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An Investigation Of Endogenous Pain Modulation And Inflammatory Biomarkers In Non-Specific Chronic Low Back Pain

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BEHAVIORAL NEUROSCIENCE PSYCHOLOGY

ABSTRACT

Chronic low back pain (cLBP) is one of the most common disabling conditions in the world, and is one of the leading contributors to medical care seeking in adults. The worldwide prevalence of activity-limiting (acute and chronic) low back pain is about 12%, which equates to approximately 933 million people globally suffering with low back pain at any given time. Despite the prevalence and frequency of medical intervention, sustained pain relief and functional restoration are rarely achieved for those with cLBP. The vast majority of cLBP is "non-specific" with no identifiable pathology of the spine or related tissues. Without a clear target for treatment of cLBP, effective pain management can be difficult to achieve. Even when pathoanatomical changes in the spine are detected, there is often poor correspondence between these diagnostic measures of cLBP and clinical symptoms. This suggests that factors above and beyond pathoanatomy, such as altered pain modulation and inflammation, may contribute to cLBP severity.

Past research examining predictors of cLBP outcomes, specifically markers of inflammation and endogenous pain modulation, has been mixed. One reason for this may be that many of the studies investigating cLBP severity have relied on measurements of pain at rest that incorporated validated self-report questionnaires as the clinically-relevant

index of pain severity. However, individuals with musculoskeletal pain conditions including cLBP often experience movement-evoked pain upon completion of physical activity. Emerging evidence has revealed distinct mechanistic differences between pain at rest and movement-evoked pain. Thus, pain at rest may fail to accurately isolate the type of pain that is most predictive of cLBP or explain contradictory outcomes. Thus, the objective of this dissertation was to identify inflammatory and endogenous pain modulatory processes that could possibly differentially predict severity of movement-evoked pain versus pain at rest in individuals with non-specific cLBP.

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INTRODUCTION

Endogenous Pain Modulation

The perception of pain is greatly influenced by the endogenous processing of ascending (i.e., incoming) nociceptive signals from peripheral afferents, which are powerfully modulated by complex descending inhibitory and facilitatory processes within the central nervous system (Ossipov et al., 2010, 2014). The descending modulation of incoming nociceptive stimuli is manifested via pathways that originate at the level of the cerebral cortex, the thalamus, and the brainstem (Heinricher et al., 2009). Activation of these descending pain modulatory processes (hereafter referred to as endogenous pain modulation or endogenous pain modulatory processes) often involves the release of inhibitory and/or excitatory neurotransmitters that can produce potent pain inhibition or facilitation, respectively (Staud, 2013). Mounting evidence has further demonstrated that emotional state, anxiety, expectations, attention and distraction, memories, stress, and many other factors can also engage endogenous pain modulatory processes that inhibit or facilitate the percept of pain (Nir & Yarnitsky, 2015; Staud, 2012). There is growing appreciation for the important role that descending (i.e., "top–down") systems within the central nervous system play in endogenous pain modulation, which can profoundly affect the percept of pain.

Endogenous Pain Modulation and Chronic Pain

An important factor to consider in the evaluation and management of chronic pain is that it is a highly variable experience across individuals. Whereas pain is generally initiated by activation of nociceptors that function to detect noxious stimuli capable of producing tissue damage, there is only limited evidence that the experiential perception of chronic pain is directly correlated with the level of nociceptor activation (Ossipov, 2012). The variability of the chronic pain experience lends credence to the presence of endogenous pain modulatory processes that can either inhibit or facilitate the percept of pain. It has been hypothesized that these endogenous pain modulatory processes yield a pain inhibitory/facilitatory balance, which places individuals on a spectrum between anti-nociception and pro-nociception (Yarnitsky et al., 2014). To illustrate, an individual expressing diminished inhibition and/or enhanced facilitation would be positioned on the pro-nociceptive side of the spectrum. As a result, this individual would express a more pain sensitive phenotype that increases the risk of developing a chronic pain condition. Conversely, an individual expressing efficient inhibition and/or non-enhanced facilitation would be positioned on the anti-nociceptive side of the spectrum, and therefore express a less pain sensitive phenotype that would help prevent the development of a chronic pain condition. It is important to note that the clinical relevance of this purported inhibitory/facilitatory balance remains hypothetical; however, recent studies have increased our understanding of endogenous pain modulatory processes in relation to clinical pain (Cruz-Almeida & Fillingim, 2014; Granovsky & Yarnitsky, 2013). In the future, it may be that engagement of these modulatory processes leads to more efficacious

therapeutics for the treatment of chronic pain, or perhaps even prevention of chronic pain development.

Quantitative Sensory Testing of Endogenous Pain Modulation

The Quantitative Sensory Testing (QST) measures are used to characterize human pain perception are frequently categorized as either "static" or "dynamic" in nature (Eisenberg et al., 2010; Olesen et al., 2012). Traditionally, QST has been used in a static fashion by measuring responses to single discrete stimuli with either fixed intensities or intensities that gradually change over time (e.g., ascending method of limits for detection of pain threshold/tolerance). More recently, advanced methods of dynamic QST have been developed whereby stimuli are applied repetitively or simultaneously to different body areas (Mackey et al., 2017). Further, dynamic QST response measures are emerging as more reliable and valid predictors of chronic pain outcomes than static measures (Arendt-Nielsen & Yarnitsky, 2009; Mackey et al., 2017). The specific dynamic measures of endogenous pain modulation for the purpose of this study are listed below.

Dynamic Response Measures

- *Temporal summation of pain* (TS) refers to a form of endogenous pain facilitation characterized by the perception of increased pain despite constant or even reduced peripheral afferent input (Staud et al., 2001). Temporal summation is presumed to be the psychophysical manifestation of wind-up (Staud et al., 2003). Wind-up is a phenomenon where repetitive stimulation of C primary afferents at rates greater than 0.3Hz produces a slowly increasing response of second-order neurons in the

spinal cord (Herrero et al., 2000), and increased magnitudes are indicative of increased central sensitization and secondary hyperalgesia.

- *Conditioned pain modulation* (CPM) refers to the reduction in pain from one stimulus (the test stimulus) produced by the application of a second pain stimulus at a remote body site (the conditioning stimulus) (Nir & Yarnitsky, 2015). Conditioned pain modulation is believed to reflect the perceptual manifestation of diffuse noxious inhibitory controls (Sprenger et al., 2011), whereby ascending projections from one noxious stimulus activate supraspinal structures that trigger descending inhibitory projections to the dorsal horn.

Dynamic forms of QST that include tests of TS of pain and CPM are likely best suited for the current study given the growing evidence base attesting to the clinical relevance of each (Mackey et al., 2017). TS of pain is a QST method that invokes neural mechanisms related to pain facilitation (Goodin et al., 2014), while CPM invokes neural mechanisms related to pain inhibition (Staud et al., 2003). Taken together, TS and CPM measures are thought to induce a process of modulation believed to reflect the "real-life" endogenous modulation exerted by patients when exposed to clinical pain (Granovsky & Yarnitsky, 2013). Typically, patients with clinical pain of various types express either less efficient CPM or enhanced TS, or both (Granovsky & Yarnitsky, 2013; Yarnitsky, 2015).

Endogenous Pain Modulation and cLBP

In recent years, a growing number of case-control studies have revealed that individuals with cLBP demonstrate greater dysfunction in endogenous pain modulatory pathways compared to controls using experimental pain protocols (i.e., quantitative sensory testing or QST) (LeResche et al., 2013; Mlekusch et al., 2013; O'Neill et al., 2007). Similarly, a cross-sectional study addressing this topic found that augmented pain sensitivity and dysfunctional endogenous pain modulation were associated with greater cLBP severity and disability (Owens et al., 2016). Emerging evidence suggests that cLBP severity is related to a pro-nociceptive pain modulatory balance characterized by enhanced facilitation (TS) and diminished inhibition (CPM) (Yarnitsky et al., 2014). As stated, much of this evidence has been cross-sectional, making it difficult to ascertain the directionality of the relationships. Whether QST-based tests of endogenous pain modulatory balance might be useful for prospectively predicting future reports of cLBP severity has received less attention.

Compared with traditional neurological assessments (e.g., evoked potential, nerve conduction velocity, electromyography), QST provides certain advantages for clinical practice and research. For instance, QST can better target small nerve fibers such as A-delta and C-fibers involved in deep-tissue pain sensation, compared to traditional neurological assessments (Mense, 1993; Uddin & MacDermid, 2016). In addition, clinical observations provide little information about the underlying mechanisms of an individual's pain experience (Uddin & MacDermid, 2016). In fact, clinical observations do not always correlate with mechanism-based appraisals as assessed by QST.

Biomarkers of Inflammation and Chronic Pain

Findings from preexisting research suggest that systemic levels of inflammatory markers such as cytokines are elevated in individuals living with chronic low back pain (Lim et al., 2020; van den Berg et al., 2018). Cytokines are small proteins released primarily by helper T cells (Th) and macrophages that can be found in peripheral nerve tissue after an injury is sustained. These proteins constitute part of the immunogenic and pathogenic recognizing systems. Cytokines act upon receptor cells that respond based on their individual physiology, genetic composition and a multitude of external stressors (Mogensen, 2009; Zhang & An, 2007). It is hypothesized that this immunomodulatory process, if persistent, can sustain pro-inflammatory pathways that may ultimately give rise to low back pain (van den Berg et al., 2018; Watkins et al., 2003; Zhang & An, 2007). Furthermore, it has been suggested that cytokines have the potential to alter neuronal activity in both the peripheral and central nervous system (van den Berg et al., 2018; Watkins et al., 2003; Zhang & An, 2007). There are pro-inflammatory cytokines that promote inflammation as well as anti-inflammatory cytokines that decrease the inflammatory response via antagonist effects, and are related to the attenuation of hyperalgesia. Pro-inflammatory cytokines are derived primarily by activated macrophages and contribute significantly to the upregulation of inflammatory reactions (Vanderwall & Milligan, 2019; Zhang & An, 2007). A considerable amount of the preexisting pain literature suggests that specific pro-inflammatory cytokines interleukin- 1 alpha (IL-1α) interleukin-1 beta (IL-1β), interleukin- 6 (IL-6), tumor necrosis factor- alpha (TNF-α), as well as non-cytokine markers of inflammation such as C-reactive protein (CRP) and

fibrinogen, contribute to the pathological process of pain development, specifically cLBP (Lim et al., 2020; Sowa et al., 2014).

Further, five previous studies have investigated the relationship between CRP and cLBP severity at rest. Of these five separate studies, four demonstrated significant findings (Gebhardt et al., 2006; D. Klyne et al., 2018; D. M. Klyne et al., 2017). Results from three of the five studies revealed positive associations between higher levels of CRP and greater self-reported cLBP severity (D. Klyne et al., 2018; D. M. Klyne et al., 2017), whilst the fourth study determined that elevated levels of CRP significantly increased the odds of experiencing non- specific cLBP (Gebhardt et al., 2006; Sowa et al., 2014). The final study reported a positive relationship between CRP and cLBP severity; however, this finding was no longer significant after appropriate confounders were incorporated into the data analysis (Stürmer et al., 2005). Taken together, the literature suggests that CRP is a clinically relevant marker of inflammation to use in future studies examining the inflammatory nature of cLBP.

Similar findings were also reported in some studies examining the relationship between select pro-inflammatory cytokines and cLBP. For example, it is suggested that TNF-α, might be a valid biomarker candidate for cLBP (Kraychete et al., 2010) though the literature is mixed. In a systematic review, six independent studies investigated the association between TNF-α and cLBP (Lim et al., 2020). Although two studies determined that the association between TNF-α and cLBP was non-significant (D. Klyne et al., 2018; D. M. Klyne et al., 2017), findings from three of the six studies revealed a positive relationship between higher TNF-α levels and greater cLBP severity at rest (de Queiroz et

al., 2016). Similarly, another study concluded that circulating levels of TNF-α was higher in participants with cLBP than controls at day 0 and again at day 180 (follow-up). That same study also reported that higher levels of TNF-α was significantly associated with greater pain severity as well as cLBP-specific disability as measured by the Oswestry Disability Index.

One of the most studied pro-inflammatory markers, IL-6, has historically been reported to be involved in the process of pathological pain. Prior research suggests that an increase in circulating concentrations of IL-6 is positively associated with cLBP severity (Heffner et al., 2011; D. Klyne et al., 2018; D. M. Klyne et al., 2017; Li et al., 2016; Lim et al., 2020; Queiroz et al., 2015) It has also been reported that cLBP severity tends to be greater in individuals who have higher concentrations of IL-6 in their blood plasma as well as serum in a separate study (Lim et al., 2020). Though findings for IL-6 in relation to low back pain are fairly consistent across studies, the literature remains inconsistent for IL-β. It has however been recently been discovered that IL-1β is expressed in nociceptive dorsal root ganglion neurons (Aydeniz et al., 2009). The cLBP literature also remains inconsistent for studies examining its association with fibrinogen (Lim et al., 2020; Zebouni et al., 1993).

In addition to the cytokines that promote inflammation, anti-inflammatory cytokines are immunomodulatory proteins that control the response of pro-inflammatory cytokines. Interleukin- 4 (IL-4) and interleukin 13 (IL-13) are reportedly among the most pain-relevant, anti-inflammatory cytokines (DeVon et al., 2014). Though not many studies exist that have examined the association between these specific anti-inflammatory

cytokines and cLBP, one study of individuals living with chronic widespread pain (CWP) concluded that reduced levels of IL-4 was significantly associated with more CWP (DeVon et al., 2014; Kindler et al., 2010). IL-13 has also been linked to painful musculoskeletal conditions. For example, it was reported that individuals living with severe rheumatoid arthritis (RA) presented with greater levels of IL-13 in blood plasma compared to individuals in the mild RA subgroup (Isomäki et al., 1996; Mao et al., 2019). More studies are needed to determine the role of both IL-13 and IL-4 in relation to cLBP.

25 hydroxy vitamin D (vitamin D) is a fat-soluble micronutrient that is well known for its critical role in calcium homeostasis (Fleet, 2017). Recently, it has been suggested that the nutrient has the hormonal potential to influence immunomodulation by downregulating the production of pro-inflammatory cytokines that are known to be associated with the pathogenesis of many inflammatory conditions (Liu et al., 2018; Mascarenhas & Mobarhan, 2004; Norman, 2008). Across many musculoskeletal conditions, insufficient levels of circulating vitamin D has been associated with various negative health outcomes including increased pain severity (Glover et al., 2012, 2015; Norman, 2008). Further, a meta-analysis determined that across 14 studies, vitamin D deficiency was highly prevalent in cLBP patients (Dahlhamer, 2018). In a study of 98 patients living with cLBP, analyses revealed not only a differences in VAS pain scores between groups based on circulating levels of vitamin D, but also a negative relationship between overall VAS scores and vitamin D in their sample, even after incorporating relevant covariates into their analysis (Gokcek & Kaydu, 2018). Ongoing research continues to examine whether vitamin D supplementation is effective for pain management; however, the results tend to suggest it is not. Interestingly, low vitamin D is

consistently associated with greater chronic pain severity; however, supplementation has not proven as helpful as originally hoped (Glover et al., 2012). Currently, no studies exist that have sought to examine the association between vitamin D and movement-evoked pain in a sample of individuals living with cLBP.

Movement-Evoked Pain and Pain at Rest

Traditionally, clinical and experimental pain research has relied primarily on static measures of pain using ratings scales and/or assessments of retrospective self-report, obtained by use of questionnaires. Though these methods have provided a plethora of information about pain at rest, it is surmised that the pain outcome itself might pose as a factor that limits the ability of researchers to investigate possible mechanisms that contribute to the pathogenesis associated with specific painful conditions (Corbett et al., 2019). For example, the predominant driver of pain in chronic musculoskeletal conditions is often physical activity, which is commonly referred to as movement-evoked pain (Palit et al., 2019). It has been hypothesized that pain at rest and movement-evoked pain could very well be facilitated by different underlying mechanisms. Historically, the driving factors that contribute to painful experiences, which are pain-sensory, motor factors, and psychological, were studied independent of each other. An evolved model of pain suggests that if the three categories of factors are integrated then we would be provided a more comprehensive understanding of the interrelationship between pain and movement (Corbett et al., 2019; Vardeh et al., 2016).

Inflammation and Movement-Evoked Pain

An exigent mechanism specific to movement- evoked pain is the activation of nociceptors (Corbett et al., 2019; Dessem & Lovering, 2011). Movement-evoked pain is typically instigated in response to cell damage via mechanisms of inflammation in peripheral neurons that facilitate sensitization (Riley & Boulis, 2006). The biomarkers included in the current study could very well serve as proxies for inflammation, providing new insight on how inflammatory markers might influence chronic pain. Some studies have alluded to psychosocial factors (e.g., negative affect, perceived injustice) predicting movement-evoked pain in cLBP, which may also amplify inflammatory processes (Bartley et al., 2019; Penn et al., 2020). Still, to our knowledge, no other study has elucidated the extent to which movement-evoked pain compared to pain at rest can be predicted by pro- and anti-inflammatory cytokines in a sample of individuals living with cLBP.

Endogenous Pain Modulation and Movement-Evoked Pain

Prior research examining factors of endogenous pain modulation, specifically CPM and TS, in relation to cLBP severity has primarily employed the use of validated self-report questionnaires as the clinically-relevant index of pain severity at rest (LeResche et al., 2013; O'Neill et al., 2007; Owens et al., 2016). However, individuals suffering from musculoskeletal pain conditions often experience movement-evoked pain following the completion of a physical task (Corbett et al., 2019). A growing body of evidence suggests that distinct differences exist between pain severity recalled on questionnaires and movement-evoked pain. In adults living with painful musculoskeletal conditions, including cLBP and knee osteoarthritis, QST measures of endogenous pain modulation (i.e., TS and CPM) were associated with movement-evoked pain during functional experimental tasks;

however, these very measures were not associated with self-reports of pain recalled on questionnaires (Rakel et al., 2015; Simon et al., 2015). Whether TS of pain and CPM might differentially predict cLBP severity of movement-evoked pain versus pain severity reported on a validated questionnaire has yet to be addressed.

SPECIFIC AIMS AND HYPOTHESES

Specific Aim 1: To examine differences in endogenous pain modulation profiles (Conditioned Pain Modulation or CPM and Temporal Summation of mechanical pain or TS) as well as markers of inflammation found in peripheral blood between people with cLBP and pain-free controls.

Hypothesis 1a: *People with cLBP will present with significantly greater TS of mechanical pain and significantly less CPM in comparison to pain-free controls.*

Hypothesis 1b: *People with cLBP will have significantly higher concentrations of pro-inflammatory markers (fibrinogen, CRP, serum amyloid A, TNF-α, IL-1α, IL-1β, IL-6) as well as a significantly lower concentrations of anti-inflammatory markers (IL-4, IL-13, Vitamin D) present in blood plasma samples in comparison to pain-free controls.*

Specific Aim 2: To investigate the extent to which endogenous pain modulation profiles (CPM & TS) are associated with pain at rest and movement-evoked pain in people with cLBP.

Hypothesis 2: *Greater TS of mechanical pain and reduced CPM will each be significantly associated with increased self-reported pain at rest and movement-evoked pain.*

Specific Aim 3: To examine the associations between markers of inflammation (pro- and anti-) and pain at rest, as well as movement-evoked pain in people with cLBP.

Hypothesis 3a: *Higher concentrations of circulating pro-inflammatory markers (fibrinogen, CRP, serum amyloid A, TNF-α, IL-1α, IL-1β, IL-6) will be significantly associated with greater pain at rest and greater movement- evoked pain in people with cLBP.*

Hypothesis 3b: *Lower concentrations of circulating anti-inflammatory markers (IL-4 and IL-13, and Vitamin D) will be significantly associated with greater pain at rest and greater movement- evoked pain in people with cLBP.*

METHODS

Study Overview

This study was part of an ongoing parent project investigating ethnic/racial and socioeconomic differences in cLBP severity and disability (Examining Racial And Socioeconomic Disparities in cLBP; ERASED). The parent project employs a biopsychosocial conceptual model that examines biobehavioral, psychological, and sociocultural factors that may help explain differences in cLBP between non-Hispanic Black and non-Hispanic White adults. The procedures and experimental methods described below are limited to those involved in the present study. A flow diagram illustrating matriculation through the current study is presented in **Figure 1** (below). Interested participants completed a telephone-based screening to determine study eligibility; health history was reviewed via electronic medical records. Eligible participants engaged in two distinct laboratory-based study sessions separated by 1-week. Participants completed a comprehensive QST battery during the first study session. Approximately 1-week later, participants returned to the laboratory to take part in the second study session, which included a blood draw and assessments of movement-evoked pain and physical function using a standardized short physical performance battery. (Guralnik et al., 1994) Participants then completed the Brief Pain Inventory – Short Form (pain at rest), which is a validated questionnaire of self-reported pain severity and interference (Mendoza et al., 2006). This study was conducted in accordance with the cLBP

research standards put forth by the Research Task Force of the NIH Pain Consortium (Deyo et al., 2014). It was reviewed and approved by the local Institutional Review Board, and was carried out in a manner consistent with ethical research guidelines.

Participants and Recruitment

Adult participants with cLBP were recruited via flyers posted at the UAB Pain Treatment Clinic and surrounding community. Individuals were included in the study if low back pain had reportedly persisted for at least three consecutive months and was present for at least half the days in the past six months (Treede et al., 2015). The participants' primary pain complaint had to be low back pain with no evidence of surgical intervention or accident/trauma within the past 12 months. The inclusion criteria for participants with cLBP were: 1) non-specific cLBP that has persisted for at least 3 months and has resulted in pain on at least half the days in the past 6 months, 2) age 19 – 85; the lower end of this age range was chosen in order to capture the growing prevalence of young adults with cLBP, and participants over 85 years are increasingly likely to meet one or more exclusion criteria, and 3) participants reported ethnic group as non-Hispanic and racial group as either Black/African American or White/Caucasian. An individual was considered ineligible for participation in this study if he or she had a medical condition that could potentially confound outcome measures (i.e., biomarkers, QST responses or self-reported pain measures). This included the following reasons: 1) Injury or low back pain surgery that occurred within the past 12 months, 2) cLBP caused by a specific pathophysiological condition of the lumbar such as compression fracture, trauma, ankylosing spondylitis, malignancy etc. 3) diagnosis of a comorbid systemic rheumatic

condition (e.g. rheumatoid arthritis, systemic lupus erythematosus, fibromyalgia), 4) uncontrolled hypertension (i.e. SBP/DBP > 150/95), 5) cardiovascular disease, 6) poorly controlled diabetes (HbA1c> 7%), 7) neurological disease, or 8) pregnancy.

Procedure

Experimental Session 1: Participants initially provided sociodemographic information that included race/ethnicity, age, sex/gender, and annual household income. Height and weight data were collected for calculation of body mass index (BMI) prior to completion of a standardized depressive symptoms measure. Next, participants completed a QST battery designed to assess endogenous pain modulatory balance. The QST battery specifically included controlled sensory stimulation procedures to assess endogenous pain facilitatory processes - TS of mechanical pain, as well as endogenous pain inhibitory processes - CPM. For this study, TS of mechanical pain was examined exclusively as a measure of endogenous pain facilitation. This is because previous research has demonstrated that TS of mechanical pain is more clinically relevant than TS of thermal pain for predicting musculoskeletal clinical pain severity (Goodin et al., 2014; Owens et al., 2016, 2019).

Questionnaires

Depression: Depressive symptoms was assessed using the Center for Epidemiological Studies-Depression Scale (CES-D) (Goodin et al., 2014). This 20-item measure assesses the frequency of experiencing depressive symptoms over the past week (0 – never or rarely to 3 – most of the time/all the time). Symptoms of depression measured by the CES-D include negative mood, guilt/worthlessness, helplessness/hopelessness,

psychomotor retardation, loss of appetite, and sleep disturbance. This measure has been shown to be reliable and valid in general populations, including when used in chronic pain populations. Responses are summed (range 0 – 60), with higher scores indicating greater severity of depression.

Dynamic QST Modalities

Temporal Summation (TS): TS of mechanical pain was assessed at the erector spinae muscles of the lumbar spine using a weighted (512 mN) pinprick stimulator (MRC Systems, Heidelberg, Germany) (van den Broeke et al., 2015). The pinprick stimulator was oriented perpendicularly and held just above the intended point of contact. The punctate probe was then lowered gently until the fine weighted probe retracts fully inside of the probe's hollow metal cylinder, creating the desired standardized stimulation. First, participants were subjected to a single contact from the pinprick stimulator and prompted to rate the pain intensity resulting from this sensation using a 0-100 numeric rating scale, where "0 = no pain and 100 = most intense pain imaginable". Next, the pinprick stimulator was applied 10 successive times at a rate of one contact per second. Participants were again asked to provide a single 0-100 rating indicating the greatest intensity of pain experienced during the 10 repeated contacts. This procedure was repeated twice at the lumbar spine. Pain ratings for the single and multiple contacts performed at each anatomical location are averaged across the two trials.

Conditioned Pain Modulation (CPM): CPM was also be tested at the erector spinae muscles of the lumbar spine using algometry as the test stimulus and hand immersion into the cold pressor as the conditioning stimulus (Yarnitsky et al., 2015). A handheld algometer (Medoc, Ltd., AlgoMed, Ramat Yishai, Israel) was applied three times

at the lumbar region to determine participants' baseline pressure pain thresholds (PPTs). Pressure gradually increased at a rate of 30 kilopascals (kPa) per seconds, and participants were to indicate when the increasing pressure stimulation first becomes painful. PPTs were measured in kilopascals (kPa). Following baseline PPT determination, participants underwent a series of two cold pressor immersions that consisted of placing the left hand, up to the wrist, into 12 degrees C circulating cold water for 1 min. The cold pressor was maintained at 12 degrees C, given previous work indicating this temperature to be best for maximizing a full 1 min hand immersion, while also producing a moderate amount of pain (~50 ± 10 on the 0-100 numeric rating scale) (Thompson et al., 2018). Immediately upon removal of the hand from the cold pressor, the algometer was used to deliver noxious mechanical stimulation to the lumbar region. Participants again were to indicate when the increasing pressure stimulation first becomes painful, which represents their conditioned PPTs. There was a 2-min rest period between each CPM trial. The three baseline PPTs were averaged, as were the two conditioned PPTs from the CPM trials.

Experimental Session 2: Approximately 1-week (7 days) after completing the first experimental session, each participant returned to the laboratory and completed the second experimental study session. This included a blood draw, as well as completion of the BPI-SF pain questionnaire and assessment of movement-evoked pain and physical function.

Blood Specimen Collection

Specimen samples were collected from each participant at the beginning of experimental session 2 as part of a single blood draw. A 23-gauge butterfly needle was inserted into the antecubital fossa by a research nurse within the Clinical Research Unit. Peripheral blood samples were stored in vacutainer tubes and processed in the CRU laboratory, then stored

in a -80C freezer. Specimens were then transported to the UAB Physiology and Metabolism core. C-reactive protein (CRP) was assayed using Cayman Chemical CRP ELISA kits. The following biomarkers: (pro-inflammatory) fibrinogen, serum amyloid A, interleukin-1 alpha (IL-1α), 1IL-1β, IL-6, Tumor necrosis factor-alpha (TNF-α), and (Anti-inflammatory) Vitamin D, IL4, IL10, IFN-a were determined with Meso Scale Discovery, which is an enzyme-linked immunosorbent assay (ELISA) that employs electrochemiluminescence to identify specific binding events. This method is advantageous because it allows for multiplexing, thus a variety of biomarkers can be assayed simultaneously.

Pain at Rest

The BPI-SF is a multidimensional pain scale used to assess self-reported pain severity and its interference with daily functioning (Mendoza et al., 2006). The questionnaire is composed of four items asking about pain severity (worst pain, least pain, average pain, and pain right now) over the past 24 hours. There are also seven items that assess the degree to which pain interferes with functioning in the following domains: general activity, mood, walking ability, normal work, relations with other people, sleep, and enjoyment of life. Each item is scored from 0 (no pain or does not interfere) to 10 (worst imaginable pain or completely interferes). Higher scores suggest great pain severity and interference. The BPI-SF is a well validated chronic pain questionnaire that has previously been used in samples with cLBP.(*Validation of the Brief Pain Inventory in Patients With Low Back Pain. - PubMed - NCBI*, n.d.)

Movement- evoked Pain

The SPPB assesses lower extremity function with three movement tasks: standing balance, 4-meter walking speed, and ability to rise from a chair.(Guralnik et al., 1994) Specifically, participants are to complete the following movement tasks in consecutive order: 1) Stand with their feet oriented in the side-by-side, semi-tandem, and tandem positions for 10 seconds each; 2) Rise from, and return to, a seated position in a chair five times; and 3) Walk a distance of four-meters, twice. For each movement, they received a score of 0–4 (total score 0–12) based on their performance. If participants did not feel safe completing any of the SPPB tasks, they were given a score of zero to denote non-participation. A lower score on the SPPB is indicative of worse physical function, and greater likelihood of disability. After completion of each movement task, participants were asked to provide a pain intensity rating for any movement-evoked pain experienced during completion of the balance, chair, and walking tests. The 0-100 numeric rating scale was again utilized for this purpose, whereby: (0 = no pain and 100 = most intense pain imaginable). The SPPB is standardized and has been well validated for use in populations with cLBP(Cruz-Almeida & Fillingim, 2014; Weiner et al., 2003) and also used to measure movement-evoked pain (Booker et al., 2019; Cruz-Almeida et al., 2017).

DATA ANALYSIS

Data Inspection

Prior to testing hypotheses, each variable was examined to identify missing values, statistical outliers, and the violation of relevant assumptions. It must be noted that 8.23% of the overall cases (across groups) were deleted listwise due to missing data. This resulted in a final sample size of 212 (n =156 cLBP participants and n = 56 pain free controls). According to (Tabachnick et al., 2019), 5 to 10% of cases with missing data is recognized as acceptable and does not threaten the study's external validity. All data were analyzed using SPSS, version 25 (IBM; Armonk, NY). Descriptive statistics were computed and represented as percentages or means (standard deviations). Group differences (cLBP vs. controls) among potential covariates of interest were examined using independent samples t-tests. Paired t-tests were used to examine differences within individuals between the 1st contact and a series of 10 contacts for TS of mechanical pain and between baseline and conditioned PPTs for CPM. The strength and direction of associations among continuous variables were examined using Pearson's correlations.

Data Reduction and Transformations

Prior to completion of Pearson correlations, TS effects (i.e., Δ change score) at the lumbar spine and dorsal aspect of the left hand were calculated by subtracting the pain intensity ratings following the first contact from the ratings following the series of 10 contacts. The presence of TS-related pain facilitation effects was observed if the final pain intensity rating was significantly greater than the initial reported pain intensity

rating. CPM effects at the lumbar spine and dorsal aspect of non-dominant forearm and hand were then calculated as a percent change from baseline, according to the following formula: ((Conditioned PPT – Baseline PPT) / Baseline PPT) * 100). The presence of CPM-related pain inhibition was observed only if the PPTs obtained via the algometer were higher when combined with a conditioned stimulus (cold water immersion) in comparison to the initial PPTs obtained at baseline. The dynamic QST data (TS & CPM variables) were skewed. These data were not transformed for the purpose of parametric analyses. According to (Treister et al., 2015)it has been suggested that parametric analyses for dynamic QST measures are routine but may be inappropriate. It has also been stated that utilizing parametric analyses on heavily skewed QST data may increase the chance of committing a Type 1 error. Further, it was determined by visual inspection that the QST variables contained no outliers or cases that were positioned 3 standard deviations above/below the mean.

Upon examining the scatterplots for all blood-based biomarkers of inflammation, it was discovered that these data were non-normally distributed and contained a substantial amount of outlier cases. According to (Treister et al., 2015) it is appropriate to perform parametric analyses on log (base 10)-transformed cytokines if this technique does indeed make the distribution of the data approximately normal (Fjell et al., 2013; Genser et al., 2007).

Inferential Statistics

Aim 1a and 1b: To examine hypotheses 1a, a series of Mann Whitney U tests were conducted to investigate dynamic QST (TS and CPM) differences between cLBP participants and pain free controls. To examine hypothesis 1b, a series of t tests was

conducted to assess the differences in circulating levels of pro- inflammatory acute phase reactants (fibrinogen, CRP, serum amyloid A), pro-inflammatory cytokines (A, TNF-α, IL-1α, IL-1β, IL-6) as well as concentrations of anti-inflammatory cytokines and (IL-4, IL-13) and Vitamin D.

Aim 2a and 2b: To examine hypotheses 2a and 2b, sequential hierarchical multiple regression models were employed to investigate the extent to which experimentally induced TS of mechanical pain and CPM predict pain at rest (BPI-SF) and movement-evoked pain (SPPB), controlling for demographic and clinical characteristics. Demographic characteristics were entered in step 1 of the hierarchical regression models, while clinical characteristics were entered in step 2, followed by TS of mechanical pain and CPM in step 3.

Aim 3a and 3b: To examine Hypotheses 3a and 3b, sequential hierarchical multiple regression models were employed to investigate the extent to which pro-inflammatory acute phase reactants (fibrinogen, CRP, Serum amyloid A), pro-inflammatory cytokines (A,TNF-α, IL-1α, IL-1β, IL-6), anti-inflammatory cytokines (IL-4, IL-13) and vitamin D were associated with pain at rest (BPI-SF) and movement-evoked pain (SPPB), controlling for demographic and clinical characteristics. Again, demographic characteristics were entered in step 1 of the hierarchical regression models, while clinical characteristics were entered in step 2, followed by the pro- and anti-inflammatory markers in step

RESULTS

Participant characteristics

Descriptive characteristics for the sample of participants living with cLBP are shown in **Table 1**. The average age for our overall sample was 43.76 (SD = 13.85) with a range of 18 to 82 years; however, participants with cLBP were significantly older than controls ($t = 3.08$, $p = .002$). This sample was comprised of more female (57.5%) than male (42.5%) participants. Most participants self-identified as Non-Hispanic Black or African American (59.4%), while the remaining participants indicated their race/ethnicity to be Non-Hispanic White or Caucasian (40.6%). Our groups did not differ significantly by race. The largest portion of the sample, both groups (26.4%), reported their annual household income to be between $0 and $19,999; however, controls reported significantly higher incomes than their cLBP counterparts $\chi^2(1, 212) = 4.76$, $p = .043$. The average BMI was significantly greater for participants with cLBP in comparison to controls ($t = 2.28$, $p = .023$). The overall average score for depressive symptoms on the CES-D was 19.93 (SD = 13.85), with a range of 0 to 50. However, depressive symptoms differed significantly between groups, such that participants with cLBP reported greater depressive symptom severity compared to controls ($t = 5.24$, $p < .001$). Medical record review and participant self-report revealed that 13.5% of participants with cLBP had a current prescription for an opioid analgesic, which was significantly greater than the 0% of controls with a current opioid prescription ($\chi2 = 8.37$, $p = .004$). Average movement-evoked pain severity on the SPPB was significantly greater for cLBP compared to

controls ($t = 6.84$, $p < .001$). Similarly, average pain severity at rest on the BPI-SF was significantly greater for cLBP compared to controls ($t = 13.69$, $p < .001$)

Specific Aim 1

TS and CPM effects across the total sample

TS (1 contact vs 10 contacts) and CPM (baseline PPT vs conditioned PPT) effects for the entire sample (i.e., both cLBP and controls) are displayed in **Table 2**. For TS of mechanical pain (512mN), the pain intensity rating elicited by the first contact was compared to the pain intensity rating elicited following 10 successive contacts. A paired t-test revealed that the mean pain intensity rating following 10 successive contacts was significantly greater than the mean pain intensity rating for the first contact at the left hand ($t = 12.85$, $p < .001$) and the lumbar spine ($t = 13.75$, $p < .001$). For analysis of CPM effects, mean baseline PPTs were compared to mean conditioned PPTs. Paired t-tests revealed statistically significant evidence of a CPM effect at the forearm ($t = 6.23$, $p < .001$), but not at the erector spinae muscles of the lumbar spine ($t = 1.50$, $p = .109$).

Hypothesis 1a: *People with cLBP will present with significantly greater TS of mechanical pain and significantly less CPM in comparison to pain-free controls.*

A series of Mann Whitney U tests were employed to assess differences in TS of mechanical pain (left hand and lumbar spine) and CPM (right forearm and lumbar spine) between cLBP and pain-free controls. Results revealed that participants with cLBP demonstrated a significantly greater amount of pain facilitation assessed by TS at the left hand ($U = 3516$, $p = 0.03$) (See **Figure 2**), but not at the lumbar spine (See **Figure 3**). There was also no significant CPM difference at the forearm or lumbar spine between cLBP and controls.

Comparing Pain Ratings and PPTs between cLBP and Controls

Despite the lack of significant differences in CPM effects and TS at the lumbar spine between cLBP and controls, subsequent analyses revealed evidence that participants with cLBP demonstrated significantly greater hyperalgesia (reflected as lower PPTs and greater pain severity ratings) compared to controls. Independent samples t- tests were conducted to determine the differences in mechanical stimulation pain ratings as well as PPTs between cases and controls; see **Table 3 and 4**. Analyses revealed that participants with cLBP rated contacts at both the left hand (1 contact: $t = 3.36$, $p < .001$; 10 contacts: $t = 4.06$, $p < .001$) and lumbar spine (1 contact: $t = 3.22$, $p = .001$; 10 contacts: $t = 3.29$, $p = .001$) as significantly more painful than controls. These differences are represented in **Figures 4 and 5**. Results further revealed that individuals with cLBP demonstrated significantly lower PPTs at both the forearm (baseline PPT: $t = 3.22$, $p = .001$; conditioned PPT: $t = 2.06$, $p = .04$) and the lumbar spine (baseline PPT: $t = 5.12$, $p < .001$; conditioned PPT: $t = 4.80$, $p < .001$); see **Figures 6 and 7**.

Hypothesis 1b: *People with cLBP will have significantly higher concentrations of pro-inflammatory markers (fibrinogen, CRP, serum amyloid A, TNF-α, IL-1α, IL-1β, IL-6) as well as a significantly lower concentrations of anti-inflammatory markers (IL-4, IL-13, Vitamin D) present in blood plasma samples in comparison to pain-free controls.*

T-tests were conducted to assess differences in log-transformed pro- and anti-inflammatory, blood-based, markers of inflammation between individuals with cLBP and controls. Analyses revealed that participants with cLBP presented with higher concentrations of CRP ($t = 2.14$, $p = .034$), fibrinogen ($t = 4.01$, $p < .001$), IL-6 ($t = 2.70$, $p = .007$), and IL-4 ($t = 2.73$, $p = .007$) compared to controls. Conversely, our control

group presented with greater levels of IL-1α ($t = 6.04$, $p < .001$) compared to participants with cLBP. Differences in these individual biomarkers can be shown in **Figures 8-12**, respectively. No significant group differences were observed among any of the other blood-based biomarkers (SAA, Vitamin D, TNF-α, IL-1β, IL-13).

Correlations among TS, CPM, and pain severity in participants with cLBP

Results from the correlation analyses are displayed in **Table 5**. Exclusively among participants with cLBP, greater movement-evoked pain severity was significantly associated with greater TS of mechanical pain at the hand ($r = .26$, $p = .001$) and lumbar spine ($r = .16$, $p = .040$). Similarly, greater severity of pain at rest, as indicated by the BPI-SF, was significantly associated with greater TS of mechanical pain at the hand ($r = .17$, $p = .027$), but not at the lumbar spine ($r = .09$, $p = .257$). Neither CPM at the lumbar spine or forearm was significantly associated with severity of pain at rest nor movement-evoked pain severity. Additionally, movement-evoked pain severity on the SPPB was significantly associated with pain severity at rest on the BPI-SF ($r = .71$, $p < .001$). Lastly, greater CPM at the lumbar spine was significantly correlated with greater TS of mechanical pain at the lumbar spine ($r = .43$, $p = .023$).

Covariates of interest

Exclusively among individuals with cLBP, male participants reported significantly greater movement-evoked pain severity than female participants ($t = 3.08$, $p = .002$). Compared to their non-Hispanic White counterparts, non-Hispanic Black participants had significantly greater movement-evoked pain severity on the SPPB ($t = 2.76$, $p = .006$) as well as greater pain severity at rest on the BPI-SF ($t = 2.80$, $p = .006$). Increasing age was significantly associated with greater movement-evoked pain severity

(r = .19, p = .020), but not pain severity at rest (r = .14, p = .077). Lower annual household income was significantly associated with greater pain severity at rest and greater movement-evoked pain severity (all p's < .001). Current opioid prescription and BMI were not significantly associated with pain severity ratings on the SPPB or BPI-SF. However, greater depressive symptoms were significantly associated with greater movement-evoked pain severity (r = .19, p = .018) as well as greater self-reported pain severity at rest (r = .34, p < .001). Given their theoretical and empirical relevance, participant sex, annual household income, age, race, BMI, current opioid prescription, and depressive symptoms were all included as statistical covariates in the hierarchical regression models presented below.

Specific Aim 2

Hypothesis 2: *Greater TS of mechanical pain and reduced CPM will each be significantly associated with increased self-reported pain at rest and movement-evoked pain.*

Three hierarchical multiple regression models were analyzed based upon the significant bivariate associations we observed among TS, CPM, and pain severity: 1) TS at the lumbar spine predicting movement- evoked pain severity, 2) TS at the lumbar spine predicting pain severity at rest, and 3) TS at the left-hand predicting movement-evoked pain severity. As presented in **Table 6**, the overall model for TS at the lumbar spine predicted approximately 29% of the variance in movement-evoked pain, which was statistically significant (R^2 = .29; $F_{1,203}$ =7.55, p < .001). As shown in **Figure 13,** after adjusting for covariates, TS at the lumbar spine accounted for a modest, yet statistically significant, 2% of the variance in movement-evoked pain severity (β = .15, p = .035).

Interestingly, sociodemographic and clinical characteristics including participant sex (male > female) (β = -.17, p = .021), lower annual household income (β = -.32, p < .001), and greater depressive symptoms (β = .23, p < .001) were more strongly associated with movement-evoked pain severity than TS at the lumbar spine.

TS at the lumbar spine was also significantly correlated with pain severity at rest prior to the inclusion of covariates. The overall model for TS at the lumbar spine predicted approximately 32% of the variance in pain severity at rest, which was statistically significant (R^2 = .32; $F_{1,203}$ = 8.48, p < .001). However, TS at the lumbar spine did not remain a significant predictor of pain severity at rest after adjusting for covariates (β = .06, p = .393). Lower annual household income (β = -.37, p < .001) and greater depressive symptom severity (β = -.33, p < .000) were robust predictors of pain severity at rest.

As shown in **Table 7,** the overall model for TS at the left hand predicted approximately 29% of the variance in movement-evoked pain, which was statistically significant (R^2 = .29; $F_{1,203}$ = 8.38, p < .001). **Figure 14** shows that TS of mechanical pain at the left hand was significantly associated with movement-evoked pain (2% of the total variance) after controlling for relevant sociodemographic and clinical covariates (β = .16, p = .034). Additionally, covariates contributed substantially to the model as well. Participant sex (male > female) (β = -.17, p = .021), lower annual household income (β = -.32, p < .001) and greater depressive symptoms (β = .21, p < .001) were all found to be statistically significantly predictive of movement- evoked pain severity in this model.

Neither CPM at the lumbar spine (β = .03, p = .659) nor CPM at the forearm (β = -.05, p = .511) were significantly associated with movement-evoked pain severity in the adjusted regression models; **see Figure 13**.

Aim 3

Correlations among inflammatory biomarkers and pain severity in cLBP

Bivariate analyses were conducted to investigate associations between the pro-inflammatory acute phase reactants (fibrinogen, CRP, serum amyloid A), pro-inflammatory cytokines (TNF-α, IL-1α, IL-1β, IL-6) and movement-evoked pain severity as well as pain severity at rest. Additional bivariate analyses were conducted to examine the associations of anti-inflammatory biomarkers (IL-4, IL-13, and Vitamin D) with pain severity (movement-evoked and at rest); see **Tables 8, 9 and 10,** respectively. A Pearson correlation analysis revealed a positive association between IL-6 and pain severity at rest, such that higher concentrations of IL-6 were associated with greater pain severity at rest for participants with cLBP (r = .17, p = .031). No other significant correlations were found among pro-inflammatory markers and pain severity at rest or movement-evoked pain severity. Vitamin D was found to be significantly and negatively associated with pain severity at rest, such that lower Vitamin D levels were associated with greater pain severity at rest (r = -.21, p = .008). There were no other significant correlations observed among anti-inflammatory markers and pain severity at rest or movement-evoked pain severity.

Hypothesis 3a: *Higher concentrations of circulating pro- inflammatory acute phase reactants (fibrinogen, CRP, serum amyloid A) and pro- inflammatory cytokines (TNF-α,*

IL-1α, IL-1β, IL-6) will be significantly associated with greater pain at rest and greater movement- evoked pain in people with cLBP.

A hierarchical multiple regression model was completed to determine whether pain severity at rest as measured with the BPI-SF remained significantly associated with IL-6 after controlling for covariates. No other regression models were completed for pro-inflammatory markers due to the lack of significant correlations described directly above. As seen in **Table 11,** Results revealed that IL-6 was no longer significantly associated with pain severity at rest after controlling for covariates ($\beta = .08$, $p = .37$). The overall model explained 31.8% of the variance in BPI-SF pain severity at rest, with income ($\beta = -.37$, $p < .001$) and depressive symptoms ($\beta = .31$, $p < .001$) presenting as the covariates most significantly associated with pain severity at rest.

Hypothesis 3b: *Lower concentrations of circulating anti-inflammatory markers (IL-4 and IL-13, and Vitamin D) will be significantly associated with greater pain at rest and greater movement- evoked pain in people with cLBP.*

A hierarchical multiple regression model was completed to determine whether pain severity at rest as measured with the BPI-SF remained significantly associated with Vitamin D after controlling for covariates. No other regression models were completed for anti-inflammatory markers due to the lack of significant correlations described above. Results revealed that Vitamin D was no longer significantly associated with pain severity at rest after controlling for covariates ($\beta = -.13$, $p = .095$); see **Table 12**. The overall model explained 32.5% of the variance in BPI-SF pain severity at rest; however, income ($\beta = -.37$, $p < .001$) and depressive symptoms ($\beta = .33$, $p < .001$) were again the covariates significantly associated with pain severity at rest.

DISCUSSION

Specific Aim One

The principal objective of study aim 1 was to identify group differences in endogenous pain modulation profiles (TS and CPM), as well as blood-based biomarkers of inflammation (pro and anti-inflammatory), between individuals living with cLBP and a cohort of individuals without pain. It was initially observed that both, participants with cLBP and controls, demonstrated an enhanced facilitatory effect; wherein the overall response of 10 successive contacts elicited a greater pain response than the initial mechanical contact made by a 512(mN) punctate probe. Specifically, our clinical subset of cLBP participants reported greater pain intensity from the mechanical stimulation at first contact and after 10 contacts (at both the dorsal aspect of the left hand and the lumbar spine). Correspondingly and as expected, pain pressure thresholds were lower for the cLBP group at baseline and after the presentation of the conditioned stimulus. Taken together, these findings are suggestive of significant hyperalgesia (i.e., increased pain ratings and PPTs) and/or allodynia (i.e., a painful response solicited from normally no painful stimuli) in participants with cLBP compared to pain-free controls. This finding is in line with previously published results of studies wherein cohorts of individuals with musculoskeletal conditions, (e.g., knee osteoarthritis) demonstrated hyperalgesic responses to experimentally induced noxious stimuli (Fingleton et al., 2015; Moss et al., 2016).

Despite observing profound differences in pain ratings in response to mechanical stimulation via punctate probe, hypothesis 1a of this study was only partially supported. Once temporal summation was calculated for body relevant sites (i.e., lumbar spine and left hand) the groups only differed significantly at the distal site (left hand). This was likely due to our controls demonstrating more pain facilitation than expected, particularly at the lumbar spine. Thus, findings in the present study provide only tentative evidence for augmented pain facilitation. Similarly, we expected the cLBP group to demonstrate a significantly different CPM effect than controls. Despite observing significant group differences in pain pressure thresholds via algometry, at the dorsal aspect of the left forearm and the lumbar spine, we found no support for significant case-control differences in CPM effect at either body site. Though non-significant, our findings are similar to those of a recent study in cLBP with a smaller sample size (Cruz-Almeida et al., 2014). Similarly, we found that both control and cLBP groups lacked a CPM effect at the forearm/distal site.

Several study limitations deserve notice. Firstly, QST session were not conducted on a specific day/time across participants, thus" time of day" may have contributed to study findings involving these dynamic assessments of endogenous pain modulation (Aviram et al., 2015; Bachmann et al., 2011). Secondly, our sample of cLBP participants was mostly comprised of individuals who identified as non-Hispanic Black. The racial composition of our sample is important to note as a limitation because racial differences in QST responses have been reported in various chronic pain conditions including cLBP (Campbell & Edwards, 2012; Cruz-Almeida et al., 2014). Lastly, there are known sex

differences in experimental pain ratings. It has been reported that females are more pain sensitive to a noxious stimulus than males (Bulls et al., 2015; Wiesenfeld-Hallin, 2005).

For study aim 1b, the researchers hypothesized that compared to controls, the cLBP group would present with a more pro-nociceptive inflammatory profile, characterized by greater circulating concentrations of pro-inflammatory acute phase reactants and cytokines in addition to lower concentrations of anti-inflammatory cytokines and vitamin D. This hypothesis was partially supported. It was found that the cLBP group did indeed present with greater basal levels of circulating CRP and fibrinogen than their pain-free counterparts. CRP was initially solely considered a biological underpinning of infection or tissue damage, produced by the liver in response to an upregulation of pro-inflammatory cytokines such as IL-6 and macrophages, but now it is speculated that this overall marker of inflammation may very well contribute to, or reflect, chronic systemic inflammation (Peck et al., 2020). It has long been reported that individuals with chronic pain disorders (e.g., fibromyalgia and knee osteoarthritis) present with greater concentrations of circulating CRP (Sebba, 2021). The specific pathophysiology associated with non-specific chronic low back pain remains a mystery among clinicians and medical scientists; however, there are reports on the possible involvement of pro-inflammatory proteins, specifically CRP in heterogeneous cohorts of patients with low back pain. (Teodorczyk-Injeyan et al., 2019). Our findings are in part consistent with the aforementioned study as we found support for IL-6 but not TNF α- and IL-1β. Our findings also complement the existing body of literature by including a measure of movement- evoked pain.

The potential role of fibrinogen has been studied less in chronic pain, but this marker is an acute phase protein involved in the cascade of coagulation that has key implications for forming fibrin, the structural foundation of clotting. Though the development and collection of fibrin polymer at the location of vessel damage is crucial to the process of hemostasis, the accumulation therein has been linked to inflammation and pain in RA (Flick et al., 2007); however the literature remains mixed as it relates to fibrinogen's role in cLBP (Lim et al., 2020) . Additionally, we found differences in two pro-inflammatory cytokines. As the researchers expected, cLBP participants presented with greater concentrations of IL-6 in their plasma at the time of blood collection. This is consistent with majority of the literature (Lim et al., 2020). Aside from the studies presented in that systematic review, findings from a 2016 study reported that a cohort of individuals with cLBP presented with greater levels of IL-6 in their blood plasma than both, their pain free counterparts as well as individuals with upper back pain. The cLBP group in their study also had lower levels of an anti-inflammatory cytokine, IL-10 (Li et al., 2016). We further hypothesized that our cLBP group would demonstrate higher concentrations of IL-1 α, but the opposite was observed. Also, divergent from the narrative that remains consistent across the few studies that report IL-4 differences, our cLBP group presented with higher levels of IL-4. This specific anti-inflammatory cytokine may have been attempting to compensate for the upregulation observed in IL-6 for our clBP group. More research is needed in this area to elucidate the role of IL-4 in chronic pain.

Some limitations exist for this specific study aim and must be noted. Firstly, our group differed significantly in terms of BMI. This variable must be mentioned because

the adipose tissue of individuals who are obese secretes a wide range of pro-inflammatory cytokines that are responsible for the stimulation of CRP stimulation (da Cruz Fernandes et al., 2018). Secondly, racial differences in CRP and IL-6 have also been reported for individuals living with pain as well as those without (Paalani et al., 2011). It has been suggested that Non- Hispanic Black individuals are negatively impacted by social disadvantages and this could possibly explain the large disparities in inflammation. It suggested in a recent paper that older age and lower socioencominc status for Non- Hispanic Black individuals further contribute to racial differences in inflammatory biomarkers (Lam et al., 2021).

Specific Aim Two

The primary objective of study aim 2 was to examine whether QST-based tests of endogenous pain modulatory balance might be useful for prospectively predicting future reports of cLBP severity – both movement-evoked pain via SPPB and pain at rest on the BPI-SF. Experimental protocols for the assessment of endogenous pain modulatory balance included TS of mechanical pain and CPM, each assessed at the lumbar spine and a remote body site (i.e., TS: hand, CPM: forearm). TS & CPM protocols were carried out in accordance with commonly recommended methods (Cruz-Almeida & Fillingim, 2014; Owens et al., 2019; Yarnitsky et al., 2010, 2015b). Our findings suggest that a pro-nociceptive pain modulatory balance, characterized by a high degree of endogenous pain facilitation (i.e., TS of mechanical pain at the site of pain and at a remote body site) may be an important contributor to future episodes of movement-evoked cLBP severity when assessed with the SPPB. CPM did not significantly predict movement-evoked pain

severity, which is likely attributable to the overall lack of a significant CPM effect observed in this study.

Previous literature has frequently reported high degrees of endogenous pain facilitation (e.g., TS), with concomitant low degrees of pain inhibition (e.g., CPM), as a common characteristic of chronic musculoskeletal pain conditions, like cLBP (Arendt-Nielsen et al., 2018; den Bandt et al., 2019). Although statistically non-significant, the CPM effect was not completely absent given that some participants with cLBP demonstrated modest CPM effects. Interestingly, CPM was significantly correlated with TS of mechanical pain in this study. This suggests that the pain inhibitory processes represented by CPM may have been trying to compensate for the increased amount of pain facilitation, represented by the statistically significant TS of mechanical pain effect. Over time, it may be that pain inhibitory processes are no longer able to remain in balance with pain facilitatory processes as chronic pain develops; thus resulting in a pro-nociceptive endogenous pain modulatory balance (Ossipov et al., 2014b). Whether the shift to a pro-nociceptive pain modulatory balance is actually antecedent or consequent to chronic pain development is an important topic for understanding the transition from acute to chronic pain. It is not possible for the current study to shed light on this question given that our participants had already developed cLBP. However, it is important to note our study provides evidence that a pro-nociceptive pain modulatory balance, particularly TS of mechanical pain at the site of pain (i.e., low back), may predict the perpetuation of cLBP over time. This study complements previous cross-sectional research correlating TS of mechanical pain to cLBP severity (Owens et al., 2016).

Findings from this study suggest that endogenous pain facilitation as measured by TS of mechanical pain may be more mechanistically relevant to movement-evoked pain severity than pain severity at rest, at least among individuals with cLBP. It has been suggested that movement-evoked pain and pain at rest are not one in the same (Corbett et al., 2019). This is because, as the name suggests, movement-evoked pain arises upon completion of some physical activity, and its severity is rated in the moment. Most validated questionnaires, like the BPI-SF, ask people to retrospectively recall the severity of their pain while at rest (e.g., rate your worst pain in the last 24 hours) (Litcher-Kelly et al., 2007). An emerging literature provides evidence of important distinctions between movement-evoked pain and pain at rest, as recalled on validated questionnaires. For example, transcutaneous electric nerve stimulation for individuals with fibromyalgia significantly improved movement-evoked pain but not pain at rest (Dailey et al., 2013). Peripheral and/or central sensitization may help explain why TS of mechanical pain was related to movement-evoked pain severity on the SPPB in this study, but not pain severity at rest as reported on the BPI-SF. Peripheral sensitization refers to a phenomena that occurs when pro-inflammatory chemical mediators (e.g., prostaglandins, bradykinin, and leukotrienes) are secreted from damaged cells in the periphery and active primary afferent nociceptors. This cascade of physiological events decreases the threshold of activation for first order neurons, ultimately increasing the rate of activation for all stimulus intensities (Dubin & Patapoutian, 2010). Additionally, peripheral sensitization has the potential to increase the intensity and overall number of nociceptive signals ascending via the spinothalamic tract to the spinal cord. This neuronal plastic change appears to play a crucial role in the development of central sensitization (Latremoliere &

Woolf, 2009). Central sensitization refers to the phenomenon whereby nociceptive afferents can trigger a prolonged increase in the excitability and synaptic efficacy of neurons in central nociceptive pathways (Latremoliere & Woolf, 2009; Woolf, 2011). TS of pain is a widely accepted QST method that has been shown to activate neural mechanisms consistent with central sensitization (Staud et al., 2001, 2003). When central sensitization is present, generally innocuous movements such as standing from a seated position become sufficient to stimulate nociceptive afferents and produce movement-evoked pain (Harte et al., 2018; Woolf, 2018), which in turn can compromise physical function. This would help explain why TS of mechanical pain was a significant predictor of movement-evoked pain on the SPPB in this study. Most validated questionnaires that retrospectively assess pain at rest, and its interference with daily living, do not include a central sensitization component. To address this shortcoming, new measures such as the Central Sensitization Inventory have been developed in an attempt to assess central sensitization via validated questionnaire, especially in studies that are not amenable to inclusion of a QST battery (Mayer et al., 2012; Neblett et al., 2013). As it relates to this study, the BPI-SF does not include any specific assessment of central sensitization. This may help explain why TS of mechanical pain was not significantly related to pain severity at rest on the BPI-SF after controlling for covariates.

Consistent with the biopsychosocial model of chronic pain (Gatchel et al., 2007), other factors besides endogenous pain modulatory balance were also found to be predictive of movement-evoked pain severity. Specifically, low annual household income and greater depressive symptoms were each found to significantly predict greater movement-evoked pain. Evidence suggests that sex (Wáng et al., 2016), limited

socioeconomic resources (Ikeda et al., 2019), and depressed mood (Tsuji et al., 2016) may each heighten risk for poor cLBP outcomes. Further, poverty (Goodin et al., 2014), and depression (Adams & Turk, 2015) may augment central sensitization, thereby exacerbating movement-evoked pain. Taken together, sex, poverty, depression, and a pro-nociceptive pain modulatory balance may represent a biopsychosocial phenotype of vulnerability for poor cLBP outcomes; however, additional research is needed to confirm this hypothesis.

TS of mechanical pain accounted for a modest 2% of the variance in movement-evoked pain and physical function, respectively. Although TS of mechanical pain was a statistically significant predictor of movement-evoked pain the modest amount of variance accounted for rightfully calls in to question the clinical relevance of TS of mechanical pain. Importantly, our findings coincide with a growing body of evidence that collectively attests to the clinical relevance of laboratory-based assessments of endogenous pain modulatory balance using TS and CPM protocols (Owens et al., 2016; Petersen et al., 2015). It appears both TS and CPM have value for prospectively predicting chronic pain development as well as the severity of chronic pain over time. For example, greater pre-surgical TS of mechanical pain predicted the development of chronic pain 12 months following total knee arthroplasty in patients with knee osteoarthritis. Our findings add to this body of clinically relevant literature by showing that TS of mechanical pain assessed at the lumbar region and left hand of people with cLBP predicts their movement-evoked pain severity 1-week later. Additionally, it appears that widespread pain facilitation may have been the phenomenon observed in the current study. This corroborates a previous study that reported widespread

musculoskeletal pain in patients with cLBP and found fibromyalgia syndrome in 15/55 of their cLBP sample (Yağcı et al., 2010).

Despite the clinical relevance described above, this study (and others like it) have practical limitations that need to be addressed. For example, many of the QST protocols for the assessment of TS and CPM require expensive equipment and protocols that are technically complex and time consuming. As such, research involving QST is often carried out in specialized laboratories with highly trained technicians who can operate the equipment. This generally precludes protocols for the assessment of endogenous pain modulatory balance from being widely implemented in the clinical settings where patients present for pain treatments. Recent research has attempted to develop a more clinic-friendly "bedside" QST protocol for use in clinical trials and clinical practice (Wasan et al., 2019); however, it remains to be determined whether this will be an acceptable approach going forward. Another limitation of this research relates to the current lack of consensus regarding how best to quantify endogenous pain modulatory balance for inclusion in predictive models of future chronic pain outcomes. Endogenous pain modulation represents a complex interplay of top-down and bottom-up inhibitory and facilitatory processes (Staud, 2013). Yet in the laboratory, researchers tend to measure these processes separately using TS and CPM protocols. Moreover, TS and CPM tend to be examined separately in data analytic models, which arguably does not capture the interactive nature of endogenous pain modulation. Whether novel experimental and/or data analytic methods might be able to better approximate the dynamic interplay of pain inhibitory and facilitatory processes in research addressing endogenous pain modulatory processes is an area in need of greater attention.

Additionally, it is not completely clear if our measures of endogenous pain modulation (TS, CPM) are reflective of central sensitization, as opposed to peripheral sensitization. However, contemporary considerations on this topic generally suggest that TS and CPM are assess central sensitization (Starkweather et al., 2016). Lastly, our cLBP sample was comprised primarily of African Americans (62.2%), and the largest proportion of the sample (32.1%) fell within the lowest annual household income bracket ($0 - $19,999). Therefore, our study findings may not generalize well to Caucasian populations, or those with higher socioeconomic status (SES). Importantly, African Americans and those with low SES tend to be the most vulnerable to the deleterious effects of chronic pain (Janevic et al., 2017). Additional cLBP research focused specifically on African Americans and individuals with low SES seems warranted.

Specific Aim Three

The primary objective of aim 3 was to investigate the nature of the associations among pro-inflammatory acute phase reactants (CRP, fibrinogen, SAA), pro-inflammatory cytokines (TNF-α, IL-1α, IL-1β, IL-6), movement evoked pain (assessed by the SPPB) and pain severity at rest (assessed using the BPI-SF). We were also interested in understanding the association between the aforementioned pain severity variables and anti-inflammatory cytokines (IL-4 and IL-13) as well as Vitamin D. While several inflammatory markers differed significantly between cLBP and pain-free controls, inflammation did not appear to contribute substantially to pain severity exclusively among our cLBP participants. Our hypotheses were only partially supported. We found evidence of a positive bivariate relationship between pro-inflammatory IL-6

and pain at rest, and an inverse association between anti-inflammatory Vitamin D and pain severity at rest. Thus, only 2 models were considered for further analyses adjusting for covariates. It stands to reason that if significant associations were not observed in an unadjusted bivariate analysis (Pearson correlation) then no significant relationship would be present in a more stringent model adjusting for covariates (i.e., hierarchical regression analysis).

Despite finding associations among pro-inflammatory IL-6 and anti-inflammatory Vitamin D, our results were not robust enough to withstand the inclusion of covariates in models predicting pain at rest. Based on the current aim, it appears that factors outside of biological markers of inflammation, such as household income and depressive symptoms may be better predictors of pain severity at rest. Both of these findings are consistent with the literature. In fact, it was reported among several studies, in a systematic review, that aspects of socioecomic status (i.e., level of education, earned income etc.) were the strongest predictors of cLBP (Karran et al., 2020). Further, depressive symptoms have consistently been associated with cLBP. In fact, it was estimated that individuals living with depression are 60% more likely to develop cLBP at some point in their life (Robertson et al., 2017).

Prior studies have examined the associations between blood-based, biomarkers and pain severity in musculoskeletal conditions. One study in particular investigating case control differences in sleep disturbance and IL-6, with a small sample size (cLBP n = 25) found a significant association between the pro-inflammatory marker and cLBP (Heffner et al., 2011). A separate systematic review reported finding moderate evidence for the association among IL-6 and CRP and cLBP severity (van den Berg et al., 2018).

Our bivariate findings compliment the literature as we also discovered a relationship between IL-6 and pain severity at rest as assessed by the BPI-SF.

Though vitamin D did not emerge as a significant predictor of cLBP as we hypothesized, the hormone is well known for its role in musculoskeletal health, immune function and cell growth (Wintermeyer et al., 2016). Recently, Vitamin D has also been linked to nociceptive and inflammatory pain. Specifically, it has been suggested that low levels of circulating vitamin D is associated with various negative health outcomes including increased pain severity. Specifically, vitamin D inhibits the release of pro-inflammatory cytokines and suppresses the response of T-cells (Helde-Frankling & Björkhem-Bergman, 2017). It is likely that our non-significant finding is due in part to the well-known racial difference in circulating levels of vitamin D. Specifically, it has been well documented that Black individuals present with lower levels of the hormone mainly due to issues in absorption (Glover et al., 2012).

This study may have been affected by several limitations. Firstly, it must be noted that our cLBP sample was primarily composed of Non-Hispanic Black participants. This composition may have hindered our ability to see a significant relationship between vitamin D and either or both measures of pain severity. Furthermore, our sample does not reflect the numerical composition of America and may threaten the external validity of our findings. Future studies should stratify based on race to better understand the relationship between blood-based biomarkers, particularly vitamin D and chronic pain. It must also be noted that though some studies report a relationship between CRP and markers of inflammation found in the CNS (Felger et al., 2020), there is a lack of clarity regarding whether inflammatory biomarkers taken from the periphery actually reflect

central inflammation. Peripheral inflammatory diseases (e.g., arthritis) can actually activate central inflammation (Lampa et al., 2012). This suggests that the two types of inflammation – peripheral and central – share an important overlap. Additionally, "time of day" for the completion of the movement evoked pain task varied across participant. It is suggested that hormones such as melatonin and cortisol may potentially have analgesic effects. This could have contributed to a diurnal effect not accounted for in this study (Aviram et al., 2015; Bachmann et al., 2011).

Implications

Several clinical implications can be drawn from present study. It was demonstrated that an aspect of dynamic quantitative sensory testing, specifically TS of mechanical pain, may have the potential to predict movement evoked pain severity in patients with non-specific cLBP. Improving prediction accuracy in cLBP would allow for the allocation of appropriate health care resources to those patients who are high risk for greater pain severity in the near future (George et al., 2020).

Furthermore, it was observed in the current study that household income was a better predictor of cLBP severity as it accounted for a large percentage of the variance in our models. The role of social determinants of health (SDH) in cLBP is poorly understood. A recent systematic review that included 41 studies, across 17 countries, found 166 relationships between SDH and cLBP. It was reported that the most robust relationships were associated with socioeconomic status and education attainment. Future studies should focus on elucidating the role of SDH in cLBP.

Findings from the current study tentatively support the suggestion that endogenous pain modulation may be more relevant in predicting movement evoked pain,

while biomarkers of inflammation may be more relevant for predicting pain at rest in patients living with cLBP; however, more research is needed to confirm this hypothesis. In essence, the inclusion of endogenous pain modulatory balance assessment, as well as consideration of sociodemographic (e.g., annual household income, age) and clinical variables (e.g., depressive symptoms), may help improve the overall ability of a clinical assessment to identify people at greatest risk for poor cLBP outcomes (Timmerman et al., 2018).

Summary

The purpose of the present study was to identify endogenous pain modulatory processes and blood-based markers of inflammation that have the potential to predict pain severity at rest as well as movement- evoked pain in individuals living with non-specific cLBP. We were able to demonstrate group differences in TS at the left hand, but not the lumbar spine. We also failed to find CPM differences between cLBP and controls. We did however, find partial support for aim 2b as our cLBP group presented with significantly greater concentrations of circulating CRP, fibrinogen, IL-6 and contrary to the directionality of our hypothesis, IL-4. Also not in line with our hypothesis, we found significantly higher concentrations of pro-inflammatory IL-1α in the control group. TS of mechanical stimulation at the left hand and lumbar spine was found to predict movement- evoked pain; however sex, household income and depressive symptoms accounted for most of the variance in our models.

Our findings add to the existing body of literature attesting to the clinical relevance of endogenous pain modulatory balance for predicting cLBP outcomes, like movement-evoked pain. Although beyond the scope of this study, future research should

consider investigating the role of race/ethnicity, sex, gender identity to further understand if, and how, these important individual difference factors affect endogenous pain modulatory balance and its impact on cLBP.

Additional research should specifically focus on the time interval between the assessment of endogenous pain modulatory balance and subsequent movement-evoked pain. In the current study it was only 1 week, but additional research could address how long into the future movement-evoked pain can be predicted (e.g., 1 month, 1 year). Lastly, more studies are needed to further elucidate the extent to which specific aspects of endogenous pain modulation interact with other underlying biopsychosocial mechanisms that contribute to poor cLBP outcomes.

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Table 1. Participant Characteristics for cLBP and controls

	cLPB (N = 156)	Controls (N = 56)
Demographic Questionnaires	Mean SD or %	Mean SD or %
Age - Years	45.48(13.81)	38.96(12.95)
Gender		
Men	40.4%	48.2%
Women	59.6%	51.8%
Race		
Non-Hispanic White	37.8%	48.2%
Non-Hispanic Black	62.2%	51.8%
Annual Household Income		
$0 – 19,000	32.1%	10.7%
$20,000 – 34,999	14.1%	17.9%
$35,000 – 49,000	12.2%	21.4%
$50,000 – 74,999	16.7%	21.4%
$75,000 – 99,999	9.6%	7.1%
$100,000 – Greater	12.2%	16.1%
Clinical Characteristics		
BMI (weight/height^2)	31.31 (7.5)	28.77 (6.03)
Current Opioid Prescription (% yes)	13.5%	0%
Depressive Symptoms (CESD)	21.69 (8.9)	15.05 (5.5)
Movement- evoked pain (SPPB)	24.9 (26.8)	.35 (1.7)
Pain Severity (BPI-SF)	4.56 (2.4)	.13

Note: BMI= Body Mass Index; CES-D = Center for Epidemiological Studies Depression Scale; SPPB = Short Physical Performance Battery; BPI = Brief Pain Inventory.

Table 2. Temporal summation (TS) of mechanical pain and conditioned pain modulation scores across total sample.

TS of mechanical pain (512mn) at the hand		
1 Contact	21.86 (25.52)	
10 Contacts	39.19 (32.43)	t = 12.85 (p < .001)
TS of mechanical pain (512mn) at the lumbar spine		
1 Contact	27.75 (28.41)	
10 Contacts	45.54 (32.45)	t = 13.78 (p < .001)
Conditioned Pain Modulation at the forearm		
Baseline PPT	319.62 (137.15)	
Conditioned PPT	367.51 (174.85)	t = -6.23 (p < .001)
Conditioned Pain Modulation at the lumbar spine		
Baseline PPT	459.29 (238.66)	
Conditioned PPT	470.92 (253.55)	t = -1.50 (p = .109)

Note: 1 Contact = pain intensity rating in response to first contact with mechanical stimuli, 10 contact = pain intensity rating in response to 10 contacts with mechanical stimuli; Pain pressure scores are measured using kilopascals (kPa).

Table 3. Temporal summation (TS) of mechanical pain between cLBP and controls according to ratings of pain intensity on the 0–100 numeric rating scale.

	cLBP Mean (SD)	**Controls Mean (SD)**
TS of mechanical pain		
Left Hand		
1 Contact	25.31(27.51)	12.27(15.39)
10 Contacts	44.43 (33.54)	24.61 (23.98)
Lumbar Spine		
1 Contact	31.56 (30.72)	17.59 (17.27)
10 Contacts	49.97 (32.75)	33.82 (27.63)

Note: 1 Contact = pain intensity rating in response to first contact with mechanical stimuli, 10 contacts = pain intensity rating in response to 10 contacts with mechanical stimuli.

Table 4. Baseline and conditioned pressure pain thresholds (PPTs) used to calculate Conditioned pain modulation (CPM) at the forearm and lumbar.

	cLBP Mean (SD)	**Controls Mean (SD)**
Pain Pressure Thresholds		
Forearm		
Baseline	308.08 (135.66)	351.75 (137.38)
10 Contacts	344.76 (164.25)	430.90 (190.83)
Lumbar Spine		
1 Contact	410.99 (220.84)	593.84 (236.67)
10 Contacts	425.79 (227.50)	596.61 (229.14)

Note: Pain pressure scores are measured using kilopascals (kPa).

Table. 5 Associations among dynamic QST measures (TS and CPM) and Pain severity measures (Movement-evoked pain severity and pain severity at rest)

Variable	1	2	3	4	5
1. TS mechanical 512mN (lumbar)	-				
2. TS Mechanical 512m (hand)	.41**	-			
3. CPM (forearm)	-.13	-.07	-		
4. CPM (lumbar)	-.02	.21**	.08	-	
5. Movement- evoked pain (SPPB)	.26**	.16*	.06	.05	-
6. Pain severity at rest (BPI-SF)	.18*	.09	.03	.00	.43**

Note: $p < 0.05$, ** $p < 0.01$, (TS) TS temporal summation, mN= milliNewton, (CPM) Conditioned pain modulation, Kpa = kilopascal (SPPB) Short Physical Performance Battery- Movement- evoked pain, (BPI-SF) Brief Pain Inventory- Pain severity at rest

Table 6. Temporal summation of mechanical pain (lumbar spine**)** predicting movement- evoked pain severity in adults with Low back pain.

	B	**SEB**	**β**	R^2	ΔR^2	**ΔF**
Step 1				.21	–	10.59**
Sex	-9.39	4.03	-.17*			
Household Income	-2.01	.48	-.32**			
Age	.23	.14	.12			
Race	-7.79	4.11	-.14			
Step 2				.27	.05	3.4*
BMI	.04	.27	.01			
Current Opioid	6.7	5.87	.09			
Depressive Symptoms	.62	.23	.21**			
Step 3				.29	.02	4.55*
TS Mechanical (512mN)- lumbar	.21	.1	.15*			

$p < 0.05$, ** $p < 0.01$, BMI body mass index, TS temporal summation, mN= milliNewton, Sex coded: 1 = Male, 2 = Female Race coded: 1 = Black, 2 = White

Table 7. Temporal summation of mechanical pain (hand) predicting movement- evoked pain severity in adults with chronic low back pain.

	B	SEB	β	R^2	ΔR^2	ΔF
Step 1				.21	–	10.59**
Sex	-9.39	4.03	-.17*			
Household Income	-2.00	.48	-.32**			
Age	.23	.14	.12			
Race	-7.8	4.11	-.14			
Step 2				.27	.05	3.4*
BMI	.04	.27	.01			
Current Opioid	6.7	5.87	.09			
Depressive Symptoms	.62	.23	.21**			
Step 3				.29	.02	4.57*
TS Mechanical (512mN)- Hand	.20	.1	.16*			

* $p < 0.05$, ** $p < 0.01$, *BMI* body mass index, TS temporal summation, mN= MilliNewton, Sex coded: 1 = Male, 2 = Female, Race coded: 1 = Black, 2 = White,

Table 8. Associations among Acute Phase Reactants, Vitamin D, movement- evoked pain severity and pain severity at rest.

Variable	1	2	3	4	5
1. C Reactive Protein	-				
2. Fibrinogen	.13	-			
3. Serum Amyloid A	.62**	.17*	-		
4. Vitamin D	-.10	.17*	-.12	-	
5. Movement- evoked pain (SPPB)	.05	-.11	-.04	-.15	- -.43**
6. Pain severity (BPI-SF)	.11	-.15	.13	-.21**	

Note: Pearson correlation matrix, *p < 0.05, **p < 0.01, SPPB-Pain = Short Physical Performance Battery- Movement- evoked pain, BPI-SF = Brief Pain Inventory – Short Form

Table 9. Associations among pro- inflammatory cytokines, movement- evoked pain severity and pain severity at rest.

Variable	1	2	3	4	5
1. TNF alpha	-				
2. IL-1 alpha	.40**	-			
3. IL-1 beta	-.04	.28**	-		
4. IL-6	.09	.03	.07	-	
5. Movement- evoked pain (SPPB)	.06	.07	-.04	-.15	-
6. Pain severity (BPI-SF)	.07	.13	-.02	.17*	-.72**

Note: Pearson correlation matrix, *p < 0.05, **p < 0.01, SPPB-Pain = Short Physical Performance Battery- Movement- evoked pain, BPI-SF = Brief Pain Inventory – Short Form

Table 10. Associations among anti-inflammatory cytokines, movement- evoked pain severity and pain severity at rest.

Variable	1	2	3	4
1. IL-4	-			
2. IL-13	-.04	-		
3. Movement- evoked pain (SPPB)	-.04	.03	-	
4. Pain severity (BPI-SF)	-.05	.02	.72**	-

Note: Pearson correlation matrix, *p < 0.05, **p < 0.01, SPPB-Pain = Short Physical Performance Battery- Movement- evoked pain, BPI-SF = Brief Pain Inventory – Short Form

Table 11. IL-6 predicting pain severity at rest (BPI) in adults with Low back pain.

* $p < 0.05$, ** $p < 0.01$, BMI body mass index, Sex coded: 1 = Male, 2 = Female, Race coded: 1 = Black, 2 = White,

	B	**SEB**	**β**	R^2	ΔR^2	**ΔF**
Step 1				.2	–	9.36**
Sex	-.11	.37	-.02			
Household Income	-.21	.04	-.37**			
Age	.02	.01	.1			
Race	-.62	.37	-.13			
Step 2				.31	.11	8.15**
BMI	.00	.02	.01			
Current Opioid	.67	.51	.1			
Depressive Symptoms	.09	.02	.33**			
Step 3				.32	.00	.83
IL-6	.53	.58	.08			

Table 12. Vitamin D predicting pain severity at rest (BPI) in adults with Low back pain.
*Note: * $p < 0.05$, ** $p < 0.01$, BMI* = Body Mass Index, Sex coded: 1 = Male, 2 = Female, Race coded: 1 = Black, 2 = White

	B	**SEB**	**β**	R^2	ΔR^2	**ΔF**
Step 1				.2	–	9.36**
Sex	-.11	.37	-.02			
Household Income	-.21	.04	-.37**			
Age	.02	.01	.1			
Race	-.62	.37	-.13			
Step 2				.31	.11	8.15**
BMI	.00	.02	.01			
Current Opioid	.67	.51	.1			
Depressive Symptoms	.09	.02	.33**			
Step 3				.33	.01	2.82
Vitamin D	-1.4	.83	-.13			

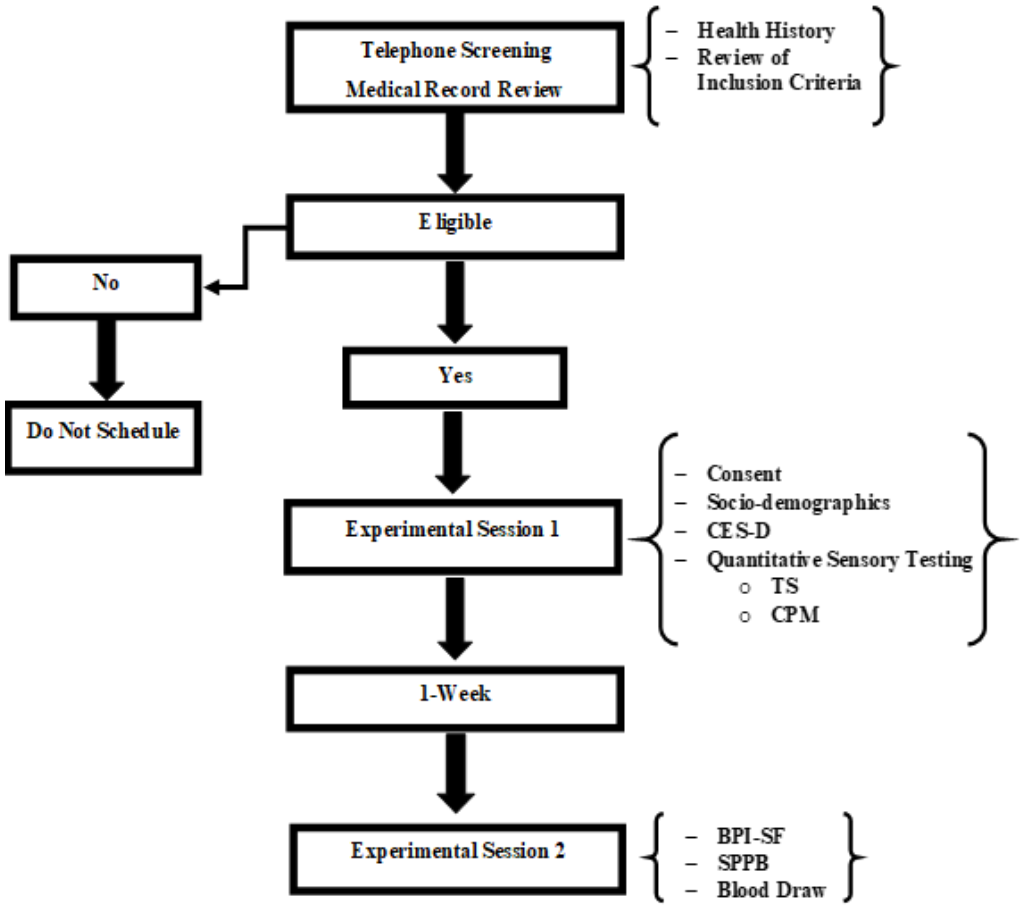

Figure 1. Timeline for participant matriculation through study protocol. *Note*: CES-D = Center for Epidemiological Studies – Depression, TS = Temporal Summation of Mechanical Stimulation (512mn), Conditioned Pain Modulation, BPI-SF = Brief Pain Inventory- Short Form, SPPB = Short Physical Performance Battery.

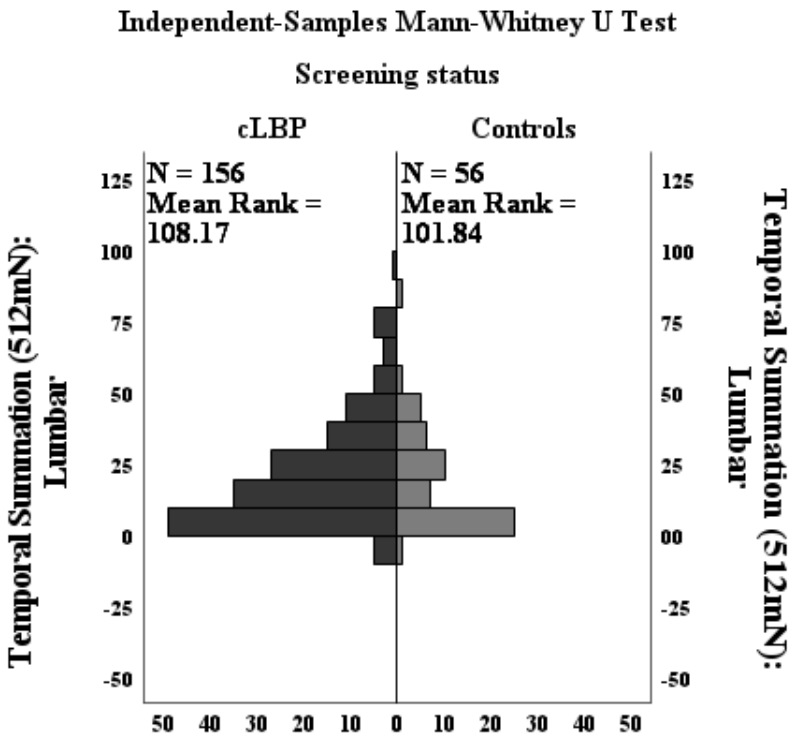

Figure 2. Sample distributions for temporal summation at the lumbar. Man- Whitney U Tests were conducted to compare differences between cLBP and controls. *Note*: mN = Millinewton

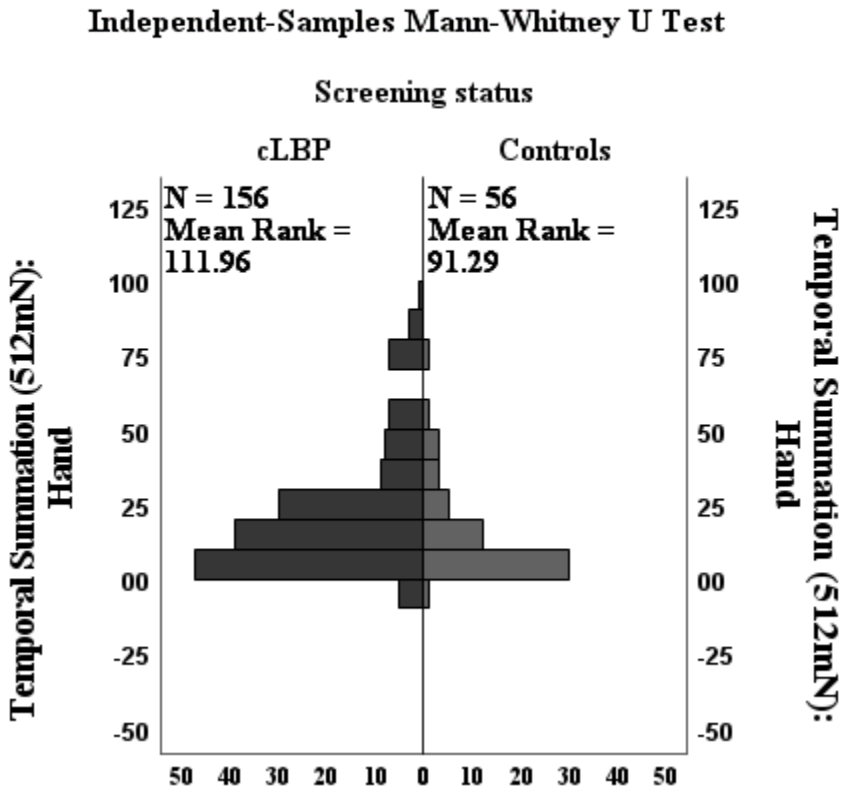

Figure 3. Sample distributions for temporal summation at the left hand. Man- Whitney U Tests were conducted to compare differences between cLBP and controls. *Note*: mN = Millinewton

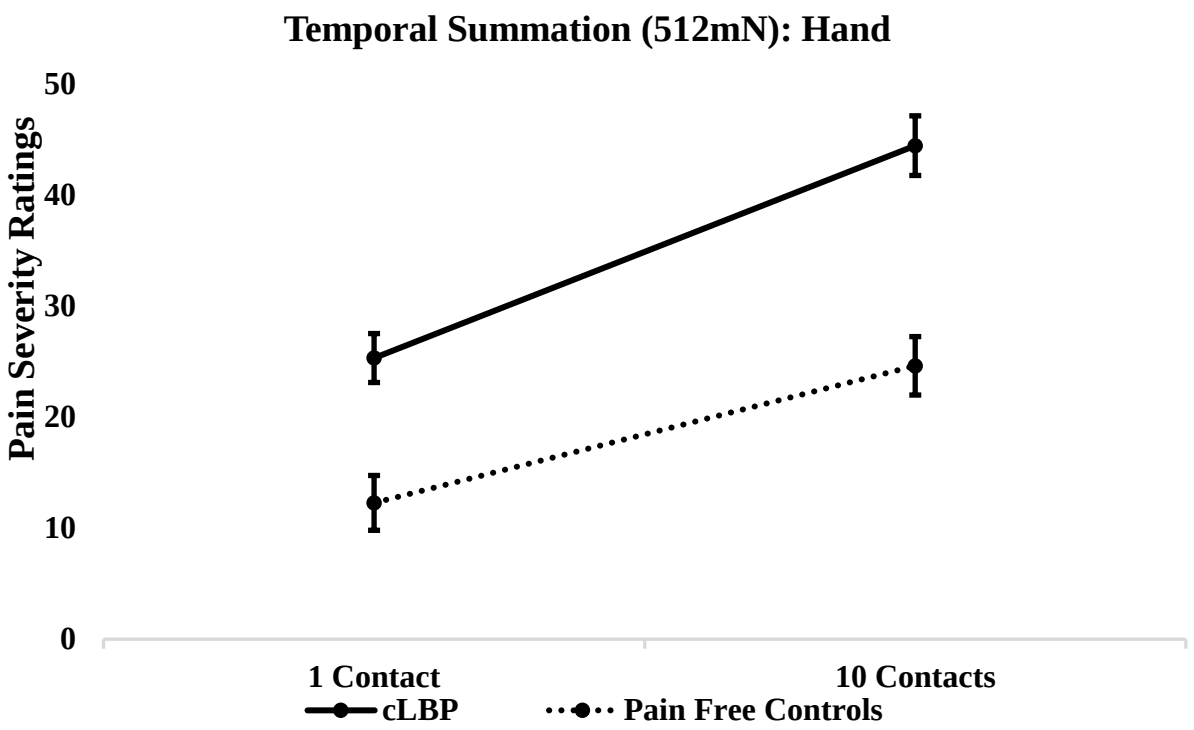

Figure 4. Pain ratings, at the left hand, in response to mechanical stimulation at first contact and after 10 contacts for cLBP and controls. *Note*: mN = Millinewton

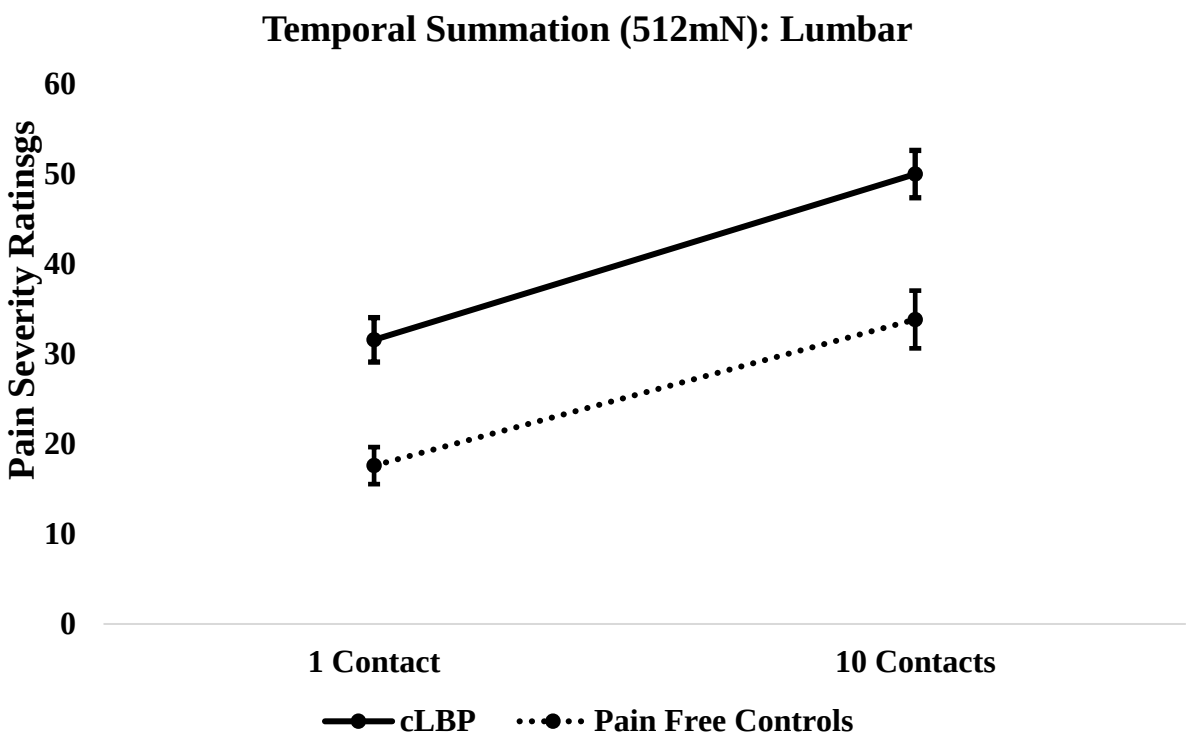

Figure 5. Pain ratings, at the lumbar spine, in response to mechanical stimulation at first contact and after 10 contacts for cLBP and controls. *Note*: mN = Millinewton.

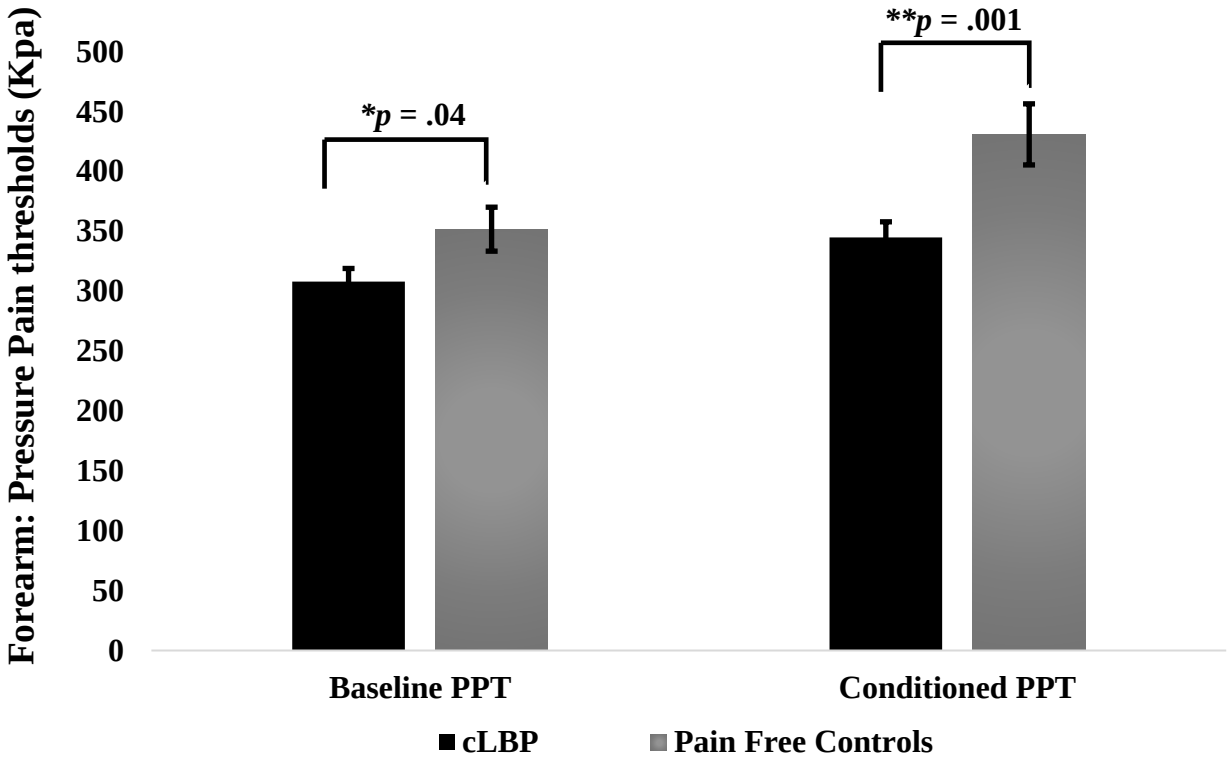

Figure 6. (PPTs) Pain pressure thresholds (forearm) at baseline and conditioning for cLBP and controls. *Note:* *p < .05, **p <.001, Kpa = Kilopascal.

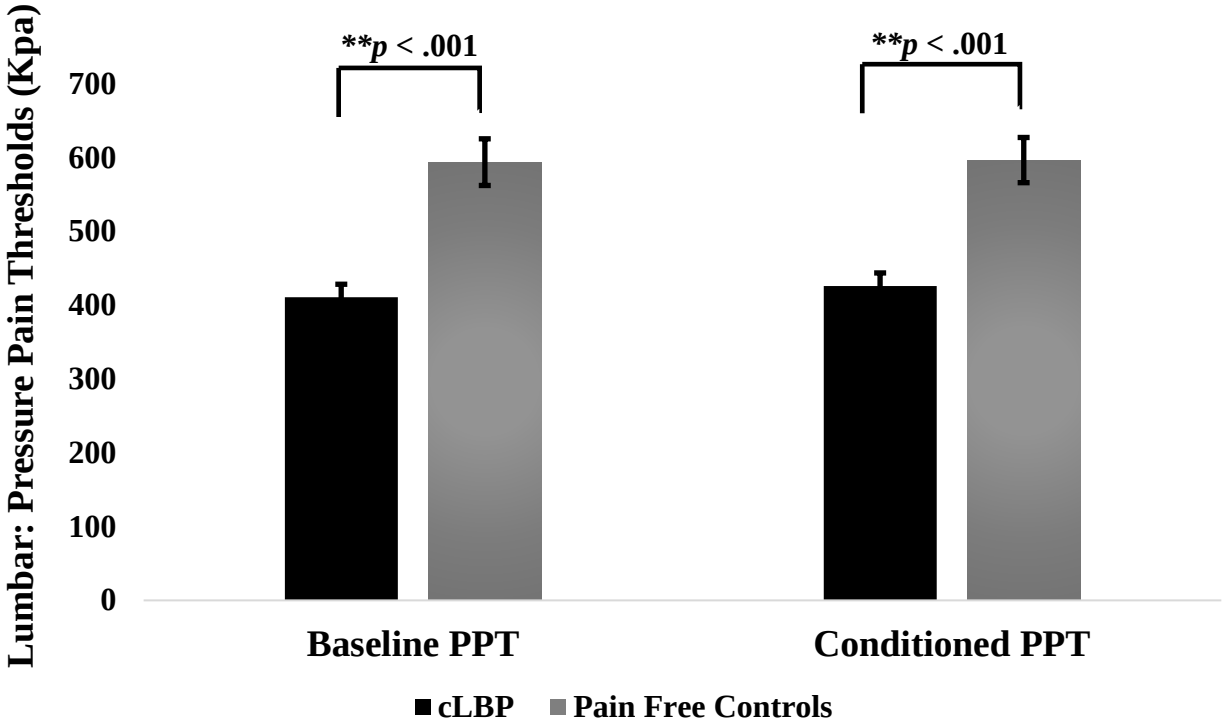

Figure 7. Pain pressure thresholds (lumbar) at baseline and conditioning for cLBP and controls. *Note:*$p < .05$, **$p < .001$, Kpa = Kilopascal.

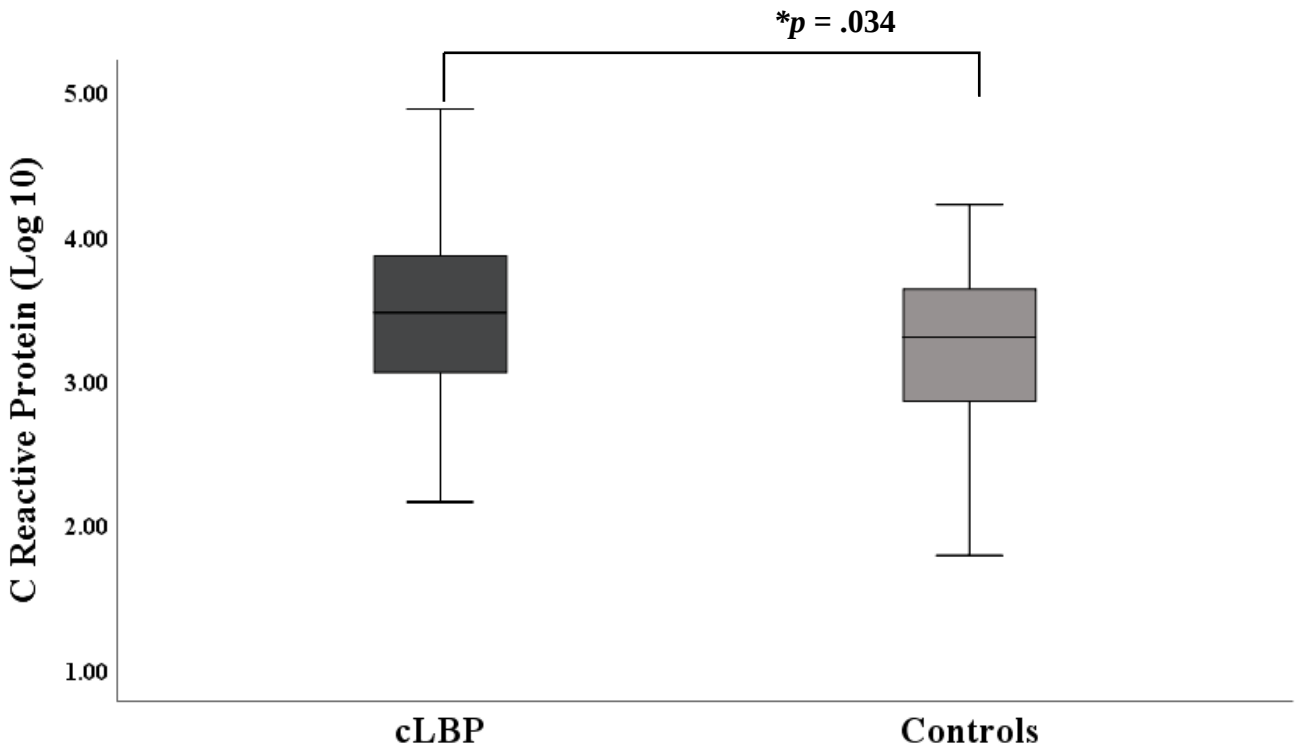

Figure 8. Graph comparing the means of circulating levels of (CRP) C reactive protein in cLBP and controls. *Note:*$p < .05$, **$p < .001$, ng/mL = Nanogram per milliliter.

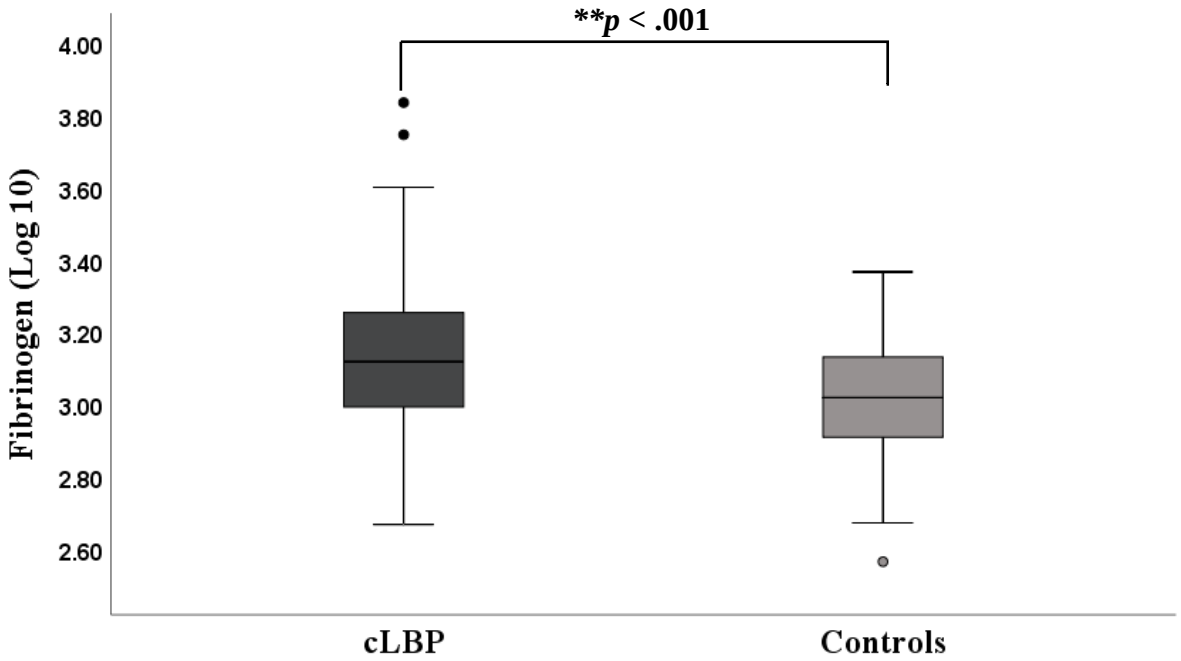

Figure 9. Graph comparing the means of circulating levels of fibrinogen in cLBP and controls.
*Note:*p* < .05, ** *p* < .001, ng/mL = Nanogram per milliliter.

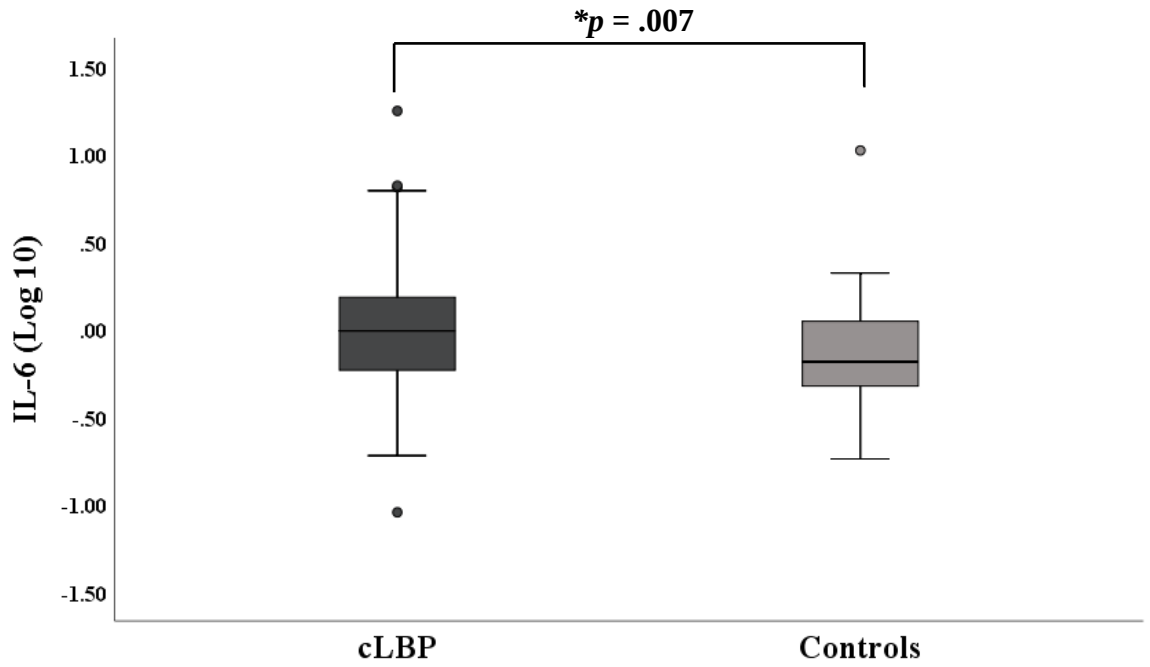

Figure 10. Graph comparing the means of circulating levels of (IL-6) interleukin 6 in cLBP and controls. *Note:*p* < .05, **p* < .001, pg/mL = Picogram per milliliter.

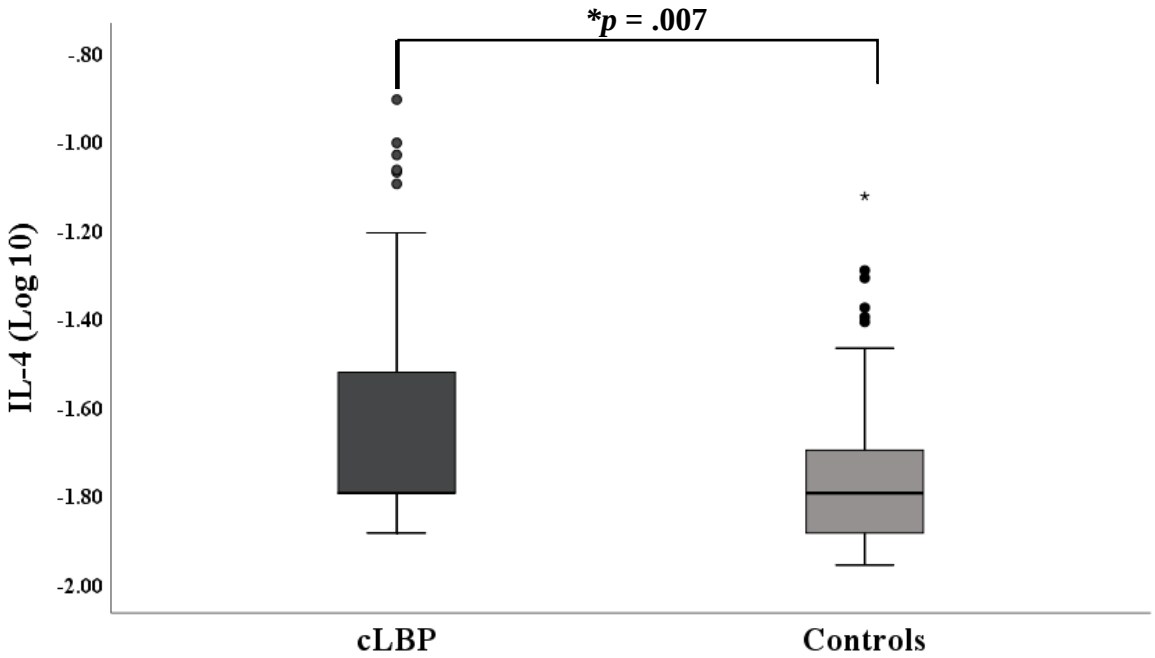

Figure 11. Graph comparing the means of circulating levels of (IL-4) interleukin 4 in cLBP and controls. *Note:*p* < .05, **p* < .01, pg/mL = Picogram per milliliter.

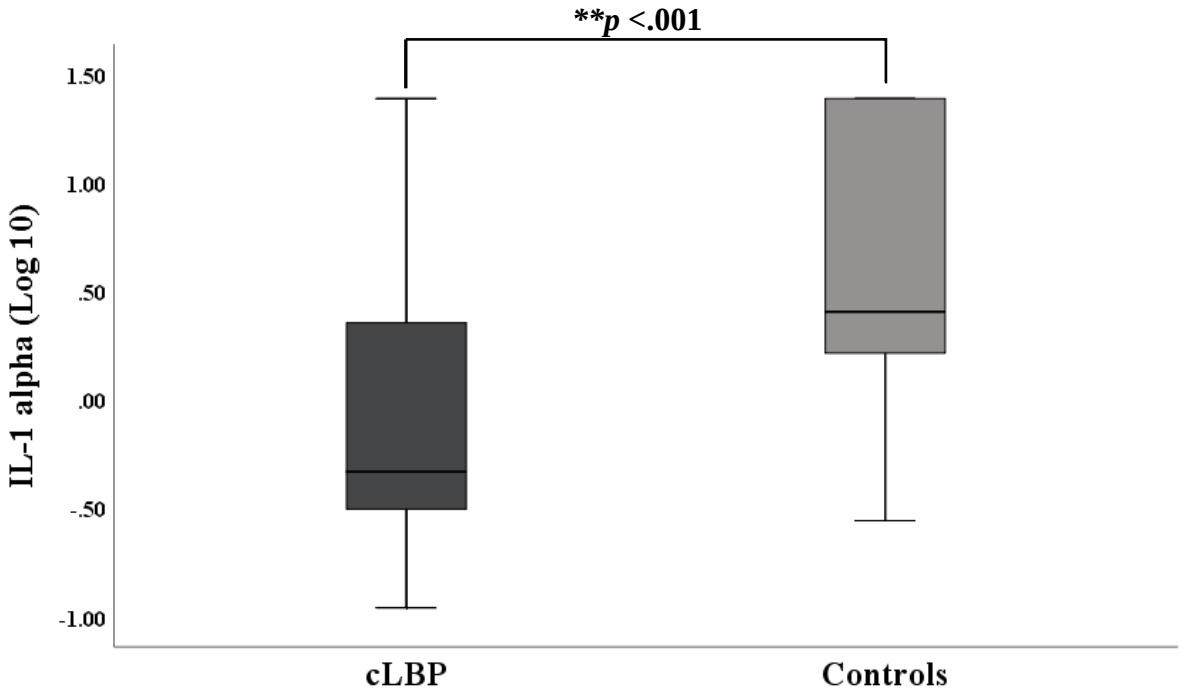

Figure 12. Graph comparing the means of circulating levels of (IL-1a) interleukin 1 alpha in cLBP and controls. *Note*: *$p < .05$, **$p < .001$. pg/mL = Picogram per milliliter.

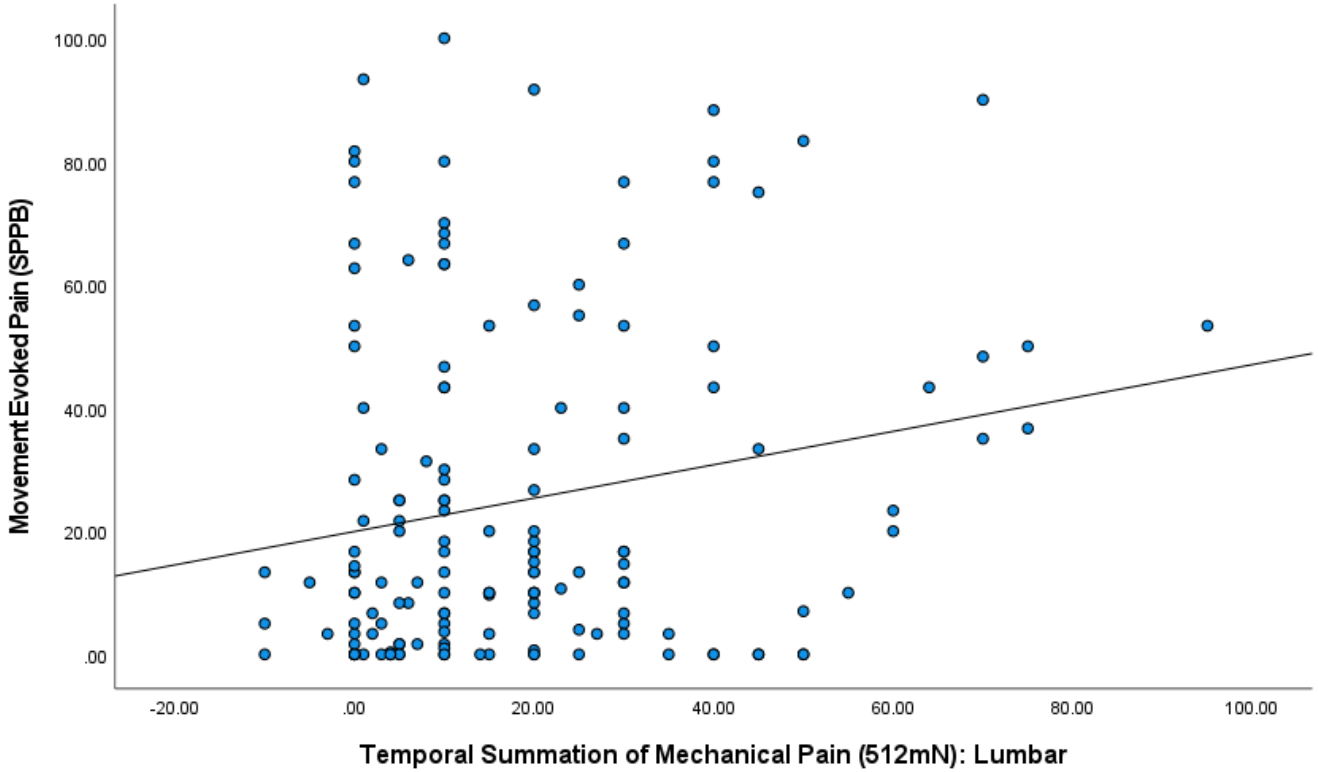

Figure 13. Correlation between temporal summation at the lumbar and movement- evoked pain severity using the (SPPB) Short Physical Performance Battery. *Note*: mN = Millinewton.

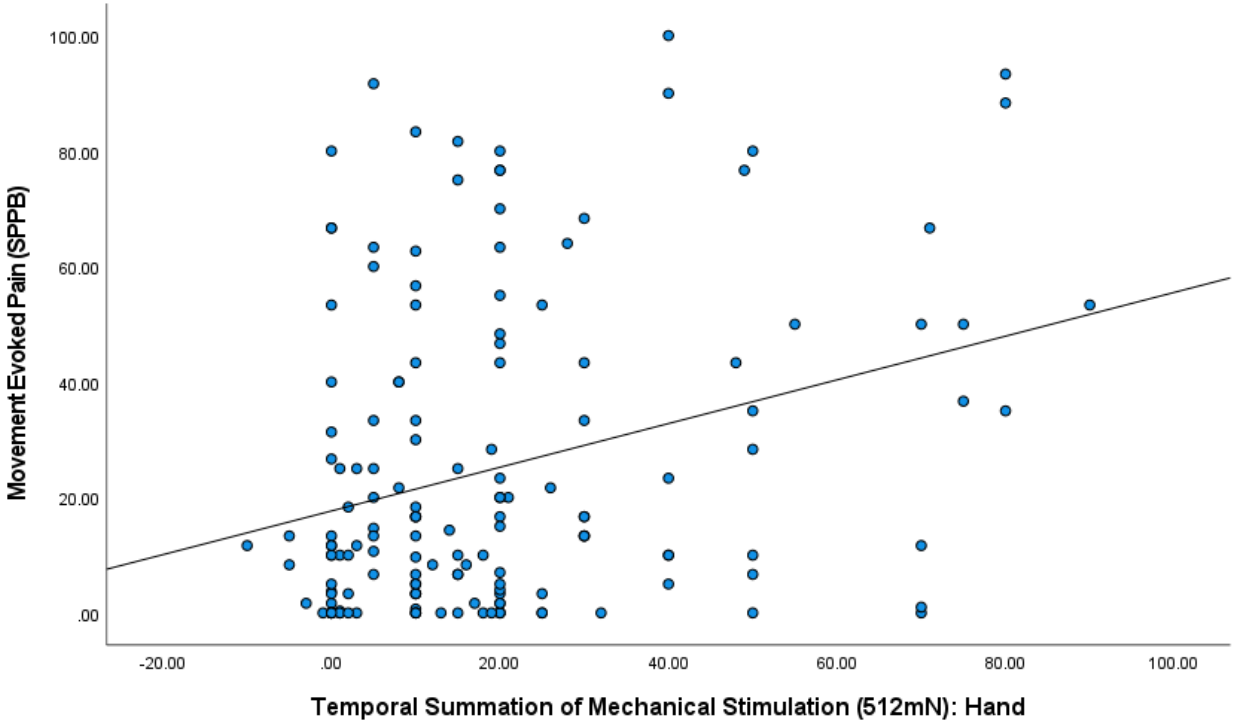

Figure 14. Correlation between temporal summation at the lumbar spine and movement- evoked pain severity using the (SPPB) Short Physical Performance battery. *Note*: mN = Millinewton.

APPENDIX A

UAB OFFICE OF THE IRB APPROVAL FORMS

THE UNIVERSITY OF ALABAMA AT BIRMINGHAM
Office of the Institutional Review Board for Human Use

470 Administration Building
701 20th Street South
Birmingham, AL 35294-0104
205.934.3789 | Fax 205.934.1301 |
irb@uab.edu

APPROVAL LETTER

TO: Goodin, Burel R

FROM: University of Alabama at Birmingham Institutional Review Board
Federalwide Assurance # FWA00005960
IORG Registration # IRB00000196 (IRB 01)
IORG Registration # IRB00000726 (IRB 02)
IORG Registration # IRB00012550 (IRB 03)

DATE: 05-Feb-2021

RE: IRB-170119003
Racial and Socioeconomic Differences in Chronic Low Back Pain

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The IRB reviewed and approved the Personnel Amendment submitted on 04-Feb-2021 for the above referenced project. The review was conducted in accordance with UAB's Assurance of Compliance approved by the Department of Health and Human Services.

Type of Review: Expedited
Expedited Categories: b2,
Determination: Approved
Approval Date: 05-Feb-2021
Expiration Date: 12-Jan-2022

The following apply to this project related to informed consent and/or assent:

- Waiver (Partial) of HIPAA

Documents Included in Review:

- IRB PERSONNEL EFORM

Name
Overstreet, Demario

Email	Mario22@uab.edu
Department	Psychology

Principal Investigator ☐

Start Date: 30-Mar-2017

End Date:

* Role: Other Personnel

Certifications

Certification	Begin	End
IRB Initial Training - CITI	03-Oct-2016	03-Oct-2019
Financial Conflict of Interest	23-Feb-2017	23-Feb-2021
IRB ICH-GCP	03-Mar-2018	03-Mar-2021
IRB CITI 2018 Refresher Training	06-Oct-2019	06-Oct-2022
Financial Conflict of Interest in Research - 4th Yr Refresher	03-Aug-2020	03-Aug-2024

Degree

Training certificates

Indicate the following activities in which this individual will be involved. If this individual is not involved in any of these activities, he/she should not be listed as key personnel on the IRB submission:

- ☐ Involved in the design of the human subjects research
- ☑ Obtaining informed consent*
- ☑ Interacting/intervening with participants for research purposes
- ☑ Obtaining private identifiable data or identifiable specimens
- ☐ Administering investigational (non-FDA-approved) product (e.g., drug, device, or biologic)
- ☐ Named on the FDA 1572 or device agreement*
- ☐ Required to complete sponsor's conflict of interest form*

Yes Is the individual named above "responsible" for the design, conduct, or reporting of the research?

Yes Will the individual named above be involved in explaining the study, risk-benefit, and/or alternatives to potential participants?

No Does this individual have a financial interest in this project (see below for definition)?

Please note: Individuals in a role of PI, Co-PI, and/or Faculty Advisor, as well as anyone who is involved in an activity marked with an asterisk, or answers yes to one of the additional questions related to responsible personnel above must file a disclosure of financial interests and complete training requirements of the UAB CIRB .