Figure 35. ***Paced Visual Serial Addition Task performance displayed as percentage correct answers across four blocks of trials.***



Note. ANS = autonomic nervous system; FM = fibromyalgia; KOA = knee osteoarthritis; PVSAT = Paced Visual Serial Addition Task; QST = quantitative sensory testing; # = significant difference from control; error bars are one standard error of the mean

Table 5. ***Change values in ANS outcome measures from baseline to immediately following, and baseline to 15 mins after the PVSAT. Data are mean (SD).***

Variable	Control *n* = 15	KOA *n* = 14	FM *n* = 13	Omnibus *p* value	ES (95% CI) KOA-control	ES (95% CI) FM-control
Δ_0HF HRV, nu	-16.55 (15.26)	0.50 (13.96)*	-7.21 (14.76)	.02#	1.16 (0.38 – 1.95)	0.62 (-0.14 – 1.38)
Δ_{15}HF HRV, nu	-8.53 (19.04)	5.66 (17.45)	-2.17 (7.75)	.07	0.78 (0.02 – 1.53)	0.43 (-0.33 – 1.18)
Δ_0PEP, ms	-10.19 (13.07)	-10.32 (13.31)	-9.59 (12.57)	.99	-0.01 (-0.74 – 0.72)	0.05 (-0.70 – 0.79)
Δ_{15}PEP, ms	1.02 (5.85)	2.95 (12.30)	-4.56 (9.50)	.17	0.20 (-0.53 – 0.93)	-0.72 (-1.49 – 0.05)
Δ_0SCL, µS	7.55 (5.93)	7.16 (6.36)	6.49 (4.51)	.89	0.06 (-0.67 – 0.79)	0.20 (-0.55 – 0.94)
Δ_{15}SCL, µS	2.50 (2.12)	3.11 (3.56)	3.10 (2.32)	.76	-0.21 (-0.94 – 0.52)	-0.27 (-1.02 – 0.48)
Δ_0SCR, spikes/min	3.99 (2.33)	4.12 (1.92)	2.94 (3.01)	.40	-0.06 (-0.79 – 0.67)	0.39 (-0.36 – 1.14)
Δ_{15}SCR, spikes/min	0.44 (0.71)	0.77 (0.91)	-0.82 (2.23)	.07	-0.41 (-1.14 – 0.33)	0.79 (0.02 – 1.56)
Δ_0HR, beats/min	7.98 (5.06)	3.73 (5.91)	1.58 (4.22)*	.01#	0.78 (0.02 – 1.53)	1.36 (0.54 – 2.19)
Δ_{15}HR, beats/min	-2.08 (3.42)	-4.79 (4.45)	-2.89 (3.59)	.17	0.69 (-0.06 – 1.44)	0.23 (-0.51 – 0.98)

Note. ANS = autonomic nervous system; CI = confidence interval; ES = effect size; FM = fibromyalgia; HF HRV = high frequency heart rate variability; HR = heart rate; KOA = knee osteoarthritis; PEP = pre-ejection period; PVSAT = Pace Visual Serial Addition Task; SCL = skin conductance level; SCR = skin conductance response; SD = standard deviation; * = significant difference from control; # = significant difference between groups; Δ_0 = difference from baseline to immediately following the PVSAT; Δ_{15} = difference from baseline to 15 mins after the PVSAT

4.4. Discussion

4.4.1. Introduction

The first aim of this study was to determine whether people with KOA and FM would exhibit ANS dysfunction at rest and, indeed, there was some evidence this was the case. The second aim of this study was to evaluate ANS function and nociceptive processing in people with KOA and FM in response to acute mental stress using mental arithmetic. The results showed people with KOA demonstrate smaller changes in ANS function and impaired descending inhibition of nociception in response to acute mental stress compared to pain free controls. Similarly, the FM group showed evidence of impaired descending inhibition of nociception. The third aim of this study was to document the recovery of the ANS and nociceptive system 15 mins after exposure to acute mental stress. The results for sympathetic function showed greater EDA activity at rest in the KOA and FM groups compared to controls, yet were largely similar to controls in response to mental arithmetic, and at the follow-up period. This section discusses these findings in more detail.

4.4.2. Parasympathetic nervous system function at baseline

The baseline measures and discussion of resting vagal tone are broadly similar to Section 3.4.3. However, some minor differences were observed. The findings of this study supported the hypothesis of reduced HF HRV at baseline in the KOA group compared to pain free controls. These results corroborate the novel findings of the first study of reduced vagal tone in OA at rest. While the FM group displayed lower HF HRV at baseline than controls, the difference between the two groups was not statistically significant.

4.4.3. Sympathetic nervous system function at baseline

Assessment of the SNS supported the hypothesis of increased SCL in the KOA and FM groups at baseline compared to controls, but the PEP and SCR outcomes were no different to controls. Several previous studies have shown elevated SCL, and a greater number of SCRs, in people with FM compared to controls [36, 118, 167, 210-212], while others have shown the opposite [421]. This is the first study to demonstrate increased SCL in people with KOA at rest compared to pain free controls. Sympathetic neurotransmitters are known to influence OA-related pain [436]. Increased sympathetic activity causes the release of endogenous catecholamines, adrenaline and noradrenaline, which are synthesised in the adrenal medulla, yet the chief source of circulating noradrenaline are the peripheral sympathetic nerve endings found in all joint tissues, except for articular cartilage [436]. Both catecholamines bind to adrenoreceptors that mediate the effects of an activated SNS; however, the influence of noradrenaline on joint tissue specifically, via adrenoreceptors, is catabolic and pro-inflammatory, potentially leading to increased pain and joint degeneration in OA [33, 436-438]. Similar to the first study, PEP values of the KOA and FM groups were statistically equivalent to controls at rest, consistent with previous studies [131, 422], one of which found no significant association with pain and PEP in people with chronic widespread pain [131]. However, three studies by Reyes del Paso et al. [31,

138, 431] found myocardial contractility to be lower in FM at rest compared to healthy controls. The authors postulate that reduced myocardial contractility at baseline, mediated by beta adrenergic activity, may be indicative of blunted cardiac sympathetic reactivity when exposed to stress that can adversely affect pain inhibition via the baroreflex system [31, 140, 421].

4.4.4. Nociceptive system response to acute mental stress

In accordance with our hypothesis, pain free controls showed significantly reduced pain ratings (HP60 scores) immediately following an acute mental stressor. Previous studies in pain free people, involving experimental pain and mental arithmetic, support these findings of mental stress-induced hypoalgesia [271, 273, 276, 439]. Mental stress-induced hypoalgesia in healthy people can be activated by several means, including opioid and non-opioid pathways [230, 273, 439, 440], and the cardiovascular system in the form of hypertension-induced hypoalgesia [140, 148]. The hypothesis of increased HP60 scores immediately following acute mental stress in the KOA and FM groups was not supported. HP60 scores were equivalent to baseline immediately following mental arithmetic in the KOA and FM groups. Mental stress can enhance pain in people with chronic pain [167]. Indeed, previous studies have found acute mental stress, by way of mental arithmetic, to increase levels of pain in chronic pain populations, such as FM [22, 31, 167], CRPS [239], and RA [22]. There is an overlap of areas in the brain that regulate stress responses and mood and that modulate pain, such that worsening of mood via mental stress can lead to greater sympathetic activation, increased inflammation, and dysfunction of descending pain modulation [22, 441, 442]. The current study found no significant increases or decreases in HP60 scores in the KOA and FM groups immediately following mental arithmetic. These results provide some evidence of impaired mental stress-induced hypoalgesia in people with KOA and FM in response to mental arithmetic. As such, this is the first study to document this finding in OA. However, the results did not demonstrate the expected increase in HP60 scores immediately following mental arithmetic in the KOA and FM groups. The reasons for this may include the intensity/duration of the stressor not being great enough, or differences in participant characteristics, e.g. depression/anxiety, compared to other studies [31]. Indeed, even though the depression and stress scores of the KOA and FM groups were significantly higher than controls, they still fell within normal ranges. Therefore, the combination of these factors may explain the lack of significant pain facilitation observed in the KOA and FM groups immediately following the mental stressor.

4.4.5. Parasympathetic nervous system response to acute mental stress

The results of this study supported the hypothesis of vagal withdrawal in response to acute mental stress in the control group, but not in the KOA and FM groups. These findings are congruent with previous studies examining HF HRV in healthy controls [31, 238, 248, 258, 264, 443-445] and people with FM [31] exposed to acute mental stress. The KOA group showed a marginal, nonsignificant increase in HF HRV immediately following mental arithmetic; and, while the FM group demonstrated vagal withdrawal, this change did not reach significance, similarly to findings reported by Reyes del Paso [31]. Only one study has previously reported opposite effects of increased HF HRV in healthy people in response to mental arithmetic (with verbalisation), which could only be explained by either

complex respiratory patterns or dual activation of the two autonomic branches [446]. This is the first study to show significantly smaller change in HF HRV in OA compared to healthy controls in response to mental arithmetic.

During mental stress, autonomic adjustments, such as vagal withdrawal and sympathetic activation, are made by regions of the cortex that modulate cardiovascular function [258]. Typically, when BP is increased, HR is decreased via vagal withdrawal [351, 447]. However, under mental stress, HR and BP are increased simultaneously because higher brain centres can blunt the baroreflex response [258, 448]. Previous studies have shown that BRS is decreased in response to mental stress, yet centrally mediated mechanisms maintain BP and HR despite vagal inhibition [448]. Therefore, if functional deficits in people with chronic pain lie within either the brain or the baroreflex system; or, vagal function is impaired at rest and/or in response to mental stress, as shown in the KOA group in this study, then a person may be at risk for aberrant cardiovascular regulation and, by extension, reduced antinociceptive effects [24, 31, 431].

4.4.6. Sympathetic nervous system response to acute mental stress

Acute psychological stress induces hypoactivity of the cortex, altering autonomic outflow from brainstem nuclei that modulate HR and BP that, in turn, activate descending inhibitory pathways [208, 237, 273, 449]. The cumulative effects of ongoing pain and chronic stress can, however, affect the function of the central autonomic network [124]. SNS hyperactivity at baseline can create a ceiling effect, whereby sympathetic reactivity to a stressor becomes dampened with reduced physiological resources for tolerating environmental demands [37, 421]. Sympathetic ceiling effects are caused by chronic stimulation of adrenergic receptors, leading to their desensitisation and downregulation [37]. This leads to hypoactivity of the prefrontal cortex, resulting in disinhibition of subcortical sympathetic circuits and, hence, further SNS hyperactivity [29, 208, 410]. Raised SCLs found at baseline in the KOA and FM groups may be indicative of a ceiling effect that can adversely impact stress and pain responses via the cardiovascular system [37, 38, 41, 167, 421].

The results for cardiac sympathetic reactivity to acute mental stress supported the hypothesis of reduced PEP in the control group, but the hypothesis of unchanged PEP in the KOA and FM groups was not supported. PEP shortened significantly across all three groups to a similar extent in response to acute mental stress. It was expected PEP would shorten during mental arithmetic in the control group, reflecting an increase in sympathetic activity, but be blunted in the KOA and FM groups. This is the first study to investigate changes in PEP in people with KOA in response to acute mental stress. It is well established that acute psychological stress raises beta adrenergic activity in healthy people, resulting in shortened PEP and increased cardiac contractility [238]. There are more studies investigating PEP in response to acute mental stress than painful physical conditioning stimuli. Five studies examined PEP in healthy people in response to mental arithmetic and the results consistently showed shortening of PEP during the stressor [165, 261, 266, 267, 433]. While PEP shortened significantly in response to mental arithmetic in the FM group in the current study, previous research has shown the opposite [431].

Mixed PEP results in people with chronic pain may be due to several reasons. Firstly, individual differences in output from cortex to heart in response to stress may exist [237], since there are direct cortical projections to the NTS [450]. Secondly, higher brain centres may reduce BRS depending on the intensity/duration of the stress [258, 448]. Therefore, if people with FM exhibit baroreflex dysfunction, via reduced reactivity to mental stress, this may result in unchanged or increased PEP [431].

The hypothesis of increased EDA immediately following acute mental stress in the control group was supported, but the hypothesis of smaller changes in EDA in the KOA and FM groups was not supported. SCL increased significantly in all three groups immediately following acute mental stress. The SCL increase in response to mental arithmetic was expected in healthy controls, as demonstrated by Fechir et al. [451]. A blunted sympathetic response was expected in the KOA and FM groups in response to stress. Indeed, Reyes del Paso et al. [421] found a dampened SCL response during breath-holding in FM compared to controls. However, two previous studies showed SCL to significantly increase from baseline in FM following mental arithmetic compared to controls [167, 283]. One study matched the findings of the KOA group with increased SCL in response to mental arithmetic in inflammatory arthritis [34], suggesting that sudomotor responses to psychological stress in people with arthritis may be no different to controls. To date, this is the first study to examine EDA in people with OA in response to acute mental stress. Similar to the control group, there were a larger number of SCRs in the KOA and FM groups in response to mental arithmetic when a blunted sympathetic response was expected based on the elevated level of SNS activity found at baseline, potentially creating a ceiling effect.

In sum, all SNS outcome measures in the current study reacted in a manner that may indicate normal sympathetic function immediately following acute mental stress in people with KOA and FM, as no differences to controls were demonstrated.

4.4.7. Nociceptive system function following recovery from acute mental stress

The hypothesis that pain ratings (HP60 scores) would return to baseline 15 mins after mental arithmetic in the control group was supported, but the hypothesis that HP60 scores would be prolonged in the KOA and FM groups was not supported. Following a significant reduction in HP60 scores during acute mental stress, HP60 scores returned to baseline 15 mins after mental arithmetic in the control group. It was expected that HP60 scores would increase in the KOA and FM groups immediately following mental arithmetic, and remain elevated for up to 15 mins after the stressor. However, HP60 scores in the KOA and FM groups were equivalent to baseline values 15 mins after mental arithmetic, following a nonsignificant reduction during mental arithmetic. There is limited evidence showing the carry-over effects on the nociceptive system following acute mental stress beyond 5 mins. The available data suggests the persistence of pain from an experimental pain stimulus following mental stress is short-lived, with pain ratings returning to baseline values within 2 – 5 mins in healthy people [239, 271] and people with chronic conditions [22, 167]. This is the first study to examine pain up to 15 mins after acute

mental stress in OA, FM, and healthy controls, and suggests any effects on the nociceptive system are short-lived.

4.4.8. Parasympathetic nervous system function following recovery from acute mental stress

The hypothesis that HF HRV would be equivalent to baseline 15 mins after mental arithmetic in the control group was supported. The hypothesis that HF HRV would remain unchanged from baseline to 15 mins after mental arithmetic in the KOA and FM groups was also supported. In a healthy person, vagal withdrawal occurs during mental stress, followed by a return of vagal activity to baseline level upon termination of the stress [218]. Laborde et al. [25] describe this phenomenon as the “vagal tank” of: rest, reactivity, and recovery. Other authors have referred to vagal recovery as “vagal rebound” [218, 266, 452]. The metaphor illustrates a person’s ability to face the demands of stress and appropriately replenish their autonomic tank during recovery. Failure to adapt to stress (i.e. to empty and refill the vagal tank) has been identified as a risk factor for disease [29, 208, 420]. Weber et al. [420] demonstrated that people with reduced HF HRV at baseline leave little room to adapt to mental stress, with no post-stress recovery, compared to people with high HF HRV at baseline rebounding within 5 mins. The control group showed rebound of vagal activity toward baseline with HF HRV being equivalent to baseline after the 15-minute recovery period. In contrast, there was no evidence of significantly altered vagal activity in the KOA and FM groups at 15 mins given the diminished change in vagal reactivity following mental arithmetic. The KOA group showed an increase in HF HRV compared to baseline after the 15-minute recovery period, but this change was not significant. The increase in HF HRV may be explained by altered breathing patterns during recovery [376, 446].

4.4.9. Sympathetic nervous system function following recovery from acute mental stress

Assessment of the SNS 15 mins after mental arithmetic showed a discrepancy in recovery of PEP and EDA outcomes. The hypothesis that PEP would return to baseline 15 mins after mental arithmetic in the control group was supported, but the hypothesis that PEP would remain unchanged or be maintained in the KOA and FM groups was not supported. PEP values were equivalent to baseline in all three groups 15 mins after mental arithmetic. The hypothesis that EDA would return to baseline 15 mins after mental arithmetic in the control group was not supported, while the hypothesis that any changes in EDA would be maintained in the KOA and FM groups was supported. EDA remained significantly elevated in all three groups after the 15-minute recovery period. Schachinger et al. [191] discuss the differences between central and peripheral SNS activation in periods following stressful events. Changes in sympathetic activity directed at supraspinal structures versus peripheral structures do not necessarily run in parallel. While central sympathetic output from the brain and brainstem may return to normal immediately following a stressor, peripheral structures can still be stimulated by circulating catecholamines [191]. Therefore, PEP values may return to baseline during recovery following acute stress, while EDA can remain elevated. Indeed, this was the case in the current study. Few studies report SNS outcome measures after a recovery period following stress. Reyes del Paso et al. [431] found PEP values to return to baseline in healthy controls and people with FM 5 mins after mental arithmetic, and Thieme et al. [167, 283] reported elevated SCLs being maintained 4 mins after

mental arithmetic in healthy controls and FM. The discrepancy in recovery times in healthy and FM populations, between previous studies and the current study, may be due to the intensity/duration of the stressor. For example, Thieme et al. [167, 283] employed mental arithmetic for 4 mins, compared to ≈12 mins in the current study.

4.4.10. Heart rate following recovery from acute mental stress

HR does not feature in the hypotheses due to its complex, multivariate nature of control from multiple sources, such as the PNS, SNS, and brain – especially during mental stress [260, 261, 284, 285]. However, with interest it was noted that HR was lower after the 15-minute recovery period than during baseline in the KOA and FM groups. Mezzacappa et al. [266] state two possibilities for this outcome. The first is that HR may be higher than it needs to be at baseline due to anticipatory stress of the experiment; and, the second possibility is that the slower HR during recovery may be from an active recovery process occurring in the KOA and FM groups but not controls.

4.5. Strengths and limitations

The strengths of this study include the use of the PVSAT, a valid and reliable stressor [248, 263, 265], and the range of validated QST and ANS measures collected in a controlled laboratory environment. This study, however, is not without its limitations. COVID-19 restrictions led to the sample size being smaller than intended, with the study potentially being underpowered to detect some differences between groups and/or over time. The PVSAT is a computerised version of the PASAT, requiring the use of a mouse and laptop. Therefore, instead of participants responding verballing to each PASAT item, they required a degree of dexterity to coordinate the mouse cursor onscreen to find the correct answer to the PVSAT. Indeed, the KOA group – the eldest group – demonstrated the poorest PVSAT performance. Age and hand-eye coordination may have been a factor in this result, potentially making the task more stressful for the KOA group than the control and FM groups. PVSAT performance results were lower during the ANS session than the QST session in all three groups, possibly due to each participant needing to remain still in a 30° recumbent position while recording ANS outcome measures. Remaining still to reduce artefacts in the ICG/ECG recordings may also have heightened the level of difficulty and stress for participants. Finally, previously undiagnosed cardiac issues in some participants resulted in the loss of ECG data. A larger sample size would accommodate for such unforeseen circumstances in future research.

4.6. Conclusion

This is the first study to demonstrate vagal dysfunction in people with KOA in response to acute mental stress. The results of the current study corroborate those of the first study: people with KOA displayed significantly reduced vagal tone at rest compared to healthy controls, but additionally showed vagal changes that were significantly smaller in the KOA group immediately following mental stress compared to controls. A floor effect of reduced vagal tone at baseline may be a potential indicator for poor adaptation to stress and increased pain sensitivity. Psychological stress induces both vagal withdrawal

and sympathetic activation to approximately equal extent [238]. This balanced ANS response was demonstrated in the control group in the current study. The KOA and FM groups exhibited significant increases in SNS activation, yet dampened vagal reactivity to acute mental stress. Autonomic imbalance shown by the KOA and FM groups in the current study has been implicated in failure to adapt to stress [420] and potential cardiovascular problems [208, 453, 454]. Altered cardiovascular control may reduce BRS and, in turn, a chronic pain person's ability to attenuate pain when exposed to stress [140, 455]. These dysfunctions may contribute to the impairment of mental stress-induced hypoalgesia seen in the KOA and FM groups compared to controls.

Chapter 5. Effects of acute isometric exercise on nociception and the autonomic nervous system in people with knee osteoarthritis and fibromyalgia

5.1. Introduction

The following chapter outlines the methodology used by describing the study aim, design, participants, procedure, outcome measures, and statistical analyses.

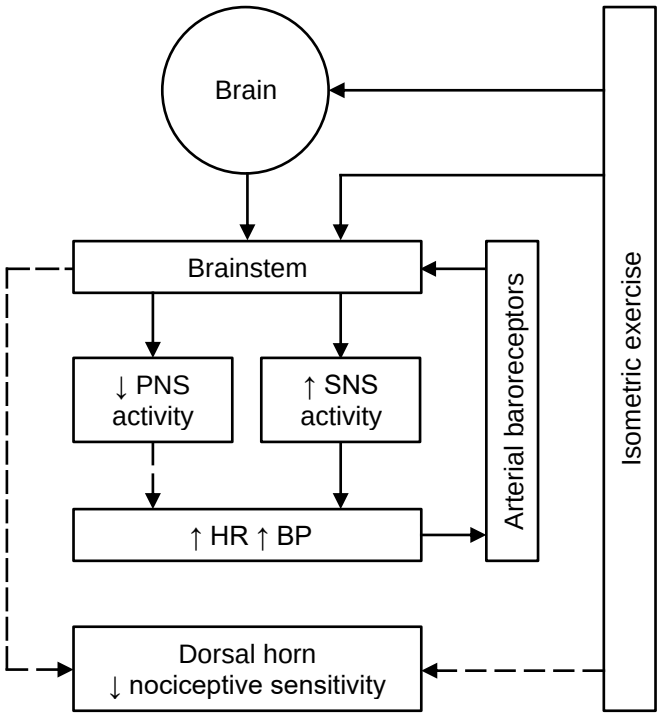
The aim of the third study was to examine the effects of acute isometric exercise on the nociceptive and autonomic nervous systems in people with KOA and FM. The following specific hypotheses were tested:

I. At rest, people with KOA and FM would exhibit reduced HF HRV, reduced PEP, increased EDA; and, reduced PPT compared to pain free controls.

II. Immediately following isometric exercise, pain free controls (Figure 36) would demonstrate a reduction in HF HRV, reduced PEP, increased EDA; and increased knee PPT (EIH). In comparison to controls, these changes will be reduced in the KOA and FM groups (Figure 37).

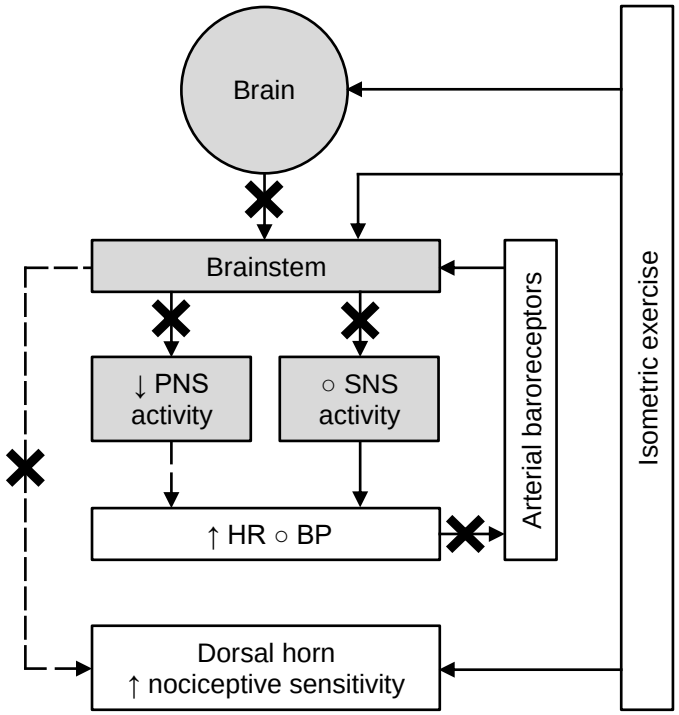
III. Fifteen minutes after isometric exercise, HF HRV, PEP, EDA, and PPT would be equivalent to baseline values in the control group. In comparison to baseline, any changes in ANS outcome measures and PPT would be maintained in the KOA and FM groups at 15 mins.

Figure 36. ***Hypothesis of a pain free control exhibiting normal autonomic function resulting in reduced nociceptive sensitivity in response to acute isometric exercise.***



Note. BP = blood pressure**;** HR = heart rate; PNS = parasympathetic nervous system; SNS = sympathetic nervous system**;** ⟶ = excitation; ⇢ = inhibition

Figure 37. ***Hypothesis of a floor effect of reduced high frequency heart rate variability, blunted sympathetic reactivity, and reduced pressure pain threshold in response to acute isometric exercise in people with knee osteoarthritis.***



Note. BP = blood pressure; grey = area of dysfunction; HR = heart rate; PNS = parasympathetic nervous system; SNS = sympathetic nervous system; ○ = blunted response; ✕ = dysfunctional pathway; ⟶ = excitation; ⇢ = inhibition

5.2. Method

5.2.1. Study design

The study was a cross-sectional, experimental design undertaken at the AUT Biomechanics Laboratory, North Shore Hospital, Auckland.

5.2.2. Sample size

A sample size for this study was powered based on a previous study examining the reliability of HRV indices to a repetitive, low-force task [456], equivalent to a maintained isometric muscle contraction. No previous studies were available to estimate the effects of acute isometric exercise on HRV in people with OA. Therefore, the effect size and power calculation was guided by the recommendations of Hallman et al. [456]. The authors found that sample sizes for detecting clinically meaningful, reliable changes of 20% of the mean of each HRV parameter (including HF HRV) between groups in response to a low-intensity physical task, using an alpha level of .05 and power of 0.8, corresponded to approximately 20 participants per group. Therefore, based on these recommendations, for three groups

with 20 participants per group, a sample size of *N* = 60 was required to detect a 20% change in HF HRV in participants between groups [456].

5.2.3. Participants

The same participant groups (KOA, FM, and pain free control) and inclusion/exclusion criteria from the first study were used. Demographic information was collected from each participant and the nature of each participant's pain was determined using the same questionnaires as the first study. Participants were asked to refrain from taking analgesic medication for 24 hours prior to data collection, and from taking caffeine and tobacco products 6 hours prior to data collection.

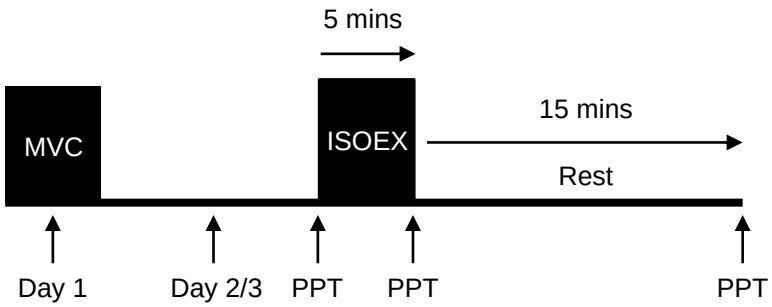
5.2.4. Ethical considerations

Ethical approval was obtained from the Health and Disability Ethics Committees (HDEC): approval number 18/CEN/45 (Appendix F). Consultation was also provided by the Matauranga Maori Committee. During the design and implementation of the study the principles of the Treaty of Waitangi, including partnership, participation and protection were applied, and the recruitment process ensured that all eligible participants had equal opportunity to take part in the study regardless of ethnicity. Each participant was fully informed with a participant information sheet (Appendix G) and consented to participation in the study by signing a consent form (Appendix H). The privacy of each participant was maintained at all times by assigning participant codes.

5.2.5. Study procedure

Data collection was conducted over three separate days. The MVC was determined separately on the first day to avoid fatigue effects. Nociceptive and ANS outcomes in response to submaximal isometric contraction were recorded on separate days, in randomised order. Outcome measures were obtained on separate days in order for the nociceptive measures not to confound the ANS recordings.

Figure 38. ***Procedure for assessing nociceptive outcome measures in response to isometric exercise.***

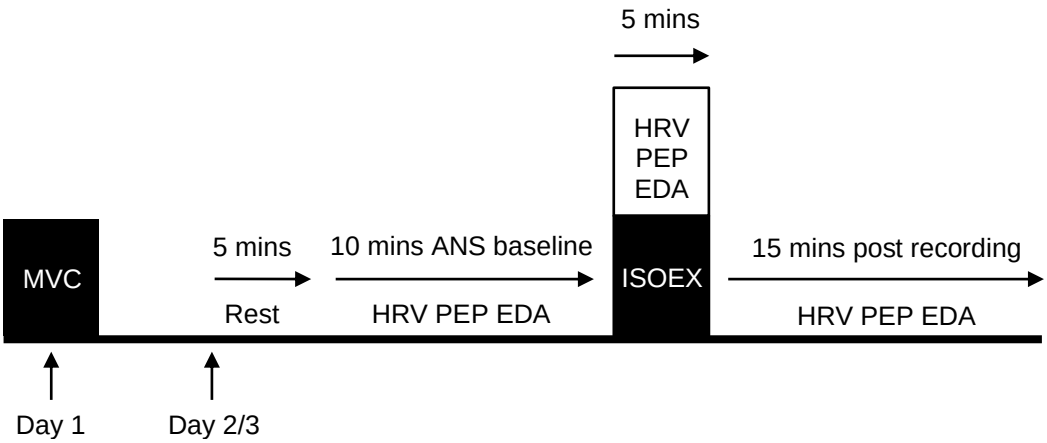


Note. ISOEX = isometric exercise; MVC = maximal voluntary contraction; PPT = pressure pain threshold

Nociceptive outcome measures were assessed in response to a submaximal isometric exercise (Figure 38). The participant was then instructed to hold an isometric knee extension at 20% of the

predetermined MVC for 5 mins. If the person was not able to maintain the contraction, they were permitted ≈10 s rest breaks as required in order to achieve 5 mins. During the isometric exercise, the participant rated their perceived level of exertion using the 6 – 20 Borg scale [457]. Every 60 s, the participant reported a single number representing their perceived exertion ranging from 6 (no exertion at all) to 20 (maximal exertion) [458]. PPTs were measured before, immediately following, and 15 mins after the isometric exercise as the participant sat quietly at rest.

Figure 39. ***Procedure for assessing autonomic nervous system outcome measures in response to isometric exercise.***



Note. ANS = autonomic nervous system; EDA = electrodermal activity; HRV = heart rate variability; ISOEX = isometric exercise; MVC = maximal voluntary contraction; PEP = pre-ejection period

ANS outcome measures were assessed in response to a submaximal isometric exercise (Figure 39). Participants were asked to recline in the supine position with the torso elevated at 30° for 5 mins before continuous resting HRV, PEP, and EDA baselines were recorded for at least 10 mins (Figure 40). The participant was then instructed to hold an isometric knee extension at 20% of the predetermined MVC for 5 mins. If the person was not able to maintain the contraction, they were permitted ≈10 s rest breaks as required in order to achieve 5 mins. During the isometric exercise, the participant rated their perceived level of exertion using the 6 – 20 Borg scale [457]. Every 60 s, the participant reported a single number representing their perceived exertion ranging from 6 (no exertion at all) to 20 (maximal exertion) [458]. The ICG/ECG and EDA data were recorded continuously during the intervention, and then for at least 15 mins following the isometric exercise as the participant quietly rested.

5.2.6. Acute exercise stress intervention

MVC of the quadriceps femoris was determined in all participants on the first day using a Biodex Multi-Joint System dynamometer (Biodex, USA). The most affected limb was used to perform the exercise in the KOA group, while the other participants used their left leg. Participants were asked to recline in the supine position with the torso elevated at 30° for the purpose of ANS measurement. Knees were flexed at 90° on the chair with straps crisscrossed over the chest to prevent excess body movement. The thigh

of the limb performing the extension contraction was strapped to the seat, and the ankle strapped to the arm of the dynamometer, just above the malleoli (Figure 40).

Figure 40. ***Participant resting at 30° on the Biodex Multi-Joint System before and during autonomic baseline recording with the ankle strapped to the arm of the dynamometer.***

A warm-up procedure was performed for familiarisation with the task involving four repetitions of graded effort: 25%, 50%, and 75% MVC twice. Four maximal voluntary contractions of 5 s were then performed with a 60 s rest between contractions. The peak torque (Nm) value in any of the four contractions was recorded as MVC. Participants were given consistent verbal encouragement during each of the MVC

contractions. The intervention on the second and third days involved sustaining a 20% MVC contraction of the quadriceps femoris muscle, in the same body position as described above, for 5 mins with ≈10 s rest breaks as required in order to achieve that time frame. The intensity and duration were chosen based on previous research in similar populations [93, 117] demonstrating that this level of contraction is generally tolerable in FM and OA populations and leads to a clear EIH response in pain free controls. Maintaining 20% MVC for the intervention was achieved by monitoring feedback from the dynamometer operation screen, using the Biodex Advantage Software program (Biodex, USA), and matching a line depicting the target force.

5.2.7. Nociceptive outcome measures

Participant's nociceptive processing was assessed using PPT before, immediately following, and 15 mins after isometric exercise (Figure 38). The algometer was applied to the skin at two locations: one test site and one control site. For the KOA group, the test site was 2 cm distal to the inferior edge of the medial patella on the involved knee [15], while the control site was the volar forearm on the ipsilateral limb, 5 cm distal to the elbow along the radial border [15]. For all other participants, PPT sites were the same location on the knee and ipsilateral volar forearm. The procedure was performed three times at each location with a 30 s interval between stimuli and the average used for further analyses [368]. EIH was calculated by subtracting the mean PPT immediately following isometric exercise from the mean PPT at baseline. Positive values indicate the presence of EIH (pain inhibited after exercise) while negative values indicate the absence of EIH (pain increased after exercise) [93].

5.2.8. Autonomic nervous system outcome measures

HF HRV, PEP, SCL, SCR, and HR were recorded 10 mins before (baseline), during, and continuously for 15 mins after isometric exercise (Figure 39) using procedures described in the first study.

5.2.9. Data analysis

Normality of distribution of data was analysed using the Kolmogorov-Smirnov test and variables that were not statistically significant were classed as having normal distribution. Normally distributed dependent variables were analysed by parametric analyses.

To compare characteristics of the three groups, continuous variables were compared between groups using one-way ANOVA. Significant main effects were followed up with two-sided Dunnett's test to compare the KOA and FM groups to the control group. Ordinal data from questionnaires was compared between groups using Kruskal-Wallis tests, with follow-up Mann-Whitney U tests for significant results. Gender was analysed using the chi-square test.

To compare the outcome measures at baseline among the three groups, one-way ANOVA was used. Significant main effects were followed up with one-sided Dunnett's test to compare the KOA and FM groups to the control group. Comparisons were not made between KOA and FM groups because this

was not an outcome of interest and the studies were not powered to detect this. To determine the effect of isometric exercise on the ANS, repeated measures ANOVAs were used to compare the outcome measures across three time periods: baseline, immediately following, and 15 mins after isometric exercise. The Huynh-Feldt correction factor was used when Epsilon < 1. Significant main effects were investigated with planned comparisons between baseline, immediately following, and 15 mins after isometric exercise. To determine the effect of isometric exercise on the nociceptive system (EIH), paired *t*-tests were used to compare baseline data with that obtained immediately following and 15 mins after isometric exercise within each group. Effect sizes and 95% confidence intervals were determined from the difference scores between the KOA-control and FM-control comparisons.

Difference values (i.e. change scores) in outcome measures from baseline to immediately following isometric exercise, and from baseline to 15 mins after isometric exercise, were compared between groups using one-way ANOVA. Significant main effects were followed up with one-sided Dunnett's test to compare the KOA and FM groups to the control group.

MVC and Borg values were compared between groups using one-way ANOVA. Significant main effects were followed up with one-sided Dunnett's test to compare the KOA and FM groups to the control group. Borg scores were analysed between groups using Kruskal-Wallis tests, with follow-up Mann-Whitney U tests for significant results.

For equivalent non-parametric analyses, between group comparisons were made using Kruskal-Wallis tests, with follow-up Mann-Whitney U tests for significant results. Comparisons over the three time periods (baseline, immediately following, and 15 mins after isometric exercise) were analysed within each group using Friedman tests, with significant results followed up with Wilcoxon Signed Rank tests. Within group comparisons were made using Wilcoxon Signed Rank tests. The alpha level for all statistical procedures was set to .05, and all statistical analyses were performed using IBM SPSS Statistics for Windows version 28 (Armonk, NY: IBM Corp).

5.3. Results

5.3.1. Recruitment

During the period of recruitment, the worldwide COVID-19 pandemic led to several lockdown periods in the Auckland region, and a general hesitancy on the part of participants to attend hospital-based appointments for research involving face-to-face data collection. As such, only forty-two of the planned 60 participants were able to be recruited: 15 pain free people, 14 people with KOA, and 13 people with FM. All 42 participants met the inclusion criteria and completed the study. It was discovered during data processing that one control participant exhibited a previously undiagnosed ectopic beat; a second control participant displayed heart beat errors; and, a third control participant experienced a coughing fit at the 15-minute mark. These anomalies impacted measurements of HF HRV; therefore, HF HRV

data was removed from data analyses for these three participants. Data collection took place from May 2019 to June 2021.

5.3.2. Participant characteristics

Participant characteristics are shown in Table 1 and described in Section 3.3.2.

5.3.3. Data distribution

SCR and PEP were not normally distributed at baseline and analysed using non-parametric methods. For comparisons over time, the following ANS variables were not normally distributed in at least one group and analysed using non-parametric methods: HF HRV, PEP, and SCR. Difference values from baseline to immediately following isometric exercise (Δ_0), and from baseline to 15 mins after isometric exercise (Δ_{15}), that were not normally distributed included: Δ_0SCL, Δ_0HR, Δ_{15}PEP, Δ_{15}SCL, and Δ_{15}SCR. The remaining ANS variables were normally distributed and analysed using parametric methods.

Arm PPT at baseline in the control group, and knee PPT 15 mins after isometric exercise in the control and KOA groups, were not normally distributed and analysed using non-parametric methods. Difference values in PPT from baseline to immediately following isometric exercise (Δ_0PPT), and from baseline to 15 mins after isometric exercise (Δ_{15}PPT), were normally distributed and analysed using parametric methods. All remaining QST variables were normally distributed and analysed using parametric analyses.

MVC values were normally distributed and analysed using parametric methods. Borg scores were not normally distributed and analysed using non-parametric methods.

5.3.4. Maximal voluntary contraction and Borg ratings of perceived exertion

Group results for MVC and Borg ratings of perceived exertion (RPE) are shown in Table 6. There were significant differences between groups in MVC ($F_{2,39}$ = 5.87, p = .01). Follow-up one-sided Dunnett's test showed that, as expected, MVC was significantly lower in both the KOA (p = .003) and FM (p = .01) groups compared to the control group. There were no significant differences between groups in Borg RPE during ANS testing (H_2 = 2.99, p = .22) or QST testing (H_2 = 5.93, p = .052).

Table 6. ***MVC values and Borg RPE. Data are mean (SD).***

Variable	Control *n* = 15	KOA *n* = 14	FM *n* = 13	Omnibus *p* value
MVC, Nm	200 (63)	135 (62)*	145 (32)*	.01[#]
20% MVC, Nm	40 (13)	27 (12)	29 (6)	
Borg RPE, 6 – 20:				
During ANS testing	16 (3)	15 (2)	16 (2)	.22
During QST testing	16 (3)	14 (2)	16 (2)	.052

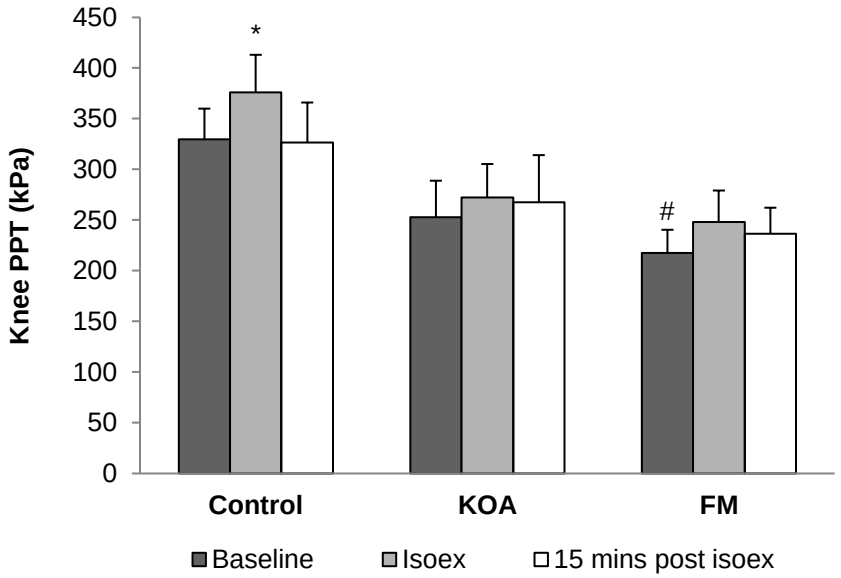
Note. ANS = autonomic nervous system; FM = fibromyalgia; KOA = knee osteoarthritis; MVC = maximal voluntary contraction; QST = quantitative sensory testing; RPE = ratings of perceived exertion; SD = standard deviation; * = significant difference from control; [#] = significant difference between groups

5.3.5. Pressure pain threshold and exercise-induced hypoalgesia

Group results for knee PTT are shown in Figure 41. There were significant differences among groups in knee PPT at baseline ($F_{2,39}$ = 3.58, p = .04). Follow-up one-sided Dunnett's test showed the FM group to have significantly lower knee PPT at baseline compared to the control group (p = .01). While knee PPT values were lower in the KOA group compared to the control group, this difference did not reach statistical significance (p = .07). Isometric exercise led to a significant increase in knee PPT (EIH) for the control group (t_{14} = -2.52, p = .03). In contrast, the KOA (t_{13} = -0.88, p = .39) and FM (t_{12} = -1.70, p = .11) groups did not show significant change. Therefore, the control group demonstrated EIH at the knee immediately following isometric exercise, while the KOA and FM groups did not. Knee PPT values were equivalent to baseline in the control group 15 mins after isometric exercise (t_{14} = 0.14, p = .89). There were no significant differences in knee PPT in the KOA (t_{13} = -0.43, p = 0.68) and FM (t_{12} = -1.14, p = .28) groups from baseline to 15 mins after isometric exercise.

Raw and change values for knee PPT are shown in Table 7. The change in knee PPT from baseline to immediately following isometric exercise was not significantly different among groups ($F_{2,39}$ = 0.51, p = .61). Similarly, the change in knee PPT from baseline to 15 mins after isometric exercise was not significantly different among groups ($F_{2,39}$ = 0.21, p = .82).

Figure 41. ***Knee pressure pain thresholds at baseline, immediately following, and 15 mins after isometric exercise.***

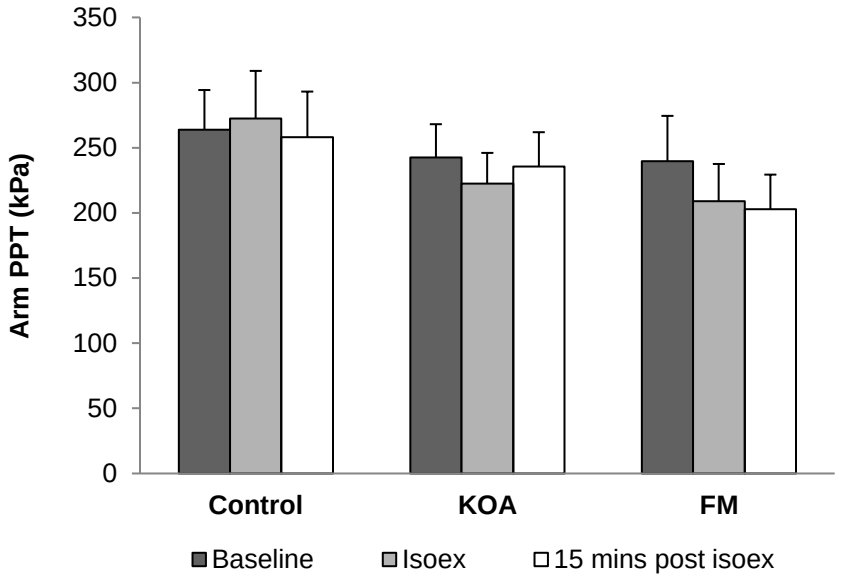


Note. FM = fibromyalgia; Isoex = isometric exercise; KOA = knee osteoarthritis; PPT = pressure pain threshold; * = significant difference from baseline; # = significant difference from control; error bars are one standard error of the mean

Group results for arm PTT are shown in Figure 42. There were no significant differences among groups in arm PPT at baseline (H_2 = 0.36, p = .84). No significant differences were found in arm PPT from baseline to immediately following isometric exercise in the control (t_{14} = -0.56, p = .58), KOA (t_{13} = 1.32, p = .21), or FM (t_{12} = 2.02, p = .07) groups. Similarly, no significant differences were found in arm PPT from baseline to 15 mins after isometric exercise in the control (t_{14} = 0.33, p = .75), KOA (t_{13} = 0.36, p = .72), or FM (t_{12} = 1.87, p = .09) groups.

Raw and change values for arm PPT are shown in Table 7. The change in arm PPT from baseline to immediately following isometric exercise was not significantly different among groups ($F_{2,39}$ = 1.79, p = .18). Similarly, the change in arm PPT from baseline to 15 mins after isometric exercise was not significantly different among groups ($F_{2,39}$ = 0.83, p = .44).

Figure 42. ***Arm pressure pain thresholds at baseline, immediately following, and 15 mins after isometric exercise.***



Note. FM = fibromyalgia; Isoex = isometric exercise; KOA = knee osteoarthritis; PPT = pressure pain threshold; error bars are one standard error of the mean

Table 7. ***Raw and change QST values. Data are mean (SD).***

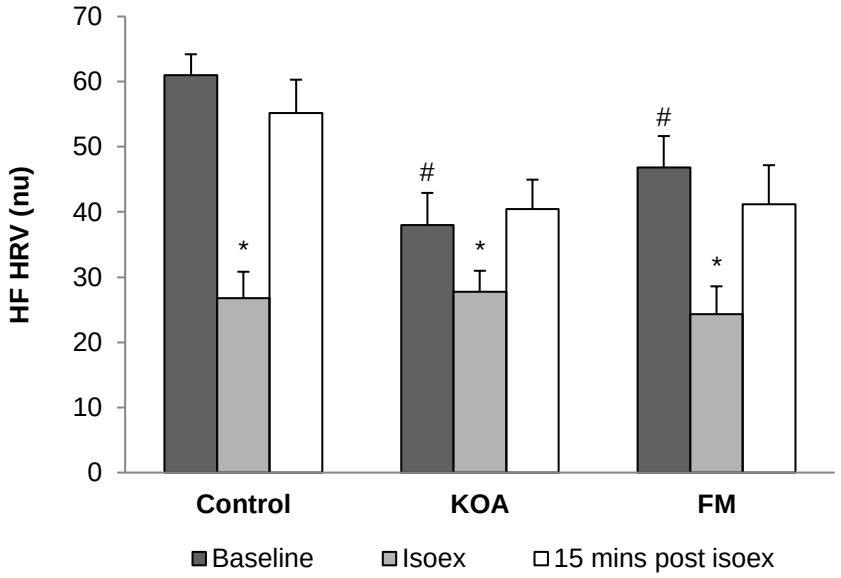
Variable	Control *n* = 15	KOA *n* = 14	FM *n* = 13	Omnibus *p* value	ES (95% CI) KOA-control	ES (95% CI) FM-control
PPT knee, kPa:						
Baseline	330 (117)	253 (134)	217 (83)*	.04[#]	0.61 (-0.13 – 1.36)	1.1 (0.30 – 1.90)
Immediately following isoex	376 (143)	272 (124)	248 (113)		0.78 (0.02 – 1.53)	0.98 (0.20 – 1.77)
Fifteen minutes after isoex	326 (154)	267 (175)	237 (92)		0.36 (-0.38 – 1.09)	0.69 (-0.08 – 1.45)
PPT arm, kPa:						
Baseline	264 (118)	243 (95)	240 (209)	.84	0.20 (-0.54 – 0.93)	0.14 (-0.60 – 0.89)
Immediately following isoex	273 (141)	223 (88)	209 (103)		0.42 (-0.31 – 1.16)	0.51 (-0.24 – 1.27)
Fifteen minutes after isoex	258 (136)	236 (99)	203 (95)		0.18 (-0.55 – 0.91)	0.46 (-0.29 – 1.22)
Δ_0PPT knee, kPa	46 (71)	19 (82)	30 (64)	.61	0.35 (-0.38 – 1.09)	0.24 (-0.51 – 0.98)
Δ_{15}PPT knee, kPa	-3 (97)	15 (127)	19 (61)	.82	-0.16 (-0.89 – 0.57)	-0.27 (-1.01 – 0.48)
Δ_0PPT arm, kPa	9 (61)	-20 (57)	-31 (55)	.18	0.49 (-0.25 – 1.23)	0.68 (-0.08 – 1.45)
Δ_{15}PPT arm, kPa	-6 (68)	-7 (73)	-37 (70)	.44	0.01 (-0.71 – 0.74)	0.45 (-0.30 – 1.20)

Note. CI = confidence interval; ES = effect size; FM = fibromyalgia; Isoex = isometric exercise; KOA = knee osteoarthritis; PPT = pressure pain threshold; QST = quantitative sensory testing; SD = standard deviation; * = significant difference from control; [#] = significant difference between groups; Δ_0 = difference from baseline to immediately following isometric exercise; Δ_{15} = difference from baseline to 15 mins after isometric exercise

5.3.6. Heart rate variability

Group results for HF HRV are shown in Figure 43. There were significant differences among groups in HF HRV at baseline ($F_{2,37}$ = 6.90, p = .003). Follow-up one-sided Dunnett's test showed both the KOA (p < .001) and FM (p = .03) groups to have significantly lower HF HRV at baseline compared to the control group. The main effect of time was significant for HF HRV in the control ($F_{2,22}$ = 28.26, p < .001), KOA ($F_{2,26}$ = 3.65, p = .040), and FM ($\chi^2(2)$ = 14.00, p < .001) groups. Compared to baseline, HF HRV was significantly lower in all three groups immediately following isometric exercise (all p < .04), but not significantly different 15 mins after isometric exercise (all p > .20).

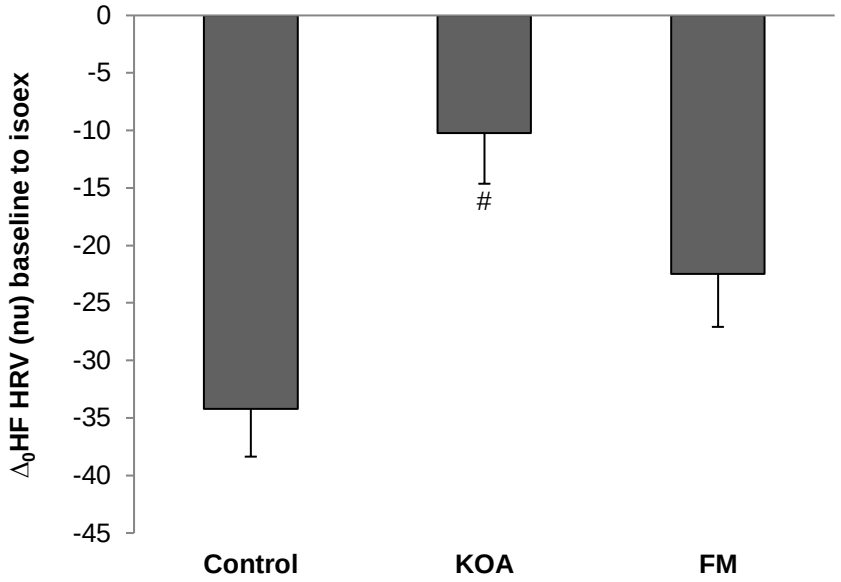
Figure 43. ***High frequency heart rate variability at baseline, immediately following, and 15 mins after isometric exercise.***



Note. FM = fibromyalgia; HF HRV = high frequency heart rate variability; Isoex = isometric exercise; KOA = knee osteoarthritis; * = significant difference from baseline; # = significant difference from control; error bars are one standard error of the mean

Change values for HF HRV are shown in Figure 44 and Table 8. Change values in HF HRV from baseline to immediately following isometric exercise were significantly different among groups ($F_{2,37}$ = 7.52, p = .002). Follow-up one-sided Dunnett's test showed change values for the KOA group were significantly smaller than the control group (p < .001). Change values in HF HRV from baseline to 15 mins after isometric exercise were not significantly different among groups ($F_{2,36}$ = 1.13, p = .34).

Figure 44. ***Change values in high frequency heart rate variability from baseline to immediately following isometric exercise.***



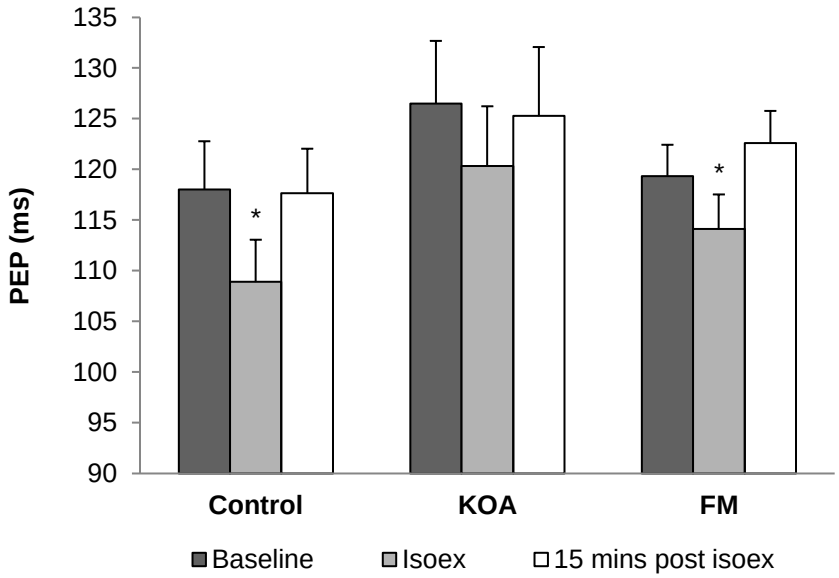
Note. FM = fibromyalgia; HF HRV = high frequency heart rate variability; Isoex = isometric exercise; KOA = knee osteoarthritis; # = significant difference from control; Δ_0 = difference from baseline to immediately following isometric exercise; error bars are one standard error of the mean

5.3.7. Pre-ejection period

Group results for PEP are shown in Figure 45. There were no significant differences among groups in PEP at baseline (H_2 = .83, p = .66). The main effect of time was significant for PEP in the control ($\chi^2(2)$ = 16.93, p < .001) and FM ($\chi^2(2)$ = 8.77, p = .01) groups. Compared to baseline, PEP was significantly lower in the control (p < .001) and FM (p = .04) groups immediately following isometric exercise, but not 15 mins after isometric exercise (both p > .06). The main effect of time was not significant for PEP in the KOA group ($F_{1.9,25.0}$ = 1.82, p = .19).

Change values for PEP are shown in Table 8. The change in PEP from baseline to immediately following isometric exercise was not significantly different among groups ($F_{2,39}$ = .64, p = .53). Similarly, the change in PEP from baseline to 15 mins after isometric exercise was not significantly different among groups (H_2 = 2.24, p = .33).

Figure 45. ***Pre-ejection period at baseline, immediately following, and 15 mins after isometric exercise.***



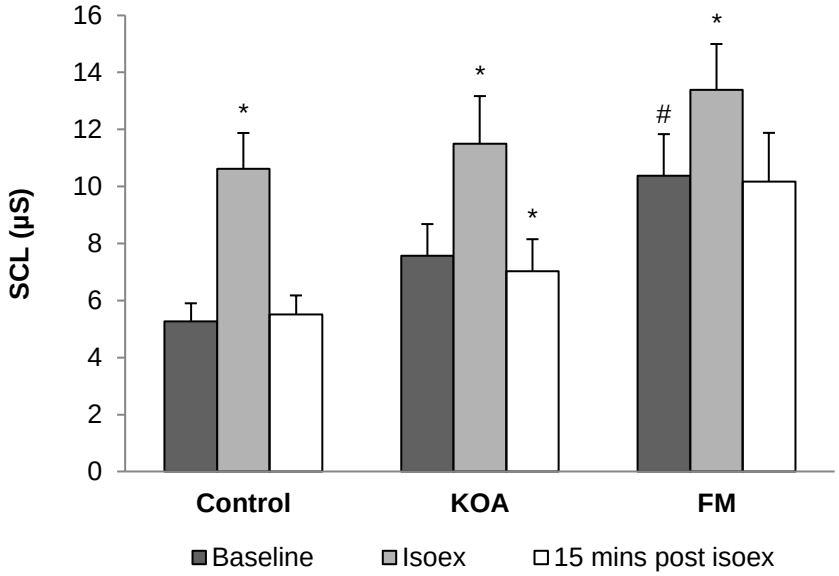
Note. FM = fibromyalgia; Isoex = isometric exercise; KOA = knee osteoarthritis; PEP = pre-ejection period; * = significant difference from baseline; error bars are one standard error of the mean

5.3.8. Skin conductance level and response

Group results for SCL are shown in Figure 46. There were significant differences among groups in SCL at baseline ($F_{2,39}$ = 5.53, p = .01). Follow-up one-sided Dunnett's test showed that SCL at baseline was significantly higher in the FM group compared to the control group (p = .002), while the KOA group were not significantly different from the control group (p = .12). The main effect of time was significant for SCL in the control ($F_{1.2,13.2}$ = 36.08, p < .001), KOA ($F_{1.1,14.5}$ = 21.37, p < .001), and FM ($F_{1.6,19.8}$ = 11.90, p < .001) groups. SCL was raised significantly from baseline in all three groups immediately following isometric exercise (all p < .007). Fifteen minutes after isometric exercise, SCL was significantly lower than baseline in the KOA group (p < .001), while SCL values were equivalent to baseline in the control (p = .29) and FM (p = .66) groups.

Change values for SCL are shown in Table 8. The change in SCL from baseline to immediately following isometric exercise was not significantly different among groups (H_2 = 4.09, p = .13). Similarly, the change in SCL from baseline to 15 mins after isometric exercise was not significantly different among groups (H_2 = 3.61, p = .16).

Figure 46. ***Skin conductance levels at baseline, immediately following, and 15 mins after isometric exercise.***

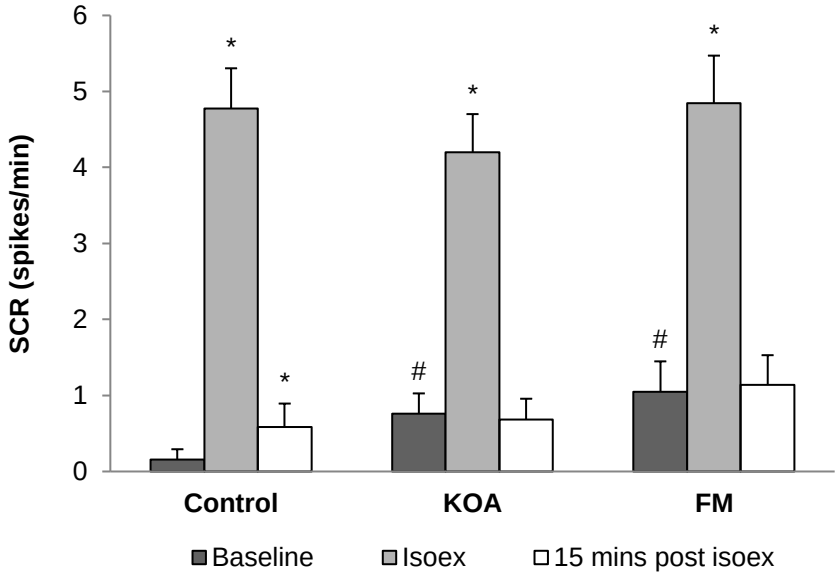


Note. FM = fibromyalgia; Isoex = isometric exercise; KOA = knee osteoarthritis; SCL = skin conductance level; * = significant difference from baseline; # = significant difference from control; error bars are one standard error of the mean

Group results for SCR are shown in Figure 47. There were significant differences among groups in SCR at baseline (H_2 = 6.71, p = .04). Follow-up Mann-Whitney U tests showed that the number of SCRs was significantly higher at baseline in both the KOA (p = .03) and FM (p = .02) groups compared to the control group. The main effect of time was significant for SCR in the control ($\chi^2(2) = 26.92$, $p < .001$), KOA ($\chi^2(2) = 22.77$, $p < .001$), and FM ($\chi^2(2) = 20.69$, $p < .001$) groups. SCR was raised significantly from baseline in all three groups immediately following isometric exercise (all $p \leq .001$). Fifteen minutes after isometric exercise, SCR was raised significantly from baseline in the control group, but not the KOA (p = 1.00) and FM (p = .72) groups.

Change values for SCR are shown in Table 8. The change in SCR from baseline to immediately following isometric exercise was not significantly different among groups ($F_{2,39}$ = 1.69, p = .20). Similarly, the change in SCR from baseline to 15 mins after isometric exercise was not significantly different among groups (H_2 = 2.08, p = .35).

Figure 47. ***Skin conductance responses at baseline, immediately following, and 15 mins after isometric exercise.***



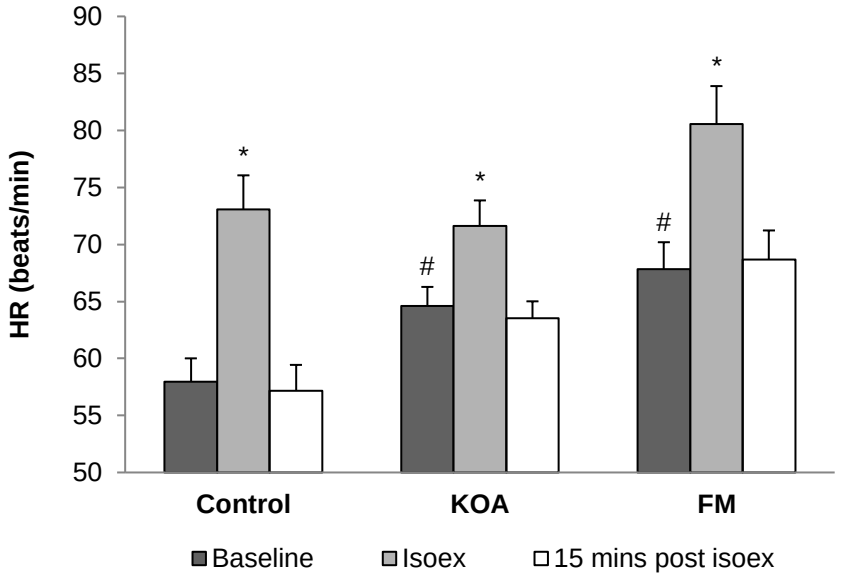
Note. FM = fibromyalgia; Isoex = isometric exercise; KOA = knee osteoarthritis; SCR = skin conductance response; * = significant difference from baseline; # = significant difference from control; error bars are one standard error of the mean

5.3.9. Heart rate

Group results for HR are shown in Figure 48. There were significant differences among groups in HR at baseline ($F_{2,39}$ = 6.15, p = .005). Follow-up one-sided Dunnett's tests showed that HR was significantly higher at baseline in the KOA (p = .02) and FM (p = .001) groups compared to the control group. The main effect of time was significant for HR in the control ($F_{1.1,12.4}$ = 77.62, p < .001), KOA ($F_{1.3,17.2}$ = 28.33, p < .001), and FM ($F_{1.1,13.5}$ = 23.71, p < .001) groups. HR was raised significantly from baseline to immediately following isometric exercise in all three groups (all p < .001). HR was equivalent to baseline values in all three groups 15 mins after isometric exercise (all p > .11).

Change values for HR are shown in Table 8. Change values in HR from baseline to immediately following isometric exercise were significantly different among groups (H_2 = 10.91, p = .004). Follow-up Mann-Whitney U tests showed the KOA group to display a significantly smaller change in HR than the control group (p = .001), while the FM group were not significantly different to controls (p = .26). The change in HR from baseline to 15 mins after isometric exercise was not significantly different among groups ($F_{2,39}$ = 3.06, p = .06).

Figure 48. ***Heart rate at baseline, immediately following, and 15 mins after isometric exercise.***



Note. FM = fibromyalgia; HR = heart rate; Isoex = isometric exercise; KOA = knee osteoarthritis; * = significant difference from baseline; # = significant difference from control; error bars are one standard error of the mean

Table 8. ***Change values in ANS outcome measures from baseline to immediately following, and baseline to 15 mins after isometric exercise. Data are mean (SD).***

Variable	Control *n* = 15	KOA *n* = 14	FM *n* = 13	Omnibus *p* value	ES (95% CI) KOA-control	ES (95% CI) FM-control
Δ_0HF HRV, nu	-34.19 (15.04)	-10.24 (16.42)*	-22.48 (16.58)	.002#	1.52 (0.70 – 2.35)	0.74 (-0.03 – 1.51)
Δ_{15}HF HRV, nu	-6.15 (15.62)	2.49 (19.62)	-5.68 (14.24)	.34	0.49 (-0.25 – 1.23)	0.03 (-0.71 – 0.77)
Δ_0PEP, ms	-9.16 (9.51)	-6.16 (10.41)	-5.25 (8.98)	.53	0.30 (-0.43 – 1.03)	0.42 (-0.33 – 1.17)
Δ_{15}PEP, ms	-0.37 (3.65)	-1.25 (15.07)	3.22 (5.81)	.33	-0.08 (-0.81 – 0.65)	0.75 (-0.02 – 1.52)
Δ_0SCL, µS	5.36 (3.31)	3.93 (3.46)	3.02 (3.05)	.13	0.42 (-0.31 – 1.16)	0.73 (-0.03 – 1.50)
Δ_{15}SCL, µS	0.25 (1.02)	-0.53 (0.83)	-0.21 (1.72)	.16	0.84 (0.08 – 1.60)	0.33 (-0.42 – 1.08)
Δ_0SCR, spikes/min	4.61 (1.86)	3.44 (1.38)	3.80 (2.00)	.20	0.71 (-0.04 – 1.46)	0.42 (-0.33 – 1.18)
Δ_{15}SCR, spikes/min	0.43 (0.73)	-0.07 (0.71)	0.09 (0.55)	.35	0.69 (-0.06 – 1.44)	0.52 (-0.24 – 1.28)
Δ_0HR, beats/min	15.11 (5.85)	7.02 (4.88)*	12.73 (8.71)	.004#	1.50 (0.67 – 2.32)	0.33 (-0.42 – 1.07)
Δ_{15}HR, beats/min	-0.80 (2.16)	-1.10 (2.15)	0.87 (2.35)	.06	0.14 (-0.59 – 0.87)	-0.74 (-1.51 – 0.03)

Note. ANS = autonomic nervous system; CI = confidence interval; ES = effect size; FM = fibromyalgia; HF HRV = high frequency heart rate variability; HR = heart rate; KOA = knee osteoarthritis; PEP = pre-ejection period; SCL = skin conductance level; SCR = skin conductance response; SD = standard deviation; * = significant difference from control; # = significant difference between groups; Δ_0 = difference from baseline to immediately following isometric exercise; Δ_{15} = difference from baseline to 15 mins after isometric exercise

5.4. Discussion

5.4.1. Introduction

The first aim of this study was to determine whether people with KOA and FM would exhibit ANS dysfunction and altered nociceptive processing at rest and, indeed, there was some evidence this was the case. The second aim of this study was to evaluate ANS function, and to assess for EIH, in people with KOA and FM in response to acute isometric exercise. The results, while mixed, suggest that people with KOA demonstrate some evidence of dampened cardiac sympathetic reactivity, smaller vagal withdrawal, and impaired EIH in response to acute isometric exercise compared to pain free controls. The third aim of this study was to document the recovery of the ANS and nociceptive system 15 mins after acute isometric exercise. The results for the KOA and FM groups showed that ANS function and nociceptive processing were largely similar to controls at the follow-up period, with some differences observed in EDA activity. This section discusses these findings in more detail.

5.4.2. Nociceptive system function at baseline

The baseline measures and discussion of knee PPT are similar to Section 3.4.2, except for differences observed in the FM-control comparison. The hypothesis was not supported for reduced knee PPT at rest in the KOA group compared to controls, but was supported in the FM group. The FM group displayed significantly lower knee PPT at baseline compared to controls. A recent meta-analysis by Amiri et al. [459] concluded that chronic pain groups, including FM, demonstrate significantly reduced PPTs compared to healthy controls. The effect size of the current study for the KOA-control comparison was similar to the first study; and, the lack of significant findings could, again, be due to participant characteristics, testing protocol, testing site, and/or small sample size and the subsequent lack of power.

The baseline measures and discussion of arm PPT are similar to Section 3.4.2. The hypothesis was not supported for reduced arm PPT at rest in the KOA and FM groups compared to controls. Arm PPTs were marginally lower in the KOA and FM groups compared to controls, but not significantly different. Given the lack of significant findings at the knee between the KOA and control groups in this study, it is unsurprising there were no significant distal effects.

5.4.3. Parasympathetic nervous system function at baseline

The baseline measures and discussion of resting vagal tone are similar to Section 3.4.3. The findings of this study supported the hypothesis of reduced HF HRV at baseline in the KOA and FM groups compared to pain free controls. These results corroborate the novel findings of the first and second studies showing reduced vagal tone in OA at rest.

5.4.4. Sympathetic nervous system function at baseline

Assessment of the SNS supported the hypothesis of increased SCL in the FM group at baseline compared to controls, but not the KOA group. The hypothesis of increased SCR at baseline in the KOA and FM groups was supported, but PEP outcomes were no different to controls. As previously discussed, studies have shown mixed results for tonic EDA in people with FM compared to controls [36, 118, 167, 210-212, 421]. PEP values in the KOA and FM groups were elevated at baseline, yet not significantly different to controls, potentially indicating increased beta adrenergic activity. These findings are consistent with previous research [131, 422]. Interestingly, HR and SCR were significantly higher in the KOA and FM groups at baseline compared to healthy controls. The SNS plays an important role in adaptation to exercise [361], and it is possible that the anticipation of the upcoming 5-minute exercise task may have resulted in increased SNS activity at baseline [266, 460]. Indeed, anticipation of an event or exercise is known to increase SCRs [129, 195] and plasma catecholamines [460]. Tod et al. [461] refer to this phenomenon as "preparatory arousal" seen in athletes when mentally preparing themselves prior to performance. Participants had previously exerted themselves with great effort using the dynamometer on the first day of the study to determine their MVC, and were aware of what was required during the proceeding isometric exercise. Since the KOA and FM groups scored significantly higher than controls in their stress and pain catastrophising scales, it is possible these two groups were more anxious about how the exercise may impact their current pain levels or whether they would be able to complete the task. People with chronic pain may perceive exercise to be stressful and/or painful [361, 460]. There is an association between pain and perceived exertion that can be explained in terms of generalised hypervigilance to noxious stimuli, which can result in increased SNS activity before exercise [240, 361, 460].

5.4.5. Exercise-induced hypoalgesic response to acute isometric exercise

EIH is characterised by an increase in pain thresholds and a decrease in pain ratings following acute exercise [292, 295, 462]. In this study, immediately following acute isometric exercise, pain free controls exhibited a significant increase in knee PPT. This result is congruent with previous research showing the presence of EIH in healthy, pain free populations by way of increased PPTs following isometric exercise [291, 297, 300, 301, 306, 312, 313, 315, 317, 319, 337, 463, 464]. In contrast, the KOA and FM groups did not show a significant increase in knee PPT immediately following acute isometric exercise. However, the change in knee PPT was not different between groups, thus, the hypothesis of impaired EIH in the KOA and FM groups was partially supported. The hypoalgesic effect of exercise occurs at peripheral, spinal, and supraspinal levels [292, 308, 462]. EIH can be the net result of increased activity in descending pain modulating pathways facilitated by the baroreflex system, endogenous opioids, cannabinoids, serotonin, and/or noradrenaline (as shown in animal studies) [81, 291, 308, 326, 462, 463]. Impairment of EIH, as demonstrated in the current study, has been shown in chronic pain populations [326, 465], including KOA [93, 308] and, specifically, FM [117, 317, 321], but is not always consistent [93, 369, 370]. Therefore, variability of the EIH response exists in people with KOA. For example, EIH may be intact or impaired depending on whether people with KOA show effective CPM [93, 368]. With this in mind, it is possible that research with small sample sizes, such as

the current study, may potentially be underpowered to detect significant differences in PPT before and after isometric exercise. Indeed, larger studies by Kosek et al. [370] and Neelapala et al. [369] found normal EIH function in people with KOA using sample sizes of 66 and 70 respectively. Variability in the EIH response in people with KOA may also result from methodological differences between studies; e.g. depending which body part is exercised [308, 466]. Impaired EIH has been shown at sites distal to the contracted muscle in people with chronic pain via decreased PPTs after isometric exercise [117, 317, 320]. However, no statistically significant differences in arm PPT were found among groups in the current study. Arm PPTs were equivalent to baseline in the KOA and FM groups immediately following isometric exercise and were no different to controls. This suggests that only a local EIH effect was observed in the current study, which is supported by previous research reporting remote EIH to be either absent, or smaller in magnitude, compared to local EIH in healthy people [340, 341] and, specifically, people with KOA [466].

5.4.6. Parasympathetic nervous system function in response to acute isometric exercise

Assessment of the PNS revealed that while HF HRV reduced significantly in all three groups, a significantly smaller change occurred in the KOA group immediately following isometric exercise compared to controls, partially supporting the hypothesis of impaired vagal function in people with KOA. It was expected that HF HRV would decrease in healthy controls immediately following isometric exercise since this has been shown in previous research [344, 345, 348, 349, 467]. Taylor et al. [467] compared HF HRV between healthy young (≈25 years old) and older adults (≈67 years old) in response to isometric exercise and suggested that reduced vagal tone at baseline, as seen in older adults, can be a factor in smaller HF HRV changes during contraction. Only one study has examined HF HRV in response to isometric exercise in a chronic pain condition and found no significant difference to controls [359]. This is the first study to demonstrate reduced vagal withdrawal in OA in response to acute isometric exercise.

As seen with other types of stressors, the typical biphasic response of cardiac vagal withdrawal and sympathetic activation is expected during isometric exercise [354]. However, isometric exercise, specifically, can elicit complex autonomic and cardiovascular reactions depending on the intensity and/or duration of the exercise [354]. Increases in both vagal and sympathetic activity have been observed during isometric exercise due to: (1) the influence of exercise-induced muscle ischemia and metaboreflexes; and (2) reduced BRS via higher brain centres [342, 347, 468, 469]. Through muscle ischemia and metaboreflex effects generated by isometric exercise, sympathetic activity is increased and, in turn, BP and HR [344, 354]. As HR and BP are increased, so too is arterial baroreflex loading [343, 346]. Higher brain centres may lower BRS at the onset of exercise, allowing vagal activity to increase at the same time as sympathetic activation [344, 469]. Brainstem nuclei in the medulla are then triggered to increase cardiac vagal activity, in order to balance increased sympathetic activity, and maintain HR [342, 346, 347, 351, 468]. In sum, isometric exercise can induce co-activation of both autonomic branches [342].

Vagal withdrawal varies depending on the metabolic demands of the physical task [25, 468]. With “less in the tank” [25] at baseline, plus reduced changes in vagal reactivity, as shown in the KOA group in this study, baroreflex activation of descending inhibitory nociceptive pathways may be negatively impacted resulting in increased pain sensitivity [140, 344, 347, 351]. Therefore, while vagal reactivity to isometric exercise is complex and varied, the current study demonstrates, for the first time, reduced vagal withdrawal following isometric in people with KOA.

5.4.7. Sympathetic nervous system function in response to acute isometric exercise

The hypothesis of reduced PEP immediately following acute isometric exercise in the control group was supported, as was the hypothesis of smaller change in PEP in the KOA group. The hypothesis of smaller change in PEP in the FM group was not supported. PEP reduced significantly in the control and FM groups immediately following isometric exercise; and, while PEP reduced in the KOA group, this change did not reach significance. This is the first study to examine PEP in response to acute isometric exercise in a chronic pain population, including KOA, providing some evidence of blunted sympathetic reactivity. Four studies have shown PEP to significantly decrease in healthy people in response to isometric exercise [345, 346, 470, 471], similar to the results in the current study. An increase in metabolic demand from isometric exercise causes sympathetic stimulation of the heart to change its rate and force of contraction [471]. Myocardial contractility decreases with age though and, in turn, physiological responsiveness to exercise stress is reduced [470, 472]. Two studies compared PEP values post isometric exercise in healthy young and old people, with the mean age of the older participants being equivalent to that of the current study – 60 years old [345, 470]. The results of both previous studies were similar insofar as the young groups exhibited significantly shorter PEP compared to the older groups following isometric exercise, implying greater beta adrenergic activity in younger people [345, 470]. Although the age difference between the control and KOA groups in the current study was not significantly different, the control group were, on average, 7 years younger. Desensitisation of beta adrenoreceptors occurs with increasing age, possibly due to chronic elevations of plasma catecholamines in older people from excess neural discharge and poor clearance over time [355]. Decreased beta adrenergic sensitivity on the myocardium can therefore cause blunting of contractility in response to exercise [345, 355, 470]. This age-related phenomenon may explain the nonsignificant decrease in PEP in the KOA group shown in the current study.

The hypothesis of increased EDA immediately following isometric exercise in the control group was supported, but the hypothesis of smaller changes in EDA in the KOA and FM groups was not supported. EDA increased significantly in all three groups immediately following acute isometric exercise, where it was expected sympathetic reactivity would be blunted in the KOA and FM groups. Evidence is limited for EDA in response to isometric exercise. Previous studies, primarily utilising microneurography, have shown that isometric contraction increases sympathetic outflow to the skin in healthy people [473-479] and people with FM [373]. To date, this is the first study to examine EDA in KOA in response to acute isometric exercise. It is generally accepted that increases in sympathetic activity in response to isometric exercise arise chiefly from the brainstem, with little influence from mechano- and

chemoreceptors in the muscle being exercised [473-478]. Rather, autonomic nuclei involved in sudomotor activity receive cortical input during exercise, which trigger the activation of sympathetic neurons [474, 477]. Therefore, the significant increase in EDA seen in all three groups in the current study could be facilitated by other physiological mechanisms, such as spontaneous respiration during exercise, which may lead to direct activation of autonomic brainstem nuclei that, in turn, activate sudomotor nerve fibres [477, 479].

5.4.8. Exercise-induced hypoalgesia following recovery from acute isometric exercise

The hypothesis that knee PPT would be equivalent to baseline 15 mins after isometric exercise in the control group was supported. The hypothesis that knee PPTs would remain unchanged from baseline to 15 mins after isometric exercise in the KOA and FM groups was also supported. It is well established that the effects of EIH can last for at least 10 mins following exercise in healthy people [295, 339]. Three studies assessing PPT in healthy people following isometric exercise, which included a recovery period, found the effects of EIH to last up to 10 [339] and 15 [306, 337] mins. Two other studies using similar methodologies demonstrated the effects of EIH to dissipate after 30 mins [313, 464]. Evidence of the effects of EIH beyond the period of isometric contraction in chronic pain populations is limited, with none involving KOA [295, 465, 480]. Indeed, a recent review of EIH involving isometric exercise in people with musculoskeletal pain pointed out the low number of studies, small sample sizes, and mixed EIH responses currently in the literature [480]. The authors suggested that the focus of future research involving isometric exercise and EIH in people with chronic pain should be protocols employing longer duration contractions of higher intensity [480].

5.4.9. Parasympathetic nervous system function following recovery from acute isometric exercise

The hypothesis that HF HRV would be equivalent to baseline 15 mins after isometric exercise in the control group was supported, but the hypothesis that HF HRV would remain unchanged or be maintained in the KOA and FM groups was not supported. Vagal rebound/recovery occurred in all three groups to a similar extent 15 mins after isometric exercise such that HF HRV values were equivalent to baseline. The recovery of HF HRV to baseline, following its reduction during isometric exercise, has been demonstrated in previous studies involving healthy people [344, 346] and typically occurs within the first few minutes after exercise [481]. As shown in the current study, HR decreases toward resting values following the cessation of exercise [482]. This decrease in HR is thought to be facilitated by the recovery of vagal activity at the sinus node level, and is a complex entanglement of neural cardiac vagal activity and respiration [376, 483]. Eckberg at al. [484] states that respiration exerts a profound influence on the “quantity, periodicity, and timing” of vagal efferent activity. Respiration produces changes in cardiac activity, known as respiratory sinus arrhythmia (RSA), mediated by multiple physiological mechanisms, one being: cardiac vagal activity. RSA is characterised by an increase in HR during inspiration, resulting from vagal withdrawal; and, a decrease in HR during expiration, due to vagal rebound facilitated by the brainstem [173, 218]. The effects of breathing on RSA operate independently of neural cardiac vagal activity [485] such that, during fast or deep breathing, HF oscillations shift to the

LF range, appearing as a reduction in HF HRV [376, 485, 486]. Breathing rate and cardiovascular responses during exercise stress are different/faster than during mental stress due to the metabolic demands of muscle mass involved, and the intensity and duration of the exercise [346, 354, 467, 481, 483, 487]. Therefore, it is likely that respiration rate normalised 15 mins after isometric exercise, and this contributed to the recovery of HF HRV observed in all three groups in the current study.

5.4.10. Sympathetic nervous system function following recovery from acute isometric exercise

The hypothesis that PEP would return to baseline 15 mins after isometric exercise in the control group was supported. The hypothesis that PEP would remain unchanged or be maintained from baseline to 15 mins after isometric exercise in the KOA group was also supported. However, the hypothesis was not supported for the FM group, with PEP shortening and lengthening similarly to controls. PEP during post-exercise recovery has rarely been examined. This is the first study to assess PEP during recovery in people with KOA following isometric exercise. Nihiyasu et al. [346] and Boutcher at al. [345] assessed follow-up PEP in healthy people at 2 and 4 mins respectively after isometric exercise. Both studies found PEP values to return to baseline in young adults within those recovery time frames. However, in older adults, Boutcher at al. [345] found PEP to be maintained from baseline through to recovery, with a nonsignificant decrease during contraction, similar to the results of the current study for the KOA group. Recovery of PEP in the control and FM groups may be due to participants being younger and exhibiting greater efficiency of beta adrenergic activity on the myocardium, while the slightly older KOA group demonstrated dampening of cardiac sympathetic activity [345, 355, 470]. Therefore, the difference in PEP results between the groups may be age-related and not necessarily condition dependent.

The hypothesis that EDA would return to baseline 15 mins after acute isometric exercise was supported for the control group, but the hypothesis that any changes in EDA would be maintained after 15 mins in the KOA and FM groups was not supported. EDA returned to baseline 15 mins after isometric exercise in the KOA and FM groups; and, while there were a significantly greater number of SCRs in the control group after 15 mins compared to baseline, the raw difference was small. Similarly, the raw difference in number of SCRs between baseline and the 15-minute recovery period in the KOA group was also small. This is the first study to evaluate EDA in people with KOA during a recovery phase. The autonomic adjustments that occur following the cessation of isometric exercise are the reverse of what happens during contraction: vagal activity is reactivated and SNS activity is withdrawn [354, 481]. The trend in EDA for all groups was a return of sudomotor activity to baseline. Indeed, previous research examining recovery of EDA in healthy people has shown sympathetic outflow to the skin to return to baseline levels in short time frames (i.e. 2 mins) [473, 474]. Vissing et al. [474] suggest that sympathetic activation by the CNS to the skin is a specific response to motor effort from isometric exercise that does not habituate; i.e. EDA continues to increase in magnitude with repeated input. Therefore, upon cessation of muscle contraction and, hence, cortical input, SNS activity rapidly withdraws and EDA returns to baseline, as shown in the current study.

5.5. Strengths and limitations

The strengths of this study include the range of validated QST and ANS measures collected in a controlled laboratory environment. ICG/ECG is highly sensitive to movement artefact, which can make accurate recording challenging during exercise [481]. In this study, participants were recumbent while performing isometric contraction of the quadriceps, which involved minimal movement of the upper body, greatly reducing the chance of ICG/ECG artefacts. MVC in the KOA group was only as great as the pain the participant's affected knee permitted, which could have been a limitation. Indeed, the KOA group exhibited the lowest MVC value. However, Borg ratings of perceived exertion were no different between groups during both ANS and QST sessions, suggesting the chosen intensity and duration, based on previous studies [93, 117], provided a sufficient level of controlled, tolerable muscle activation for all groups. This study, however, is not without its limitations. COVID-19 restrictions led to the sample size being smaller than intended, with the study potentially being underpowered to detect some differences between groups and/or over time. The literature shows variability of EIH responses to isometric exercise in people with KOA. This variability means that it may be less likely to find significant changes in PPT (intact EIH) in studies with smaller sample sizes, such as the current study, compared to larger studies [369, 370]. Unlike with mental stress, exercise stress results in a change in respiration that can create two potential issues. Firstly, heavy breathing can cause movement of the thorax, negatively influencing thoracic impedance [481]. Secondly, faster and/or deeper breathing changes the amplitude of RSA that can potentially confound HRV results, as discussed in Section 5.4.9 [375, 376]. Appelhans et al. [173] have suggested including respiration rate as a covariate in statistical analyses to rule out these potentially confounding effects. Finally, previously undiagnosed cardiac issues in some participants resulted in the loss of ECG data. A larger sample size would accommodate for such unforeseen circumstances in future research.

5.6. Conclusion

The current study reiterated the findings of the first study of reduced resting vagal tone in people with KOA and FM compared to controls. In addition, this is the first study to show significantly smaller vagal withdrawal in people with KOA immediately following isometric exercise compared to controls. This sample of people with KOA also showed impairment of EIH in response to acute isometric exercise. A floor effect of reduced vagal tone at baseline may be a factor in diminished autonomic reactivity during isometric exercise and increased pain sensitivity. Isometric exercise induces concomitant vagal withdrawal and sympathetic activation [354]. This balanced autonomic response was demonstrated in the control group, but not the KOA group. The KOA group exhibited blunted cardiac sympathetic reactivity to isometric exercise that may lead to reduced BRS and, in turn, diminished descending inhibition of nociceptive pathways [140, 148, 345, 347].

Chapter 6. Summary

6.1. Introduction

The aim of this thesis was to assess the function of the ANS in a novel chronic pain population, namely people with KOA, where autonomic function had not previously been investigated [33]; and, how any evidence of ANS dysfunction may be associated with nociceptive processing. In three separate studies, people with KOA and people with FM – a population known to exhibit ANS dysfunction [31, 421, 431] – were compared to healthy, pain free controls. The nociceptive and autonomic nervous systems were first examined at rest. Then, the relationships between the two systems were assessed by manipulating one system and examining the response in the other. Three different types of stressors were applied to examine the responses in the two systems: (1) nociceptive stress, (2) mental stress, and (3) exercise stress. This chapter summarises the key findings of each study, points to ideas for future research, and draws conclusions across all three studies.

6.2. Key findings

6.2.1. Chapter 3. Nociceptive and autonomic function in people with knee osteoarthritis and fibromyalgia

The aim of the first study (Chapter 3) was to assess for evidence of resting ANS dysfunction and altered nociceptive processing in people with KOA and FM compared to controls. In addition, all three groups were exposed to a nociceptive stressor, in the form of cold water conditioning, to examine the effects on the nociceptive and autonomic nervous systems.

Knee PPTs were compared between the KOA and control groups; and, even though results showed the two groups to be statistically equivalent, knee PPT for the KOA group was lower than controls. Previous studies consistently show reduced PPTs in people with OA [97]. Therefore, the lack of significant differences between groups could be explained by either interindividual variability, methodological differences between studies, or insufficient power of the current study. There do not appear to be meaningful differences in thermal thresholds in OA. Indeed, no significant differences were found between groups for HPT and CPT. The current study showed no evidence of a sensitised nociceptive system in the KOA and FM groups, although there were some trends towards sensitisation for local PPT.

There was evidence of resting ANS dysfunction in people with KOA and FM in this study. The FM group showed elevated EDA compared to controls, suggesting SNS hyperactivity at rest. In contrast to the control group, both the KOA and FM groups showed impaired vagal function at rest via reduced HF HRV. This may have important clinical implications. Firstly, a reduction in tonic vagal activity may attenuate tonic descending inhibition of nociception. Indeed, the KOA and FM groups showed evidence of impaired CPM. In contrast to controls, pain ratings in the KOA and FM groups were unchanged

immediately following cold water conditioning. This suggests impairment of descending inhibition and/or increased descending facilitation of nociceptive pathways in people with KOA and FM. Secondly, reduced resting vagal tone may set up a floor effect within the ANS when a person is exposed to a stressor. Indeed, the KOA and FM groups displayed reduced vagal withdrawal in response to cold water conditioning compared to controls, highlighting the impact of a vagal floor effect in response to a noxious stimulus. The KOA and FM groups also showed dampened cardiac sympathetic reactivity to nociceptive stress via a smaller reduction in PEP compared to controls. Aberrant cardiovascular responses in people with chronic pain may contribute to reduced baroreceptor activation and, in turn, diminished descending inhibition of nociceptive pathways. The combination of reduced vagal withdrawal and blunted SNS reactivity may be associated with the impairment of CPM seen in the KOA and FM groups in this study.

6.2.2. Chapter 4. Effects of acute mental stress on nociception and the autonomic nervous system in people with knee osteoarthritis and fibromyalgia

The aim of the second study (Chapter 4) was to examine the effects of acute mental stress on the nociceptive and autonomic nervous systems in people with KOA and FM immediately following, and 15 mins after the stressor. Resting ANS function was also assessed in the KOA and FM groups in comparison to controls.

This study supports the findings of the first study of resting ANS dysfunction in people with KOA and FM. Both the KOA and FM groups showed signs of SNS hyperactivity at rest via elevated EDA. Resting vagal tone was shown to be reduced in the KOA group compared to controls, potentially creating a floor effect upon exposure to acute mental stress. Indeed, in response to mental arithmetic, the KOA group showed no significant change in vagal activity compared to controls. The FM group also demonstrated reduced resting vagal tone and a smaller change in vagal withdrawal in response to mental arithmetic compared to controls; however, the differences between groups did not reach significance. A vagal floor effect may lead to diminished cardiovascular regulation via reduced baroreflex activation and, in turn, diminished descending modulation of pain. Indeed, immediately following mental arithmetic, the control group showed a significant reduction in pain ratings, while the KOA and FM groups demonstrated no significant change. These results suggest a potential impairment of the vagally mediated baroreflex response in the KOA and FM groups, since cardiac sympathetic reactivity (reduced PEP) in the two pain groups was no different to controls. This study provided some evidence of impaired mental stress-induced hypoalgesia in people with KOA and FM.

Following a 15-minute recovery period, pain ratings returned to baseline in the control group, while remaining unchanged from baseline to 15 mins after mental arithmetic in the KOA and FM groups. Similarly, the control group demonstrated normal vagal rebound, with HF HRV values returning to baseline after 15 mins, while the KOA and FM groups showed vagal activity to be unchanged from baseline to 15 mins after mental arithmetic. No noteworthy differences in SNS activity were observed

between all three groups at the 15-minute recovery period. PEP values returned to baseline, and EDA remained elevated to a similar extent in all three groups 15 mins after mental arithmetic.

6.2.3. Chapter 5. Effects of acute isometric exercise on nociception and the autonomic nervous system in people with knee osteoarthritis and fibromyalgia

The aim of the third study (Chapter 5) was to examine the effects of acute isometric exercise on the nociceptive and autonomic nervous systems in people with KOA and FM immediately following, and 15 mins after the stressor. Resting ANS function was also assessed in the KOA and FM groups in comparison to controls.

PPTs were assessed at the knee and arm at baseline using the same methodology as the first study, with the exception of including knee PPT in the FM group. Knee PPT was significantly reduced in the FM group at baseline, while no other significant differences for knee or arm PPTs were found in the KOA and FM groups compared to controls.

This study supports the findings of the first and second studies of resting ANS dysfunction in people with KOA and FM. Resting vagal tone was reduced in the KOA and FM groups compared to controls, with some evidence of SNS hyperactivity via elevated EDA. These differences in tonic ANS function may potentially limit autonomic reactivity of the KOA and FM groups with vagal floor and a sympathetic ceiling effects when undertaking isometric exercise. Indeed, while significant vagal withdrawal occurred in all three groups immediately following isometric exercise, the KOA group showed a significantly smaller change compared to controls. Furthermore, only the KOA group showed a smaller reduction in PEP immediately following isometric exercise, which suggests dampening of cardiac sympathetic reactivity. Vagal dysfunction and blunted cardiac sympathetic reactivity may be, at least partly, responsible for reduced BRS and, in turn, diminished descending inhibition of nociceptive pathways. Indeed, immediately following acute isometric exercise, knee PPT was significantly increased in the control group, while knee PPTs in the KOA and FM groups were equivalent to baseline. Therefore, this study provided some evidence of EIH in the control group, and impaired EIH in the KOA and FM groups.

Following a 15-minute recovery period, knee PPT was equivalent to baseline in the control group, while PPTs were unchanged in the KOA and FM groups from baseline to 15 mins after isometric exercise. EDA significantly rose and fell to a similar extent in all three groups from immediately following isometric exercise to 15 mins after.

6.3. Future research

6.3.1. Introduction

The following sections present three suggestions for future research based on the findings of this thesis: (1) The assessment of BRS and how it may relate to pain in people with KOA, (2) the manipulation of

vagal tone and its potential impact on the nociceptive system in people with KOA, and (3) the impact of pain relief on the ANS in people with KOA.

6.3.2. Associations between the nociceptive and baroreflex systems in people with knee osteoarthritis

Vagal dysfunction, as shown in people with KOA in the current studies, may reduce BRS and, in turn, diminish the responsivity of descending pain inhibitory pathways [138, 150]. This is because BRS is primarily under cardiac vagal control [146, 147] and is another index of ANS function, alongside HRV [141, 150, 488, 489]. BRS has been raised as a concept throughout this thesis (Section 2.5.3) because of its importance in BP homeostasis [150], and its relationship to pain perception [143]. Baroreflex impairment can occur secondary to ANS dysfunction, resulting in changes in pain sensitivity [143, 490]. However, to date, BRS has not been directly measured in people with KOA. As discussed in Section 2.7.1, reduced resting vagal tone has been shown in people with RA compared to controls [206]. Studies have also shown BRS to be reduced in people with RA [491, 492], and FM [138], compared to healthy controls, which suggests baroreflex dysfunction may be associated with vagal dysfunction and chronic pain. It would be of interest to assess BRS as a natural progression from the discovery of vagal dysfunction seen in people with KOA in the current studies. If baroreflex function were found to be impaired in people with KOA, interventions aimed at increasing BRS, e.g. vagal nerve stimulation [493-495], may have a positive impact on their pain.

6.3.3. Impact of parasympathetic nervous system manipulation on the nociceptive system in people with knee osteoarthritis

The results of the current studies showed people with KOA to exhibit vagal dysfunction at rest and in response to various stressors. Therefore, the manipulation of vagal tone may provide further insight into improving pain in people with KOA. Clonidine is an alpha-2 adrenoceptor agonist that can be administered by intra-articular injection in people with KOA to produce local anaesthetic effects via inhibition of C and A-delta fibres [496, 497]. Clonidine has also been used in the assessment of BRS, as the medication is known to reduce SNS activity and directly increase vagal tone [147, 498]. In healthy people, oral clonidine has been shown to decrease HR and BP [416, 498], and increase RMSSD [416] and BRS [147, 499]. Previous research has found associations between increased efficiency of pain modulation and decreased HR when taking oral clonidine in a placebo controlled trial with healthy people [416]. Therefore, it would be of interest to examine the effects of vagal tone manipulation, via oral clonidine, for example, on nociception and descending pain inhibitory systems in people with KOA who exhibit vagal dysfunction. It is expected that clonidine would increase vagal activity, increase BRS and, in turn, decrease pain sensitivity in people with KOA.

6.3.4. Impact of local pain relief on the nociceptive and autonomic nervous systems in people with knee osteoarthritis

There are few studies that have examined the effects of pain relief on ANS function in people with chronic pain conditions. It may be relevant to determine whether ongoing joint nociceptive input contributes to ANS dysfunction in people with KOA since the current studies have shown this dysfunction to exist. Changes in vagal function have been shown in people with chronic pain following interventions such as: deep brain stimulation [84], acupressure [500], and compression of myofascial trigger points [501]. The results across all three studies consistently showed that these interventions increased vagal activity, which was associated with hypoalgesia. Two main objectives for the treatment of KOA are to reduce pain and improve function. People that are unresponsive to conservative management are often offered intra-articular injections in order to delay the need for TKA [502]. Studies evaluating different types of intra-articular injections for OA-related knee pain, such as corticosteroid [503, 504], lidocaine [502], or ketorolac [505], have shown intra-articular injections to be beneficial in improving these two objectives, at least in the short term. However, to date, no studies have examined the effects of local pain relief on the ANS due to intra-articular injection in people with KOA. If local pain relief were shown to modulate and/or improve ANS function, this may hold clinical relevance for how OA-related pain may contribute to other factors, such as cardiovascular health.

6.4. Associations between the autonomic nervous system and chronic pain

The findings of the current research support existing evidence that ANS dysfunction is a feature of chronic pain conditions, such as FM [19, 31], and implicate that OA may be included in this list. The interactions between the nociceptive, autonomic, and cardiovascular systems are extensive, from cortex to end-organ [26, 27, 135, 506]. Alterations in one or more of these systems will impact the others, resulting in reduced adaptability to stress or noxious stimuli and, ultimately, lead to an increase in nociception and pain [130, 140]. The role of the ANS is to maintain homeostasis and modulate pain [26, 135]. Autonomic nuclei within the brainstem comprise the gateway between processing noxious stimuli, and commanding changes of the cardiovascular system and endogenous descending nociceptive pathways [73]. Homeostatic balance is maintained in pain free people by the flexibility of a healthy ANS, which is defined by a system of high vagal, and low sympathetic tone [29, 453]. Results of the current studies corroborate growing evidence that reduced vagal tone, and dampened cardiac sympathetic reactivity, places a person at risk of developing or worsening chronic pain due to a decreased capacity to respond to noxious stimuli, and mental or physical stress [23, 25]. There is an inverse relationship between pain sensitivity and cardiovascular control [140], and since the heart is primarily under vagal control [146, 147], alterations in vagal function will directly impact nociceptive processing and the subjective experience of pain [131, 140]. Therefore, impaired vagal function, reflected by a decrease in BRS, may correspond to alterations in descending inhibitory control of nociception, giving rise to states of chronic pain [23, 131, 140].

6.5. Clinical implications

Understanding the ANS status of a person can potentially serve as a guide for pain management and rehabilitation. HRV and, specifically, HF HRV, can be used as a biomarker for people with chronic pain [507]. If a person has been shown to exhibit low vagal tone, or decreased vagal recovery following exercise, tailored therapies can be implemented to offer better support for pain. Interventions such as mindfulness [508] and physical training [509] have been shown to improve vagal function, in addition to psychological well-being. However, when diminished HRV is found, individualised, lower intensity training programs will be more favourable for that person to maintain an active lifestyle and adhere to clinical recommendations [510]. As ANS research continues, treatments that specifically target vagal dysfunction should be included to support people with chronic pain conditions, including OA, using ANS indices as outcomes measures for the management of pain and rehabilitation [23, 130].

6.6. Conclusion

This thesis aimed to examine the function of the ANS in people with KOA for the first time. OA is common as people age and has the potential to reduce quality of life. One role of the ANS is to adapt to stress and modulate pain through its inextricable associations with the nociceptive system. ANS dysfunction is common in other chronic pain conditions and could be related to altered nociceptive processing. As such, ANS dysfunction may be associated with impaired nociceptive modulation in people with KOA. Three studies were designed to investigate ANS and nociceptive function at rest in people with KOA; and, to investigate the relationship between the ANS and nociceptive systems by manipulating one system and examining the response in the other. This was achieved via different types of stressors: nociceptive, mental, and exercise stress. One chronic pain condition that has consistently been associated with altered ANS function is FM. Therefore, people with FM were included in the studies to demonstrate that the experimental procedures were able to detect ANS dysfunction. Several novel findings were produced from these three studies.

There is evidence of resting ANS dysfunction in people with KOA, and people with FM. Tonic vagal dysfunction was exhibited by the KOA group consistently across all three studies for the first time. The level of resting vagal activity determines the degree of adaptation to stress and, in part, changes in perception of pain. Vagal withdrawal is the first response of the ANS to a noxious stimulus, mental stress, or physical stress. Thus, in order for an adequate reduction in vagal activity to occur, its base tone needs to be sufficiently elevated prior to stress exposure. The baroreflex system – a feedback loop of cardiovascular, autonomic, and pain modulation – is primarily under cardiac vagal control. Therefore, reduced tonic vagal activity may create a floor effect of little room for vagal withdrawal in response to stress and, in turn, lead to attenuated descending inhibition of nociceptive pathways. This vagal floor effect was found in all three studies. In response to nociceptive, mental, and exercise stress, reduced vagal withdrawal was shown in the KOA and FM groups immediately following each stressor compared to the control group. Therefore, people with KOA may be less adept at responding to mental and physical stress due to the diminished flexibility of the vagal system, and at risk of impaired modulation of nociception.

There was some evidence of SNS hyperactivity at rest in the KOA and FM groups. Elevated SNS activity at rest may create a ceiling effect of blunted SNS reactivity when a person is faced with acute stress. While SNS responses via sudomotor activity were mostly similar across all three groups in response to the three stressors, there were two significant results for cardiac sympathetic reactivity. Cardiac sympathetic reactivity was found to be dampened in the KOA group in response to nociceptive stress, and exercise stress. Acute stress typically elicits a biphasic ANS response of cardiac vagal withdrawal and sympathetic activation, depending on the intensity and/or duration of the stress. Failure of the ANS to adapt and follow this pattern – to an extent such that the baroreflex system is sufficiently activated – will likely lead to diminished descending inhibition of nociceptive pathways. An impairment of descending inhibition and/or increased descending facilitation of nociceptive pathways was observed in the KOA and FM groups in all three studies. In response to nociceptive stress, acute mental stress, and acute exercise stress, people with KOA and people with FM demonstrated an impairment of CPM, mental stress-induced hypoalgesia, and EIH, respectively. Therefore, ANS dysfunction may be associated with impaired modulation of nociception, such that people with KOA may be at risk of greater pain sensitivity when exposed to acute stress.

Chapter 7. References

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Appendix A. Advertisement

A study at Auckland University of Technology

How does stress affect chronic pain?

- Have you been diagnosed with osteoarthritis of the knee?
- Have you been diagnosed with fibromyalgia?
- Or, are you a healthy, pain free person who would like to participate in a study about stress and its effects on pain?

If so, we would love to hear from you.

A PhD study is underway at AUT's Health and Rehabilitation Research Institute (North Shore Hospital) investigating whether pain due to knee osteoarthritis is influenced by stress. People with long term pain conditions such as fibromyalgia are known to have problems with their stress response, which can contribute to chronic pain. We want to know if this is the case in people with knee osteoarthritis. This study will compare people with painful knee osteoarthritis, and people with fibromyalgia, to people who are pain free.

If you meet the study criteria, and want to take part, you will:

- Attend 3 sessions (120 minutes each) on 3 separate days at the AUT Biomechanics Laboratory, Whenua Pupuke Waitemata Clinical Skills Centre, North Shore Hospital.
- Fill in questionnaires about your pain and stress levels.
- Undergo various sensation tests, including assessing sensitivity to heat, cold, and pressure.
- Perform basic mental arithmetic.
- Perform a controlled, safe, leg extension exercise of your knee.
- Have the activity of your stress system continuously measured from electrodes placed on your neck, chest and fingers.

The study outcomes will tell us more about the relationship between pain and stress in people with painful knee osteoarthritis. This may provide us with ideas on how to improve pain in people with osteoarthritis.

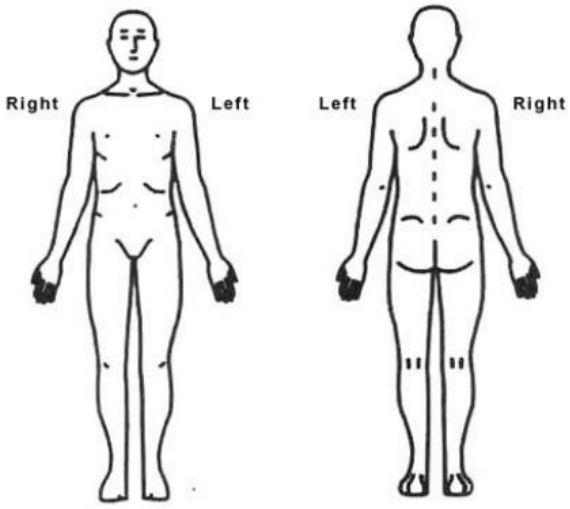
To obtain more information about this study, please contact:
Neil Bossenger ■ 09 522 0025 ■ neil.bossenger@aut.ac.nz
Health and Rehabilitation Research Institute, AUT

Appendix B. Brief Pain Inventory

Brief Pain Inventory **ID:** _____ **Date:** _____

1. Throughout our lives, most of us have had pain from time to time such as minor headaches, sprains, and toothaches. Have you had pain other than these everyday kinds of pain today? **YES / NO**

2. On the diagram, shade in the areas where you feel pain. Put an **X** on the area that hurts the most.



3. Please rate your pain by circling the one number that best describes your pain at its **WORST** in the past 24 hours.

No pain	0	1	2	3	4	5	6	7	8	9	10	*Worst pain imaginable*

4. Please rate your pain by circling the one number that best describes your pain at its **LEAST** in the past 24 hours.

No pain	0	1	2	3	4	5	6	7	8	9	10	*Worst pain imaginable*

5. Please rate your pain by circling the one number that best describes your pain on the **AVERAGE**.

No pain	0	1	2	3	4	5	6	7	8	9	10	*Worst pain imaginable*

6. Please rate your pain by circling the one number that tells how much pain you have **RIGHT NOW**.

No pain	0	1	2	3	4	5	6	7	8	9	10	*Worst pain imaginable*

7. What treatments or medications are you receiving for your pain?

_____

_____

_____

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8. In the past 24 hours, how much relief have pain treatments or medications provided? Please circle the one percentage that most shows how much **RELIEF** you have received.

0%	10%	20%	30%	40%	50%	60%	70%	80%	90%	100%
None										*Complete relief*

9. Circle the one number that describes how, during the past 24 hours, pain has interfered with your:

A. General activity

Does not interfere	0	1	2	3	4	5	6	7	8	9	10	*Completely interferes*

B. Mood

Does not interfere	0	1	2	3	4	5	6	7	8	9	10	*Completely interferes*

C. Walking ability

Does not interfere	0	1	2	3	4	5	6	7	8	9	10	*Completely interferes*

D. Normal work, including both work outside the home and housework

Does not interfere	0	1	2	3	4	5	6	7	8	9	10	*Completely interferes*

E. Relations with other people

Does not interfere	0	1	2	3	4	5	6	7	8	9	10	*Completely interferes*

F. Sleep

Does not interfere	0	1	2	3	4	5	6	7	8	9	10	*Completely interferes*

G. Enjoyment of life

Does not interfere	0	1	2	3	4	5	6	7	8	9	10	*Completely interferes*

Appendix C. Pain Catastrophising Scale

Pain Catastrophising Scale **ID: _____** **Date: _____**

Everyone experiences painful situations at some point in their lives. Such experiences may include headaches, tooth pain, joint or muscle pain. People are often exposed to situations that may cause pain such as illness, injury, dental procedures or surgery. We are interested in the types of thoughts and feelings that you have when you are in pain.

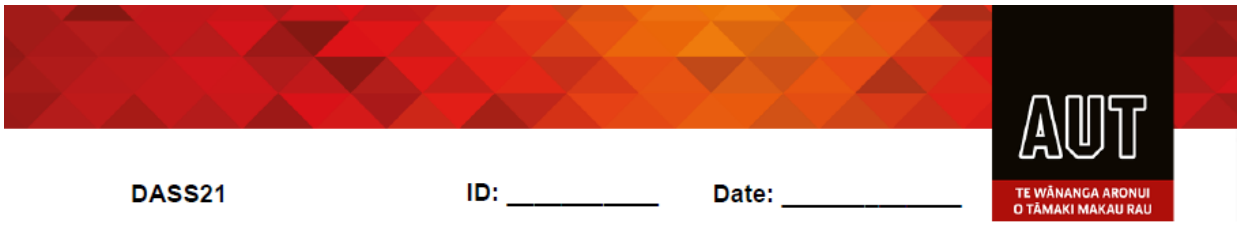
Listed below are 13 statements describing different thoughts and feelings that may be associated with pain. Using the following scale, indicate the degree to which you have these thoughts and feelings when you are experiencing pain.

Rating	0	1	2	3	4
Meaning	Not at all	To a slight degree	To a moderate degree	To a great degree	All the time

When I'm in pain…

Number	Statement	Rating
1	I worry all the time about whether the pain will end.	
2	I feel I can't go on.	
3	It's terrible and I think it's never going to get any better.	
4	It's awful and I feel that it overwhelms me.	
5	I feel I can't stand it anymore.	
6	I become afraid that the pain will get worse.	
7	I keep thinking of other painful events.	
8	I anxiously want the pain to go away.	
9	I can't seem to keep it out of my mind.	
10	I keep thinking about how much it hurts.	
11	I keep thinking about how badly I want the pain to stop.	
12	There's nothing I can do to reduce the intensity of the pain.	
13	I wonder whether something serious may happen.	
Total		

Appendix D. Depression, Anxiety, and Stress Scales



DASS21 **ID:** _____ **Date:** _____

Depression Anxiety Stress Scales

Please read each statement and circle a number (0, 1, 2 or 3) that indicates how much the statement applied to you **over the past week**. There are no right or wrong answers. Do not spend too much time on any statement.

The rating scale is as follows:

0 Did not apply to me at all
1 Applied to me to some degree, or some of the time
2 Applied to me to a considerable degree, or a good part of time
3 Applied to me very much, or most of the time

1	I found it hard to wind down	0	1	2	3
2	I was aware of dryness of my mouth	0	1	2	3
3	I couldn't seem to experience any positive feeling at all	0	1	2	3
4	I experienced breathing difficulty (e.g., excessively rapid breathing, breathlessness in the absence of physical exertion)	0	1	2	3
5	I found it difficult to work up the initiative to do things	0	1	2	3
6	I tended to over-react to situations	0	1	2	3
7	I experienced trembling (e.g., in the hands)	0	1	2	3
8	I felt that I was using a lot of nervous energy	0	1	2	3
9	I was worried about situations in which I might panic and make a fool of myself	0	1	2	3
10	I felt that I had nothing to look forward to	0	1	2	3
11	I found myself getting agitated	0	1	2	3
12	I found it difficult to relax	0	1	2	3
13	I felt down-hearted and blue	0	1	2	3
14	I was intolerant of anything that kept me from getting on with what I was doing	0	1	2	3
15	I felt I was close to panic	0	1	2	3
16	I was unable to become enthusiastic about anything	0	1	2	3
17	I felt I wasn't worth much as a person	0	1	2	3
18	I felt that I was rather touchy	0	1	2	3
19	I was aware of the action of my heart in the absence of physical exertion (e.g., sense of heart rate increase, heart missing a beat)	0	1	2	3
20	I felt scared without any good reason	0	1	2	3
21	I felt that life was meaningless	0	1	2	3

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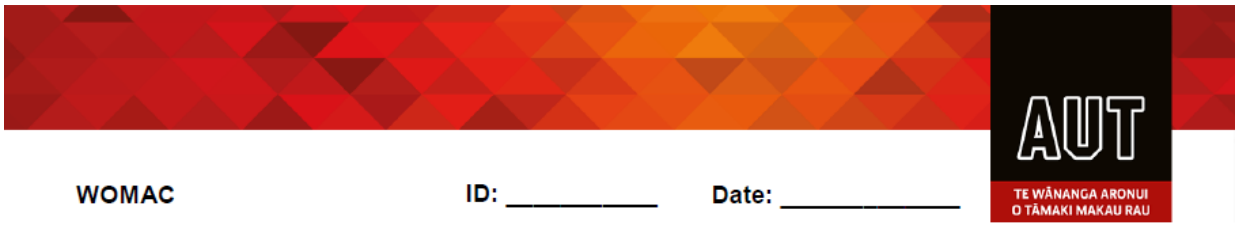
Appendix E. Western Ontario and McMaster Universities Osteoarthritis Index



WOMAC **ID:** _____ **Date:** _____

Western Ontario and McMaster Universities Osteoarthritis Index

Please rate and circle **one number** per activity in each category according to the following scale of difficulty:

Rating	0	1	2	3	4
Meaning	None	Mild	Moderate	Severe	Extreme

The following questions concern the amount of **pain** you are currently experiencing in your knee(s). For each situation, please enter the amount of pain you have experienced in the past 48 hours.

1	Walking on a flat surface	0	1	2	3	4
2	Going up or down stairs	0	1	2	3	4
3	At night while in bed	0	1	2	3	4
4	Sitting or lying	0	1	2	3	4
5	Standing upright	0	1	2	3	4

The following questions concern the amount of **stiffness** you are currently experiencing in your knee(s).

1	How severe is your stiffness after first awakening in the morning?	0	1	2	3	4
2	How severe is your stiffness after sitting, lying, or resting later in the day?	0	1	2	3	4

The following questions concern your **physical function**, i.e. your ability to move around and to look after yourself. For each of the following activities, please indicate the degree of difficulty you have experienced in the last 48 hours with your knee(s).

1	Descending (going down) stairs	0	1	2	3	4
2	Ascending (going up) stairs	0	1	2	3	4
3	Rising from sitting	0	1	2	3	4
4	Standing	0	1	2	3	4
5	Bending to floor	0	1	2	3	4
6	Walking on a flat surface	0	1	2	3	4
7	Getting in/out of car	0	1	2	3	4
8	Going shopping	0	1	2	3	4
9	Putting on socks/stockings	0	1	2	3	4
10	Rising from bed	0	1	2	3	4
11	Taking off socks/stockings	0	1	2	3	4
12	Lying in bed	0	1	2	3	4
13	Getting in/out of bath	0	1	2	3	4
14	Sitting	0	1	2	3	4
15	Getting on/off toilet	0	1	2	3	4
16	Heavy domestic duties (mowing the lawn, lifting heavy grocery bags)	0	1	2	3	4
17	Light domestic duties (such as tidying a room, dusting, cooking)	0	1	2	3	4

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Appendix F. Ethical Approval

Health and Disability Ethics Committees

Health and Disability Ethics Committees
Ministry of Health
133 Molesworth Street
PO Box 5013
Wellington
6011

0800 4 ETHICS
hdecs@moh.govt.nz

09 April 2019

Mr Neil Bossenger
18A Sealy Road
Torbay 0630

Dear Mr Bossenger

Re:	**Ethics ref:**	**18/CEN/45/AM01**
	Study title:	The interaction of the nociceptive and autonomic nervous systems in people with knee osteoarthritis.

I am pleased to advise that this amendment has been *approved* by the Central Health and Disability Ethics Committee. This decision was made through the HDEC Expedited Review pathway.

Non-standard conditions:

1. Please check contact details at the end of the information sheet. There should be details for the Health Disability Commission, it should note the HDECs 0800 contact number and a contact for a Maori support person(s). For perspective, please refer to HDEC's Participant Information Sheet template at https://ethics.health.govt.nz/home in the Quick Links section.

Non-standard conditions must be completed before commencing any changes as a result of this amendment, however they do not need to be submitted to or reviewed by HDEC.

If you would like an acknowledgement of completion of your non-standard conditions you may submit a post approval form amendment through Online Forms. Please clearly identify in the amendment form that the changes relate to non-standard conditions and ensure that supporting documents (if requested) are tracked/highlighted with changes.

For information on non-standard conditions please see section 128 and 129 of the *Standard Operating Procedures for Health and Disability Ethics Committees* (available on www.ethics.health.govt.nz)

Please don't hesitate to contact the HDEC secretariat for further information. We wish you all the best for your study.

Yours sincerely,

Mrs Helen Walker
Chairperson
Central Health and Disability Ethics Committee

Appendix G. Participant Information Sheet

Participant Information Sheet

Study title:	**Associations between pain and stress in healthy people and people with chronic pain**		
Locality:	**AUT Lab, NSH**	HDEC reference:	**18/CEN/45/AM01**
Lead investigator:	**Dr Gwyn Lewis**	Contact number:	**09 921 9999 x7621**

Date information sheet produced

9 April 2019

Project title

What are the associations between pain and stress in healthy people and people with chronic pain?

An invitation

My name is Neil Bossenger and I work in the Health and Rehabilitation Research Institute at Auckland University of Technology. I would like to invite you to participate in our research study called: *What are the associations between pain and stress in healthy people and people with chronic pain?* Your participation in this study is voluntary and you may withdraw at any time prior to the completion of data collection.

What is the purpose of this research?

The purpose of this study is to determine if pain due to knee osteoarthritis is influenced by the autonomic nervous system. The autonomic nervous system controls the heart, organs, glands and blood vessels, and controls the response to stress (fight/flight). People with painful conditions such as fibromyalgia are known to have problems with the autonomic nervous system, which can contribute to their pain. We want to know if this is the case in people with knee osteoarthritis. This study will compare people with painful knee osteoarthritis, and people with fibromyalgia, to people who are pain free. The study will be written up for publication in an international journal and will be used for a PhD thesis.

How was I identified and why am I being invited to participate in this research?

You have been identified because you responded to an advertisement and have painful osteoarthritis of the knee; or, you have been diagnosed with fibromyalgia; or, you have no pain in your body and identify as healthy. You may be excluded from participating if you have heart problems, are taking medications that affect the cardiovascular system, or have uncorrected hearing loss. You may be excluded from the pain free group if you have experienced pain in the last 3 months. Taking part in this study is voluntary (your choice) and you may withdraw at any time without being disadvantaged in any way. If you withdraw from the study then you will be offered the choice between having any identifiable information removed or allow it to continue to be used. However, once the findings have been produced, removal of any data may not be possible.

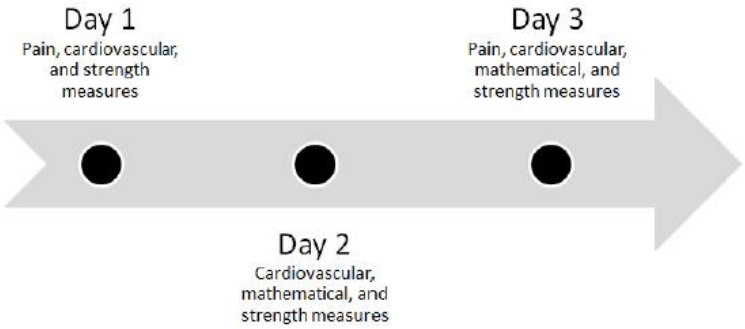
What will happen in this research?

If you participate in this study you will be asked to attend three data collection sessions over three separate days (see flowchart below) at the AUT Biomechanics Laboratory, Whenua Pupuke Waitemata Clinical Skills Centre, North Shore Hospital. Each session will last no more than 120 minutes and will not take place on consecutive days. In each session, we will test your pain system, including determining your pain threshold. This will involve the following:

- Placing a temperature probe on your forearm and knee that will heat up and cool down until you report that it is painful (left).
- Pressing a rubber-tip probe into your forearm and knee until you report that is painful (right).

- Asking you to rate your pain while the probes are applied.
- Immersing your arm up to the elbow in ice-cold water for up to 5 minutes.

During two of the sessions we will be testing the strength of your leg in the form of a leg extension exercise. In one of the sessions you will be asked to perform a series of simple mathematical calculations by way of addition only. During all three sessions, we will be recording the activity of your cardiovascular and fight/flight systems. This will involve putting electrodes on your neck, chest, and fingers. Participation in this study will require you to withhold all usual pain relief medications for 24 hours prior to each session.



What are the discomforts and risks?

You will be exposed to pain since measuring pain thresholds is important for the outcomes of this study. The effects of pain will be short-lived, i.e. less than 5 minutes. However, you may stop any test at any time. There is a risk that you may have minor skin irritation where electrodes are placed. As this study requires participants to withhold pain relief medication for 24 hours prior to testing, you may experience more pain than usual during the course of the day.

How will these discomforts and risks be alleviated?

No painful testing procedure will exceed levels that you cannot tolerate. You will be able to stop any of the tests at any time. Hypoallergenic medical tape will be used to secure electrodes to the skin and aloe vera cream will be available in the laboratory.

What are the benefits?

You will receive no direct benefit from participating in this research. The study outcomes will tell us more about the relationship between pain and the fight/flight system. This may provide us with ideas on how to improve pain in people with chronic osteoarthritic pain.

What compensation is available for injury or negligence?

In the unlikely event of a physical injury as a result of your participation in this study, rehabilitation and compensation for injury by accident may be available from the Accident Compensation Corporation, providing the incident details satisfy the requirements of the law and the Corporation's regulations.

How will my privacy be protected?

You will be given a code upon entry to the study and your name will not be used. The Consent Form that contains your name and your code will be stored in a locked filing cabinet. No individual results will be identifiable in the study.

What are the costs of participating in this research?

The cost of participating in this project will be your time. The three data collection sessions are expected to last no longer than 120 minutes each. Parking will be available on the premises and travel expenses will be reimbursed by way of petrol voucher.

What opportunity do I have to consider this invitation?

You will have one week to consider this invitation after receiving the Information Sheet. We will call you at the end of the week to see if you would like to participate.

How do I agree to participate in this research?

You will need to complete a Consent Form that will be provided at the first data collection session. This session will be scheduled after you have told us that you would like to participate.

Will I receive feedback on the results of this research?

You will have the opportunity to receive a one page summary of the study results at the conclusion of the study. There will be a section in the Consent Form to indicate if you would like to receive this summary.

What do I do if I have concerns about this research?

Any concerns regarding the nature of this study should be notified in the first instance to the researcher, Gwyn Lewis, gwyn.lewis@aut.ac.nz, 921 9999 x7621. Concerns regarding the conduct of the research should be notified to the Executive Secretary, AUTEC, Kate O'Connor, ethics@aut.ac.nz, 09 921 9999 x6038.

Whom do I contact for further information about this research?

Researcher contact details:
Neil Bossenger, Auckland University of Technology, North Campus
Landline: 09 522 0025 E-mail: neil.bossenger@aut.ac.nz Mobile: 0212397623

Project Supervisor Contact Details:
Dr Gwyn Lewis, Rm AB112, Auckland University of Technology, North Campus
Landline: 921 9999 x7621 E-mail: gwyn.lewis@aut.ac.nz

Approved by the Health and Disability Ethics Committee (HDEC): 9 April 2019

HDEC reference number: 18/CEN/45/AM01

Appendix H. Consent Form

Consent Form

Project title: **Associations between pain and stress in healthy people and people with chronic pain**

Project supervisor: **Dr Gwyn Lewis**

Researcher: **Neil Bossenger**

Declaration by participant

- ○ I have read and understood the information provided about this research project in the Participant Information Sheet dated 9 April 2019.
- ○ I have had an opportunity to ask questions and to have them answered.
- ○ I understand that taking part in this study is voluntary (my choice) and that I may withdraw from the study at any time without being disadvantaged in any way.
- ○ I understand that if I withdraw from the study then I will be offered the choice between having any data that is identifiable as belonging to me removed or allowing it to continue to be used. However, once the findings have been produced, removal of my data may not be possible.
- ○ I am not suffering from heart problems, medications that affect cardiovascular activity, or uncorrected hearing loss.
- ○ I agree to take part in this research.
- ○ I wish to receive a summary of the research findings (please tick one):

  Yes ○ No ○
- ○ I wish to be contacted regarding further research projects (please tick one):

  Yes ○ No ○

Participant's name: _____

Participant's signature: _____ **Date:** _____

Participant's contact details

Postal address: _____

E-mail address: _____

Best contact number: _____

Declaration by member of research team

I have given a verbal explanation of the research project to the participant and have answered the participant's questions about it. I believe that the participant understands the study and has given informed consent to participate.

Researcher's name: Neil Bossenger

Researcher's signature: **Date:**

Approved by the Health and Disability Ethics Committee (HDEC): 9 April 2019

HDEC reference number: 18/CEN/45/AM01