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Faculteit Revalidatiewetenschappen

master in de revalidatiewetenschappen en de kinesitherapie

Masterthesis

The Relationship Between Heart Rate Variability and Pain Processing in Individuals with Chronic Non-Specific Low Back Pain

Melanie Gavriilakis
Lynn Theunissen

Scriptie ingediend tot het behalen van de graad van master in de revalidatiewetenschappen en de kinesitherapie, afstudeerrichting revalidatiewetenschappen en kinesitherapie bij musculoskeletale aandoeningen

PROMOTOR :
Prof. dr. Annick TIMMERMANS

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2025

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We would also like to express our sincere appreciation to the participants for their involvement in the study. Their contribution was essential to the success of the study.

Lastly, we want to thank our families and partners for their unwavering support.

RESEARCH CONTEXT

This master's thesis belongs to the domain of Chronic Musculoskeletal Pain Rehabilitation and focuses on individuals with chronic non-specific low back pain (CNSLBP), defined as musculoskeletal discomfort in the lumbar area persisting for over three months. CNSLBP is a major contributor to disability, pain and diminished quality of life across all age groups.

The thesis is situated within ongoing research projects, specifically the Techno-HIT Trial ('Technology-Supported High-Intensity Training in Chronic Non-Specific Low Back Pain') and the HIT-BACK-HEART study ('Effects of High Intensity Training on Mechanistic Pain Profiling and Heart Rate Variability in Persons with Chronic Low Back Pain'). The Techno-HIT Trial is funded by the Research Foundation Flanders (FWO_TBM) under project number T000822N. The HIT-BACK-HEART project is supported by the Special Research Fund (BOF) of Hasselt University (grant number: BOF23DOC40).

This preliminary analysis of the larger longitudinal study (HIT-BACK-HEART) investigates the relationship between heart rate variability (HRV) and conditioned pain modulation (CPM) in individuals with CNSLBP. HRV and CPM are physiological markers associated with the functioning of the autonomic nervous system and endogenous pain inhibitory pathways, respectively. Understanding the interaction between these systems may contribute to a better understanding of the complex biopsychosocial mechanisms underlying CNSLBP.

The initial research question and protocol were developed by the supervisors and were further adapted to align with the specific aims of this thesis. This final protocol was submitted to the Medical Ethics Committee of the University of Hasselt. Recruitment was conducted by informing participants through their physician at the University Hospital Antwerp and Jessa Hospital.

This study, including the academic writing process, was carried out by second-degree master's students Melanie Gavriilakis and Lynn Theunissen, under the supervision of promotor Prof. Dr. Annick Timmermans and co-promotor Dr. Jonas Verbrugghe. Both students contributed equally to the preparation and analysis for this trial. For academic writing and paraphrasing the information from scientific articles, we used ChatGPT.

The Relationship Between Heart Rate Variability and Pain Processing in Individuals with Chronic Non-Specific Low Back Pain.

ABSTRACT

Background: Chronic non-specific low back pain (CNSLBP) is a prevalent condition associated with reduced heart rate variability (HRV) and impaired pain modulation. Thermal quantitative sensory testing (QST) is used to evaluate altered sensory perception and indicates central sensitization as a major contributor to pain amplification in CNSLBP. However, evidence on the relationship between HRV and pain responses in these patients remains limited and inconclusive.

Objectives: The study examined the relationship between HRV and conditioned pain modulation (CPM) in individuals with CNSLBP.

Methods: This cross-sectional study utilized data from a larger multicenter, double-blinded randomized clinical trial (NCT06491121) with CNSLBP participants aged 18 to 65 years. HRV and QST were assessed using standardized protocols. Their associations were explored through multiple simple linear regression (SLR) analyses.

Results: No associations were found between HRV metrics (time and frequency domains) and CPM responses. In a sample of 11 participants with CNSLBP, subsequent SLR analyses confirmed no significant predictive relationships of root mean square of successive differences (RMSSD), low-frequency (LF) and high-frequency (HF) for CPM outcome in persons with CNSLBP, with RMSSD showing $\beta = 0.14$ (95% *CI* [-0.86, 1.15], $p = 0.754$), LF $\beta = 0.13$ (95% *CI* [-0.52, 0.79], $p = 0.659$) and HF $\beta = 0.17$ (95% *CI* [-0.44, 0.79], $p = 0.541$).

Conclusion: This study found no relationship between HRV parameters and CPM in individuals with CNSLBP. Larger-scale studies are necessary to strengthen the evidence base.

Keywords: chronic non-specific low back pain, vagal modulation, quantitative sensory testing, autonomic nervous system.

1. INTRODUCTION

Chronic low back pain (CLBP) is a global disease affecting all ages (Airaksinen et al., 2006). The term chronic refers to the presence of musculoskeletal discomfort for over three months (Last & Hulbert, 2009). CLBP is not only characterized by pain, but also by muscle tightness or stiffness in the lower back, i.e. the area below the ribcage and above the buttocks (den Bandt et al., 2019; Koes et al., 2006). Furthermore, among individuals diagnosed with CLBP, approximately 85% experience chronic 'non-specific' low back pain (CNSLBP), which refers to the fact that no pathoanatomical etiology is identified (Neelapala et al., 2020). CNSLBP not only causes physical discomfort but also affects psychological well-being and social functioning (den Bandt et al., 2019; Kamper et al., 2015). Recognition of the multifactorial nature of CNSLBP highlights the formulation of the biopsychosocial model, which emphasizes the interplay between biological, psychological and social factors (Kamper et al., 2015). Within this framework, increasing attention is directed towards the cardiac autonomic nervous system, specifically heart rate variability (HRV), as a biological mechanism influenced by psychological and social factors such as stress, emotional regulation and social interactions (Kim et al., 2018; Shahrestani et al., 2015; Williams et al., 2015).

Indeed, CNSLBP is associated with reduced HRV (Bandeira et al., 2021), referring to the fluctuations in time intervals between consecutive heartbeats (Turcu et al., 2023). HRV is closely linked to endogenous pain modulation, playing a major role in the onset and persistence of chronic pain (Forte et al., 2022; Van Den Houte et al., 2018). In persons with CNSLBP, reduced HRV is further characterized by decreased parasympathetic activation and sympathetic dominance (Bandeira et al., 2021). Additionally, HRV serves as an indicator of the body's self-regulatory capacity, with lower HRV signaling self-regulatory exhaustion (Allen et al., 2018; Koenig et al., 2016; Reynard et al., 2011).

As mentioned above, there is a link between autonomic functioning and pain processing. Autonomic dysfunction, indicated by reduced HRV, can lead to impaired emotional and pain response regulation (Bandeira et al., 2021; Gibler & Jastrowski Mano, 2021; Greenspan et al., 2013; Mostoufi et al., 2012). Although individual HRV components such as low-frequency (LF) and high-frequency (HF) power have been examined in relation to pain sensitivity, findings remain inconsistent. For instance, higher LF power is associated with higher pain thresholds and lower pain unpleasantness (Appelhans & Luecken, 2008; Tracy et al., 2018). However,

the interpretation of LF remains controversial, as it may reflect both sympathetic and parasympathetic influences, complicating its use as a clear biomarker (Goldstein et al., 2011; Laborde et al., 2017). Conversely, HF power, typically associated with parasympathetic activity, does not consistently predict pain sensitivity measures (Appelhans & Luecken, 2008). Similarly, some studies find no significant association between resting HVR indices such as standard deviation of normal-to-normal intervals (SDNN) and pain responses (Meeuse et al., 2013) or between HF power and cold pressor tests (CPT) results (Appelhans & Luecken, 2008).

Research on pain modulation highlights the role of psychological and social factors in amplifying pain signals through the central nervous system (Zusman, 2002). Thermal quantitative sensory testing (QST) objectively measures somatosensory function and pain processing (Neblett et al., 2024). It evaluates thermal sensory function, offering insights into potential mechanisms influencing chronic pain (Neblett et al., 2024; Weaver et al., 2021). This aids in identifying sensory deficiencies such as hypoesthesia or hypoalgesia, hyperalgesia or allodynia (Weaver et al., 2021). Research using thermal QST shows that individuals with CNSLBP experience significantly lower cold pain thresholds both at the site of pain and at remote locations, reflecting altered pain processing. This includes mechanisms such as reduced conditioned pain modulation (CPM), reflecting impaired endogenous pain inhibition (Hübscher et al., 2014). Moreover, central sensitization is notable in patients with persistent low back pain, which may be an important risk factor for CNSLBP (Giesecke et al., 2004; Weaver et al., 2021). Thus, thermal QST is valuable for identifying altered sensory perception and heightened pain sensitivity in CNSLBP patients, contributing to the understanding of the physiological mechanisms underlying this chronic pain condition (Meints et al., 2019).

Despite growing evidence supporting a link between autonomic functioning and pain processing, the potential of HRV to serve as a reliable physiological marker of pain modulation remains uncertain. Various HRV components, such as LF and HF power, have been explored in relation to pain sensitivity, yet the findings are inconsistent. Consequently, further investigation is necessary to clarify whether specific HRV parameters are associated with altered pain modulation. Therefore, this study aims to evaluate the relationship between HRV in the time and frequency domains and CPM in individuals with CNSLBP.

2. METHOD

2.1 Study design & study setting

The data used in this preliminary cross-sectional analysis originated from a larger multicenter, double-blinded, three-armed parallel-group clinical trial (registration number: NCT06491121). Collaborating institutions included the University Hospital Antwerp (UZA), Jessa Hospital Hasselt (Jessa), University of Hasselt (UH) and University of Antwerp (UA).

Participation in the study required written informed consent from each participant. Ethical approval for the study protocol was obtained from FAGG (consolidated opinion Federal Agency for Medicine and Health Products (AMHP) (Ref. CIV-23-12-045154)) and the respective medical ethics committees of UZA, Jessa, UH and UA.

2.2 Inclusion and exclusion criteria

Eligible participants for this study were individuals aged between 18 and 65 years who had received a diagnosis of CNSLBP. This was defined as chronic primary musculoskeletal pain located below the costal margin and above the inferior gluteal folds. Table 1 contains all inclusion and exclusion criteria.

Table 1

Inclusion and Exclusion Criteria

Inclusion	Exclusion
18-65 years old	Spinal fusion surgery
Speak Dutch	Musculoskeletal and/or chronic disorder aside from CLBP
Non-specific CLBP for >12 weeks	Severe comorbidities (e.g., paresis, sensory disturbances by neurological causes, diabetes mellitus, rheumatoid arthritis)
≥20% on the MODI	Pregnancy
	Ongoing compensation claims
	Inability to attend regular therapy appointments

Abbreviations: CLBP = chronic low back pain, MODI = Modified Oswestry Disability Index

2.3 Recruitment

Participants deemed eligible for the study during their consultation at UZA/Jessa were informed about the study by their physician. If they expressed interest, the physician provided them with a study flyer and a consent form for further contact (via email and/or telephone, depending on their preference). The researchers then contacted the potential participant within two to seven days to answer initial questions, review the inclusion criteria and provide the informed consent form (available online or as a hard copy, depending on preference). Participants who signed and returned the informed consent form within two weeks were contacted for potential enrollment in the study.

2.4 Outcome measures

Sociodemographic variables, such as age and gender were included in the analysis.

2.4.1 Heart rate variability

HRV is a non-invasive method for analyzing cardiac autonomic function (Turcu et al., 2023). Participants followed a 30-minute standardized protocol in a low-stimulation, quiet room with a controlled temperature of 20 to 24°C and a humidity between 40 and 60%. To ensure comparability of test results, the examiner read standardized and consistent instructions aloud during the test. The examiner first verified the laboratory environment conditions using a standardized checklist (Catai et al., 2020). After the participant entered the room, the examiner confirmed compliance with the participant's conditions by questioning them. The participant and laboratory environment conditions are outlined in Tables 2 and 3 (Catai et al., 2020).

A H10 heart rate band monitor (Polar Electro, Finland) was placed around the participant's chest. The Ignite wristwatch (Polar Electro, Finland) was positioned on the examiner's desk rather than being placed on the participant's wrist. This eliminated the inconvenience of starting and stopping the watch on the patient's wrist, as discomfort could potentially affect HRV. The sensor was synchronized with the wristwatch to record beat-to-beat intervals (R–R). If signal issues arose, the Polar H10 sensor was repositioned to the left side to obtain reliable measurements and accurate registration. After placing the sensor and watch, the examiner informed the participant that sleeping or engaging in conversations was not permitted during the measurement. The participant was instructed to lie quietly in supine position on the treatment table. The recording began at the start of the session. Any occurrence of sneezing, coughing, movement or falling asleep (e.g. snoring or a sudden change in head position) during the measurement was carefully noted, as these events could cause disruptions in the data. However, data analysis was performed exclusively on the final 7 minutes, during which the patient remained relaxed and breathed spontaneously. This allowed the body to reach a stable resting rate, ensuring that the measurement accurately reflected the proper autonomic balance. After this, the examiner stopped the protocol and verbally informed the participant accordingly. The examiner removed the sensor, completing the test procedure. Moreover, a minimal degree of artifact correction was applied to the R-R data obtained

during the measurements using Kubios HRV software. The time-domain parameter assessed was RMSSD, while the frequency-domain parameters measured were HF (ms^2) and LF (ms^2) (Catai et al., 2020).

Table 2

Conditions for The Laboratory Environment

Conditions for the laboratory environment	
Calm Environment	The room should be quiet and still
Consistent Timing	The measurement should be conducted at the same time as the previous one
Room Temperature	The temperature should be between 20-24 degrees Celsius
Time of Day	The time of day should be recorded to account for potential variations in circadian rhythm and their impact on heart rate variability

Table 3

Conditions for The Participants

Conditions for the participants	
Hydration	The participant should drink sufficient water
Restrictions in the last 48 hours	No alcohol
Restrictions on the day of the test	No caffeine, no nicotine, no soft drinks, no energy drinks, no chocolate
Physical Activity Restrictions in the Last 24 Hours	No intense physical activity (>70% of maximum heart rate). Meaning the participant will still be able to hold a conversation without getting out of breath.

2.4.2 Dynamic quantitative sensory testing

Dynamic QST is a non-invasive method widely used in pain diagnostics to evaluate peripheral and central somatosensory systems (Rolke et al., 2006; Uddin & MacDermid, 2016). By following a standardized 30-minute test protocol, it was possible to identify patterns related to sensory loss and adaptations (Mücke et al., 2021). Advanced thermal stimulation was conducted using a Peltier-based computerized thermal stimulator from the TSA-2 device (Medoc Ltd., Ramat-Ishay, Israel), to perform QST measurements. The TSA-2 evaluated small nerve fiber function and provided information about the physiological and psychological mechanisms underlying pain and sensation responses throughout thermal stimulation (Sergooris et al., 2023). All participants completed the standardized QST protocol, which measured CPM. A thermode measuring 30 x 30 mm was used to apply the cold and heat stimuli. All measurements were taken at a base temperature of 32°C.

2.4.2.1 Conditioned pain modulation

First, a visual analogue scale (VAS) search protocol was used at the anterior aspect of the dominant forearm to ascertain the temperature corresponding to a VAS score of 60 out of 100. The thermode continuously warmed up, reaching temperatures between 39 and 50°C from the starting point of 32°C. For each stimulus intensity, participants were required to rate their pain level on a scale from zero, meaning "no pain", to 100, meaning "worst imaginable pain". The thermode warmed up to a higher or lower temperature based on the participant's pain level until they reported a VAS of 60 out of 100. A maximum of 45°C was determined to be the temperature corresponding to a VAS of 60 in the dynamic QST protocols for assessing CPM.

Then, a dual-thermode program was used to assess CPM (Levy et al., 2018). This assessment investigated the endogenous analgesic system by observing changes in perceived pain in one body region (the anterior side of the dominant forearm) in response to pain induced in another body region (the anterior side of the non-dominant forearm) (Rolke et al., 2006; Sergooris et al., 2023). As the test stimulus, a heat stimulus was applied twice to the anterior aspect of the dominant forearm. The first application occurred before the conditioning heat stimulus and the second application occurred after it, with an interstimulus interval of 10

seconds. After the first test stimulus was applied, a heat stimulus was applied to the anterior aspect of the non-dominant wrist as the conditioning stimulus. A VAS with a range of zero to 100 measured pain intensity; zero represented "no pain" and 100 represented "worst imaginable pain". The difference in pain intensity at the dominant wrist between the test stimulus during the conditioning and the test stimulus during the stand-alone stimulus was calculated.

2.5 Data-analysis

Data were analyzed through JMP Pro (version 17.0). A priori power analysis indicated a requirement of 133 participants to detect a small effect size (Cohen's d = 0.06) in the RMSSD, with a statistical power of 80% and a significance level of 0.05 (Calderón-García et al., 2024; Tousignant-Laflamme & Marchand, 2006). HRV metrics were processed through Kubios HRV software (version 4.1.2), applying artifact correction (<10% beat modification) and analyzing minutes 7-14 of baseline recordings, with RMSSD, LF and HF values across two baseline measurements and exported to JMP Pro (Gronwald et al., 2024). QST data focused exclusively on target-side measurements, calculating CPM as the difference between the first and second numeric VAS pain values.

Before statistical analysis in JMP, logarithmic transformations were applied to normalize skewed distributions. Because assumptions of normality, based on the Shapiro-Wilk test, homoscedasticity and linearity were not initially satisfied, Spearman's rank correlation (ρ) was used to assess nonparametric relationships between each HRV variable (RMSSD, LF and HF) and CPM. After this, a decision tree (Appendix I) approach was followed to analyze the influence of the continuous independent variables on the continuous dependent variable. At this stage, the assumptions of normality, homoscedasticity and linearity were all satisfied, allowing a simple linear regression (SLR) model to be applied. As a result, a series of SLR analyses were conducted, each focusing on the relationship between CPM and one independent HRV variable at a time. To account for multiple comparisons, a Bonferroni correction was applied, adjusting the significance threshold to $\alpha = 0.0167$. Reported p-values were unadjusted but were interpreted relative to this corrected threshold to reduce the risk of Type I errors (false positives).

3. RESULTS

3.1 Participant characteristics

This preliminary analysis included a final sample size comprising 12 enrolled participants, with the subsequent exclusion of one participant due to a data error (acquisition artifacts), reducing the final sample to *n* = 11. Descriptive statistics were used to present the baseline characteristics, which are summarized in Table 4.

Table 4

Characteristics of Participants

Characteristics	
Age, mean (SD) (y)	38.2 (8.78)
Sex, n (%)	
Women	10 (90.9)
Men	1 (9.1)
HRV baseline	
RMSSD (ms) (SD)	28.1 (17.3)
Duration of LBP, mean (m)	69.19 (66.1)
MODI, n (%)	
Mild[a]	3 (27.3)
Moderate[b]	7 (63.6)
Severe[c]	1 (9.1)

Abbreviations: SD = standard deviation, n = sample size, y = years, HRV = heart rate variability, RMSSD = root mean square of successive differences, ms = milliseconds, LBP = low back pain, m = months, MODI = Modified Oswestry Disability Index

[a]20-40% disability, [b]40-60% disability, [c]60-80% disability

3.2 Associations between HRV components and CPM

3.2.1 RMSSD and CPM

Spearman's rank correlation showed no significant association between RMSSD and CPM (ρ = 0.1789, p = 0.5986). The SLR analysis was also non-significant (p = 0.7544), with an F ratio of 0.1041 and an R^2 value of 0.01143, suggesting that RMSSD explained only 1.14% of the variance in CPM (Figure 1).

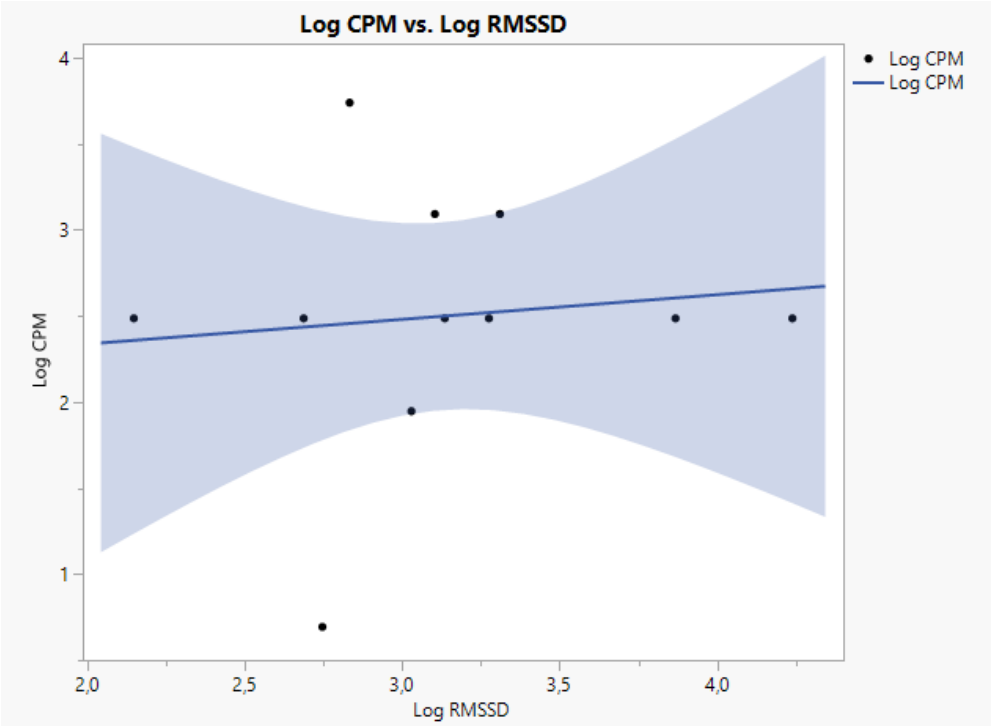
3.2.2 LF and CPM

Spearman's rank correlation showed no significant association between LF and CPM (ρ = 0.1392, p = 0.6832). The SLR analysis was also non-significant (p = 0.6588), with an F ratio of 0.2085 and an R^2 value of 0.02263, indicating that LF accounted for just 2.26% of the variance in CPM (Figure 2).

3.2.3 HF and CPM

Spearman's rank correlation showed no significant association between HF and CPM (ρ = 0.0895, p = 0.7936). Similarly, the SLR analysis was also non-significant (p = 0.5411), with an F ratio of 0.4034 and an R^2 value of 0.0429, suggesting that HF explained 4.29% of the variance in CPM (Figure 3).

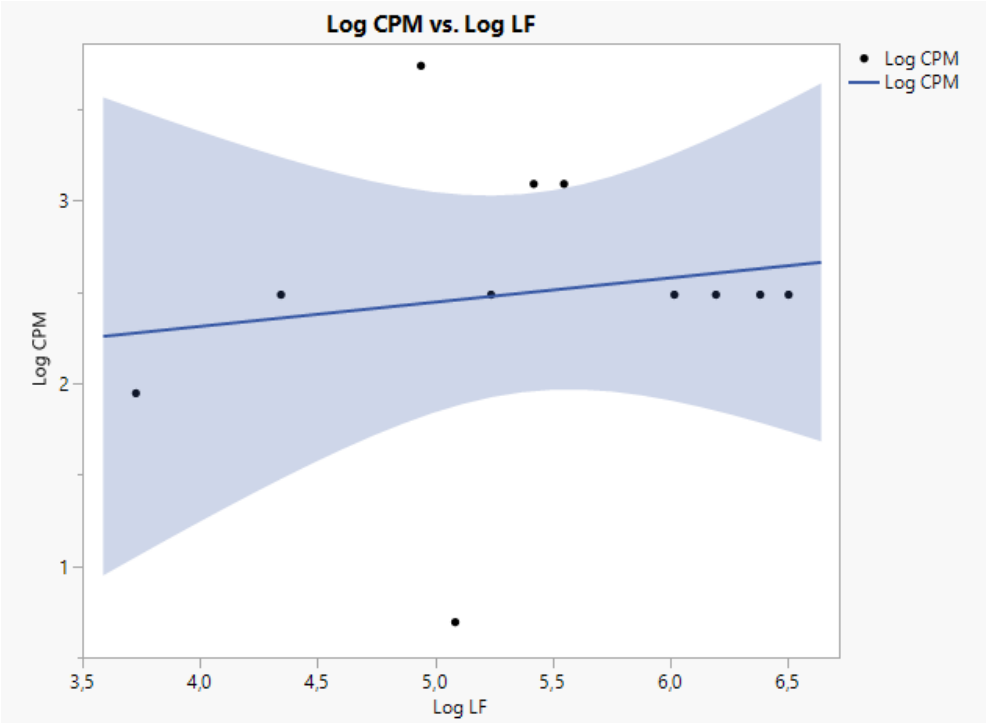
Figure 1



Scatterplot Matrix of the Relationship between Log CPM and Log RMSSD

Note. The scatterplot matrix displays the relationship between Log CPM and Log RMSSD. The blue regression line indicates the trend, while the shaded area represents the confidence interval.

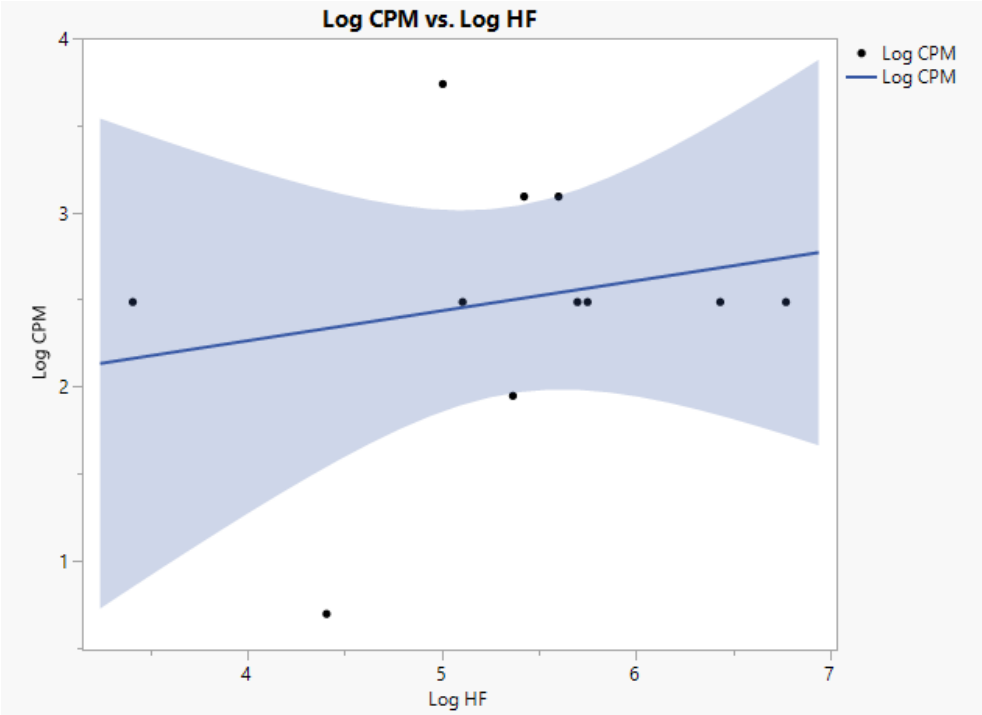
Figure 2



Scatterplot Matrix of the Relationship between Log CPM and Log LF

Note. The scatterplot matrix displays the relationship between Log CPM and Log LF. The blue regression line indicates the trend, while the shaded area represents the confidence interval.

Figure 3



Scatterplot Matrix of the Relationship between Log CPM and Log HF

Note. The scatterplot matrix displays the relationship between Log CPM and Log HF. The blue regression line indicates the trend, while the shaded area represents the confidence interval.

4. DISCUSSION

The primary objective of this study was to examine whether a relationship exists between HRV, assessed in both time (RMSSD) and frequency domains (LF, HF) and CPM in individuals with CNSLBP. The results indicated no significant correlations between RMSSD, LF, HF and CPM. Similarly, no significant linear associations were found between HRV measures and CPM. These findings suggest that, within this preliminary sample of individuals with CNSLBP, variations in HRV do not predict or explain differences in CPM.

It is important to consider that the sample was predominantly female. Prior research has demonstrated significant sex differences in autonomic control, with women exhibiting greater parasympathetic activity and men showing relative sympathetic dominance. This was observed despite women having a higher mean heart rate (HR), a decreased R-R interval and reduced total variability in heartbeat time-series compared to men (Koenig & Thayer, 2016). As a result, the overrepresentation of women may influence the findings, as women tend to exhibit higher RMSSD, lower LF/HF ratio and higher overall HRV (Calderón-García et al., 2024), indicating lower sympathetic activation and higher parasympathetic dominance at rest (Kim et al., 2018). These potentially higher HRV measures might have limited our ability to detect meaningful associations between HRV and CPM. Therefore, the lack of significant findings in this study should be interpreted with caution, as they may reflect sample composition rather than the absence of a true relationship between HRV and CPM.

The varying associations between HRV indices and CPM emphasize the need to consider the physiological mechanisms underlying each measure. Although RMSSD and HF are highly correlated and both considered indicators of parasympathetic (vagal) activity (Kleiger et al., 2005; Porges, 2001), they are not interchangeable. For example, RMSSD is less affected by respiratory influences, while HF is more influenced by respiratory sinus arrhythmia (RSA) and thus may capture not only vagal tone but also reflect respiratory patterns (Hill & Siebenbrock, 2009). On the other hand, LF does not reliably represent sympathetic activity alone, but instead captures a complex mix of sympathetic, parasympathetic and other unidentified factors (Billman, 2013). Despite the differences, the consistent direction of associations among RMSSD, HF and LF suggests a coherent and complementary picture of autonomic functioning, even though each measure emphasizes different aspects of autonomic activity.

4.1 Comparison with other studies

Various studies showed that the relationship between HRV and pain is more complex than a straightforward link to pain intensity. Gockel et al. (2008) emphasized that not pain intensity itself, but the perceived impact of pain on daily functioning correlated with changes in HRV, suggesting that the mental and emotional response to pain was more relevant to the physiological stress system than the pain itself. Conversely, Barakat et al. (2012) found that autonomic nervous system activity was not directly associated with the presence of chronic widespread pain, but that lower parasympathetic activity, as measured by SDNN and RSA, was related to higher pain intensity in these individuals. Supporting this, Rodrigues et al. (2018) reported that patients with chronic musculoskeletal pain and impaired descending nociceptive inhibitory system (DNIS) showed significantly lower HRV (RMSSD, HF, LF) compared to those with normal DNIS function. Hallman et al. (2011) also observed an inverse relationship between pain intensity and resting SDNN in patients with chronic neck and shoulder pain, again suggesting that increased pain is associated with reduced HRV.

In contrast, De Kooning et al. (2015) challenged the theory that a dysregulated autonomic response (such as stress system overactivation) was a primary cause of prolonged pain in whiplash-associated disorders (WAD). In their study, no relationship was found between the parasympathetic branch and the CPM mechanism in whiplash patients. Interestingly, in the chronic WAD group, a higher resting HR was associated with a higher pain threshold in the shoulder and a stronger pain inhibitory effect (CPM) was associated with lower LF values. Likewise, Rampazo et al. (2024) reported no significant group differences in CPM test outcomes, HRV time-domain indices or HR during and after the CPT. Their findings contradicted the hypothesis that patients with chronic neck pain exhibited a weaker cardiac autonomic response to painful stimuli than healthy individuals. Finally, Kyrosis et al. (2024) and Moens et al. (2023) also found no relationship between pain intensity and HRV indices in patients with chronic pain.

4.2 Strengths and limitations

The present study had several methodological strengths. The use of well-defined selection criteria and a validated score (MODI ≥ 20%) enhanced internal validity and ensured that the study focused on a well-defined population. For both HRV and QST assessments, highly detailed and standardized procedures were followed (e.g., standardized instructions, participant and laboratory environment conditions), ensuring the reliability and reproducibility of the measurements. Moreover, the use of reliable equipment, such as the Polar H10 and TSA-2 thermode, along with analysis via Kubios HRV software, which are known for their reliability, further increased the technical validity of the measurements.

The results of this study should be interpreted with caution due to several important limitations. Firstly, CNSLBP's heterogeneity made it difficult to identify subgroups with different pathophysiological profiles, which poses a challenge for interpreting and comparing clinical intervention outcomes. Secondly, the small sample size (n = 11) reduced the study's statistical power and generalizability, thereby increasing the risk of Type II errors. Thirdly, generalizability was also affected by the predominantly female sample. Fourthly, the absence of a healthy control group made it unclear whether the observed relationships were specific to individuals with CNSLBP or also presented in healthy populations. Comparisons with existing literature on HRV and CPM provide only an indirect alternative. Finally, conducting multiple simple linear regression analyses increased the risk of both Type I and Type II errors. To reduce this, a Bonferroni correction was applied to control for the inflated risk of Type I errors. However, this method is known to be highly conservative, which can increase the likelihood of Type II errors, as true effects may no longer reach statistical significance. This compromise should be considered when interpreting the absence of significant findings in this study.

4.3 Future directions

As this was a preliminary cross-sectional study of the relationship between HRV variables and CPM in patients with CNSLBP, causal and temporal associations could not be established. Future research should include larger and more diverse samples and adopt longitudinal designs to investigate the potential temporal link between reductions in vagal tone and changes in pain processing. Addressing the limitations of the current study will help clarify the mechanisms underlying the interaction between autonomic functioning and pain modulation.

5. CONCLUSION

In conclusion, this study found no relationship between heart rate variability (HRV) parameters (RMSSD, LF, HF) and conditioned pain modulation (CPM) in individuals with chronic non-specific low back pain (CNSLBP). Given the limitations of the study, including the small and predominantly female sample, the absence of a healthy control group and the heterogeneity of CNSLBP, further research with larger and more diverse populations is needed to explore these relationships more comprehensively.

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APPENDIX I: Decision Tree