Exploring the Mechanisms Underlying the Transient Sitting-induced Pain Response in Healthy Individuals

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Abstract

Background: Prolonged sitting has been shown to induce clinically relevant levels of transient low back pain in some back-healthy individuals. However, where this pain signal is coming from is unknown. Tissue loading instigating inflammation, or a baseline sensitivity to pain are two potential mechanisms that could explain this response.

Methods: 50 pain-free healthy adults were exposed to 60-minutes of sitting. Perceived pain ratings were taken to classify pain groups. Biomarkers of inflammation and peak brain wave alpha frequency were taken immediately before and after the trial.

Results: 29 participants were identified as pain developers and 21 as non-pain developers. Peak alpha frequency, and spine posture were not significantly different between groups. Non-pain developers had significantly higher concentrations of IL-6 and IL-10 and displayed more frequent shifts in during sitting compared to those without pain.

Conclusion: These results suggest that neither inflammation or pain sensitivity are likely explanations for the transient pain groups identified. However, findings suggest underlying differences between pain groups. Although adopting similar back postures during sitting, non-pain developers moved more during sitting, which may suggest a protective behaviour. Future research should explore the differences in inflammatory markers as well as longer sitting durations.

General Summary

A short-lived back pain response to standing and sitting has been identified in healthy individuals and may be an early predictor of a future case of clinical low back pain. Yet we do not fully understand where this pain signal is coming from. Exploring potential explanations for this pain signal, we measured inflammatory markers and brain waves for 50 healthy individuals during 60-minutes of sitting. Participants were classified into pain groups by pain ratings and spine posture was monitored. Non-pain developers moved more during sitting and had higher levels of IL-6 and IL-10 blood biomarkers which may be a protective factor against pain. Future research, building on the finding of this study, is needed to better understand the mechanisms behind this pain response.

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List of Abbreviations

ANOVA: Analysis of variance

EEG: Electroencephalography

HREB: Health Research Ethics Board

IASP: International Association for the Study of Pain

IL-10: Interleukin-10

IL-1β: Interleukin-1β

IL-6: Interleukin-6

LBP: Low back pain

NPD: Non-pain developers

NRS: Numeric pain rating scale

PD: Pain developers

TNF-α: Tumor necrosis factor alpha

VAS: Visual analog scale

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Chapter 1: Introduction and Rationale

1.1 Introduction

Low back pain (LBP) is a universal problem. It impacts up to 80-85% of the population at some point in their life and is reported to be among the leading causes of disability in most countries [1,2]. In 2015 the point prevalence of LBP was stated to be 7.3% of the global population, increasing to 7.5% in 2017 [2,3]. The global point prevalence of chronic low back pain (CLBP) in adults aged 21-69 years was reported to be 13.1% in 2009-2010 [4]. The prevalence of LBP and its impact on function renders it one of the leading causes for individuals requesting time off work globally [5]. Despite its prevalence, causative factors for LBP are not fully understood [5–7]. Musculoskeletal disorders such as LBP are often overlooked in the eyes of society as a result of social reasons, therefore, they do not garner as much attention and funding as other diseases such as cancer [8]. Despite this misguided assumption, LBP is a musculoskeletal disease which is one of the world's leading health care issues, resulting in reduced capacity for activities of daily living and imposing a substantial economic burden on society.

Societal pressures and the shift to less physically demanding desk jobs have resulted in a global epidemic of sedentary lifestyles. Although causative factors of LBP are not fully understood, we do know that prolonged postures such as spine flexion during sitting can be an aggravating factor for LBP. These postures have also been identified to induce transient LBP in individuals who are otherwise healthy [9–11]. In a systematic review, De Carvalho and colleagues (2020) have shown that sitting for 1 to 6.69 hours over five days at desk-based jobs is enough to induce clinically relevant levels of back pain, which is a cause for concern [12]. Studies included in this review included both laboratory and field settings as well as individuals with and without LBP. Evidence

suggest that individuals classified as pain developers (PD) in response to prolonged standing have a higher risk of presenting with an episode of clinical LBP within a three-year period. Currently the risk of developing a future case of LBP between sitting-induced pain groups is not known, however, this work is currently underway by our team. Given that those who report clinically relevant levels of LBP following prolonged standing have an increased likelihood of presenting to a clinic with LBP, it may be possible to use the induced pain response to identify those who may benefit from LBP prevention strategies. Therefore, it is hypothesized that this postural-induced pain response may have a role in the early identification and prevention of LBP [11].

While we know that prolonged postures such as sitting and standing can induce symptoms of back pain in some individuals, we do not fully understand why some individuals develop this response and others do not. Perhaps it has to do with individual anatomy, posture, or susceptibility to experiencing pain. The method of classifying individuals as PD versus non-pain developers (NPD) based on their subjective pain while participating in a prolonged posture study that induces LBP has been shown to be effective in pre-establishing individuals who will suffer from LBP in the future [11,13]. Furthermore, it was observed that individuals classified as PD developed LBP within three years following the study [11]. Pre-establishing individuals who will develop pain has the potential to aid in determining treatment options and pain management plans that would be personalized to an individual's expected experience with pain. In addition, determining individuals who will develop LBP following prolonged posture exposures may allow us to prevent pain development or strategize treatment options.

1.2 Purpose

This thesis will aim to explore two potential associative factors for the transient pain response throughout a laboratory-controlled prolonged sitting exposure. Specifically, this study will explore whether inflammation, instigated by tissue loading, and/or a general sensitivity to experiencing pain, play a role in the pain response. The results of this work will help to improve our understanding of the mechanisms behind the experience of pain in response to sitting in otherwise healthy individuals. In combination with additional research, this study will contribute to the ongoing body of research which aims to further investigate the development of LBP and help to inform prevention and management strategies to minimize the impact of LBP on the population.

1.3 Research Questions

1.3.1 Primary Research Questions

The primary research question asked which blood biomarkers of inflammation best reflect the transient-sitting induced pain response displayed by individuals classified as pain developers, either at baseline or after the exposure? To test this question, we exposed a sample of participants to a sitting exposure known to induce transient-sitting pain in subset of people (approximately 40%). We compared serum biomarkers of pain, pro-inflammatory cytokines, between groups of PD and NPD.

1.3.2 Secondary Research Questions

There are three secondary research aims investigated in this thesis. First, we investigated if sitting-induced PD are more sensitive to pain than NPD? To test this research question, we

compared the peak alpha-wave frequency between pain groups. Recent literature suggested that individuals who display a slower resting peak alpha-wave frequency are more sensitive to experiencing pain than those who do not [14–16].

Secondly, we were interested in understanding potential mechanisms behind the pain response displayed by individuals classified as PD. Is viscoelastic creep of the passive back tissues, evidenced by an increase in functional range of spine motion in forward flexion, occurring throughout the sitting exposure? Since repetitive flexion of the spine has been shown in animal models to result in viscoelastic creep and the presence of inflammatory markers [17], our second question explored whether inflammation was present during an exposure to prolonged sitting and may have contributed to the transient sitting-induced pain response. To indirectly test for the presence of low back creep we measured active range of spine motion using forward flexion trials before and after the sitting exposure. An increase in the range of motion would suggest that lengthening of the back tissue has occurred in response to the constant stress imparted on the spine by the effect of gravity acting on the body during sitting.

Finally, we wanted to replicate the methods of previous studies in order to better understand the questions do PD differ from NPD in the way they sit? It is currently unknown if individuals move more frequently to avoid or prevent the experience of pain or if movement precedes the pain response and potentially is an aggravating factor contributing to the development of pain. To test this, we instrumented the participants with accelerometers at the top and bottom of the low back curve (spinous processes L1 and S2) to continuously measure spine angle, and movements of the angle (shifts and fidgets) throughout the sitting exposure. We then compared the results between PD and NPD groups.

1.4 Hypotheses

1.4.1 Primary Hypothesis

Based on the literature described in section 2.6.1 Biomarkers of Inflammation we would expect there to be a significant difference in the concentration of blood biomarkers in PD compared to NPD. More specifically we would expect the concentration of pro-inflammatory cytokines: IL-1β, IL-6 and TNF-α to have elevated concentrations in individuals classified as PD. We would also expect levels of anti-inflammatory marker IL-10 to be elevated in NPD compared to PD.

1.4.2 Secondary Hypotheses

1. We expect that PD will have a slower peak alpha frequency at rest when compared to NPD. Based on the literature we know that individuals who display a slower than normal peak alpha frequency (<9.5 Hz) at rest may be more susceptible to experiencing pain than those who display at normal peak alpha frequency at rest [14–16].

2. We expect those who experience pain and are classified as PD to have a greater increase in range of motion following the sitting exposure when compared to range of motion measured before the exposure. This would provide evidence that low back creep, and resulting tissue inflammation, could be a potential source of the pain developed throughout the sitting exposure.

3. There is conflicting evidence in the literature concerning the frequency of spine angle shifts and fidgets in individuals who display a pain response. It is not fully understood if individuals experiencing pain move more to prevent the pain or if the increased movement is

contributing to the pain experience. As a secondary objective we wish to explore this conflict in the literature further to observe if PD or NPD have increased micromovements.

Chapter 2: Literature Review

2.1 Functional Anatomy of the Low Back

The spine is a crucial system for the body. It directly provides protection to the spinal cord and peripheral nerve roots, and by stabilizing the trunk segment, it facilitates motion and flexibility of the arms, legs, and head. Thus, the spine is considered to be the main supportive structure in the back [18]. The back and spinal cord are a fundamental structure for function as its structure impacts function and vice versa. The low back is responsible for nearly all of our day-to-day activities including but not limited to spinal support, upper body support, carrying loads and motion. The low back is composed mainly of bones, discs, blood vessels, ligaments, nerves, and muscle. The vertebral body is the main portion of the vertebra in the back which protects against spinal compression and allows for connection to the muscles and ligaments [19]. The vertebra are separated by fibrocartilaginous discs which are comprised of a soft nucleus pulposus centre and enclosed by tough criss-crossing layers of cartilage called lamellae [19]. The discs are important for movement and shock absorption of the vertebral body. Ligaments which extend from the vertebral body help to control joint movements, like spine bending, keeping them within safe ranges of motion to protect against tissue damage [19]. The back contains many complicated structures which are linked to function and motor control. All of the anatomical parts in the back work to stabilize the torso so that the legs and arms have a solid support from which they can generate forces and movement. Thus, optimal functioning of the back is essential for activities of daily living that include walking, bending, lifting, standing, and sitting. Thus, when affected by injury or pain, it is easy to see how many issues and complications for proper function arise when the back is not working at optimal capacity.

2.2 Pain

The definition of pain was established by the International Association for the Study of Pain (IASP) in 1979 and began revisions in 2018. Pain is now defined by the IASP as " An unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage" [20]. Pain plays an important and essential role in the protective function of the body. Nociception allows the body to sense an external stimulus which is referred to as a noxious stimulus, which creates the sensation of pain as a warning signal [21]. When we interpret this pain signal we can act to protect ourselves from the impending tissue damage by removing the noxious stimulus or ourselves from the situation [21]. Following tissue damage pain continues to play a crucial function as it aids in the healing of our tissue. Experiencing pain or tenderness when our body moves a certain way can warn us to stop this activity as it is causing harm to the tissue [21]. This is where the concept of innocuous stimuli comes in as this is a form of pain created by the immune system which helps to prevent further injury to injured or infected tissue which is defined as inflammatory pain [21]. Acute pain is an activation of a noxious stimulus which causes tissue damage that recovers. The transition from acute to chronic pain is when the pain continues to persist and there is repetitive stimuli whereas the acute period would have terminated once the tissue healed [22]. The process of repetitive activation of the stimuli in chronic pain can lead to central sensitization which leads to increased sensitization of the pain response. The increase sensitization of the pain response can lead to increased stimuli signals and increased associated responses in the brain [23].

Our individualized experience with pain is largely subjective and it has been established in the literature that our pain perception can be altered based on our mood, attention and our expectations [24]. Distracting an individual from their pain or having a positive mood has been

shown to decrease the experience of pain where on the contrary having a negative mood is shown to increase our experience with pain [25]. When we consider what pain is we have to consider the individual experience of this pain. Our experience with pain is what codes this as an unpleasant experience in our brains. Not all painful experiences are associated with negative mood or unpleasant outcomes for example a deep massage [24]. Central sensitization helps to describe the relationship between intensity of a stimulus and the way we perceive the associated pain which can contribute to the development of chronic pain in some individuals [22,26].

2.3 Clinical Low Back pain

Low back pain is generally defined as pain located between the inferior border of the ribs and the gluteal folds [27]. Pain radiating down the legs can be present or absent with LBP [27]. There is substantial heterogeneity concerning the development, progression, duration, and pathology of LBP for each individual. Numerous conditions are associated with the development of LBP as a symptom which can result from various locations of pain development. More commonly individuals who suffer from LBP are diagnosed with non-specific LBP, on average 90% of individuals will have non-specific LBP [28,29]. Non-specific LBP is when the source of the pain cannot be identified or attributed to a specific cause. When undergoing a diagnosis for non-specific LBP the conclusion is commonly reached when no other pathology can be identified, there is no concrete classification system for the diagnosis of LBP [29]. The alternative to non-specific LBP is specific where the source of the pain is known for example a tumour, fracture, or inflammatory arthritis. In addition to this, LBP can be classified as chronic or acute. The classification of acute or chronic LBP is based on the time experiencing pain, however this notion has been debated [30]. It is believed that shifting to a focus on patterns of pain versus timeframe may be more useful for classifying individuals [30]. Chronic LBP was commonly defined in the

past as pain in the lower back which persist for more than 3 months and acute is when the pain lasts for less than 6 weeks [29]. The amount of time that LBP persists varies for each individual and the majority of individuals will recover prior to the 3 months period. The duration of LBP can vary from one day to numerous years [31]. Individuals who suffer from debilitating LBP often have reoccurring episodes which persist for the majority of their life [32].

Causative factors contributing to the development of non-specific LBP are not fully understood but we do know non-specific back pain can be elicited because of numerous biomechanical factors. For instance, high external and internal forces acting on and within the back could stimulate pain sensors and/or damage tissue leading to injury. Repetitive motions such as bending and lifting can result in back pain. The risk of injury when bending has been shown to be related to the rate at which we bend and the history of repetitive bending [33]. A case-control study looked at the impact of lifting and back pain development, they found that individuals who lifted more than 25 pounds a day more than 25 times were three times more likely to have a prolapsed disc [34]. Even low magnitude forces could lead to problems if they are sustained for long durations. Prolonged postures, like sitting and standing, are common in many occupations and could lead to scenarios where tissues tolerance lowers over time leading to injury. Indeed, prolonged flexed postures have been associated with LBP and injuries [35]. Remaining in a flexed posture for periods of time can introduce viscoelastic tissue creep [35]. Viscoelastic tissue creep is a phenomenon where constant loading such as prolonged flexion on the spine causes the tissue to deform, following the spine being in a flexed state and deformed tissue it becomes more unstable which results in the back being more susceptible to an injury [36]. In order to prevent individuals from passing their normal range of motion as a result of tissue deformation the muscles work to provide a stable body for the back, however when the muscles are exposed to

the creep phenomenon inflammation and delayed muscle activity may occur which could result in injury [17,37].

2.4 Epidemiology of Low Back Pain

Low back pain is a musculoskeletal disorder that is extremely prevalent in today's society impacting the entire population at some point in their life [1]. Low back pain impacts both female and male adults but is slightly more prevalent in females impacting 47.0 per 1,000 person-years versus 42.2 males per 1,000 person-years [38,39]. The likelihood of developing LBP is reported to increase with age and males experience a peak in LBP between the ages of 45-64 years [39–41]. Although LBP is common and highly researched its heterogenous nature and numerous etiologies makes it challenging to accurately identify and predict. Based on the statistics that are reported we know that LBP is a global concern. In Canada alone it has been reported that the incidence rate of individuals who are suffering from LBP aged 20-69 is 18.9% [42]. The United States reports that 15% of their population is impacted by LBP at a point in time and 65%-80% of these individuals will have lifelong reoccurrence and struggles such as reduced quality of life as a cause of LBP [43]. A systematic literature review by Hoy and colleagues (2010) reported that the prevalence of LBP has a very wide range. Point prevalence ranging from 1% to 58.1% and one-year reoccurrence ranging from 0.8% to 82.5% [1]. In addition to this they stated that the incident rate for first time LBP episodes ranged from 6.3% to 15.4% [1]. It was previously believed that LBP was largely confined to high-income countries, however, it has now been reported that middle- and low-income counties are equally impacted by the musculoskeletal disorder [44]. It is likely that the causes and impact of LBP differ between countries as a result of socioeconomic status, health care systems and also common occupations [45]. The percentage of individuals who suffer from LBP worldwide is a major epidemiological concern as it is debilitating and has a

profound impact on daily living. In a study by De Souza and Frank (2006) investigating the impact of chronic back pain they found that participants reported lack of sleep, inability to stand, inability to climb stairs, mobility issues, feelings of reduced independency, and reduced ability to participate in leisure activities [46].

Musculoskeletal disorders are often a direct cause of severe pain [47]. As a result of its increasing incidence rate LBP is now among the main reasons people seek out healthcare services and visit a physician [48]. Low back pain is a major contributor to limited activity and is ranked as one of the main reasons for needing time off work [47]. Wynne-Jones et al. (2008) conducted a study on 935 individuals experiencing LBP aged 30-59 years. They reported that 11% of individuals were limited in their employment duties and 22% of participants were taking time off work as a result of LBP [49]. Amongst the participants who reported that they were unemployed, 37% of them stated they did not work as a result of their LBP [49]. Additionally a cross-sectional study conducted by Dutmer et al., (2019) on 1502 participants suffering from LBP reported that 17% of the individuals were permanently off work as a result of LBP [50]. The financial burden of LBP is immense and include work related costs, personal costs, and healthcare costs [45]. Luo et al. (2003) conducted a secondary analysis to determine the financial burden back pain causes in the United States. They discovered that the United States spent 90.7 billion dollars in 1998 as a direct result of back pain [51]. They also reported that individuals who suffer from back pain spend nearly 60% more when compared to individuals who are pain free, likely due to all the indirect costs such as medication, health care and massage therapy [51].

LBP has a high rate of reoccurrence which elevates its burden. In a systematic review by Hestbaek et al., (2003) they reported that 50% of individuals who experience an episode of LBP have a reoccurring episode within one year, 60% within 2 years and 70% within 5 years [32]. The

reoccurrence rate contributes to the ongoing financial burden that society faces due to LBP. Individuals who suffer from LBP recurrently will often continue to be affected for the remainder of their lives [32]. Once an individual suffers from reoccurring LBP there are biomechanical factors which have been reported to contribute to the ongoing reoccurrence of LBP. Individuals who suffer with back pain may also form maladaptive movement control and coping skills which can lead to the further development of injury [52]. In addition to the biomechanical factors there are psychological factors which may play a role in the ongoing reoccurrence of LBP. For instance individuals who have an episode of LBP may alter their movement as a fear response to the pain and may be reluctant to participate in movement or tasks in their normal way which result in fear-avoidance behaviours [53].

Many interventions have been studied for back pain and early intervention is shown to be effective in determining the outcome for individuals suffering from LBP [29]. The likelihood of recovery for individuals suffering from LBP decreases the longer they are living with the pain [29]. The United States guidelines for the treatment of LBP favour non-surgical and nonpharmacological interventions [54]. The guidelines recommend massage, acupuncture, heat therapy or spinal manipulation. In the event pharmacological intervention is required non-steroidal anti-inflammatory drugs are recommended or muscle relaxants [54]. In addition to this various exercises have been shown to be effective in reducing LBP and are recommended by the United States guidelines [54]. Evidence-based research has reported that certain exercise regimes can be more beneficial for individuals suffering with chronic LBP than usual care such as medication and physical therapy [55]. In a systematic review by Gordon and Bloxham, they concluded that exercises which target muscular strength and flexibility in the back are the best for chronic LBP [56]. Another systematic review conducted by Hayden et al (2005) was in

agreement stating that exercises which prioritize stretching and strengthening under direct supervision show improvement for chronic non-specific LBP [57]. Treatment is complex and there is no cure, therefore, prevention should be the top priority and there is a gap in the research concerning prevention.

2.5 Association of Sitting and LBP

Sedentary behaviour such as sitting has been observed to be an increasingly concerning issue over the past number of years. It is reported that before the Industrial Revolution individuals sat for approximately 5 hours a day compared to 15 hours a day presently for office workers [58,59]. Sitting has become an integrated part of our society within industrialized countries. Cars, trains, workplaces, and our homes have been designed to incorporate sitting. Sitting was incorporated into our daily lives to give the body and muscles time to relax, however, too much time spent sitting has been linked to a number of chronic health conditions including an increased risk of obesity, diabetes and heart disease [60,61]. Among the concerns of increased sitting time, seated postures are linked to the development of back pain and aggravation of LBP [9–11]. There are numerous biomechanical factors which are thought to play a role in the association of sedentary behaviour and back pain. Remaining in a static position over time causes static loads on the tissues, muscles tendons and ligaments and repeated loads can cause inflammation and the development of pain [62]. Chronic LBP is estimated to be 2.5 times more likely to occur in individuals who work in an office when compared to those who do not [63]. It has been reported that 63% of self-reported musculoskeletal pain is thought to be a direct result of occupational demands [64]. Of the individuals who self-report musculoskeletal pain attributed to their occupation, 34% reported the development or reoccurring LBP [64]. It is likely the increase of LBP in those who work compared to those who do not is a direct cause of prolonged flexed spine

postures that have been reported during sitting. Individuals who work in an office or at a desk are often exposed to static postures, awkward postures and low force repetitive movements [65]. Additionally, the demands, stressful atmosphere and pressure which is associated with work constraints contribute to psychosocial factors which are shown to be associated with the development of back pain [66].

2.6 Potential Pathways to Pain in Sitting

Various biomechanical processes may play a role in the development of LBP while in a static seated posture. A concept which has been noted in the literature to contribute to the development of LBP is the flexion-creep phenomenon. The flexion-creep response is the concept that the tissues stretch and deform in response to a static load over time, increasing tissue laxity and normal joint ranges of motion [67]. The deformation of the tissue is not severe enough to result in tissue failure, however, it has been hypothesized that this reduced stiffness could render the lumbar spine functionally unstable and susceptible to injury and trauma [36,67,68]. Evidence in the literature does demonstrate that creep causes desensitization of the mechanoreceptors which detect stimuli in the spinal tissues which decreases muscle activity, leaving the tissue susceptible to injury [69]. Work by Adams et al (1987) measured the lumbar range of motion of individuals in the morning and in the afternoon. They found that the range of motion in the lumbar spine increased by 5° throughout the day [68]. It was suggested that the increase in range of motion was a result of ligament viscoelasticity and the narrowing of discs due to a loss of fluid. Additional work by Adams and Dolan (1996) found that after two hours of compressive creep loading the range of motion of participants was increased by 12% [33]. Another laboratory-controlled study conducted by Sánchez-Zuriaga et al. (2010) exposed participants to a one-hour maximal flexion sitting exposure and found the overall range of motion to increase by 2.3°± 2.5°

[70]. They concluded that prolonged spinal flexion left the spine without protection and confirmed the increased range of motion was a result of passive tissue creep. Similar results were found with 30-minutes of maximal flexion [71]. More recently, a laboratory-controlled study conducted by Kang et al (2023) exposed participants to sub-maximal flexion sitting and also found evidence of creep deformation of viscoelastic lumbar tissue [72].

Another avenue which has been studied for the development of LBP while in a static posture is micromovements of the spine such as fidgets (movement where the spine posture changes and then goes back to its original posture) and shifts (movement where the spine posture changes and does not return to its original posture). A study evaluating the spinal micromovements in individuals suffering with sitting-aggravated LBP and comparing to asymptomatic individuals found that those with LBP displayed significantly more shifts [73]. They concluded that the increase in movement in the LBP group did help to decrease their level of pain. It has also been observed that individuals more susceptible to developing LBP (PD) display an increase in spinal movements and fidgets, therefore those classified as PD have increased muscle activity compared to NPD [13,74,75]. Contradictory to this some studies have found the opposite where NPD have increased movement. It is possible that the increased movement has a protective nature and decreases the risk of developing back pain. A recent study conducted on 60 healthy office workers evaluating the impact of shifting on discomfort while seated for one-hour found shifting to decrease discomfort [76]. In a study conducted by Greene et al., (2019) where participants were exposed to one-hour of sitting, they found that PD had significantly less spine fidgets when compared to NPD [13]. Adding to this a study by Gallagher & Callaghan (2015) evaluated spinal micromovements over 2-hours of standing in PD and NPD. They found that NPD had a higher frequency of fidgets and shifts [77]. There is conflicting evidence in this area we still do not

know what causes these micromovements and if they are a result of pain or is it protective in nature.

2.7 Sitting and the Transient Pain Phenomenon

Not to be confused with the clinical condition of LBP, a transient (i.e., goes away when getting up and moving around) sitting-induced pain response has been identified in the literature. First identified in prolonged standing studies, when a clinical threshold of meaningful increases in perceived pain were applied to datasets, two distinct groups of responses were found: individuals who exhibit no pain in response to the exposure whatsoever, and individuals who are experiencing clinically relevant levels of pain. This subtlety was historically missed when perceived discomfort scores were averaged across a population, showing increasing, but not worrisome, levels of discomfort in response to longer exposures to static postures. Currently, several research groups have consistently demonstrated the presence of these groups and a systematic review in 2020 showed that this response is present regardless of setting (laboratory versus field) or clinical status (healthy versus LBP) [12].

Various studies have classified individuals as PD and NPD [11,74,78–80]. The classification of PD and NPD uses a clinical minimal difference in the change of pain experienced throughout the postural exposure. Participants were classified as PD if they reported a 10mm or greater increase in pain throughout the sitting exposure. It has been found in the literature that 40% of individuals will be classified as PD and 60% as NPD throughout the standing exposure. A similar portion of PD and NPD were found throughout prolonged sitting exposures [74,80]. These studies have also shown a significant difference between pain groups which shows that PD have a significantly greater pain response. In a large laboratory-controlled study there were no differences in the

seated muscle activity and spine posture, or pre/post flexion range of motion found between PD and NPD groups [13]. Thus, it is unclear where this pain originates from nor what it might signal. Despite this uncertainty, classifying healthy individuals as PD and NPD might be useful in understanding sitting-induced pain as a potential pre-clinical state of LBP. A study conducted by Nelson-Wong and Callaghan (2014) followed participants over the course of three years and found that participants who were classified as PD in a standing-induced pain exposure were at greater risk of developing acute LBP within the follow up period [11] and we hypothesize this holds true for sitting-induced pain groups.

2.8 Subjective Measures of Pain

As stated above, pain is largely subjective and dependent on the individual's experience. Because the nature of pain is so individualized, it can be difficult to accurately measure pain [81]. Despite this it is not always feasible to measure an individual's pain objectively so there are subjective non-invasive ways of measuring pain. Clinical pain assessment is frequently measured using the visual analog scale (VAS), verbal rating scale (VRS) and the numerical rating scale (NRS)[82]. The VAS is an established tool for measuring pain and is reported to be both valid and reliable [82]. The VAS consists of a 100mm line where the zero on the left side means no pain, and 100 on the right side means the worst pain imaginable. These anchors are important to define as it allows for the individual rating their pain to understand where their experienced pain would be on this continuum. The VAS can be used digitally and also on paper. One would mark a line across the continuum where they feel their experienced pain is, however, they cannot see what number between 0 and 100 they are choosing.

2.9 Objective Measures Relevant to the Experience of Pain

2.9.1 Biomarkers of Inflammation

Biomarkers can be measured within the body to help inform on certain bodily functions and characteristics. Specific biomarkers can be associated with certain outcomes or diseases which provide information about the functionality or state of the body. Many disorders and diseases associated with chronic pain have been linked to an increase in proinflammatory biomarkers in the plasma of the blood [83]. Proinflammatory cytokines can play a role in modulating nociception [84]. Upon the release of inflammatory markers they can excite or cause sensitization to the nociceptors which result in the experience of pain [85]. It has been established in the literature that classic inflammatory biomarkers such as cytokines or C-reactive proteins are elevated in individuals suffering from non-specific LBP [83,86,87]. Despite the ongoing research known biomarkers which can accurately predict LBP do not currently exist.

Common cytokines involved in the inflammatory process and following injury are interleukin-1B (IL-1β), interleukin-6 (IL-6), nerve growth factor and tumor necrosis factor-alpha (TNF-α), interferon-γ and transforming growth factor-β [22,88]. IL-6 and various other cytokines are involved in the acute inflammatory response. One particular role of IL-6 specifically is that it can be both harmful and also have a protective nature [89]. In an acute response to stimuli IL-6 can suppress proinflammatory cytokines and it can also increase the concentration of IL-1 which is an anti-inflammatory cytokine. The inflammatory cytokine, IL-6 has a large variety of biological purposes. Elevated IL-6 has been observed in pathological pain models and the administration of IL-6 has been reported to cause pain-related behaviours [90]. Both IL-6 and TNF-α are able to impact each other's levels of production [91]. TNF-α is another proinflammatory marker which

is produced by various types of cells as a response to environmental stimuli, inflammation and injury [92]. Blocking TNF can be useful for chronic inflammatory disease [93]. IL-1β is another highly studied proinflammatory biomarker and is the main cytokine which is studied in the IL-1 family because of its role in inflammation [94]. In addition to proinflammatory markers which are found in inflammation there are anti-inflammatory markers which do the opposite. Anti-inflammatory cytokines such as interleukin-10 (IL-10) work to inhibit and eliminate inflammatory markers [95].

Various studies have looked at the concentration of proinflammatory cytokines to inform on their role in the experience and development of pain. A study which compared the levels of proinflammatory and anti-inflammatory markers in patients with painless neuropathy, painful neuropathy and healthy controls found that patients with painful neuropathy had significantly higher levels of TNF compared to controls [96]. They also evaluated other cytokines including the anti-inflammatory marker IL-10 which they found to be elevated in patients with painless neuropathy compared to painful neuropathy.

There are also various studies which have researched the role of proinflammatory and anti-inflammatory markers in individuals experiencing nonspecific LBP. A recent systematic review discovered that there is a positive correlation between the severity of non-specific LBP and proinflammatory biomarkers [97]. More specifically they found moderate evidence yet conflicting evidence that as the severity of non-specific LBP increased so did the concentration of C-reactive proteins, TNF-α and IL-6. Furthermore a recent systematic review conducted by Lim et al., (2020) supported the systematic review by van den Berg et al., (2018) that nonspecific LBP was positively correlated with C-reactive proteins, TNF-α and IL-6 [87]. Additional research in

the area confirmed the thought that IL-6 increases in individuals experiencing LBP. A study that compared individuals with chronic LBP compared to age-matched controls found that IL-6 was significantly higher in individuals with LBP [98]. They also found that the anti-inflammatory marker IL-10 was lower in individuals who were experiencing LBP. Adding to this base of knowledge Teodorczyk-Injeyan et al., (2019) took blood samples from individuals suffering from both chronic and acute LBP and asymptomatic controls. The results showed elevated levels of proinflammatory markers, Il-6, IL-1β and TNF-α and their ratio to anti-inflammatory marker IL-10 in both acute and chronic LBP groups when compared to controls [99].

Although much of the research which looks at individuals who already have experienced LBP or are suffering from chronic LBP found similar findings a recent study by Carter et al. (2021) investigated inflammation in healthy asymptomatic desk workers after sitting uninterrupted for 6-hours. They found contradicting evidence to what is previously identified in the context of LBP. They found no significant differences in inflammation for IL-6 and TNF-α following the sitting exposure [86]. The findings in this study contradicted their hypothesis, they reported that the inflammatory marker results may have been due to the use of saliva testing as opposed venous. They did not stratify their sample based on PD and NPD which could have been a potential explanation for their findings. The response may have been different in individuals who were experiencing pain (PD) when compared to those who were not (NPD).

2.9.2 Electroencephalography

Electroencephalography (EEG) is reported to be among one of the greatest instruments for non-invasively observing activity in the brain which can tell us a lot about both cognition functioning and disease [100]. Neurons in the human brain communicate with one another using electrical

signals and this electrical activity can be measured and recorded using EEG [101]. The EEG measures the electric fields generated by populations of active neurons [101]. Neural oscillations or brainwaves can be observed in the brain with the EEG which can help to inform us of what is going on in the brain. These oscillations are rhythmic patterns of the neurons activity [100]. We can measure neural oscillations at different frequencies. Different neural oscillation can be associated with different processes that are occurring in the brain. Some of these brain processes associated with specific wave patterns are alertness, deep sleep, dreaming and cognitive functioning [102]. These frequencies are characterized as delta (<4Hz), theta (4-8 Hz), alpha (8-13 Hz), beta (13-30 Hz), and gamma (50-90 Hz). To measure the electrical activity from outside the brain, electrodes are placed on the scalp using a fitted cap. Electrodes placement on the EEG cap commonly follows the international 10-20 system. The 10-20 system for electrode placement is based on brain structure landmarks which place the electrodes in fixed locations to cover all brain regions and take into account differing head sizes [103].

More recently in the literature it has been observed that an individual's peak alpha frequency recorded in the brain's cortex has been associated with an individual's sensitivity to chronic pain [15,16]. The alpha band is one of the most commonly researched brain oscillations because of its stability and unique aspect between individuals [104,105]. The alpha frequency is the primary frequency for a relaxed state and is related to cognitive function and the central nervous system [106]. The alpha band frequency is mainly recorded at resting state with an EEG and is predominate in the occipital and somatosensory cortices [107,108]. Changes in the resting alpha band frequency have been observed in individuals experiencing both chronic pain and experimentally induced pain [14,15,109–111]. Nir et al. (2010) conducted a study with the aim of investigating peak alpha frequency in the central, temporal, and frontal brain regions as an

objective measure of pain compared to a subjective measure of pain [15]. To do so they measured peak alpha using EEG in a resting state, innocuous temperature, and a noxious temperature. They found the subjective and objective pain measures were correlated in the noxious condition, concluding that resting state peak alpha may be a good indicator of pain sensitivity. In addition to this Furman et al. (2018) observed peak alpha frequency in a pain-free state preceding a pain induction state using the heat capsaicin model [14]. They found that a slower peak alpha frequency in the pain-free state were linked to higher pain development in the pain state, concluding that a slower peak alpha frequency at rest was correlated with heightened pain sensitivity. To add to this body of research Furman et al. (2020) then observed peak alpha frequency using EEG while their participants were induced with pain using the heat-capsaicin and phasic heat models to investigate if heightened pain sensitivity could also be a predisposition to pain development [16]. They concluded that the peak alpha frequency at rest was negatively correlated with subjective pain rating, thus peak alpha frequency was a reliable marker for short and long periods [16]. In addition to experimentally induced pain, individuals with chronic LBP were observed to have a negative correlation between subjective pain ratings and peak alpha frequency and indicated peak alpha frequency as a possible useful tool for clinical LBP measurement [112]. Therefore, peak alpha frequency may indicate individuals who are more susceptible to experiencing chronic pain as indicated by both clinical and experimental models.

2.10 Purpose of the Study

This was the first study to look at two potential theories explaining the transient pain response. The purpose of the current study is to explore these two potential explanations for the transient pain response in sitting: inflammation secondary to viscoelastic creep and an inherent increased sensitivity to pain as indicated by a lower peak alpha frequency.

2.11 Research Objectives

The main objective of the current study was to explore the transient sitting-induced pain response throughout an established prolonged sitting exposure. To do so, we used a pre-established 60-minute sitting exposure, which has been shown to induce transient but clinically meaningful levels of perceived back pain in a proportion of individuals [13]. We explored a potential mechanism for the participant's subjective experience of pain using biological markers of pain (EEG and blood biomarkers of inflammation). This allowed us to test whether those classified as PD display a different inflammatory profile than NPDs, and/or are more susceptible to pain. A secondary objective of the current study was to measure spine angles and their movements, and changes in spine flexion range of motion across the sitting exposure and compare the results between the PD and NPD groups.

2.12 Significance of Study

This was the first study to examine potential exploratory factors for the development of transient sitting-induced pain during the 60-minute siting exposure. Understanding this mechanism will help researchers learn what might be different between individuals identified as PD and NPD, and may be advantageous for understanding why some individuals develop back pain while sitting while others do not. In addition, group establishment will allow us to investigate if the transient sitting-induced phenomenon is related to some individuals' sensitivity to pain. The ability to identify the reasons why the pain response occurs may allow us to control the experience and implement tailored prevention and care strategies.

Chapter 3: Methods

3.1 Study Design

This study is a observational analytic design and therefore we followed the strengthening the reporting of observational studies in epidemiology (STROBE) checklist for this manuscript [113].

3.2 Setting

This study took place in a laboratory in the Health Science Centre, St. John's, Newfoundland, and Labrador, Canada. A portion of the data analysis was conducted by EVE Technologies in Calgary, Alberta, Canada. Participants were recruited from January 2023 to April 2023. Data collection was conducted from February 2023 to April 2023.

3.3 Participants

Participants were recruited from the local community (St. John’s, NL) and university (Memorial University) population. Our justification for largely recruiting from a local and university population was mainly for convenience. Additionally, university students are committed to participating in research and are typically familiar with seated deskwork without interruption which resembles the sitting exposure in this study. We used the Health Research Ethics Board (HREB) template poster advertisements on announcement boards around campus, advertisements to the general university population, social media recruitment (Facebook and Twitter), and in-class verbal script recruitment. Fifty healthy, pain free adults (≥18 years) participated in this study. “Pain free” was defined as individuals who reported less than 10 mm on a digital VAS at the time of the study session. We targeted pain free individuals so that there was no pre-existing

pain which would interfere with the subjective or objective pain ratings obtained throughout the sitting exposure.

Our exclusion criteria were evaluated using a health screening form (Appendix A) which screened for any individuals reporting a subjective pain rating greater than 10 mm on a 100mm digital VAS, diabetes, high blood pressure or inflammatory disease, severe chronic pain (> 4 pain days/month for at least three months), neurological disease, disorder or injury, psychiatric disorder, cognitive impairment, pregnancy, or communicable disease (hepatitis C, HIV). Eligible participants were scheduled for one 2.5-hour session at our Laboratory.

Ethics approval was received from the Health Research Ethics Board of Newfoundland and Labrador (#2021.126) (Appendix B). All of the participants reviewed a study information letter and completed a consent form and a health screening form upon arrival prior to commencing the study to ensure their eligibility to participate.

3.4 Sample Size Calculation

The sample size calculation was performed based on the inflammatory marker dataset size, which is needed, this was described as the required number of participants to detect a clinically significant difference between the concentration of blood biomarkers of inflammation between two groups with an alpha level of 0.05 [114]. The calculation found a need for 27 participants. We recruited 50 participants to account for unbalanced PD and NPD groups. We recruited 50 participants to ensure we had at minimum 60% NPD and 40% PD but ideally we wanted to get 50% NPD and 50% PD. The increase in sample size increased our chances of matched groups.

3.5 Remuneration

Participants received $20 as compensation for volunteering for this study. The remuneration was provided to assist with travel and parking costs associated with participating in the study. Participants were not required to complete the entire protocol in order to receive the compensation.

3.6 Instrumentation

3.6.1 Setup

Prior to arrival at the laboratory, participants were emailed the health screening form to confirm their eligibility. Once their eligibility to participate was confirmed we booked them in for a one-time session at the laboratory. In preparation for the session participants were asked to refrain from smoking or consuming alcohol and/or caffeine for 8-hours prior to the session. They were also asked to avoid pain or anti-inflammatory medications such as ibuprofen or acetaminophen, and strenuous exercise for 24-hours prior to the session. We also asked that participants have clean, dry, and product free hair on the day of the session. Finally we asked that participants not chew gum throughout the session.

Upon arrival at the laboratory participants were asked to complete the consent form and details of the collection were provided. Participants were given the opportunity to ask any questions they may have had prior to starting the collection. We then asked the participants to provide their age, height, and mass and these were recorded by a member of the research team. We also measured the participants head upon arrival so that a member of the research team could place electrodes in the appropriate cap. Participants were then familiarized with the lab space, set up at an ergonomic

workstation (adjusting seat, desk, and monitor height to match ergonomic standards and individual preference) and introduced to the VAS and typing programs. After the introduction to the VAS we asked the participants to complete a baseline pain rating.

3.6.2 Ergonomic Workstation

Participants were set up with an ergonomic workstation to complete the 60-minute typing task upon arrival at the laboratory (Figure 1). The typing task (Figure 2) was presented on a monitor in front of the participants at the ergonomic workstation using a custom-written software (MATLAB 2017, The MathWorks Inc., Natick, MA, USA). Participants were given the choice between four stories. A portion of the story text was presented when pressing “start” and they would copy the text into the space below. Once they pressed “enter” on the keyboard the story would continue and the next paragraph would appear.

The workstation consisted of a height adjustable backless chair, office desk, and monitor. The desktop computer was set up with a wired mouse and keyboard for the participant to use. The height of all components were adjusted specifically to the participant following the Canadian Center for Occupational Health and Safety and the methods in Green et al. (2019) [13,115]. A member of the research team ensured the participant kept their feet on the floor with their knees at a 90° angle (did not stand, cross their legs, or sit on their legs) and had their elbows at a 90° angle. A backless adjustable office chair (Figure 3) was used to accommodate the accelerometer sensors which were affixed on the participants back.

Figure 1: A side view and frontal view of the ergonomic workstation with height adjustable chair (covered in plastic to meet biohazard regulations), monitor, and desk.

ID: [Figure on the left is a side view of the ergonomic workstation containing a seat pain covered in plastic, a desk, a monitor, keyboard, and mouse.]

Figure 2: Typing program used for the 60-minute sitting exposure. This typing task was intended to be similar to a typical typing task at an office.

[Typing program shown on a monitor screen, words with a start and stop button]

Figure 3: Backless office chair used for the 60-minute sitting exposure covered in plastic to conform to biohazard regulations in the laboratory.

ID: [Seat pan covered in plastic wrapped]

3.6.3 Visual Analog Scale

Participants rated their pain using the digital 100mm VAS (Figure 4). The VAS was presented on a monitor in front of the participants using a custom-written software (Matlab version 2017, The MathWorks Inc., Natick, MA, USA). When a member of the research team instructed the participant to rate their pain they would slide the bar along a 100mm scale. The location of the bar corresponded to their perceived level of pain at that time with anchors 0mm = no pain and 100mm = worst pain imaginable. Once they rated their pain and hit save the ratings disappeared so that when they rated their pain the next time they could not see their previous ratings. They rated their pain for 9 body locations: neck, left upper back (1), right upper back (2), left lower back (3), right lower back (4), left glute (5), right glute (6), left upper leg (7) and right upper leg (8). The body locations were presented as a number shown on a diagram on the presented VAS.

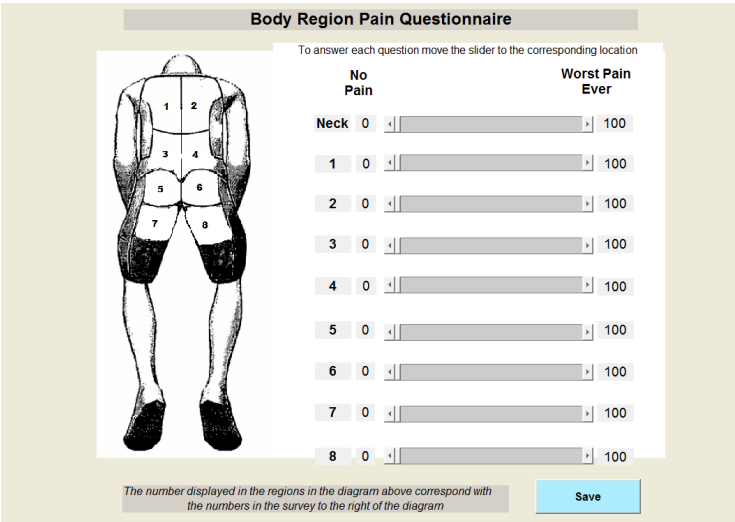

Figure 4: Digital VAS program presented in the monitor for the participant to rate their pain by sliding a bar across the line from 0mm to 100mm. Each number on the program corresponds to a location of the neck, back, buttock and thighs.

ID: [Pain rating visual analog scale presented on a monitor screen]

3.6.4 Blood Biomarkers of Inflammation

A research nurse collected blood from the participants to assess the changes in concentration of proinflammatory and anti-inflammatory cytokines from baseline to post-pain induction. The research nurse first assessed both cubital veins to determine the best side for the blood withdrawal. In the event the participant requested a specific arm the nurse assessed that one first. Once the arm was chosen, a tourniquet (Lat free 1x8 NLTB118BX, Eastern Health Stores) was placed to increase pressure, and the skin area of the cubital area was prepped with an alcohol pad (Loris antiseptic isopropyl alcohol pad, Eastern Health Stores). Blood samples (5ml each before and after the sitting trial) were extracted using a catheter (BX/50 Insyte Vialon Peripheral Venous IV Catheter, 24g X0.75" Yellow., Life Supply., Surrey, BC, Canada) placed in the cubital vein (Figure 5) by the research nurse and placed in vacutainers with EDTA anti-coagulant (Vacutainers 6ml Lavender, Life Supply, Surrey, BC, Canada). The catheter was left in for the sitting trial. Prior to collecting the second blood sample, the vein was flushed with saline (Syringe N/S 10ml Sterile Path, Eastern Health Stores, St. John's, NL, Canada) and the saline was extracted from the vein and discarded.

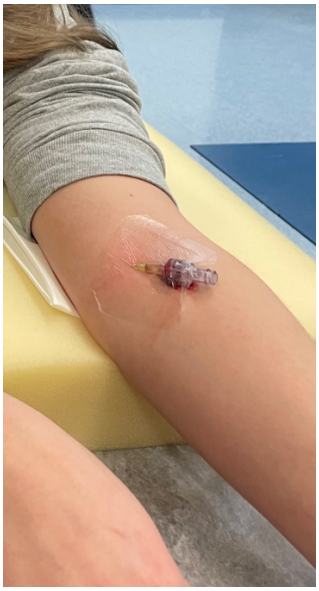

Figure 5: Catheter placed in the cubital vein of the participant.

ID: [Catheter inserted in the participants arm filled with blood and covered in clear tape]

3.6.5 Electroencephalography

EEG was used to measure brain activity in order to analyze peak alpha frequencies. Head circumference of the participant was measured upon arrival by placing a measuring tape around the forehead and to the back of the head crossing over the inion. Based on this measurement, we chose a cap (standard 64Ch actiCAP Electrode cap) from a selection of four (54cm, 56cm, 58cm and 60cm). When the participant's head was between cap sizes we choose to size down to optimize the fit and improve signal quality. The appropriate cap was instrumented with 32-electrodes (green bundle shown in Figure 6) and positioned according to the 10-20 system for electrode placement. We placed three dots on the forehead with a dry erase marker which marked where electrodes Fp1, Fp2 and the ground were placed. To place the ground in the correct location we measured the distance from the nasal bridge to the inion located on the back of the

head. We divided this value by 10 and used it as the distance from the nasal bridge to the center of the forehead and marked a dot with a dry erase marker. We then divided the head circumference by 20 and used it to mark a dot from the previously marked central dot to the left and right using this value. We placed the cap on the participant's head and ensured electrodes Fp1, Fp2 and the ground lined up with the dots marked on the forehead.

Once the cap was correctly placed on the participant's head and all the active electrodes (Brain Products GmbH, Gilching, Germany) were inserted into the cap, two members of the research team started to apply the electrolyte gel (SuperVisc 1000g High Viscosity Gel, 10% salt concentration, EasyCap, Wörthsee, GER) through the electrodes to the scalp using a blunt needle (1" Blunt needles – 16 gauge, Brain Vision Solutions INC, MTL, CAN) and syringe (syringe 10ml Luer-lock, Brain Vision INC Solutions, MTL, CAN). The gel provided a connection between the scalp and electrodes. We first inserted gel into the ground electrode and the reference electrode. Once both the reference and the ground electrode displayed an impedance less than 10kΩ in the Brain Vision Recorder software we continued to apply gel to the remaining electrodes. Lower impedance reflects a better connection between the electrode and the scalp, and will result in higher quality signal being recorded. We kept impedance below 10kΩ for each electrode before recording the 5-minute sessions. We continued to monitor the impedance for the entirety of the session, inserting more gel or adjusting the electrodes when necessary. The 5-minute sessions were recorded using the Brain Visions Recorder software and an ActiChamp EEG amplifier (ActiChamp, Brain Products GmbH, Gilching, Germany). EEG signals were recorded at a sampling rate of 500 Hz.

Figure 6: Electrode placement on the EEG cap (green bundle).

ID: [A map of the electrode placement for the electroencephalography cap with electrodes shown in numbers and bundles in yellow and green colour]

3.6.6 Accelerometers

Accelerometer signals were used to calculate time-varying measures of the relative lumbar spine angle, shifts and fidgets of the spine angle throughout the sitting exposure, and range of motion before and after the sitting exposure. Two tri-axal accelerometers (ADXL335, Analog Devices, Norwood, MA, USA) were positioned over the L1 and S2 spinous processes (Figure 7). The accelerometers were placed in the +y down, +z anterior orientation, fixed with double sided (Scotch, 3M, St. Paul, MN, USA.) and secured with medical fabric tape (Hypafix®, BSN Medical, Hamburg, Germany). Signals were sampled at 1024 Hz with a 16-bit A/D board

(Optotrak Data Acquisition System, 3D Investigator, Northern Digital Inc., Waterloo, ON, Canada).

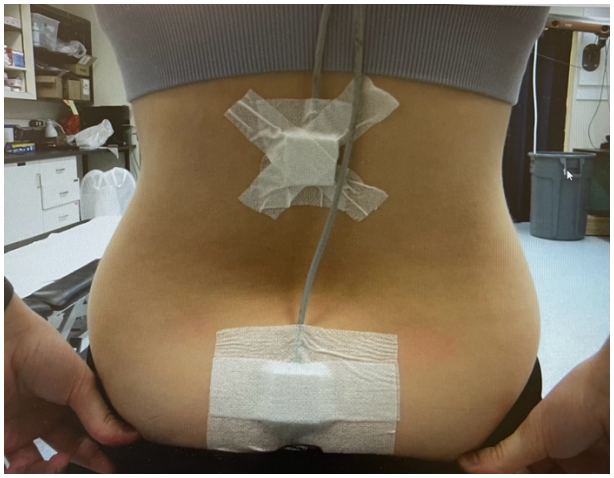

Figure 7: Accelerometer placement on L1 and S2 spinous processes.

ID: [Accelerometer sensors placed on the spinous processes directly on the skin with the cords taped to the shoulder of the participant and the sensors covered in medical tape]

3.7 Data Collection

Upon arrival to the laboratory a member of the research team explained to the participant the protocol and asked them to complete the consent form. The participant was then asked to sit at the ergonomic workstation and the height of the desk, chair and monitor was adjusted to the participant. We then introduced the VAS and allowed the participant to complete a baseline pain rating. Following the pain rating the participant laid prone on the therapy plinth where a member of the research team palpated their back to locate L1 and S2. Once the vertebrae were located a mark was placed to indicate where the accelerometer should be placed. The participant was then asked to stand up so that the accelerometer sensors could be placed on L1 and S2 and secured

with Hypafix® (BSN Medical, Hamburg, Germany) tape. Once the accelerometers were securely placed we commenced the calibration trials. Calibration trials were collected to normalize to the end range of flexion motion so that the data could be compared between participants. A total of four 5-second calibration trials were recorded: upright standing, maximum spine extension (Figure 8) where the participant was instructed to arch their back to their maximal limit without bending their legs or knees, maximum spine flexion in the standing position (Figure 9) where the participant was instructed to arch as far forward aiming to touch their toes without bending their hips or knees and maximum seated flexion (Figure 10) where the participant was instructed to arch as far forward while seated as they could. We collected each calibration trial 3 times and all of the movements were first demonstrated by a member of the research team.

Figure 8: Maximum spine extension positioning for calibration trial.

ID: [Participant extending their back to their maximum position]

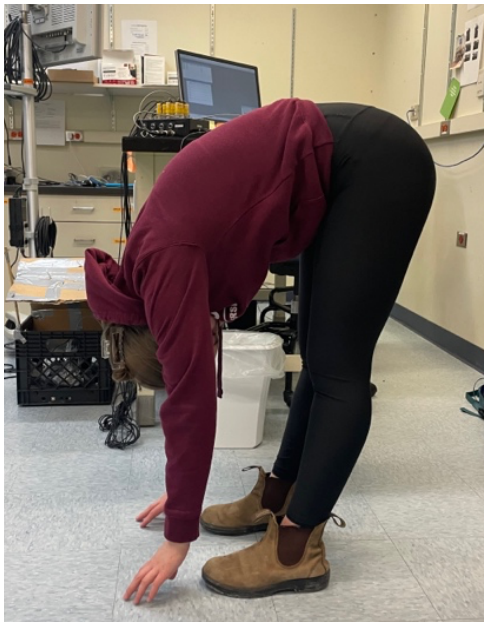

Figure 9: Maximum spine flexion in the standing position for calibration trial.

ID: [Participant bending forward to their maximum position with their neck tucked in and hands touching the floor]

Figure 10: Maximum spine flexion in the seated position for calibration trial.

ID: [Participant seated on a seat pan covered in plastic flexing forward with their hands touching the floor and extended back to their maximum position]

Once the flexion trials were completed, we asked the participant to sit down as we instrumented the EEG cap (Figure 11). We first marked out the placements for the electrodes on the forehead then placed the cap on the participant's head. We allowed the participant to secure the straps of the cap themselves and instructed them to secure it tightly but to ensure they were comfortable. We then measured the placement of the cap to ensure it was situated properly and before inserting the ground electrode we lined it up with the markings on the forehead. Once the cap was appropriately situated we started to insert the gel, we first inserted the gel into the ground, FP1 and FP2 electrodes. Once these electrodes were less than 10kΩ impedance we continued inserting gel into the remaining electrodes. Once all of the electrodes were less than 10kΩ impedance we stopped and recorded the brain waves. We recorded for 5 minutes while the lights

in the laboratory were off and the room was silent. We instructed the participant to stay seated with their eyes closed during this time.

Following the EEG recording the research nurse took 5ml of blood from the participant's cubital vein. If the blood withdrawal was not successful, the nurse would attempt multiple times until extracting blood or until the nurse deemed it unnecessary to continue or the participant opted out. We then directed the participant to the ergonomic workstation where they rated their pain on the VAS for a second time before starting the typing task. We asked the participant to choose between four standard report text options to transcribe. The participant was informed that during the sitting trial they would not be able to stand up or sit on their feet, otherwise they were free to sit and type as they normally would. The 60-minute sitting trial was then started. Throughout this trial we collected continuous data from the accelerometers. At 30 and 60 minutes we asked the participant to complete a third and fourth pain rating on the VAS and we recorded the brain waves for another 5 minutes under the same conditions and instructions as the first recording. At 60-minutes we took another 5ml of blood. Finally, we asked the participant to stay seated and completed the maximum spine flexion in the seated position trials 3 times and then the maximum spine flexion in the standing position trials 3 times.

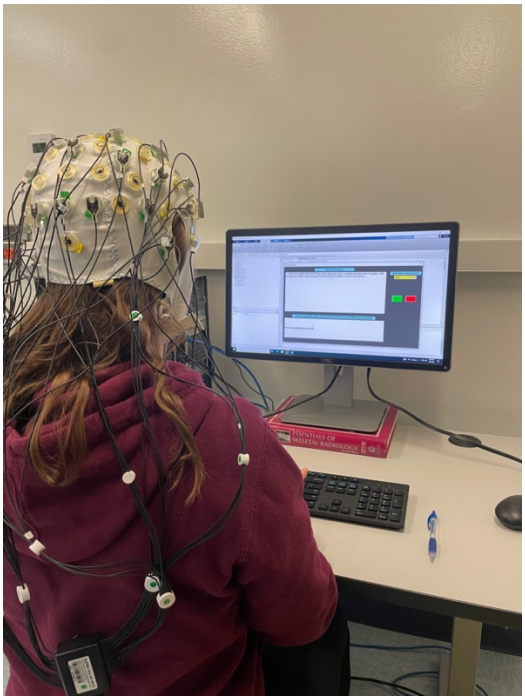

Figure 11: Participant seated at the ergonomic workstation instrumented with the EEG and accelerometers.

ID: [Participant seated at the ergonomic workstation facing the monitor which has the typing task on it and keyboard on the desk. The participant's head is instrumented with the EEG cap.]

3.8 Data Analysis

3.8.1 Self-reported Pain Ratings

Self-reported pain ratings were extracted from the digital VAS. The numbers were automatically extracted by the program and exported to a Microsoft Excel (Version 16.66) spreadsheet using MATLAB. The pain rating from each time point corresponded to the distance in millimetres (mm) between 0 mm and the point to which the participant had dragged the slider on the horizontal scale. Group classification of PD and NPD were based on these subjective pain

ratings. A change in pain from baseline greater than or equal to 10mm, from any body region and at any point during the trial was considered a clinically relevant increase in pain and established the individual as a PD. Pain rating that remained below 10 mm established the individual as a NPD. This is modified slightly from the literature [116] which uses the back region only. This decision was made because we assume that biomarkers of inflammation and peak alpha frequency would be less specific to body location versus pain intensity.

3.8.2 Concentration of Biomarkers of Inflammation

At the end of the data collection, once the participant left the laboratory, blood samples were centrifuged at 4 degrees Celsius at a speed of 3000 rpm for 15-minutes to separate plasma from cells. The EDTA plasma was then transferred into 0.6ml MCT tubes (Low-Retention 0.6ml MCT Snap Tops, Fisher Scientific Company., Ottawa, ON, Canada). We stored and froze one sample of 0.2ml and three samples of 0.6ml in a freezer safe MCT box (Argos Technologies PolarSafe, Fisher Scientific Company, Ottawa, ON, Canada). The samples were then stored in an onsite Thermo scientific freezer at -80 degrees Celsius until the end of the study.

At the end of the study, the 0.2ml pre and post samples were packaged in dry ice and shipped by courier to Eve Technologies Corporation in Calgary, Alberta. Human high sensitivity T-helper cells custom assay for interleukin 10 (IL-10), interleukin-1β (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α) was conducted. The results of the concentration of blood biomarkers of inflammation were determined in picograms per millilitre (pg/ml). The remaining blood samples were kept in the freezer at Memorial University for future analysis and then disposed of according to the Memorial University's Biosafety Standard Operating Procedures (BSOP-01).

3.8.3 Electroencephalography Data

The 5-minute EEG recordings were analyzed using the Letswave (version 7) toolbox in Matlab (Version 2017, The MathWorks, Nattick, MA, USA). EEG data files were read into the software and processed. A high pass filter with a 0.1Hz cut-off was applied and data were re-referenced by using the electrodes to the common average. Last, a Fast Fourier Transform was applied to the signal and the frequency at which peak alpha occurred within the alpha range (8-12 Hz) was extracted. The 9 electrodes (F3, Fz, F4, C3, Cz, C4, P3, Pz and P4) of interest in the somatosensory cortex were used for analysis.

3.8.4 Accelerometer

The accelerometer data were processed using a custom code (Matlab Version 2017, The MathWorks Inc., Natick, MA, USA). The custom code extracted spine angles, shifts and fidgets from the collected data according to methods previously outlined in Green et al. (2019) [13]. Accelerometers were calibrated with respect to gravity and the data were converted from voltages to accelerations\and the data were smoothed using a dual-pass 2^{nd} order Butterworth filter with a 1 Hz cut-off filter [117]. Spine angles were calculated from the absolute inclinations. Lumbar angles were then calculated as the relative angle between L1 and S2, and the pelvic angle was taken as the inclination of S2 with respect to vertical. Lumbar angle was normalized using the maximum low back range of motion which was obtained in the calibration trials. From the processed time-varying data, average low back flexion angle, and frequency (#/time block) of low back fidgets (movement where the spine posture changes and then goes back to its original posture) and shifts (movement where the spine posture changes and does not return to its original posture) were calculated for each 15-minute block of the 60-minute sitting. The threshold for a

fidget was set at 3 standard deviations, a window length of 60 seconds and a fidget length of 5 seconds. The threshold for a shift was 10 degrees and the width of the reference baseline was set to 60 seconds. The accelerometer data was analyzed using 15-minute time blocks where each 15-minutes was considered a trial to equal a total of 4 trials throughout the 60-minute sitting exposure.

3.9 Statistical Analysis

Descriptive statistics (mean and standard deviation) for age, height, and weight were computed for PD and NPD groups. A two-way univariate analysis of variance (ANOVA) was completed for peak pain rating in any body region with a between-factor of pain group (NPD, PD) and a within-factor of time (baseline, 30-minutes, and 60-minutes). A two-way multivariate ANOVA was completed for biomarker concentration (IL-1β, IL-6, IL-10, and TNF-α) with a between-factor of pain group (NPD, PD) and within-factor of time (baseline and post-exposure). An independent t-test was completed to compare the peak alpha frequency at each electrode of interest at baseline with pain group as a between factor (PD and NPD). Separate two-way univariate ANOVAs were completed for average normalized lumbar flexion angle, and the average fidget and shift frequency each with a between-factor of pain group (NPD, PD) and a within-factor of time (four 15-minute time blocks). Tukey post hoc tests was completed for significant main effects. A two-way univariate ANOVA were completed for maximum range of flexion with a between-factor of pain group (NPD, PD) and a within-factor of time (baseline and post-exposure). All statistical analysis were completed using SPSS (v#27, IBM SPSS Statistics), with significance accepted at $p<0.05$.

Chapter 4: Results

4.1 Participant Characteristics

Data from a total of 50 participants were collected for this study. 28 participants were females and 22 were males. The average age was 30.58 years (± 14.41), the average height was 171.52cm (± 10.20) and the average mass was 74.92 kg (± 17.30).

4.2 Missing data

Details concerning the missing data are displayed in Table 1. We were unable to retrieve a blood sample from one participant, therefore, the blood analysis is only including 49 participants. Technical issues resulted in some lost accelerometer data (one 15-minute block each for four participants). We did not exclude these participants from the analyses, instead, four participants only had three 15-minute accelerometer trials analyzed compared to the four that were recorded.

Table 1: Missing data for all outcome measures collected.

Blood Analysis		
Number of participants implicated	Number of participants included in the analysis	Group Classification of excluded participant
1	49	NPD
Prolonged Sitting Exposure Analysis		
Number of participants implicated	Number of participants included in the analysis	Group Classification of affected participant
4	50	PD: 2, NPD: 2

4.3 Pain Ratings

The average baseline pain rating upon entry into the lab before commencing the session was 0.1mm (± 0.6) left upper back, 0.3mm (± 1.2) right upper back, 0.35mm (± 1.3) left lower back, 0.6mm (± 1.7) right lower back, 0.4mm (± 1.8) left gluteal region, 0.4 (± 1.9) right gluteal region, 0mm (± 0.1) right upper leg, 0mm (± 0) right upper leg and 1.3mm (± 4.1) neck.

4.3.1 Pain Group Classification

Twenty-nine participants were classified as PD, and 21 were classified as NPD. The group characteristics for each are displayed in Table 2 and peak pain ratings for each group and their associated location are shown in Table 3.

Table 2: Group characteristics for PD and NPD.

	Pain Developers	**Non Pain Developers**
Sample size (% of total n)	29 (58%)	21(42%)
Sex	Females: 18 Males: 11	Females: 10 Males: 11
Age	31.28 years ± 15.60	29.62 years ± 12.92
Height (cm)	170.21 cm ± 8.05	173.33 cm ± 12.59
Mass (kg)	72.10 kg ± 15.39	78.81 kg ± 19.34

Table 3: Average peak pain rating for PD and NPD and their associated location.

Pain Developers					
Upper Back	**Lower Back**	**Buttock**	**Upper Legs**	**Neck**	**Total Peak Rating**
9.9mm ± 10.3	13.1mm ± 11.6	5.2mm ± 8.6	1.4mm ± 4.1	5.2mm ± 7.5	19.5mm ± 9.1
Non Pain Developers					
Upper Back	**Lower Back**	**Buttock**	**Upper Legs**	**Neck**	**Total Peak Rating**
3.1mm ± 3.7	2.7mm ± 3.2	1.0mm ± 2.0	0.3mm ± 0.7	1.1mm ± 2.3	5.1mm ± 3.3

There was a significant interaction between pain group and time for peak pain rating ($F(2,3) = 18.836$, $p < .001$, $\eta^2 = .207$). PDs continued to increase their pain between timepoints 2 and 3 whereas NPDs did not (Figure 12).

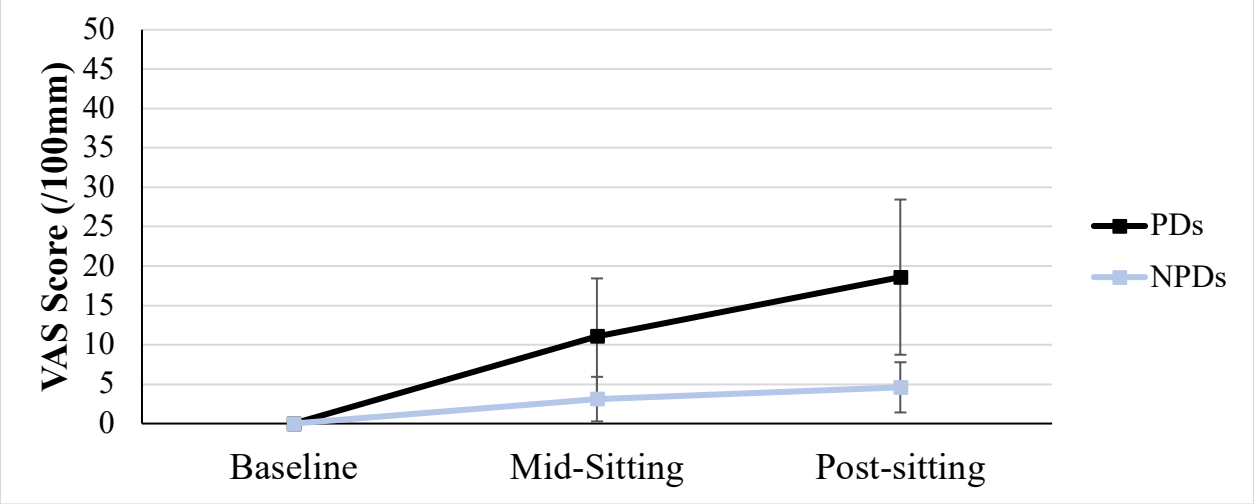

Figure 12: Baseline removed peak change in VAS scores between PD and NPD at three time points of the one-hour sitting exposure.

4.4 Biomarkers of Inflammation

No significant interactions between pain group and time, and no main effects for time were found for the biomarkers of inflammation (Table 4). Specifically, concentrations of biomarkers of inflammation did not significantly change throughout the sitting trial. However, there was a significant main effect for pain group for both IL-6 ($p = 0.004$) and IL-10 ($p = 0.001$). As displayed in Figure 13, NPDs had significantly higher levels of IL-6 (7.71 ±10.56) and IL-10 (24.41 ± 29.01) compared to PD levels of IL-6 (3.08 ± 4.26) and IL-10 (10.18 ± 10.65), which indicates group differences. There were no significant main effects for pain group for IL-1β and TNF-α (Figure 13).

Table 4: ANOVA test results for biomarkers.

	Biomarker	**df**	**F**	**p-value**	**Partial Eta Squared**
Time	**IL-1β**	1	.088	.767	.001
	IL-6	1	.185	.668	.002
	IL-10	1	.026	.871	.000
	TNF-α	1	.016	.900	.000
Pain Group	**IL-1β**	1	3.819	.054	.039
	IL-6	1	8.872	.004*	.086
	IL-10	1	11.478	.001*	.109
	TNF-α	1	.176	.676	.002
Pain Group * Time	**IL-1β**	1	.000	.984	.000
	IL-6	1	.050	.823	.001
	IL-10	1	.013	.909	.000
	TNF-α	1	.018	.893	.000

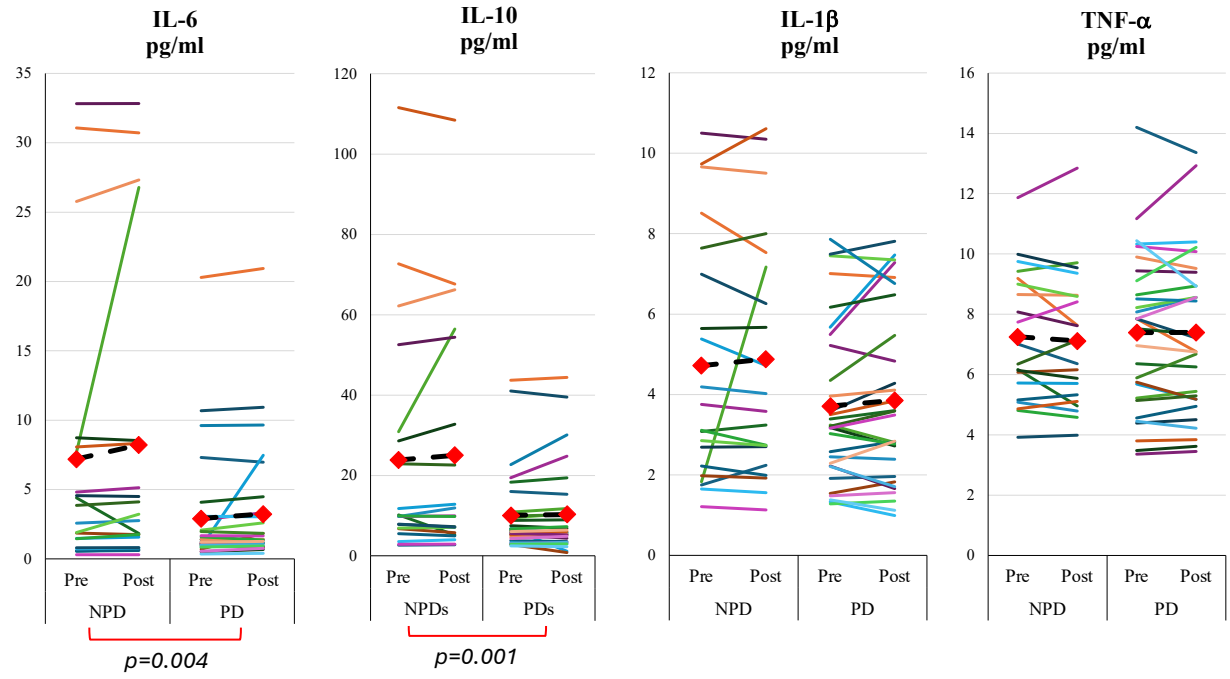

Figure 13: Individual data points for the concentration (pg/mL) of IL-6, IL-10, IL-1β and TNF-α, pre- and post-exposure for both NPD and PD. The black dotted line and diamond data points is a representation of the group average at each timepoint (pre and post sitting).

4.5 EEG Results

4.5.1 Peak Alpha Frequency at Baseline

A Q-Q plot was created for each electrode of interest to assess if the EEG data followed a normal distribution (Appendix C). The Q-Q plots suggest normality was met as only a small amount of data points in each Q-Q plot deviated substantially from the normality line. The independent sample t-test did not reveal any significant differences between peak alpha frequency for pain groups and the nine electrodes of interest in the sensorimotor cortex at baseline. The result of the independent t-test are displayed in Table 5 and graphed in Figure 14.

Table 5: The t-test results for peak alpha frequency at baseline for each sensorimotor cortex electrode.

Electrode	*t*	df	p-value	95% Confidence Interval
F3	.650	48	.519	-4.5533, .89059
Fz	-.075	48	.940	-.69842, .64793
F4	.327	48	.745	-.53390, .74127
C3	-.001	48	.999	-.69144, .69092
Cz	.103	48	.919	-.67006, .74218
C4	.461	48	.647	-.53326, .85052
P3	-.267	48	.791	-.79446, .60830
Pz	-.974	48	.335	-1.10131, .38229
P4	-.872	48	.388	-1.00304, .39627

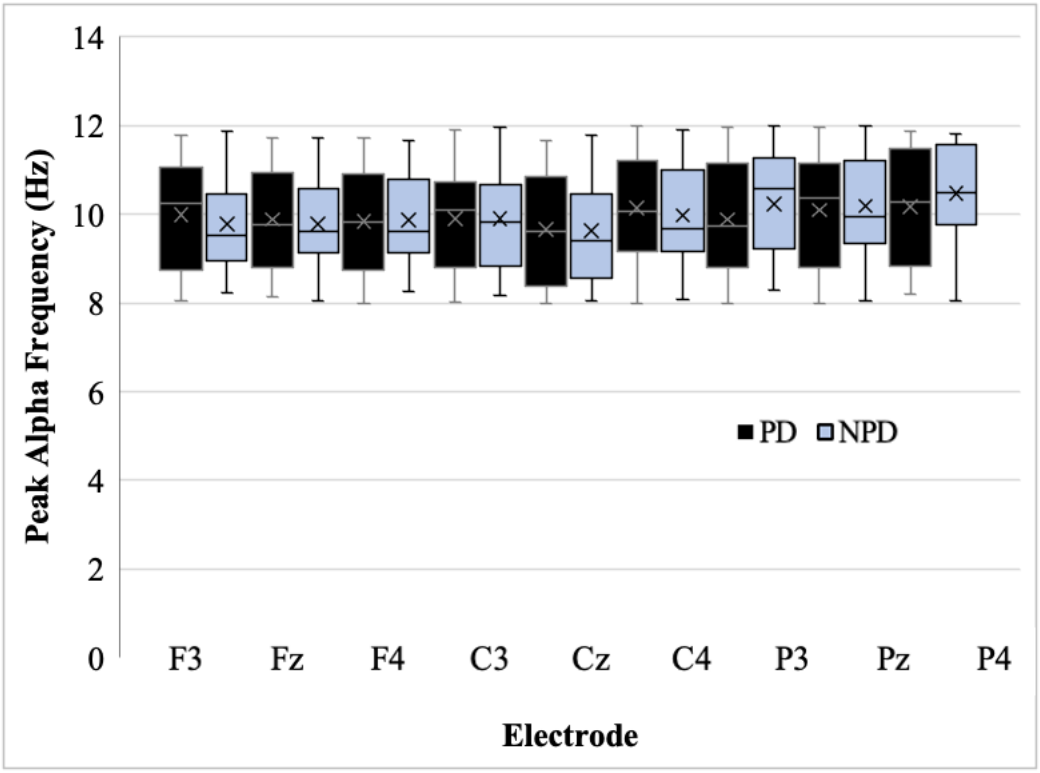

Figure 14: Peak alpha frequency at baseline for each electrode of the sensorimotor cortex between PD and NPD.

4.6 Accelerometers

4.6.1 Normalized Lumbar Flexion Angles

No significant interactions between time or group (p=0.994), or main effects of group (p=0.098), or time (p=0.960) (Figure 15) were found for the normalized lumbar flexion angle between PD and NPD (Table 6).

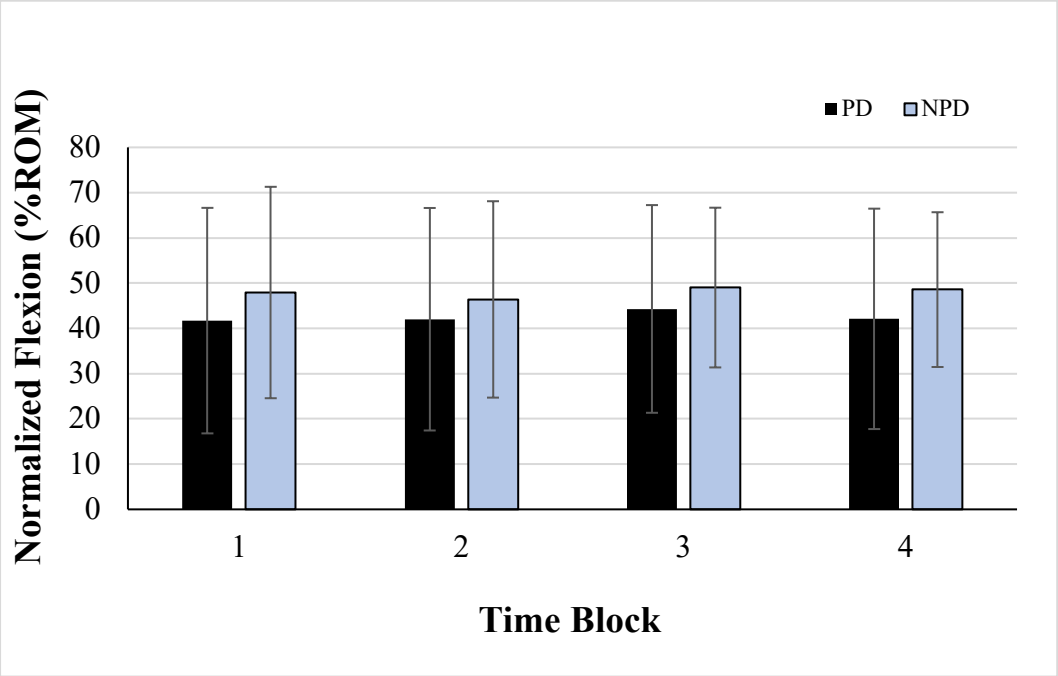

Figure 15: Normalized lumbar flexion angle for each time block throughout the 60-minute sitting trial between PD and NPD.

Table 6: ANOVA test result comparing the normalized lumber angle between PD and NPD and time.

	df	F	p-value	Partial Eta Squared
Pain Group	1	2.762	.098	.014
Trial	3	.100	.960	.002
Pain Group * Trial	3	.026	.994	.000

4.6.2 Range of Motion

There were no significant interactions between time and pain group, and no significant main effects of time or pain group for the change in maximum flexion angle pre and post sitting (Figure 16, Table 7). Specifically, maximum range of motion in flexion pre-sitting was 21.13 ± 7.71 for PDs and 20.49 ± 11.82 for NPDs and post-sitting was 21.29 ± 8.72 for PDs and 20.39 ± 11.19 for NPDs.

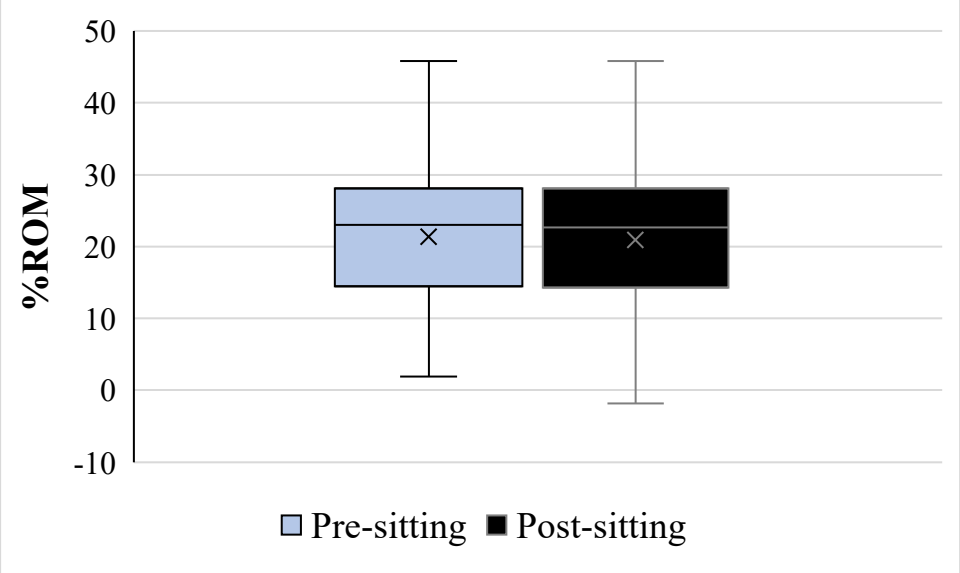

Figure 16: The average change in maximum range of motion in flexion pre and post sitting.

Table 7: ANOVA results comparing the flexion angle before and after the 60-minute sitting exposure between PD and NPD.

	df	F	p-value	Partial Eta Squared
Pain Group	1	.152	.697	.002
Time	1	.000	.991	.000
Pain Group * Time	1	.004	.948	.000

4.6.3 Fidget Frequency

The univariate ANOVA revealed no significant interaction between pain group and time ($F(3,196) = .453$, $p = .716$, $\eta^2 = .007$), and no significant main effects of pain groups for the frequency of fidgets throughout the 60-minute sitting exposure ($F(1,2) = 1.143$, $p = .286$, $\eta^2 = .006$). There was a significant main effect for time ($F(3,4) = 5.782$, $p = <.001$, $\eta^2 = .084$) (Figure 17). The post hoc test displaying the differences between the four trials is listed in Table 8. Time block 3 significantly differed from 1 and 2 indicating that in the first three time blocks of the sitting trial fidget frequency decreased.

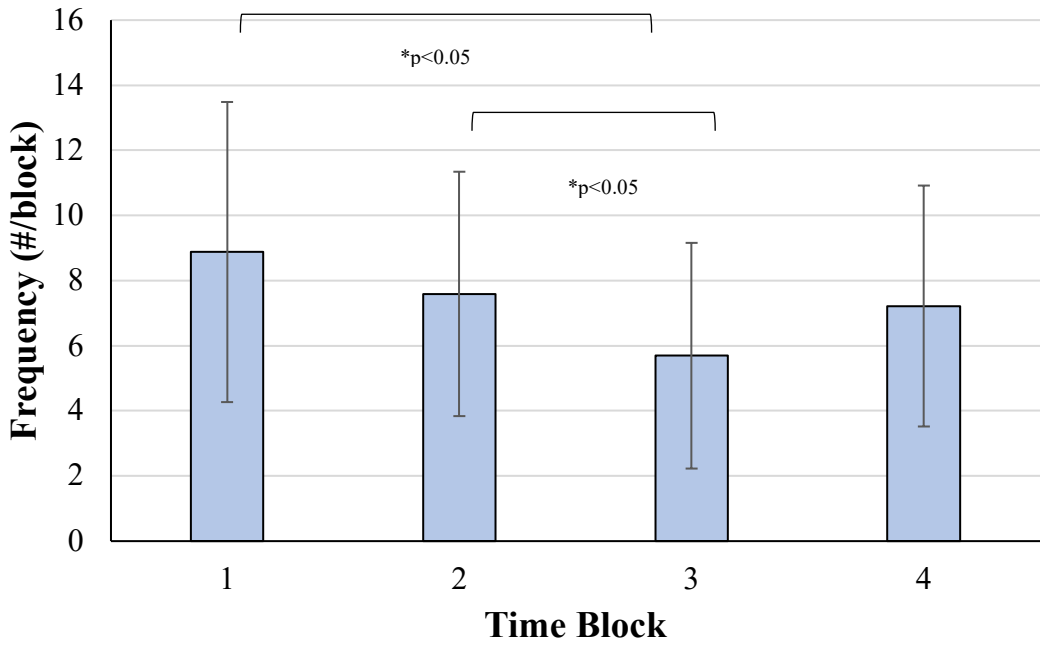

Figure 17: Average collapsed data frequency of fidgets (number per 15-minute time block).

Table 8: Post hoc test results for fidget frequency for each 15-minute time block throughout the sitting exposure.

Time Block	Trial	Mean Difference	Std. Error	p-value	95% Confidence Interval
1 (0-15 minutes)	2	1.1276	1	.484	-.9202, 3.1753
	3	3.1837*	1	<.001	1.1465, 5.2208
	4	1.6576	1	.151	-.3694, 3.6845
2 (15-30 minutes)	1	-1.1276	1	.484	-3.1753, .9202
	3	2.0561*	1	.049	.0084, 4.1039
	4	.5300	1	.907	-1.5076, 2.5676
3 (30-45 minutes)	1	-3.1837*	1	<.001	-5.2208, -1.1465
	2	-2.0561*	1	.049	-4.1039, -.0084
	4	-1.5261	1	.210	-3.5531, .5008
4 (45-60 minutes)	1	-1.6576	1	.151	-3.6845, .3694
	2	-.5300	1	.907	-2.5676, 1.5076
	3	1.5261	1	.210	-.5008, 3.5531

4.6.4 Shift Frequency

The univariate ANOVA revealed no significant interaction (F (3,195) = .542, p = .989, η^2 =.001) for the frequency of shifts throughout the 60-minute sitting exposure. There was a significant main effect of shift between pain groups (F (1,2) = 17.307, p = <.001, η^2 =.085). NPDs had significantly more shifts throughout the 60-minute sitting exposure when compared to the PD. There was also a significant main effect for time (F (3,4) = 3.978, p = .009, η^2 =.060) (Figure 18). The post hoc test displaying the differences between the four trials is listed in Table 9. Time block 3 significantly differed from blocks 1 and 2 indicating that in the first three time blocks shift frequency increased.

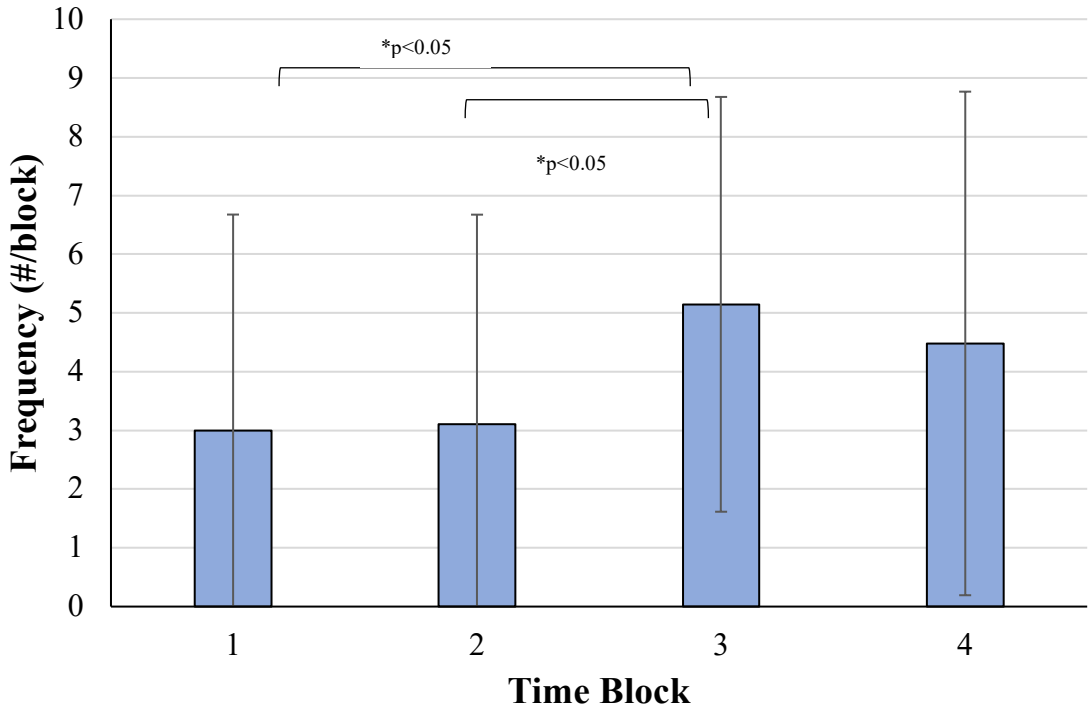

Figure 18: Average collapsed data frequency of shifts (number per 15-minute time block) for PD and NPD presented by time block.

Table 9: Post hoc test results for fidget frequency for each 15-minute time block throughout the sitting exposure.

Trial	Trial	Mean Difference	Std. Error	p-value	95% Confidence Interval
1 (0-15 minutes)	2	-.1042	1	.999	-2.0289, 1.8205
	3	-2.1458*	1	.022	-4.0705, -.2211
	4	-1.4800	1	.187	-3.3852, .4252
2 (15-30 minutes)	1	.1042	1	.999	-1.8205, 2.0289
	3	-2.0417*	1	.034	-3.9763, -.1071
	4	-1.3758	1	.248	-3.2910, .5393
3 (30-45 minutes)	1	2.1458*	1	.022	.2211, 4.0705
	2	2.0417*	1	.034	.1071, 3.9763
	4	.6658	1	.804	-1.2493, 2.5810
4 (45-60 minutes)	1	1.4800	1	.187	-.4252, 3.3852
	2	1.3758	1	.248	-.5393, 3.2910
	3	-.6658	1	.804	-2.5810, 1.2493

Chapter 5: Discussion

This study was the first in the literature to use blood marker and EEG outcomes to explore mechanisms that might explain the transient pain response that occurs in some people during sitting. Specifically, we aimed to explore the potential hypotheses that the transient pain response in sitting is related to inflammation secondary to viscoelastic creep and that those who develop this response may have an increased sensitivity to pain as indicated by a lower peak alpha frequency recorded using EEG. We investigated the potential avenue that inflammation was present as a result of the transient LBP which is developed throughout 60-minutes of sitting. In addition to the main research question we investigated if a sub-group of individuals classified as PD based on their subjective ratings of LBP throughout the 60-minute sitting trial are more susceptible to experiencing pain. Based on what is currently known in the literature we analyzed the peak alpha frequency at rest between PD and NPD to test this question. Finally, we investigated the development of viscoelastic creep and spinal micromovements in participants throughout the 60-minutes of sitting. We measured the change in range of motion before and after the exposure and also the frequency of micromovements throughout the exposure. We found that the pain groups did not significantly differ in peak alpha frequency, lumbar sitting angles, or spine flexion range of motion (an indirect indicator of passive tissue viscoelastic creep); however, the NPD displayed significantly more postural shifts throughout the 60-minute sitting exposure when compared to PD. Based on the inflammatory markers present between the groups, NPD had significantly higher levels of IL-6 and IL-10.

5.1 Pain Group Membership

The proportion of PD and NPD in this study slightly differed from that in the literature. Previous studies have found that 40% of their sample have been classified as PD and 60% as NPD when exposed to a prolonged static posture [11,74,80]. In our sample of 50 healthy individuals we classified 58% as PD and 42% as NPD. We did include all body regions of pain to be classified as PD whereas other studies in the literature only classified those who developed back pain to be considered PD. There were 4 (13.8%) individuals classified as PD who did not experience back pain and rated pain for other (neck and buttocks) body regions. This may explain the increased number of PD in this study compared to others in the literature. Furthermore, although part of the exclusion criteria was back pain, because this study was advertised as a study exploring sitting related back pain, it is possible we inadvertently attracted individuals who do not have clinical LBP, but might self-identify that they experience sitting-related pain and this might have increased their interest for participating in the study.

5.2 Biomarkers of Inflammation

The primary research question was concerned about the presence of inflammatory markers in the PD compared to the NPD which would help to inform on the presence of experienced pain as a result of the prolonged sitting exposure. The indication of an objective measure of pain shows a reflection on the presence of pain and allows us to better understand the origin of the pain and its relation to inflammation. We did not observe any significant changes in proinflammatory cytokines IL-6, TNF-α and IL-1β or anti-inflammatory cytokine IL-10 during the sitting exposure. This finding does not support our hypothesis that the PD group would display greater inflammation in response to sitting. This is also not consistent with the literature surrounding

clinical LBP. Studies looking at inflammatory markers in clinical LBP have found elevated levels of proinflammatory markers in individuals suffering with LBP [87,97–99]. Additionally, individuals who suffer from clinical LBP have been found to have lower levels of IL-10 an anti-inflammatory cytokine which works to control the concentration of proinflammatory cytokines [95,98]. It is possible that the experimental pain exposure was not long enough or not painful enough to induce a detectable inflammatory response which is similar to that found in clinical LBP or that inflammation may not be the source of experienced transient LBP in this sitting trial. In addition to this is possible that the inflammatory response is local which would not have been detectable due to the fact we used a systemic measure. Despite some methodological differences (laboratory setting versus field, university population versus office workers), our findings are consistent with the study by Carter et al., (2021) where they tested IL-6 and TNF-α in healthy office workers after sitting for 6-hours and found no elevation in the concentration of either marker [86]. Taken together it appears that sitting exposures on their own do not directly impact inflammatory markers, regardless of pain group class.

We did not see changes in concentrations in response to the sitting exposure itself, however, we did identify a significant difference in the concentration of IL-6 and IL-10 between pain groups. NPDs had significantly higher concentrations of both biomarkers. This is interesting, as it suggests that there may be a fundamental physiological difference between individuals classified as NPD and PD. Both cytokines (IL-6 and IL-10) can be classified as anti-inflammatory although IL-6 can also be pro-inflammatory in certain conditions. The difference between groups may be due to a variety of factors. While we controlled for alcohol, pain medication and physical activity 48 hours prior to the data collection, we did not control for all potential factors that can impact inflammatory markers. Thus, future directions for this work may consider exploring factors that

affect these inflammatory markers with the goal of understanding what is different between individuals that have a differential pain response to sitting.

5.3 Peak Alpha Frequency

We investigated three additional research questions in the study. First we investigated if sitting-induced PD are more sensitive to pain than NPD by comparing their peak alpha frequency at rest. We hypothesized that PD would have a slower peak alpha frequency at baseline compared to NPD, indicating a marker for pain sensitivity like that found in the literature [14–16]. We found no significant differences between the peak alpha frequency in the sensorimotor cortex between PD and NPD at baseline which was inconsistent with our hypothesis. In addition, this is not consistent with the literature in the area which has found the peak alpha frequency to be slower at rest in individuals who are more sensitive to pain or predisposed to experiencing pain [14–16]. We investigated peak alpha frequency in the sensorimotor cortex at resting state similar to methods used in the literature. However, Furman and Nir induced pain with the heat-capsaicin model whereas our "pain induction" was exposure to sitting [14,15]. It is possible that pain sensitivity does not explain the group differences or the threshold used in this study was too low and that experiencing pain may only be determined with higher levels of pain ratings and a higher pain threshold. Both our study and the study by Furman et al., (2018) used a subjective pain rating of 10 as the threshold for PD and NPD group establishment, however the average pain rating for PD in the study by Furman et al., (2018) was 56.01 (±16.96) and the average pain rating for PD in this study was 19.5 (± 9.1) [14]. In addition to this the study by Nir et al., (2010) reported average pain ratings between 54.27 and 60.91 throughout their experimental pain induction using tonic pain which is similar to the ratings found by Furman. Both studies by

Furman and Nir used relatively small samples which were smaller than the samples used in this study which should also be taken in account when interpreting these results. Therefore, in the context of this literature, we can make three potential conclusions for our findings. First, that sensitivity to pain, as defined by alpha wave frequency, does not explain the pain group differences in response to sitting. Secondly, the threshold used to classify pain development might be too low. Since pain ratings in the Furman group were substantially higher than our study, it is possible that the "true" threshold defining pain may be higher and that was captured in their study but not ours. Finally, peak alpha frequency may not be a reliable marker of pain sensitivity and the results observed by the Furman and Nir studies may be attributable to the small sample sizes used.

5.4 Viscoelastic Creep

There was no evidence of passive tissue creep induced by the sitting exposure in this study. Both the PD and NPD did not demonstrate an increase in spine flexion angles after the prolonged sitting exposure compared to before. Therefore, we reject our hypothesis that office chair sitting (even without a backrest) can induce passive tissue stiffness changes in a 60-minute exposure. Further, it may explain why we did not see changes in the inflammatory markers as we hypothesized that it may be inflammation secondary to tissue loading that is responsible for the experience of pain during sitting. The normalized lumbar flexion angles for both PD and NPD indicated that the participants were sitting at 40-50% of their maximum range of motion so we can assume that the magnitude of flexion and duration the posture was held is not high enough to induce passive tissue creep. This finding is consistent with the study by Green et al., (2019) which found no differences in the range of motion between PD and NPD after 60-minutes of sitting [13] where participants (both PD and NPD) adopted seated postures between 48-51%

maximum range of motion. If the participants had been in a more flexed position throughout the sitting trial it is possible there would have been evidence of viscoelastic creep occurring. For instance, there is evidence that increase in range of motion after a 60-minutes flexed sitting exposure where 15 healthy participants sat in a low easy chair at 70% of their range of motion to induce viscoelastic creep [70]. Therefore, it may be worth exploring the inflammatory marker hypothesis in contexts of greater magnitudes and/or durations of spine flexion. However, we can conclude that 60-minutes of office chair sitting does not appreciably change back stiffness, which is an important finding, in the context of worker safety and injury prevention.

5.6 Spine Micromovement

While analyzing the test results for spine micromovements it was revealed that over time participants went from making smaller micromovements (fidgets) to larger movements (shifts). This finding has not been observed before in previous studies and warrants further research. We also found a significant difference between PD and NPD groups for shift frequency (NPD having significantly higher shift frequency compared to PD) and no differences between groups for fidget frequency. This finding contradicts our hypothesis where we expected to find PD to display higher micromovements compared to NPD based on the literature where we expect that movement is reactive to pain. However, this is contradictory in the literature where some studies have found increased micromovements in individuals who experience LBP [73–75]. Whereas other studies have found an increase in micromovements in NPD [13]. The differences between studies are displayed in Table 10. The study by Nairn et al., (2013) and De Carvalho and Callaghan (2021) had participants seated for 2-hours and Dunk & Callaghan had participants seated for 90-minutes which is longer than the current study and the study by Green et al., (2019) which only had participants seated for 60-minutes [13,74,75,118]. Participants in the current

study, the study by Nairn et al., (2013) and by De Carvalho and Callaghan (2021) were healthy asymptomatic individuals, the study by Greene et al., (2019) included both individuals with a health history and without and the study by Dunk & Callaghan (2008) included individuals with sitting-aggravated LBP and without [13,74,118]. Both studies which found contradictory evidence to this study and the one by Greene et al., (2019) had a longer sitting exposure so it is possible time is an impacting factor when concerning spinal micromovements. However, although the study by Gallagher & Callaghan (2015) is not directly comparable to the current study as it investigated micromovements of PD and NPD throughout 2-hours of standing, they also found increased fidgets and shift in NPD which is consistent with what was found in the current study and also had an increased time frame [77]. The results supporting both angles make sense, regardless we still do not know what causes the movements and if it is protective or reactive. It is possible that individuals who experience LBP move more as a reactive mechanism to their experience of pain. It is also possible that movement is a protective mechanism to developing pain which is observed more in NPD. Given that both directions are supported in the literature this area still warrants further study, specifically, larger studies or a meta-analysis of similar studies could be conducted in the future to better estimate this effect.

5.7 Limitations

To reduce the number of limitations and biases present in the current study, we thought through and implemented strategies to minimize the risk. Not unexpected, based on the evidence in the literature, one limitation is the unequal number of participants classified as PD and NPD. As mentioned in the sample size calculation presented in section 3.4, we aimed to have equal groups or, at minimum, 60% NPD and 40% PD. To mitigate this limitation, we increased the sample from the calculated size to account for this. We had expected more individuals would be

classified as NPD compared to PD, however, that was not the case. We ended up having more PD than NPD which is not consistent with the literature, and might have been an artefact of our recruitment strategy or the use of pain in all body regions compared to just the back. However, all studies in this area have unbalanced group sizes (albeit with higher NPD than PD) and the statistical analysis used (ANOVA) is robust in terms of unequal groups which does account for this limitation.

Secondly, the sample size may be a potential limitation. We based our estimated sample size on the inflammatory marker dataset and this might not have been high enough for the rest of the outcome variables. A small sample is a limitation because it decreases the power of the study and increases the margin of error. However, it was not feasible to increase the sample of this study for several logistical reasons. First, because the study design included blood samples, it was resource intensive, requiring a research nurse, freezer space, and an expensive analysis. Therefore, increasing the sample would have exponentially increased the costs for the study. Secondly, time becomes a factor when samples are only able to be frozen for so long before analysis. Therefore, a higher sample would have lengthened the time to completion (considering that it does take longer to recruit participants for a study with blood draws).

To minimize scheduling issues, we did not control for time of day of the experiment, rather scheduled participants when they were able to come in. This could have impacted our outcome measures, particularly the inflammatory markers (which are known to change throughout the day) and spine range of motion (which also can change throughout the day). Thus, this may have limited the responsiveness of these outcome variables making them less likely to change during our experiment. Future studies should take this into consideration in their design.

The study population itself is also a limitation, as it was comprised mainly university students and staff. We expected this because the study was conducted on campus at Memorial University, and our recruitment documents were mainly available to students and faculty on campus. This population minimizes the generalizability of our results, however, it is a limitation shared with most of the studies conducted in this area to date. Future work should endeavour to broaden samples by re-evaluating recruitment strategies and reducing barriers to participation for off-campus individuals.

To minimize the risk of not being able to get a second sample at the end of the collection, our team decided to use an IV which remained in the cubital vein of the participant throughout the typing task. It was noted that some of the participants felt uncomfortable with it in their arm which may have impacted their normal typing posture. They still remained in a comfortable seated posture but chose to not type with the arm where the IV was inserted may have altered their posture, how they moved, and/or altered their experience of pain. However, considering our outcomes for posture (spine angles), movement (fidgets/shifts) and pain levels are comparable to past literature, it does not appear that this was the case.

Repeatedly asking the participants their experienced pain level may have resulted in them altering their response. To mitigate this, we limited the number of times we asked the participant for their pain rating from the frequency typically used in the literature (7.5 minutes). It does not look like this is the case as we had some individuals who did not rate any pain.

5.8 Future Research

One of the noted limitations listed above is the limited knowledge of the participants (i.e., factors that may impact inflammatory markers like stress, habits etc.) and their daily lives and activities

(age, fitness level, disease presence) prior to the experiment. A future study similar to this one with an additional survey asking the participant about these factors would be advantageous. As previously mentioned our population consisted largely of individuals who were familiar with prolonged sitting in their day to day lives. It is probable that a participant who was seated for the majority of the day prior to attending the study session may have had a different experience compared to an individual who was not seated for the majority of the day prior to the session.

It would be useful to consider exploring the most appropriate pain threshold to classify PD and NPD groups in a future study. It is possible that differences between the groups in terms of inflammation and peak alpha frequency if the experienced pain was more severe than what was found in this study. In this study the average pain rating for the PD group was below 20mm on the VAS, it would be useful to repeat this study for a longer duration and higher pain threshold with the expectation of have the PD group develop higher levels of pain.

We did not find differences between the PD and NPD that we expected to find. It is possible that the induction of experimental pain through the prolonged sitting was not long enough or painful enough to induce changes in blood biomarkers of inflammation, or it could mean that these factors are not important for these groups (or they were not the best outcomes to look at). Future research in the area should focus on what makes PD and NPD different so that we can use the groups to identify how people will experience pain and target them for interventions.

5.9 Conclusion and Impact

In conclusion, this study was the first in the literature to explore potential mechanistic hypotheses explaining the transient pain response in sitting. We aimed to explore whether sitting-induced pain was related to inflammation secondary to viscoelastic creep and/or an inherent increased

sensitivity to pain as indicated by a lower peak alpha frequency. We did not detect any systemic changes in inflammatory markers in PD compared to NPD therefore, while we have not captured what may be occurring locally at the tissue level, it does not appear that inflammation plays a large role in the development of pain during short sitting exposures. There were significant differences in IL-6 and IL-10 concentrations in PD and NPD, indicating some underlying differences between groups which should be explored further. Additionally, we aimed to investigate if some individuals are simply more susceptible to experiencing pain or developing chronic pain by comparing peak alpha frequency at rest on the basis of novel research in this area. We did not find any differences which support this hypothesis. Finally, we investigated if there was indirect evidence of viscoelastic creep and/or any evidence of differences in spinal movement between groups. Our indirect measure of creep, change in spine flexion range of motion, did not support the presence of viscoelastic changes in the passive tissues throughout the 60-minute sitting exposure. Participants sat with similar magnitudes of spine flexion and the only difference between groups was that NPD displayed greater postural shifts compared to PD. This is the first step and first study in the literature to better understand objective measures of pain throughout a sitting exposure which studies the development of a form of transient low back pain and whether some individuals might simply be more susceptible to experiencing pain. This study began the process of exploring potential hypothesis for the transient pain response sitting. Future research should focus on exploring other potential avenues for the transient pain response in sitting. In combination with additional research, this study will contribute to the ongoing body of research which aims to further investigate the phenomenon of transient development of sitting-induced pain: what causes it, what it means, and what it may be used for.

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Appendices

Appendix A: Health screening form

Health Screening Form

Subject Code: _____ Date: _____

This questionnaire asks some questions about your health status. This Information is used to guide us with your entry into the study.

Relevant Clinical History:

Please check all that apply to you (diagnosed or suspected).

☐ **Inflammatory Disorder**
- Spondyloarthropathy
- Rheumatoid Arthritis
- Other: _____

☐ **Chronic Pain Disorders**
- Fibromyalgia
- Complex Regional Pain Syndrome
- Chronic Low Back Pain
- Chronic Pain (>4 pain days/ month for at least 3 month)
- Other: _____

☐ **Neurological Disease, disorder or injury**
- Epilepsy and/or Seizures
- Alzheimer's Disease
- Stroke
- Multiple Sclerosis
- Other: _____

☐ **Psychiatric Disorder or Cognitive Impairment**
- Autism
- Bipolar Disorder
- Major Depressive Disorder
- Schizophrenia
- Attention Deficit-Hyperactivty Disorder (ADHD)
- Memory Loss
- Other: _____

☐ **Pregnancy**

☐ **Communicable Disease**
- Hepatitis C
- HIV
- Other: _____

At this moment, rate the level of pain you feel in your low back. Mark a vertical dash along the line.

no pain	worst pain imaginable
0	100

_____

Appendix B: Ethics approval

NEWFOUNDLAND AND LABRADOR
HREB
HEALTH RESEARCH ETHICS BOARD

Research Ethics Office
Suite 200, Eastern Trust Building
95 Bonaventure Avenue
St. John's, NL
A1B 2X5

August 31, 2021

230 Elizabeth Ave
Health Sciences Centre, Room 5315
St. John's, NL
A1B 3X9

DearDr De Carvalho:

Researcher Portal File # 20220467
Reference # 2021.126

RE: Validation of Induced Pain Models: Heat Capsaicin and Sitting

Your application was reviewed by the Health Research Ethics Board (HREB) at the meeting held on July 15, 2021 and your response was reviewed by the Co-Chair under the direction of the HREB and the following decision was rendered:

X	Approval
	Approval subject to changes
	Rejection

Ethics approval is granted for one year effective August 25, 2021. This ethics approval will be reported to the board at the next scheduled HREB meeting.

This is to confirm that the HREB reviewed and approved or acknowledged the following documents (as indicated):

- Application, approved
- Proposal (Part A & B), approved
- Recruitment email Part A, approved
- Recruitment email Part B, approved
- Informed Consent Form Part A, approved
- Informed Consent Form Part B, approved
- Social media communication Part A & B, approved

- Budget Part B, approved
- Budget Part A (HC), approved
- Recruitment Poster Part A (HC), approved
- Verbal Recruitment Script Part B, approved
- Verbal Recruitment Script Part A, approved
- Health Screening Form (Part A & B), approved
- Take Home Sheet (Part A), approved
- Recruitment poster for part B, approved

Please note the following:

- This ethics approval will lapse on August 25, 2022. It is your responsibility to ensure that the Ethics Renewal form is submitted prior to the renewal date.
- This is your ethics approval only. Organizational approval may also be required. It is your responsibility to seek the necessary organizational approvals.
- Modifications of the study are not permitted without prior approval from the HREB. Request for modification to the study must be outlined on the relevant Event Form available on the Researcher Portal website.
- Though this research has received HREB approval, you are responsible for the ethical conduct of this research.
- If you have any questions please contact info@hrea.ca or 709 777 6974.

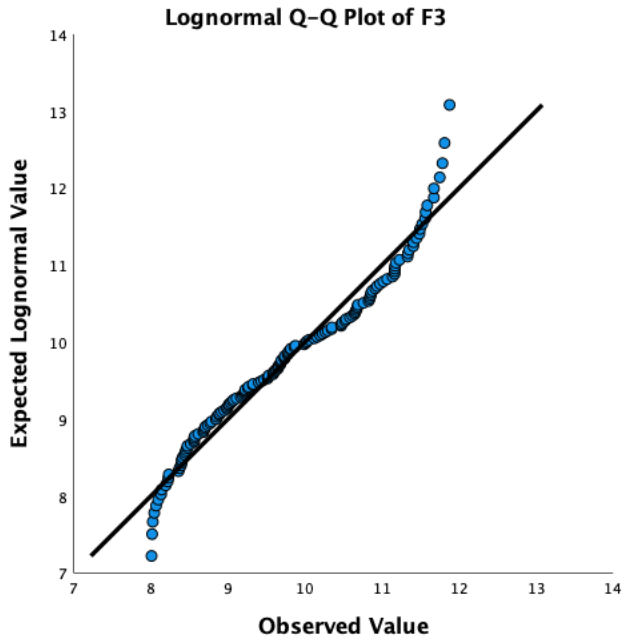
The HREB operates according to the Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans (TCPS2), ICH Guidance E6: Good Clinical Practice Guidelines (GCP), the Health Research Ethics Authority Act (HREA Act) and applicable laws and regulations.

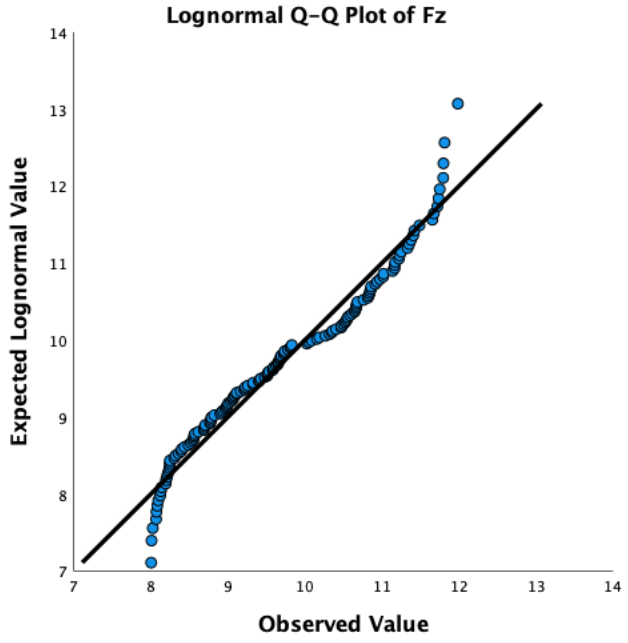
We wish you every success with your study.

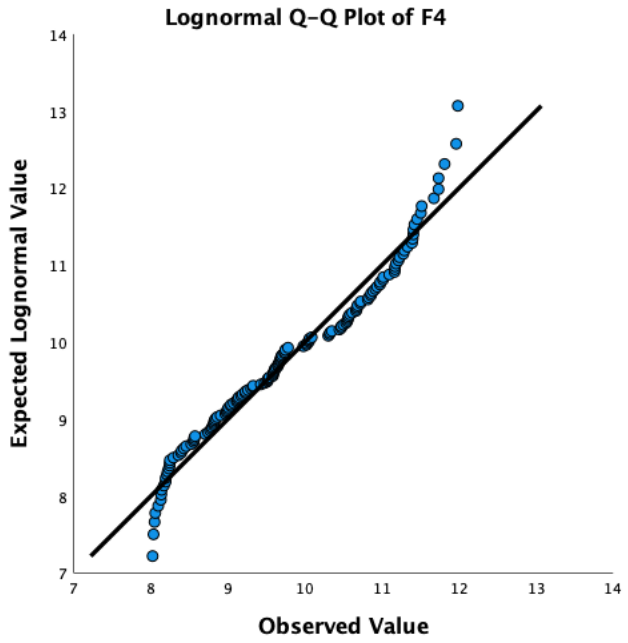
Sincerely,

Dr Robert Mercer, Acting Chair Non-Clinical Trials Committee
Health Research Ethics Board

Appendix C: Q-Q plot for electrodes of interest to check for assumption of normality of the alpha frequency data






Lognormal Q-Q Plot of F4
Expected Lognormal Value
Observed Value
14
13
12
11
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7
7
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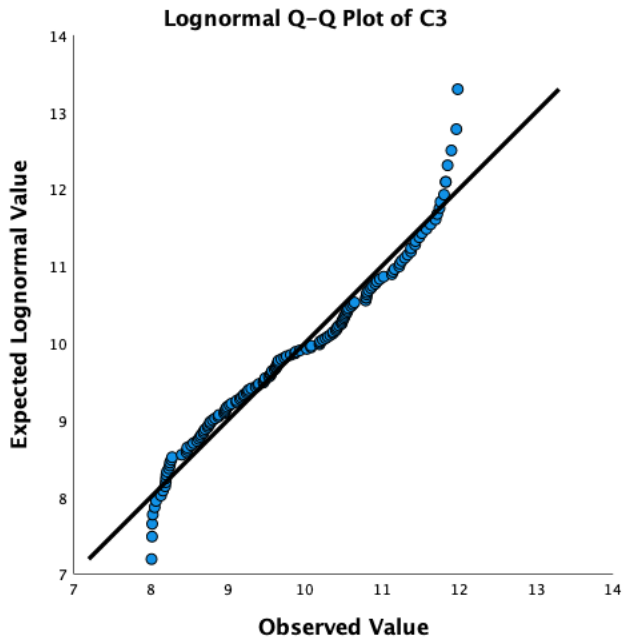
10
11
12
13
14


Lognormal Q-Q Plot of C3
Expected Lognormal Value
Observed Value
14
13
12
11
10
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7
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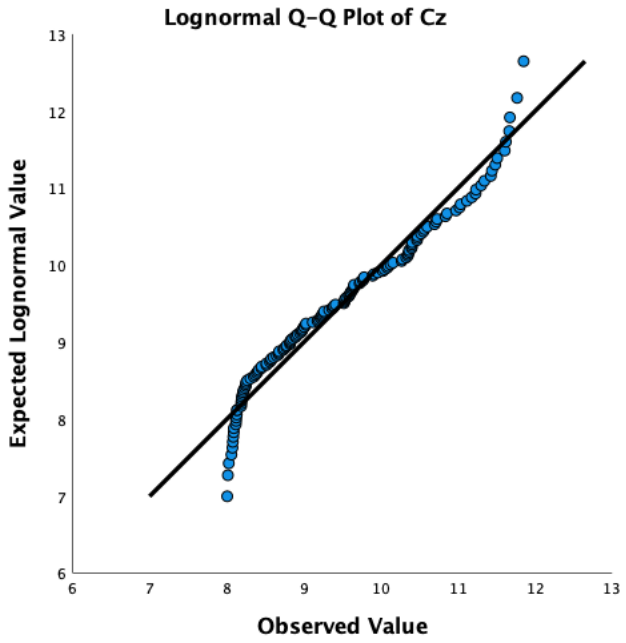
12
13
14


Lognormal Q-Q Plot of Cz
Expected Lognormal Value
Observed Value
13
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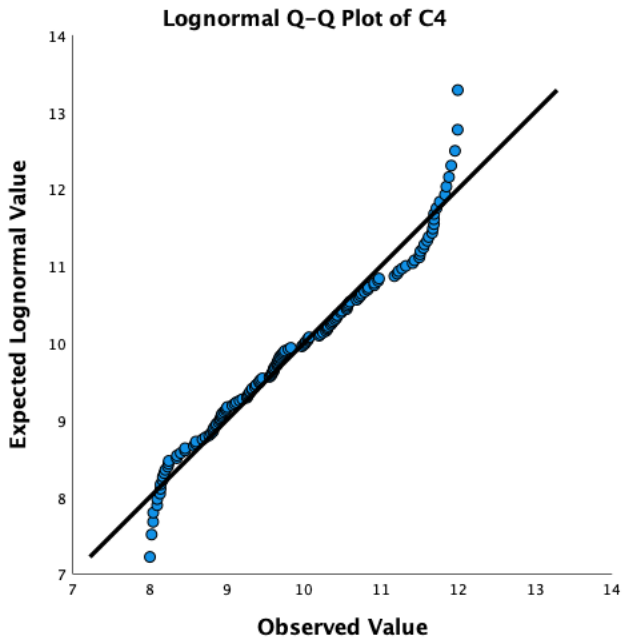
11
12
13


Lognormal Q-Q Plot of C4
Expected Lognormal Value
Observed Value
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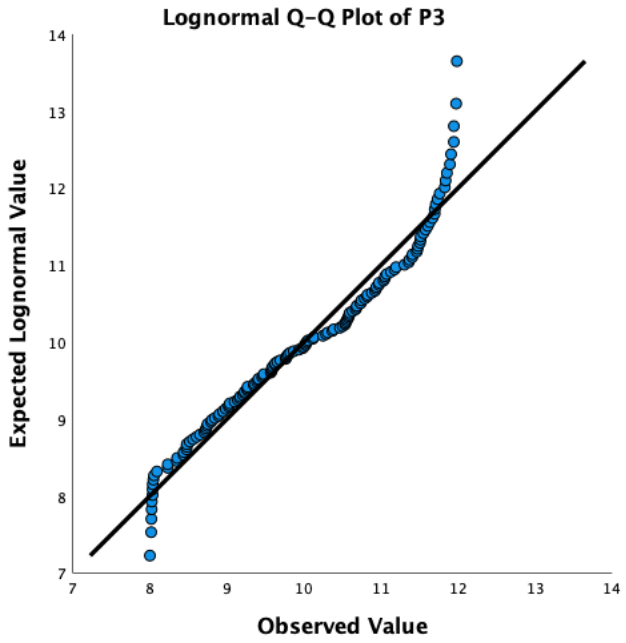
Lognormal Q-Q Plot of P3
Expected Lognormal Value
Observed Value
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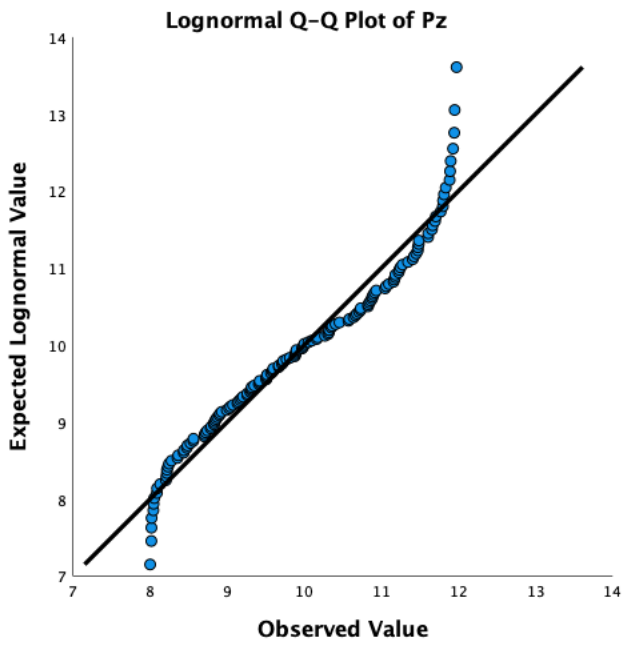
14


Lognormal Q-Q Plot of Pz
Expected Lognormal Value
Observed Value
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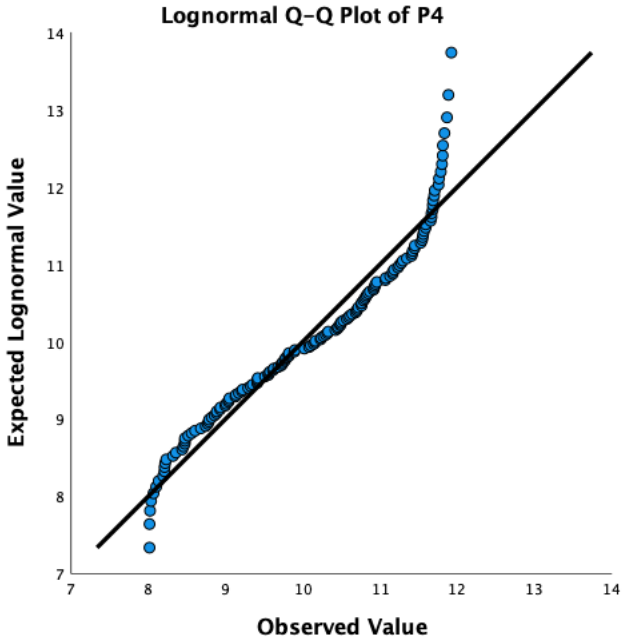
Lognormal Q-Q Plot of P4
Expected Lognormal Value
Observed Value
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