6-2024

The Association of Acute Stress and Single Leg Balance

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The Association of Acute Stress and Single Leg Balance

by

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A Dissertation submitted in partial satisfaction by the requirements for the degree

Doctorate in Philosophy in Physical Therapy

June 2024

Each person whose signature appears below certifies that this dissertation in his/ her opinion is adequate, in scope and quality, as a dissertation for the degree Doctor of Philosophy.

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ACKNOWLEDGEMENTS

I want to express gratitude to my dissertation Chairman, Dr. Everett Lohman, and my committee for their contributions to this work: Dr. Lee Berk, Dr. Robert Dudley, Dr. Lida Gharibvand, and Dr. Jo Armour Smith. This project is a testament to your patience, expertise, and faithful encouragement of me over the past five and a half years. Thank you for the ways you have impacted me and expanded my scientific understanding. Thank you for guiding me in my pursuit.

DEDICATION

This dissertation is dedicated to:

Charlotte Gehrig, without whom this wouldn't be possible

And

Tucker, Tad, Ford, and Jack

CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT OF THE DISSERTATION

The Association of Acute Stress and Single Leg Balance
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Doctor of Philosophy, School of Allied Health Professions, Department of Physical Therapy
Loma Linda University, June 2024
Dr. Everett B. Lohman, III, Chairman

Chronic low back pain is a widespread and expensive societal burden that is routinely near the top of the list of reasons people live with a disability. There is an undeniable connection between low back pain and psychological stress, and it has long been accepted that stress comes as a consequence of the burden of pain. Our group sought to determine if the inverse of this paradigm might be true: that stress may actually play a role in the etiology of low back pain through its influence on neuromuscular control and strategies for balance stability. In this dissertation, we include a brief review of the literature regarding the complex interplay of stress physiology, low back pain, and neuromuscular trunk control. In Chapters 2 and 3 we have included two manuscripts, the first of which is a published protocol for the Feigned Annoyance and Frustration Test—a novel modality that we have determined to be valid for inducing stress in a lab setting.

The second manuscript includes our analysis of the neuromuscular impact of stress on a single leg balance task. In brief: individuals demonstrated decreased activation of key trunk muscles after exposure to stress and individuals with low back pain exhibited a greater number of differences in muscle activation compared to healthy

controls. The final chapter includes a summary of suggestions for future research based on the components in our dataset that have yet to be explored.

CHAPTER ONE

THE ASSOCIATION OF ACUTE STRESS AND SINGLE LEG BALANCE: A LITERATURE REVIEW

Stress

Stress is defined as a maladaptive state in which the sympathetic nervous system is over-activated, causing acute or chronic physical, psychological, and behavioral impairment [1]. Social-evaluative threat (SET) is a robust type of cognitive load characterized by the possibility of negative judgment from others [2]. Social-evaluative threat has been described as one of the strongest stimuli to experimentally trigger autonomic and endocrine changes in human studies resulting in what is ultimately observed as physiologic and psychological stress [2]. Acutely, stress triggers an increase in sympathetic nervous system activity through the sympatho-adrenal medullary (SAM) system and the release of catecholamines. This facilitates a second neuroendocrine cascade known as the hypothalamic pituitary adrenal (HPA) axis. The HPA axis ultimately triggers the release of stress hormones (namely glucocorticoids) that facilitate the increased metabolism of fat and carbohydrates in order to mobilize glucose to accommodate for the increased physiologic demand of a "fight or flight" situation [3].

Stress can be measured with subjective report outcome measures as well as several key biometrics [4]. The Perceived Stress Scale (PSS) is a 10-item questionnaire designed to reflect an individual's perception of stress over the past 30 days. The PSS has been shown to be valid and reliable when used to measure stress [5]. The State-Trait

Anxiety Inventory (STAI) is a 40-item questionnaire designed to quantify an individual's current anxiety level (state) and tendency toward anxiety (trait). It has been determined to be valid and reliable [6].

Select physiological stress biomarkers can be easily measured and recorded and may be considered the most objective way to quantify the magnitude of a stress response. Salivary α-amylase (sAA) and heart rate are physiologic biomarkers that have been correlated with laboratory induced stress [4, 7-9].

Salivary α-amylase has been determined to be an acceptable representation of the SAM system [9-11]. It is important to consider several variables when designing a study using sAA. Time of day must be considered when conducting studies related to sAA since normal salivary levels rise and fall throughout the day promoting arousal and productivity [12, 13]. SAA levels are known to reach their nadir about 30 minutes after awakening and gradually increase throughout the day [13]. Additionally, any study that includes sAA must consider the time it takes to reach a maximum concentration in the saliva. Since sAA directly peaks in conjunction with sympathetic nervous activity, the peak salivary concentration is normally seen within five minutes of the application of the stressful stimulus [9, 10].

It has been argued that while changes in heart rate are thought to represent changes in autonomic activity, the influence of the parasympathetic nervous system holds greater sway over its function than sympathetic input [14]. Still, heart rate changes have been seen to directly correlate with acute stress [8, 9, 12, 15, 16].

When using heart rate as a biometric for stress, it becomes prudent to utilize a stressful stimulus that does not disproportionately affect the cardiovascular system, excluding exercise performance tasks such as the bicycle ergometer or a treadmill task. It is also critical that the stressful task is sufficiently robust. Cognitive load tasks, such as the Stroop Color-Word Interference task, have been shown to elicit a weak stress response when compared to other stimuli [9]. Since pain has been shown to interfere with trunk motor control [16, 17] it is important to include a non-painful stress stimulus, excluding an electric shock task or the cold pressor test. Stress tasks that involve SET have been accepted as the most robust and effective at inducing a neuroendocrine response in patients [9, 18]. However, the protocol for the standard SET task, the Trier Social Stress Test [8, 15] relies on lab resources that are not always readily available, namely space, time, and multiple researchers. Repeatedly, studies have shown that the components considered most stressful are (1) the uncontrollable nature of the stimulus, (2) the social-evaluative component of the stimulus, and (3) the threatening or challenging nature of the task [9, 18, 19]. For all these reasons, we attempted to develop our own protocol for the Feigned Annoyance and Frustration Test, based on a task previously described [16].

While the effect of stress on body systems and overall health have been extensively researched [20, 21], minimal literature exists on the correlation of acute stress with neuromuscular physiology and function and the role it may play in the etiology of low back pain (LBP).

Low Back Pain

The complexity of pain translates into a widespread and expensive societal burden. Between 1996 and 2013, the annual cost of spine pain alone exceeded $134.5 billion [22]. As many as one in five people will experience low back pain [23], making it arguably the most common source of pain and causing it to be consistently ranked as one of the leading causes for disability worldwide [24, 25].

There is a well-documented association between several cognitive affective tendencies and trunk motor control strategies in individuals with LBP. Several research groups have demonstrated decreased activation of the deep abdominal musculature in individuals with high pain-related fear and fear-avoidant tendencies [26, 27] even in the presence of focused neuromuscular re-education intervention [26]. In addition to fear-avoidance, kinesiophobia has been correlated with increased trunk stiffness during movement for individuals with LBP [28]. One small study that utilized SET in comparison to cognitive load and pain demonstrated altered timing of activation in deep trunk musculature during an arm raising task [16].

One theory that has been presented dichotomizes trunk coordination into two biomechanical strategies: “tight” and “loose” control [29]. “Tight” and “loose” control are grossly defined as increased or decreased trunk muscle excitability and co-contraction, respectively [29]. While the etiology of each strategy is likely complex and multifaceted, one suggestion is that they are influenced by cognitive-affective tendencies. Specifically, “tight” control is suggested to be influenced by anxiety and fear resulting in an increase in trunk muscle co-contraction, while “loose” control may come from

impaired motor coordination and decreased muscle activation in response to pain [30, 31].

Regarding balance stability, individuals with LBP who score higher on pain-related anxiety scales have demonstrated reduced postural sway during single leg balance [32]. Additionally, as the balance task became more challenging, the same individuals demonstrated increased reaction time when placed under a cognitive load [32].

In another study on balance stability and cognitive load, Ge et al. 2021 reported that individuals with LBP showed greater variations in center of pressure while standing [33]. Additionally, a review by Xiao et al. 2023 explores the current perspective on the interaction of dual-task processing and postural control in individuals with LBP. The authors note that while dual-task processing may at times enhance postural stability by providing an attentional redirection away from mild pain, more commonly, it introduces an additional cognitive load on the already-taxed attentional resources of individuals with LBP contributing to decreased stance stability and postural coordination [34].

Purpose and Hypotheses

The purpose of the graduate student research study is first, to validate a protocol for the Feigned Annoyance and Frustration Test, designed to induce acute stress in the lab that is low cost, has a low resource demand, is effective, and does not utilize pain or exercise as a modality. We expect the FAF Test to be an effective and reliable test that will stimulate the SAM and meet these design specifications. A second purpose of this graduate student research study is to determine whether acute stress alters the biomechanics associated with single leg balance by influencing trunk and lower

extremity muscle activation and center of pressure variations in individuals with and without LBP. As a part of this second study, another question to be explored is whether LBP interacts with stress regarding these effects. The hypothesis is that stress will alter trunk and lower extremity muscle activation and stance stability and that these changes will be more robust in individuals with LBP.

Bibliography

[1] M. Campkin, Stress management in primary care, Family Practice 17(1) (2000) 98-99.

[2] E.S. Poppelaars, J. Klackl, B. Pletzer, F.H. Wilhelm, E. Jonas, Social-evaluative threat: Stress response stages and influences of biological sex and neuroticism, Psychoneuroendocrinology 109 (2019) 104378.

[3] K.E. Hannibal, M.D. Bishop, Chronic stress, cortisol dysfunction, and pain: a psychoneuroendocrine rationale for stress management in pain rehabilitation, Phys Ther 94(12) (2014) 1816-25.

[4] D.H. Hellhammer, A.A. Stone, J. Hellhammer, J. Broderick, G.F. Koob, M.L. Moal, et al., Measuring Stress, Encyclopedia of Behavioral Neuroscience, Academic Press, Oxford, 2010, pp. 186-191.

[5] S. Cohen, T. Kamarck, R. Mermelstein, A global measure of perceived stress, J Health Soc Behav 24(4) (1983) 385-96.

[6] L.J. Julian, Measures of anxiety: State-Trait Anxiety Inventory (STAI), Beck Anxiety Inventory (BAI), and Hospital Anxiety and Depression Scale-Anxiety (HADS-A), Arthritis Care Res (Hoboken) 63 Suppl 11(0 11) (2011) S467-72.

[7] W.J. Inder, G. Dimeski, A. Russell, Measurement of salivary cortisol in 2012 - laboratory techniques and clinical indications, Clin Endocrinol (Oxf) 77(5) (2012) 645-51.

[8] C. Kirschbaum, K.M. Pirke, D.H. Hellhammer, The 'Trier Social Stress Test'--a tool for investigating psychobiological stress responses in a laboratory setting, Neuropsychobiology 28(1-2) (1993) 76-81.

[9] N. Skoluda, J. Strahler, W. Schlotz, L. Niederberger, S. Marques, S. Fischer, et al., Intra-individual psychological and physiological responses to acute laboratory stressors of different intensity, Psychoneuroendocrinology 51 (2015) 227-36.

[10] N. Takai, M. Yamaguchi, T. Aragaki, K. Eto, K. Uchihashi, Y. Nishikawa, Effect of psychological stress on the salivary cortisol and amylase levels in healthy young adults, Arch Oral Biol 49(12) (2004) 963-8.

[11] K. An, J. Salyer, R.E. Brown, H.F. Kao, A. Starkweather, I. Shim, Salivary Biomarkers of Chronic Psychosocial Stress and CVD Risks: A Systematic Review, Biol Res Nurs 18(3) (2016) 241-63.

[12] B.M. Kudielka, N.C. Schommer, D.H. Hellhammer, C. Kirschbaum, Acute HPA axis responses, heart rate, and mood changes to psychosocial stress (TSST) in humans at different times of day, Psychoneuroendocrinology 29(8) (2004) 983-92.

[13] U.M. Nater, N. Rohleder, W. Schlotz, U. Ehlert, C. Kirschbaum, Determinants of the diurnal course of salivary alpha-amylase, Psychoneuroendocrinology 32(4) (2007) 392-401.

[14] G.A. Reyes del Paso, W. Langewitz, L.J. Mulder, A. van Roon, S. Duschek, The utility of low frequency heart rate variability as an index of sympathetic cardiac tone: a review with emphasis on a reanalysis of previous studies, Psychophysiology 50(5) (2013) 477-87.

[15] B.M. Kudielka, D.H. Hellhammer, C. Kirschbaum, Ten years of research with the Trier Social Stress Test (TSST) - revisited, in: E. Harmon-Jones, P. Winkielman (Eds.), Social Neuroscience: Integrating biological and psychological explanations of social behavior, Guilford Press, New York, 2007.

[16] G.L. Moseley, M.K. Nicholas, P.W. Hodges, Pain differs from non-painful attention-demanding or stressful tasks in its effect on postural control patterns of trunk muscles, Exp Brain Res 156(1) (2004) 64-71.

[17] L.H. Larsen, R.P. Hirata, T. Graven-Nielsen, Experimental Low Back Pain Decreased Trunk Muscle Activity in Currently Asymptomatic Recurrent Low Back Pain Patients During Step Tasks, The journal of pain : official journal of the American Pain Society 19(5) (2018) 542-551.

[18] S.S. Dickerson, M.E. Kemeny, Acute stressors and cortisol responses: a theoretical integration and synthesis of laboratory research, Psychol Bull 130(3) (2004) 355-91.

[19] R. Lazarus, S. Folkman, Stress, Appraisal, and Coping, Springer Publishing Company1984.

[20] B.S. McEwen, Allostasis and allostatic load: implications for neuropsychopharmacology, Neuropsychopharmacology 22(2) (2000) 108-24.

[21] H. Yaribeygi, Y. Panahi, H. Sahraei, T.P. Johnston, A. Sahebkar, The impact of stress on body function: A review, Excli j 16 (2017) 1057-1072.

[22] J.L. Dieleman, R. Baral, M. Birger, A.L. Bui, A. Bulchis, A. Chapin, et al., US Spending on Personal Health Care and Public Health, 1996-2013, Jama 316(24) (2016) 2627-2646.

[23] F. Fatoye, T. Gebrye, I. Odeyemi, Real-world incidence and prevalence of low back pain using routinely collected data, Rheumatol Int 39(4) (2019) 619-626.

[24] G.D.a.H. Collaborators, Global, regional, and national disability-adjusted life-years (DALYs) for 359 diseases and injuries and healthy life expectancy (HALE) for 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017, Lancet 392(10159) (2018) 1859-1922.

[25] C.J. Murray, C. Atkinson, K. Bhalla, G. Birbeck, R. Burstein, D. Chou, et al., The state of US health, 1990-2010: burden of diseases, injuries, and risk factors, Jama 310(6) (2013) 591-608.

[26] M. Unsgaard-Tøndel, T.I. Nilsen, J. Magnussen, O. Vasseljen, Are fear avoidance beliefs associated with abdominal muscle activation outcome for patients with low back pain?, Physiotherapy research international : the journal for researchers and clinicians in physical therapy 18(3) (2013) 131-9.

[27] B. Van Damme, V. Stevens, D. Van Tiggelen, C. Perneel, G. Crombez, L. Danneels, Performance based on sEMG activity is related to psychosocial components: differences between back and abdominal endurance tests, J Electromyogr Kinesiol 24(5) (2014) 636-44.

[28] N.V. Karayannis, R.J. Smeets, W. van den Hoorn, P.W. Hodges, Fear of Movement Is Related to Trunk Stiffness in Low Back Pain, PloS one 8(6) (2013) e67779.

[29] J.H. van Dieën, N.P. Reeves, G. Kawchuk, L.R. van Dillen, P.W. Hodges, Motor Control Changes in Low Back Pain: Divergence in Presentations and Mechanisms, The Journal of orthopaedic and sports physical therapy 49(6) (2019) 370-379.

[30] P.W. Hodges, M.W. Coppieters, D. MacDonald, J. Cholewicki, New insight into motor adaptation to pain revealed by a combination of modelling and empirical approaches, European journal of pain (London, England) 17(8) (2013) 1138-46.

[31] J.H. van Dieën, J. Cholewicki, A. Radebold, Trunk muscle recruitment patterns in patients with low back pain enhance the stability of the lumbar spine, Spine 28(8) (2003) 834-41.

[32] S. Shanbehzadeh, M. Salavati, S. Talebian, K. Khademi-Kalantari, M. Tavahomi, Attention demands of postural control in non-specific chronic low back pain subjects with low and high pain-related anxiety, Exp Brain Res 236(7) (2018) 1927-1938.

[33] L. Ge, Q. Yu, C. Wang, H. Huang, X. Li, S. Zhang, et al., How cognitive loads modulate the postural control of older women with low back pain?, BMC Geriatr 21(1) (2021) 82.

[34] W. Xiao, H. Yang, Z. Wang, H. Mao, H. Wang, Z. Hao, et al., Postural Control of Patients with Low Back Pain Under Dual-Task Conditions, J Pain Res 16 (2023) 71-82.

CHAPTER TWO

THE FEIGNED ANNOYANCE AND FRUSTRATION TEST TO ACTIVATE THE SYMPATHOADRENAL MEDULLARY SYSTEM

Abstract

When perceived as threatening, social interactions have been shown to trigger the sympathoadrenal medullary system as well as the hypothalamic-pituitary-adrenal axis resulting in a physiologic stress response. The allostatic load placed on human health and physiology in the context of acute and chronic stress can have profound health consequences. The purpose of this study was to develop a protocol for a lab-based stress stimulus using social-evaluative threat. While several valid, stress-stimulating protocols exist, we sought to develop one that triggered a physiologic response, did not require significant lab resources, and could be completed in around 10 minutes. We included 53 participants (29 men and 24 women) and exposed them to a modified version of the Stroop Color-Word Interference Task during which the participants were made to feel they were performing the task poorly while the lead researcher feigned annoyance and frustration. After exposure to this Feigned Annoyance and Frustration (FAF) Test, both the men and women in this study demonstrated a statistically significant and clinically meaningful increase in subjective stress on the visual analog scale. Additionally, the men in this study demonstrated a statistically significant increase in heart rate and salivary α-amylase concentrations after exposure to the test. The women in this study did not demonstrate a statistically significant increase in the physiologic stress biomarkers. This

protocol for the FAF Test shows promise to researchers with limited time and resources who are interested in experimentally activating the sympathoadrenal medullary system.

Key words: acute stress; α-amylase; Stroop; social evaluative threat; sympathoadrenal medullary system

Introduction

It is well-established that stress is associated with increased risk of disease [1], increased severity of disease [1], increased rate of aging [1], higher likelihood of pain chronicity [2], and poorer disease prognosis [3]. The toll of psychosocial stress on the body has been repeatedly studied in the field of psychoneuroendocrinology and social-evaluative threat (SET) has been one of the key laboratory modalities used to trigger and study stress [4].

Acutely, stress triggers an increase in sympathetic nervous system activity through the sympathoadrenal medullary (SAM) system and the release of catecholamines. The intracellular norepinephrine triggers a cascade that results in the release of salivary α-amylase (sAA) [5]. This facilitates a second neuroendocrine cascade known as the hypothalamic-pituitary adrenal (HPA) axis. The HPA axis ultimately triggers the release of stress hormones – namely glucocorticoids – that facilitate the increased metabolism of fat and carbohydrates in order to mobilize glucose to accommodate for the increased physiologic demand of a “fight or flight” situation [6]. It is generally accepted that sAA is a valid representation of the SAM system [7-9].

Because of the negative impact stress has on overall health, it is critical to have a variety of valid laboratory stress tests to study the mechanism for the association between

stress and disease. Many tests have been utilized with their appropriateness dependent on the focus of the research question. Studies have shown that the components of the most stressful triggers are (1) the uncontrollable nature of the stimulus, (2) the social-evaluative component of the stimulus, and (3) the threatening or challenging nature of the task [10-12].

Our group was interested in a stress test that did not rely on cardiovascular stress, excluding exercise performance tasks such as the bicycle ergometer task. We also were in search of a task that was sufficiently robust, excluding stress tasks that are based solely on cognitive load, such as the Stroop Color-Word Interference Task [13] or the Serial Subtraction Task [14]. Additionally, we were interested in a task that did not involve physiologic pain, excluding an electric shock task [15] or the Cold Pressor Test [16]. Stress tasks that involve SET have been accepted as the most robust and effective at inducing a neuroendocrine response in research participants [4, 10, 12]. However, the protocol for the standard SET task, the Trier Social Stress Test [17, 18] relies on additional lab resources that often are not available, namely space, time, and multiple researchers. For all these reasons, we attempted to develop our own protocol based on a previously published stress task, the modified Stroop Color-Word Interference Task [19]. In the 2004 study, participants were instructed in a cognitive load task based on the Stroop effect. To create additional stress, participants were misinformed that the task would be "on the second easiest setting" and that "they were expected to be excellent at the task." As participants performed the task, they were informed that they were not performing well. Additionally, the lead researcher attempted to passively communicate

frustration with the participants' poor performance as well as annoyance with the other laboratory workers while participants' stress was measured.

Stress can be assessed in several ways [20]. Heart rate (HR) and sAA are physiologic biomarkers that have been correlated with laboratory induced stress [12, 17, 20, 21]. It has been argued that while changes in HR are generally accepted to represent changes in autonomic activity, the influence of the parasympathetic nervous system holds greater sway over its function than sympathetic input [22]. Still, HR changes have been seen to directly correlate with acute stress [12, 17-19, 23].

It is important to consider several variables when designing a study using a neuroendocrine biomarker such as sAA. Time of day must be considered since normal sAA levels rise and fall throughout the day promoting arousal and productivity [23, 24]. SAA levels are known to reach their nadir about 30 minutes after awakening and gradually increase throughout the day [24]. Additionally, since sAA directly peaks in conjunction with sympathetic nervous activity, the peak salivary concentration is normally seen within 5 minutes of the application of the stressful stimulus [9, 12].

Mixed findings have been reported regarding the effect of sex on sAA after exposure to stress. Some investigators report that men and women demonstrate similar changes in sAA in responses to stress [25-27]. However, others have documented the influence sex or menstrual phase can have on sAA levels [28]. Because of these mixed reports, it is recommended to consider both male and female participants in the recruitment and analysis of stress research [29].

The purpose of this research study is to develop a protocol for the Feigned Annoyance and Frustration (FAF) Test designed to stimulate the SAM using SET and to test the validity of that protocol. A second purpose of this study is to determine if the FAF Test is effective in both men and women. We hypothesize that the exposure to the FAF Test will induce stress in the study population and that this change will be demonstrated by increases in HR, sAA, and subjective stress (VAS_{stress}). Additionally, we hypothesize that the FAF Test will be effective at inducing stress in male and female participants.

Methods

Participants

This study was approved by the Internal Review Board (IRB) at Loma Linda University (IRB # 5210188) and occurred as part of a study on the effect of stress on balance strategies for individuals with and without low back pain. Sixty participants were recruited from a convenience sample at Loma Linda University and the surrounding area. All participants in the study were consented before being enrolled. Participants were included if they were between the ages of 18 and 45 years of age, could balance on one leg, and did not have: a diagnosed anxiety disorder, history of low back surgery, current pregnancy (or pregnancy in the past 12 months), severe pain (current pain >6/10), or color blindness. All participants were compensated with a $25 gift card at the completion of the study. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Quiescence Period and Subjective Report Outcome Measures (SROMs)

All data collection was conducted between 2:30 pm and 7:00 pm to account for the diurnal variation in sAA levels. Upon arriving at the research facility, participants were seated to be consented. After being consented, participants were fitted with a polar H10 HR sensor which was then connected via Bluetooth to an iPad. Participants remained seated to allow their HR and stress levels to settle at baseline and to complete several self-report outcome measures: the Spielberger State-Trait Anxiety Inventory (STAI), the Perceived Stress Scale (PSS), and the Pittsburgh Sleep Quality Index (PSQI). The STAI is a 40-item questionnaire designed to quantify an individual's current anxiety level (state) and tendency toward anxiety (trait). It has been determined to be valid and reliable [30]. The PSS is a 10-item questionnaire designed to quantify an individual's perceived stress over the past 30 days as it relates to being overwhelming, unpredictable, and uncontrollable. The PSS has been determined to be valid and reliable [31]. The PSQI was developed as a representative quantification of patients' sleep experience over the past month. It is made up of seven component scores which are then combined to form the global score. It has been determined valid and reliable when used to distinguish good quality sleepers from poor quality sleepers [32].

Heart Rate

HR was recorded using the mobile application, EliteHRV (Version 5.5.4, mobile app for IOS, EliteHRV.com, USA). HR data was exported from Elite HRV as raw inter-beat interval data. It was imported into Kubios HRV Scientific (v 4.0.1) where it was filtered for artifact and ectopic beats using the previously validated Kubios HRV algorithm [33, 34]. HR data was visually inspected for missing data or erroneously

marked beats. Average HR was calculated for 4 distinct time periods defined by 4 distinct saliva collection times: HR_1 (mean HR from the beginning of the study period until T_1), HR_2 (mean HR from T_1 to T_2), HR_3 (mean HR from T_2 to T_3), and HR_4 (mean HR from T_3 to T_4) (T_1= time immediately following the completion of the SROM paperwork; T_2= time immediately before the beginning of the stress stimulus; T_3= time immediately after the completion of the stress stimulus; T_4= 10 minutes after the completion of the stress stimulus; Figure 1).

Visual Analog Scale

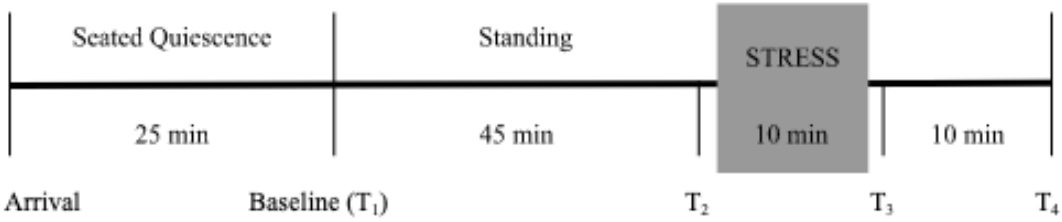
During the 25-minute quiescence period, participants were asked to annotate their current stress level on a 10 cm line with one side of the line reading, "None", and the other side of the line reading, "As bad as it could be." At the end of the 90-minute trial, participants were then again asked to rate their current stress on the same scale during the final 90-second saliva collection. The Visual Analog Scale (VAS) has been widely used in the literature and has been validated as a measure for subjective stress [35]. The minimal clinically important difference has not been determined for VAS_{stress}, however for pain it has been reported at 1.0 cm [36] and for anxiety it has been recommended between 1.2-1.3 cm [37]. We decided to use 1.2 cm as a cutoff for meaningful change in subjective stress appraisal.

Saliva Collection and Analysis

Participants were instructed to avoid rigorous physical activity within 24 hours of the saliva collection. Additionally, they were instructed to abstain from alcohol 24 hours before saliva collection. On the day of the scheduled session in the lab, participants were

requested to avoid eating or drinking anything (including caffeine) for 1 hour before coming in [5]. Plain water was permitted. Other than the aforementioned instructions, participants were advised to keep their regular routine regarding sleep, mealtimes, and daily activity. Saliva samples were collected, stored, shipped, and processed in accordance with the tier 1 BRISQ criteria [38]. Samples of saliva were collected using SalivaBio Oral Swab, 10x30mm (Item No. 5001.02). After being consented, each participant was instructed to rinse their mouth with a sip of plain, filtered water. After the quiescence period the participants were instructed to place the swab beneath the tongue directly from the packaging so as to not contaminate the swab with their hands. Next, participants were instructed to avoid swallowing, allowing saliva to pool while the swab was in place for 90 seconds. The swab was then removed, again without using the hands, by placing it directly from the mouth into a Swab Storage Tube, 17x100mm (Item No. 5001.05). Tubes were placed in a cooler during the trial and then stored in a freezer and kept at -80°C until the time of processing (2 weeks -71 weeks). Saliva was collected at T_1, T_2, T_3, and T_4. Since this protocol took place as a part of another study on stress and balance, all participants performed two single leg balance tasks that took place in two identical 10-minute trials: one just before T_2 and one between T_3 and T_4 (Figure 1). None of the participants reported the balance task to induce significant fatigue.

Figure 1: FAF timeline for the 90-minute session



Samples were shipped frozen and packaged with dry ice in accordance with the instructions provided by Salimetrics (Carlsbad, CA). Samples were assayed at the Salimetrics SalivaLab using the Salimetrics Salivary α-Amylase Assay Kit (Cat. No. 1-1902), without modifications to the manufacturer's protocol. Samples were thawed to room temperature, vortexed, and then centrifuged for 15 minutes at approximately 3,000 RPM (1,500 x g) immediately before performing the assay. Samples were tested for sAA using a kinetic enzyme immunoassay (Cat. No. 1-1902). Sample test volume was 8 µl of 200X diluted saliva per determination. The assay had a lower limit of sensitivity of 0.4 U/mL, samples exceeding 400 U/mL needed further dilution, an average intra-assay coefficient of variation of 5.47%, and an average inter-assay coefficient of variation 4.7%, which met the manufacturer's criteria for accuracy and repeatability in salivary bioscience and exceeded the applicable NIH guidelines for enhancing reproducibility through rigor and transparency.

FAF Test Protocol

Figure 1 contains the study timeline for each participant. Participants received standardized, verbal instruction in how to perform an application-based cognitive load

task (Brain Test - Stroop Effect, Copyright Attila Hegedus) on an iPad. Standardized patient instructions are listed below:

> *Your task is to tap on the appropriate label at the bottom of the screen whose text denotes the ink color of the top label. Give as many correct answers as you can in 60 seconds. Correct answers are +1 and incorrect answers are -1. This task is the easiest setting, and most people don't have any trouble. The app measures your ability and quantifies your proficiency.*

After participants began the first trial, the instructions were repeated when multiple incorrect attempts were made. No false feedback was given to the participants at any time. Examiner comments included:

> *Don't overthink it, it should be a lot easier than this. Just try to focus.*
>
> *Sorry, hang on a second. Would it be helpful if I explained the instructions again?*

If the participant answered Yes, the instructions were repeated. If the participant answered No:

> *Ok, let's start over and really try to focus this time.*

At this time, the instructor made an effort to express frustration, disappointment, and annoyance at the participant's performance by heavily sighing, changing the tone of voice, and coarsely redirecting banter back to the task. This response was used for all participants regardless of the accuracy of their answers. If the participant was performing the task with a relatively high degree of accuracy, the researcher stated:

You're doing okay, but I need you to speed up a little. Actually, I need you to speed up a lot if we're going to be able to use any of this.

After completing the second attempt, the researcher expressed further disappointment, stating:

I'm not sure we're going to be able to use any of that. Let's try this instead: Starting from 999, subtract 7 out loud. For every incorrect answer you will hear a sound.

It was then clarified that a certain speed needed to be maintained in order to have an effective trial. A bell was rung for every incorrect answer and the researcher appeared to be marking a sheet of paper and checking the time throughout the trial. The researcher would express frustration with other lab personnel present during the study and make indirect comments about the success of the performance (i.e. "Do you know of any other participants who can come in tonight?") The same frustrated tone was maintained through the end of the research session. After the collection of the final saliva sample, all participants were debriefed and a full explanation of the protocol was provided. The protocol was carried out by the same male investigator for all trials (TG).

Data Analysis

Mean and standard deviation were computed for quantitative variables and frequency (percentage) for categorical variables at baseline. Normality of quantitative variables were assessed using the Shapiro-Wilk test and box plots. Log transformation was applied to raw sAA concentrations to address non-normality and skewness. The independent t-test was used for quantitative variables at baseline and the Mann-Whitney

U test was used for non-normal and ordinal data. The independent chi square test was used for categorical variables at baseline. Linear mixed effects models (repeated measures) were used to examine the effect of the between-group factor (sex) and within-group factor (time) on the dependent variables (HR, sAA, and VAS_{stress}) [39]. A Bonferroni correction was used to adjust for multiple post-hoc comparisons. A power calculation was performed using G*Power (Version 3.1.9.2; Heinrich-Heine Universität, Düsseldorf, Germany) with a similar method as has been previously reported [10, 26]. A minimum sample size of n = 52 was required to provide 80% power at the 5% level of significance to capture a small effect size of 0.20 or higher. The data was analyzed using SPSS Statistics Software version 29.0 (SPSS Inc, Chicago, IL, USA). All analyses were performed at an alpha level of .05.

Results

Of the 60 participants who were recruited, consented, and completed the FAF Test protocol, 53 participants (29 men and 24 women) were included in the final analysis due to missing data. No participants opted to terminate the trial before completion. The average age of the men was significantly higher than the average age of the women (mean ± SD: men: 30.2 ± 4.5 years, women: 27.9 ± 5.2 years, p=.033). Mean BMI for the men was also significantly higher than the mean BMI for the women (men: 25.5 ± 3.3 kg/m^2, women: 23.6 ± 4.0 kg/m^2, p=.037). Resting HR for the men was lower than the resting HR for the women (men: 71.4 ± 8.6 bpm, women: 83.1 ± 10.6 bpm, p<.001). All other demographic data was not significantly different between groups at baseline (p>.05) (Table 1).

Table 1: Demographics and baseline data

Characteristics	Total (n=53)	Men (n_1=29)	Women (n_2=24)	P – value
Age (years)	29.2 ± 4.9	30.2 ± 4.5	27.9 ± 5.2	**0.033**
BMI (kg/m^2)	24.6 ± 3.7	25.5 ± 3.3	23.6 ± 4.0	**0.037**
NPRS[a]	0 (0, 5)	0 (0, 4)	0 (0, 5)	0.581
Occupation[b]				0.063
Medical	9 (17)	6 (21)	3 (12)	
Student	36 (68)	16 (55)	20 (83)	
Other	8 (15)	7 (24)	1 (4)	
STAI-State Subscale	29.6 ± 7.9	29.1 ± 7.0	30.2 ± 9.1	0.986
STAI-Trait Subscale	37.1 ± 10.2	34.6 ± 8.6	40.2 ± 11.3	0.093
PSQI	5.3 ± 2.4	5.2 ± 2.6	5.4 ± 2.2	0.639
PSS	14.8 ± 5.9	13.6 ± 5.0	16.2 ± 6.7	0.109
VAS_{stress} (cm)[a]	1.1 (0, 7)	1.0 (0, 5)	1.6 (0, 7)	0.180
Resting HR (bpm)	76.6 ± 11.1	71.4 ± 8.6	83.1 ± 10.6	**<.001**
sAA (U/mL)	111.9 ± 101.5	113.5 ± 87.7	110 ± 118.1	.437[c]

Abbreviations: BMI: Body Mass Index, NPRS: Numerical Pain Rating Scale, STAI: State-Trait Anxiety Inventory; TSK: Tampa Scale of Kinesiophobia, PSQI: Pittsburgh Sleep Quality Index; PSS: Perceived Stress Scale; VAS_{stress}: Visual Analog Scale for Stress; ODI: Oswestry Disability Index; HR: Heart Rate; sAA: Salivary α-Amylase
Values are presented as mean ± SD unless otherwise indicated.

[a] Median (min, max)
[b] Frequency (percentage)
[c] p-values for log-transformed data

There was a significant increase in HR for both groups over time (p<.001) (Table 2 and Figure 2a). There was no interaction between time and sex, however the between groups analysis revealed a significant difference between men and women (p=.003) (Table 2). For men there was a statistically significant increase in HR after stress (HR_4 compared to HR_2, p=.040). For women, HR was not significantly different after stress (HR_4 compared to HR_2, p=.059). However, HR_4 (after stress) increased significantly

compared to HR_3 (during the stress task) ($p=.001$) (Table 2 and Figure 2a). Both groups exhibited a significant increase in sAA over time ($p<.001$) (Table 2 and Figure 2b). Men demonstrated a significant increase sAA concentrations after stress (T_4 compared to T_2, $p<.001$). While women demonstrated a significant increase in sAA concentrations compared to baseline (T_4 compared to T_1, $p=.003$), there was no statistically significant increase in sAA concentration comparing T_4 and T_2 ($p=.086$). There was no significant difference between groups ($p=.294$) (Table 2 and Figure 2b). There was a significant increase in VAS_{stress} for both the men and women over time ($p<.001$) (Table 2 and Figure 2c). There was no significant difference between groups ($p=.233$) (Table 2).

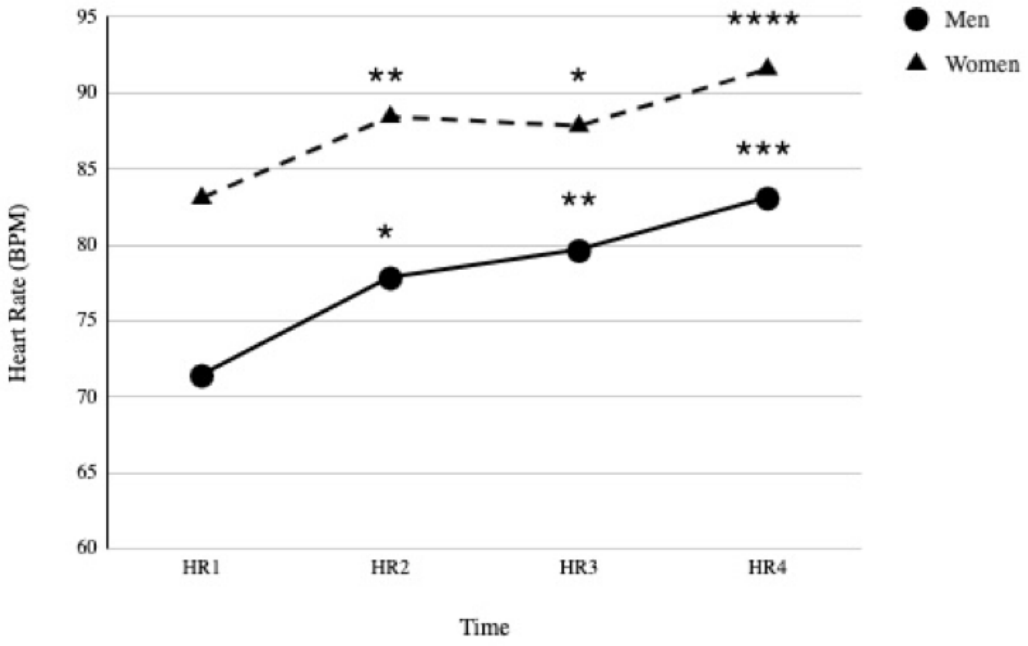
Table 2: Stress reactivity to the FAF overall with within (time) and between (sex) groups comparison

			Total (n=53)	**Men (n_1=29)**	**Women (n_2=24)**	**p◇◇**
			Mean±STD	**Mean±STD**	**Mean±STD**	
HR (BPM)		HR_1	76.6±11.1	71.4± 8.6	83.1 ±10.6	**0.003**
		HR_2	82.6±11.4*	77.8 ± 9.5*	88.4 ± 11.0**	
		HR_3	83.3±13.8*	79.6 ± 14.0**	87.8 ±12.5*	
		HR_4	86.9±14.8***	83.0 ± 16.0†	91.6 ± 11.9††	
	p◇		**<.001**	**<.001**	**<.001**	
sAA[a]		T_1	4.4 ± 0.9	4.5 ± 0.8	4.2 ±1.0	0.294
		T_2	4.5 ± 1.0†††	4.5 ± 0.9†††	4.4 ± 1.2	
		T_3	4.6 ± 0.9*	4.9 ± 0.7†††	4.4 ± 1.0	
		T_4	4.9 ± 0.8***	5.0 ± 0.6***	4.7 ± 1.0**	
	p◇		**<.001**	**<.001**	**<.001**	
VAS_{stress} (cm)		T_1	1.8 ± 2.0	1.3 ± 1.3	2.4 ± 2.5	0.233
		T_4	3.6 ± 2.2**	3.5 ± 2.0**	3.7 ± 2.4**	
	p◇		**<.001**	**<.001**	**<.001**	

Abbreviations: VAS_{stress}: Visual Analog Scale for stress; HR: Heart Rate, BPM: Beats Per Minute; sAA: Salivary α-Amylase; HR_1: mean Heart Rate from beginning of the collection until T_1; HR_2: mean Heart Rate from T_1 to T_2; HR_3: mean Heart Rate from T_2 to T_3; HR_4: mean Heart Rate from T_3 to T_4; T_1: after 25-minute quiescence period, T_2: immediately pre-stress, T_3: immediately post-stress, T_4: 10-minutes post-stress

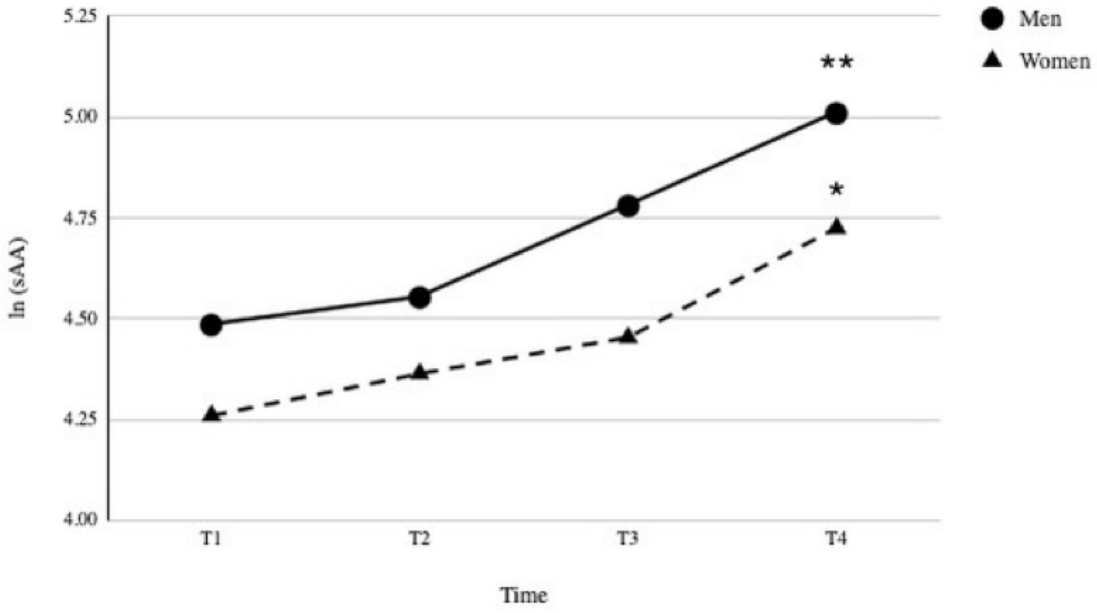
[a] log transformations of raw α-Amylase concentrations in U/mL
*p-value<.05 for within groups compared to T_1 and T_4
**p-value <.003 for within groups compared to T_1
***p-value<.05 for within groups compared to T_1, T_2, and T_3
†p-value<.05 for within groups compared to T_1 and T_2
††p-value<.05 for within groups compared to T_1 and T_3
†††p-value<.05 for within groups compared to T_4
◇ p-value for the null hypothesis that there is no significant difference within groups (variable x time)
◇◇ p-value for the null hypothesis that there is no significant difference between men and women

Figure 2a: Heart rate changes over time for men and women in response to the FAF



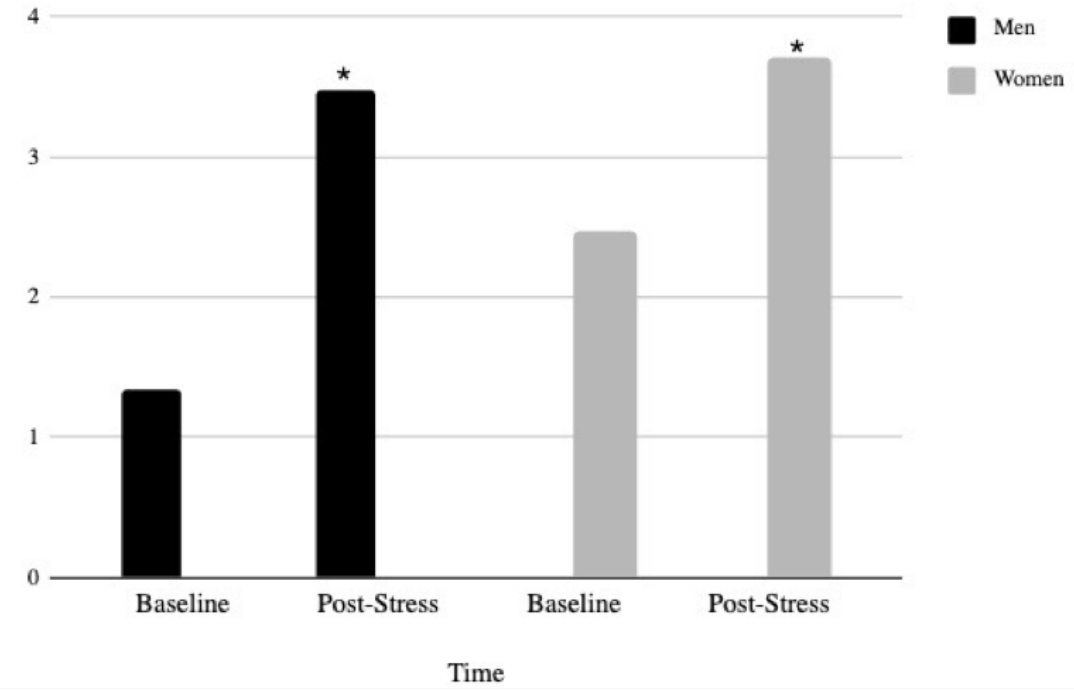
*p<.05 compared to HR_1 and HR_4
**p<.001 compared to HR_1
***p<.05 compared to HR_1 and HR_2
****p<.01 compared to HR_1 and HR_3

Figure 2b: SAA changes over time for men and women in response to the FAF



*p<.05 compared to T_1
**p<.05 compared to T_1, T_2, T_3

Figure 2c: VAS changes over time for men and women in response to the FAF



*p<.001 compared to baseline

Discussion

In this study, we sought to develop a valid protocol to induce acute stress in men and women. We introduced the FAF Test as a feasible stress stimulus that offers several advantages. Unlike other methods reliant on SET, the FAF Test can be efficiently administered in 10 minutes and requires minimal resources, making it highly suitable for most laboratory settings. While a similarly modified version of the Stroop Color-Word Interference Task has been previously described in brief [19], we felt it would offer greater utility if we sought to validate a scripted protocol to maximize reproducibility for future investigation. Our proposed protocol for the FAF Test appeared to be effective at

stimulating the SAM in men, as evidenced by the significant increase in sAA concentrations and HR after exposure to the FAF Test. The protocol was not as robust in activating the SAM in women as the changes in HR (HR_4 compared to HR_2) and sAA (T_4 compared to T_2) after the FAF Test failed to reach significance (p=.086 and p=.059, respectively). Despite the lack of physiologic change for women, both men and women reported a significant increase in subjective stress on the VAS_{stress}. It has been recommended and is of critical importance to include the subjective report of stress alongside biomarkers when assessing the negative impact of stress [40].

Our findings suggest that men and women were both influenced by the FAF Test. Other studies on stress reactivity in women often control for menstrual phase or for the use of oral contraceptives. We intentionally did not control for these variables to broaden the applicability of our study findings. In retrospect, we may have encountered a more robust physiologic change for the female group had we provided this control since women have been shown to demonstrate blunted changes in sAA concentrations during the follicular phase of the menstrual cycle [28]. It has also been suggested that menstrual phase may influence subjective stress appraisal [41], however our findings do not suggest that this was the case in our study. Our findings for women may have been more robust had we only considered individuals on oral contraceptives or in the luteal phase of the menstrual cycle.

We did find that the female participants exhibited a higher HR than the men at all 4 time points during the study period. From this, we propose that the difference in HR between groups may be due to sex and not differences in reactivity to SET. When

examining generalized HR differences in men and women, women have been shown to have a higher HR than men [42] perhaps due to the anatomical size difference and other autonomic discrepancies between male and female physiology [43]. It is important to note that the investigator administering the FAF Test protocol in this study was male. It has been suggested that stress reactivity to SET is more robust when the examiner is of the opposite sex than the participant [44], however we did not appear to demonstrate this phenomenon in our results.

The HR changes demonstrating stress reactivity for our participants was small. Other studies that use HR as a biomarker for stress compare baseline to peak HR [12] which may introduce a bias exaggerating HR reactivity. We chose to quantify HR by calculating the mean for a given time epoch which we feel was a more accurate representation of the participants' physiologic state for a given time period. Additionally, 4 mean HRs fits well with our repeated-measures design for the saliva analysis. Other studies have quantified HR by calculating minute-to-minute averages [17, 19] which may demonstrate a higher peak HR than the means we reported. Future studies may consider the role of HR variability in this analysis which may provide greater insight into autonomic reactivity than mean HR.

A common biomarker used in other studies on SET is salivary cortisol. We chose not to include this in our analysis due to the additional time requirements it would add to our protocol. We were interested in a relatively short stress stimulus (10 minutes) with a short post-stress reassessment period (10 minutes). Studies that have documented the cortisol response to SET suggest post-stress peaks in cortisol between 15 and 35 minutes

[4, 12, 17, 45]. Other researchers even recommend the use of sAA over the use of cortisol to capture overall stress reactivity [9].

Several limitations should be considered when interpreting the findings of this study. First, this validation study was based on a subset of data from another study on the effects of stress and balance. As such, there are some components of the study design that may have been different had the primary goal during data collection been to validate this protocol. For instance, the validity of this protocol would be greater if a control group was included in the study design.

To increase generalizability of our findings, both men and women were included in this study. However, we acknowledge that the baseline heterogeneity of the two groups exceeded what would be considered ideal. The men were a slightly older cohort than the women, perhaps having to do with the higher number of students in the female cohort. The lack of change in stress biomarkers for the women in this study limit the implications that can be made for females regarding the FAF Test. It may strengthen the findings of this study to further investigate inter-rater reliability of the protocol, perhaps carried out by an investigator who is not male.

For researchers interested in utilizing this protocol for future projects, we have a few small recommendations that may further enhance the sympathetic response to the FAF Test. First, the serial subtraction task that took place at the end of the FAF Test may induce a more robust stress response if participants were instructed to start the task over after every incorrect answer, as is the protocol during the Trier Social Stress Test [18].

A second recommendation is that baseline posture should be considered. HR_1 was calculated while participants were sitting to complete the paperwork. Since the FAF Test was conducted in standing, the baseline physiologic measures may be better contextualized if the paperwork was completed while the participants were standing. This would allow any changes in the SAM to be free from postural influence and may better demonstrate isolated changes due to the SET of the FAF Test. Since it has been recommended that both psychological and physiological responses to stress should be considered in the validation protocol [44], future researchers may do well to incorporate a repeat administration of the STAI-S at T_4 which would corroborate the subjective response already captured with the VAS_{stress}.

Conclusion

This protocol for the FAF Test appears to be a valid stimulus to trigger an increase in the SAM in men. While the same physiologic increase in women was not demonstrated, both sexes subjectively reported a statistically significant and clinically meaningful increase in stress after the protocol. If women are to be included in future studies utilizing the FAF Test, investigators may do well to be more selective in their recruitment of participants regarding menstruation and the use of oral contraceptives. The FAF Test may be helpful for researchers interested in triggering the SAM system without using pain or exercise. Additionally, this protocol can be completed in 10-minutes, requires little space, and relies on few personnel.

Declaration of Competing Interest

The authors declare no conflicts of interest affecting the findings of this paper

Bibliography

[1] B.S. McEwen, Allostasis and allostatic load: implications for neuropsychopharmacology, Neuropsychopharmacology 22(2) (2000) 108-24.

[2] N.N. Burke, D.P. Finn, B.E. McGuire, M. Roche, Psychological stress in early life as a predisposing factor for the development of chronic pain: Clinical and preclinical evidence and neurobiological mechanisms, J Neurosci Res 95(6) (2017) 1257-1270.

[3] A.I. Turner, N. Smyth, S.J. Hall, S.J. Torres, M. Hussein, S.U. Jayasinghe, et al., Psychological stress reactivity and future health and disease outcomes: A systematic review of prospective evidence, Psychoneuroendocrinology 114 (2020) 104599.

[4] A. Woody, E.D. Hooker, P.M. Zoccola, S.S. Dickerson, Social-evaluative threat, cognitive load, and the cortisol and cardiovascular stress response, Psychoneuroendocrinology 97 (2018) 149-155.

[5] J. Strahler, N. Skoluda, M.B. Kappert, U.M. Nater, Simultaneous measurement of salivary cortisol and alpha-amylase: Application and recommendations, Neurosci Biobehav Rev 83 (2017) 657-677.

[6] K.E. Hannibal, M.D. Bishop, Chronic stress, cortisol dysfunction, and pain: a psychoneuroendocrine rationale for stress management in pain rehabilitation, Phys Ther 94(12) (2014) 1816-25.

[7] K. An, J. Salyer, R.E. Brown, H.F. Kao, A. Starkweather, I. Shim, Salivary Biomarkers of Chronic Psychosocial Stress and CVD Risks: A Systematic Review, Biol Res Nurs 18(3) (2016) 241-63.

[8] N. Skoluda, R. La Marca, M. Gollwitzer, A. Muller, H. Limm, B. Marten-Mittag, et al., Long-term stability of diurnal salivary cortisol and alpha-amylase secretion patterns, Physiol Behav 175 (2017) 1-8.

[9] N. Takai, M. Yamaguchi, T. Aragaki, K. Eto, K. Uchihashi, Y. Nishikawa, Effect of psychological stress on the salivary cortisol and amylase levels in healthy young adults, Arch Oral Biol 49(12) (2004) 963-8.

[10] S.S. Dickerson, M.E. Kemeny, Acute stressors and cortisol responses: a theoretical integration and synthesis of laboratory research, Psychol Bull 130(3) (2004) 355-91.

[11] R. Lazarus, S. Folkman, Stress, Appraisal, and Coping, Springer Publishing Company1984.

[12] N. Skoluda, J. Strahler, W. Schlotz, L. Niederberger, S. Marques, S. Fischer, et al., Intra-individual psychological and physiological responses to acute laboratory stressors of different intensity, Psychoneuroendocrinology 51 (2015) 227-36.

[13] J.R. Stroop, Studies of interference in serial verbal reactions, Journal of Experimental Psychology 18(6) (1935) 643-662.

[14] C.A. Reed, C.K. DuBois, K.A. Hutchison, T.J. Huppert, S.M. Monfort, Influence of serial subtraction tasks on transient characteristics of postural control, Hum Mov Sci 83 (2022) 102950.

[15] P.W. Hodges, H. Tsao, K. Sims, Gain of postural responses increases in response to real and anticipated pain, Exp Brain Res 233(9) (2015) 2745-52.

[16] G. Lamotte, C.J. Boes, P.A. Low, E.A. Coon, The expanding role of the cold pressor test: a brief history, Clin Auton Res 31(2) (2021) 153-155.

[17] C. Kirschbaum, K.M. Pirke, D.H. Hellhammer, The 'Trier Social Stress Test'--a tool for investigating psychobiological stress responses in a laboratory setting, Neuropsychobiology 28(1-2) (1993) 76-81.

[18] B.M. Kudielka, D.H. Hellhammer, C. Kirschbaum, Ten years of research with the Trier Social Stress Test (TSST) - revisited, in: E. Harmon-Jones, P. Winkielman (Eds.), Social Neuroscience: Integrating biological and psychological explanations of sicial behavior, Guilford Press, New York, 2007.

[19] G.L. Moseley, M.K. Nicholas, P.W. Hodges, Pain differs from non-painful attention-demanding or stressful tasks in its effect on postural control patterns of trunk muscles, Exp Brain Res 156(1) (2004) 64-71.

[20] D.H. Hellhammer, A.A. Stone, J. Hellhammer, J. Broderick, G.F. Koob, M.L. Moal, et al., Measuring Stress, Encyclopedia of Behavioral Neuroscience, Academic Press, Oxford, 2010, pp. 186-191.

[21] W.J. Inder, G. Dimeski, A. Russell, Measurement of salivary cortisol in 2012 - laboratory techniques and clinical indications, Clin Endocrinol (Oxf) 77(5) (2012) 645-51.

[22] G.A. Reyes del Paso, W. Langewitz, L.J. Mulder, A. van Roon, S. Duschek, The utility of low frequency heart rate variability as an index of sympathetic cardiac tone: a review with emphasis on a reanalysis of previous studies, Psychophysiology 50(5) (2013) 477-87.

[23] B.M. Kudielka, N.C. Schommer, D.H. Hellhammer, C. Kirschbaum, Acute HPA axis responses, heart rate, and mood changes to psychosocial stress (TSST) in humans at different times of day, Psychoneuroendocrinology 29(8) (2004) 983-92.

[24] U.M. Nater, N. Rohleder, W. Schlotz, U. Ehlert, C. Kirschbaum, Determinants of the diurnal course of salivary alpha-amylase, Psychoneuroendocrinology 32(4) (2007) 392-401.

[25] L. Helpman, J. Penso, O. Zagoory-Sharon, R. Feldman, E. Gilboa-Schechtman, Endocrine and emotional response to exclusion among women and men; cortisol, salivary alpha amylase, and mood, Anxiety Stress Coping 30(3) (2017) 253-263.

[26] D. Schoofs, R. Hartmann, O.T. Wolf, Neuroendocrine stress responses to an oral academic examination: No strong influence of sex, repeated participation and personality traits, Stress 11(1) (2008) 52-61.

[27] A.H. van Stegeren, O.T. Wolf, M. Kindt, Salivary alpha amylase and cortisol responses to different stress tasks: impact of sex, Int J Psychophysiol 69(1) (2008) 33-40.

[28] L. Espin, C. Villada, V. Hidalgo, A. Salvador, Effects of sex and menstrual cycle phase on cardiac response and alpha- amylase levels in psychosocial stress, Biol Psychol 140 (2019) 141-148.

[29] E. Barel, R. Abu-Shkara, R. Colodner, R. Masalha, L. Mahagna, O.C. Zemel, et al., Gonadal hormones modulate the HPA-axis and the SNS in response to psychosocial stress, J Neurosci Res 96(8) (2018) 1388-1397.

[30] L.J. Julian, Measures of anxiety: State-Trait Anxiety Inventory (STAI), Beck Anxiety Inventory (BAI), and Hospital Anxiety and Depression Scale-Anxiety (HADS-A), Arthritis Care Res (Hoboken) 63 Suppl 11(0 11) (2011) S467-72.

[31] S. Cohen, T. Kamarck, R. Mermelstein, A global measure of perceived stress, J Health Soc Behav 24(4) (1983) 385-96.

[32] D.J. Buysse, C.F. Reynolds, 3rd, T.H. Monk, S.R. Berman, D.J. Kupfer, The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research, Psychiatry Res 28(2) (1989) 193-213.

[33] J.A. Lipponen, M.P. Tarvainen, A robust algorithm for heart rate variability time series artefact correction using novel beat classification, J Med Eng Technol 43(3) (2019) 173-181.

[34] M.P. Tarvainen, J.P. Niskanen, J.A. Lipponen, P.O. Ranta-Aho, P.A. Karjalainen, Kubios HRV--heart rate variability analysis software, Comput Methods Programs Biomed 113(1) (2014) 210-20.

[35] F.X. Lesage, S. Berjot, F. Deschamps, Clinical stress assessment using a visual analogue scale, Occup Med (Lond) 62(8) (2012) 600-5.

[36] P.S. Myles, D.B. Myles, W. Galagher, D. Boyd, C. Chew, N. MacDonald, et al., Measuring acute postoperative pain using the visual analog scale: the minimal clinically important difference and patient acceptable symptom state, Br J Anaesth 118(3) (2017) 424-429.

[37] V.S. Williams, R.J. Morlock, D. Feltner, Psychometric evaluation of a visual analog scale for the assessment of anxiety, Health Qual Life Outcomes 8 (2010) 57.

[38] H.M. Moore, A.B. Kelly, S.D. Jewell, L.M. McShane, D.P. Clark, R. Greenspan, et al., Biospecimen reporting for improved study quality (BRISQ), Cancer Cytopathol 119(2) (2011) 92-101.

[39] Z. Yu, M. Guindani, S.F. Grieco, L. Chen, T.C. Holmes, X. Xu, Beyond t test and ANOVA: applications of mixed-effects models for more rigorous statistical analysis in neuroscience research, Neuron 110(1) (2022) 21-35.

[40] A.D. Crosswell, K.G. Lockwood, Best practices for stress measurement: How to measure psychological stress in health research, Health Psychology Open 7(2) (2020) 2055102920933072.

[41] A. Duchesne, J.C. Pruessner, Association between subjective and cortisol stress response depends on the menstrual cycle phase, Psychoneuroendocrinology 38(12) (2013) 3155-9.

[42] K. Prabhavathi, K.T. Selvi, K.N. Poornima, A. Sarvanan, Role of biological sex in normal cardiac function and in its disease outcome - a review, J Clin Diagn Res 8(8) (2014) BE01-4.

[43] D. Ramaekers, H. Ector, A.E. Aubert, A. Rubens, F. Van de Werf, Heart rate variability and heart rate in healthy volunteers: is the female autonomic nervous system cardioprotective?, European Heart Journal 19(9) (1998) 1334-1341.

[44] A.P. Allen, P.J. Kennedy, J.F. Cryan, T.G. Dinan, G. Clarke, Biological and psychological markers of stress in humans: focus on the Trier Social Stress Test, Neurosci Biobehav Rev 38 (2014) 94-124.

[45] W.R. Lovallo, Farag, Noha H., Vincent, Andrea S., Thomas, Terrie L., Wilson, Michael F., Cortisol responses to mental stress, exercise, and meals following caffeine intake in men and women, Pharmacology, Biochemistry and Behavior 83 (2006) 441-447.

CHAPTER THREE

THE ASSOCIATION OF ACUTE STRESS AND LOWER QUARTER EMG DURING A SINGLE LEG BALANCE DEXTERITY TASK

Abstract

Background

There is a well-established connection between psychosocial stress and low back pain, however studies demonstrating a connection between motor control and acute stress are lacking.

Research Question

What is the potential interaction of acute stress (the Feigned Annoyance and Frustration Test, or the FAF Test) with lower quarter motor control and stance stability during a standing lower-extremity dexterity task in individuals with and without low back pain?

Methods

This prospective cohort study included 30 individuals with low back pain (15 men and 15 women) with an equal number of sex-matched controls for a total sample of 60 participants. Participants were fitted with surface electromyography sensors over the trunk and lower extremities. The lower extremity dexterity task was performed on 2 in-ground force plates while center of pressure data was collected. Participants were then exposed to the FAF Test and the lower extremity dexterity task was repeated.

Results

Both the control and low back pain groups showed a significant increase in stress following exposure to the FAF Test. Both groups showed altered trunk muscle activation while stressed, with a larger number of motor control deviations existing in the low back pain cohort. The participants with low back pain also demonstrated less total excursion of the center of pressure and decreased center of pressure velocity after exposure to acute stress.

Significance

Acute stress appears to alter trunk postural control during a standing lower extremity dexterity task, with more pronounced changes apparent in participants with low back pain. These findings provide novel insight into the direct influence of stress on trunk motor control and balance stability.

Introduction

The interaction of low back pain (LBP) and postural control has been widely studied. Van Dieën et al. 2019 described two strategies of spinal mechanics in individuals with LBP: “tight control” and “loose control” [1]. Each pattern of activation is grossly defined as increased (“tight”) or decreased (“loose”) excitability and co-contraction of trunk muscles [1]. The etiology of these changes have been theorized to be associated with various cognitive affective tendencies such as kinesiophobia [2] and fear-avoidance [3, 4].

Studies on cognitive load (CL) and dual-tasking have been shown to have a complex interaction with center of pressure (CoP) stability while standing [5, 6]. It has

been shown that in individuals with a pre-existing musculoskeletal injury, dual-tasking accentuates alterations in movement strategies [7]. In a study on balance stability and CL, investigators reported that individuals with LBP showed greater variations in CoP velocity, sway area, and displacement while standing [5]. In contrast, others have demonstrated compensatory stiffening of the trunk through reduced postural sway in participants scoring higher on pain-related anxiety scales [8].

Social Evaluative Threat (SET)—a robust type of CL characterized by the possibility of negative judgment from others—has the potential to elicit a significant stress response across various systems [9]. While the effect of stress on overall health has been extensively researched [10], minimal literature exists on the correlation of acute stress with motor control. One group of investigators demonstrated distinct alterations in the timing of the deep lumbar multifidi activation during preparatory movement with exposure to SET [11].

The primary aim of this graduate student research study is to determine whether acute stress alters the biomechanics associated with single leg balance by influencing trunk and lower extremity muscle activation in individuals with and without LBP. The secondary aim of this study is to assess whether LBP interacts with stress regarding these effects. Our hypothesis is that stress will alter muscle activation and that these changes will be more robust in individuals with LBP.

Methods

Participants

This study was approved by the internal review board at Loma Linda University (IRB# 5210188). Sixty participants were recruited from a convenience sample at Loma Linda University and the surrounding area. Inclusion criteria for the control group were: age between 18 and 45 and the ability to balance on one leg. Exclusion criteria for the control group were: a diagnosed anxiety disorder, history of low back surgery, current pregnancy (or pregnancy in the past 12 months), severe pain (current pain >6/10), and color blindness. Inclusion and exclusion criteria for the group with LBP were the same as the control group with the additional inclusion criterion of chronic or recurrent LBP, defined as pain >3 months located between T12 and the gluteal fold. All participants who completed the study were compensated with a $25 gift card.

Quiescence period and Subjective Report Outcome Measures

Figure 1 represents a graphical timeline for the study protocol. All trials took place between 2:30 pm and 7:00 pm. After arriving at the lab, participants were seated at a desk for a quiescence period. During this time, participants were consented and fitted with a polar H10 HR sensor which was then connected via Bluetooth to an iPad. Participants remained seated while they completed a list of self-report outcome measures. We used this time to establish baseline HR and stress levels.

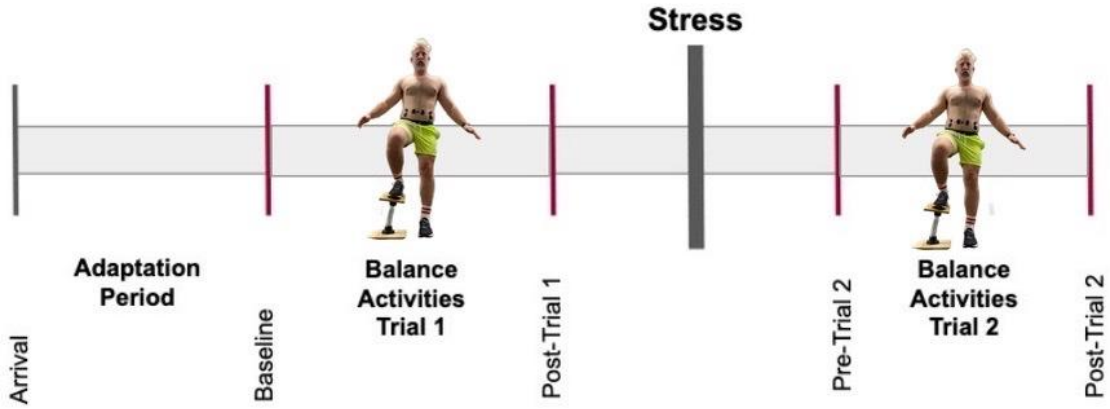


Figure 1: Graphical representation of study timeline

Visual Analog Scale (VAS)

Participants were instructed to rate their current stress level on a 10-centimeter line with the anchors: "None" and "As bad as it could be." Participants repeated this same self-assessment at the end of the trial. The VAS has been validated as a measure for subjective stress [12]. Additionally, while the minimal clinically important difference for the VAS_{stress} has not been established, it has been reported for anxiety between 1.2 and 1.3 [13].

Heart Rate

Heart Rate (HR) was recorded using the mobile application, EliteHRV (Version 5.5.4, mobile app for IOS, EliteHRV.com, USA). HR data was exported from Elite HRV as raw inter-beat interval data. It was imported into Kubios HRV Scientific version 4.0.1 [14] where it was filtered for artifact and ectopic beats using the previously validated Kubios algorithm [15]. The HRs were then visually inspected for missing data and

erroneously marked beats. Average HR was calculated for two distinct time periods (HR_1=time after completion of paperwork until after the lower extremity dexterity task (LEDT) trial 1 and HR_2=time after completion of the FAF test until the time after completion of the LEDT trial 2).

Surface Electromyography (sEMG)

Participants were instrumented with twelve Delsys Trigno Avanti sEMG electrodes (Delays Incorporated, Natick, MA) with a sampling frequency of 2000Hz. Skin was shaved, abraded, and cleaned with isopropyl alcohol before the placement of the sensors. Electrodes were placed bilaterally over the rectus abdominis (RA), obliquus externus (EO), multifidus lumborum (ML), and obliquus internus/transversus abdominis (IOTA). Sensor locations were determined in accordance with previously validated methods [16, 17]. Electrodes were named for laterality based on foot dominance [18]. Electrodes were placed unilaterally over the gluteus maximus (GMAX), gluteus medius (GMED), and biceps femoris (BF) on the dominant leg and over the rectus femoris (RF) on the non-dominant leg (leg dominance defined below). Extremity electrodes and ML electrodes were placed based on SENIAM guidelines [19].

Lower Extremity Dexterity Task

The LEDT was modeled after a task previously described and was determined to be an acceptably reliable task to test balance, lower extremity dexterity, and trunk coordination [20]. For the task, leg dominance was determined based on the participants' preferred stance leg when kicking a soccer ball [18]. Participants were instructed to stand with the dominant leg on an in-ground force plate (AMTI Gen5 force plate, AMTI,

Watertown, MA, capture frequency 1000Hz) as they placed the non-dominant leg on a 12-inch box on an identical, adjacent force plate. The vertical force vector corresponding to the force plate with the box was then displayed on a monitor as feedback for the participants. Participants were instructed to press their foot onto the box until the feedback line on the monitor was as high and then as stable as possible. Participants were instructed not to shift their weight onto the box, but rather to maintain their dominant leg as the primary stance leg. Two trials were completed with the box. SEMG data collected during these static trials were used for normalization of the EMG signals collected during the LEDT. The trials lasted 30 seconds.

After the normalization trials, the box was removed and replaced with a custom-built compressible spring platform. The apparatus was constructed by affixing a hollowed out, 3D printed cylinder (CAD file available upon request) to a 13-inch square by ½-inch pine board base. An identical 3D-printed cylinder was secured to a 13-inch by 8-inch by ½-inch pine board platform. A 12-inch spring was placed in the cylinders to complete the construction of the device Compression Spring model 805; Century Spring Corporation, Commerce, CA) (Figure 2). Participants were given the same instructions they received during the box trials. Participants were given one familiarization trial and three practice trials, during which the target force was determined from the participants' maximum stable force based on the researcher's visual appraisal of the real time feedback graph. The participants were then cued to maintain a stable force output to match the maximum stable force target level. After a short break, the participants performed three, 30-second trials, with the goal of compressing the spring platform until the feedback line reached

the target force set during the practice trials. Each of the spring trials were trimmed to preserve the middle 50% for analysis. EMG data was bandpass filtered from 20-350 Hz using a Butterworth filter and an RMS (root mean squared) with a 50 ms window. Mean EMG amplitude was extracted from the middle 50% of the normalization trials. All subsequent EMG data was normalized to that mean amplitude and expressed as a percentage of the normalized value. The value from each of the three LEDT trials was then averaged and used to represent muscle activation during the task. The force plate data was filtered with a lowpass Butterworth filter with a frequency cutoff of 50Hz [18]. Center of pressure (CoP) calculations in both the anterior-posterior and medial-lateral directions were based on previously defined variables [21]: distance (D: average distance from the mean CoP), range (R: maximum distance between any two points in the CoP path), RMS distance (Root mean squared of the resultant distance between mean CoP and CoP coordinates in A-P and M-L), total excursion (TE: mean velocity of the CoP/Time), and velocity (V: average velocity of the CoP).

Figure 2: (a) 12-inch box for sEMG normalization trials (b) Spring apparatus used for the LEDT

Feigned Annoyance and Frustration (FAF) Test

A protocol for the FAF Test has been previously published and has been validated for inducing stress in men and women [22]. In brief, the application used was based on the Stroop Effect (Brain Test - Stroop Effect, Copyright Attila Hegedus). It consists of a series of color words (“red” or “green” in matching or contrasting color text) appearing on a screen while the participants are instructed to tap the word on bottom of the screen

that correlated with the ink color of the word at the top of the screen. As the task progressed, the participants were made to believe they were performing the task poorly through the investigator's feigned annoyance and frustration. During the FAF Test, it was emphasized that the trial could be terminated at any time at the request of the participant, however all participants completed the full protocol. The protocol was executed by the same male researcher (TG) for all participants.

Data Analysis

Mean and standard deviation (SD) were computed for quantitative variables, median (min, max) was computed for ordinal variables, and frequency (percentage) was computed for nominal variables. Normality of quantitative variables was assessed using the Shapiro-Wilk test and box plots. Raw data for EMG values and CoP variables were log transformed to address non-normality and outliers. The independent χ^2 test was used to compare nominal variables between the two groups at baseline. The independent t-test was used to compare continuous independent variables for both groups at baseline. The Mann-Whitney U test was used to compare the ordinal variables and variables with non-normal distribution. A linear mixed effects model (repeated measures) was used to examine the effect of the between-group factor (control and LBP) and within-group factor (time) on the dependent variables (HR and VAS_{stress}) [23]. A Bonferroni correction was used to adjust for multiple post-hoc comparisons. Management and analysis of data was performed using the statistical package SPSS for Mac version 29.0 (SPSS Inc, Chicago, IL, USA). The level of statistical significance was set at $p<.05$.

Results

Sixty participants were included in the study. Participants without back pain were assigned to the control group (n= 30, 15 females, 15 males) and participants who met the inclusion criteria for LBP were assigned to the group with LBP (n=30, 15 females, 15 males.) None of the demographic variables were significantly different between groups at baseline (Table 1). There was no significant difference for between group comparisons of muscle activation, CoP variables, or force output during the LEDT during the pre-stress trial, with the exception of IIOTA activation (p=.049) (Appendix 1: Table 3).

Table 1: Demographics and baseline data

Characteristics	Control (n_1=30)	LBP (n_2=30)	p – value
Age (years)	29.1 ± 5.4	28.4 ± 4.3	0.598
Sex[a] (Female/Male)	50/50	50/50	0.602
BMI	24.0 ± 4.0	25.4 ± 3.6	0.143
Leg Dominance[a] (Left/Right)	90/10	87/13	1.000
Duration of Low Back Pain (months)		51.4 ± 58.1	
STAI-State Subscale	28.7 ± 7.9	31.9 ± 9.0	0.210
STAI-Trait Subscale	36.0 ± 8.0	38.6 ± 11.6	0.367
TSK	28.7 ± 5.8	31.3 ± 5.4	0.090
PSQI	4.8 ± 2.4	5.8 ± 2.4	0.174
PSS	13.9 ± 6.0	15.8 ± 5.6	0.208
ODI[b]		8 (0, 24)	
NPRS[c]		1.7 (0, 5)	
VAS_{stress} (cm)[b]	1.30 (0,9)	1.27 (0,7)	0.796
Heart Rate (bpm)	76.0 ± 13.3	78.0 ± 11.4	0.566

Abbreviations: BMI: body mass index; NPRS: numerical pain rating scale, STAI: state-trait anxiety inventory; TSK: Tampa scale of kinesiophobia, PSQI: Pittsburgh sleep quality index; PSS: perceived stress scale; VAS_{stress}: visual analog scale for stress; ODI: Oswestry disability index
Values are presented as mean ± SD unless otherwise indicated.

[a] frequency (percentage)
[b] Median (min, max)
[c] p-values for log-transformed data

Seven subjects did not have post-stress VAS_{stress} ratings, and 3 subjects had missing HR data. Both the control group and group with LBP demonstrated a significant increase in stress over time after exposure to the FAF based on the VAS_{stress} (p=.001 and p=.001, respectively) and HR (p=.001 and p=.001, respectively. Figure 3). However, there was no statistically significant difference between groups (Table 2).

Table 2: Stress reactivity to the FAF overall with within (time) and between groups comparison

Variable			Control (n_1=29)	LBP (n_2=28)	$p^{\diamond\diamond}$
			Mean ± SD	**Mean±SD**	
HR (BPM)		HR_1	76.1 ± 13.3$^{\dagger ab}$	78.0 ±11.4$^{\dagger ab}$	.873
		HR_2	82.7 ± 13.0*	82.7 ± 12.2*^{b}	
		HR_3	83.0 ± 14.9*^{b}	83.7 ±15.0*^{b}	
		HR_4	87.0 ± 17.6*$^{\dagger a}$	86.7 ± 14.5*$^{\dagger a}$	
	$p^{\diamond}$		**<.001**	**<.001**	
VAS_{stress} (cm)		T_1	1.6 ± 1.8	2.0 ± 2.2	.193
		T_4	3.6 ± 1.9	3.5 ± 2.4	
	$p^{\diamond}$		**<.001**	**<.001**	

Abbreviations: VAS_{stress}: Visual Analog Scale for stress; HR: Heart Rate, BPM: Beats Per Minute; HR_1: mean Heart Rate from beginning of the collection until T_1; HR_2: mean Heart Rate from T_1 to T_2; HR_3: mean Heart Rate from T_2 to T_3; HR_4: mean Heart Rate from T_3 to T_4; T_1: after 25-minute quiescence period, T_2: immediately pre-stress, T_3: immediately post-stress, T_4: 10-minutes post-stress

*p-value<.05 for within groups compared to HR_1
†p-value <.05 for within groups compared to HR_2
^{a}p-value <.05 for within groups compared to HR_3
^{b}p-value <.05 for within groups compared to HR_4

$^{\diamond}$ p-value for the null hypothesis that there is no significant difference within groups (variable x time)
$^{\diamond\diamond}$ p-value for the null hypothesis that there is no significant difference between control group and group with LBP

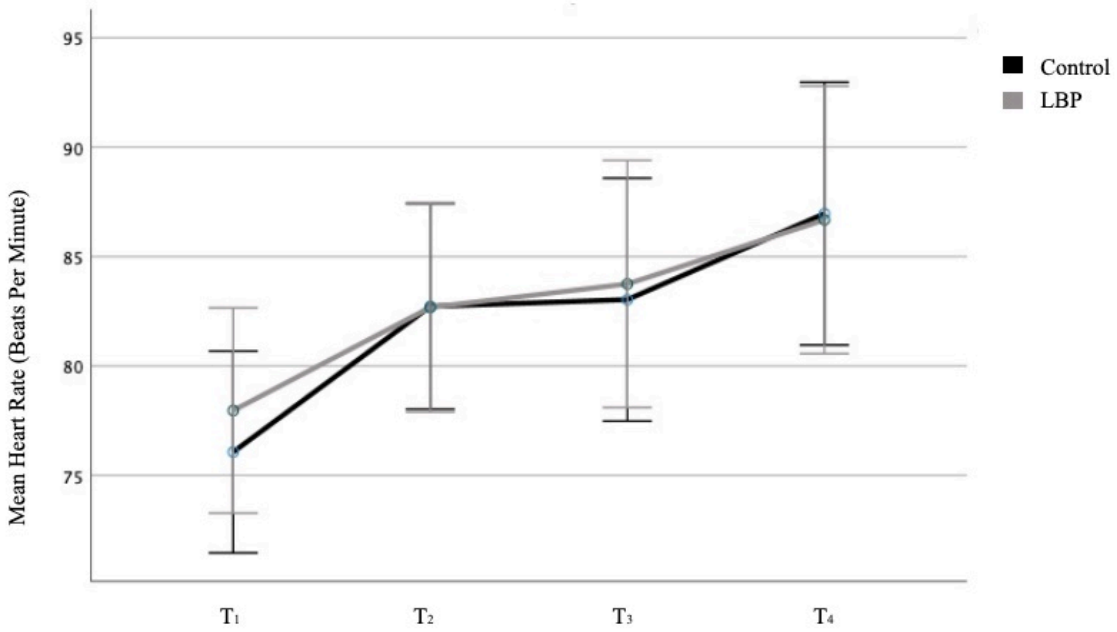


Figure 3: Heart rate changes over time for control group and group with LBP

T_1: after 25-minute quiescence period, T_2: immediately pre-stress, T_3: immediately post-stress, T_4: 10-minutes post-stress

Error bars represent 95% CI

Within the control group, only the CML and the CRA showed a statistically significant decrease in activation post stress (p=.007 and p=.025, respectively). However, within the group with LBP all of the trunk and gluteal muscles demonstrated a significant decrease in activation when comparing pre- and post-stress trials (CEO: p=.007; IEO: p=.001; CIOTA: p=.001; IIOTA: p=.016; CML: p=.013; IML: p=.002; CRA: p=.003; IRA: p=.002; GMAX: p=.013; GMED: p=.006). There were no statistically significant differences in activation between groups (Figure 4; Appendix: Table 4).

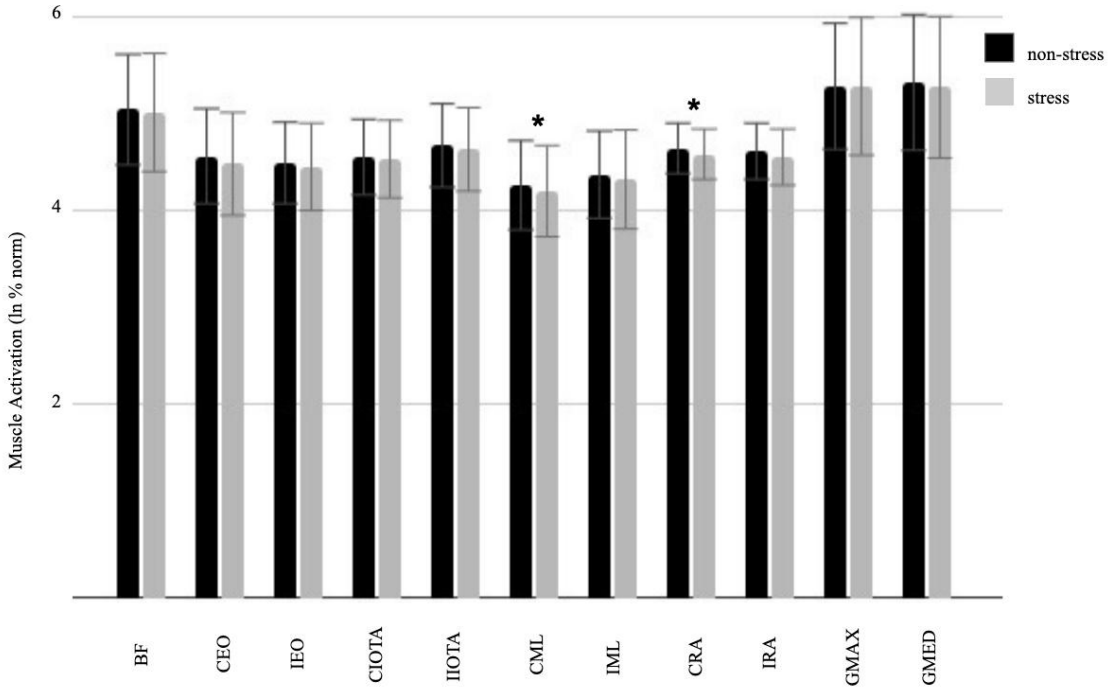


Figure 4a: Mean muscle activation in % normalization for control group during non-stress and stress trials.

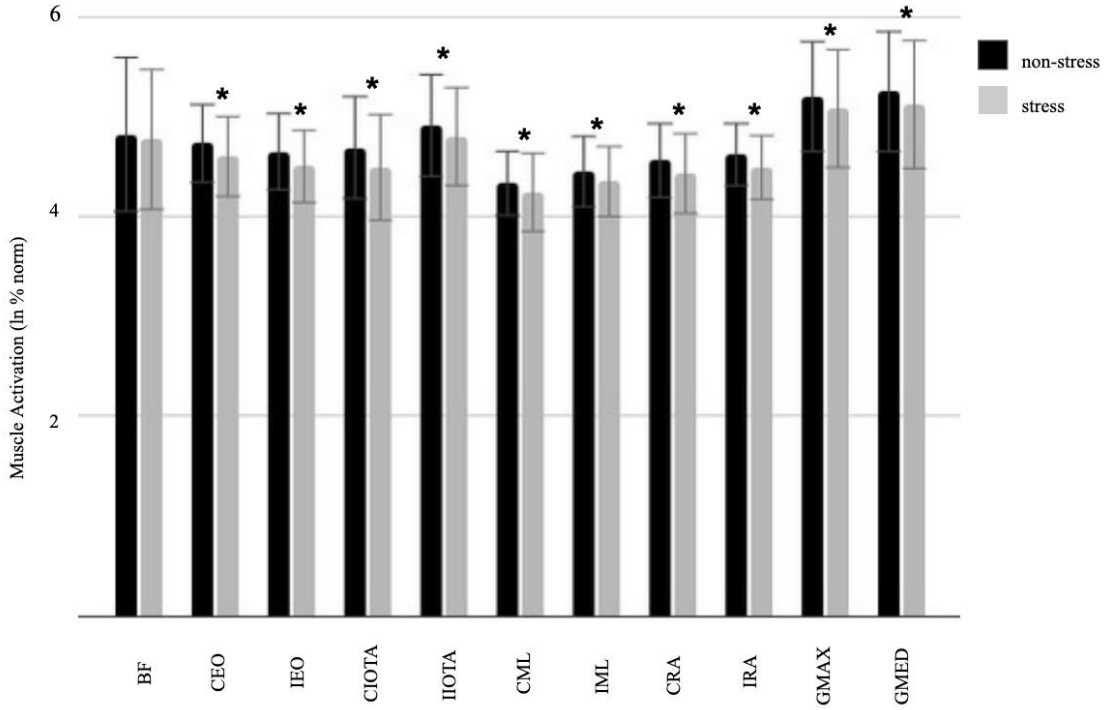


Figure 4b: Mean muscle activation in % normalization for LBP group during non-stress and stress trials

Abbreviations: BF: Biceps femoris; CEO: contralateral obliquus externus; IEO: ipsilateral obliquus externus; CIOTA: contralateral obliquus internus/transversus abdominis; IIOTA: ipsilateral obliquus internus/transversus abdominis; CML: contralateral multifidus lumborum; IML: ipsilateral multifidus lumborum; CRA: contralateral rectus abdominis; IRA: ipsilateral rectus abdominis; GMAX: gluteus maximus; GMED: gluteus medius
*p<.05. Error bars represent standard deviation. Values displayed have been log-transformed.

Within the control group, none of the variables related to CoP exhibited a significant difference pre- and post-stress. For pre- and post-stress comparison of CoP variables within the group with LBP, there was a statistically significant decrease in TE in both the medial-lateral and anterior-posterior plane (p=.010 and p=.003, respectively) as well as a significant decrease in the velocity of the CoP in the medial-lateral and anterior-posterior planes (p=.010 and p=.003, respectively). There were no statistically significant differences between the groups. (Figure 4; Appendix: Table 5).

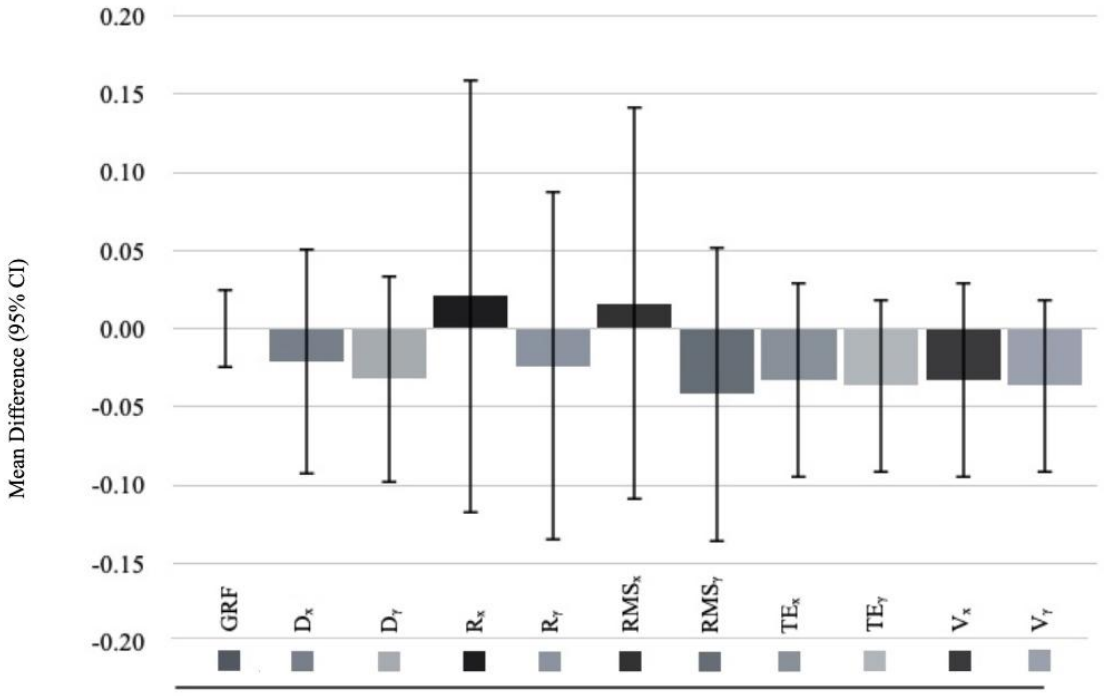


Figure 5a: Mean difference of center of pressure variables for control group comparing non-stress and stress trials

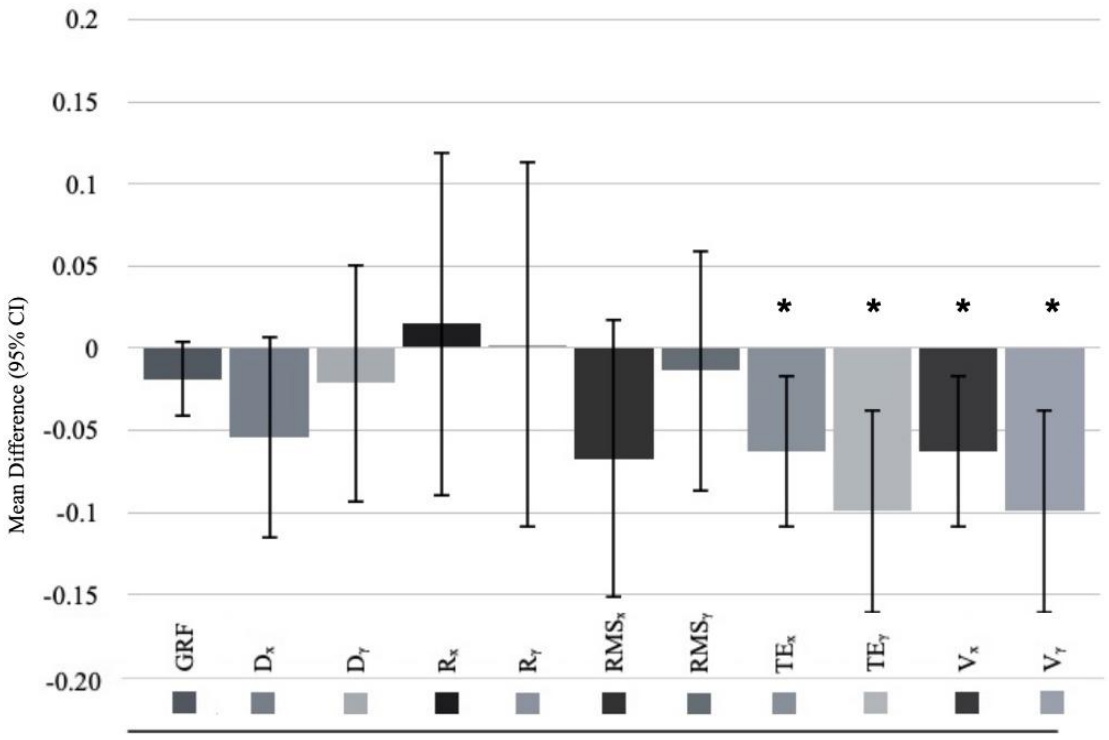


Figure 5b: Mean difference of center of pressure variables for LBP group comparing non-stress and stress trials

Abbreviations: GRF: ground reaction force, D_X: frontal plane distance travelled; D_Y: sagittal plane distance travelled;, R_X: frontal plane range;R_Y: sagittal plane range; RMS_X:Root Mean Squared of the distance travelled in the frontal plane; RMS_Y: root mean squared of the distance travelled in the sagittal plane; TE_X: total excursion of the center of pressure in the frontal plane; TE_Y:total excursion of the center of pressure in the sagittal plane; V_X: velocity of the center of pressure in the frontal plane; V_Y: velocity of the center of pressure in the sagittal plane *p<.05. Error bars represent 95% confidence interval. Values displayed have been log-transformed.

Discussion

Both groups demonstrated altered strategies for trunk and lower quarter muscle activation under stress, with a greater number of alterations seen in individuals who have chronic or recurrent LBP. This is consistent with the originally stated hypothesis. At baseline, both groups demonstrated muscle recruitment strategies for trunk and lower extremity control that were not significantly different, except for the activation of the IIOTA for which the group with LBP demonstrated significantly increased activation

compared to the control group (Appendix: Table 4). Overall, there is inconsistency in the literature when comparing strategies for trunk muscle activation for individuals with LBP. Several studies suggest patterns of increased activation for individuals with LBP [24-30]. Others contend that the strategies are exceptionally variable and perhaps more based on task type, intensity, and other psychosocial factors [1, 31, 32].

Participants were adequately stressed by the FAF Test as evidenced by the significant increase in the VAS_{stress} and HR. While both groups in our study demonstrated a difference in trunk motor control during the LEDT after exposure to the FAF Test, participants with LBP demonstrated a greater number of deviations compared to the control group. Most notably, in the group with LBP every trunk muscle measured exhibited a significant decrease in activation despite participants' maintaining the same overall force output. In addition to changes in trunk muscle activation, individuals with LBP demonstrated decreased activation of the GMED and GMAX after being exposed to the FAF Test. This change was not apparent in the control group.

We measured muscle activation using SEMG and were therefore largely observing activation of superficial muscle groups. Additional insight may be gained from observing the effect of stress on deep trunk musculature using fine wire EMG. Moseley, Nicolas, and Hodges observed a delay in the activation of deep trunk muscles due to stress distinct from what has been demonstrated due to pain . [11]

Stress did not have an effect on the stance stability of individuals without LBP. However, participants with LBP demonstrated reduced CoP velocity and TE during the task. Other researchers have similarly demonstrated decreased CoP velocity in

individuals with LBP [33, 34]. Xiao et al. discussed this phenomenon where individuals with LBP demonstrate a paradoxical improvement in stance stability when exposed to additional CL [6]. This may be due to redirecting postural control from a cognitive process to a more reflexive and unconscious control, which has been described as a "posture first" principle [35]. Other researchers suggest that increased velocity of center of pressure may be due to exploratory strategies to enhance postural control and should not be interpreted as decreased stability [36]. Still others hypothesize that these changes are representative of deviations in balance strategy altogether (i.e. moving away from a "hip strategy" to an "ankle strategy") [37]. Regardless, this represents a novel finding suggestive of altered biomechanics in the LEDT in response to acute stress.

Our study had some limitations that should be addressed. Due to the study design, we were unable to control any potential effect of fatigue on task performance. And while trunk extensors and GMAX have been shown to have greater fatiguability in individuals with LBP, the dynamometry-based, maximal exertion tasks that have been studied and used to support this finding [38, 39], have very different mechanical demands than our upright, sub-maximal, LEDT. Additionally, for our task, there was an extended recovery time allowed between trials to mitigate the effect of fatigue. Another limitation previously described was our use of SEMG instead of fine wire, limiting our observation to superficial fibers of the target muscles. And while the electrode placement protocol was previously validated, it is well-documented that measurement of isolated activation of the abdominal muscles is not feasible with SEMG [17]. It is worth noting, however, that

some investigators recommend the use of SEMG instead of intramuscular fine wire EMG for motor tasks involving high levels of muscle activation [40].

The neuromuscular changes noted in the findings of this study should be considered in the context of physical therapy practice as it relates to patient education and pain etiology. Additionally, physical therapists should consider the role of stress mitigation and behavioral modification strategies for stress management in their physical therapy practice considering how psychosocial stress may interact with neuromuscular control during the therapeutic process. At the very least, the findings in this study should inform physical therapists in practice of the need to address psychosocial stress in their patients through referrals and interdisciplinary integration with experts who are better prepared to utilize formal strategies to treat the psychological distress. While it is unclear whether the neuromuscular changes demonstrated in this study are maladaptive, it may provide insight to patients and therapists regarding movement strategies in the presence of social stress.

Future investigation may consider the role task novelty plays in task performance and stress response. While the LEDT was not designed to be overly challenging, it was novel to all of the participants and therefore placed a degree of CL that likely wouldn't be present in the context of a familiar task. Additionally, studies that better control for trunk muscle fatigue may be warranted to further validate the interaction of acute stress and trunk motor control.

Conclusion

Exposure to SET alters trunk and lower extremity muscle activation during a sub-maximal LEDT. These effects as well as changes in CoP stability are more notable in individuals with chronic LBP. The findings of this study further demonstrate the complex interaction of trunk and lower extremity motor control with LBP. Additionally, these findings suggest that psychosocial stress as it relates to trunk and lower extremity muscle activation should be considered in the context of neuromuscular reeducation and patient education for the treatment of individuals with LBP.

Acknowledgements

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Special thanks to:

Charlotte Gehrig for assistance with proof-reading and graphics

Ted Gehrig, Jr. for assistance with proof-reading and data management

Mike Davidson for biomedical engineering contributions

Lab assistants: Simran Jaisinghani, Kezia Marceline, Owee Mulay, Aaditi Gokhale, and Raaga Mullapati

Bibliography

[1] J.H. van Dieën, N.P. Reeves, G. Kawchuk, L.R. van Dillen, P.W. Hodges, Motor Control Changes in Low Back Pain: Divergence in Presentations and Mechanisms, The Journal of orthopaedic and sports physical therapy 49(6) (2019) 370-379.

[2] N.V. Karayannis, R.J. Smeets, W. van den Hoorn, P.W. Hodges, Fear of Movement Is Related to Trunk Stiffness in Low Back Pain, PloS one 8(6) (2013) e67779.

[3] M. Unsgaard-Tøndel, T.I. Nilsen, J. Magnussen, O. Vasseljen, Are fear avoidance beliefs associated with abdominal muscle activation outcome for patients with low back pain?, Physiotherapy research international : the journal for researchers and clinicians in physical therapy 18(3) (2013) 131-9.

[4] B. Van Damme, V. Stevens, D. Van Tiggelen, C. Perneel, G. Crombez, L. Danneels, Performance based on sEMG activity is related to psychosocial components: differences between back and abdominal endurance tests, J Electromyogr Kinesiol 24(5) (2014) 636-44.

[5] L. Ge, Q. Yu, C. Wang, H. Huang, X. Li, S. Zhang, et al., How cognitive loads modulate the postural control of older women with low back pain?, BMC Geriatr 21(1) (2021) 82.

[6] W. Xiao, H. Yang, Z. Wang, H. Mao, H. Wang, Z. Hao, et al., Postural Control of Patients with Low Back Pain Under Dual-Task Conditions, J Pain Res 16 (2023) 71-82.

[7] C.J. Burcal, A.R. Needle, L. Custer, A.B. Rosen, The Effects of Cognitive Loading on Motor Behavior in Injured Individuals: A Systematic Review, Sports Med 49(8) (2019) 1233-1253.

[8] S. Shanbehzadeh, M. Salavati, S. Talebian, K. Khademi-Kalantari, M. Tavahomi, Attention demands of postural control in non-specific chronic low back pain subjects with low and high pain-related anxiety, Exp Brain Res 236(7) (2018) 1927-1938.

[9] E.S. Poppelaars, J. Klackl, B. Pletzer, F.H. Wilhelm, E. Jonas, Social-evaluative threat: Stress response stages and influences of biological sex and neuroticism, Psychoneuroendocrinology 109 (2019) 104378.

[10] H. Yaribeygi, Y. Panahi, H. Sahraei, T.P. Johnston, A. Sahebkar, The impact of stress on body function: A review, Excli j 16 (2017) 1057-1072.

[11] G.L. Moseley, M.K. Nicholas, P.W. Hodges, Pain differs from non-painful attention-demanding or stressful tasks in its effect on postural control patterns of trunk muscles, Exp Brain Res 156(1) (2004) 64-71.

[12] F.X. Lesage, S. Berjot, F. Deschamps, Clinical stress assessment using a visual analogue scale, Occup Med (Lond) 62(8) (2012) 600-5.

[13] V.S. Williams, R.J. Morlock, D. Feltner, Psychometric evaluation of a visual analog scale for the assessment of anxiety, Health Qual Life Outcomes 8 (2010) 57..

[14] M.P. Tarvainen, J.P. Niskanen, J.A. Lipponen, P.O. Ranta-Aho, P.A. Karjalainen, Kubios HRV--heart rate variability analysis software, Comput Methods Programs Biomed 113(1) (2014) 210-20.

[15] J.A. Lipponen, M.P. Tarvainen, A robust algorithm for heart rate variability time series artefact correction using novel beat classification, J Med Eng Technol 43(3) (2019) 173-181.

[16] S.M. McGill, Electromyographic activity of the abdominal and low back musculature during the generation of isometric and dynamic axial trunk torque: implications for lumbar mechanics, J Orthop Res 9(1) (1991) 91-103.

[17] S. McGill, D. Juker, P. Kropf, Appropriately placed surface EMG electrodes reflect deep muscle activity (psoas, quadratus lumborum, abdominal wall) in the lumbar spine, J Biomech 29(11) (1996) 1503-7.

[18] K.M. Rowley, J.A. Smith, K. Kulig, Reduced Trunk Coupling in Persons With Recurrent Low Back Pain Is Associated With Greater Deep-to-Superficial Trunk Muscle Activation Ratios During the Balance-Dexterity Task, The Journal of orthopaedic and sports physical therapy 49(12) (2019) 887-898.

[19] H.J. Hermens, B. Freriks, C. Disselhorst-Klug, G. Rau, Development of recommendations for SEMG sensors and sensor placement procedures, Journal of Electromyography and Kinesiology 10 (2000) 361-374.

[20] K.M. Rowley, J. Gordon, K. Kulig, Characterizing the balance-dexterity task as a concurrent bipedal task to investigate trunk control during dynamic balance, J Biomech 77 (2018) 211-217.

[21] T.E. Prieto, J.B. Myklebust, R.G. Hoffmann, E.G. Lovett, B.M. Myklebust, Measures of postural steadiness: differences between healthy young and elderly adults, IEEE Transactions on Biomedical Engineering 43(9) (1996) 956-66.

[22] T.W. Gehrig, L.S. Berk, R.I. Dudley, J.A. Smith, L. Gharibvand, E.B. Lohman, The feigned annoyance and frustration test to activate the sympathoadrenal medullary system, Comprehensive Psychoneuroendocrinology 18 (2024).

[23] Z. Yu, M. Guindani, S.F. Grieco, L. Chen, T.C. Holmes, X. Xu, Beyond t test and ANOVA: applications of mixed-effects models for more rigorous statistical analysis in neuroscience research, Neuron 110(1) (2022) 21-35.

[24] F. Ehsani, A.M. Arab, S. Jaberzadeh, M. Salavati, Ultrasound measurement of deep and superficial abdominal muscles thickness during standing postural tasks in participants with and without chronic low back pain, Manual therapy 23 (2016) 98-105.

[25] M. Freddolini, S. Strike, R. Lee, Dynamic stability of the trunk during unstable sitting in people with low back pain, Spine 39(10) (2014) 785-90.

[26] M. Freddolini, S. Strike, R.Y. Lee, Stiffness properties of the trunk in people with low back pain, Hum Mov Sci 36 (2014) 70-9.

[27] P. Hodges, W. van den Hoorn, A. Dawson, J. Cholewicki, Changes in the mechanical properties of the trunk in low back pain may be associated with recurrence, J Biomech 42(1) (2009) 61-6.

[28] J.H. van Dieën, J. Cholewicki, A. Radebold, Trunk muscle recruitment patterns in patients with low back pain enhance the stability of the lumbar spine, Spine 28(8) (2003) 834-41.

[29] S.L. Jones, S.M. Henry, C.C. Raasch, J.R. Hitt, J.Y. Bunn, Individuals with non-specific low back pain use a trunk stiffening strategy to maintain upright posture, J Electromyogr Kinesiol 22(1) (2012) 13-20.

[30] A. Radebold, J. Cholewicki, M.M. Panjabi, C.P. Tushar, Muscle response pattern to sudden trunk loading in healthy individuals and in patients with chronic low back pain, Spine 25(8) (2000) 947-954.

[31] P.W. Hodges, Pain and motor control: From the laboratory to rehabilitation, J Electromyogr Kinesiol 21(2) (2011) 220-8.

[32] P.W. Hodges, K. Tucker, Moving differently in pain: a new theory to explain the adaptation to pain, Pain 152(3 Suppl) (2011) S90-S98.

[33] N.W. Mok, S.G. Brauer, P.W. Hodges, Hip strategy for balance control in quiet standing is reduced in people with low back pain, Spine 29(6) E107-E112.

[34] M. Salavati, M. Mazaheri, H. Negahban, I. Ebrahimi, A.H. Jafari, A. Kazemnejad, et al., Effect of dual-tasking on postural control in subjects with nonspecific low back pain., Spine 34(13) (2009) 1415-1421.

[35] A. Shumway-Cook, M. Woollacott, K.A. Kerns, M. Baldwin, The effects of two types of cognitive tasks on postural stability in older adults with and without a history of falls, Journals of Gerontology. Series A, biological sciences and medical sciences 52A(4) (1997) M232-M240.

[36] K. Davids, D. Kingsbury, K. George, M. O'Connell, D. Stock, Interacting constraints and the emergence of postural behavior in ACL-deficient subjects, Journal of Motor Behavior 31(4) (1999) 358-366.

[37] N.W. Mok, S.G. Brauer, P.W. Hodges, Hip strategy for balance control in quiet standing is reduced in people with low back pain, Spine 29(6) (2004) E107-E112.

[38] G.J. Beneck, L.L. Baker, K. Kulig, Spectral analysis of EMG using intramuscular electrodes reveals non-linear fatigability characteristics in persons with chronic low back pain, J Electromyogr Kinesiol 23(1) (2013) 70-7.

[39] M. Kankaanpaa, S. Taimela, D. Laaksonen, O. Hanninen, O. Airaksinen, Back and hip extensor fatigability in chronic low back pain patients and controls, Archives of physical medicine and rehabilitation 79(4) (1998) P412-417.

[40] S.P. Lee, V. Dinglasan, A. Duong, R. Totten, J.A. Smith, Individuals With Recurrent Low Back Pain Exhibit Significant Changes in Paraspinal Muscle Strength After Intramuscular Fine Wire Electrode Insertion, PM R 12(8) (2020) 775-782.

Appendix 1: Supplemental Tables

Table 3: Baseline means and standard deviations for muscle activation and center of pressure variables

Lower Extremity Muscles	**Control (n_1=30)**	**LBP (n_2=30)**	**p – value[a]**
BF	183.1 ± 118.7	162.6 ± 128.4	0.205
GMAX	242.7 ± 163.5	209.8 ± 121.8	0.590
GMED	266.0 ± 218.6	232.2 ± 177.7	0.651
Trunk Muscles			
CEO	111.3 ± 90.6	122.9 ± 56.5	0.137
IEO	97.7 ± 52.6	112.4 ± 49.7	0.127
CIOTA	102.6 ± 43.4	124.9 ± 73.2	0.240
IIOTA	115.7 ± 46.0	155.0 ± 87.1	**0.049**
CML	76.9 ± 26.8	79.9 ± 24.8	0.494
IML	87.7 ± 41.6	91.8 ± 36.4	0.435
CRA	108.1 ± 40.5	101.6 ± 39.7	0.315
IRA	105.7 ± 40.0	107.6 ± 43.4	0.901
Center of Pressure Variables			
D_X (mm)	6.9 ± 1.8	6.4 ± 1.9	0.295
D_Y (mm)	9.3 ± 2.4	9.2 ± 2.8	0.684
R_X (mm)	49.1 ± 28.2	40.8 ± 16.2	0.105
R_Y (mm)	72.0 ± 29.1	69.5 ± 27.6	0.601
RMS_X	5.3 ± 1.7	4.9 ± 2.0	0.223
RMS_Y	7.8 ± 2.6	7.3 ± 2.4	0.436
TE_X (mm)	999.7 ± 310.0	925.9 ± 328.3	0.256
TE_Y (mm)	1131.1 ± 379.9	1213.8 ± 471.1	0.796
V_X (m/s)	66.6 ± 20.7	61.7 ± 21.9	0.256
V_Y (m/s)	75.4 ± 25.3	80.9 ± 31.4	0.796
GRF (N)	120.9 ± 26.0	123.4 ± 17.7	0.501

Abbreviations: BF: Biceps femoris; GMAX: gluteus maximus; GMED: gluteus medius; CEO: contralateral obliquus externus; IEO: ipsilateral obliquus externus; CIOTA: contralateral obliquus internus/transversus abdominis; IIOTA: ipsilateral obliquus internus/transversus abdominis; CML: contralateral multifidus lumborum; IML: ipsilateral multifidus lumborum; CRA: contralateral rectus abdominis; IRA: ipsilateral rectus abdominis; GRF: ground reaction force, D_X: frontal plane distance travelled; D_Y: sagittal plane distance travelled;, R_X: frontal plane range;R_Y: sagittal plane range; RMS_X:Root Mean Squared of the distance travelled in the frontal plane; RMS_Y: root mean squared of the distance travelled in the sagittal plane; TE_X: total excursion of the center of pressure in the frontal plane; TE_Y:total excursion of the center of pressure in the sagittal plane; V_X: velocity of the center of pressure in the frontal plane; V_Y: velocity of the center of pressure in the sagittal plane

[a]All p-values for log-transformed data

Table 4: Mean and standard deviation for pre- and post-stress muscle activation expressed as percent normalization

	Control (n_1=30)			LBP (n_2=30)			
Muscle	**Pre**	**Post**	**MD (p^a)**	**Pre**	**Post**	**MD (p^a)**	**p^b**
BF	183.1 ± 118.7	182.2 ± 134.4	-.9 (.543)	162.6 ± 128.4	153.1 ± 121.9	-9.4 (.341)	0.178
CEO	111.3 ± 90.6	105.8 ± 98.6	-5.5 (.060)	122.9 ± 56.5	110.2 ± 60.8	-12.7 **(.007)**	0.197
IEO	97.7 ± 52.6	96.5 ± 62.1	-1.2 (.205)	112.4 ± 49.7	98.3 ± 51.1	-14.0 **(.001)**	0.319
CIOTA	102.6 ± 43.4	100.8 ± 46.8	-1.8 (.363)	124.9 ± 73.2	104.9 ± 72.2	-20.0 **(.001)**	0.663
IIOTA	115.7 ± 46.0	112.3 ± 49.1	-3.4 (.290)	155.0 ± 87.1	141.8 ± 98.2	-13.2 **(.016)**	0.082
CML	76.9 ± 26.8	73.2 ± 27.7	-3.7 **(.007)**	79.9 ± 24.8	75.3 ± 28.1	-4.6 **(.013)**	0.615
IML	87.7 ± 41.6	85.1 ± 44.4	-2.6 (.157)	91.8 ± 36.4	84.1 ± 38.8	-7.7 **(.002)**	0.599
CRA	108.1 ± 40.5	101.1 ± 34.1	-7.0 **(.025)**	101.6 ± 39.7	91.8 ± 37.5	-9.8 **(.003)**	0.171
IRA	105.7 ± 40.0	99.1 ± 32.5	-6.5 (.094)	107.6± 43.4	95.4 ± 40.0	-12.2 **(.002)**	0.735
GMAX	242.7 ± 163.5	253.2 ± 206.5	10.5 (.835)	209.8 ± 121.8	197.9 ± 142.2	-11.9 **(.013)**	0.391
GMED	266.0 ± 218.6	259.8 ± 225.4	-6.1 (.202)	232.2 ± 177.7	214.2 ± 191.9	-18.0 **(.006)**	0.499

Abbreviations: BF: Biceps femoris; CEO: contralateral obliquus externus; IEO: ipsilateral obliquus externus; CIOTA: contralateral obliquus internus/transversus abdominis; IIOTA: ipsilateral obliquus internus/transversus abdominis; CML: contralateral multifidus lumborum; IML: ipsilateral multifidus lumborum; CRA: contralateral rectus abdominis; IRA: ipsilateral rectus abdominis; GMAX: gluteus maximus; GMED: gluteus medius

MD: Mean Difference

Values are presented as mean ± SD

[a] p- values for the null hypothesis that there is no difference between pre and post. All P-values for log-transformed data

[b] p- values for the null hypothesis that there is no difference between groups.

All values are expressed as a percent of activation during normalization trial

Table 5: Mean and standard deviation for pre- and post-stress center of pressure data

	Control (n_1=30)			LBP (n_2=30)			
	Pre	**Post**	**MD (p^a)**	**Pre**	**Post**	**MD (p^a)**	**p^b**
GRF (N)	120.9 ± 26.0	121.0 ± 26.4	.1 (.982)	123.4 ± 17.7	121.5 ± 19.6	-2.0 (.110)	0.638
D_X (mm)	6.9 ± 1.8	6.9 ± 2.3	.002 (.556)	6.4 ± 1.9	6.0 ± 1.8	-.4 (.085)	0.206
D_Y (mm)	9.3 ± 2.4	9.1 ± 2.3	-.2 (.329)	9.2 ± 2.8	9.0 ± 2.7	-.1 (.557)	0.736
R_X (mm)	49.1 ± 28.2	52.3 ± 35.0	3.2 (.763)	40.8 ± 16.2	41.2 ± 15.9	.4 (.768)	0.085
R_Y (mm)	72.0 ± 29.1	70.4 ± 22.9	-1.6 (.669)	69.5 ± 27.6	69.8 ± 26.9	.3 (.967)	0.683
RMS_X	5.3 ± 1.7	5.7 ± 3.4	.5 (.799)	4.9 ± 2.0	4.5 ± 1.4	-.4 (.120)	0.088
RMS_Y	7.8 ± 2.6	7.5 ± 2.2	-.3 (.371)	7.3 ± 2.4	7.2 ± 2.1	-.3 (.710)	0.530
TE_X (mm)	999.7 ± 310.0	979.8 ± 341.3	-19.9 (.289)	925.9 ± 328.3	872.8 ± 310.4	-53.1 **(.010)**	0.253
TE_Y (mm)	1131.1 ± 379.9	1100.3 ± 385.7	-30.8 (.193)	1213.8 ± 471.1	1087.6 ± 387.8	-126.2 **(.003)**	0.976
V_X (m/s)	66.6 ± 20.7	65.3 ± 22.7	-1.3 (.289)	61.7 ± 21.9	58.2 ± 20.7	-3.5 **(.010)**	0.253
V_Y (m/s)	75.4 ± 25.3	73.3 ± 25.7	-2.0 (.193)	80.9 ± 31.4	72.5 ± 25.8	-8.4 **(.003)**	0.976

Abbreviations: GRF: ground reaction force, D_X: frontal plane distance travelled; D_Y: sagittal plane distance travelled;, R_X: frontal plane range;R_Y: sagittal plane range; RMS_X:Root Mean Squared of the distance travelled in the frontal plane; RMS_Y: root mean squared of the distance travelled in the sagittal plane; TE_X: total excursion of the center of pressure in the frontal plane; TE_Y:total excursion of the center of pressure in the sagittal plane; V_X: velocity of the center of pressure in the frontal plane; V_Y: velocity of the center of pressure in the sagittal plane

MD: Mean Difference

Values are presented as mean ± SD

[a] p- values for the null hypothesis that there is no difference between pre and post. All P-values for log-transformed data

[b] p- values for the null hypothesis that there is no difference between groups.

CHAPTER FOUR

THE ASSOCIATION OF ACUTE STRESS AND SINGLE LEG BALANCE: RECOMMENDATIONS FOR FUTURE INVESTIGATION

Introduction

To maximize the efficiency of our data collection period, we collected multiple variables on each subject with the expectation that we would be able to perform additional analyses separate from what has already been described, submitted, and published. Below is a list of unexplored investigative questions based on our current data set.

Cued Movement

Key to motor control and postural coordination in the trunk are anticipatory postural adjustments (APAs). APAs provide preparatory trunk support preceding a movement by activating trunk musculature before the task [1]. Experimentally induced acute pain as well as chronic low back pain have both been associated with delayed APA onset [2, 3]. The addition of a cognitive load task has been shown to accentuate APA onset delay in individuals with chronic low back pain [4]. A review by Xiao et al. 2023 explores the current perspective on the interaction of cognitive load and postural control in individuals with low back pain. The authors surmise that dual-task processing introduces an additional cognitive load on the already-taxed attentional resources of individuals with low back pain contributing to decreased stance stability and postural coordination [5]. Some have argued that the stress response to SET has been shown to be

distinct from cognitive load [6] but still, the question arises: what effect might SET have on APAs and motor control?

We anticipate that our future investigation will be directed toward the potential influence of acute stress on cued movement (i.e. standing hip flexion) in individuals with and without low back pain (LBP). Analysis will include the latency of activation of the prime mover (rectus femoris) and timing of trunk and stance limb muscle activation during the APA window. Additionally, we will examine the amplitude of muscle activation for each of the target trunk and lower extremity muscles. Finally, we will examine if low back pain plays a role in accentuating any neuromuscular changes due to stress.

Heart Rate Variability

There is a growing interest in heart rate variability (HRV) and the implications for disease incidence and prognosis. HRV is the fluctuation of the length of heartbeat intervals [7] and represents the capacity of the cardiovascular system to respond to environmental stimuli [8]. Low HRV suggests impaired parasympathetic activity, while high HRV suggests physiological capacity to cope with increased stress [8].

Future investigation should be directed toward whether a relationship exists between specific HRV parameters and the Feigned Annoyance and Frustration (FAF) Test. Additionally, it may be insightful to examine whether a relationship exists between specific HRV parameters and whether individuals' motor control strategy changes when exposed to the FAF Test. Additionally, given that HRV is generally used as a metric for parasympathetic activity [9] and that salivary α-amylase (sAA) is a proxy for sympathetic

activity [10-12], it may be insightful to observe the relationship between HRV and sAA in response to the FAF Test.

Menstrual Phase

It has been suggested that menstrual phase influences women's change in sAA concentration after acute stress exposure [13]. In our study, women failed to demonstrate a significant increase in sAA concentrations after exposure to the FAF Test [14]. It may provide additional insight to perform a deeper analysis to determine which phase of the menstrual cycle each individual was in when they participated in our study and determine if that uncovers any unexplored statistical relationships.

FAF and LBP

Acute pain has been utilized as a stimulus for stress in a research context [15-17] and the role of psychosocial stress in the etiology and chronification of LBP continues to be a common topic for investigation. A question that was unexplored by our group was whether individuals with low back pain demonstrated a change in sAA after the FAF Test that was different than the change demonstrated by individuals without low back pain. And while we did not assess subjective markers for emotional resilience, it has been suggested that characteristics such as hope and optimism may improve outcomes in individuals with LBP [18].

Subjective Report Outcome Measures

We collected data on five subjective outcome measures: the State-Trait Anxiety Inventory (STAI), the Pittsburgh Sleep Quality Index (PSQI), the Perceived Stress Scale (PSS), the Tampa Scale of Kinesiophobia (TSK), and the Oswestry Disability Index

(ODI). In general, implications from these measures was largely unexplored regarding the responsiveness of participants to the FAF Test and the participants' tendency toward altered trunk coordination and balance stability strategies. Items such as the TSK and the STAI may give insight into nuanced changes associated with stress and trunk muscle recruitment strategies. The PSQI and the PSS may give insight into why some participants were minimally responsive in the sAA changes.

Conclusion

We acknowledge that it is immensely optimistic to consider that each of these topics will serve as the foundations for standalone papers. However, we took great care in our data collection process and believe that further analysis will uncover additional novel findings. We recognize the critical implications associated with the findings from our investigation. The role of psychosocial stress and its influence on motor control in the context of rehabilitation and LBP must be integrated into future research. We will work to do just that as we strive to fulfill our role as Physical Therapists, optimizing movement and seeking to bring an end to the suffering that comes from chronic pain.

Bibliography

[1] P.J. Cordo, L.M. Nashner, Properties of postural adjustments associated with rapid arm movements, Journal of Neurophysiology 47(2) (1982) 287-302.

[2] G.L. Moseley, M.K. Nicholas, P.W. Hodges, Pain differs from non-painful attention-demanding or stressful tasks in its effect on postural control patterns of trunk muscles, Exp Brain Res 156(1) (2004) 64-71.

[3] M.F. Knox, L.S. Chipchase, S.M. Schabrun, R.J. Romero, P.W.M. Marshall, Anticipatory and compensatory postural adjustments in people with low back pain: a systematic review and meta-analysis, The spine journal: official journal of the North American Spine Society 18(10) (2018) 1934-1949.

[4] H.C. Yang, W.W. Xiao, Y.X. Guan, H.A. Mao, Z.M. Hao, C.H. Wang, Effect of Cognitive Load on Anticipatory Postural Adjustment Latency and its Relationship with Pain-Related Dysfunction in Non-specific Chronic Low Back Pain: A Cross-Sectional Study, Pain Ther 12(3) (2023) 723-735.

[5] W. Xiao, H. Yang, Z. Wang, H. Mao, H. Wang, Z. Hao, et al., Postural Control of Patients with Low Back Pain Under Dual-Task Conditions, J Pain Res 16 (2023) 71-82.

[6] A. Woody, E.D. Hooker, P.M. Zoccola, S.S. Dickerson, Social-evaluative threat, cognitive load, and the cortisol and cardiovascular stress response, Psychoneuroendocrinology 97 (2018) 149-155.

[7] M. Malik, A.J. Camm, Heart Rate Variability, 1995.

[8] H.G. Kim, E.J. Cheon, D.S. Bai, Y.H. Lee, B.H. Koo, Stress and Heart Rate Variability: A Meta-Analysis and Review of the Literature, Psychiatry Investig 15(3) (2018) 235-245.

[9] G.A. Reyes del Paso, W. Langewitz, L.J. Mulder, A. van Roon, S. Duschek, The utility of low frequency heart rate variability as an index of sympathetic cardiac tone: a review with emphasis on a reanalysis of previous studies, Psychophysiology 50(5) (2013) 477-87.

[10] N. Takai, M. Yamaguchi, T. Aragaki, K. Eto, K. Uchihashi, Y. Nishikawa, Effect of psychological stress on the salivary cortisol and amylase levels in healthy young adults, Arch Oral Biol 49(12) (2004) 963-8.

[11] K. An, J. Salyer, R.E. Brown, H.F. Kao, A. Starkweather, I. Shim, Salivary Biomarkers of Chronic Psychosocial Stress and CVD Risks: A Systematic Review, Biol Res Nurs 18(3) (2016) 241-63.

[12] N. Skoluda, R. La Marca, M. Gollwitzer, A. Muller, H. Limm, B. Marten-Mittag, et al., Long-term stability of diurnal salivary cortisol and alpha-amylase secretion patterns, Physiol Behav 175 (2017) 1-8.

[13] L. Espin, C. Villada, V. Hidalgo, A. Salvador, Effects of sex and menstrual cycle phase on cardiac response and alpha- amylase levels in psychosocial stress, Biol Psychol 140 (2019) 141-148.

[14] T.W. Gehrig, L.S. Berk, R.I. Dudley, J.A. Smith, L. Gharibvand, E.B. Lohman, The feigned annoyance and frustration test to activate the sympathoadrenal medullary system, Comprehensive Psychoneuroendocrinology 18 (2024).

[15] A.H. van Stegeren, O.T. Wolf, M. Kindt, Salivary alpha amylase and cortisol responses to different stress tasks: impact of sex, Int J Psychophysiol 69(1) (2008) 33-40.

[16] N. Skoluda, J. Strahler, W. Schlotz, L. Niederberger, S. Marques, S. Fischer, et al., Intra-individual psychological and physiological responses to acute laboratory stressors of different intensity, Psychoneuroendocrinology 51 (2015) 227-36.

[17] G. Lamotte, C.J. Boes, P.A. Low, E.A. Coon, The expanding role of the cold pressor test: a brief history, Clin Auton Res 31(2) (2021) 153-155.

[18] E.L. Terry, L. Meng, Z. Huo, E.J. Bartley, Examining Reactivity and Recovery Patterns of Pain-Evoked Cortisol and Alpha-Amylase Trajectories: Relations Between Psychological Markers of Risk and Resilience, Journal of Pain (2024).