SOUTH BAYLO UNIVERSITY

Comparative Effectiveness of Experimental Back Acupuncture to Traditional Distal Acupuncture for Improving Heart-Rate-Variability (HRVs) in Adults with Toxic Stress and ACE-Associated Health Conditions (AAHCs): A Pilot Randomized Single-Blinded Active-Controlled Trial

by

Jennifer So

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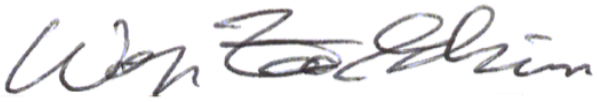

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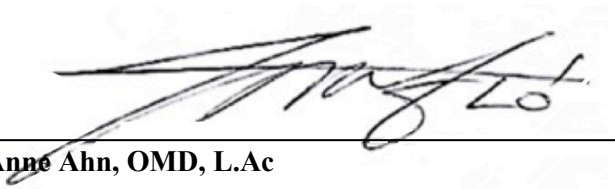

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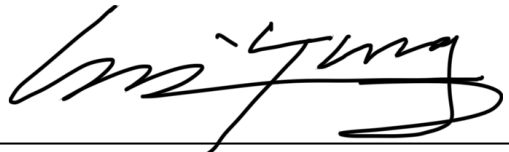

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Comparative Effectiveness of Experimental Back Acupuncture to Traditional Distal Acupuncture for Improving Heart-Rate-Variability (HRVs) in Adults with Toxic Stress and ACE-Associated Health Conditions (AAHCs): A Pilot Randomized Single-Blinded Active-Controlled Trial

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ABSTRACT

The objective of this clinical trial was to establish an evidence-based acupuncture protocol to help improve Heart Rate Variability (HRVs) for adult participants struggling with Toxic Stress, Adverse Childhood Experiences (ACEs) and ACE-Associated Health Conditions (AAHCs). Effectiveness of Experimental 'EG' Group's back acupuncture (Back-shu and Psychic-aspect points) was compared with Active-control 'CG' Group's traditional distal acupuncture (ML-10 points), for twelve weekly treatments. Inclusion criterias: adults ages 18–80 who scored 1 or higher on ACEs Questionnaire and scored a 27 or higher on Perceived Stress Scale (PSS), and exclusion criterias, all needed to be met for enrollment. Investigator blinded and randomly assigned each participant (n=35) to either EG (n=18) or CG (n=17), with an even distribution of females, males, ages 18-50 and ages 51-80 in both groups. Throughout the trial,

all participants's HRVs (RMSSD, SDNN, pNN20, pNN50, LF, HF and LF/HF) were recorded weekly, while PSS Scores and Quality of Life (QOL) Scores were collected three times. After 12th treatment: RMSSD for EG was 60.6±21.92 and for CG was 45.8±26.20 (p=0.001). SDNN for EG was 123.7±31.56 and for CG was 123.0±30.03 (p= 0.000). pNN20 for EG was 33.4±6.16 and for CG was 27.9±17.44 (p=0.008). pNN50 for EG was 19.3±8.26 and for CG was 13.4±12.43 (p=0.008). LF for EG was 782.1±311.43 and for CG was 689.0±408.10 (p=0.000). HF for EG was 667.5±335.26 and for CG was 628.4±396.77 (p=0.026). LF/HF for EG was 1.3±0.50 and for CG was 1.5±1.17 (p=0.111). PSS for EG was 23.2±2.79 and for CG was 27.4±3.32 (p=0.000). QOL for EG was 61.3±12.12 and for CG was 57.62±10.70 (p=0.000). In conclusion: CG's acupuncture showed effectiveness in improving HRVs (RMSSD, SDNN, pNN50, LF), reducing stress (PSS), and making a difference in quality of life (QOL) after 12th treatment. However, EG's acupuncture shows more effectiveness on improving the same measured variables earlier-on, after 6th and 12th treatments. Therefore, EG's Back-shu and Psychic-Aspect points hold potential as a valid acupuncture protocol for Toxic Stress and AAHCs.

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Dr. Nadine Burke Harris, MD, from The Center for Youth Wellness— for sharing her decades of research on Adverse Childhood Experiences (ACEs), and unknowingly, for giving me the strength to pursue this acupuncture research project in search for an acupuncture treatment protocol for toxic stress and ACE-Associated Health Conditions (AAHCs).

HYS Engineering Team—for loaning their electrocardiogram (ECG) recording device and heart rate variability (HRV) calculating software application, which allowed me to collect HRV data for this research project. Without their generosity and user-friendly device, I would not be able to efficiently measure and collect multiple types of time-domain HRVs and frequency-domain HRVs measurements from my research participants.

My research participants/patients—for trusting me and giving their commitment to participate in this pilot clinical trial. From my research participants, I learned many lessons and gained more understanding in HRVs and Yin Yang Theory, and more perspective in treatments for chronic stress and trauma.

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Dr. Joseph Suh, Ph.D, OMD, L.Ac, from South Baylo University—for advising me and helping me complete this research project. Without his expertises in clinical research and biostatistics, and his willingness to help me, I would not have been able to competently articulate my last four years of acupuncture and HRV research, organize nine different measured variables with more than 3,780 data points, and interpret my research findings in a logical and comprehensive manner. Thank you.

March 2024

DEDICATION

I dedicate this:

To the Children, and To Anyone, who was once a kid.

I. INTRODUCTION

1.1 Opening Statement:

Since the original Adverse Childhood Experiences (ACEs) Study was conducted in 1998, more than 250 studies have followed, and on average, participants that scored 6 or more ACEs (on a scale of 0 to 10) died almost two decades earlier than participants without ACEs.[1, 2,3] All top 10 leading causes of death in the United States are associated with having ACEs.[8,9,10,11] Amongst the recent 998,870 ACEs Screenings conducted between January 2020 to March 2022 in California, 699,209 (or 70%) of those participants reported to have experienced 1 or more type of ACEs, with ACE Score of 1 or higher. Also, 17% of those participants scored 4 or more ACEs. Interestingly, 704,614 participants (or 71%) from the total ACEs screening data collected, reside in Los Angeles, Orange, San Bernardino and Riverside counties alone. Even so, more than 97% of Californians have not yet been screened for ACEs, and many are unaware that exposure to ACEs is associated with increased vulnerability to Toxic Stress, disguised as chronic physical

and mental health problems (known as ACE-Associated Health Conditions ‘AAHCs’) throughout lifespan. [4,5]

As an acupuncturist and mental health ally, the primary investigator is committed to bringing awareness to ACEs, and to contributing solutions to remedy the stress epidemic. Because of this, the purpose of this clinical research project was, first (1) to introduce background and concepts of Heart Rate Variability (HRV), Toxic Stress, ACEs, AAHCs, next (2) to critically assess the theory-based and evidence-based literatures, concerning acupuncture interventions for Toxic Stress and AAHCs, then (3) to formulate hypothesis for testing by comparing effectiveness of existing ‘active-control’ and experimental acupuncture protocols on real-life participants, and finally (4) to present the outcomes, findings, remaining questions and promising solutions for intervening Toxic Stress and AAHCs.

Table 1. Association between ACEs and 2021 Leading Causes of Death in the U.S. [8, 9, 10, 11]

Leading Causes of Death in the U.S.	**ACE-Associated Health Condition (AAHCs)**	**Odds Ratio for ACEs Score of 4 or more** (relative to no ACEs)
1. Heart disease	✓	2.1
2. Cancer	✓	2.3
3. Covid-19	✓	12
4. Accidents (unintentional injuries)	✓	2.6
5. Stroke	✓	2.0
6. Chronic lower respiratory disease	✓	3.1
7. Alzheimer’s disease or dementia	✓	11.2
8. Diabetes	✓	1.4
9. Chronic Liver disease, cirrosis	✓	2.6
10. Kidney disease	✓	1.7

Figure 1: The 10 Adverse Childhood Experiences (ACEs) investigated in the original 1998 ACEs Study by CDC & Kaiser Permanente.

1.2 Background: HRVs, Toxic Stress, ACEs and AAHCs

Heart Rate Variability (HRVs) biomarkers measure the relative balance of our autonomic nervous systems (ANS), indicating the activity of the parasympathetic (PNS) and sympathetic (SNS) nervous systems. When our ANS is unbalanced and dysregulated, our body's capabilities in regulating stress, hormones secretions, immune system, blood circulation, respiration, digestion, sleep and basic homeostasis functions are all disrupted. In a healthy person, respiratory rate is slow, regular and in-sync, with fluctuations in their heart rate, as indicated in normal HRV variable measurements. In a chronically stressed person, respiratory rate can be too slow or too fast, irregular, and out of sync with their heart rate, where their heart rate tends to have low fluctuations (low HRVs) or very high fluctuations (high HRVs), see Figure 2. For a person to face stress too frequently and too long, this not only causes chronic stress but also

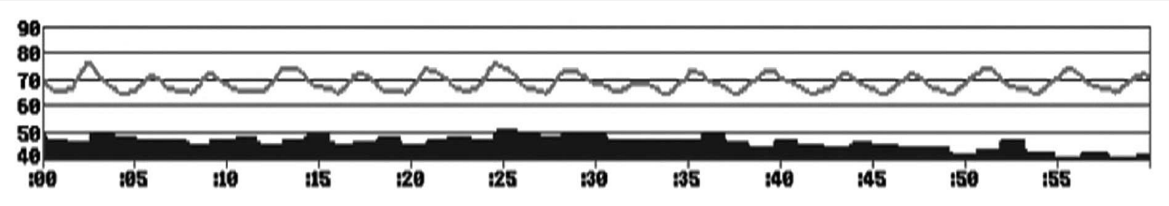
causes a dysregulated stress response, called Toxic Stress. Having toxic stress and abnormal HRVs, are increased in adults with childhood trauma or Adverse Childhood Experiences (ACEs).[12, 13,14, 15, 16, 17] With dysregulated stress response system or an unbalanced autonomic nervous system, people with ACEs are unfortunately more vulnerable to ACE-Associated Health Conditions (AAHCs)—a variety of physical and mental health conditions, such as cardiovascular diseases, cancer, PTSD, depression & anxiety disorders.

Measuring the health of the autonomic nervous system's sympathetic and parasympathetic-vagal tones, HRV variables can be a useful biomarker to track the progress for adults undergoing treatment(s) for managing mental and physical illnesses. Since the advancements in signal processing technology for electrocardiogram (ECG) in early 1960s, investigations of HRVs and its relationship to health and disease have gained popularity amongst cardiologists, neurologists, rheumatologists, kinesiologists and psychologists. Currently, there are over 60 parameters or types of HRV variable measurements applied in research studies, and in general, there are three main categories: time-domain HRVs, frequency-domain HRVs, and nonlinear HRVs. For this pilot clinical trial, the investigator measured time-domain and frequency domain HRV measurements from its participants. Considering that HRV is still an uncommon biomarker in clinical and research settings, the investigator selected seven of the most researched HRV variables with the significant physiological indicators to measure.[13] There were four time-domain HRVs (RMSSD, SDNN, pNN50, pNN20) and three frequency-domain HRVs (LF power, HF power, LF/HF ratio). Refer to Table 2. For monitoring HRV in for clinical applications, it is essential to not look at just one HRV variable, but a minimum of four different HRV variables should be examined together for valid and potential diagnostic interpretations.[18, 19]

RESPIRATION, HEART RATE AND HEART RATE VARIABILITY (HRV)

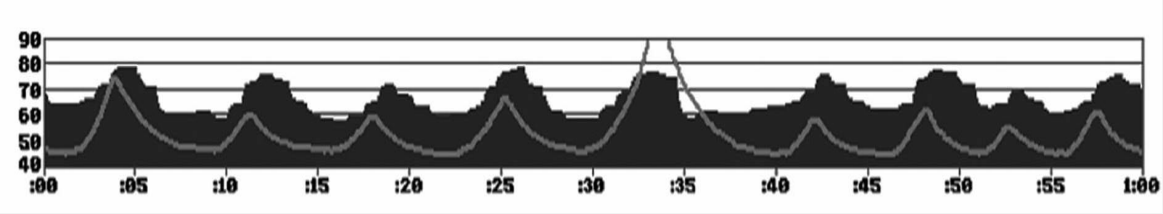
Abnormally Low HRV in a person with depression, or toxic stress.

Gray wavy line represents breathing, in this case, breathing is rapid and shallow. This inhibits their vagal tone and overstimulates their sympathetic tone, shown in the black areas— producing low variations in their heart rate that can be indicated with abnormally low HRV values. Heart rate is typically slow and out of sync with the breath. This is a typical pattern of a shut-down person with chronic depression.



Normal HRV in a healthy person with well-regulated stress response.

Healthy breathing, shown with gray wavy line, is slow and regular inhalations and exhalations. Their vagal tone is balanced with sympathetic tone, producing a steady fluctuation (black areas) of low and high heart rates that can be indicated with normal HRV values.Whenever this individual inhales, the heart rate goes up; during exhalations the heart slows down. This pattern of HRV reflects good physiological health.



Abnormally High HRV in a person with severe anxiety, or toxic stress.

Breathing rapidly and irregularly (gray wave line), as does heart rate, is due to overstimulated sympathetic tone and sometimes overactive vagal tone, producing extremely high variations in heart rate more frequently than normal that can be indicated with abnormally high HRV values (black areas). Heart rate and respiration no longer stay perfectly in sync. This is typically a normal pattern of response for a person responding to temporary stress, however, it becomes a problem when it happens too often and frequently, making this pattern common in a person with chronic anxiety.

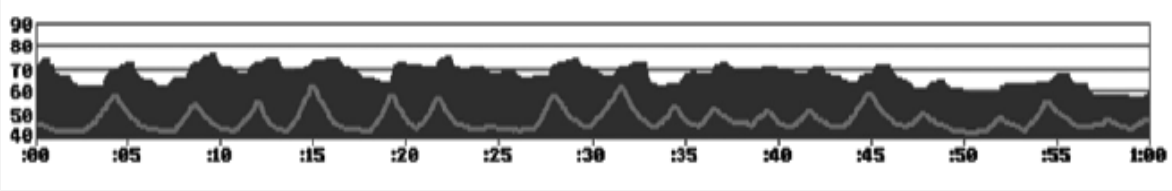


Figure 2. Visual Comparison of Normal and Abnormal HRVs

Table 2. The Seven Types of Heart Rate Variability (HRV) Variables collected & studied in this clinical research project

Heart Rate Variability (HRV) Variables			
HRV Categories	**HRV Variables**	**Description:**	**Physiological Significance:**
Time - Domain HRV	**RMSSD** (ms):	Root mean square of successive RR interval differences	Parasympathetic-Vague tone, Respiratory Sinus Arrhythmia
	SDNN (ms):	Standard deviation of NN intervals	Sympathetic-Vagal tone
	pNN20 (%):	% of successive RR intervals that differ by more than 20 ms	Parasympathetic-Vague tone, Respiratory Sinus Arrhythmia
	pNN50 (%):	% of successive RR intervals that differ by more than 50 ms	Parasympathetic-Vague tone, Respiratory Sinus Arrhythmia
Frequency - Domain HRV	**LF Power** (ms^2) :	Absolute power of low frequency band (0.04 - 0.15 Hz)	Sympathetic-Vagal tone; Baroreflex, Vasomotor
	HF Power (ms^2) :	Absolute power of high frequency band (0.15 - 0.4 Hz)	Parasympathetic-Vague tone, Respiratory Sinus Arrhythmia
	LF/HF (ratio) :	Ratio of LF Power to HF Power	Parasympathetic or Sympathetic predominance

1.3 Importance of this Research:

ACEs prevention early-on is a better solution than having to seek AAHCs treatments later-on. Preventing ACEs in the child's life could help reduce the number of adults with chronic depression, anxiety, obesity, headaches, drug addiction and other AAHCs, by as much as 44%.[11] For many adults with ACEs, they do not have ACEs prevention as an option, but they could seek treatment. According to the Center of Disease Control and Prevention, 113 billions of dollars are spent every year in California treating patients with ACE-Associated Health Conditions (AAHCs).[25,26] Acupuncturists' scope of practice is essentially restoring balance of systems in

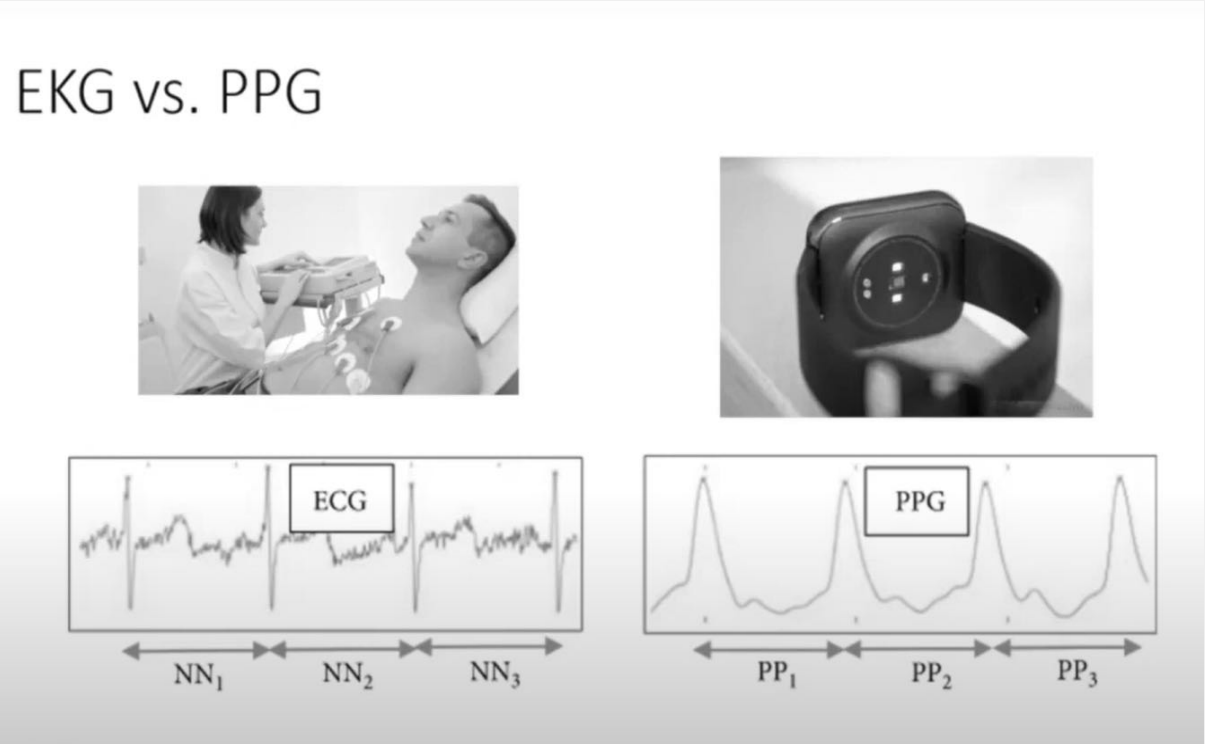
the human body. Acupuncture's foundational theory is balancing the yin and the yang, which can coincide with parasympathetic and sympathetic activity measurements from HRV. When there is dysregulated stress response, either: (1) the Yang overwhelms Yin, Yin cannot cool/slow/settle Yang. or (2) the Yin overwhelms Yang, Yang can no longer warm / mobilize / activate Yin.[27,33] With HRV measurements, acupuncturists can potentially supplement their TCM diagnosis and track the process of their patients' acupuncture treatments quantitatively in the future. There can be various combinations in acupuncture points for treating toxic stress and childhood trauma, hence the intentions of this pilot clinical trial was to examine and establish an evidence-based acupuncture protocol for improving HRVs, balancing autonomic nervous system and Yin and Yang for the many adults seeking the help and treatments.

1.4 Key Issues in Existing Research

According to the National Institute of Health, as of February 2024, there were 22 studies involving acupuncture treatments for stress and trauma related disorders with HRV outcome measurements. The studies were done with acupuncture points on the distal extremities (arms and legs) or on the ears, and their HRV outcome measurements showed some HRV differences between their experimental acupuncture and control groups, however, their HRV changes were inconclusive and/or not statistically significant before and after acupuncture treatments.[29, 30, 31, 32]

Four factors can lead to inconclusive HRV data collection in research: (1) Since most of the published clinical studies were done in less than 4 weeks time, that leaves limitations in collecting enough HRV data points to see significant changes. (2) Since young adults tend to have higher HRVs than the elderly, there needs to be a good distribution of age groups to collect HRV data. (3) Furthermore, many Acupuncture with HRV clinical trials collected data using

fitness watches that don't collect electrocardiogram (EKG) for HRV, instead they are collecting photoplethysmography (PPG) for HRV, see Figure 3. PPG measures the flow through microvasculature on the wrist, where there is poor contact, a lot of movement and noise and that can produce irregular sinus rhythms that falsely produce higher HRV readings. Using EKG for HRV calculations allows for filtering out noise that may present falsely high HRV measurements.[13] (4) Most acupuncture with HRV research only measured RMSSD, pNN50, pNN20 HRV variables, that are indicators of only the parasympathetic activity. To measure the overall autonomic nervous system activity or sympathovagal tone, it's essential to also measure SDNN, LF power, HF power types of HRVs.[13,18,19,29, 30, 31, 32]



* Image from HRV Lecture by Dr. A. Ahn (2021).[13]

Figure 3. Electrocardiogram (ECG) and Photoplethysmography (PPG) for Measuring Heart Rate Variability (HRVs)

1.5. Rationale for this Clinical Trial

Compared to studies examining distal acupuncture points on the limbs, there was a lack of studies done with Back-Shu (back) or Front-Mu (abdominal) acupuncture points for treating physical and mental stress and improving HRV. In a survey conducted on California licensed acupuncturists during a continuing education webinar in 2022, acupuncture points on the back were also not commonly chosen for treating chronic stress and mental illnesses. This was surprising, considering chronic types of disease tend to affect the internal organs, and Back-Shu points can directly regulate the internal organs. Biomedical literature regarding the Autonomic Nervous System (ANS) map overlapping on the spinal roots and internal organs, led the investigator to examine using Back-Shu or Urinary Bladder channel points for treating chronic stress or dysregulated sympathetic and parasympathetic nervous systems. Figure 4 shows two branches of the ANS: SNS and PNS working together constantly.[34] Figure 5 shows how the Urinary Bladder channel acupuncture points on the back overlap with the ANS. From the investigator's observations, distal (extremity) acupuncture points tend to work well on patients with acute stress; however, they were less effective on patients with more chronic, prolonged, or Toxic Stress. Hence, this design of study intended to further investigate Urinary Bladder channel acupuncture points for treatment of Toxic Stress and AAHCs. By designing an experimental acupuncture treatment protocol and comparing to existing traditional acupuncture treatments, backed with quantitatively measurable HRV biomarkers to determine acupuncture's effectiveness, this clinical project could potentially provide to people with more accessible interventions and long-term solutions to treating their dysregulated stress systems and ACE-Associated Health Conditions. This pilot clinical trial could carry potential impact on medical care, safety and economy for California residents, as well as external validity for future clinical research outside of the California population.

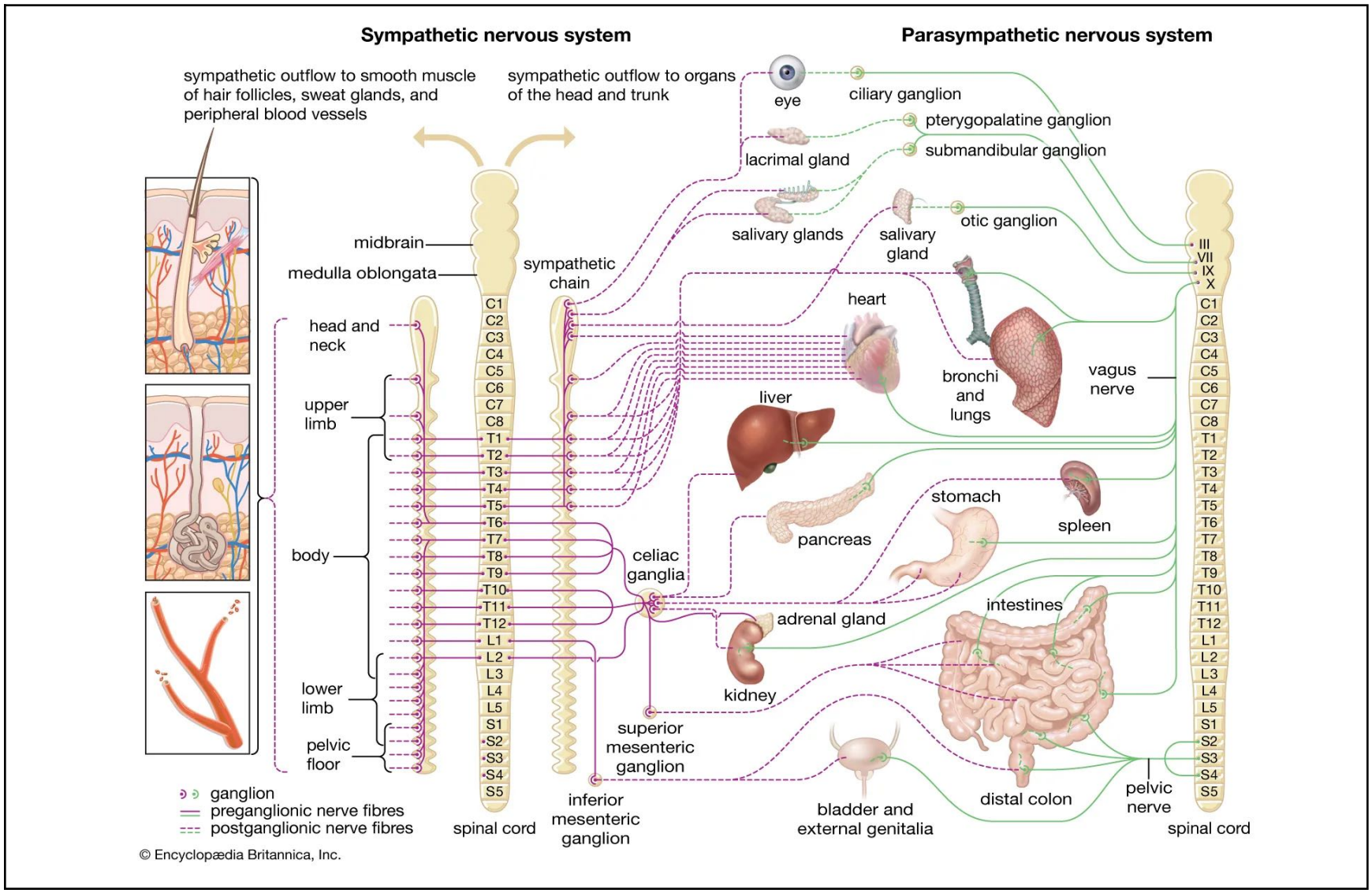


Figure 4. Autonomic Nervous System (ANS) map along the spine.

⬛**Black Squares** show the Urinary Bladder (Bladder) channel acupuncture points, that overlap with the autonomic nervous system's SNS ganglia and spinal nerves (T1 to L2) and PNS pelvic splanchnic nerve (S2 to S4) :

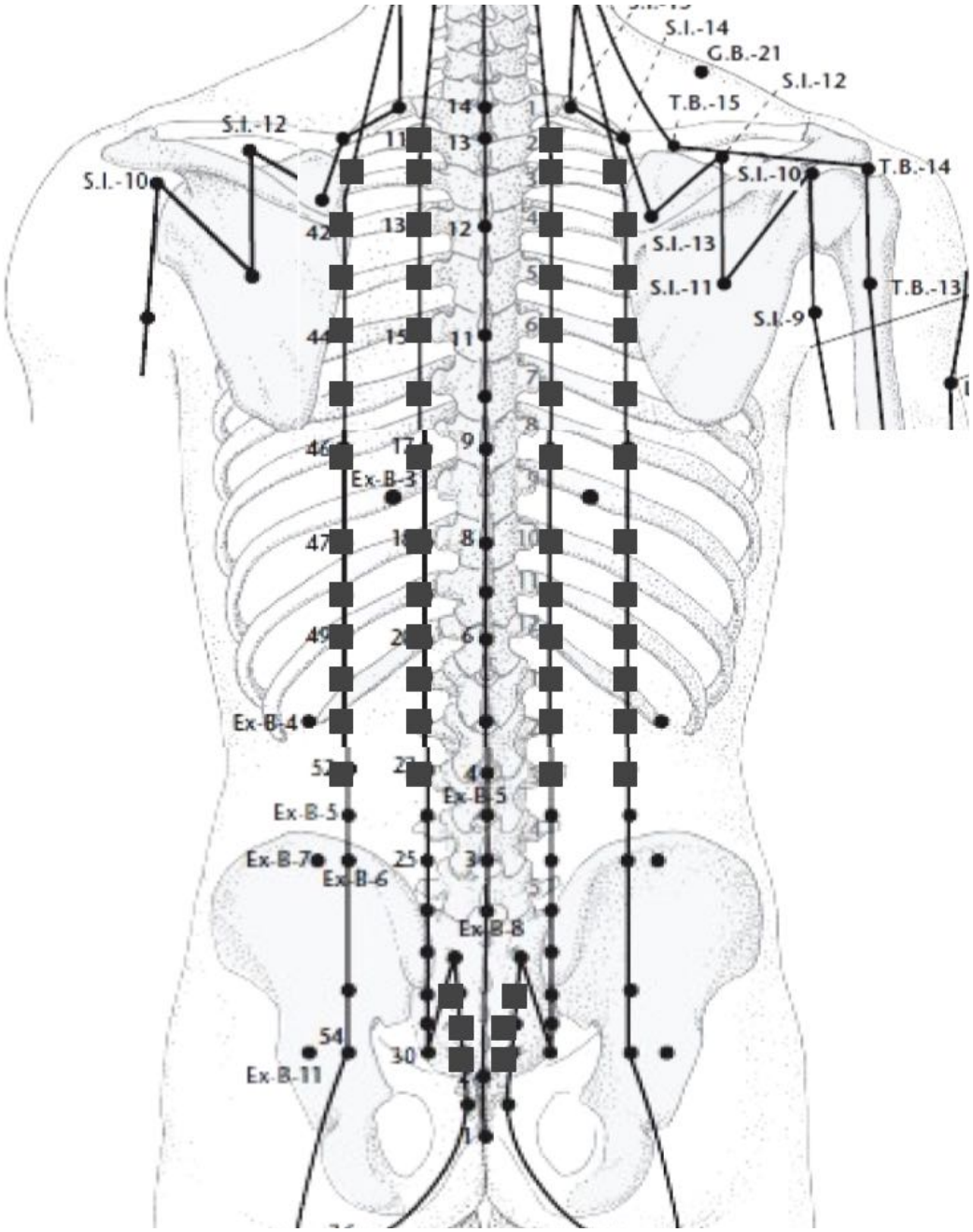


Figure 5. ANS Overlapping Urinary Bladder Channel Acupuncture Points on Back

OBJECTIVES

Target Disease: Toxic Stress and ACE-Associated Health Conditions (AAHCs), see Table 3.

Primary Objective: Using Experimental Back Acupuncture to improve the autonomic nervous system's biomarker – Heart Rate Variability (HRV) in adult participants with Toxic Stress and ACE-Associated Health Conditions (AAHCs).

> Hypothesis 1: Applying Experimental Back Acupuncture treatment will improve the participant's Heart Rate Variability (HRVs), compared to participants with active-control–Traditional Distal Acupuncture treatment.

Secondary Objective: Using Experimental Back Acupuncture treatment to improve Quality of Life (WHOQOL-BREF / QOL) Scores and to reduce Perceived Stress Scale (PSS) Scores in adult participants with Toxic Stress and ACE-Associated Health Conditions (AAHCs).

> Hypothesis 2: Applying Experimental Back Acupuncture will improve the participant's QOL Scores and to reduce PSS Scores, compared to participants with active-control–Traditional Distal Acupuncture treatment.

LITERATURE REVIEW

A. Overview of Literature Review

The purpose of this integrative literature review was to develop a comprehensive understanding on the topic of Toxic Stress, and to determine the effectiveness of theory-driven and evidence-based interventions, in order to finalize this study's objectives and methodology. This chapter focused on laying out the pathophysiology of toxic stress from Western Medicine and Traditional Chinese Medicine (TCM) perspectives, and analyzing the key findings related to the treatments for toxic stress. Since 'toxic stress' terminology has only been used for the last two decades of research, the terms 'chronic stress' and 'childhood trauma' was also used in search engines, and be used interchangeably with 'toxic stress' throughout this research paper.[23] Critical western and eastern theories associated with toxic stress are the Polyvagal Theory, Yin-Yang Theory, Zang-Fu Organ Theory and Five Psychic 'Spirit' Aspects. Investigating how the stress response system is embedded in multiple biological systems, plays an essential role in understanding Toxic Stress as a disease and its inseparable relation to ACE-Associated Health Conditions (AAHCs). Finally, this review will emphasize why treatment of toxic stress goes hand in hand while treating any AAHCs. The central questions guiding this literature review are:

- What is Toxic Stress and how is it related to having ACEs?
- How does the Toxic Stress response involve multiple organ systems according to Western Medicine and Traditional Chinese Medicine?
- What does the existing research and data conclude on using acupuncture for improving HRVs, treating chronic stress and childhood trauma?

B. Review— What is Toxic Stress?

There are three categories of biological stress— positive stress, tolerable stress and toxic stress. Some stress is necessary and essential for development and survival, so not all stress is detrimental. When responding to routine stress in life, heart rate, blood pressure and stress hormones adrenaline and cortisol can be briefly spiked, known as the Positive Stress response. Furthermore with more severe, longer lasting difficulties, such as facing natural disasters and death of loved ones, the body's Tolerable Stress response will activate. The child's brain and organs can recover easily with tolerable stress, as it is only activated for a short period of time, and it is ameliorated by supportive relationships and healthy adults that are capable of helping the child adapt and process the stressful situation. When the stress response system is activated too frequently for long periods, this is known as the Toxic Stress response, which disrupts cerebral development and many organ systems, increasing risk of stress-related disease and lifelong physical and mental illnesses into adulthood. The advantage of recognizing toxic stress over just 'chronic stress' is that the term 'toxic stress' recognizes the connection between root cause and manifestations of diseases, in other words, the association between childhood adversity and chronic health conditions.[4,6,20]

How a person's stress response system reacts internally in the body, differs from how the person perceives a stressful event or experience. In the stress response system, the amygdala, hypothalamus, pituitary gland and adrenal gland are already circulating stress hormones before the person can decide what is stressful or not. The body's stress response system can be activated even if the person perceives their stress as low. Some stress is necessary for survival (positive stress), and sometimes stressful events are unavoidable, but tolerable by the stress response system as long as it happens in moderation (tolerable stress). It becomes problematic when the stress response system is activated too often and too long, where the autonomic nervous system and many organ systems of the body become dysregulated (toxic stress). Having experienced abuse, neglect and familial dysfunction in childhood will overwork the stress response system

and other organ systems to the point of malfunction, which is how Adverse Childhood Experiences (ACEs) form Toxic Stress and chronic illnesses (AAHCs).

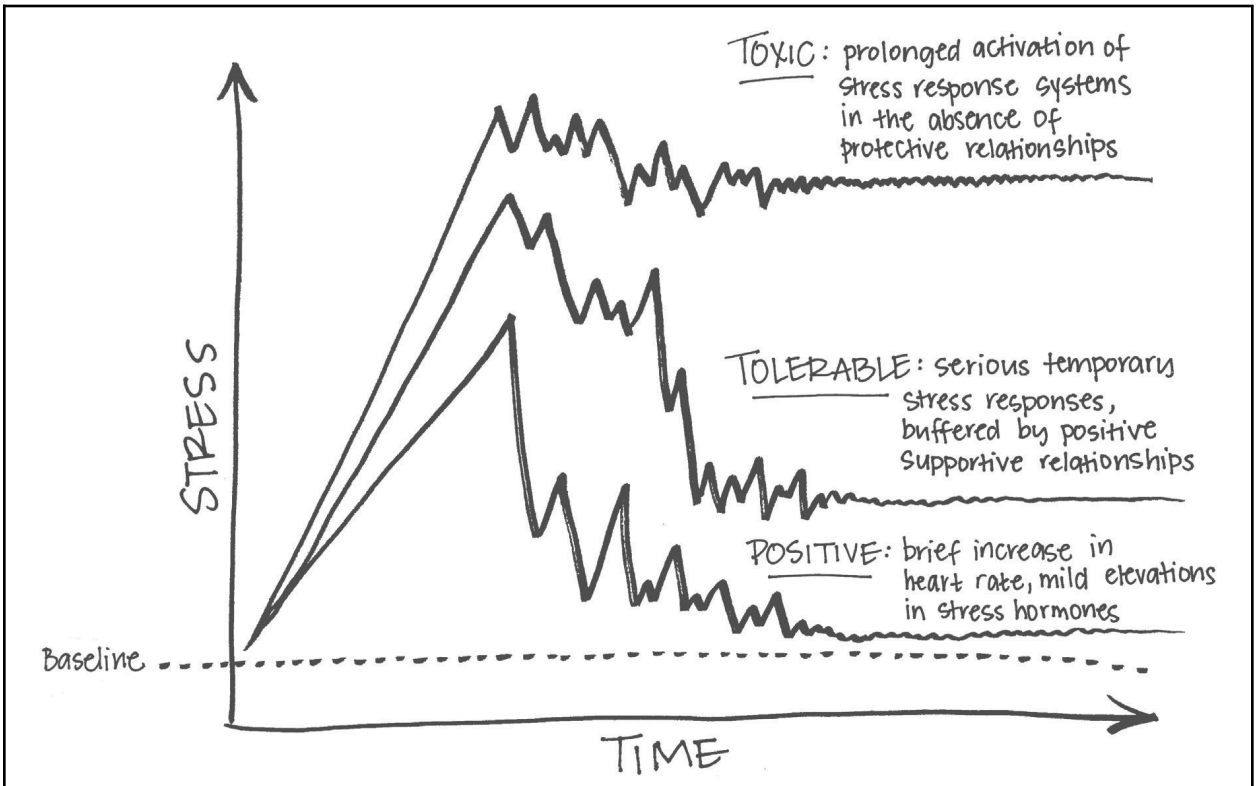


**Image from Colker and Erdmann's book Trauma and Young Children (2020).* [20]

Figure 6. Three categories of biological stress responses

B1. History of ACEs and Toxic Stress

Adverse Childhood Experiences, more commonly known as ACEs, are harmful or traumatic experiences that happen during the ages 0 to 18. ACE score refers to the total reported exposure(s) to the 10 ACE categories, consisting of excessive abuse (physical, emotional, sexual), neglect (physical, emotional), and household challenges (familial violence, separations, substance abuse, mental illness, incarceration). Refer back to Figure 1. Each category experienced counts as 1 point, and with 10 categories, the highest ACE score is 10 (on a scale from 0 to 10). Patients screened for ACEs, with ACE Score of 0 to 3

are considered low-risk, and ACE Score of 4 or more are considered high-risk for ACE-Associated Health Conditions (AAHCs), see Table 3. [4,5]

Table 3. ACE-Associated Health Conditions (AAHCs) in Adults

ACE-Associated Health Conditions: Adults

Symptom or Health Condition	Odds Ratio (excluding outliers)
Cardiovascular disease[21] (CAD, MI, ischemic heart disease)	2.1
Tachycardia[37]	≥ 1 ACE: 1.4
Stroke[20]	2.0
Chronic obstructive pulmonary disease (emphysema, bronchitis)[21]	3.1
Asthma[43]	2.2
Diabetes[21]	1.4
Obesity[20]	2.1
Hepatitis or jaundice[1]	2.4
Cancer, any[21]	2.3
Arthritis[32, 7] (self-reported)	3 ACEs, HR: 1.5 ≥ 1 ACE: 1.3
Memory impairment[20] (all causes, including dementias)	4.9
Kidney disease[43]	1.7
Headaches[11]	≥ 5 ACEs: 2.1
Chronic pain, any[38] (using trauma z-score)	1.2
Chronic back pain[38] (using trauma z-score)	1.3
Fibromyalgia[37]	≥ 1 ACE: 1.8
Unexplained somatic symptoms, including somatic pain, headaches[20, 2]	2.0 - 2.7
Skeletal fracture[1]	1.6 - 2.6[20]
Physical disability requiring assistive equipment[23]	1.8
Depression[21]	4.7
Suicide attempts[21]	37.5
Suicidal ideation[20]	10.5
Sleep disturbance[20]	1.6
Anxiety[21]	3.7
Panic and anxiety[20]	6.8
Post-traumatic stress disorder[37]	4.5
Illicit drug use[21] (any)	5.2
Injected drug, crack cocaine, or heroin use[21]	10.2
Alcohol use[21]	6.9
Cigarettes or e-cigarettes use[35]	6.1
Cannabis use[35]	11.0
Teen pregnancy[21]	4.2
Sexually transmitted infections, lifetime[21]	5.9
Violence victimization[21] (intimate partner violence, sexual assault)	7.5
Violence perpetration[21]	8.1

*This data table was compiled by ACEs Aware and the CA Department of Healthcare Services in 2020. Odds ratios compare outcomes in individuals with >4 ACEs to those with 0 ACEs, except where specified.

Anyone as young as a newborn and of any age at any time, can be screened for ACEs and may already have AAHCs. Fortunately, implementing ACEs screenings during initial medical check-ups allow for healthcare providers to refocus on preventative care and/or on treatment of the root cause rather than just covering the symptoms and manifestations. Another benefit of the ACEs questionnaire is that the patients are not required to share which type of ACE(s) they have, or disclose the specifics of their past traumatic histories to their treating physician, instead, they can keep their ACEs questionnaire sheet and only have to share their ACEs Score (number) during their intake. See Appendix B, for ACEs Screening Questions.[6]

Between 1995 and 1997, Dr. Vincent Felitti, Dr. Robert Anda and their research team conducted the first ever ACEs Study on 17,421 Kaiser patients in San Diego, in hopes to understand how childhood experiences affected health by first determining each patient's level of exposure (ACEs Score). At this point, they discovered 67% of the population had at least one type of ACE, and 13% had four or more ACEs. Following the discovery of the dose-response relationship between ACEs and poor health conditions, it has become evident that the higher the ACEs Score, the higher the risk of developing physical and mental illnesses (AAHCs).[1] For instance, a child with a ACEs Score of 4 is 2 times more likely to develop asthma, is 32 times more likely to develop learning and behavior disorders, anxiety, depression, and is 9 times more likely to develop unexplained nausea/vomiting/irritable bowels. An adult with ACEs Score of 7 or higher is 3 times more likely to get lung cancer and 3.5 times more likely to have ischemic heart disease.[1,5,6] Having ACEs increases the risk factors for toxic stress, and untreated toxic stress leads to ACE-Associated Health Conditions (AAHCs). Keep in mind, ACE exposure alone does

not guarantee a person's future health or outcomes, because AAHCs are also influenced by multiple factors. Factors include the overall health of biological systems, and the amount of protection and support from personal relationships and community resources. Building up positive experiences during childhood, builds the person's resilience and the body's capability to face and recover from stress later on in adulthood. If a positive environment is not nurtured in childhood, as an adult, they will be more vulnerable to toxic stress and have greater difficulty in regulating emotions and in recovery from any type of illnesses.[21,22]

Many people, including medical providers, are unaware that ACEs are NOT "a poor people's problem." The original ACEs Study was actually done on upper middle class residents of San Diego, with 70% college-educated Caucasians in the mid-90s. And with more than hundreds of follow-up studies from across the U.S, ACEs have been proven to happen regardless of income, race and poor access to healthcare. Another argument attacking ACEs studies, was that there was a belief that the increased risks has everything to do with one's behavior, and that if one can control the behavior one could rise above and not let their past adversities affect their future. Unfortunately that is only 50% truth, according to two 2004 studies on the associations between ACEs and ischemic heart disease. By avoiding traditional ischemic heart disease risk factors (smoking, sedentary habits, obesity), one could only protect themself only 50% of having a stroke, because psychological risk factors outweigh traditional ischemic stroke factors. Meaning, even if one does not engage in 'bad behaviors,' one could still be more likely to develop heart disease, especially if one has not worked on improving their psychology or mental health. [7,8]

C. Review— Western Medicine Perspective for Toxic Stress

Understanding how toxic stress is embedded in biological systems or different organ systems, can help visualize how Adverse Childhood Experiences (ACEs) and Toxic Stress lead to ACE-Associated Health Conditions (AAHCs). Table 4 is a brief overview of the biological systems disrupted when an individual has toxic stress or a dysregulated stress response. Toxic stress affects the Nervous, Immune Metabolic, and Epigenetic/genetic systems.

Table 4. How Toxic Stress Disrupts Biological Systems

Systems	Mechanism(s)	Health Impact
Nervous System	Dysregulation of sympatho– adrenal–medullary (SAM) and hypothalamic–pituitary–adrenal (HPA) axes, with long-term changes in regulation of key hormones, including cortisol, adrenaline; autonomic imbalance	Difficulty modulating, sustaining, or dampening the stress response; heightened or blunted stress sensitivity
	Altered reactivity and size of the amygdala	Increased fear responsiveness, impulsivity, and aggression
	Inhibition of the prefrontal cortex	Impaired executive function, with poorer planning, decision-making, impulse control & emotion regulation
	Hippocampal neurotoxicity	Difficulty with learning & memory
	Ventral tegmental area (VTA) and reward processing dysregulation	Increased risky behaviors and risk of addiction
Immune System	Increased inflammatory mediators and markers, especially of the Th2 response; inhibition of anti-inflammatory pathways; gut microbiome dysbiosis	Increased risk of infection, autoimmune disorders, cancers, chronic inflammation; cardiometabolic disorders
	Changes in growth hormone, thyroid hormone, and pubertal hormonal axes	Changes in growth, development, basal metabolism, and pubertal events
Metabolic System	Changes to leptin, ghrelin, lipid and glucose metabolism, and other metabolic pathways	Increased risk of overweight, obesity, cardiometabolic disorders, and insulin resistance
	Sustained changes to the way DNA is read and transcribed	Mediates all aspects of the toxic stress response
Epigenetic / Genetics	Sustained changes to the way DNA is read and transcribed	Mediates all aspects of the toxic stress response
	Telomere erosion, altered cell replication, and premature cell death	Increased risk for disease, cancer, and early mortality

C1. Toxic Stress on the Nervous System According to Polyvagal Theory

In 1994, neuroscientist and psychologist Dr. Stephen Porges introduced the Polyvagal Theory (PVT). PVT is a way of understanding how the nervous system responds to stress, threats or safety. In other words, PVT can help understand how the brain and body collaborate to respond to: stressors that are a part of daily life (positive and tolerable stress), versus significant stressors that tend to cause trauma (toxic stress). PVT consists of three states of stress response: (1) Mobilization "fight or flight," (2) Immobilization "freeze or collapse," and (3) Social Engagement "I'm safe."

First, Mobilization state is the sympathetic (SNS) "fight or flight" response that is activated when a person's situation becomes potentially threatening or dangerous. Next, if person feels overwhelmed or powerless toward fighting the danger, the Immobilization state will be activated, activating the Dorsal Vagal Complex (DVC), causing their body to 'give up,' 'play dead,' freeze, faint, collapse, or become numb or dissociated from their surrounding environment. Finally, when person recognizes that they're out of danger and are safe, their Social Engagement state is activated, activating the Ventral Vagal Complex (VVC), causing s/he to be relaxed and open to social interaction (with good eye contact, facial countenance, and tone of voice) in the surrounding environment.[36,37] On a daily basis, it is healthy for these three PVT states to operate in a continuum, not separately. This is what is known to have a normal stress response system (with Positive Stress or Tolerable Stress responses), however, it also depends on the frequency and duration the individual stays in certain states. When the individual stays in the Mobilization and/or Immobilization states too often and too long, unable to merge over to their Social Engagement state, this is an unhealthy and dysregulated stress response system, or Toxic

Stress response. Staying in the unhealthy state of toxic stress disrupts the important neurologic and neuroendocrine systems, heightening pain sensitivities, increasing fear response, impulsivity and aggression, impair cognition, memory and learning.[20,36,37,38]

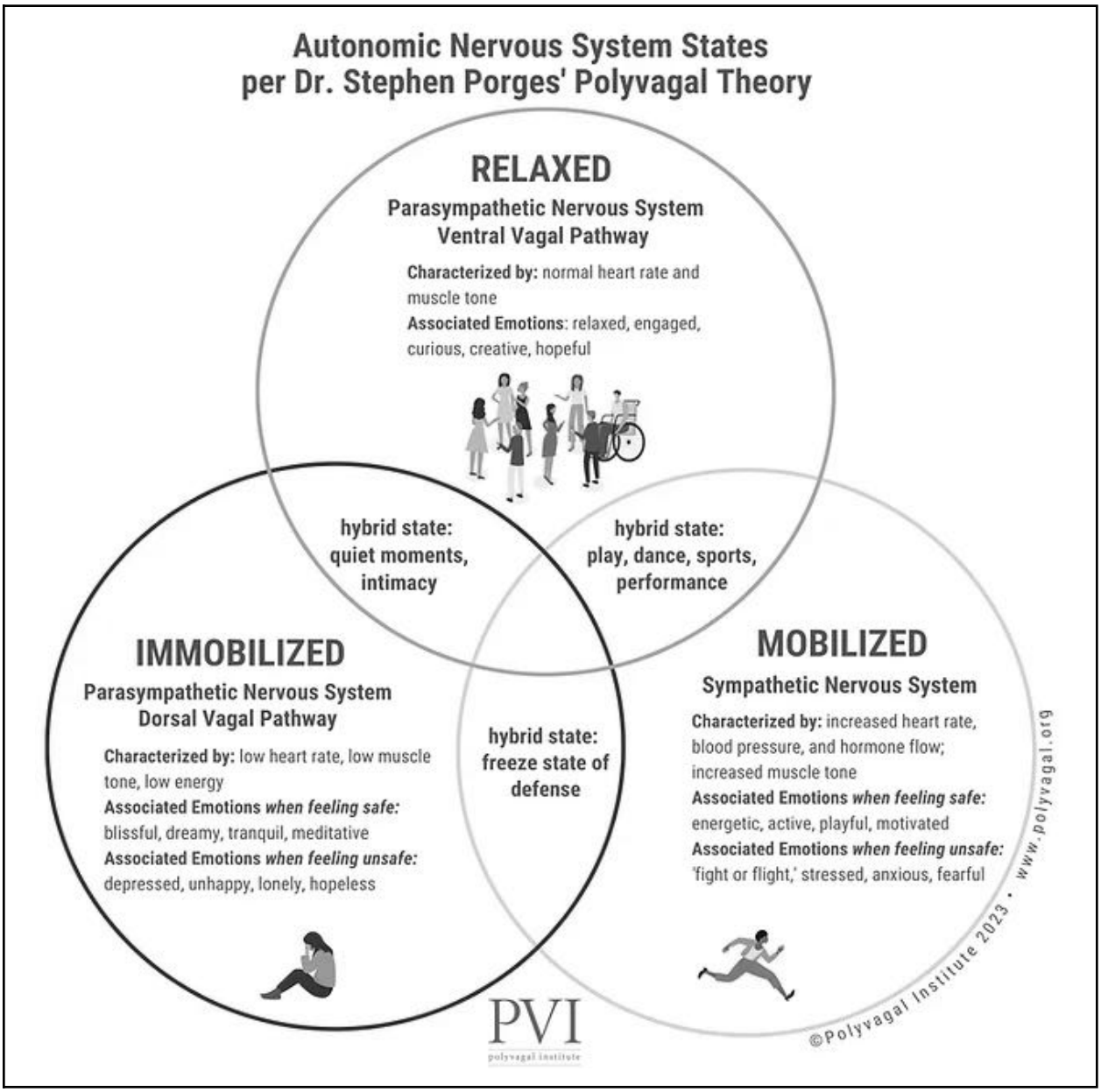


Figure 7. Polyvagal Theory Diagram[37]

Normal stress response regulation functions to maintain homeostasis. High levels of adrenaline and cortisol trigger the brain's hypothalamus and pituitary gland to signal to

adrenal glands to lower its production in a normal process called feedback inhibition. When chronically stressed, the stress response is activated repeatedly for very long periods of time (stuck in Mobilization and Immobilization states), and gradually impairs the feedback inhibition process. Meaning, toxic stress compromises a person's capacity to recover back to baseline (Social Engagement state) even when in reality there is no actual danger, the brain and body is still perceiving there is danger. [38] When exposed to ACEs and trauma, glucocorticoid resistance can occur, reducing responsiveness of cortisol. With glucocorticoid resistance, the glucocorticoid receptors cannot bind with cortisol and cannot respond normally to the negative feedback inhibition, because the receptor cells have been damaged or aged by by the abnormally high amounts of cortisol (from diseases that spike the cortisol levels, such as ACE-Associated Health Conditions–diabetes, obesity, cardiovascular diseases).[39,40]

Furthermore, structural and functional changes to the brain occur with toxic stress. Specifically, the amygdala (who governs fear and emotions, and works as "smoke detector" alarm for threats) become larger in size and overactive in adults who have anxiety with a history of childhood traumas (ACEs). Along with, the prefrontal cortex (the executive or "watch tower" for seeing the reality and making decisions on plans) and hippocampus (memory storage) are smaller and underactive, shown in functional neuroimaging studies of individuals with ACEs.[12,41] Also in the limbic system (responsible for reward and motivation) of individuals with trauma and/or depression, the dopamine receptors are less responsive to rewards, leading to the person having less intrinsic motivation to perform daily tasks.[42,43] As a result of adversities in childhood, the brain's structural and functional circuits are hypersensitive, causing the individual to have

higher perceived pain, increasing susceptibility to chronic pain disorders (such as AAHCs– fibromyalgia, chronic back pain, migraines).[44.45] See Table 3 on AAHCs.

C2. Toxic Stress on the Immune System

Toxic stress can impair the immune response, causing increased risks of infections, autoimmune disorders, cancers and inflammatory diseases. Immune dysregulation can affect both innate and acquired immunity, that involve either the immune system is underactive or overactive. When the immune system is underactive, it is less likely to fight off pathogenic invasions, such as from respiratory Corona viruses, intestinal Escherichia Coli (E. coli) bacterias, and dermatological Candida fungal infections. When the immune system is overactive, it can lead to greater vulnerability to inflammatory diseases and autoimmune conditions, where the immune system starts attacking its own body host. People with high Adverse Childhood Experiences (ACEs) and toxic stress, along with a dysfunctional immune system, are more likely to develop inflammatory diseases—arthritis, asthma, allergies, dermatitis.[46,47,48]

With high perceived stress scale (PSS) scores, these individuals were six times more likely to become infected with and/or express more severe symptoms of respiratory viruses, including Covid-19 virus, than those with low PSS.[49] Another study indicated high stress triggers the liver to increase inflammation biomarkers, like C-reactive proteins, that can lead to lung inflammation, elevating the levels of virus antibodies for Epsten-Barr virus found in highly stressed people.With chronic stress and a dysfunctional immune response system, chronic inflammatory diseases arise, leading to increasing DNA mutation rate and angiogenesis for tumors with potential cancerous cells.[50,51,52,53]

C3. Toxic Stress on Metabolic Systems

Dysregulated stress response systems can disrupt the metabolic systems, increasing risk of gastro-intestinal diseases, obesity, diabetes mellitus, cardiovascular diseases, etc.[54] Frequent and prolonged stress response changes the brain's reward signaling pathways governing satiety cues—the hormone leptin. During heightened sympathetic 'fight-flight' state, leptin barely secretes to signal appropriate fullness and/or leptin resistance occurs, contributing to overeating. Because the body thinks it's in survival mode, the person is also biologically wired to over consume high-fat and high-carbohydrate foods for longer storage, as the body is in a state of uncertainty of when they'll be eating next.[55,56] The overconsumption exhausts the pancreas from constantly secreting insulin to move glucose from the bloodstream into cells to make energy. Because of the overwhelming concentration of glucose in the bloodstream, the insulin receptors of the cells can become damaged, causing insulin resistance (Diabetes Mellitus - Type II). With excess glucose and insulin remaining in the bloodstream, the cells are unable to generate energy, the person will become chronically fatigued if not treated.[57,58,59,60,61] Exhaustion discourages physical exercise and encourages sedentary habits that are linked to increasing anxiety, cardiovascular diseases, and stroke. Not only does toxic stress lead to cardiometabolic disorders, the predominance of sympathetic tone also indicates the parasympathetic-vagus tone is decreased, meaning there is lower peristalsis that can lead to dysphagia, poor gag reflex, poor bowel movements, bloating, constipation, irregular bowel syndrome, intestinal polyps, diverticulitis, etc.[62,63]

C4. Toxic Stress on Epigenetic and Genetic Systems

Experiencing toxic stress early-on in childhood (ACEs) can alter gene expression, that is responsible for a series of biological processes throughout life. Epigenetics is the study of how animal behavior and environment can cause changes to how genes are read (gene expression). ACEs and toxic stress cannot change a person's genomes (DNA), but they can alter gene expression and epigenetic markers (epigenomes). Epigenetic markers and DNA are passed down from parent to child. Luckily, the offspring's gene expression is not limited to just their parent's DNA and epigenetic markers, but also can be altered by nurture (safe or dangerous environments).[64,65,66] On the other hand, if the individual inherited their parent's long and healthy telomeres, they should want to keep those telomeres as those can work as a protective buffer against future stressors encountered in life. In recent studies, exposure to environmental stressors (abuse, neglect, challenges) shortened the telomeres— the protective ends of a DNA strand that make sure the DNA gets replicated correctly. When telomeres are damaged or shorten, they signal to the cells to either retire early (for cell death) or become precancerous (compromising DNA from copying correctly, causing uncontrollable replications or mutations).[67,68] In short, for people with toxic stress, their cells are more prone to aging faster or mutating. This may lead to higher rates of Alzhimer's disease, dementia, and cognitive decline—all signs of cellular premature aging.[69] Fortunately, if the parents of trauma survivors passed down long telomeres to them, they may be able to buffer some of the premature cellular aging. Furthermore, some epigenetic changes can be reversible when ameliorated and treated early-on. Recent studies on ACEs treatments show resiliency from epigenetic changes. The treatments will be discussed in the next section.[70,71,72]

D. Review— Western Medicine Treatments for Toxic Stress

D1. Stress-mitigation Strategies

The western medicine strategy uses a framework of stress-mitigation strategies that enhances the effectiveness of treating toxic stress. Behavior therapies are commonly prescribed as treatment for toxic stress, in conjunction with the stress-mitigation strategies. In many cases, if the stress-mitigation framework is not addressed first, or not applied to the patients with traumas, the behavior therapies and psychiatric medications are not as effective or not helpful at all.[4,6,12] Depending on the patient's ACE score and current severity of symptoms, the ACE-informed practitioner will prescribe a different protocol of intervention.

For example, an adult patient found to be at low risk of toxic stress (ACE score of 3 with no associated symptoms or conditions) and with a number of protective factors may not need any additional interventions or referrals beyond patient education. However, with an ACE score of 1, and symptoms of depression and uncontrollable asthma and with lack of social support, the patient would be considered to be at intermediate risk of toxic stress. In that case, they may benefit from specific interventions that target the toxic stress response, as well as referrals for community and/or mental health resources. See Figure 8.[73]

The stress-mitigation strategies listed below are the current recommended care for toxic stress. Reducing stress hormones, inflammation, and enhancing neuroplasticity could help counteract the toxic stress response and improve overall health and well-being. These strategies offer an integrative approach to ACEs and toxic stress intervention:

Healthy familial and social relationships, Quality sleep, Nutritious diet, Regular physical activity, Mindfulness meditation, Time in nature, Mental Health Care Therapies (Bottom-up or Top-down regulation types).

Healthy relationships establish safe, stable and nurturing environments for the ACEs survivor. This is the first step in treatment for toxic stress from ACEs, because even if the doctors prescribed behavior therapies as treatment, without a safe and supportive environment for the survivor, the treatments will not be effective in protecting the survivor from remaining environmental stressors and triggers.[74] Supportive relationships not only provide safe housing for the survivors, but also they give release of hormone oxytocin. Oxytocin helps protect the stress response system, reduces inflammation on the blood vessels, and brings homeostasis to metabolism.[75] Next, training for good sleep gives their biological systems opportunities to repair its damages from toxic stress on a cellular level. Poor sleep impairs cognitive functions, causes traffic accidents, and triggers inflammation. Healthy sleep can help reduce the inflammatory C-reactive proteins, high cortisol and blood pressure levels.[76,77] Most importantly for system repair, is having fresh, nutritious and whole foods to consume. Consuming whole foods rich in enzymes and antioxidants have shown to repair the gastrointestinal tract or gut microbiome, allowing for the exhausted brain and organ systems to be supported and nourished.[78, 79, 80] Following with daily exercise provides movement and blood oxygen circulation for the whole body, and can mitigate the negative consequence of mental illnesses such as depression.[81,82] Meditation and behavior therapy can help patients build tools and capacities for resilience, and for what to do to reduce the anxiety or trauma triggers.[83,84,85,86,87]

D2. Bottom-up or Top-down Regulation Therapies

Speaking of mental health care therapies, it is important for the therapist or psychologist to differentiate what type of therapy to apply to their patients depending on their current state. These therapies can be categorized into two general types: Top-down regulation and Bottom-up regulation therapies.[12] Top-down regulation therapies involve strengthening the capacity of the prefrontal cortex "watch tower" to monitor the body's sensations. Talk therapy (cognitive behavior therapy, dialectical behavior therapy) and mindfulness meditation can help with this top-down regulation.[87] However, in many cases for patients with trauma, top-down therapy will not be helpful in the initial treatments because the patient's basic physiological needs are not addressed, hence, bottom-up therapy would be recommended. Bottom-up regulation therapies involve recalibrating the autonomic nervous system, or restoring the brainstem and hypothalamus's basic housekeeping functions (temperature regulation, breathing, eating, sleeping, bowel movements, urination) back to homeostasis. Breathing and movement exercises, Tai-chi, Qi-qong, Yoga,[84] Emotional Freedom Technique (ETP tapping therapy)[88,89,90] and acupuncture,[85,91,92,93,94,95] are shown to be effective Bottom-up regulation therapies for patients with trauma history. Establishing safe housing, balanced nutrition and good sleep hygiene are also considered Bottom-up regulation treatments. Understanding the difference between top-down and bottom-up regulation, and identifying which type of invention is best for an individual patient's current state, is essential for treating patients with childhood trauma and Toxic Stress.[4,12,41,90]

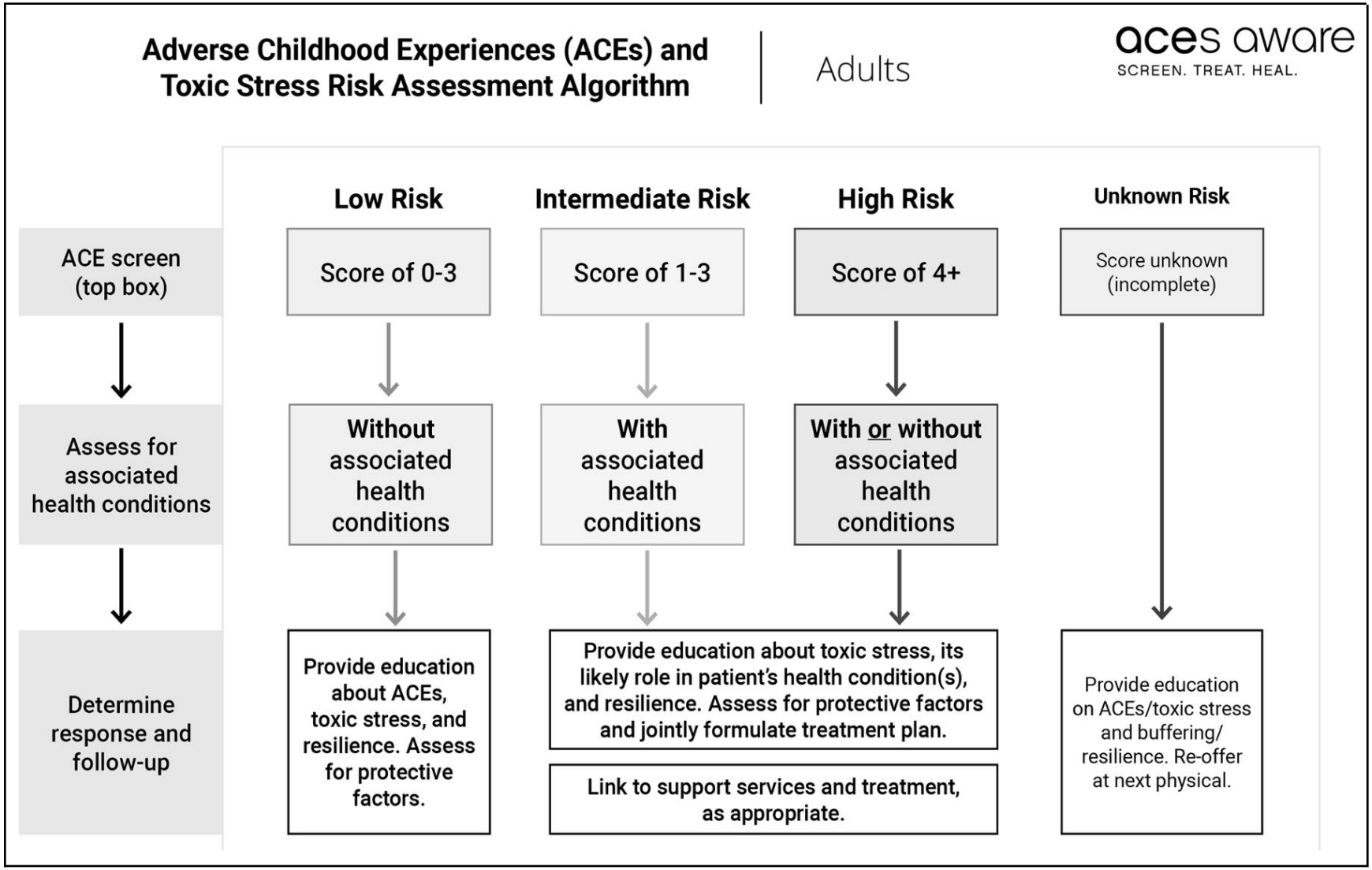


Figure 8. Western Treatment Protocol for Toxic Stress Developed by ACEsAware [73]

E. Review— Traditional Chinese Medicine (TCM) Perspective of Toxic Stress

E1. Zang Fu Theory

The concept of Toxic Stress is closely associated with mental and emotional problems in Traditional Chinese Medicine (TCM), that cannot be treated effectively without understanding Zang Fu (Internal Organ) Theory and its relationship to emotions. There are twelve Zang Fu (internal) organs, however, more particularly, the five Zang organs (Liver, Heart, Spleen, Lung and Kidney) have more emphasis on emotions and will be the organs further discussed here.[97,98] In Chapter 1 of Huang Di Nei Jing 'Su Wen' (黃帝內經素問), the unity of the body and mind was said to be what keeps a person alive, in other words, by keeping the body's Zang Organs and mind's Spirit in mutual relationship, the person can live "the entire lifespan of one hundred years." It is when the person's body and mind are both no longer healthy and cooperating together, that is when the person "dies at the age of fifty," as mentioned in Chapter 34 of Su Wen.[96] Qi is the foundation of all physiological processes, and that same Qi is the basis for emotional and mental processes.

In Chapter 9 of Huang Di Nei Jing 'Lin Shu' (黃帝內經靈樞), the emotional and mental aspects are part of the action of internal organs. Each internal organ is related to a particular emotion. The state of the organ will affect the emotions, vice versa, the emotions will affect the state of the organ. Liver relates to anger, Heart to joy, Spleen to pensiveness, Lung to sadness and worrying and Kidneys to fear. In reverse: Too much anger can damage the Liver, too much joy (disillusion) can impair the Heart, overthinking

stresses the Spleen, excessive grief depletes the Lungs, and too much fear exhausts the Kidneys.[101,104] Table 5 describes the Zang organs and their associated emotions.

Table 5. TCM Internal Organs and their associated Emotions

Zang Organs	Seven Emotions
Liver	**Anger** affects the qi direction. Too much anger injures the liver organ, and causes qi to rise upward, building up tension in the head, neck and shoulder areas. Anger can present as headaches, red face, dizziness, tinnitus, stiff neck and shoulders.
Heart	**Joy** affects the qi speed. Too much joy can slow down the qi, injuring the heart organ, head, causing insomnia, overexcitement, palpitations.
Spleen	**Pensiveness** affects the qi speed; too much can stagnant qi. Pensiveness injures lungs and spleen, causing chest discomfort, breathlessness, tense shoulders, epigastric discomfort and abdominal distension.
Lung	**Sorrow and Worry** affect the qi amount, because too much sadness can consume qi, leading to qi deficiency, or fatigue. Sadness can injure the lung organ, causing breathlessness and chest discomfort. Worrying knots the Qi, causing shoulder and breast tension. Both sadness and worry together can cause chest tightness and breathlessness.
Kidney	**Fear** affects qi direction, causing qi to descend. Constant fear injures the brain, Heart and kidneys, causing urination problems and diarrhea, with issues of sleep and palpitations. **Shock**, similar to sorrow, affects the qi amount. Being in shock scatters the qi, causing problems with the kidneys, heart and head. Shock can lead to insomnia, palpitations and breathlessness.

E2. Five Psychic 'Spirit' Aspects and Seven Emotions

The concept of toxic stress is most closely associated with mental and emotional problems in TCM, that cannot be treated effectively without understanding the Five Psyche Aspects and their associated Seven Emotions. Five Psyche 'Spirit' Aspects include: Corporeal Soul (Po), Spirit (Shen), Ethereal Soul (Hun), Intent (Cang Yi), and Will (Zhi). Again, the Seven Emotions are Anger, Joy, Pensiveness, Worry, Sadness, Fear and Shock. There is a difference between the five psychic aspects and the seven emotions. In the case of the spleen, the associated psychic aspect is intent, while the associated emotion is pensiveness/though. While the two are related, the first is considered to be a fundamental aspect of the subconscious mind while the second involves a response to external stimuli. In other words, intent is a capacity while pensiveness/thought is an activity.[102]

Psych Aspects are prenatal (deep-seated) aspects of personality or character, and Emotions are post-natal manifestations of the interaction of the individual with the world. Psyche is with a person from birth, inherent in their organs themselves. How organs can be strengthened and weakened overtime, so do the psychic aspects. In case of disease, both psychic aspects and seven emotions can be affected.

Table 6 describes the association between the psyche aspects and emotions. Psyche aspects— Ethereal Soul-Hun is associated with emotion Anger, Spirit-Shen with Joy, Intent-Yi with Pensiveness, Corporal Soul with Sadness/Worry, and Will-Zhi with Fear/Shock.[100,104]

Table 6. Relationship between Seven Emotions and Five Psyche Aspects

7 Emotions	5 Psyche Aspects
Anger	**<u>Ethereal Soul - Hun</u> :** Ethereal Soul is the psychic aspect associated with a person's courage capacity, that is dependent on their liver organ health and function. When the liver is healthy, it makes courageous or fearless yet cautious decisions to remove toxins and distribute blood, strengthening the person's ethereal soul. This is the type of courage, allowing the facilitation of a person's ability to wait and observe situations and emotional conditions until the time is appropriate for action. Ethereal Soul courage is balanced with rashness while maintaining a certain degree of caution. When the person displays excessive courage with 'nothing to lose' attitude, that is a sign of an unbalanced ethereal soul and liver, then it's appropriate to apply treatment to nourish the liver blood because liver blood houses the ethereal soul. Ethereal Soul-Hun gives movement (coming and going), ideas, inspiration, intuition and creativity to Spirit-Shen. When Hun is coming and going too much, Shen becomes overwhelmed and fails to control Hun, causing the person to become manic. When Hun is not coming and going enough, because Shen is over-controlling, the person can become depressed.
Joy	**<u>Spirit - Shen</u> :** Spirit refers to the intelligence in living things. In humans, spirit is the capacity for us to understand, compare and contrast concepts. In plants, spirit is the plants' ability to adapt or change with the seasons and weather conditions. Spirit-Shen controls and integrates to Ethereal Soul-Hun. When Shen is over-controlling Hun, the person can become depressed. When Shen fails to control Hun, the person can become manic.

Pensiveness	**<u>Intent - Cang Yi</u> :** A well-regulated spleen helps a person form random ideas into organized intent. Similarly, good digestion with even pace transforms raw foods into useful nutrition. Both intent and digestion are healthy when they both move at an even pace. Excessive thinking or excessive intent creates imbalance or unhealthy obsessions, however, a lack of intent produces scattered and disorganized thoughts. With a healthy amount and rhythm of thoughts, this not only supports one's subconscious mind, but also helps improve spleen's transformation and transportation functions for providing nourishment for the body. When one overthinks, one tends to become more sedentary and less mobile with fitness. Regular exercise and body movement help support the spleen's action and nourishment to the whole body. Strong spleen function allows a person to have better capacity to focus with intent, without overthinking.
Sorrow / Worry	**<u>Corporeal Soul - Po</u> :** Physical Strength of the body is reflected in one's corporeal soul capacity, in other words, this is also one's confidence that can be sensed by others. The relationship amongst sorrow, corporeal soul, and lung function are multifaceted. The lung is associated with emotion of sorrow or pessimism. When the lung is in a healthy state, the person's physical strength and corporal soul are also strong, therefore, one can more easily maintain an optimistic outlook, and is less susceptible to pessimism or sorrow. Experiencing sorrow can impair the lung's qi transformation function, impairing one's corporal soul and ability to stick with physically challenging work over a long period of time. Sorrow dispels qi in the upper burner, compromising lung and spleen's natural down-up movement and causing lack of movement, therefore creating heat in the spleen. The spleen is responsible for postnatal qi production, and without its function, the corporeal soul

	strength weakens. Weak lungs or deficiency in corporeal soul strength may leave one more susceptible to sorrow, however, excessive sorrow, from life situations or passing loved ones, can also damage the strongest lungs. Lung governs the qi of the entire body, therefore, it's important to address the lung in treatment in even disease patterns from other organs.
Fear / Shock	**<u>Will - Zhi</u> :** The associated psychic aspect of the kidney is will, that is a person's ability to make and follow through with plans. Will is determined by the quality, quantity and metabolism of essence. It is important for the heart and kidney to maintain communication, because essence from the kidneys root the heart's spirit, so that a person's abundance of ideas and plans can be followed through properly into reality. The will to live is supported by the body's essence, so as long as the person has an adequate supply of essence, they will have a strong will to live, regardless of life challenges—chronic diseases, grief, economic hardship, etc. In order to preserve their pre-heaven essence, one must not engage in excessive sensuous activities, compromise sleep, etc; in addition, to build post-heaven essence, one should enhance their spleen by eating nutritional foods and maintaining good digestion.

E3. Yin Yang Theory

Toxic stress response is dysregulation of the biological stress response system, and can be interpreted as disharmony of Yin Yang in Traditional Chinese Medicine (TCM) theories. In Chapter 39 of Huang Di Nei Jing 'Su Wen' (黃帝內經素問), the Yin and Yang Doctrine consists of five components: Opposition of yin and yang, Interdependence of yin and yang, Mutual Consumption of yin and yang, Inter-Transformation of yin and yang, and Infinite Divisibility of yin and yang.[96] A general example of the Mutual Consumption of Yin and Yang Theory would be that the decrease of yang energy leads to yin excess, causing cold and chillness symptoms. The same Yin and Yang theory of Mutual Consumption can also be applied in the relationship between the yang-aspect character Hun and yin-aspect character Shen: When Hun is coming and going in excess, Shen becomes exhausted (deficient) and fails to control Hun, causing the person to become manic. When Hun is not coming and going enough (deficient), Shen can be over-controlling (excess), the person can become depressed. Mutual consumption is said to be in balance when the Hun gives movement (coming and going), ideas, inspiration, intuition and creativity to Shen. [100] Furthermore, Yin and Yang Theory can also be applied in Western Medicine, such that our stress response system can be regulated by balancing the autonomic nervous system. In other words, by balancing the parasympathetic (PNS) and sympathetic (SNS) nervous systems, the Yin and Yang respectively can be working together in harmony. When Yin and Yang are in balance, the stress response system is healthy and functioning normally in the state of Positive Stress or Tolerable Stress, instead of in a dysfunctional state of Toxic Stress. Table 7 summarizes the Yin-Yang Theory and its relationship to the autonomic nervous system, along with the five aspects of Yin-Yang Theory.

Table 7. Relationship between Yin-Yang Theory and the Autonomic Nervous System

Yin-Yang Theory		**In Relation to Autonomic Nervous System** (PNS and SNS)
1. Opposition of Yin-Yang	All things have Yin and Yang aspects.	Examples: The Heart has both PNS and SNS states. Under PNS state, heart muscle slows heart rate and decreases contraction force, and also the heart coronaries dilate. Under SNS state, heart muscle increases heart rate and increases contraction force, and also the heart coronaries constrict (alpha cells) and dilate (beta cells).
2. Inter-Dependence of Yin-Yang	Yin and Yang cannot exist without the other.	PNS and SNS cannot exist without the other. Their actions are synergistic, and they rely on each other to maintain a balanced sympathovagal tone.
3. Mutual Consumption of Yin-Yang	Yin and Yang are in a constant state of change for preserving the balance.	PNS and SNS are in constant state of change in regulating balance of the organ systems. When PNS vagus nerve causes the blood pressure drop too low, the baroreceptors and medulla cardiovascular center know to signal the SNS to excite and PNS to inhibit. This causes increased heart rate, increased stroke volume, therefore increasing cardiac output, and also increased resistance from vein constriction. (Blood pressure = cardiac output x resistance). This results in blood pressure rising. When the blood rises too high, the reverse occurs, telling the PNS to excite and SNS to inhibit, in order to preserve balance of normal blood pressure.
4. Inter - transformation of Yin-Yang	Yin and Yang can change into one another.	When a person is relaxed, their PNS predominates, but when a person senses danger, the PNS switches to SNS predominance.Vice versa, SNS predominance can switch back to PNS predominance.
5. Infinite Divisibility of Yin-Yang	Yin and Yang aspect can be further divided into Yin Yang.	PNS controls can be further divided into exciting and inhibiting functions, such as for contraction (constriction, depolarization) and relaxation (dilation, repolarization). SNS controls can also be further divided with exciting and inhibiting functions.

F. Review— TCM Acupuncture Treatments for Toxic Stress

F1. He-Sea, Yuan-Source, Luo-Connecting and Group-Luo Points

Distal (extremities) acupuncture points are commonly used in clinics for treatment of various physical and mental stress conditions. Most popular amongst American, European, Australian and English-speaking acupuncturists are the 'ML-10' acupuncture points: He-Sea points (ST-36, LI-11), Yuan-Source (LI-4), Luo-Connecting points (LU-7), and Group-Luo point (SP-6). The ML-10 points are named after Dr. Miriam Lee, OMD, the author of the 1991 published book "Insights of a Senior Acupuncturist." Dr. Miriam Lee, trained as an acupuncturist in China prior to liberation period in 1949, and practiced in California for 60 years until her passing in 2009, she was the one of the pioneering acupuncturists in the US, and was responsible for the 1976 legislation passed to legalize acupuncture in California.[106]

Dr. Miriam Lee formulated and practiced the distal ML-10 point combination with exceptional results in treating most internal organ diseases. The rationale is that the nerve endings on the extremities when needled cause strong reflex action to the subcortex. On the other hand, when needles are applied on the location of the pain or disease, the reflex action to the brain is smaller. There is research indicating the further away the needle stimulation from the brain, the site of pain or disease, the stronger the reflex action to the subcortex.[107] For this reason and after testing on thousands of patients, Dr. Lee has narrowed down the most convenient, effective and versatile acupuncture points to needle on the extremities to be used to treat most diseases by acupuncturists of any experience level. Refer to Table 8 for more detailed understanding

of these distal points. Because ML-10's He-Sea points (ST-36, LI-11), Yuan-Source (LI-4), Luo-Connecting points (LU-7), and Group-Luo point (SP-6) have well-established research showing results in treating stress, these traditional distal acupuncture points will be applied on the participants in the Active-Control group for this pilot trial. Another benefit of selecting ML-10 points for this clinical trial's Active-Control acupuncture group (CG) was that they are not located on the same dermatomes as the back acupuncture points in the Experimental back acupuncture group (EG). By selecting points not overlapping dermatomes in acupuncture comparison trials, according to sham-acupuncture research theory, it will make a more distinguishable comparison and have have more potential statistical significance between the two acupuncture groups.[108]

Table 8: Miriam Lee's ML-10 Acupuncture Point Protocol

CG's Active-Control Group Acupuncture Points (ML-10)	
Acupuncture Points	Explanation
ST-36 **Zu san li** 足三里 Leg Three Miles	**He-Sea Points**: These points located at the elbows and knee joints regulate the physiological activity of the internal organs, especially when the disease is caused by Yang organs or poor and irregular dietary habits. Combining ST-36 and LI-11 increases metabolism, digestive function and respiration. They have a long history of traditional anecdotes and research literature backing the strong effectiveness in strengthening the whole body health.[109,97] Of the twelve he-sea points, ST-36 is the most powerful he-sea point, because it is located on the yang-ming channel, it has the fullest of qi and blood, and is prominent in adjusting the qi and blood of all channels. ST-36 strengthens the spleen and stomach, and when the body has good digestion it has enough nutrition to keep calming the spirit, reducing stress. Also located on the yang-ming channel is he-sea point LI-1l, which treats diseases of the yang organs and intestinal bowel. In combination with ST-36, LI-11 dispels winds and pathogens, boosting the body's immune system and protecting the body from external pathogenic factors.
LI-11 **Qu chi** 曲池 Pool at the Crook	
LI-4 **He gu** 合谷 Joining Valley, Yuan-Source point	**Yuan-Source and Luo-Connecting Points, or "Host-Guest" Points**: These points located at the wrist and forearm, are important paired Yin and Yang channel points, where LI-4 is the (Yuan-source) host and LU-7 is the (Luo-connecting) guest. In "Great Compendium of Acupuncture" of 1601 and now in clinical practice, Yuan-source point is taken as the main point to treat the affected channel; Luo-connecting point of internally-exteriorly related channel is added to supplement the treatment.[98] Dr. Miriam Lee selected LI-4 for its influence on the brain,

LU-7 **Li que** 列缺 Broken Sequence, Luo-Connecting point Confluent point of the Ren channel	and also its ability to regulate qi of the three burners, allowing the point to reach the whole body, especially below the navel for treating intestine problems. LI-4 in combination with LU-7, they can treat diseases that occur above the neck. In the clinic, LU-7 area's appearance can be used for diagnosis of deficiency (when sunken) or excess (when puffy) conditions. Lung meridian is an aspect of the metal element that can relate to feelings of grief, shame, and disembodiment—as well as to overtly lung-related symptoms such as asthma. As an Eight Confluent point, LU-7 can access the Ren meridian (conception vessel), which has a strong relationship with early development, and so may be particularly helpful for certain aspects of loss or disruption in infancy and early childhood. LU-7 point name 'Broken Sequence' can refer to the ruptures experienced as the result of intergenerational trauma.
SP-6 **San yin jiao** 三陰交 Three Yin Intersection	**Great-Luo Point**: Three Yin Intersection SP-6 gives its name for being the crossing point of three yin channels of the leg, that being the spleen, kidney and liver channels all cross here at SP-6. So while SP-6 is on the spleen channel, it can also address problems on the kidney and liver channel. SP-6 mainly treats reproductive system problems, but needling this point also strengthens kidney qi for improving memory functions. SP-6 can also address Heart indirectly via treating the Kidney and Spleen, the Heart is automatically regulated (Kidney is the controlling grandmother of Heart, and Spleen is the son of Heart).[97,98]

F2. Back-Shu and Psychic Aspects Points

Back-Shu acupuncture points are located parallel to the vertebra and coincide with twelve Urinary Bladder channel points, primarily used for treating internal organ diseases directly, where the points' names overlap with their respective internal organs. The primary twelve Back-shu Urinary Bladder (UB) channel points are—UB-13 Fei 'Lung' Shu, UB-14 Xin Bao 'Pericardium' Shu, UB-15 Xin 'Heart' Shu, UB-18 Gan 'Liver' Shu, UB-19 Dan 'Gallbladder' Shu, UB-20 Pi 'Spleen' Shu, UB-21 Wei 'Stomach' Shu, UB-22 San Jiao 'Triple Burner' Shu, UB-23 Shen 'Kidney' Shu, UB-25 Da Chang 'Large Intestine' Shu, UB-27 Xiao Chang 'Small Intestine' Shu and UB-28 Pang Guan 'Urinary Bladder' Shu. Back-shu points are applicable for treating both deficiency and excess types of diseases, which makes these points versatile in treatment management of various mental and physical illnesses, such as AAHCs.

Psychic Aspect acupuncture points are also located parallel to the vertebra and coincide with the five Urinary Bladder channel points, mainly used for treating mental and emotional health conditions. The Five Psychic points are—UB-42, UB-44, UB-47, UB-49 and UB52, located on the outermost line of the Urinary Bladder channel, lateral to the Back-shu points mentioned previously. Acupuncturing these psychic points regulate the mental capacities of Confidence & Optimism (UB-42 Po Hu), Intelligence & Morality (UB-44 Shen Tang), Courage & Decisiveness (UB-47 Hun Men), Focus & Intent (UB-49 Yi She), and Willpower & Commitment (UB-52 Zhi Shi).[100,102,103]

While distal extremities acupuncture points can treat internal organ diseases indirectly, Urinary Bladder channel points can treat internal organ diseases directly. Although Back-shu points are located on the Yang posterior portion of the body, they are

predominately used to treat Zang (Yin) organ diseases. In Chapter 5 of Huang Di Ba Shi Yi Nan Jing (黃帝八十一難經) , the 67th Difficult Issue states “Yin disease moves to yang [area], and Yang diseases move to the Yin [area]..... “[Treat] From the yang pull the yin; [treat] from the yin pull the yang.” With this in consideration, when diseases are from the Yin organs located on the front side, such as the Liver, Heart, Spleen, Lung and Kidney, then Back-shu point can be needled. Yin diseases are also considered more chronic diseases and Yang diseases are more acute diseases.[105] Hence, in the Great Compendium of Acupuncture “Zhen Jiu Da Cheng” (針灸大成) of 1601, Dr. Ji Zhou Yang stated one cannot treat chronic diseases without needling back-shu points, because the internal organs can be exhausted and deficient in chronic states of illnesses and need to be strengthened. This can be done by tonifying the back-shu points corresponding to the diseased organs.[103]

Internal Zang organs (Liver, Heart, Spleen, Lung and Kidney) house the Psychic ‘Spirit’ Aspects. When mental and emotional disease is chronic, indicating the stress response system is also chronically dysfunctional, the internal organs are said to be exhausted and depleted. This stressful state can deplete Psychic Aspects’s capacities to manage their person’s emotions.[96] For example, when Liver blood is deficient, Hun cannot be rooted and cannot have a sense of direction in life. In theory, the Back-shu of the Lung, Heart, Liver, Spleen and Kidneys can be needled to tonify the organs directly, along with repairing the housing and replenishing the capacities for the Psychic Aspects. In doing so, continuing from the previous example, when Liver organ is replenished, the Liver blood is normal, therefore, the Hun can be firmly rooted and can help give the person a secure sense of direction.

G. Research Gaps and Conclusion of Literature Review

By exploring relevant literature, this review intended to bridge the gaps between the Western perspective of biological stress and TCM perspective of Internal Organs and Psychic 'Spirit' Aspects. Despite the extensive research throughout classical and modern texts, a noticeable gap of information remains regarding the TCM interventions for chronic stress and childhood trauma. The classical and current literatures lack a consensus on the application of Five Psychic Aspect acupuncture points, located on the bilateral outer lines of Urinary Bladder channel points, for clinical treatment. There is presence of theory-based literature on Back-shu acupuncture applications, however, there is also a notable limitation of evidence-based studies regarding the general application of Back-Shu acupuncture points for any disease interventions. The investigator struggled to find well documented case studies, series and/or clinical trials regarding the usage of back (Urinary Bladder channel) acupuncture points for not only treatment of chronic stress but also for treatments for any chronic diseases. Perhaps this was due to the challenges in finding enough consenting participants to commit to consistent treatments, and also due to the inconveniences of accessing back acupuncture points in busy clinical settings.

Furthermore, there is a need for further research to address the inconsistencies found in the Heart Rate Variability (HRV) data collected from acupuncture research studies. The fact is a standardized HRV guideline for clinical diagnosis cannot be developed when acupuncturists/investigators all collect different types of HRV data. Some collect only pNN50–HRV, some collect RMSSD–HRV, others collect LF and HF power–HRVs in their clinical research; with all investigators collecting different HRVs and without at least four types of HRV measurements recorded from each participant, this makes it nearly impossible to form a

conclusion of the health and abnormalities in patients' autonomic nervous systems, in which HRVs are essential biomarkers of biological stress.

Future studies should investigate the impacts of acupuncture on a minimum of 4 types of HRV variables, and experiment with not just distal acupuncture points but also back acupuncture points for the treatment of chronic conditions, as recommended by classical TCM theories. Building on the insights gained from Huang Di Nei Jing Ling Su, Su Wen, Nan Jing and modern scientific literatures, future research should prioritize designing their studies to test and record the outcomes of back (yang side) acupuncture points for treatment of chronic conditions (yin diseases). Since there is already plenty of qualitative or subjective data collected from acupuncture studies, such as questionnaire scores and patient feedback, outcome measurements should include and collect more quantitative or objective data, such as HRV and other numerical vital sign measurements, in order to contribute data of higher statistical power in acupuncture clinical research. Conclusively, this comprehensive examination of the existing literature laid the foundation for this Pilot Randomized Single-Blinded Active-Control Trial, on comparing the effectiveness of Back-shu and Psychic Aspect acupuncture, and Distal acupuncture for the intervention of chronic disease of Toxic Stress and ACE-Associated Health Conditions (AAHCs).

II. MATERIALS AND METHODS

2.1 Materials

Acupuncture Needles (Single-use, Sterilized, Stainless Steel):

***For Experimental Group (EG) with Back Acupuncture**:

Needle Size and Length: 0.30mm gauge, 1 inch needles

Needle Depth and Angle: 0.5-0.8 inches obliquely

Manufacturer: DBC

Quantity: 20 needles per treatment, 1 treatment per week for 12 weeks, 240 needles total per participant needed

***For Active-Control Group (CG) with Traditional Distal 'ML-10' Acupuncture**:

Needle Size and Length: 0.25mm gauge, 0.5 inch needles

Needle Depth and Angle: less than 0.4 inch perpendicularly/obliquely

Manufacturer: DBC

Quantity: 10 needles per treatment, 1 treatment per week for 12 weeks, 120 needles total per participant needed

Electrocardiogram (EKG) equipment:

3-Lead ECG device consists of one positive for the left inner wrist, one negative for right inner wrist, and one ground for left medial ankle.

Heart Rate Variability (HRV) Software Application:

Extracts the EKG's electric conductivity readings for normal R-R intervals to generate time-domain HRV data, and then applies Fast Fourier Transform 'FFT' algorithm for frequent-domain HRV data. This software reads four 'time-domain' HRV measurements (RMSSD, SDNN, pNN50, pNN20), and three 'frequency-domain' HRV measurements (LF power, HF power, LF/HF ratio).

Manufacturer: HYS

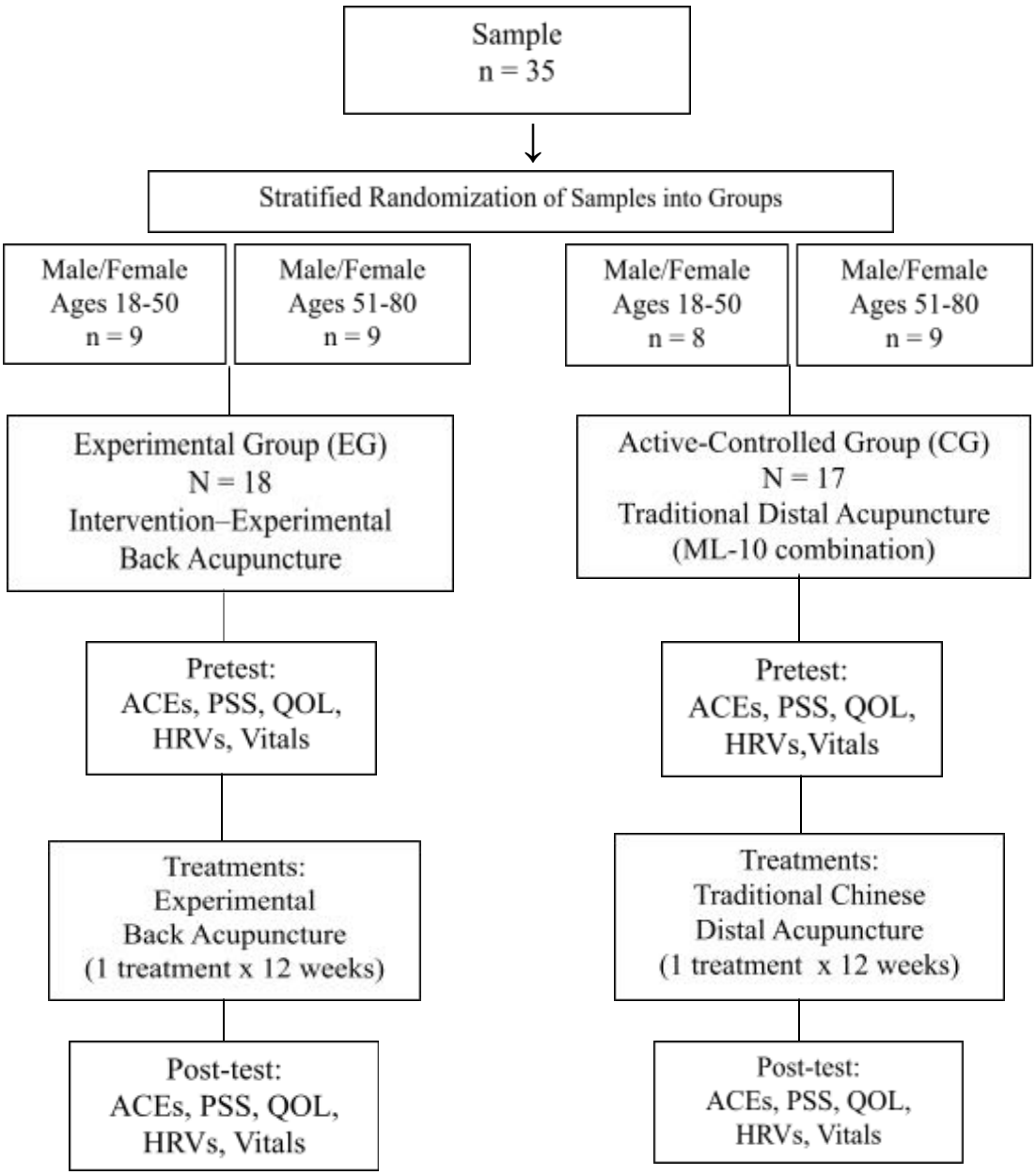
EKG Leads/Electrodes/Pads: 3 pads designated per participant for all 12 weeks of recordings. Manufacturer: 3M
Sphygmomanometer Blood Pressure Monitor, and Stethoscope: For weekly Vital Signs Intake. Manufacturers: GreaterGood and MDF
Treatment Supplies: Treatment bed, paper, Clean Needle Technique (CNT) Supplies Kit.
Questionnaires: Adverse Childhood Experiences (ACEs) Screening, by Felitti et al, 1998. Perceived Stress Scale (PSS-10), by Colen et al, 1983. Quality of Life Assessment (WHOQOL-BREF), by World Health Organization, 2012. See Appendix 2.
Google Forms and Sheets: Google Forms, used to create and collect questionnaire answers from participants. Google Sheets, used to record weekly data and intakes.

2.2 Methods - Study Design

This pilot randomized single blind active-controlled trial applied Experimental Back Acupuncture, and compared its effectiveness in improving heart rate variability (HRVs) with Active-control Traditional Distal Acupuncture, on a weekly basis for twelve weeks. Participants' inclusion criterias: adults, ages 18–80, who scored a 1 or higher on Adverse Childhood Experiences (ACE) Questionnaire and scored a 27 or higher on Perceived Stress Scale (PSS), and exclusion criterias, all needed to be met to qualified for enrollment. The investigator blinded and randomly assigned each participant (n=35) to either the Experimental 'EG' Group (n=18) or the Active-control 'CG' group (n=17), with a fairly even distribution of females, males, ages 18-50 and ages 51-80 in both groups.

Both groups' participants received acupuncture treatments. EG participants receive the Experimental Back Acupuncture, consisting of 10 bilateral acupuncture points along the posterior spine, 20 needles total inserted once per week for 12 weeks. CG participants received the Active-control Traditional Distal Acupuncture, consisting of 5 bilateral points on arms and legs, 10 needles total inserted once per week for 12 weeks.

For all participants' measurements: ACEs Questionnaire Scores were only collected once in their initial screening. Perceived Stress Scale (PSS) Scores and Quality of Life (QOL) Scores were collected three times throughout treatments. HRV variables (RMSSD, SDNN, pNN20, pNN50, LF, HF, LF/HF) were measured once per week for twelve consecutive weeks. Additionally, vital signs (i.e. blood pressure, heart rate, respiratory rate, tongue, pulse) were monitored weekly for participants' safety throughout treatments. After all participants completed the 12th treatments, HRV and questionnaire data were analyzed.



*ACEs : Adverse Childhood Experiences (10 - question scale)

*PSS: Perceived Stress Scale (10 - question scale)

*QOL / WHOQOL-BREF : World Health Organization Quality of Life - Brief Version (26 - questions)

*HRVs : Heart Rate Variability (RMSSD, SDNN, pNN20, pNN50, LF, HF, LF/HF)

*Vitals (for safety monitoring): blood pressure, heart rate, respiratory rate, TCM pulse & tongue

Figure 9. Study Design

2.3 Methods - Participants

Advertisements, via word of mouth, electronic mail and Facebook, were shared to Southern California residents and communities, including university campuses, community centers, public libraries, athletics centers, gyms, churches, behavior therapist offices, acupuncture clinics, and local businesses.

2.3.1. Inclusion Criteria

- Between ages 18 to 80, AND
- Met the initial screening scores from both Questionnaires:
    - Scoring 1 or higher on ACEs Screening, AND
    - Scoring 27 or higher on PSS Questionnaire for Stress Severity, AND
- Completed and submitted Consent Forms

2.3.2. Exclusion Criterias

- Minors
- Inability to consent
- Serious non-stable medical illness
- Pacemaker or other implantable electronic device
- Pregnant, or planning to be pregnant for next 3-6 months,
- Has excessive coughing, excessive uncontrollable body movements, or cannot sit still during ECG/HRV recording process
- Active suicidal risk, self-injury, or aggression towards others within the past year
- Unlikely to Complete Follow-up due to moving, time or schedule conflicts
- Undergoing other acupuncture treatments

- Unable to access email and internet (if not, then the participant needs to come early before their appointment times to use investigator's computers/internet);
- Not literate in either English/Spanish/Mandarin/Cantonese, (if not, then the participant needs to bring their translator/interpreter during appointment visits.)

2.3.3. Randomization and Blinding Procedures

- **Randomization:** A stratified randomization method was applied, which intended for a fairly equal distribution of females, males, ages 18-50 and ages 51-80 in the two groups: experimental group (EG) and active-control group (CG). Participants were blinded and assigned a Participant Identification Number (Participant ID) from 23001 to 23038 randomly, which also coincided with the order the participants enrolled. Participants with odd number IDs were assigned to EG in their respective gender and age blocks; and even number IDs were assigned to the CG in their respective gender and age blocks.

- **Blinding:** All participants were 'unmasked' during acupuncture treatments, but they were all blinded from knowing if they've been assigned to EG or CG. Since this was a pilot trial and participant numbers were uncertain, the investigator accepted on-going enrollment at different treatment start-times. Although the investigator was unblinded throughout the participants' treatments, however, the investigator was partially blinded from the weekly HRV variable measurements until every participant completed the 12th treatment.

2.3.4. Sample Size

Due to the challenges of pandemic restrictions, time constraints, economical barriers and unpredictable situations, this clinical trial was only able to enroll 38 participants (n=38) initially, and finished with 35 total participants (n=35), instead of the recommended sample size estimation of 62 participants. Participants consisted of university students, athletes, teachers, clerks, engineers, artists, healthcare professionals, business owners, retired seniors, all residing in Los Angeles, Orange, San Bernardino and/or Riverside counties.

2.4 Methods - Acupuncture Treatment Protocols

All acupuncture points in this study are well documented acupuncture points from the 2009's Chinese Acupuncture and Moxibustion (CAM) 3rd edition textbook, commonly used by most Eastern Medicine universities in the United States.[97] All of the experimental group (EG) and active-control group (CG) participants received acupuncture treatments, once per week for twelve consecutive weeks. Each acupuncture appointment with research participants lasted approximately 60 to 70 minutes, which consisted of: 5 minutes of general patient intake, 2 to 5 minutes of acupuncture needle inserting time, 25 minutes of retention for acupuncture needles, 2 to 5 minutes of acupuncture needle removing time and ECG preparation time, and 20 minutes of ECG recording time for HRVs measurements.

2.4.1 Experimental Back Acupuncture (EG) Points

EG participants received the Experimental Back Acupuncture points combination, consisting of Back-shu and Psychic Aspect points, located bilaterally on the posterior spine. EG points were UB-13, UB-15, UB-18, UB-20, UB-23, UB-42, UB-44, UB-47, UB-49 and UB-52.

2.4.2 Active-control Traditional Distal Acupuncture (CG) Points

CG participants received the Traditional Distal Acupuncture points combination, popularly known as Miriam Lee's 10-point combination (ML-10), located bilaterally on the arms and legs. CG points were ST36, SP6, LU7, LI4 and LI11.

Gray Boxes are the locations of Experimental Back acupuncture point combination or Urinary Bladder (Bladder) channel points applied, consisting of the five Back-shu and the five Psychic Aspects points: (Ten points bilaterally = Twenty points)

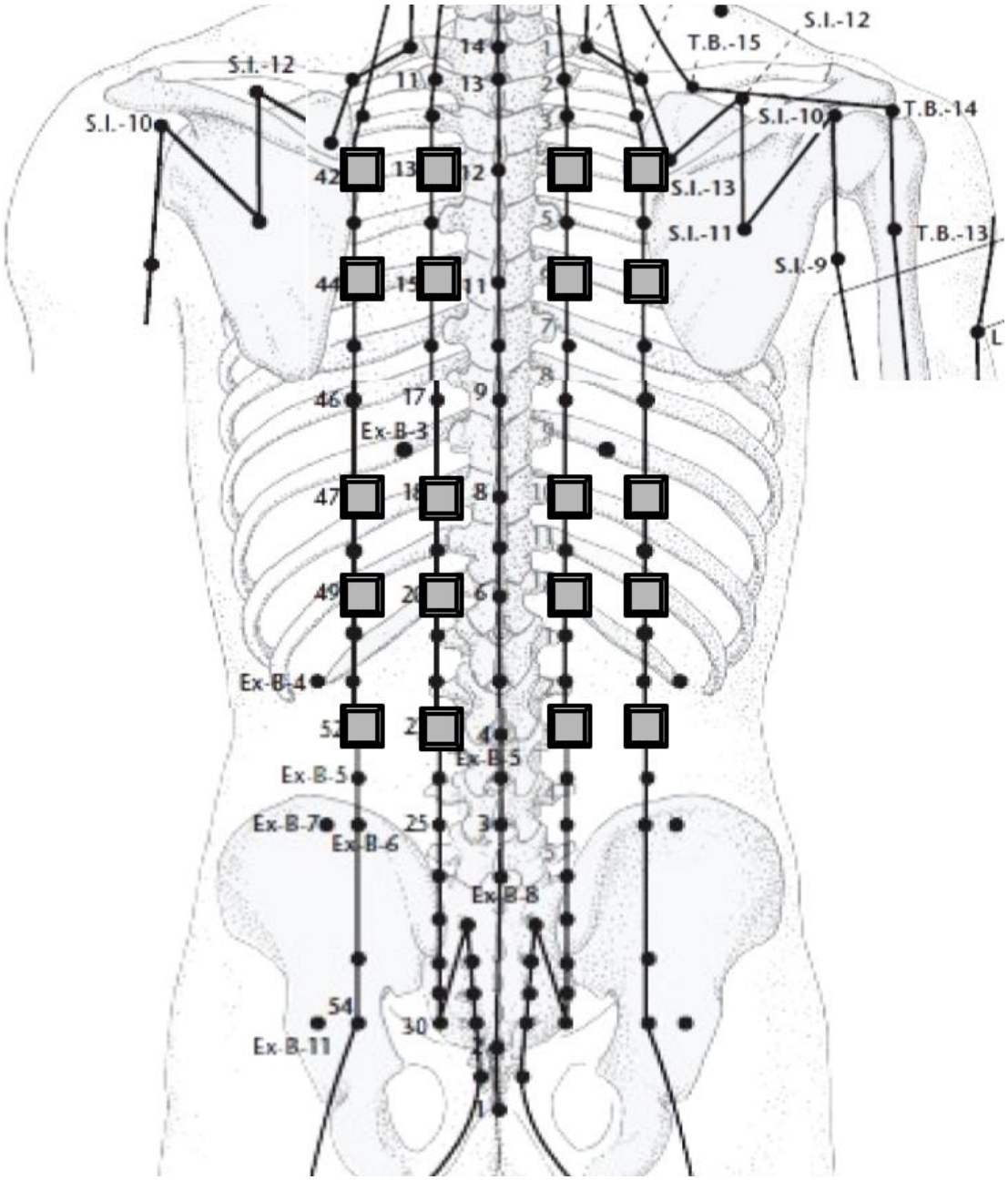


Figure 10. Visual Map of Experimental Back Acupuncture (EG)

Table 9 shares the Back-shu acupuncture points for EG treatments, and Table 10 shares the Psychic Aspect acupuncture points also for EG treatments; with acupuncture point names, location and indications.

Table 9. Back-Shu Acupuncture Points for Experimental Group (EG)

EG's Experimental Back Acupuncture Points (First Line)		
Points	Locations	Indications
UB13 **(Fei shu -** 肺俞 **)** Back-Shu point of the lung	1.5 cun lateral to the lower border of the spinous process of third thoracic vertebra (T3).	Cough, asthma, fullness of the chest, shortness of breath with no desire to speak, excessive grief, lung atrophy, consumption, mania, heat in the body
UB15 **(Xin shu -** 心俞 **)** Back-Shu point of the heart	1.5 cun lateral to the lower border of the spinous process of fifth thoracic vertebra (T5).	Heart pain, chest pain, palpitations, fright, palpitations, irregular pulse, Poor memory, anxiety, weeping with grief, insomnia, excessive dreaming
UB18 **(Gan shu -** 肝俞 **)** Back-Shu point of the Liver	1.5 cun lateral to the lower border of the spinous process of ninth thoracic vertebra (T9).	Distension and pain of the lateral costal region, epigastric pain, abdominal masses, focal distension, hypogastric fullness and pain, jaundice, dry mouth, anger, blurred vision, eye redness, night blindness, excessive lacrimation, visual dizziness, pain of the supraorbital region.
UB20 **(Pi shu -** 脾俞 **)** Back-Shu point of the Spleen	1.5 cun lateral to the lower border of the spinous process of 11th thoracic vertebra (T11).	Distension and pain of the abdomen, focal distension, abdominal masses, lack of appetite, undigested food in the stools, diarrhea, dysentery, nutritional impairment
UB23 **(Shen shu -** 腎俞**)** Back-Shu point of the kidneys	1.5 cun lateral to the lower border of the spinous process of the second lumbar vertebra (L2).	Pain, soreness and/or cold sensation of the lumbar region and knees, cold legs, hot and cold sensations of bones, stroke, hemiplegia, edema, difficult urination, enuresis, frequent urination, dripping urination, blood in the urine, acute hypogastric pain, irregular menstruation, leucorrhoea, deafness, tinnitus, dizziness, night blindness

Table 10. Psychic Aspect Acupuncture Points for Experimental Group (EG)

EG's Experimental Back Acupuncture Points (Second Line)		
Points	Location	Indications
UB-42 **(Po hu -** 魄戶 **)** Door of Corporeal Soul	3 cun lateral to the midline, level with the lower border of the spinous process of the third thoracic vertebra (T3) and level with Feishu UB-13.	Excessive grief, inability to express grief from trauma, Lung atrophy, Lung consumption, cough, asthma, Pain of the shoulder, scapula, back, chest, back, neck, Vomiting with agitation and fullness, loss of consciousness.
UB-44 **(Shen tang -** 神堂 **)** Hall of the Spirit	3 cun lateral to the midline, level with the lower border of the spinous process of the fifth thoracic vertebra (T5) and level with Xinshu UB-15.	Excessive joy, inability to express joy, cough, asthma, dyspnoea, fullness of the chest with rebellious qi, stiffness and pain of the back shoulder radiating to the chest, esophageal constriction, headache, intrascapular pain
UB-47 **(Hunmen -** 魂門 **)** Gate of Ethereal Soul	3 cun lateral to the midline, level with the lower border of the spinous process of the ninth thoracic vertebra (T9) and level with Ganshu UB-18.	Fullness and distension of the chest and lateral costal region, back pain, contraction of the sinews, bone and joint pain of the whole body, Anger, resentment, lack of sense of purpose in life, depression, drugs or alcohol abuse issues.
UB-49 **(Yishe -** 意舍 **)** Abode of Thought	3 cun lateral to the midline, level with the lower border of the spinous process of the eleventh thoracic vertebra (T11) and level with Pishu UB-20.	Distension and fullness of the abdomen, distension and pain of the chest and lateral costal region, slippery diarrhea, difficult ingestion, vomiting, heat in the body with yellow face and eyes, reddish-yellow urine, Inability to think or focus, obsessive thoughts, overthinking, eating disorders
UB-52 **(Zhi shi -** 志室**)** Residence of Will	3 cun lateral to the midline, level with the lower border of the spinous process of the second lumbar vertebra (L2) and level with Shenshu UB-23.	Excessive fear, lack of willpower, sense of hopelessness, weakness from overwork, Lumbar pain and stiffness, back pain, dribbling urination, difficult urination, oedema, impotence, premature ejaculation, pain of the genitals, fullness and pain of the lateral costal region, vomiting, difficult defecation, hardness of the abdomen and hypogastrium.

Black squares are the locations of Traditional distal acupuncture points or ML-10 protocol combination applied on CG participants: Five points bilaterally = Ten points

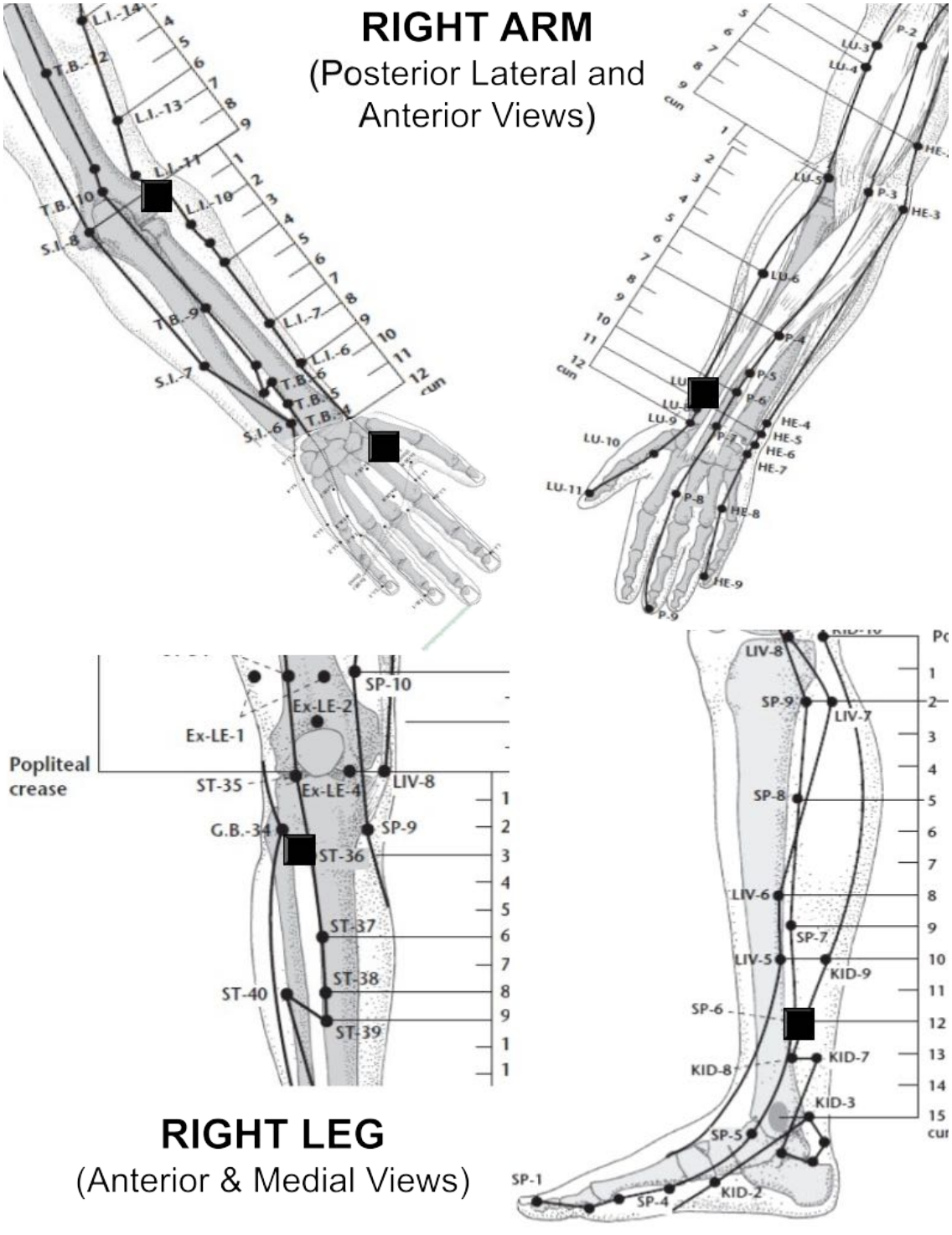


Figure 11. Visual Map of Active-Control Traditional Distal Acupuncture (CG)

Table 11 shares the traditional distal acupuncture for CG; with acupuncture point names, locations and indications.

Table 11. Traditional Distal Acupuncture Points for Active-control Group (CG)

CG's Traditional Distal Acupuncture Points		
Points	Locations	Indications
ST36 **(Zu san li -** 足三里**)** Leg Three Miles	Below the knee, 3 cun inferior to ST-35 Du Bi, one fingerbreadth lateral to the anterior crest of the tibia.	Epigastric pain, nausea, vomiting, hiccup, pain of the abdomen, fullness and distension, propensity to hunger, hunger without desire to eat, poor appetite, difficult ingestion, diarrhea, dysentery, undigested food in the stool, cold in the intestines, palpitations, hypertension, anger and fright, tendency to sadness, outrageous laughter, agitation, forehead headache, pain of the knee and shin, pain of the brain, pain of the lateral costal region, pain of the thigh and shin, hemiplegia, muscle pain, chronic pain.
SP6 **San yin jiao** 三陰交 Three Yin Intersection	On the medial side of the lower leg, 3 cun superior to the prominence of the medial malleolus, in a depression close to the medial crest of the tibia.	Spleen and Stomach deficiency, heavy body with heaviness of the four limbs, edema, diarrhea, undigested food in the stool, abdominal distension, irregular menstruation, uterine bleeding, menorrhagia, amenorrhoea, dysmenorrhoea, abdominal masses in women, leucorrhoea, uterine prolapse, infertility, impotence, difficult urination, enuresis, palpitations, insomnia, blurred vision, tinnitus, hypertension, leg pain, hemiplegia, heat in the soles of the feet, shin pain, eczema, urticaria, counterflow cold of the foot and hand.
LU7 **(Li que -** 列缺 **)** Broken Sequence, Luo-Connecting	On the radial aspect of the forearm, approximately 1.5 cun proximal to Yang Xi L.I.-5, in the cleft	Chills and fever, nasal congestion and discharge, cough, asthma, diminished qi and shortness of breath, Headache and stiffness of the neck and nape, one-sided headache,

point, Confluent point of the Ren channel	between the tendons of brachioradialis and abductor pollicis longus.	lockjaw, hemiplegia, deviation of the mouth and eye, toothache, epilepsy, loss of consciousness, vomiting of saliva, hypertension, Retention of fetus, postpartum inability to speak, Poor memory, palpitations, propensity to laughter, tension of the chest and back, fullness of the lateral costal region, breast abscess, Weakness or pain of the wrist and hand, pain of the thumb, shoulder pain, heat in the palm, Malarial.
LI4 **(He gu -** 合谷 **)** Joining Valley, Yuan-Source point	On the dorsum of the hand, between the first and second metacarpal bones, at the midpoint of the second metacarpal bone and close to its radial border	Chills and fever, febrile disease, Malarial, Headache, hypertension, pain of the eyes, dimness of vision, Nosebleed, nasal congestion, rhinitis,Toothache, mouth ulcers,mouth motor control, mumps, loss of voice, Swelling of the face, deviation of the face and mouth, lockjaw, deafness, tinnitus. Amenorrhoea, prolonged labor, delayed labor, mania, hemiplegia, pain of the sinews, bones, arm, fingers, lumbar spine.
LI11 **(Quchi -** 曲池 **)** Pool at the Crook	At the elbow, midway between Chize LU-5 and the lateral epicondyle of the humerus at the lateral end of the transverse cubital crease	High fever, Malarial, toothache, redness and pain of the eyes, lacrimation, pain of the ear, Agitation and oppression of the chest, manic disorders, poor memory, tongue thrusting, dizziness, hypertension, goiter, scrofula, urticaria, wind rash, dry skin, scaly skin, itching of the skin, shingles, pain and itching of the whole body from insects, pain of the abdomen, vomiting and diarrhea, Dysentery, amenorrhoea, Numbness of the upper arm, hemiplegia, clonic spasm, contraction, immobility and pain of the elbow and shoulder, emaciation and weakness of the elbow, atrophy disorder of the lower limbs, pain and swelling of the ankle.

2.4.3. Methods - Dosage Modification/Adverse Effects

According to statistics published from the Council of Colleges of Acupuncture and Herbal Medicine (CCAHM), acupuncture performed by trained and licensed acupuncturist practitioners using Clean Needle Technique is generally safe, and has less than 4.5% chance of adverse effects. During acupuncture treatment, the possible adverse effects include: fainting, stuck needle, bent needle, broken needle, hematoma, bruising, and/or post-treatment-effects, such as soreness after withdrawal of the needle which may persist for a period of time. In case of adverse effects, the participant/patients were instructed to report the adverse effect(s) to the investigator/acupuncturist immediately, and the investigator should follow the safety actions instructed in Clean Needle Technique (CNT) Manual 7th Edition.[113] If the adverse effect were serious, the investigator needed to file a Serious Adverse Events (SAEs) form to the IRB of South Baylo University, and submit an incident report to the CA Acupuncture Board.

2.5 Methods - Measurements of Outcome Variables

The primary outcome variables measured in this trial are Heart Rate Variability (HRV) variables, and the additional variables collected are considered the secondary outcome variables (PSS and QOL questionnaire scores). HRV variables (RMSSD, SDNN, pNN20, pNN50, LF, HF, LF/HF) were measured once per week for twelve consecutive weeks—where HRVs were measured before treatments for 1st treatment, and the following HRVs measured after treatments for 2nd to 12th treatments. Perceived Stress Scale (PSS) Scores and Quality of Life (QOL) Scores were collected three times throughout treatments (before 1st treatment at initial screening, after 6th treatment and after final 12th treatment). Additionally, vital signs (i.e. blood pressure, heart rate, respiratory rate, tongue, pulse) were monitored weekly for participants' safety throughout treatments.

2.5.1. Primary Outcome Variables : Seven HRV Parameters

Heart Rate Variability (HRV) measures the relative balance between the sympathetic and parasympathetic systems. Poor HRV, usually low, means a lack of fluctuation in heart rate in response to breathing, contributing to negative effects on thinking and feeling, along with how our body responds to stress. Lack of coherence between breathing and heart rate makes people vulnerable to a variety of physical and mental illnesses. Each day, an increase in HRV is not always 'good' and a decrease is not always 'bad,' hence it's important to not focus on specific numerical valves, instead what should be focused on is the overall fluctuations in heart rate in response to breathing. Generally, there are over 60 parameters of HRV types that have been measured for previous HRV clinical studies combined. For this trial, seven parameters of HRV were

collected from each participant, once per week for 12 weeks, in order to determine the overall HRV progress sufficiently. The seven of the most researched HRV variables are the four time-domain HRV measurements (RMSSD, SDNN, pNN50, pNN20) and three frequency-domain HRVs (LF power, HF power, LF/HF ratio).

Table 12. Seven Heart Rate Variability (HRV) Variables Measured and Their Indications

Heart Rate Variability (HRVs)				
HRV Categories	**HRV Variables**	**Physiological Significance**	**Normal**	**Abnormal**
TIME-DOMAIN HRV	**RMSSD** (ms): Root mean square of successive RR interval differences	Parasympathetic-Vague tone, Respiratory Sinus Arrhythmia	35 ms to 107 ms	Below 35 ms, or Above 107 ms
	SDNN (ms): Standard deviation of NN intervals	Sympathetic-Vagal tone	100 ms to 180 ms	Below 100 ms, or Above 180 ms
	pNN20 (%): % of successive RR intervals that differ by more than 20 ms	Parasympathetic-Vague tone, Respiratory Sinus Arrhythmia	20% to 40%	Below 20%, or Above 40%
	pNN50 (%): % of successive RR intervals that differ by more than 50 ms	Parasympathetic-Vague tone, Respiratory Sinus Arrhythmia	5% to 30 %	Below 5%, or Above 30%
FREQUENCY-DOMAIN HRV	**LF Power** (ms^2) : Absolute power of low frequency band (0.04 - 0.15 Hz)	Sympathetic-Vagal tone; Baroreflex, Vasomotor	$650ms^2$ to $1500ms^2$	Below $650ms^2$, or Above $1500\ ms^2$
	HF Power (ms^2) : Absolute power of high frequency band (0.15 - 0.4 Hz)	Parasympathetic-Vague tone, Respiratory Sinus Arrhythmia	$220\ ms^2$ to $1200ms^2$	Below $220ms^2$ or Above $1200\ ms^2$
	LF/HF (ratio) : Ratio of LF Power to HF Power	Parasympathetic or Sympathetic predominance	Between 1 to 2	Below 1, or Above 2

2.5.1.1. How were the HRVs calculated?

There are two primary ways to collect HRV datas—with photoplethysmography (PPG) or electrocardiogram (ECG or EKG).This clinical trial used an ECG device to determine HRV measurements. Using ECG for HRV can collect P-Q-R-S-T waves, and using HRV software can detect the irregular heart rate, remove those irregular waveform segments from HRV calculations and filter out noise that may present falsely high HRV measurements, commonly found in HRV readings from PPG devices (such as smartwatches). In Figure 12 is an image of the combined ECG and HRV software used on the participants weekly to collect all seven HRV parameters: four time-domain HRVs (RMSSD, SDNN, pNN20, pNN50) and three frequency-domain HRVs (LF Power, HF Power, LF/HF ratio) .

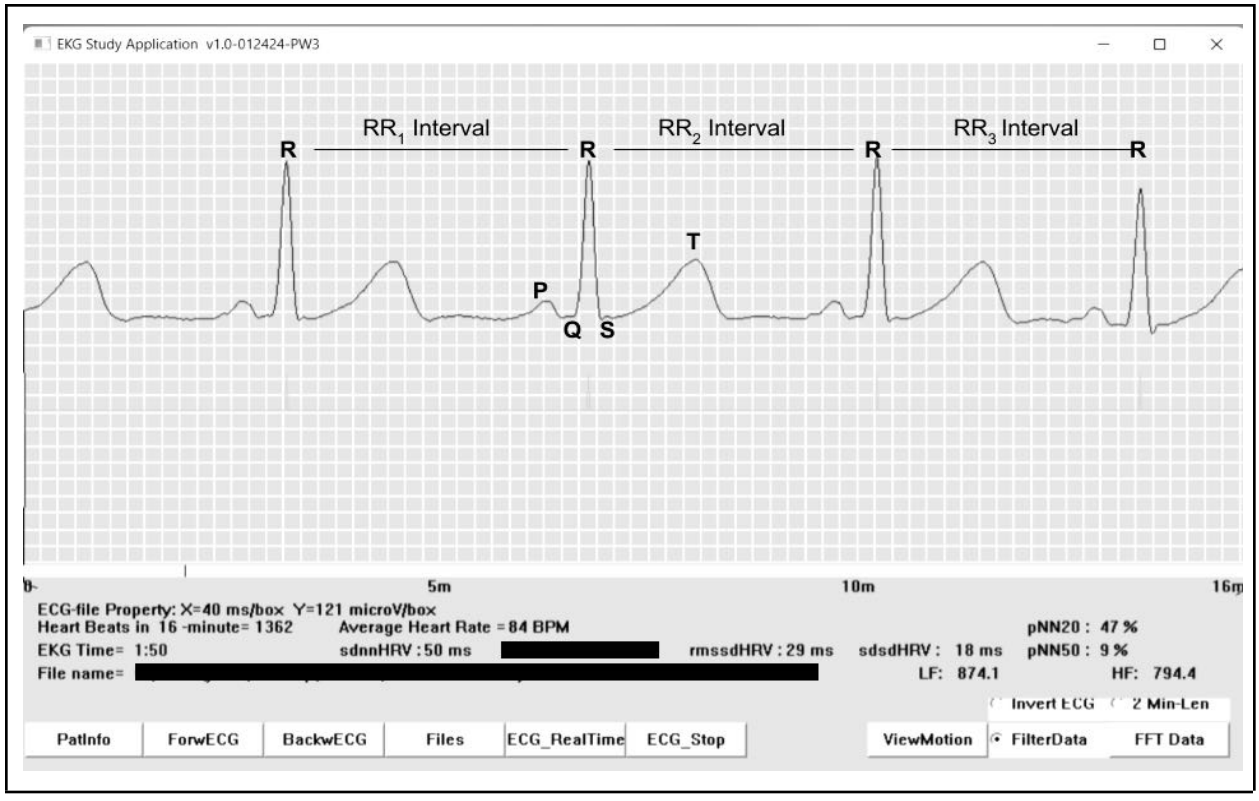


Figure 12. Electrocardiogram Study Application Software for HRVs

Participants have their ECG data recorded and HRV calculated via the software's assistance. When viewing the ECG files after each participants' recordings, click on the 'ViewMotion' button, and the application will automatically find the ECG segments with the least amount of noise and with normal sinus rhythms out of each of the 20-minute total recordings. For calculating time-domain HRVs measurements (RMSSD, SDNN, pNN20 and pNN50): **RMSSD** calculations take the root mean square data of successive R–R interval differences in a 5-minute raw ECG segment, while **SDNN** calculations take the standard deviation data of the normal R–R intervals from a 15-minute segment. Also, for **pNN20** calculations, the software finds the percent of successive R–R intervals within the same 5-minute segment that differ by at least 20 ms; and similarly for **pNN50** calculations, the software finds the percent of successive R–R intervals within the same 5-minute segment that differ by at least 50 ms. See Figure 13. For calculating frequency-domain HRVs measurements (LF Power, HF power and LF/HF Power): the software further converts the P-Q-R-S-T time-domain waveform (ms) to frequency-domain waveform (ms^2) using an advance computational tool called Fast Fourier Transform (FFT). Applying FFT to the time-domain waveform generates the Power Density Spectra (PSD) or (ms^2/Hz). For **LF Power** (ms^2) calculations, the software applies a low frequency filter of 0.04–0.15 Hz to the PSD (ms^2/Hz) generated to determine the Low Frequency Power (ms^2) HRV measurement. For **HF Power** (ms^2) calculations, the software applies a higher frequency filter of 0.15–0.4 Hz to the PSD (ms^2/Hz) generated to determine the High Frequency Power (ms^2) HRV measurement. **LF/HF ratio** calculations are done manually dividing the LF power by HR power values, resulting in a ratio of less than 1, exactly 1 or greater than 1.

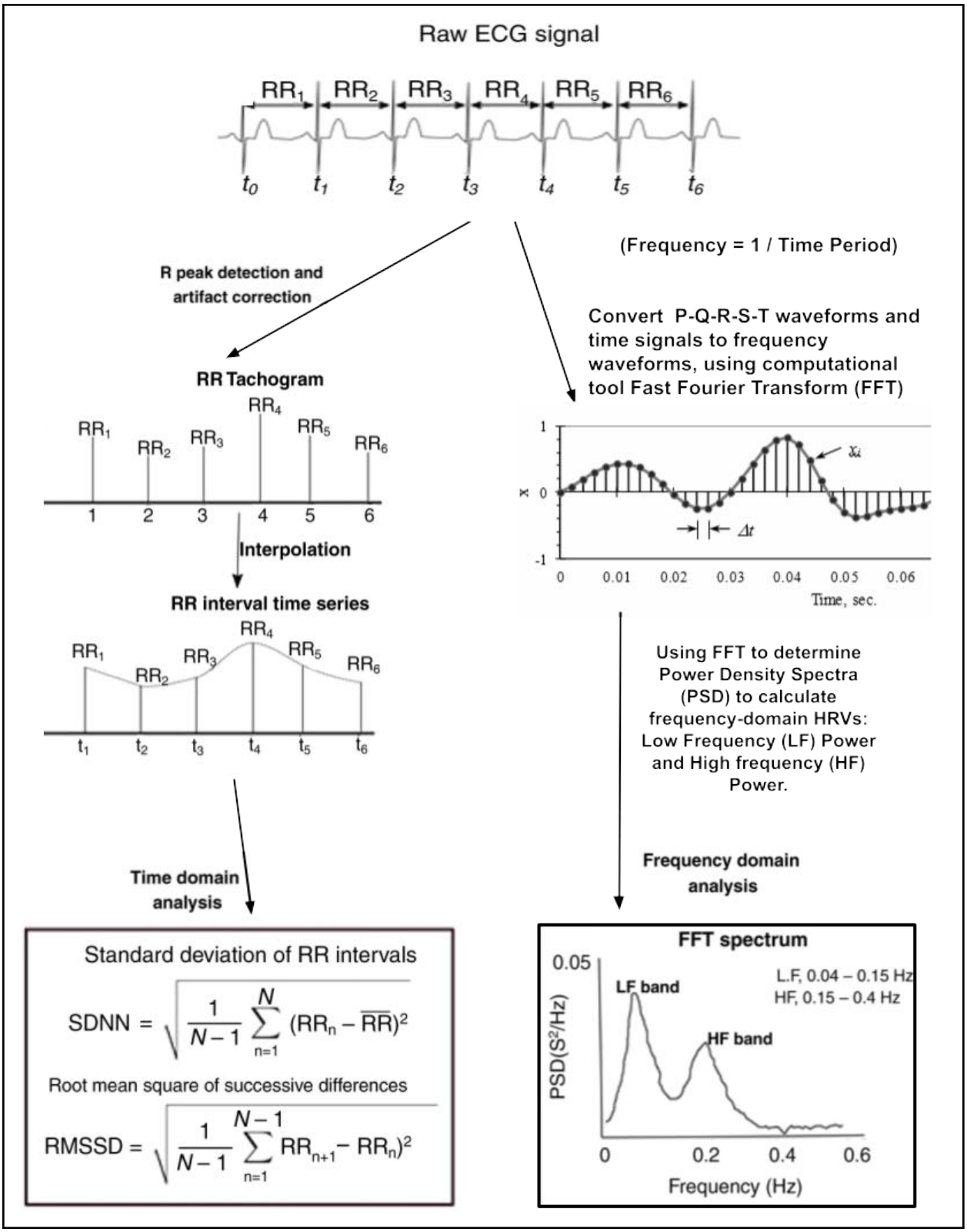


Figure 13. Calculations of Time-Domain and Frequency-Domain HRVs from ECG Data

2.5.1.2. Potential Confounding Variables

Factors that can influence HRV measurements are caffeine, heart medications, alcohol, exercise, food intake, time of day, water intake, and urinary bladder fullness. Hence, it's important to advise participants to avoid coffee and large meals, 3 hours before HRV recordings. Avoid drinking more than 1 cup of water 2 hours before and use the restroom before HRV recordings. Alcohol should be avoided 2 days before HRV recordings. Since time of day during HRV recording, it was best to schedule participants to visit on the same day and time every week for treatment and HRV recording. Medications do affect HRVs, however for safety, participants should continue to take their prescribed medications as prescribed by their medical provider. With instructions for participants to follow before weekly visit, and with consistent settings and procedures for the investigator to follow to record participant's weekly HRVs, this intended to reduce dramatic fluctuation in HRV readings.[13, 110]

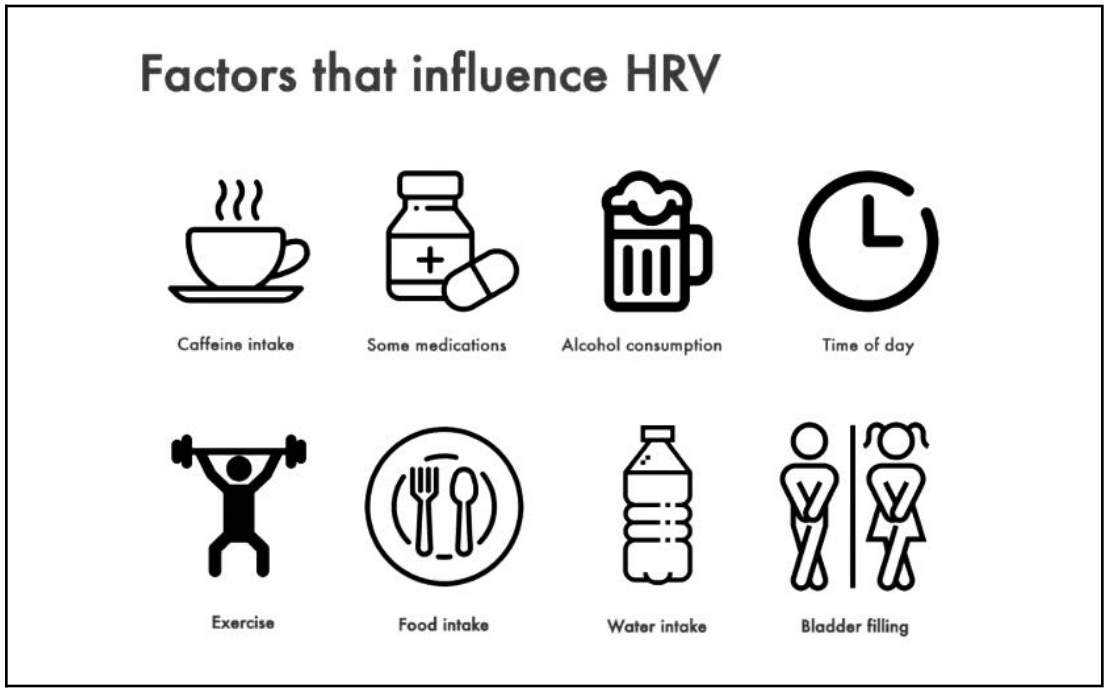


Figure 14. Potential Confounder Variables in HRV studies [110]

2.5.2. Secondary Outcome Variables: Two Questionnaire Scores

- **PSS-10 / PSS :** Perceived Stress Scale Scores is determined with 10 questions, ranging from 0 to 40. Scoring 0 to 13 is low stress; Scoring 14 to 26 is moderate stress; Scoring 27 to 40 is high perceived stress. See Appendix B for the full PSS Questionnaire.[111]

- **WHOQOL-BREF / QOL :** World Health Organization Quality of Life - Brief Version Scores, are percentage scores determined by 26 questions that examine individuals' evaluations on their physical health, psychology, social relationships and environments. Scores range from 0% being the worst possible state of health, to 100% points being the best possible state of health. See Appendix B for the full WHOQOL-BREF or QOL Questionnaire.[112]

- **Vital Signs:** As a way to monitor for safety and adverse effects, blood pressure, heart rate, respiratory rate, TCM pulse and tongue readings were also collected before every acupuncture treatment. Also, participant's subjective reportings of signs and symptoms from their ACE-Associated Health Conditions–AAHCs (such as, abnormal pain, sleep, appetite, energy level, bowel movements, urination, etc.) experienced were asked and noted. These Vital Signs were not part of the hypotheses, but they were important to monitor for the general health and safety of the participants during acupuncture trials. See Appendix C for Case Report Forms (CRFs).

2.6. Methods—Data Management

2.6.1. Data Recording

The investigator used a modified SOAP-Notes Patient Intake Form and Participants' Data Collection Form as the Case Report Forms (CRF) to manage this clinical trial's data. See Appendix C. CRFs are protected by the principal investigator of this study. Before and during the 12-week treatment period, only the participant's initial Perceived Stress (PSS) score, Adverse Childhood Experience (ACE) screening score, ACE-Associated Health Condition(s) or AAHCs symptoms, AAHCs onset, and weekly vital signs (blood pressure, heart rate, TCM tongue and pulse, symptoms) were recorded onto the CRFs. Throughout the trial, all the participant's raw EKG data for HRV calculation were recorded and stored on a designated laptop and backup drive, used only for conducting this research project. After final treatment was completed, before Data Analysis could be conducted, the investigator performed a Data Extraction Step, in order to calculate and record the questionnaire scores and HRVs values onto the CRFs.

2.6.2. Data Extraction Step and Partial Blinding

In order to reduce bias during the treatments, the Investigator applied a Data Extraction step, by being blinded partially until the end of acupuncture trial from knowing the Primary outcome measurements (seven HRVs–RMSSD, SDNN, pNN20, pNN50, LF power, HF power and LF/HF) and Secondary outcome measurements (PSS and QOL scores). This was possible because while the investigator was closely monitoring and recording the participants' electrocardiogram (ECG) waveform data, the investigator could not see the participants' HRV data during the ECG recordings. HRV data calculations could only be revealed when reopening the ECG files after the ECG

recordings were completed. In this study, the investigator did not reopen or extract the HRV data calculations until after all participants completed the 12th treatment. Another part of the Data Extraction step was to revisit the answers from questionnaire forms (PSS and QOL) completed by the participants from after 6th and 12th treatments, and input their answers into the questionnaires' formulas, in order to calculate the PSS and QOF scores for each of the participants.

Investigator was not blinded from the initial ACE screenings, the initial PSS questionnaires and the weekly Vital Sign data. Reasons were (1) because investigator needed to determine if participants qualified for the inclusion criterias from the ACEs and PSS questionnaires; and (2) because the Investigator needed to monitor the participant's weekly vital signs (heart rate, blood pressure, TCM tongue/pulse) in case of any adverse side effects or health complications arise during the 12-weeks acupuncture treatment period. If so, the investigator needed to report to proper authorities and send the patient to urgent/emergency care.

2.6.3. Data Storage and Data Integrity

A designated password protected computer laptop was assigned to run the ECG/HRV Software. The investigator collected and recorded the participants' weekly vital signs, HRVs, questionnaire scores onto CRFs in Google Forms and Google Sheets applications. A designated Google email account with HIPAA compliance was used for this trial's data and participant management. Two-step verification access was activated for the account's patient information safety and security. Any changes could be tracked by CRF to ensure the accuracy of the data. Also, the investigator backuped ECG data on a

weekly basis to ensure the data collected was not lost in case the laptop was ever lost/damaged.

2.6.4. Statistical Data Analysis Procedure

For initial homogeneity tests, statistical analysis was performed using the formula input functions on Google Sheets, and verified using calculators on SocSciStatistics.com. Fisher's Exact Tests and Chi-square Tests were performed to determine whether there were significant differences in the groups' participants' general characteristics from the beginning. Also, Chi-Square Tests were performed to determine whether there were significant differences in the groups' participants' initial variables before any acupuncture treatment.

For outcome variables, statistical analysis and graphs were performed using the R program. R version 4.3.1 (2023-06-16) -- "Beagle Scouts" Copyright ©2023 The R Foundation for Statistical Computing. A paired-sample t-test was performed to determine the significance of differences within groups, and the Mann-Whitney U test was performed to determine the significance of differences between groups. At a 95% confidence level, a p-value < 0.05 is considered to be a statistically significant difference. After the final treatment, heterogeneity tests on the final variables were also performed using Chi-Square Tests, to show a categorical comparison on final variables, in comparison to the initial variables.

2.7. Methods–Ethical Considerations

This research follows the Declaration of Helsinki, and complies with Common Rule in the Belmont Report established in 1978. The proposal for this study was submitted to the Institutional Review Board at the South Baylo University in September 2023 for review and validation. This research project includes the participants' Informed Consent Forms, see Appendix A. Potential participants and enrolled participants were all given the opportunity to read the consent documents fully, and ask questions about anything they did not understand. All participants were given the following information:

- Purpose and objective of the trial
- How long it is expected to take
- All procedures and tests that would be completed during the enrollment into the trial
- How participant's information would be kept private during trial and used for future research publications
- Whether any compensation or medical treatment will be available if injury occurs, or where that info will be found
- The research participant's right, such as the right to refuse treatment or stop in clinical trials at any time.
- How adverse events will be reported, following Council of Colleges of Acupuncture and Herbal Medicine (CCAHM) and CA Acupuncture Board guidelines.
- Data Management process. Refer to the Data Management section regarding Participant Data handling, access, storage and integrity.

III. RESULTS

3.1. Overview of Data Collected

3.2. Homogeneity Tests

3.2.1. Homogeneity Tests for Participants' General Characteristics
Gender, Age, ACEs Score, AAHCs, Onset

3.2.2. Homogeneity Tests for Variables Before (Initial) Treatment
PSS, QOL, RMSSD, SDNN, pNN20, pNN50, LF, HF, LF/HF

3.3. RMSSD

3.3.1. RMSSD Change throughout Treatment
3.3.2. RMSSD Difference throughout Treatment

3.4. SDNN

3.4.1. SDNN Change throughout Treatment
3.4.2. SDNN Difference throughout Treatment

3.5. pNN20

3.5.1. pNN20 Change throughout Treatment
3.5.2. pNN20 Difference throughout Treatment

3.6. pNN50

3.6.1. pNN50 Change throughout Treatment
3.6.2. pNN50 Difference throughout Treatment

3.7. LF

3.7.1. LF Change throughout Treatment
3.7.2. LF Difference throughout Treatment

3.8. HF

3.8.1. HF Change throughout Treatment
5.8.2. HF Difference throughout Treatment

3.9. LF/HF

3.9.1. LF/HF Change throughout Treatment
3.9.2. LF/HF Difference throughout Treatment

3.10. Perceived Stress Score (PSS)

3.10.1. PSS Change throughout Treatment
3.10.2. PSS Difference throughout Treatment

3.11. Quality of Life (QOL) Score

3.11.1. QOL Change throughout Treatment
3.11.2. QOL Difference throughout Treatment

3.12. Summary of Results

3.1. Overview of Data Collected

The following data collection in this chapter is to support this clinical trial's primary and secondary hypotheses. Nine outcome variables were collected from two acupuncture groups: Experimental Group 'EG' (n=18) and Active-Control Group 'CG' (n=17). The primary quantitative outcome variables collected were the seven Heart Rate Variability (HRVs): RMSSD, SDNN, pNN20, pNN50, LF Power, HF Power and LF/HF Ratio, that were measured weekly from 35 participants over the course of 12-weeks. The secondary quantitative variables collected were the Perceived Stress Scale (PSS) Scores and the Quality of Life (QOL) Scores, from the same 35 participants, before 1st treatment, after 6th and 12th treatments.

Carrying on, the first statistical tests presented are homogeneity tests results to illustrate whether the sample of participants selected in both groups were statistically significant to begin with, which can determine if this clinical trial's primary and secondary hypotheses were tested on groups with similar characteristics and variables from the beginning. Moreover, non-parametric and parametric statistical tests will be presented to reveal whether there were statistically significant changes between groups, differences within groups, and differences between groups. Throughout these tests, the *p*-value premises were as follows: if $p > 0.05$, then the data were not significantly different, while if the $p < 0.05$, then the data were significantly different.[114] Finally, in order to reveal the sizes of effect, Cohen's Distances (*d*) were also calculated, following Cohen's 1992 guidance, where: $d < 0.2$ are considered negligible, $d < 0.5$ are considered small, $d < 0.8$ are considered medium, and $d > 0.8$ are considered large.[115]

3.2. Homogeneity Tests

3.2.1. Homogeneity Tests for Participants' General Characteristics

Table 13 portrays the results of the homogeneity test conducted to compare the participants' general characteristics of the two groups: the Experimental Group (EG) and the Active-Control (CG). Fisher's Exact Tests and Chi-SquareTests were applied to test for homogeneity, in other words, whether there are significant differences between the groups' participants' general characteristics.

Table 13. **Homogeneity Tests for Participants' General Characteristics**

		EG (n=18)	**CG** (n=17)	***p*-value**	
Gender	Male	7	9		
	Female	11	8	0.505	*
Age	18-30	4	5		
	31-40	4	2		
	41-50	1	1		
	51-60	4	4		
	61-70	2	2		
	71-80	3	3	0.980	**
ACEs Score	1-2	2	4		
	3-5	8	8		
	6-10	8	5	0.515	**
AAHC(s)	0	0	0		
	1	4	3		
	>1	14	14	0.735	**
Onset	<1 year	0	1		
	≥1 year	18	16	0.486	*

*Fisher Exact Test
**Chi-square Test

- Characteristics: The left-hand column lists the participants' general characteristics or attributes— gender, age, ACEs Score, AAHCs and onset, that are being

examined to determine homogeneity between the two groups.

- EG: This column represents the Experiment Group with 18 total participants.
- CG: This column represents the Active-Control Group with 17 total participants.
- *p*-value: This column lists the *p*-values, used to determine whether there are any differences and statistical significance observed between the groups. If the *p*-value is greater than 0.05, then the null hypothesis is true and the alternative hypothesis is false. In the case of $p > 0.05$, there is no significant difference between EG and CG, and the treatments were performed on two groups with similar conditions.
- Gender: While in EG, there were 7 males and 11 females, in CG, there were 9 males and 8 females. The *p*-value of 0.505 is greater than 0.05, indicating that the groups have similar amounts of male and female participants.
- Age: In EG, there were 4 participants ages 18-30, 4 participants ages 31-40, 1 participant ages 41-50, 4 participants ages 51-60, 2 participants ages 61-70 and 3 participants ages 71-80. Similarly in CG, there were 5 participants ages 18-30, 2 participants ages 31-40, 1 participant ages 41-50, 4 participants ages 51-60, 2 participants ages 61-70 and 3 participants ages 71-80. The *p*-value of 0.980 is greater than 0.05, indicating that the groups have similar distribution of ages participating.
- ACEs Score: In EG, there were 2 participants with 1-2 adverse childhood experiences (ACEs), 8 participants with 3-4 ACEs, and 8 participants with 6-10 ACEs. In CG, there were 4 participants with 1-2 ACEs, 8 participants with 3-4 ACEs, and 5 participants with 6-10 ACEs. The *p*-value of 0.515 is greater than 0.05, suggesting that the groups have similar participants distribution numbers of

adverse childhood experiences.

- AAHCs: In both EG and CG, there were 0 participants with no ACE-Associated Health Conditions (AAHCs). In EG, there were 4 participants with 1 AAHC, and 14 participants with more than one AAHCs. In CG, there were 3 participants with 1 AAHC, and also14 participants with more than one AAHCs. The *p*-value of 0.735 is greater than 0.05, suggesting that the groups have similar participants distribution numbers of ACE-Associated Health Conditions.
- Onset: In EG, 0 participants had their onset of AAHCs for less than 1 year, instead, all 18 participants had their onset of AAHCs for a year or greater. In CG, 1 participant had their onset of AAHCs for less than 1 year, and the remaining 16 participants had their onset of AAHCs for a year or greater. The *p*-value of 0.486 is greater than 0.05, suggesting that the groups have similar onset characteristics.

3.2.2. Homogeneity Tests for Variables Before (Initial) Treatment

Table 14 shows homogeneity tests conducted to compare the participants' initial variable measurements before any treatments in the two groups: the Experimental Group (EG) and the Active-Control (CG). Chi Square Tests were applied to test for homogeneity, in other words, whether there were differences between the groups' participants' initial variables.

Table 14. **Homogeneity Tests for Variables Before (Initial) Treatment**

		EG n=18	**CG** n=17	*p*-value*
PSS	27-31	6	8	
	32-35	7	5	
	36-40	5	4	0.704
QOL	0-50	7	5	
	51-100	11	12	0.555
RMSSD	Low (0-34)	12	12	
	Normal (35-107)	4	5	
	High (108+)	2	0	0.353
SDNN	Low (0-99)	10	6	
	Normal (100-180)	7	9	
	High (181+)	1	2	0.459
pNN20	Low (0-19)	7	8	
	Normal (20-40)	8	4	
	High (41+)	3	5	0.392
pNN50	Low (0-4)	7	9	
	Normal (5-30)	8	5	
	High (31+)	3	3	0.633
LF	Low (0-649)	10	8	
	Normal (650-1500)	7	8	
	High (1501+)	1	1	0.878
HF	Low (0-219)	2	4	
	Normal (220-1200)	11	11	
	High (1201+)	5	2	0.382
LF/HF	Low (0-0.99)	9	9	
	Normal (1-2)	6	4	
	High (2.01+)	3	4	0.773

* Chi-Square Test

- Variables: The left-hand column lists the participants' initial variables measured—questionnaire scores: PSS and QOL, and Heart Rate Variability parameters: RMSSD, SDNN, pNN20, pNN50, LF, HF, and LF/HF.
- EG: This column represents the Experiment Group with 18 total participants.
- CG: This column represents the Active-Control Group with 17 total participants.
- *p*-valve: This column lists the *p*-values used to determine whether there are any differences and statistical significance observed between the groups' initial variables. If the *p*-value is greater than 0.05, then the null hypothesis is true and the alternative hypothesis is false. In the case of $p > 0.05$, there is no significant difference between EG and CG, and the treatments were performed on two groups with similar initial variables.
- PSS Score: In EG, there were 6 participants PSS of 27-31 , 7 participants with PSS of 31-35 , and 5 participants with PSS of 36-40. In CG, there were 8 participants PSS of 27-31 , 5 participants with PSS of 31-35 , and 4 participants with PSS of 36-40. The *p*-value of 0.704 is greater than 0.05, suggesting that the groups have similar participants distribution scores for Perceived Stress Score.
- QOL Score: In EG, there were 7 participants with QOL of 0-50 , and 11 participants with QOL of 50-100. In CG, there were 5 participants with QOL of 0-50 , and 12 participants with QOL of 50-100. The *p*-value of 0.555 is greater than 0.05, suggesting that the groups have similar participants distribution scores for Quality of Life Score.
- RMSSD: In EG, there were 12 participants with low RMSSD from 0 to 34 ms, 4 participants with normal RMSSD from 35-107 ms, and 2 participants with high

RMSSD of 108 ms or more. In CG, there were 12 participants with low RMSSD from 0 to 34 ms, 5 participants with normal RMSSD from 35-107 ms, and 0 participants with high RMSSD of 108 ms or more. The *p*-value of 0.353 is greater than 0.05, suggesting that the groups have similar categorical RMSSD (HRV) distributions.

- SDNN: In EG, there were 10 participants with low SDNN ranging from 0 to 90 ms, 7 participants with normal SDNN from 100-180 ms, and 1 participant with high SDNN of 180 ms or more. In CG, there were 6 participants with low SDNN ranging from 0 to 90 ms, 7 participants with normal SDNN from 100-180 ms, and 2 participants with high SDNN of 180 ms or more. The *p*-value of 0.459 is greater than 0.05, suggesting that the groups have similar categorical SDNN (HRV) distributions before treatment.
- pNN20: In EG, there were 7 participants with low pNN20 ranging from 0 to 19 %, 8 participants with normal pNN20 ranging from 20 to 40 %, and 1 participant with high pNN20 ranging from 41 % or more. In CG, there were 8 participants with low pNN20 ranging from 0 to 19 %, 4 participants with normal pNN20 ranging from 20 to 40 %, and 5 participants with high pNN20 ranging from 41 % or more. The *p*-value of 0.392 is greater than 0.05, suggesting that the groups have similar categorical pNN20 (HRV) distributions.
- pNN50: In EG, there were 7 participants with low pNN50 ranging from 0 to 4 %, 8 participants with normal pNN50 ranging from 5 to 30 %, and 3 participants with high pNN50 ranging from 31 % or more. In CG, there were 9 participants with low pNN50 ranging from 0 to 4 %, 5 participants with normal pNN50 ranging from 5 to

30 %, and 3 participants with high pNN50 ranging from 31 % or more. The *p*-value of 0.633 is greater than 0.05, suggesting that the groups have similar categorical pNN50 (HRV) distributions.

- LF: In EG, there were 10 participants with low LF ranging from 0 to 649 ms^2, 7 participants with normal LF ranging from 650 to 1500 ms^2, and 1 participant with high LF ranging 1501 ms^2 or more. In CG, there were 8 participants with low LF ranging from 0 to 649 ms^2, 8 participants with normal LF ranging from 650 to 1500 ms^2, and 1 participant with high LF ranging 1501 ms^2 or more. The *p*-value of 0.878 is greater than 0.05, suggesting that the groups have similar categorical Low Frequency Power (HRV) distributions before treatment.
- HF: In EG, there were 2 participants with low HF ranging from 0 to 219 ms^2, 11 participants with normal HF ranging from 220 to 1200 ms^2, and 5 participants with high HF ranging 1201 ms^2 or more. In CG, there were 4 participants with low HF ranging from 0 to 219 ms^2, 11 participants with normal HF ranging from 220 to 1200 ms^2, and 2 participants with high HF ranging 1201 ms^2 or more. The *p*-value of 0.382 is greater than 0.05, suggesting that the groups have similar categorical High Frequency Power (HRV) distributions before treatment.
- LF/HF: In EG, there were 9 participants with a low LF/HF ratio ranging from 0 to 0.99, 6 participants with normal LF/HF ratio ranging from 1 to 2, and 3 participants with a high LF/HF ratio of 2.01 or more. In CG, there were 9 participants with a low LF/HF ratio ranging from 0 to 0.99, 4 participants with normal LF/HF ratio ranging from 1 to 2, and 4 participants with a high LF/HF ratio of 2.01 or more. The *p*-value of 0.773 is greater than 0.05, suggesting that the groups have similar categorical LF/HF ratio (HRV) distributions before treatment.

3.3. RMSSD

RMSSD was the 1st of the seven Heart Rate Variability (HRV) parameters data collected. RMSSD below 35 ms is low, between 35-107 ms is normal range, and above 107 ms is high.

3.3.1. RMSSD Change throughout Treatment

Figure 15 provides a line graph comparing the mean RMSSD change throughout the 12-weeks of treatment, between two groups—the 'EG' (Experimental Group) and 'CG' (Active-Control Group).

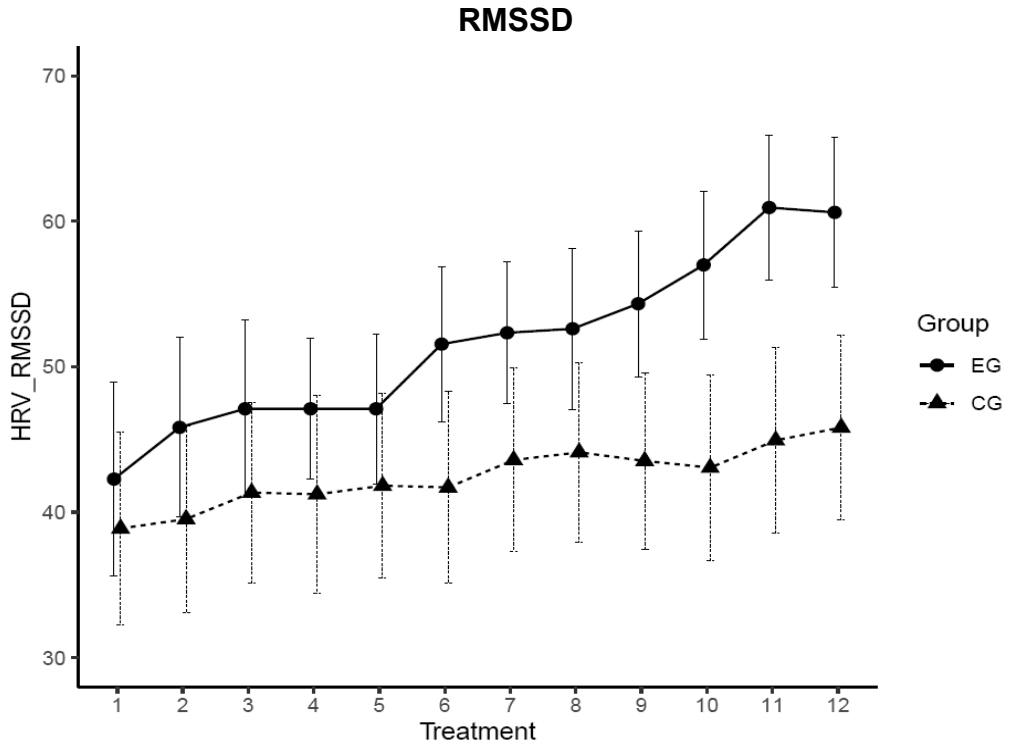


Figure 15. **Line Graph of RMSSD change throughout treatment**

- EG's RMSSD change throughout treatment weeks had a positive trend with a slope value of 1.609, while CG's RMSSD change also had a positive trend with a lower slope value of 0.575.

Table 15 provides a comparison of data between two groups, the 'EG' (Experimental Group) and 'CG' (Active-Control Group), regarding their RMSSD measurements throughout the 12-week of treatments from: before 1st treatment, after 6th treatment and after 12th treatment. Additionally, it includes *p*-values and Cohen's *d* effect size values to assess the significance and magnitude of observed change between groups.

Table 15. **Comparison of RMSSD change throughout treatment between groups**

	RMSSD			
Treatment	EG	CG	*p*-value	Cohen's *d*
1st	42.3 ± 28.34	38.9 ± 27.25	0.519	0.122
6th	51.6 ± 22.65	41.7 ± 27.16	0.057	0.396
12th	60.6 ± 21.92	45.8 ± 26.20	0.033	0.613

* Mann-Whitney U Test

- Experimental Group (EG):

  Before 1st treatment, the group's mean RMSSD measurement was 42.3 ± 28.34, and after 6th treatment, it increased to 51.6 ± 22.65. After final 12th treatment, mean RMSSD of EG increased even further to 60.6 ± 21.92.

- Active-Control (CG):

  Before 1st treatment, the group's mean RMSSD measurement was 38.9 ± 27.25, and after 6th treatment, it increased to 41.7 ± 27.16. After final 12th treatment, mean RMSSD of CG increased further to 45.8 ± 26.20.

- *p*-value*:

  The *p*-values in this column are indicative of whether the two groups' RMSSD measurements are statistically significant. From the RMSSD measured before 1st

treatment, *p*-value was 0.519 and was greater than 0.05, indicating that the two groups were not statistically significant from each other. From the RMSSD measured after 6th treatment and 12th treatment, *p*-values were 0.057 and 0.033 respectively, and the *p*-values here were less than 0.05, suggesting that the two groups were starting to show greater statistically significant results from each other as they go further into the treatment weeks.

- Cohen's *d*:

  Larger Cohen's *d* values indicate larger effect size, suggesting greater difference between the groups. Before 1st treatment, Cohen's *d* was 0.122, indicating negligible effect size. After 6th treatment, Cohen's *d* value was 0.396, indicating small effect size. After the final 12th treatment, there was a significant difference (p=0.033) with at least 95% confidence interval and medium magnitude of effect (d = 0.613) between the groups' RMSSD changes.

Summarizing the RMSSD changes through treatment, Table 15 compared the changes of RMSSD through the treatment weeks between EG and CG, along with their associated statistical significance (*p*-values) and effect size (Cohen's *d*). Before the 1st treatment, results indicated that EG's and CG's RMSSD measurements were not statistically significant and have very small effect size, however, after the 6th and 12th treatments, they became statistically significant, with small and medium effect sizes respectively. Final average RMSSD change from EG is 0.613 standard deviations greater than the average RMSSD change from CG. At least 73% of CG participant's average RMSSD change would be below the average RMSSD change in EG's participants, making RMSSD change between groups fairly obvious to the eye.

Figure 16 provides a bar graph comparing the mean RMSSD change between two groups, the ‘EG’ (Experimental Group) and ‘CG’ (Active-Control Group), throughout treatments (from before 1st, after 6th and after 12th treatments). This is a visual representation of the data from Table 15.

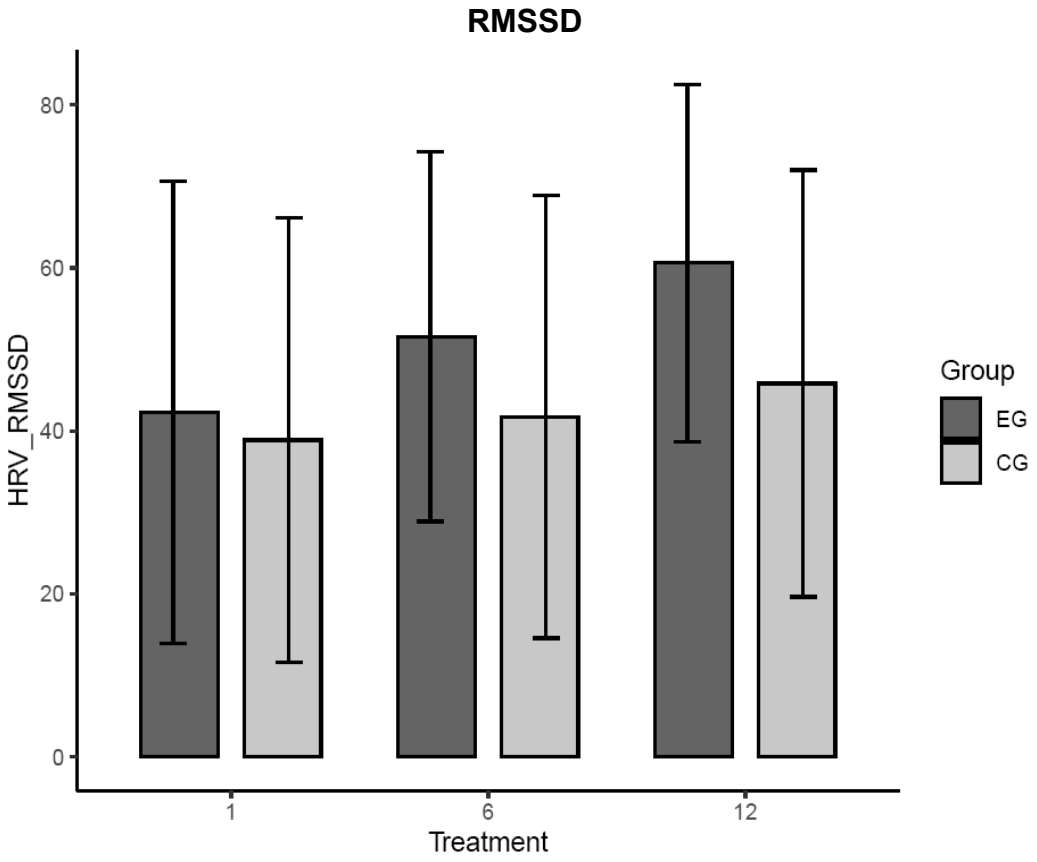


Figure 16. **Bar Graph of RMSSD change throughout treatment**

Figure 17 provides a boxplot graph that visually compares the distribution of numerical RMSSD data and skewness throughout treatment (from before 1st, after 6th and after 12th treatments), between two groups: the 'EG' (Experimental Group) and 'CG' (Active-Control Group).

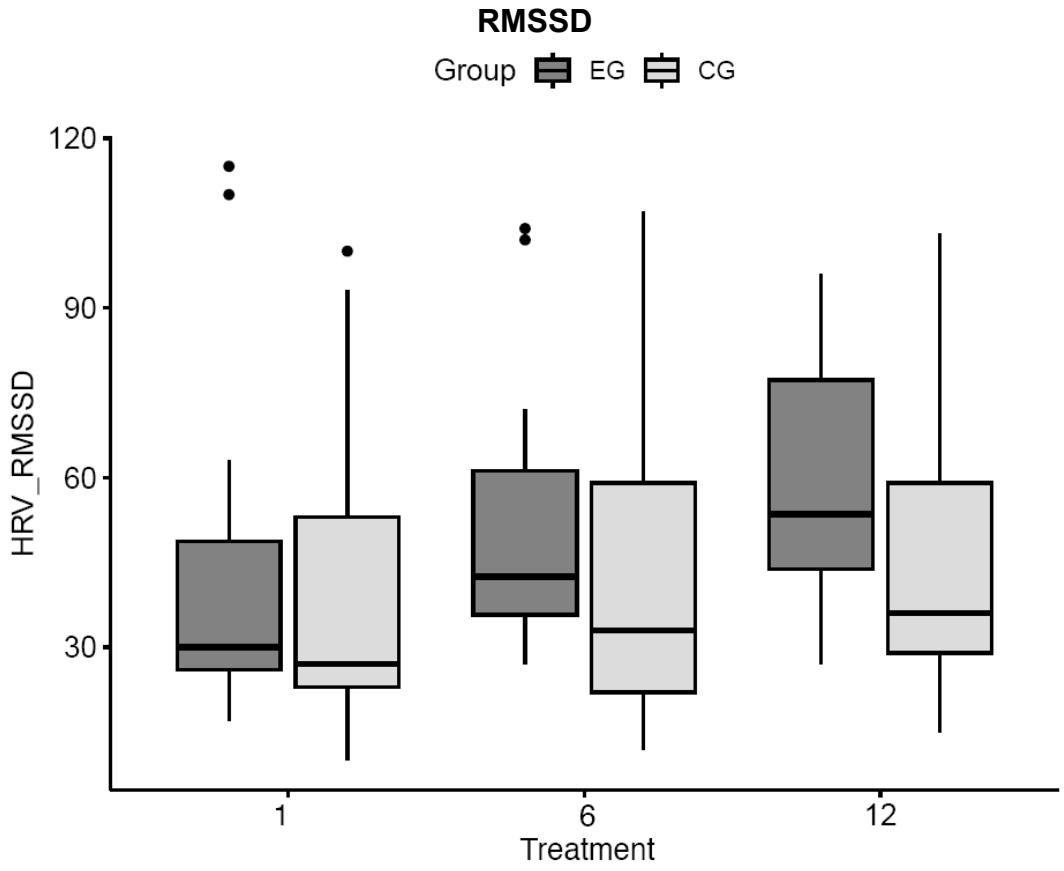


Figure 17. **Boxplot of RMSSD change throughout treatment**

- Before 1st treatment, positions of the Interquartile Range (IQR) boxes were aligned side by side about the same level, suggesting that the group's RMSSD were similar from the beginning. After 12th treatment, the position of groups' IQR boxes shifted, suggesting that the group's RMSSD changes were different.

3.3.2. RMSSD Difference throughout Treatment

Table 16 provides a comparison of RMSSD differences within each groups' RMSSD before and after treatment. Specifically, it compares each groups' RMSSD from before 1st week treatment with after 6th treatment, following with, comparing data between each groups' RMSSD from before 1st week treatment with after 12th treatment. Additionally, it includes *p*-values to assess the significance between the RMSSD difference before and after treatment within each group.

Table 16. **Comparison of RMSSD difference within groups**

		RMSSD	
Group	Treatment	Difference	*p*-value*
EG	6th - 1st	9.3 ± 7.93	0.003
	12th - 1st	18.3 ± 15.44	0.001
CG	6th - 1st	2.8 ± 4.11	0.012
	12th - 1st	6.9 ± 3.53	0.000

* Paired samples t-Test

- Experimental Group (EG): Between the 1st and 6th treatments, EG's RMSSD difference was 9.3 ± 7.93. Between the 1st and 12th treatments, EG's RMSSD difference increased to 18.3 ± 15.44. The *p*-values were 0.003 and 0.001, where both *p*-values were less than 0.05, indicating there were statistical significant differences within EG's before and after treatment.
- Active-Control (CG): Between the 1st and 6th treatments, CG's RMSSD difference was 2.8 ± 4.11. Between the 1st and 12th treatments, CG's RMSSD difference increased to 6.9 ± 3.53. The *p*-values were 0.012 and 0.000, where both *p*-values

were less than 0.05, indicating there were statistical significant differences within CG's before and after treatment.

Table 17 provides a comparison for the RMSSD differences before and after treatments between groups. First, it compares EG's and CG's difference between RMSSD from before 1st and after 6th treatment. Second, it compares EG's and CG's difference between RMSSD from before 1st and after 12th treatment. Additionally, it includes *p*-values to assess the significance between each groups' RMSSD difference.

Table 17. **Comparison of RMSSD difference between groups**

	RMSSD			
Treatment	EG	CG	*p*-value*	Cohen's *d*
6th - 1st	9.3 ± 7.93	2.8 ± 4.11	0.001	1.013
12th - 1st	18.3 ± 15.44	6.9 ± 3.53	0.001	1.004

* Mann-Whitney U Test

- Between the 1st and 6th treatments, EG's RMSSD mean difference was 9.3 ± 7.93, and CG's RMSSD mean difference was 2.8 ± 4.11; showing EG's mean RMSSD difference was 6.5 points higher than CG's mean RMSSD difference. Between the 1st and 12th treatments, EG's RMSSD mean difference increased to 18.3 ± 15.44, and CG's RMSSD mean difference increased to 6.9 ± 3.53; showing EG's mean RMSSD difference was 11.4 points higher than CG's mean RMSSD difference.
- *p*-values: Between groups, *p*-value was 0.001 from 1st–6th treatment, and *p*-value was again 0.001 from 1st–12th treatment. Both *p*-values are less than 0.05,

indicating these were statistically significant differences between the groups' RMSSD difference value.

- Cohen's *d*: From 1st to 6th treatment, Cohen's *d* was 1.013, indicating very large effect size. Following Cohen's *d* value was 1.004 from 1st to 12th treatment, also indicating large effect size.

Summarizing the RMSSD difference through treatment, Tables 16 compared the RMSSD differences within each groups' before and after treatment, and Table 17 compared the RMSSD differences between Experimental Group (EG) and Active-Control Group (CG). For the RMSSD difference within each groups' before and after treatment, the results indicated that both groups' treatments showed statistical significance. Between the two groups' RMSSD difference throughout treatment, the groups results were also statistically different, with a large magnitude of difference. With EG averaging 11.4 higher in RMSSD difference than CG, this indicated that EG had the significantly greater RMSSD difference out of the two groups.

Figure 18 provides a bar graph comparing the mean RMSSD difference between two groups, the ‘EG’ (Experimental Group) and ‘CG’ (Active-Control Group), throughout the 12-week of treatments. This is a visual representation of the data displayed from Tables 16 and Table 17.

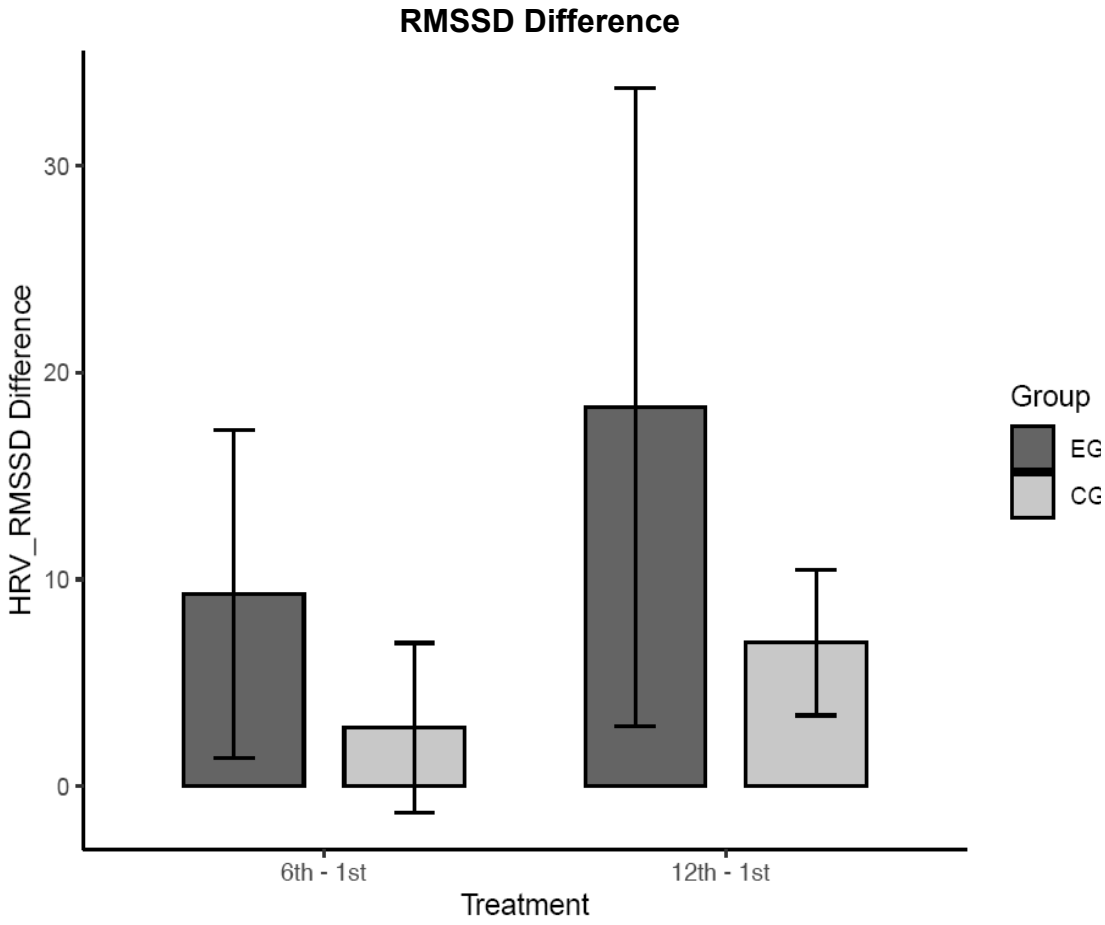


Figure 18. **Bar Graph of RMSSD Difference throughout treatment**

Figure 19 provides a boxplot graph that visually compares the skewness and distribution of numerical RMSSD difference values throughout treatment, between two groups: the 'EG' (Experimental Group) and 'CG' (Active-Control Group).

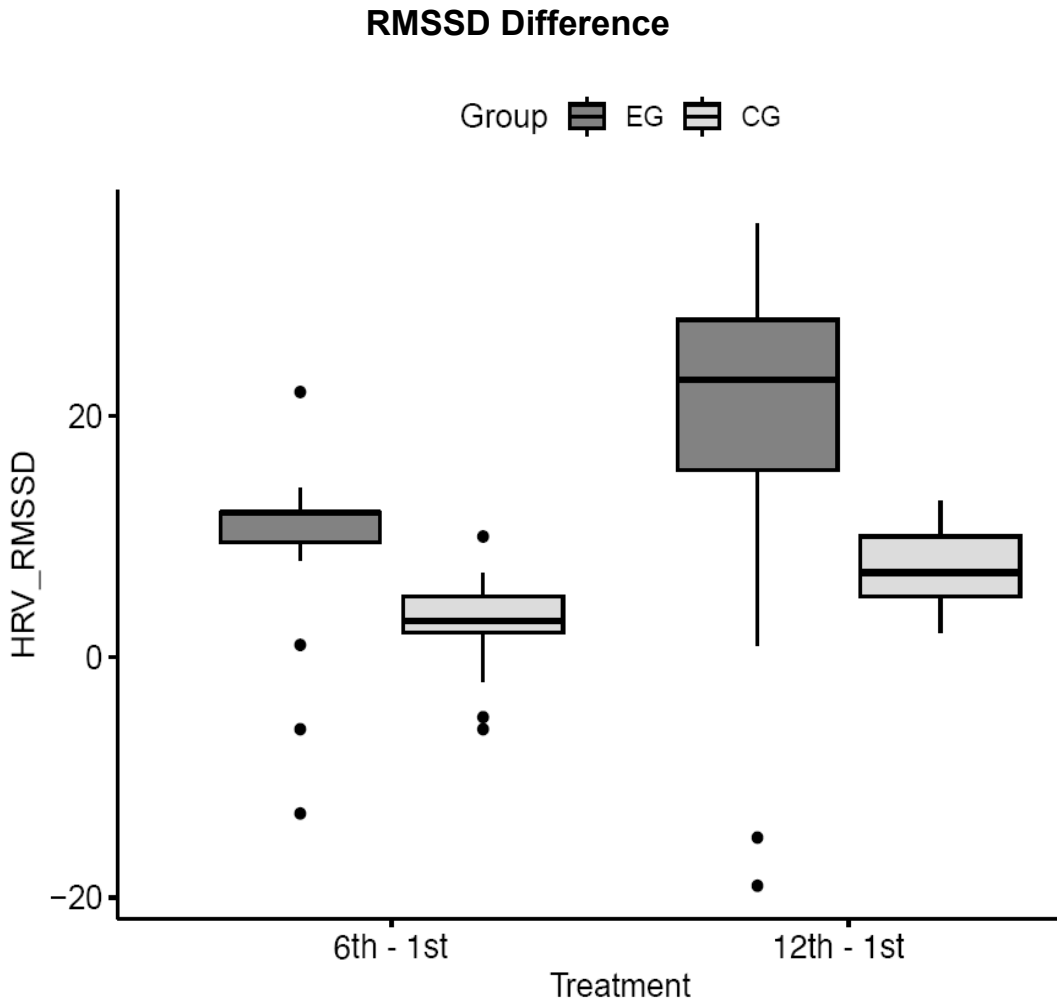


Figure 19. **Boxplot of RMSSD Difference throughout treatment**

- In both time instances, both groups' positions of the Interquartile Range (IQR) boxes were not at level, suggesting that the groups' RMSSD mean differences were actually different after the 6th treatment and after 12th treatment.

3.4. SDNN

SDNN was the 2nd of the seven Heart Rate Variability (HRV) parameters data collected. SDNN below 100 ms is low, between 100-180 ms is normal range, and above 180 ms is high.

3.4.1. SDNN Change throughout Treatment

Figure 20 provides a line graph comparing the mean SDNN change throughout the 12-weeks of treatment, between two groups— 'EG' (Experimental Group) and 'CG' (Active-Control Group).

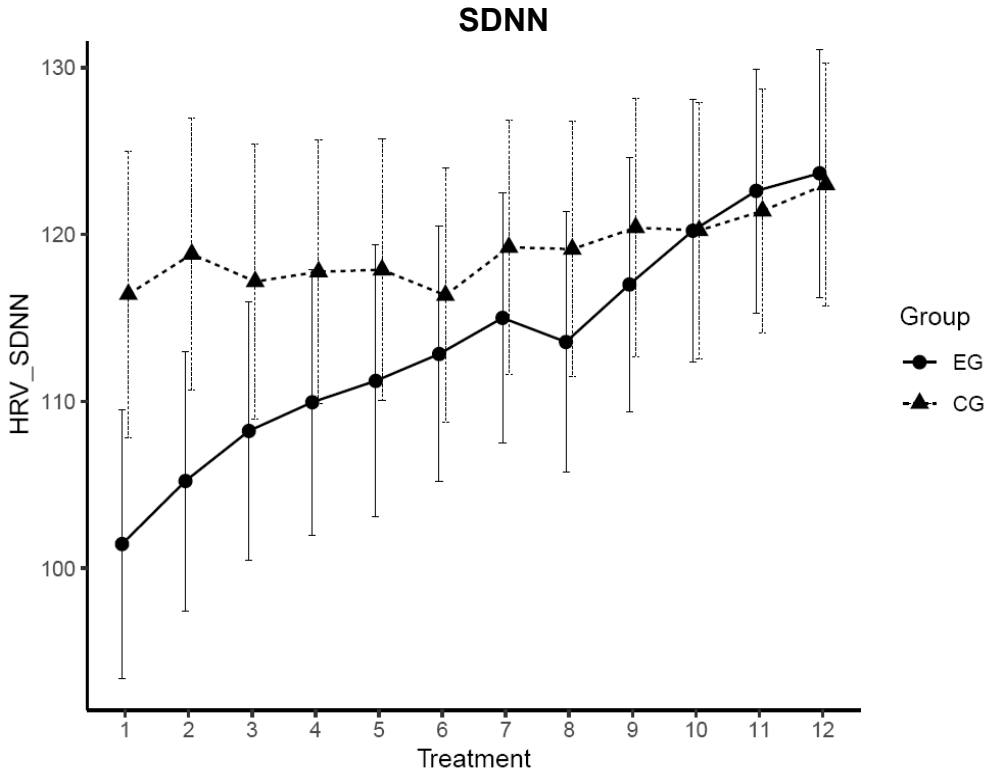


Figure 20. **Line Graph of SDNN change throughout treatment**

- EG's SDNN change throughout treatment weeks had a positive trend with a slope value of 1.851, while CG's SDNN change also had a positive trend with a lower slope value of 0.479.

Table 18 provides a comparison of data between two groups, the 'EG' (Experimental Group) and 'CG' (Active-Control Group), regarding their SDNN measurements throughout the 12-week of treatments from: before 1st treatment, after 6th treatment and after 12th treatment. Additionally, it includes *p*-values and Cohen's *d* effect size values to assess the significance and magnitude of observed change between groups.

Table 18. **Comparison of SDNN change throughout treatment between groups**

	SDNN			
Treatment	EG	CG	*p*-value	Cohen's *d*
1st	101.4 ± 34.25	116.4 ± 35.34	0.176	0.431
6th	112.8 ± 32.49	116.4 ± 31.44	0.747	0.113
12th	123.7 ± 31.56	123.0 ± 30.03	0.949	0.023

* Mann-Whitney U Test

- Experimental Group (EG):

  Before 1st treatment, the group's mean SDNN measurement was 101.4 ± 34.25, and after 6th treatment, it increased to 112.8 ± 32.49. After final 12th treatment, mean SDNN of EG increased further to 123.7 ± 31.56.

- Active-Control (CG):

  Before 1st treatment, the group's mean SDNN measurement was 116.4 ± 35.34, and after 6th treatment, it stayed the same at around 116.4 ± 31.44. After final 12th treatment, mean SDNN of CG increased to 123.0 ± 30.03.

- *p*-value*:

  The *p*-values in this column are indicative of whether the two groups' SDNN measurements are statistically significant. From the SDNN measured before 1st

treatment, *p*-value was 0.176 and was greater than 0.05, indicating that the two groups were not statistically significant from each other. From the SDNN measured after 6th treatment and 12th treatment, *p*-values were 0.747 and 0.949 respectively, and the *p*-values here were greater than 0.05, indicating that the two groups' SDNN changes were still not statistically significant from each other.

- Cohen's *d*:

  Before 1st treatment, Cohen's *d* was 0.431, indicating small effect size. Following 6th treatment, Cohen's *d* value was 0.113 , indicating negligible effect size, and then after final 12th treatment, Cohen's *d* value was even lower at 0.02, indicating also negligible effect sizes in SDNN measurements between the groups. (The reasoning will be discussed in the next chapter.)

Summarizing the SDNN changes through treatment, Table 18 compared the changes of SDNN through the treatment weeks between EG and CG, along with their associated statistical significance (*p*-values) and effect size (Cohen's *d*). The table's results indicated that EG's and CG's SDNN changes were not statistically significant and had negligible magnitude of differences.

Figure 21 provides a bar graph comparing the mean SDNN change between two groups, the ‘EG’ (Experimental Group) and ‘CG’ (Active-Control Group), throughout treatments (from before 1st, after 6th and after 12th treatments). This is a visual representation of the data from Table 18.

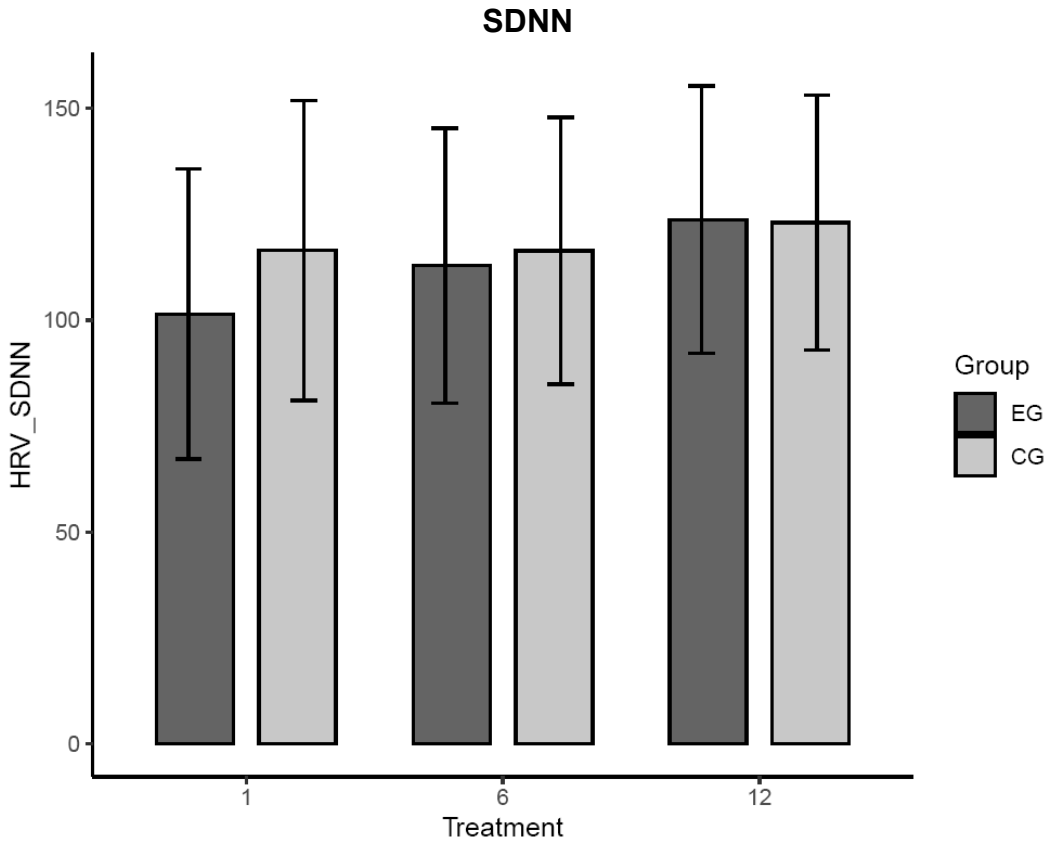


Figure 21. **Bar Graph of SDNN change throughout treatment**

Figure 22 provides a boxplot graph that visually compares the distribution of numerical SDNN data and skewness throughout treatment (from before 1st, after 6th and after 12th treatments), between two groups: the ‘EG’ (Experimental Group) and ‘CG’ (Active-Control Group).

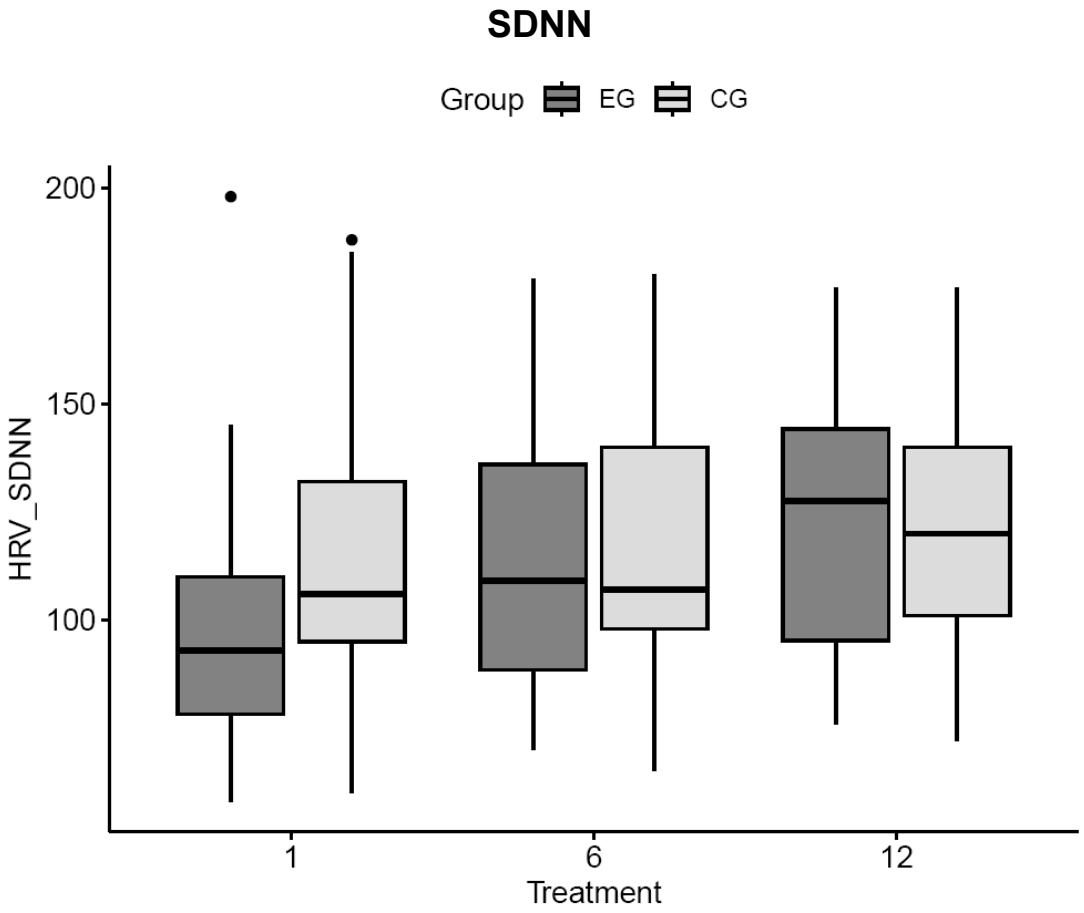


Figure 22. **Boxplot of SDNN change throughout treatment**

- Before 1st treatment, positions of the Interquartile Range (IQR) boxes were misaligned, suggesting that the group’s SDNN were slightly dissimilar from the beginning. After 12th treatment, the position of groups’ IQR boxes shifted to side

by side, suggesting that the group's SDNN were more similar. (The reasoning will be discussed in the next chapter.)

3.4.2. SDNN Difference throughout Treatment

Table 19 provides a comparison of SDNN differences within each groups' SDNN before and after treatment. Specifically, it compares each groups' SDNN from before 1st week treatment with after 6th treatment, following with, comparing data between each groups' SDNN from before 1st week treatment with after 12th treatment. Additionally, it includes *p*-values to assess the significance between the SDNN difference before and after treatment within each group.

Table 19. **Comparison of SDNN difference within groups**

		SDNN	
Group	Treatment	Difference	*p*-value*
EG	6th - 1st	11.4 ± 11.95	0.001
	12th - 1st	22.2 ± 15.84	0.000
CG	6th - 1st	-0.1 ± 10.54	0.982
	12th - 1st	6.6 ± 7.67	0.009

* Paired samples t-Test

- Experimental Group (EG): Between the 1st and 6th treatments, EG's SDNN difference was 11.4 ± 11.95. Between the 1st and 12th treatments, EG's SDNN difference increased to 22.2 ± 15.84. The *p*-values were 0.001 and 0.000, where both *p*-values were less than 0.05, indicating there were statistically significant differences within EG's before and after treatment.

- Active-Control (CG): Between the 1st and 6th treatments, CG's SDNN difference was -0.1 ± 10.54. *P*-value was 0.982, where *p*-value was greater than 0.05, indicating there were no statistical significant differences within CG's before and after treatment from 1st to 6th treatments. However, between the 1st and 12th treatments, CG's SDNN difference increased to 6.6 ± 7.67, *p*-value was 0.009, with *p*-value was less than 0.05, indicating there was statistical significant difference within CG's before and after treatment from 1st to 12th treatment.

Table 20 provides a comparison for the SDNN differences before and after treatments between groups. First, it compares EG's and CG's difference between SDNN from before 1st and after 6th treatment. Second, it compares EG's and CG's difference between SDNN from before 1st and after 12th treatment. Additionally, it includes *p*-values to assess the significance between each groups' SDNN difference.

Table 20. **Comparison of SDNN difference between groups**

	SDNN			
Treatment	EG	CG	*p*-value*	Cohen's *d*
6th - 1st	11.4 ± 11.95	-0.1 ± 10.54	0.005	1.014
12th - 1st	22.2 ± 15.84	6.6 ± 7.67	0.000	1.245

* Mann-Whitney U Test

- Between the 1st and 6th treatments, EG's SDNN mean difference was 11.4 ± 11.95, and CG's SDNN mean difference was -0.1 ± 10.54; showing EG's mean SDNN difference was 11.5 points higher than CG's mean SDNN difference. Between the

1st and 12th treatments, EG's SDNN mean difference increased to 22.2 ± 15.84, and CG's SDNN mean difference increased to 6.6 ± 7.67; showing EG's mean SDNN difference was 15.6 points higher than CG's mean SDNN difference.

- *p*-values: Between groups, *p*-value was 0.005 from 1st–6th treatment, and *p*-value was 0.000 from 1st–12th treatment. Both *p*-values are less than 0.05, indicating these were statistically significant differences between the groups' SDNN difference value.
- Cohen's *d*: From 1st to 6th treatment, Cohen's *d* was 1.014, indicating large effect size. Following Cohen's *d* value was 1.245 from 1st to 12th treatment, also indicating even larger effect size or greater magnitude of difference.

Summarizing the SDNN difference through treatment, Tables 19 compared the SDNN differences within each groups' before and after treatment, and Table 20 compared the SDNN differences between Experimental Group (EG) and Active-Control Group (CG). Between the two groups' SDNN difference throughout treatment, the groups results were statistically different, and there was a large magnitude of difference.

Figure 23 provides a bar graph comparing the mean SDNN difference between two groups, the ‘EG’ (Experimental Group) and ‘CG’ (Active-Control Group), throughout the 12-week of treatments. This is a visual representation of the data displayed from Table 19 and Table 20.

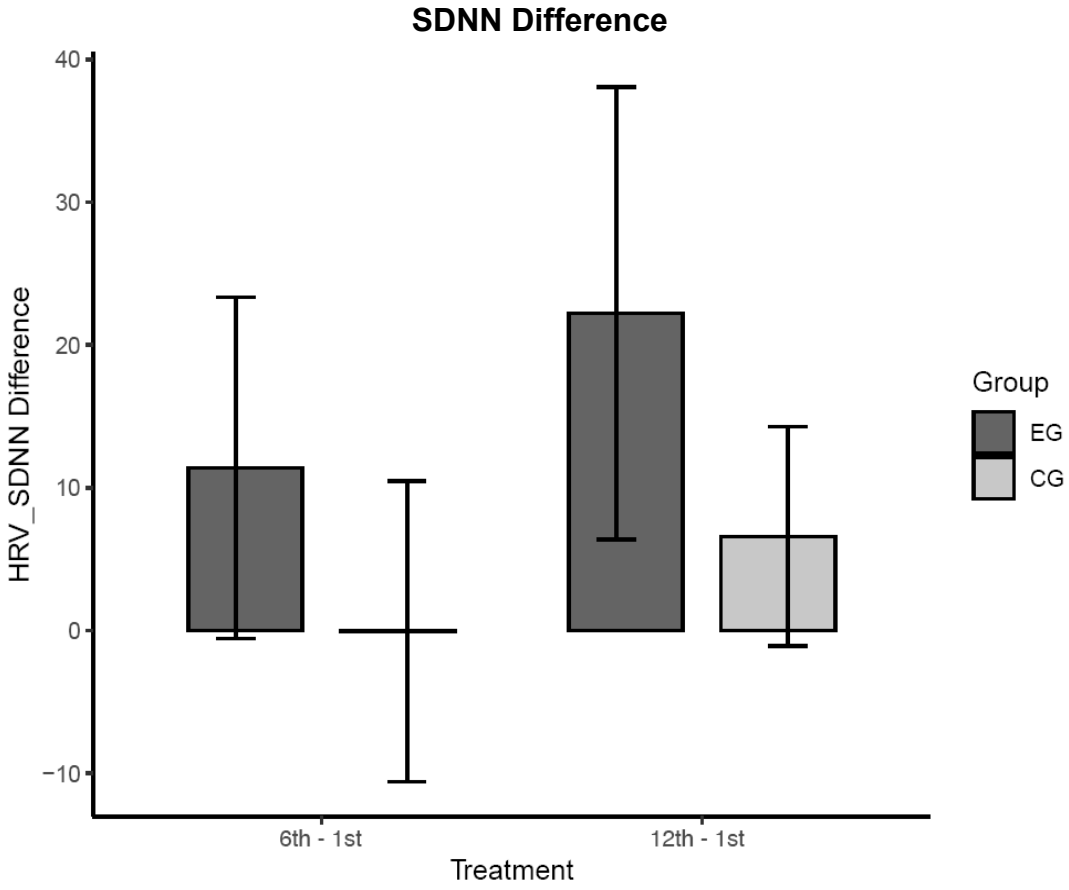


Figure 23. **Bar Graph of SDNN Difference throughout treatment**

Figure 24 provides a boxplot graph that visually compares the skewness and distribution of numerical SDNN difference values throughout treatment, between two groups: the 'EG' (Experimental Group) and 'CG' (Active-Control Group).

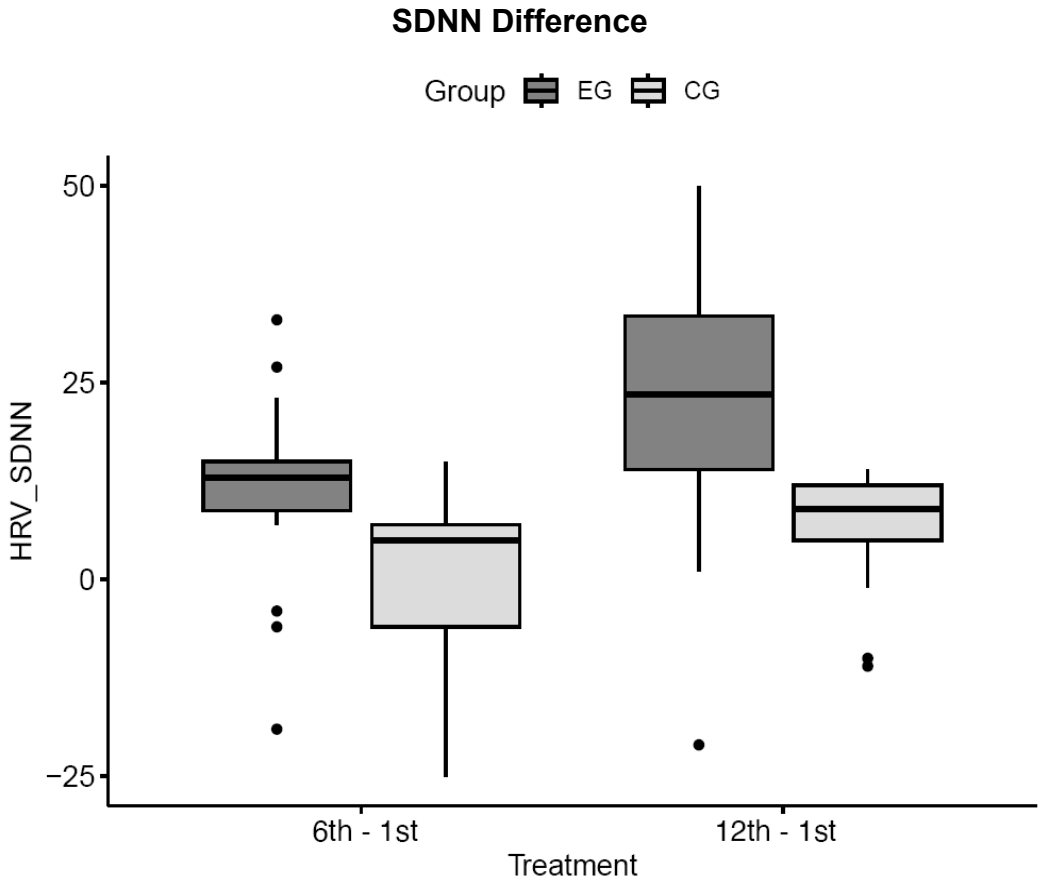


Figure 24. **Boxplot of SDNN Difference throughout treatment**

- In both time instances, both groups' positions of the Interquartile Range (IQR) boxes were not at level, suggesting that the groups' SDNN mean differences were actually different after the 6th treatment and after 12th treatment.

3.5. pNN20

pNN20 was the 3rd of the seven Heart Rate Variability (HRV) parameters data collected. pNN20 below 20% is low, between 20-40% is normal range, and above 40% is high.

3.5.1. pNN20 Change throughout Treatment

Figure 25 provides a line graph comparing the mean pNN20 change throughout the 12-weeks of treatment, between two groups—the 'EG' (Experimental Group) and 'CG' (Active-Control Group).

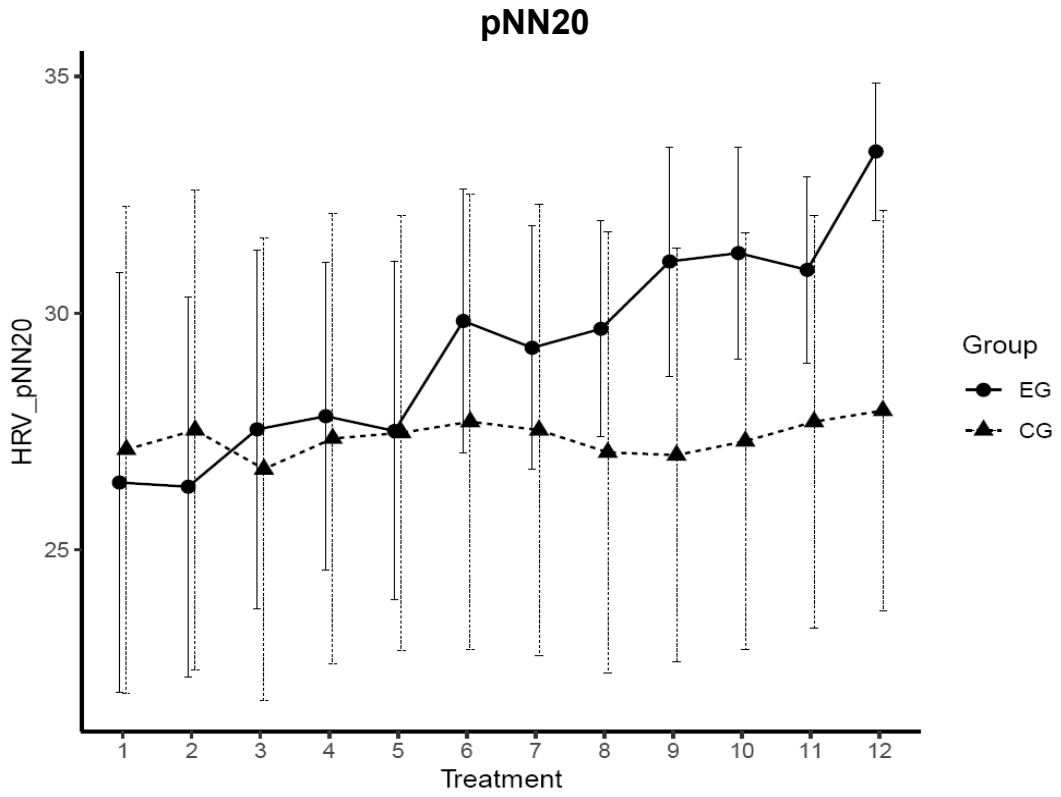


Figure 25. **Line Graph of pNN20 change throughout treatment**

- EG's pNN20 change throughout treatment weeks had a positive trend with a slope value of 0.582, while CG's pNN20 change had a flatter trend with a lower slope value of 0.041.

Table 21 provides a comparison of data between two groups, the 'EG' (Experimental Group) and 'CG' (Active-Control Group), regarding their pNN20 measurements throughout the 12-week of treatments from: before 1st treatment, after 6th treatment and after 12th treatment. Additionally, it includes *p*-values and Cohen's *d* effect size values to assess the significance and magnitude of observed change between groups.

Table 21. **Comparison of pNN20 change throughout treatment between groups**

	pNN20			
Treatment	EG	CG	*p*-value*	Cohen's *d*
1st	26.4 ± 18.82	27.1 ± 21.17	0.817	0.035
6th	29.8 ± 11.81	27.7 ± 19.81	0.355	0.129
12th	33.4 ± 6.16	27.9 ± 17.44	0.068	0.421

* Mann-Whitney U Test

- Experimental Group (EG):

  Before 1st treatment, the group's mean pNN20 measurement was 26.4 ± 18.82, and after 6th treatment, it increased to 29.8 ± 11.81. After final 12th treatment, mean pNN20 of EG increased further to 33.4 ± 6.16.

- Active-Control (CG):

  Before 1st treatment, the group's mean pNN20 measurement was 27.1 ± 21.17, and after 6th treatment, it stayed the same at around 27.7 ± 19.81. After final 12th treatment, the mean pNN20 of CG increased to 27.9 ± 17.44.

- *p*-value*:

  The *p*-values in this column are indicative of whether the two groups' pNN20 measurements are statistically significant. From the pNN20 measured before 1st

treatment and after 6th treatment, *p*-values were 0.817 and 0.355, both cases were greater than 0.05, indicating that the two groups were not statistically significant from each other. From the pNN20 measured after 12th treatment, *p*-value was 0.068, and the *p*-value here was still greater than 0.05, indicating that the two groups' pNN20 changes were still not statistically significant from each other.

- Cohen's *d*:

  Before 1st treatment, Cohen's *d* was 0.035, indicating negligible effect size. Following Cohen's *d* value of 0.129 after 6th treatment, also indicating negligible effect size, and then Cohen's *d* value of 0.421 after final 12th treatment, indicating small effect size in pNN20 measurements between the groups.

Summarizing the pNN20 changes through treatment, Table 21 compared the changes of pNN20 through the treatment weeks between EG and CG, along with their associated statistical significance (*p*-values) and effect size (Cohen's *d*). The table's results indicated that EG's and CG's pNN20 changes were not statistically significant but have small magnitudes of difference after 12th treatment. (The reasoning will be discussed in the next chapter.)

Figure 26 provides a bar graph comparing the mean pNN20 change between two groups, the ‘EG’ (Experimental Group) and ‘CG’ (Active-Control Group), throughout treatments (from before 1st, after 6th and after 12th treatments). This is a visual representation of the data from Table 21.

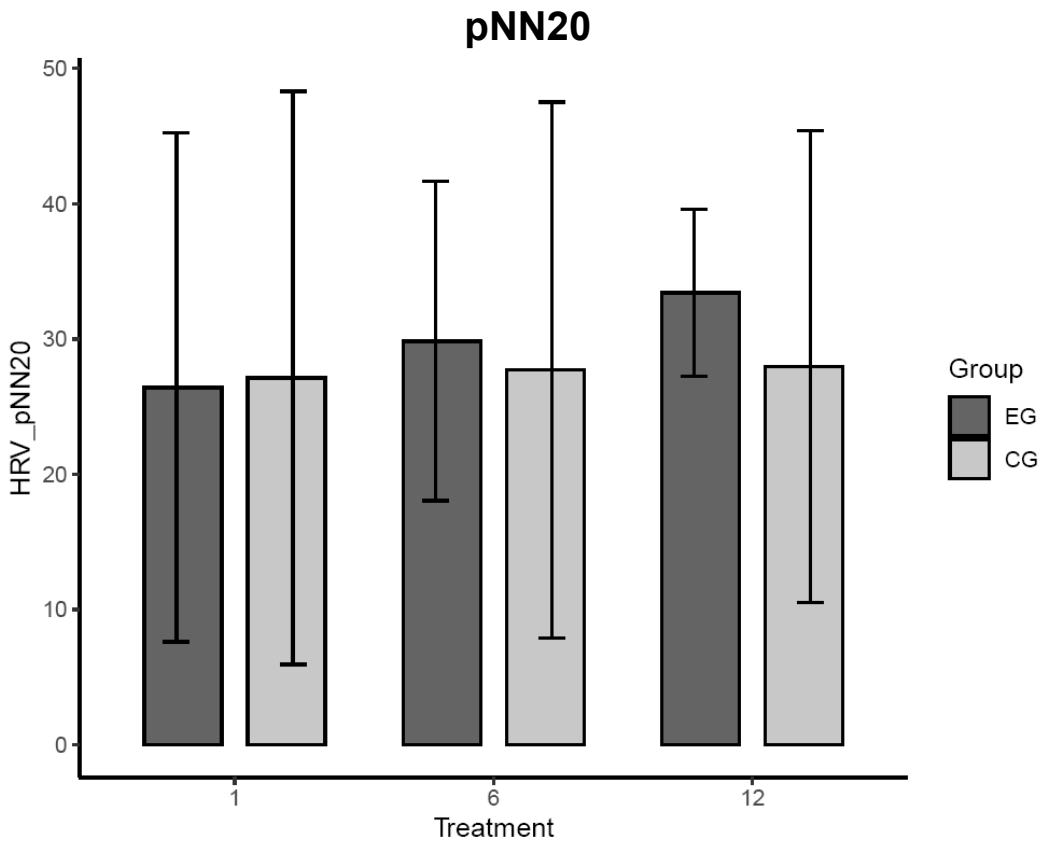


Figure 26. **Bar Graph of pNN20 change throughout treatment**

Figure 27 provides a boxplot graph that visually compares the distribution of numerical pNN20 data and skewness throughout treatment (from before 1st, after 6th and after 12th treatments), between two groups: the ‘EG’ (Experimental Group) and ‘CG’ (Active-Control Group).

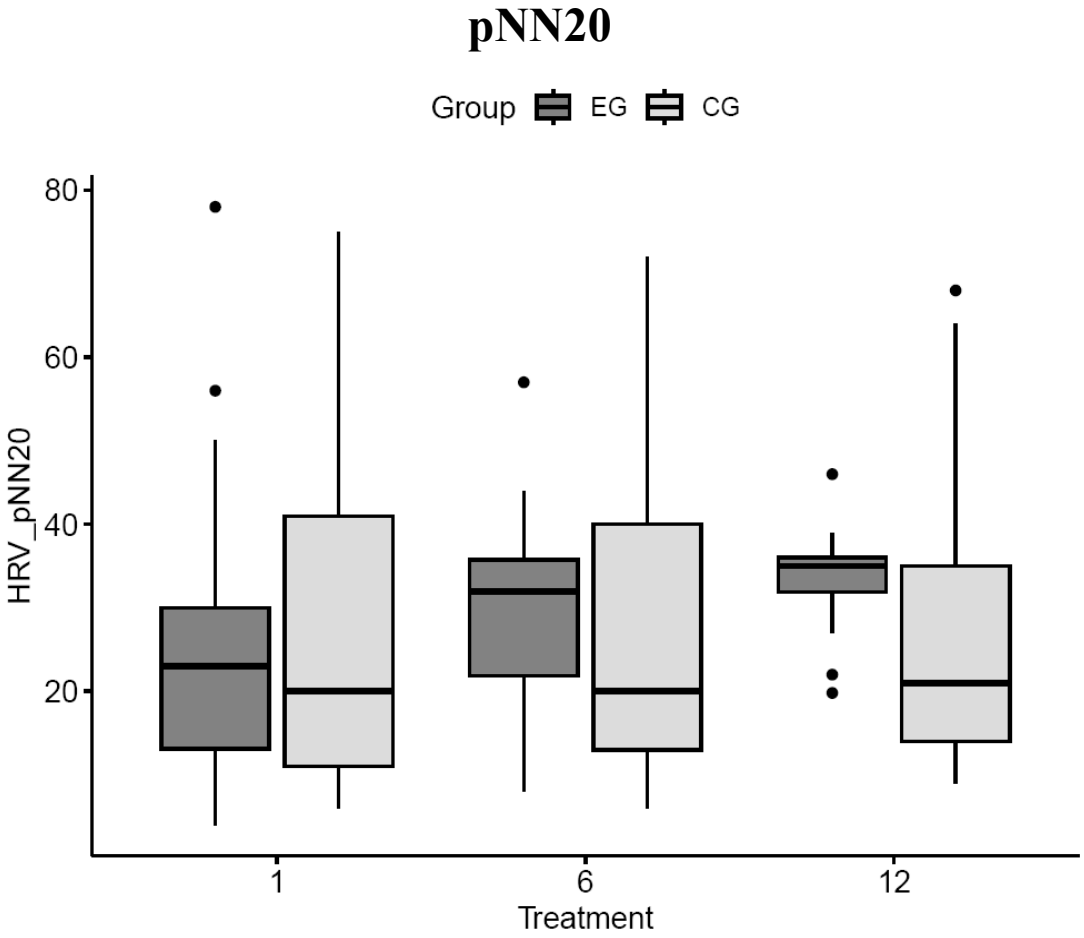


Figure 27. **Boxplot of pNN20 change throughout treatment**

- Before 1st treatment, positions of the Interquartile Range (IQR) boxes were aligned, suggesting that the group’s pNN20 were similar from the beginning. After 12th treatment, the position of groups’ IQR boxes shifted, suggesting that the group’s pNN20 changes were different.

3.5.2. pNN20 Difference throughout Treatment

Table 22 provides a comparison of pNN20 differences within each groups' pNN20 before and after treatment. Specifically, it compares each groups' pNN20 from before 1st week treatment with after 6th treatment, following with, comparing data between each groups' pNN20 from before 1st week treatment with after 12th treatment. Additionally, it includes *p*-values to assess the significance between the pNN20 difference before and after treatment within each group.

Table 22. **Comparison of pNN20 difference within groups**

		pNN20	
Group	Treatment	Difference	*p*-value*
EG	6th - 1st	3.4 ± 9.10	0.130
	12th - 1st	7.0 ± 14.75	0.058
CG	6th - 1st	0.6 ± 2.72	0.385
	12th - 1st	0.8 ± 5.55	0.549

* Paired samples t-Test

- Experimental Group (EG): Between the 1st and 6th treatments, EG's pNN20 difference was 3.4 ± 9.10. Between the 1st and 12th treatments, EG's pNN20 difference increased to 7.0 ± 14.75. The *p*-value was 0.130 from before 1st and after 6th treatment, where *p*-value was greater than 0.05, indicating there was not yet a statistically significant difference within EG's before and after treatment. However, *p*-value was 0.058 from before 1st and after 12th treatment, where *p*-value was equal to 0.05, indicating there was a marginally statistical significant difference within EG's before and after treatment.

- Active-Control (CG): Between the 1st and 6th treatments, CG's pNN20 difference was 0.6 ± 2.72. *P*-value was 0.385, where *p*-value was greater than 0.05, indicating there were no statistical significant differences within CG's before and after treatment from 1st to 6th treatments. Between the 1st and 12th treatments, CG's pNN20 difference increased slightly to 0.8 ± 5.55, *p*-value was 0.549, with *p*-value was still greater than 0.05, indicating there was no statistical significant difference within CG's before and after treatment from 1st to 12th treatment.

Table 23 provides a comparison for the pNN20 differences before and after treatments between groups. First, it compares EG's and CG's difference between pNN20 from before 1st and after 6th treatment. Second, it compares EG's and CG's difference between pNN20 from before 1st and after 12th treatment. Additionally, it includes *p*-values to assess the significance between each groups' pNN20 difference.

Table 23. **Comparison of pNN20 difference between groups**

	pNN20			
Treatment	EG	CG	*p*-value*	Cohen's *d*
6th - 1st	3.4 ± 9.10	0.6 ± 2.72	0.014	0.415
12th - 1st	7.0 ± 14.75	0.8 ± 5.55	0.008	0.547

* Mann-Whitney U Test

- Between the 1st and 6th treatments, EG's pNN20 mean difference was 3.4 ± 9.10, and CG's pNN20 mean difference was 0.6 ± 2.72; showing EG's mean pNN20 difference was 2.8 points higher than CG's mean pNN20 difference. Between the

1st and 12th treatments, EG's pNN20 mean difference increased to 7.0 ± 14.75, and CG's pNN20 mean difference increased slightly to 0.8 ± 5.55; showing EG's mean pNN20 difference was 6.2 points higher than CG's mean pNN20 difference.

- *p*-values: Between groups, *p*-value was 0.014 from 1st–6th treatment, and *p*-value was 0.008 from 1st–12th treatment. Both instances' *p*-values are less than 0.05, indicating these were statistically significant differences between the groups' pNN20 difference value.
- Cohen's *d*: From 1st to 6th treatment, Cohen's *d* was 0.415, indicating small effect size. Following Cohen's *d* value was 0.547 from 1st to 12th treatment, indicating medium effect size.

Summarizing the pNN20 difference through treatment, Tables 22 compared the pNN20 differences within each groups' before and after treatment, and Table 23 compared the pNN20 differences between Experimental Group (EG) and Active-Control Group (CG). For pNN20 differences within each groups' before and after treatment, results indicated that both groups' treatments did not have statistical significance. (Note, results from EG's pNN20 difference within the group did show marginal statistical significance after 12th treatment.) Between the two groups' pNN20 difference after 12th treatment, the results were significant, with a medium magnitude of difference. Comparing the pNN20 difference between EG and CG, EG had the higher pNN20 difference throughout treatment.

Figure 28 provides a bar graph comparing the mean pNN20 difference between two groups, the ‘EG’ (Experimental Group) and ‘CG’ (Active-Control Group), throughout the 12-week of treatments. This is a visual representation of the data displayed from Table 22 and Table 23.

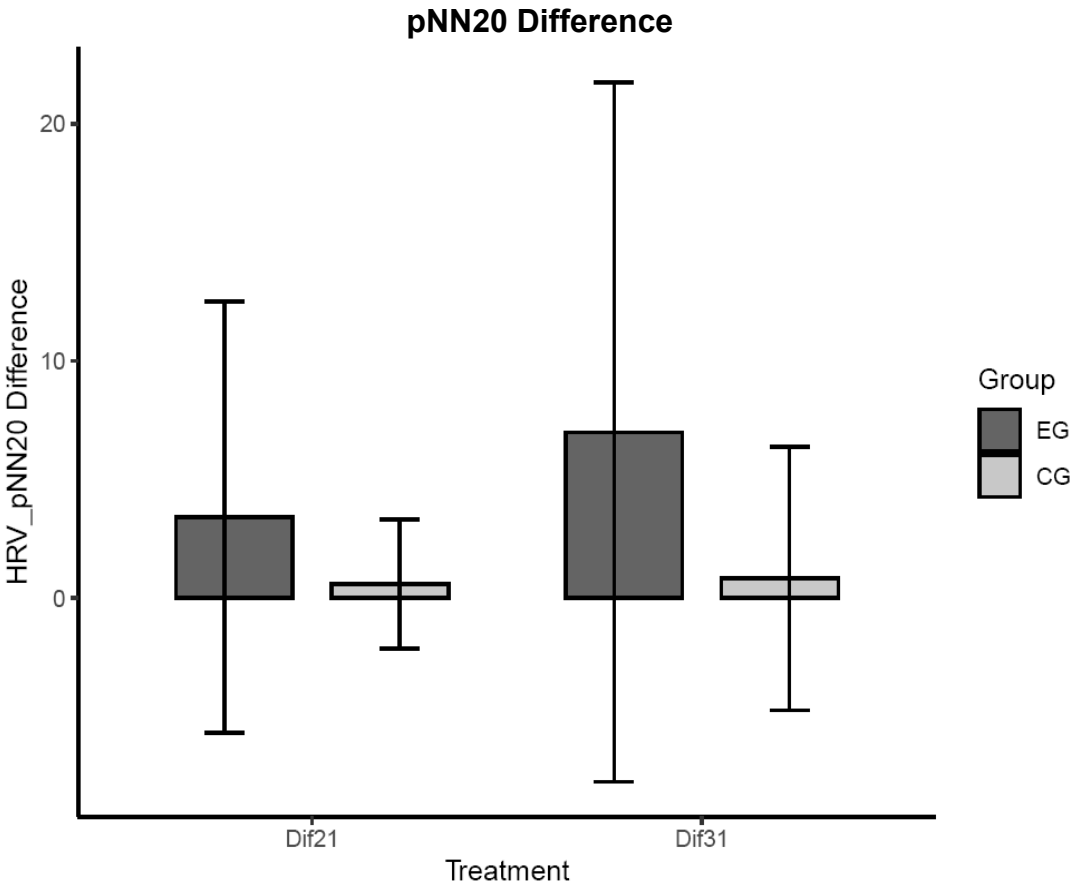


Figure 28. **Bar Graph of pNN20 Difference throughout treatment**

Figure 29 provides a boxplot graph that visually compares the skewness and distribution of numerical pNN20 difference values throughout treatment, between two groups: the 'EG' (Experimental Group) and 'CG' (Active-Control Group).

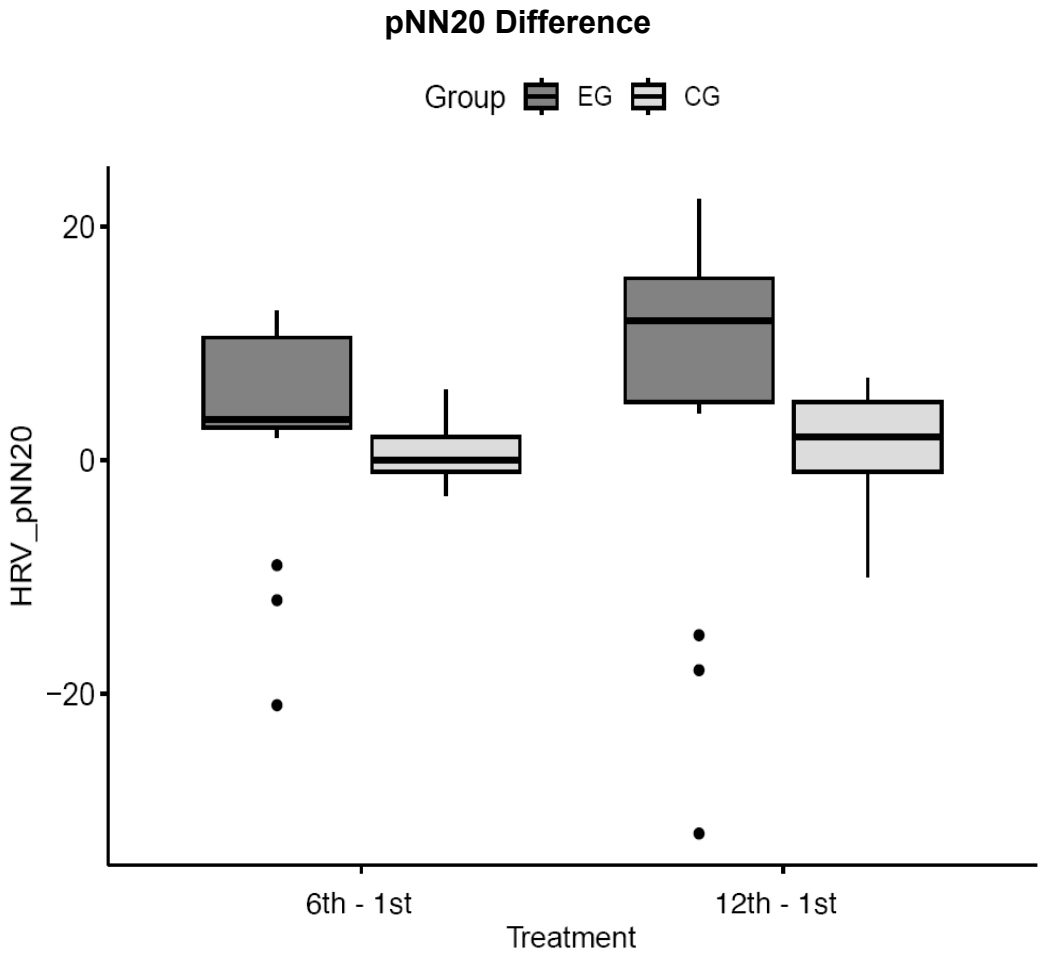


Figure 29. **Boxplot of pNN20 Difference throughout treatment**

- In both time instances, both groups' positions of the Interquartile Range (IQR) boxes were not at level, suggesting that the groups' pNN20 mean differences were actually different after the 6th treatment and after 12th treatment.

3.6. pNN50

pNN50 was the 4th of the seven Heart Rate Variability (HRV) parameters data collected. pNN50 below 5% is low, between 5-30% is normal range, and above 30% is high.

3.6.1. pNN50 Change throughout Treatment

Figure 30 provides a line graph comparing the mean pNN50 change throughout the 12-weeks of treatment, between two groups—the 'EG' (Experimental Group) and 'CG' (Active-Control Group).

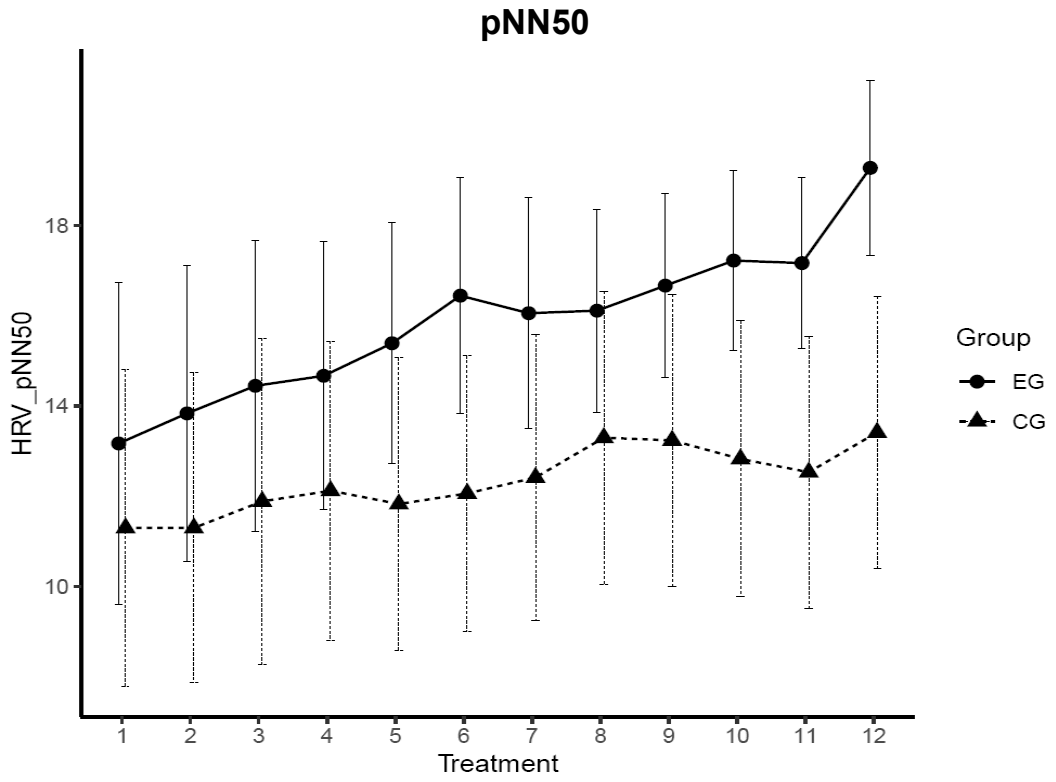


Figure 30. **Line Graph of pNN50 change throughout treatment**

- EG's pNN50 change throughout treatment weeks had a positive trend with a slope value of 0.449, while CG's pNN50 change had a slight positive trend with a lower slope value of 0.180.

Table 24 provides a comparison of data between two groups, the 'EG' (Experimental Group) and 'CG' (Active-Control Group), regarding their pNN50 measurements throughout the 12-week of treatments from: before 1st treatment, after 6th treatment and after 12th treatment. Additionally, it includes *p*-values and Cohen's *d* effect size values to assess the significance and magnitude of observed change between groups.

Table 24. **Comparison of pNN50 change throughout treatment between groups**

	pNN50			
Treatment	EG	CG	*p*-value*	Cohen's *d*
1st	13.2 ± 15.12	11.3 ± 14.51	0.370	0.128
6th	16.4 ± 11.12	12.1 ± 12.60	0.080	0.362
12th	19.3 ± 8.26	13.4 ± 12.43	0.034	0.559

* Mann-Whitney U Test

- Experimental Group (EG):

  Before 1st treatment, the group's mean pNN50 measurement was 13.2 ± 15.12, and after 6th treatment, it increased to 16.4 ± 11.12. After final 12th treatment, mean pNN50 of EG increased further to 19.3 ± 8.26.

- Active-Control (CG):

  Before 1st treatment, the group's mean pNN50 measurement was 11.3 ± 14.51, and after 6th treatment, it increased to 12.1 ± 12.60. After final 12th treatment, the mean pNN50 of CG increased slightly to 13.4 ± 12.43.

- *p*-value*:

  The *p*-values in this column are indicative of whether the two groups' pNN50 measurements are statistically significant. From the pNN50 measured before 1st

treatment and after 6th treatment, *p*-values were 0.370 and 0.080, both cases were greater than 0.05, indicating that the two groups were not statistically significant from each other. From the pNN50 measured after 12th treatment, *p*-value was 0.034, and the *p*-value here was less than 0.05, indicating that the two groups' pNN50 changes were statistically significant from each other.

- Cohen's *d*:

  Before 1st treatment, Cohen's *d* was 0.128, indicating negligible effect size. Following Cohen's *d* value of 0.362 after 6th treatment, indicating small effect size, and then Cohen's *d* value of 0.559 after final 12th treatment, indicating medium effect size in pNN50 measurements between the groups.

Summarizing the pNN50 changes through treatment, Table 24 compared the changes of pNN50 through the treatment weeks between EG and CG, along with their associated statistical significance (*p*-values) and effect size (Cohen's *d*). The table's results indicated that EG's and CG's pNN50 changes were statistically significant and have medium effect size after the 12th treatments.

Figure 31 provides a bar graph comparing the mean pNN50 change between two groups, the ‘EG’ (Experimental Group) and ‘CG’ (Active-Control Group), throughout treatments (from before 1st, after 6th and after 12th treatments). This is a visual representation of the data from Table 24.

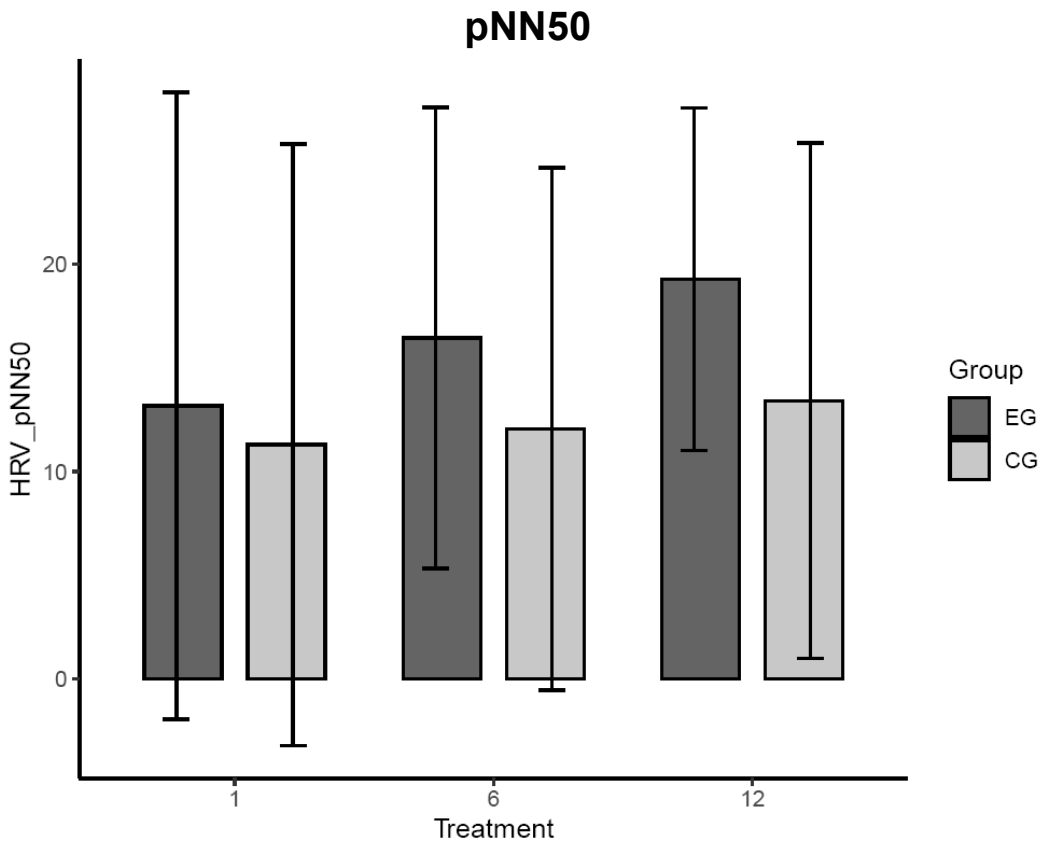


Figure 31. **Bar Graph of pNN50 change throughout treatment**

Figure 32 provides a boxplot graph that visually compares the distribution of numerical pNN50 data and skewness throughout treatment (from before 1st, after 6th and after 12th treatments), between two groups: the ‘EG’ (Experimental Group) and ‘CG’ (Active-Control Group).

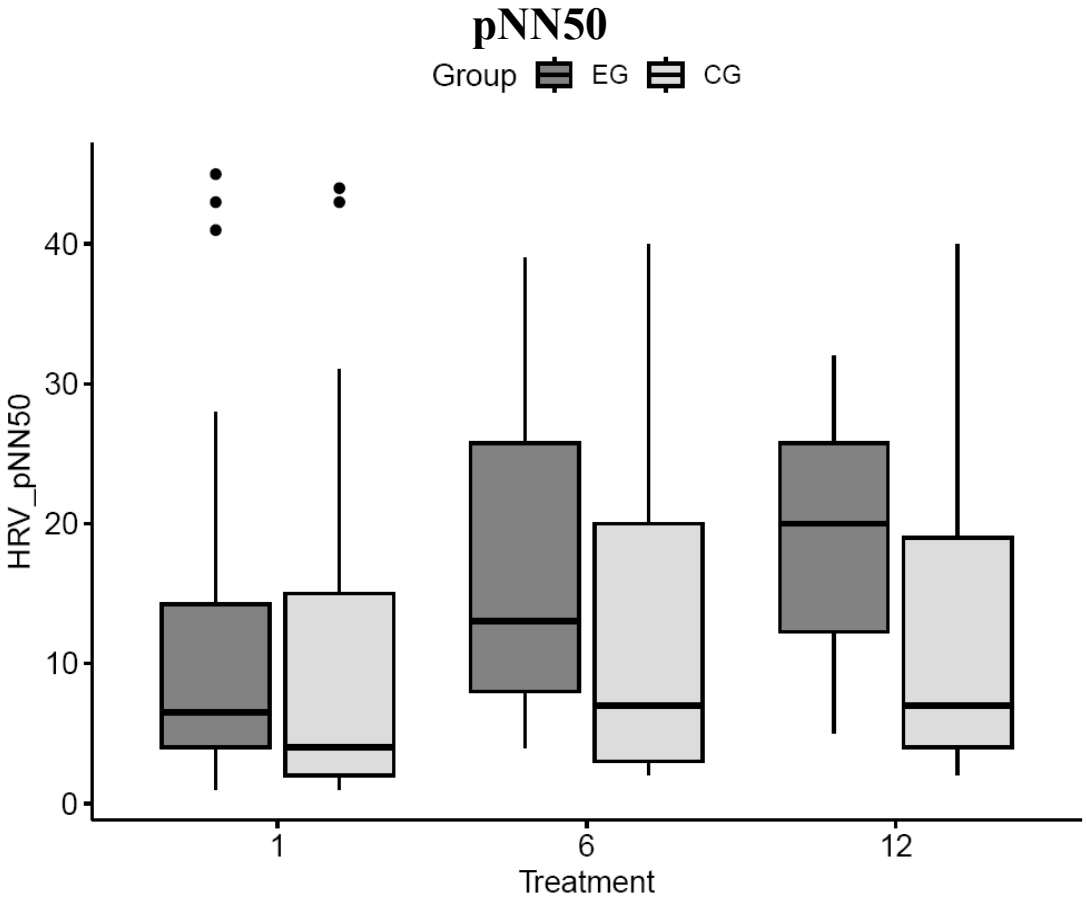


Figure 32. **Boxplot of pNN50 change throughout treatment**

- Before 1st treatment, positions of the Interquartile Range (IQR) boxes were aligned, suggesting that the group’s pNN50 were similar from the beginning. After 12th treatment, the position of groups’ IQR boxes slightly shifted out of alignment, suggesting that the group’s pNN50 changes were different.

3.6.2. pNN50 Difference throughout Treatment

Table 25 provides a comparison of pNN50 differences within each groups' pNN50 before and after treatment. Specifically, it compares each groups' pNN50 from before 1st week treatment with after 6th treatment, following with, comparing data between each groups' pNN50 from before 1st week treatment with after 12th treatment. Additionally, it includes *p*-values to assess the significance between the pNN50 difference before and after treatment within each group.

Table 25. **Comparison of pNN50 difference within groups**

		pNN50	
Group	Treatment	Difference	*p*-value*
EG	6th - 1st	3.3 ± 6.46	0.046
	12th - 1st	6.1 ± 10.6	0.026
CG	6th - 1st	0.8 ± 2.70	0.261
	12th - 1st	2.1 ± 3.41	0.021

* Paired samples t-Test

- Experimental Group (EG): Between the 1st and 6th treatments, EG's pNN50 difference was 3.3 ± 6.46. Between the 1st and 12th treatments, EG's pNN50 difference increased to 6.1 ± 10.6. The *p*-value was 0.046 from before 1st and after 6th treatment, where *p*-value was less than 0.05, indicating there was statistical significant difference within EG's before and after treatment. From before 1st and after 12th treatment, the *p*-value was 0.026, where *p*-value was less than 0.05, indicating there was a statistical significant difference within EG's before and after treatment.

- Active-Control (CG): Between the 1st and 6th treatments, CG's pNN50 difference was 0.8 ± 2.70. *P*-value was 0.261, where *p*-value was greater than 0.05, indicating there were no statistical significant differences within CG's before and after treatment from 1st to 6th treatments. Between the 1st and 12th treatments, CG's pNN50 difference increased slightly to 2.1 ± 3.41, *p*-value was 0.021, with *p*-value here lesser than 0.05, indicating there was statistical significant difference within CG's before and after treatment from 1st to 12th treatment.

Table 26 provides a comparison for the pNN50 differences before and after treatments between groups. First, it compares EG's and CG's difference between pNN50 from before 1st and after 6th treatment. Second, it compares EG's and CG's difference between pNN50 from before 1st and after 12th treatment. Additionally, it includes *p*-values to assess the significance between each groups' pNN50 difference.

Table 26. **Comparison of pNN50 difference between groups**

	pNN50			
Treatment	EG	CG	*p*-value*	Cohen's *d*
6th - 1st	3.3 ± 6.46	0.8 ± 2.70	0.058	0.502
12th - 1st	6.1 ± 10.6	2.1 ± 3.41	0.008	0.500

* Mann-Whitney U Test

- Between the 1st and 6th treatments, EG's pNN50 mean difference was 3.3 ± 6.46, and CG's pNN50 mean difference was 0.8 ± 2.70; showing EG's mean pNN50 difference was 2.5 points higher than CG's mean pNN50 difference. Between the

1st and 12th treatments, EG's pNN50 mean difference increased to 6.1 ± 10.6, and CG's pNN50 mean difference increased to 2.1 ± 3.41; showing EG's mean pNN50 difference was 4 points higher than CG's mean pNN50 difference.

- *p*-values: Between groups from 1st–6th treatment, *p*-value was 0.058, where *p*-value was equal to 0.05, indicating there was a marginal statistically significant difference between the groups' pNN50 difference value. Between groups from 1st–12th treatment, *p*-value was 0.008, where *p*-value was less than 0.05, indicating these were statistically significant differences between the groups' pNN50 difference value.
- Cohen's *d*: From 1st to 6th treatment, Cohen's *d* was 0.502, indicating medium effect size. Following Cohen's *d* value was 0.500 from 1st to 12th treatment, also indicating medium effect size.

Summarizing the pNN50 difference through treatment, Tables 25 compared the pNN50 differences within each groups' before and after treatment, and Table 26 compared the pNN50 differences between Experimental Group (EG) and Active-Control Group (CG). For pNN50 differences within each groups' before and after treatment, results indicated that both groups' treatments did have statistical significance. For between the two groups' pNN50 difference throughout treatment, the groups' results were statistically significant, with a medium magnitude of difference. Comparing the pNN50 difference between EG and CG, EG had the higher pNN50 difference throughout treatment.

Figure 33 provides a bar graph comparing the mean pNN50 difference between two groups, the ‘EG’ (Experimental Group) and ‘CG’ (Active-Control Group), throughout the 12-week of treatments. This is a visual representation of the data displayed from Table 25 and Table 26.

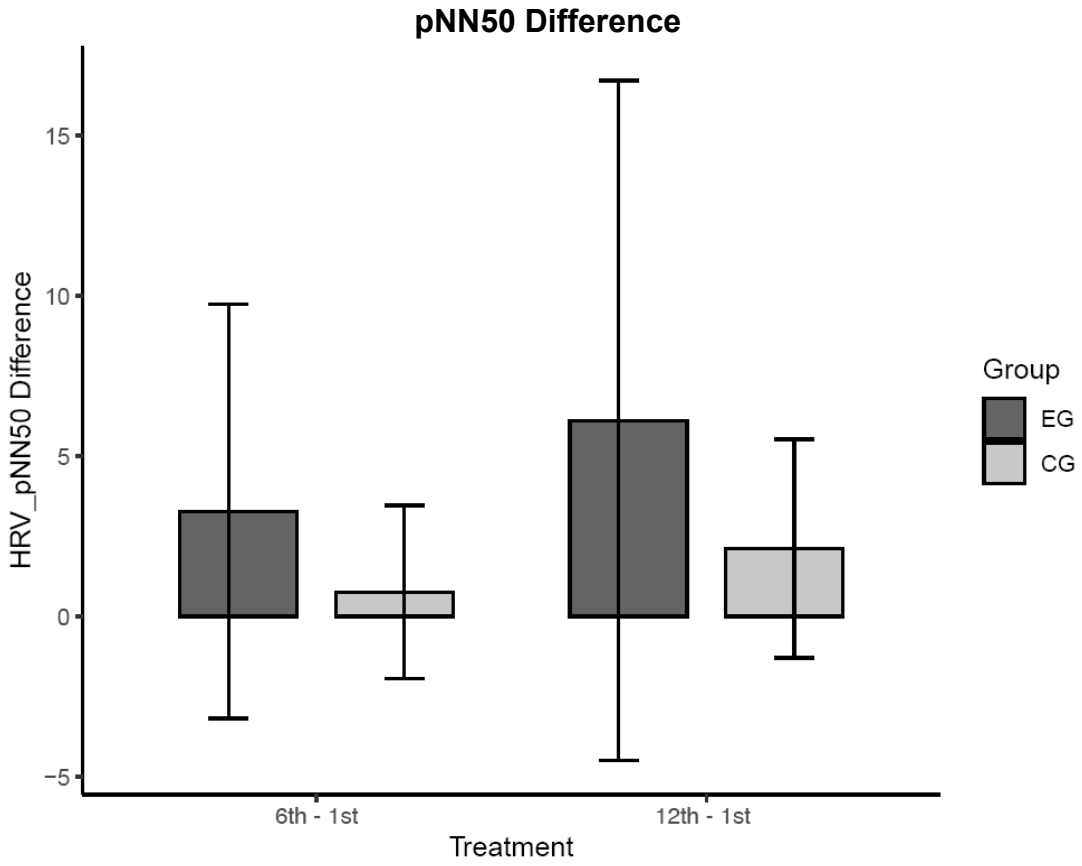


Figure 33. **Bar Graph of pNN50 Difference throughout treatment**

Figure 34 provides a boxplot graph that visually compares the skewness and distribution of numerical pNN50 difference values throughout treatment, between two groups: the 'EG' (Experimental Group) and 'CG' (Active-Control Group).

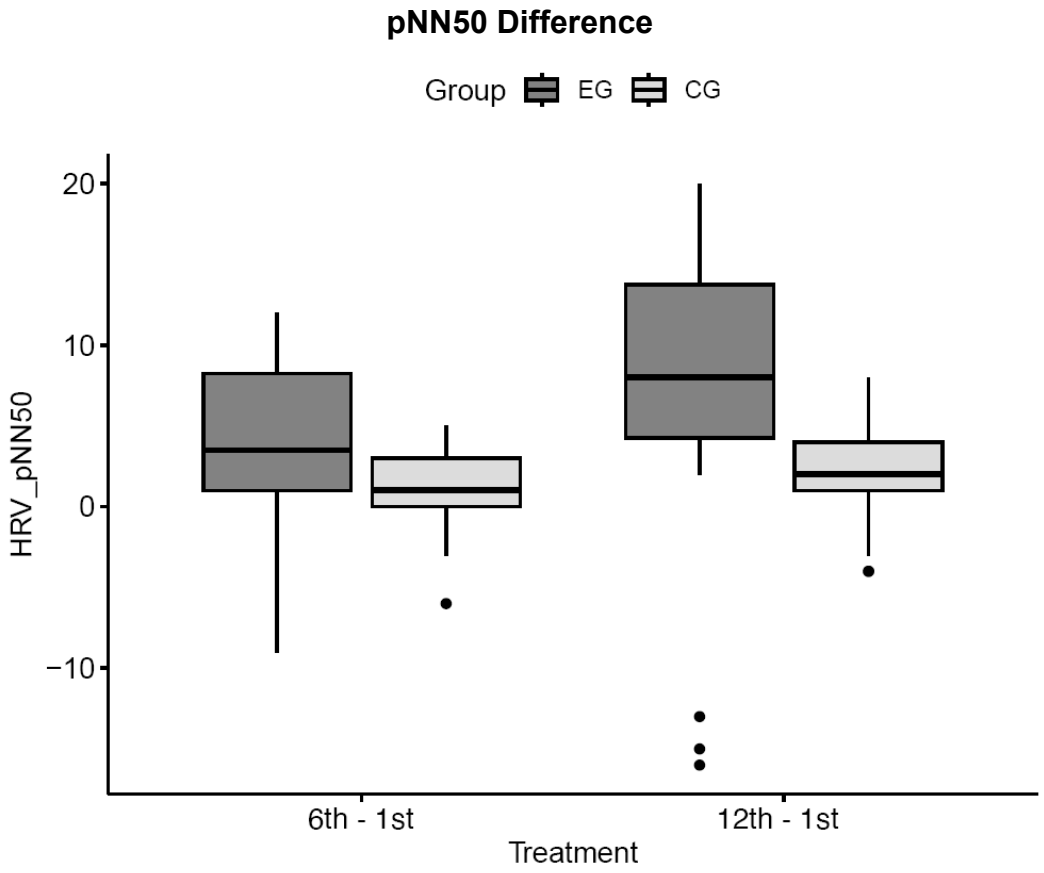


Figure 34. **Boxplot of pNN50 Difference throughout treatment**

- From 1st to 6th treatments, positions of the Interquartile Range (IQR) boxes were aligned side by side, suggesting that the group's pNN50 differences were still similar. After 12th treatment, the position of groups' IQR boxes shifted away from each other, suggesting that the groups' pNN50 differences were different from each other at the end of trial

3.7. LF

LF (known as Low Frequency Power) was the 5th of the seven Heart Rate Variability (HRV) parameters data collected. LF below 650 ms^2 is low, between 650-1500 ms^2 is normal range, and above 1500 ms^2 is high.

3.7.1. LF Change throughout Treatment

Figure 35 provides a line graph comparing the mean LF change throughout the 12-weeks of treatment, between two groups—the 'EG' (Experimental Group) and 'CG' (Active-Control Group).

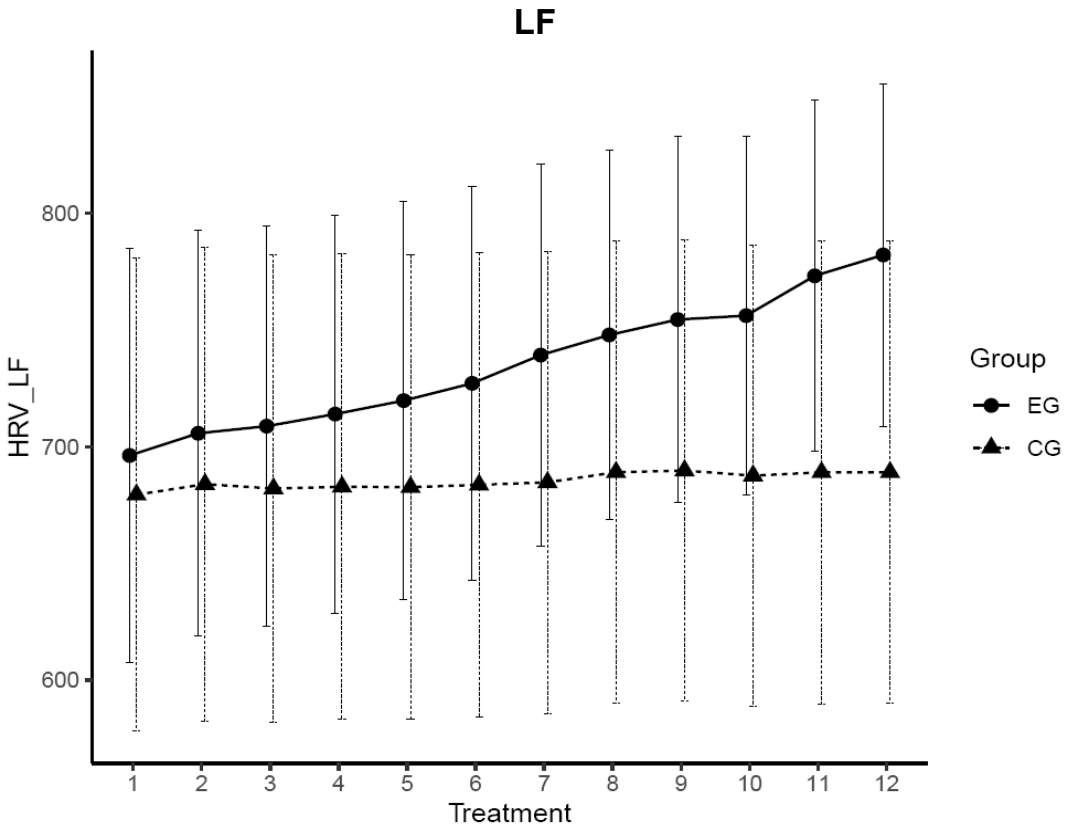


Figure 35. **Line Graph of LF change throughout treatment**

- EG's LF change throughout treatment weeks had a positive trend with a slope value of 7.630, while CG's LF change had a flat trend with a low slope value of 0.856.

Table 27 provides a comparison of data between two groups, the 'EG' (Experimental Group) and 'CG' (Active-Control Group), regarding their LF measurements throughout the 12-week of treatments from: before 1st treatment, after 6th treatment and after 12th treatment. Additionally, it includes *p*-values and Cohen's *d* effect size values to assess the significance and magnitude of observed change between groups.

Table 27. **Comparison of LF change throughout treatment between groups**

	LF			
Treatment	EG	CG	*p*-value*	Cohen's *d*
1st	696.2 ± 376.10	679.4 ± 417.23	0.987	0.042
6th	727.1 ± 357.49	683.6 ± 410.78	0.830	0.113
12th	782.1 ± 311.43	689.0 ± 408.10	0.452	0.256

* Mann-Whitney U Test

- Experimental Group (EG):

  Before 1st treatment, the group's mean LF measurement was 696.2 ± 376.10, and after 6th treatment, it increased to 727.1 ± 357.49. After the final 12th treatment, mean LF of EG increased further to 782.1 ± 311.43.

- Active-Control (CG):

  Before 1st treatment, the group's mean LF measurement was 679.4 ± 417.23, and after 6th treatment, it increased to 683.6 ± 410.78. After final 12th treatment, the mean LF of CG increased slightly to 689.0 ± 408.10.

- *p*-value*:

  The *p*-values in this column are indicative of whether the two groups' LF measurements are statistically significant. From the LF change measured before 1st

treatment, after 6th treatment and after 12th treatment, *p*-values were 0.370, 0.080 and 0.452, with all *p*-values greater than 0.05, indicating that the two groups were not statistically significant from each other.

- Cohen's *d*:

  Before 1st treatment, Cohen's *d* was 0.042, indicating negligible effect size. Following Cohen's *d* value of 0.113 after 6th treatment, still indicating negligible effect size, and then Cohen's *d* value of 0.256 after final 12th treatment, indicating small effect size in LF change between the groups.

Summarizing the LF changes through treatment, Table 27 compared the changes of LF through the treatment weeks between EG and CG, along with their associated statistical significance (*p*-values) and effect size (Cohen's *d*). The table's results indicated that EG's and CG's LF changes were not statistically significant, but they have small magnitudes of difference after the 12th treatments. (Reason will be discussed in the next chapter.)

Figure 36 provides a bar graph comparing the mean LF change between two groups, the 'EG' (Experimental Group) and 'CG' (Active-Control Group), throughout treatments (from before 1st, after 6th and after 12th treatments). This is a visual representation of the data from Table 27.

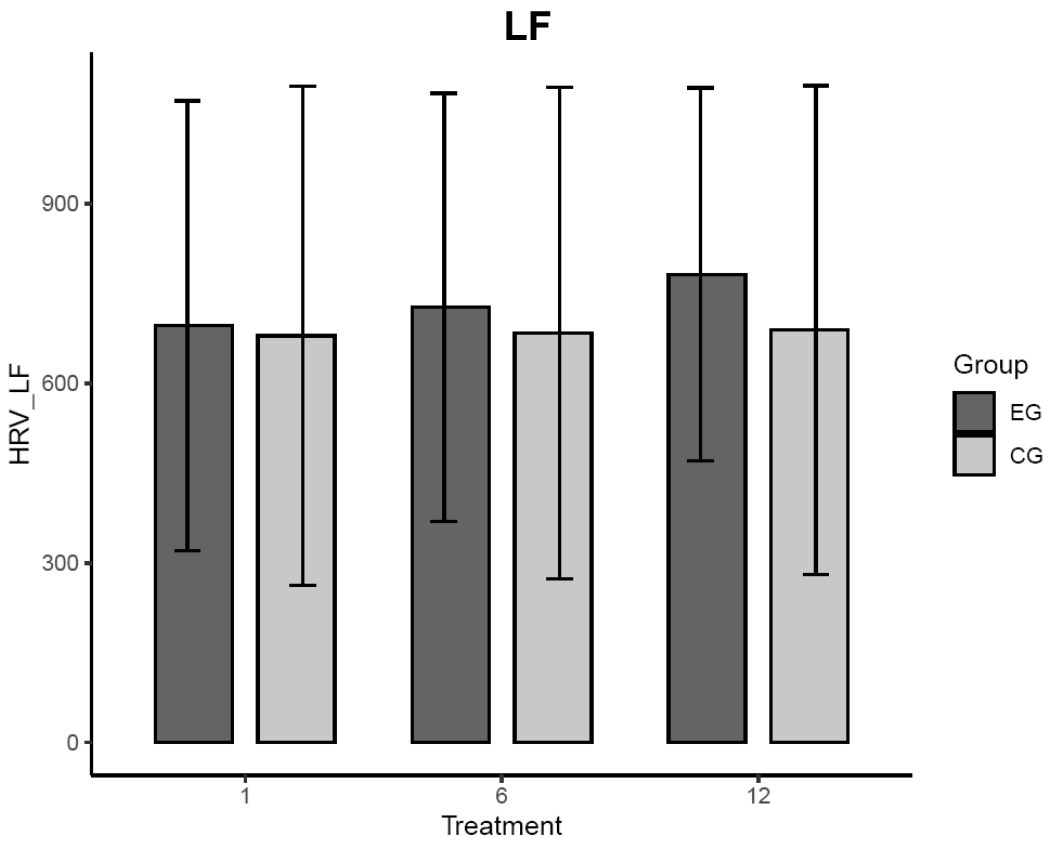


Figure 36. **Bar Graph of LF change throughout treatment**

Figure 37 provides a boxplot graph that visually compares the distribution of numerical LF data and skewness throughout treatment (from before 1st, after 6th and after 12th treatments), between two groups: the 'EG' (Experimental Group) and 'CG' (Active-Control Group).

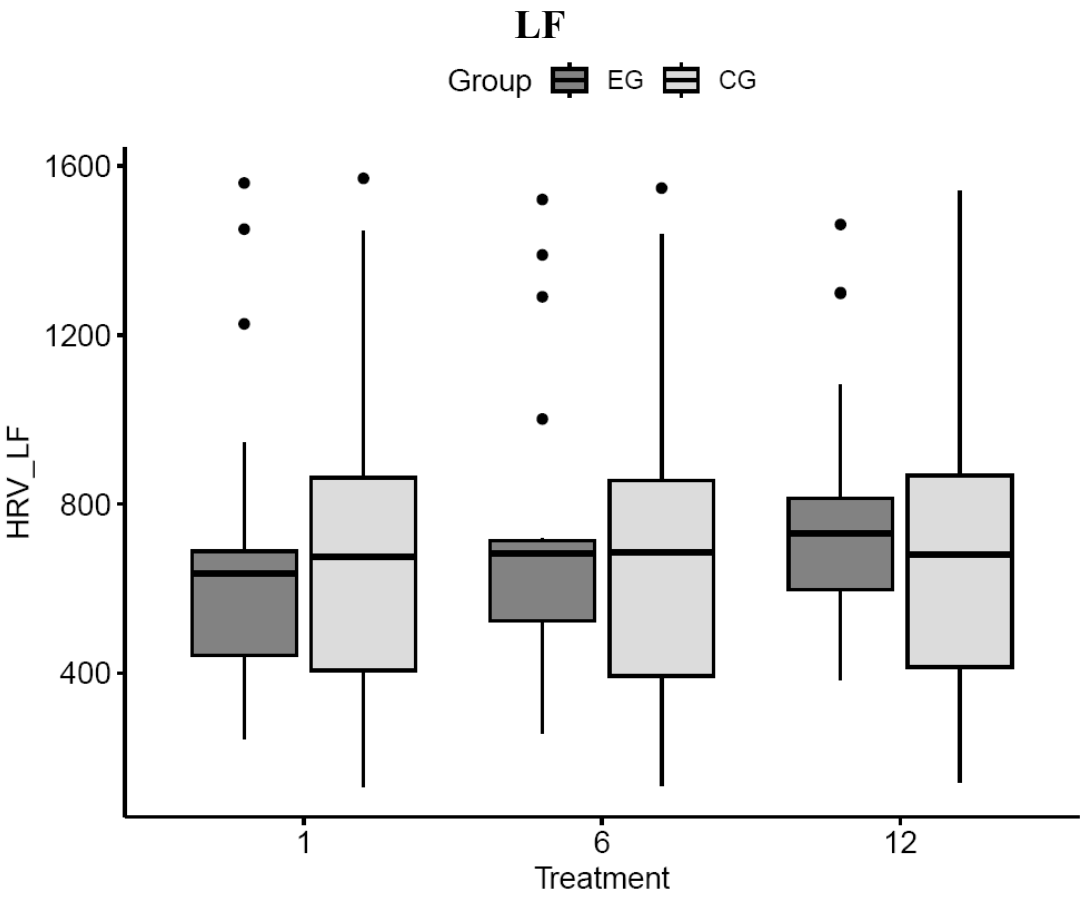


Figure 37. **Boxplot of LF change throughout treatment**

- Before 1st treatments, the positions of the groups' Interquartile Range (IQR) boxes were aligned side by side, suggesting that the group's LF were similar in the beginning. After 12th treatment, the position of groups' IQR boxes shifted slightly but are still at the same level, suggesting that the groups' LF changes were still not significantly different from each other at the end of trial.

3.7.2. LF Difference throughout Treatment

Table 28 provides a comparison of LF differences within each groups' LF before and after treatment. Specifically, it compares each groups' LF from before 1st week treatment with after 6th treatment, following with, comparing data between each groups' LF from before 1st week treatment with after 12th treatment. Additionally, it includes *p*-values to assess the significance between the LF difference before and after treatment within each group.

Table 28. **Comparison of LF difference within groups**

		LF	
Group	Treatment	Difference	*p*-value*
EG	6th - 1st	30.9 ± 37.38	0.003
	12th - 1st	85.9 ± 92.14	0.005
CG	6th - 1st	4.2 ± 12.03	0.166
	12th - 1st	9.6 ± 14.95	0.022

* Paired samples t-Test

- Experimental Group (EG): Between the 1st and 6th treatments, EG's LF difference was 30.9 ± 37.38. Between the 1st and 12th treatments, EG's LF difference increased to 85.9 ± 92.14. The *p*-values were 0.003 from before 1st and after 6th treatment and 0.005, where *p*-values were both less than 0.05, indicating there was statistical significant difference within EG's before and after treatment.
- Active-Control (CG): Between the 1st and 6th treatments, CG's LF difference was 4.2 ± 12.03. *P*-value was 0.166, where *p*-value was greater than 0.05, indicating there were no statistical significant differences within CG's before and after

treatment from 1st to 6th treatments. Between the 1st and 12th treatments, CG's LF difference increased slightly to 9.6 ± 14.95, *p*-value was 0.022, with *p*-value here lesser than 0.05, indicating there was statistical significant difference within CG's before and after treatment from 1st to 12th treatment.

Table 29 provides a comparison for the LF differences before and after treatments between groups. First, it compares EG's and CG's difference between LF from before 1st and after 6th treatment. Second, it compares EG's and CG's difference between LF from before 1st and after 12th treatment. Additionally, it includes *p*-values to assess the significance between each groups' LF difference.

Table 29. **Comparison of LF difference between groups**

	LF			
Treatment	EG	CG	*p*-value*	Cohen's *d*
6th - 1st	30.9 ± 37.38	4.2 ± 12.03	0.002	0.948
12th - 1st	85.9 ± 92.14	9.6 ± 14.95	0.000	1.140

* Mann-Whitney U Test

- Between the 1st and 6th treatments, EG's LF mean difference was 30.9 ± 37.38, and CG's LF mean difference was 4.2 ± 12.03; showing EG's mean LF difference was 26.7 points higher than CG's mean LF difference. Between the 1st and 12th treatments, EG's LF mean difference increased to 85.9 ± 92.14, and CG's LF mean difference increased to 9.6 ± 14.95; showing EG's mean LF difference was 76.3 points higher than CG's mean LF difference.

- *p*-values: Between groups from 1st–6th treatment and from 1st–12th treatment, *p*-values were 0.002 and 0.000, where *p*-values were both less than 0.05, indicating these were statistically significant differences between the groups' LF difference valves.
- Cohen's *d*: From 1st to 6th treatment, Cohen's *d* was 0.948, indicating large effect size. Following Cohen's *d* value was 1.140 from 1st to 12th treatment, also indicating very large effect size.

Summarizing the LF difference through treatment, Tables 28 compared the LF differences within each groups' before and after treatment, and Table 29 compared the LF differences between Experimental Group (EG) and Active-Control Group (CG). For LF differences within each groups' before and after treatment, results indicated that both groups' treatments did have statistical significance. For between the two groups' LF difference throughout treatment, the groups' results were statistically significant, with a very large magnitude of difference. Comparing the LF difference between EG and CG, EG had the higher LF difference throughout treatment.

Figure 38 provides a bar graph comparing the mean LF difference between two groups, the ‘EG’ (Experimental Group) and ‘CG’ (Active-Control Group), throughout the 12-week of treatments. This is a visual representation of the data displayed from Table 28 and Table 29.

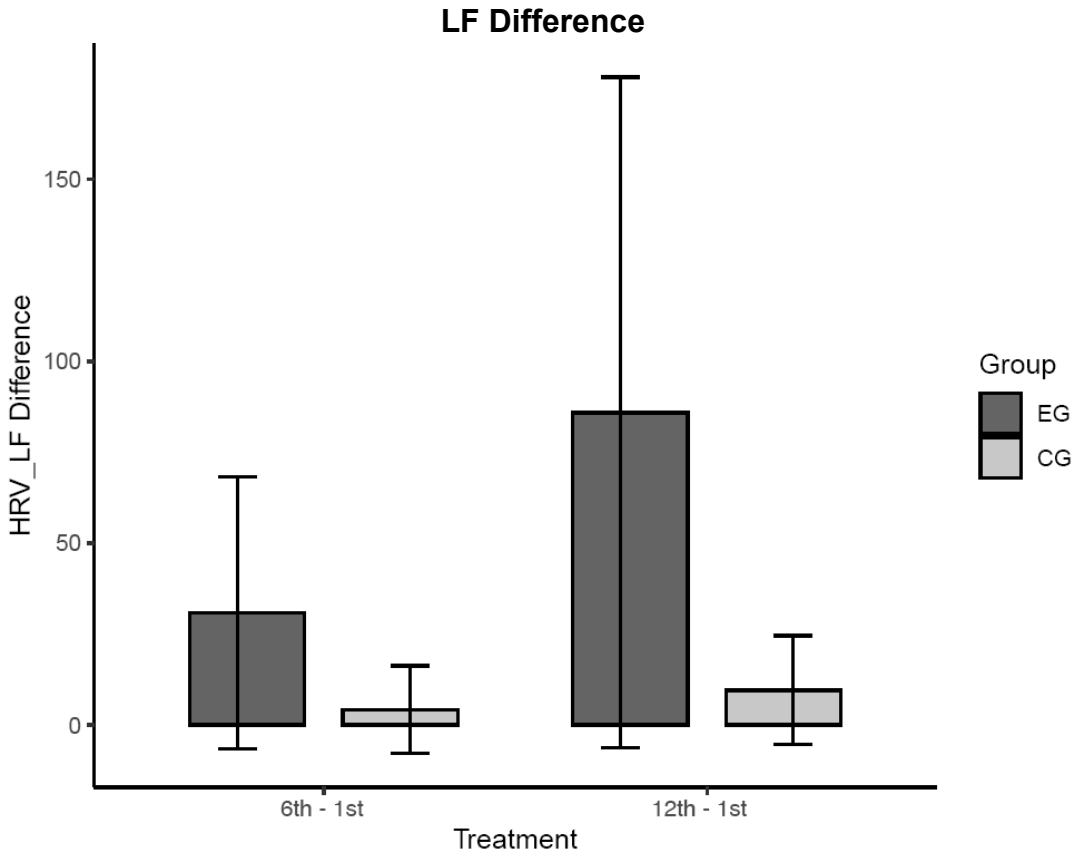


Figure 38. **Bar Graph of LF Difference throughout treatment**

Figure 39 provides a boxplot graph that visually compares the skewness and distribution of numerical LF difference values throughout treatment, between two groups: the ‘EG’ (Experimental Group) and ‘CG’ (Active-Control Group).

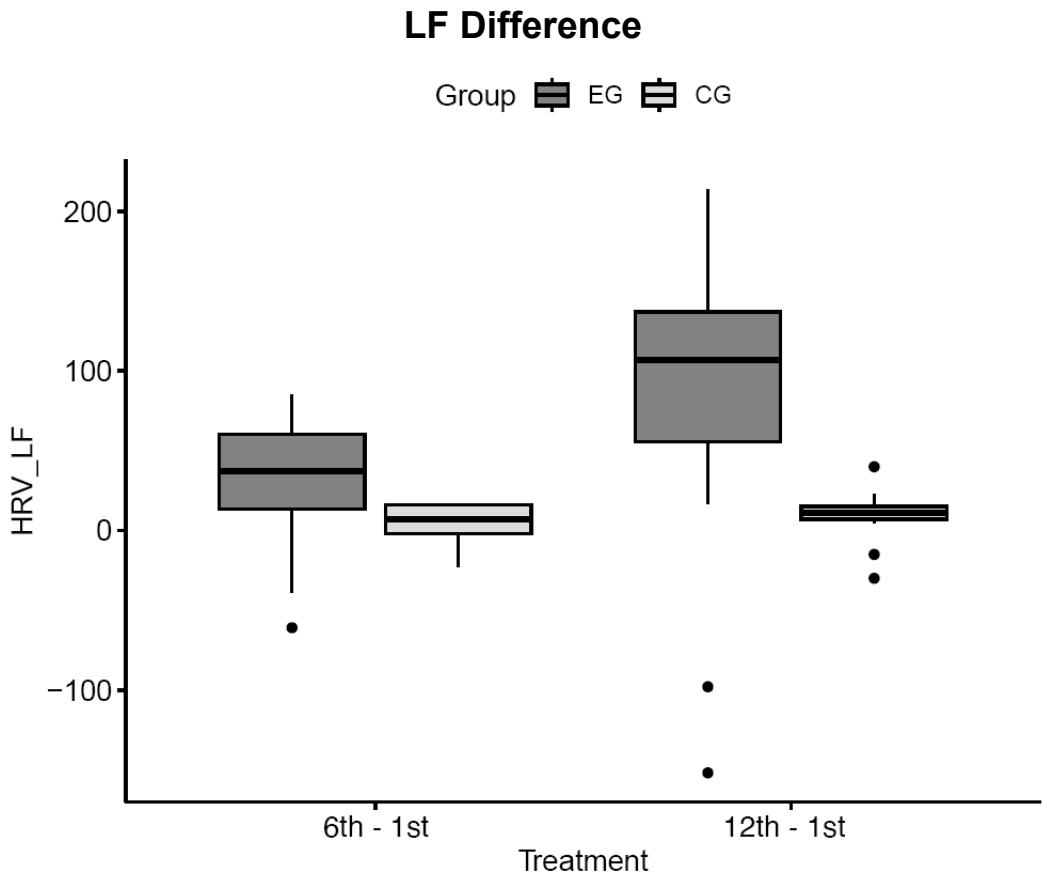


Figure 39. **Boxplot of LF Difference throughout treatment**

- In both time instances, both groups’ positions of the Interquartile Range (IQR) boxes were not at level, suggesting that the groups’ LF mean differences were actually different after the 6th treatment and after 12th treatment.

3.8. HF

HF (known as High Frequency Power) was the 6th of the seven Heart Rate Variability (HRV) parameters data collected. HF below 220 ms^2 is low, between 220-1200 ms^2 is normal range, and above 1200 ms^2 is high.

3.8.1. HF Change throughout Treatment

Figure 40 provides a line graph comparing the mean HF change throughout the 12-weeks of treatment, between two groups—the 'EG' (Experimental Group) and 'CG' (Active-Control Group).

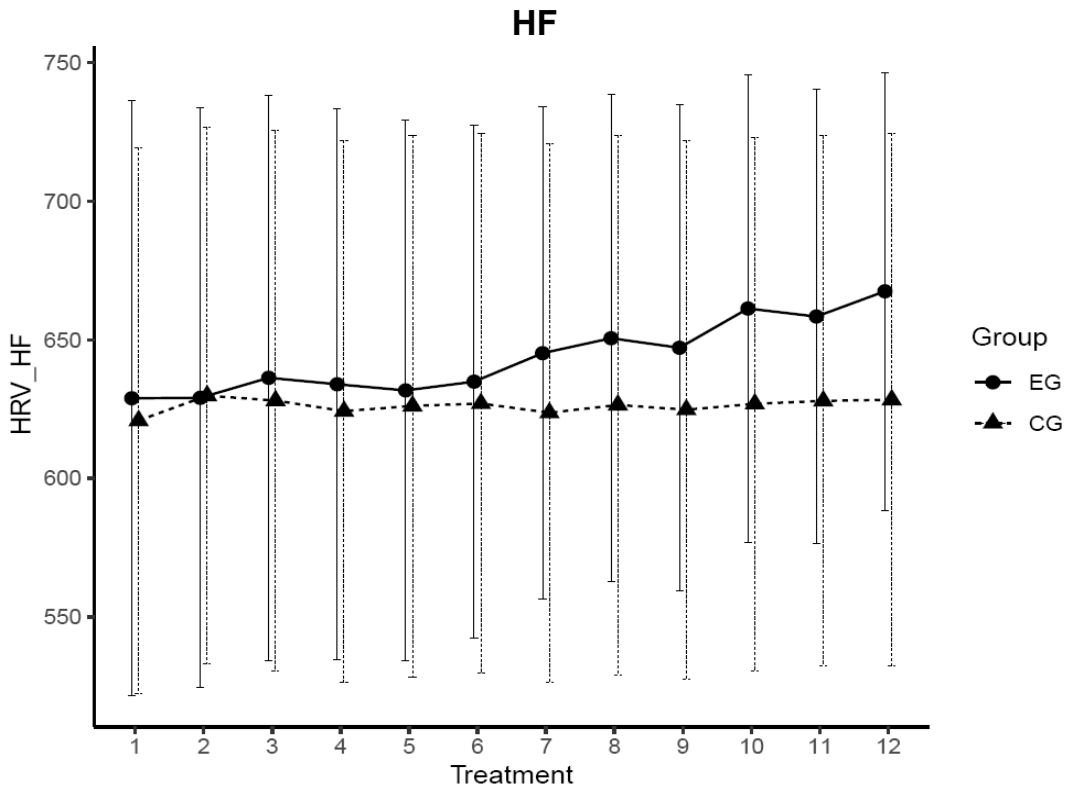


Figure 40. **Line Graph of HF change throughout treatment**

- EG's HF change throughout treatment weeks had a positive trend with a slope value of 3.484, while CG's HF change had a flat trend with a low slope value of 0.202.

Table 30 provides a comparison of data between two groups, the 'EG' (Experimental Group) and 'CG' (Active-Control Group), regarding their HF measurements throughout the 12-week of treatments from: before 1st treatment, after 6th treatment and after 12th treatment. Additionally, it includes *p*-values and Cohen's *d* effect size values to assess the significance and magnitude of observed change between groups.

Table 30. **Comparison of HF change throughout treatment between groups**

	HF			
Treatment	EG	CG	*p*-value*	Cohen's *d*
1st	628.9 ± 455.63	620.8 ± 405.95	0.804	0.019
6th	643.9 ± 393.05	627.0 ± 401.25	0.729	0.043
12th	667.5 ± 335.26	628.4 ± 396.77	0.428	0.106

* Mann-Whitney U Test

- Experimental Group (EG):

  Before 1st treatment, the group's mean HF measurement was 628.9 ± 455.63, and after 6th treatment, it increased to 643.9 ± 393.05. After the final 12th treatment, mean HF of EG increased slightly to 667.5 ± 335.26.

- Active-Control (CG):

  Before 1st treatment, the group's mean HF measurement was 620.8 ± 405.95, and after 6th treatment, it increased to 627.0 ± 401.25. After final 12th treatment, the mean HF of CG increased slightly to 628.4 ± 396.77.

- *p*-value*:

  The *p*-values in this column are indicative of whether the two groups' HF measurements are statistically significant. From the HF change measured before 1st

treatment, after 6th treatment and after 12th treatment, *p*-values were 0.804, 0.729 and 0.428, with all *p*-values greater than 0.05, indicating that the two groups were not statistically significant from each other.

- Cohen's *d*:

  Before 1st treatment, Cohen's *d* was 0.019, indicating negligible effect size. Following Cohen's *d* value of 0.043 after 6th treatment, also indicating negligible effect size, and then Cohen's *d* value of 0.106 after final 12th treatment, indicating still negligible effect size in HF measurements between the groups.

Summarizing the HF changes through treatment, Table 30 compared the changes of HF through the treatment weeks between EG and CG, along with their associated statistical significance (*p*-values) and effect size (Cohen's *d*). The table's results indicated that EG's and CG's HF changes were not statistically significant and have negligible magnitude of difference after the 12th treatments.

Figure 41 provides a bar graph comparing the mean HF change between two groups, the 'EG' (Experimental Group) and 'CG' (Active-Control Group), throughout treatments (from before 1st, after 6th and after 12th treatments). This is a visual representation of the data from Table 30.

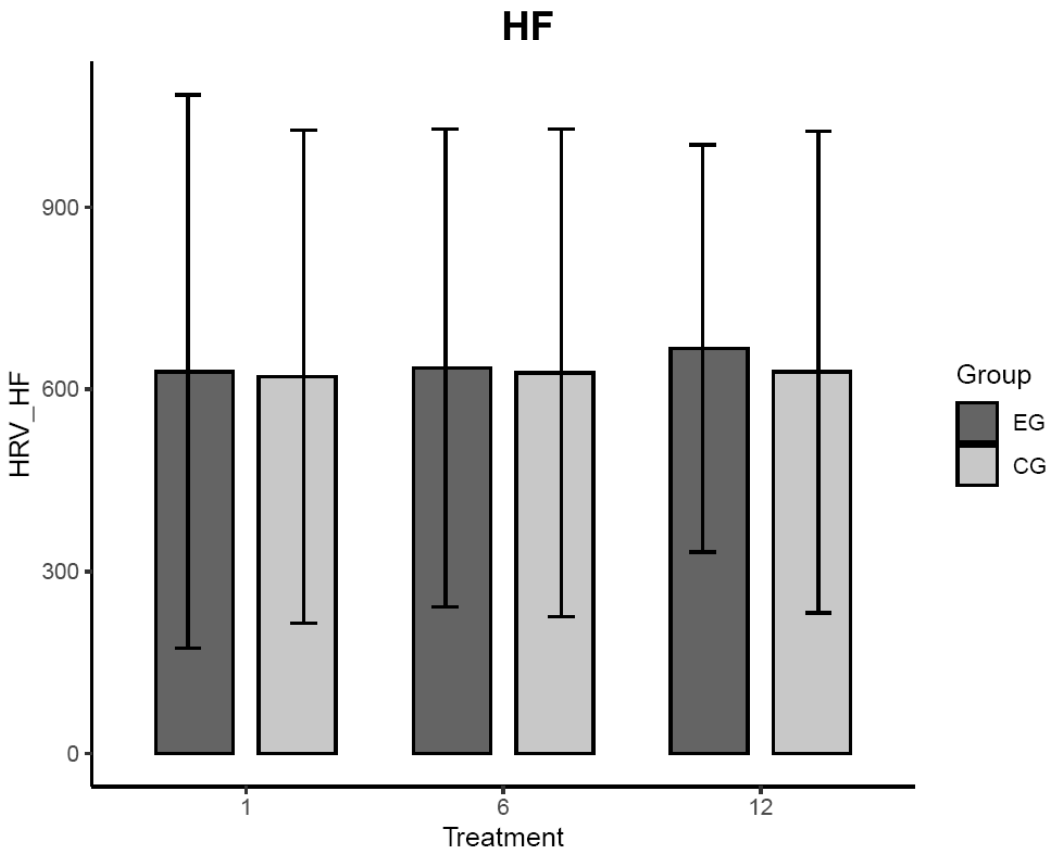


Figure 41. **Bar Graph of HF change throughout treatment**

Figure 42 provides a boxplot graph that visually compares the distribution of numerical HF data and skewness throughout treatment (from before 1st, after 6th and after 12th treatments), between two groups: the ‘EG’ (Experimental Group) and ‘CG’ (Active-Control Group).

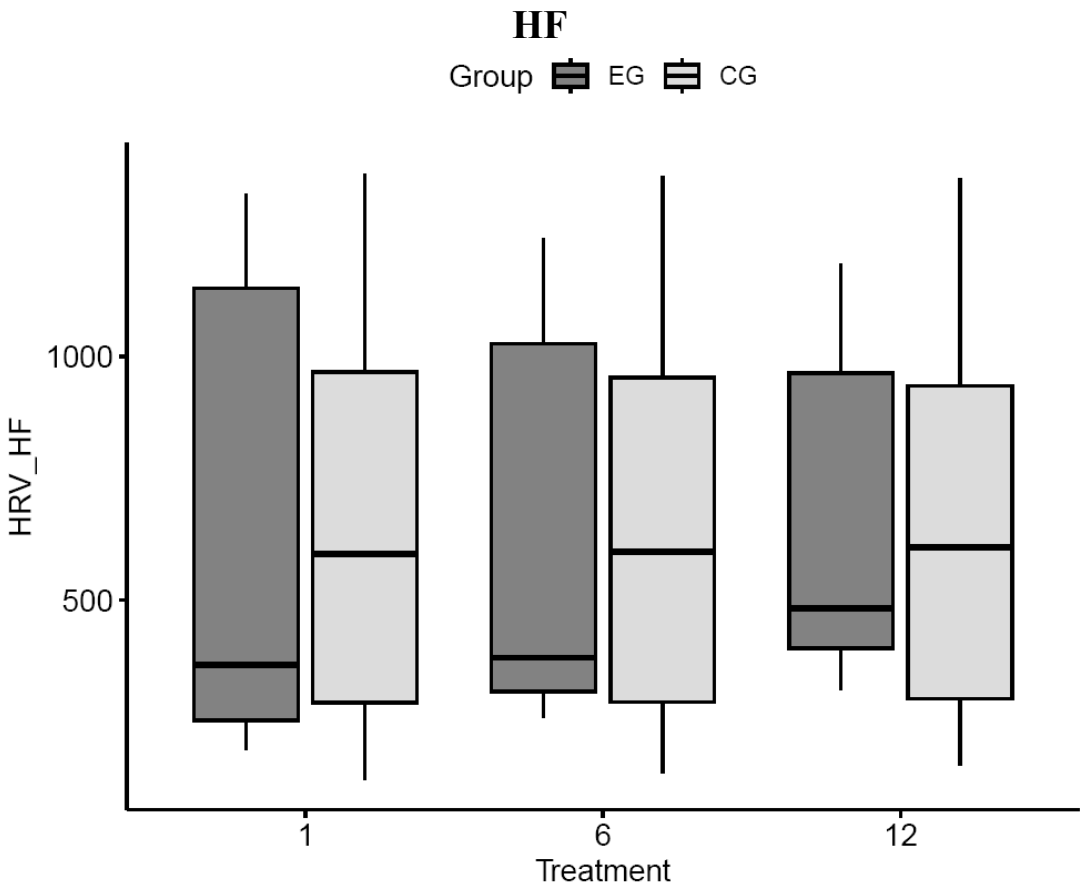


Figure 42. **Boxplot of HF change throughout treatment**

- Before 1st treatments, the positions of the groups’ Interquartile Range (IQR) boxes were aligned side by side, suggesting that the group’s HF were similar in the beginning. After 12th treatment, the position of groups’ IQR boxes were still at the same level, suggesting that the groups’ HF changes were still not significantly different from each other at the end of trial.

3.8.2. HF Difference throughout Treatment

Table 31 provides a comparison of HF differences within each groups' HF before and after treatment. Specifically, it compares each groups' HF from before 1st week treatment with after 6th treatment, following with, comparing data between each groups' HF from before 1st week treatment with after 12th treatment. Additionally, it includes *p*-values to assess the significance between the HF difference before and after treatment within each group.

Table 31. **Comparison of HF difference within groups**

		HF	
Group	Treatment	Difference	*p*-value*
EG	6th - 1st	6.0 ± 73.91	0.571
	12th - 1st	38.6 ± 138.34	0.316
CG	6th - 1st	6.2 ± 17.02	0.155
	12th - 1st	7.5 ± 20.09	0.058

* Paired samples t-Test

- Experimental Group (EG): Between the 1st and 6th treatments, EG's HF difference was 6.0 ± 73.91. Between the 1st and 12th treatments, EG's HF difference increased to 38.6 ± 138.34. The *p*-values were 0.571 from before 1st and after 6th treatment and 0.316, where *p*-values were both greater than 0.05, indicating there were no statistical significant differences within EG's before and after treatment.
- Active-Control (CG): Between the 1st and 6th treatments, CG's HF difference was 6.2 ± 17.02. *P*-value was 0.155, where *p*-value was greater than 0.05, indicating there were no statistical significant differences within CG's before and after

treatment from 1st to 6th treatments. Between the 1st and 12th treatments, CG's HF difference increased slightly to 7.5 ± 20.09, *p*-value was 0.058, with *p*-value here is equal to 0.05, indicating there was a marginal statistical significant difference within CG's before and after treatment from 1st to 12th treatment.

Table 32 provides a comparison for the HF differences before and after treatments between groups. First, it compares EG's and CG's difference between HF from before 1st and after 6th treatment. Second, it compares EG's and CG's difference between HF from before 1st and after 12th treatment. Additionally, it includes *p*-values to assess the significance between each groups' HF difference.

Table 32. **Comparison of HF difference between groups**

	HF			
Treatment	EG	CG	*p*-value*	Cohen's *d*
6th - 1st	6.0 ± 73.91	6.2 ± 17.02	0.117	0.003
12th - 1st	38.6 ± 138.34	7.5 ± 20.09	0.026	0.310

* Mann-Whitney U Test

- Between the 1st and 6th treatments, EG's HF mean difference was 6.0 ± 73.91, and CG's HF mean difference was 6.2 ± 17.02; showing CG's mean HF difference was 0.2 points higher than EG's mean HF difference. Between the 1st and 12th treatments, EG's HF mean difference increased to 38.6 ± 138.34, and CG's HF mean difference increased to 7.5 ± 20.09; showing now EG's mean HF difference was 31.1 points higher than CG's mean HF difference.

- *p*-values: Between groups from 1st–6th treatment and from 1st–12th treatment, *p*-values were 0.117 and 0.026. From 1st–6th treatment, *p*-value was greater than 0.05, indicating there was not a statistically significant difference between the groups' HF difference valves. On the contrary, from 1st–12th treatment, *p*-value was lesser than 0.05, indicating there was a statistically significant difference between the groups' HF difference valves.
- Cohen's *d*: From 1st to 6th treatment, Cohen's *d* was 0.003, indicating negligible effect size. Following Cohen's *d* value was 0.310 from 1st to 12th treatment, also indicating small effect size.

Summarizing the HF difference through treatment, Tables 31 compared the HF differences within each groups' before and after treatment, and Table 32 compared the HF differences between Experimental Group (EG) and Active-Control Group (CG). For HF differences within each groups' before and after treatment, results indicated that EG's treatments did not have statistical significance, but CG's treatments did have statistical significance. For between the two groups' HF difference throughout treatment, the groups' results were not statistically significant from 1st-6th treatment, but the groups' results were statistically significant from 1st-12th treatment, with a small magnitude of difference. Comparing the HF difference between EG and CG, EG had the higher HF difference at 12th treatment.

Figure 43 provides a bar graph comparing the mean HF difference between two groups, the ‘EG’ (Experimental Group) and ‘CG’ (Active-Control Group), throughout the 12-week of treatments. This is a visual representation of the data displayed from Table 31 and Table 32.

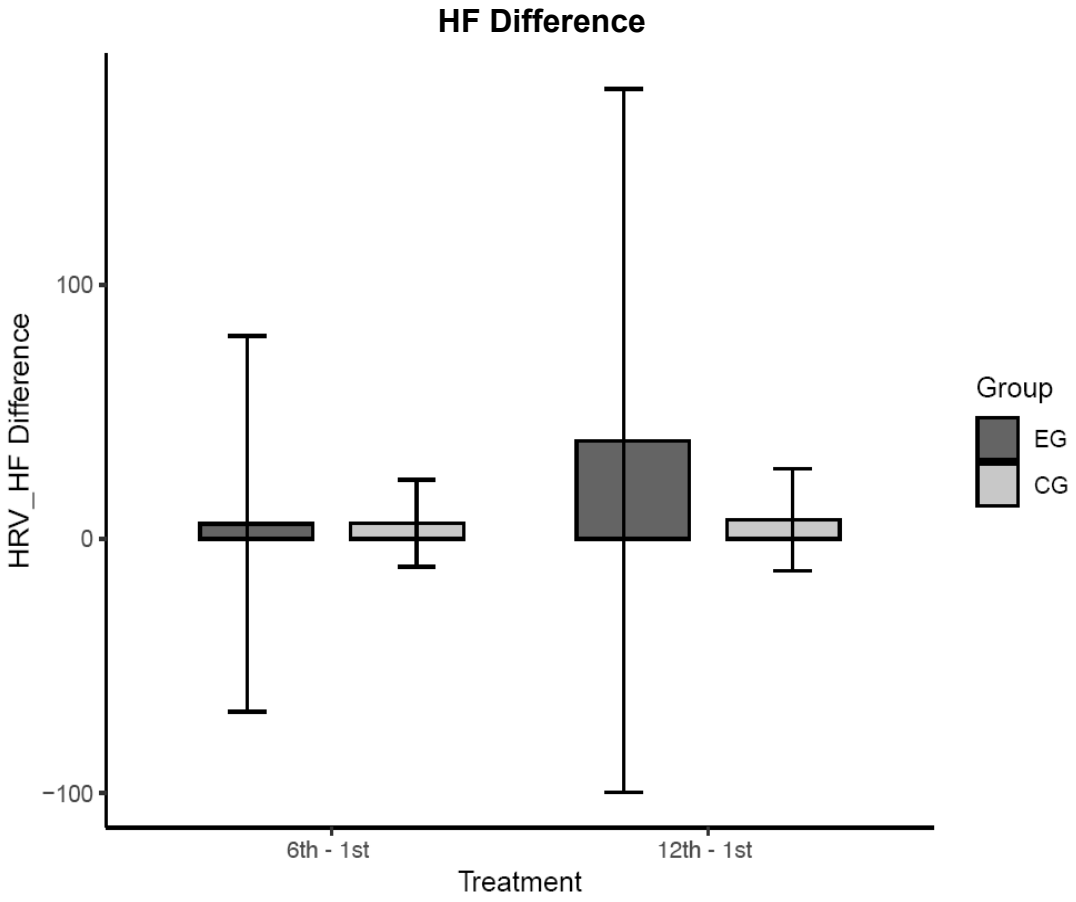


Figure 43. **Bar Graph of HF Difference throughout treatment**

Figure 44 provides a boxplot graph that visually compares the skewness and distribution of numerical HF difference values throughout treatment, between two groups: the ‘EG’ (Experimental Group) and ‘CG’ (Active-Control Group).

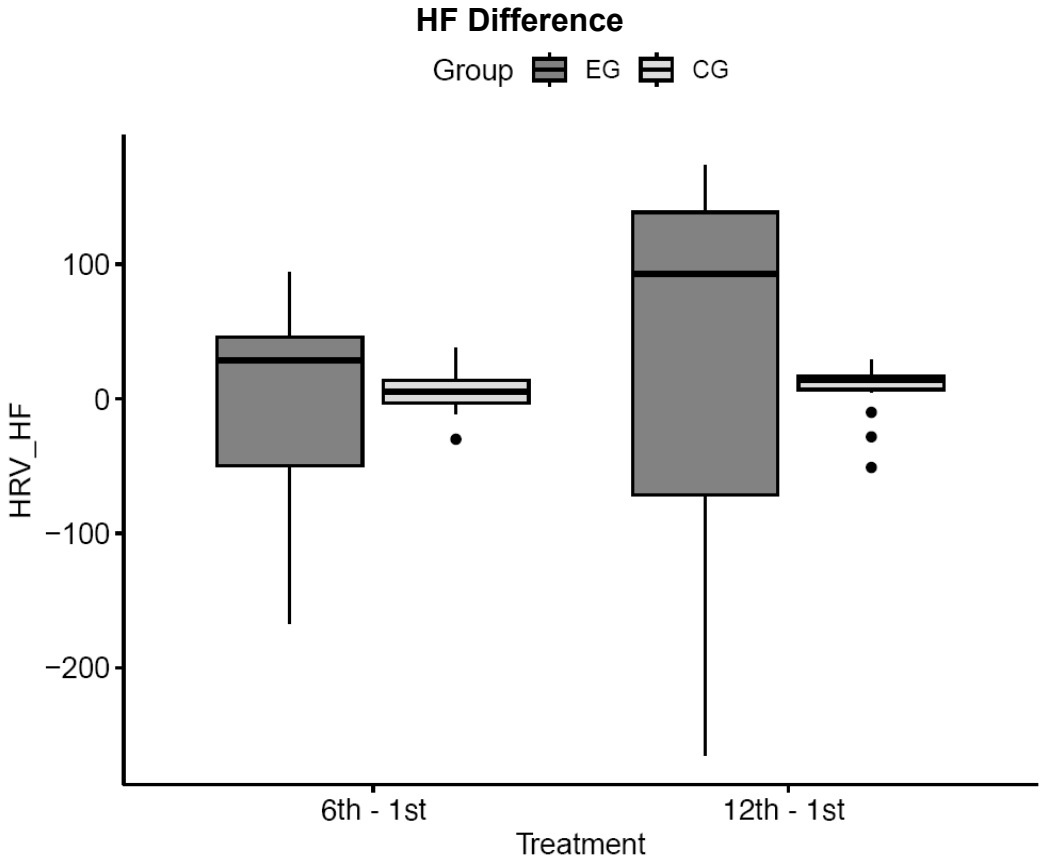


Figure 44. **Boxplot of HF Difference throughout treatment**

- In both time instances, both groups’ positions of the Interquartile Range (IQR) boxes were at level, suggesting that the groups’ HF mean differences were actually not different from each other, after the 6th treatment and after 12th treatment.

3.9. LF/HF

LF/HF (known as Low-to-High Frequency Power Ratio) was the last of the seven Heart Rate Variability (HRV) parameters data collected. LF/HF below 1 is low, between 1-2 is normal range, and above 2 is high.

3.9.1. LF/HF Change throughout Treatment

Figure 45 provides a line graph comparing the mean LF/HF change throughout the 12-weeks of treatment, between two groups—the 'EG' (Experimental Group) and 'CG' (Active-Control Group).

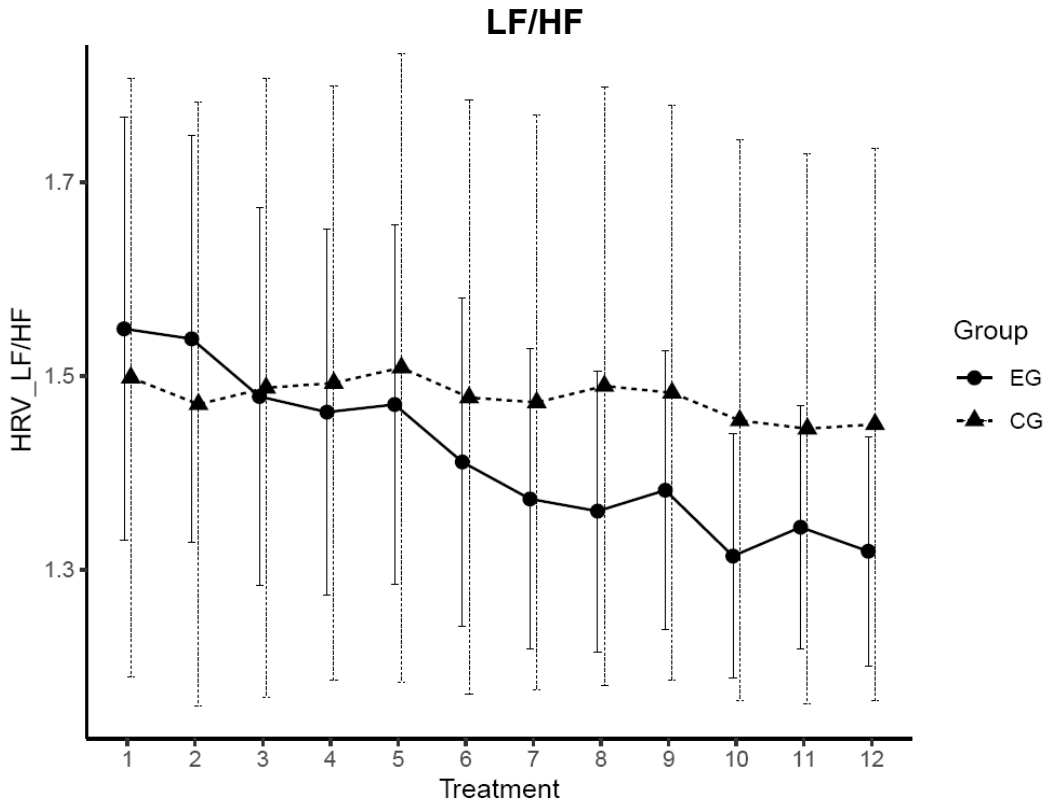


Figure 45. **Line Graph of LF/HF change throughout treatment**

- EG's LF/HF change throughout treatment weeks had a negative trend with a slope value of -0.022, while CG's LF/HF had a negative trend with slope of -0.004.

Table 33 provides a comparison of data between two groups, the 'EG' (Experimental Group) and 'CG' (Active-Control Group), regarding their LF/HF measurements throughout the 12-week of treatments from: before 1st treatment, after 6th treatment and after 12th treatment. Additionally, it includes *p*-values and Cohen's *d* effect size values to assess the significance and magnitude of observed change between groups.

Table 33. **Comparison of LF/HF change throughout treatment between groups**

	LF/HF			
Treatment	EG	CG	*p*-value*	Cohen's d
1st	1.5 ± 0.93	1.5 ± 1.27	0.632	0.000
6th	1.4 ± 0.72	1.5 ± 1.26	0.668	0.100
12th	1.3 ± 0.50	1.5 ± 1.17	0.478	0.222

* Mann-Whitney U Test

- Experimental Group (EG):

  Before 1st treatment, the group's mean LF/HF measurement was 1.5 ± 0.93, and after 6th treatment, it dropped to 1.4 ± 0.72. After the final 12th treatment, mean LF/HF of EG decreased slightly to 1.3 ± 0.50.

- Active-Control (CG):

  Before 1st treatment, the group's mean LF/HF measurement was 1.5 ± 1.27, and after 6th treatment, it stayed the same around 1.5 ± 1.26. After final 12th treatment, the mean LF/HF of CG again stayed the same around 1.5 ± 1.17.

- *p*-value*:

  The *p*-values in this column are indicative of whether the two groups' LF/HF measurements are statistically significant. From the LF/HF change measured before

1st treatment, after 6th treatment and after 12th treatment, *p*-values were 0.632, 0.668 and 0.478, with all *p*-values greater than 0.05, indicating that the two groups were not statistically significant from each other.

- Cohen's *d*:

  Before 1st treatment, Cohen's *d* was 0.000, indicating no effect size. Following Cohen's *d* value of 0.100 after 6th treatment, also indicating negligible effect size, and then Cohen's *d* value of 0.222 after final 12th treatment, indicating small effect size in LF/HF measurements between the groups.

Summarizing the LF/HF changes through treatment, Table 33 compared the changes of LF/HF through the treatment weeks between EG and CG, along with their associated statistical significance (*p*-values) and effect size (Cohen's *d*). The table's results indicated that EG's and CG's LF/HF changes were not statistically significant and have small effect size after the 12th treatments.

Figure 46 provides a bar graph comparing the mean LF/HF change between two groups, the ‘EG’ (Experimental Group) and ‘CG’ (Active-Control Group), throughout treatments (from before 1st, after 6th and after 12th treatments). This is a visual representation of the data from Table 33.

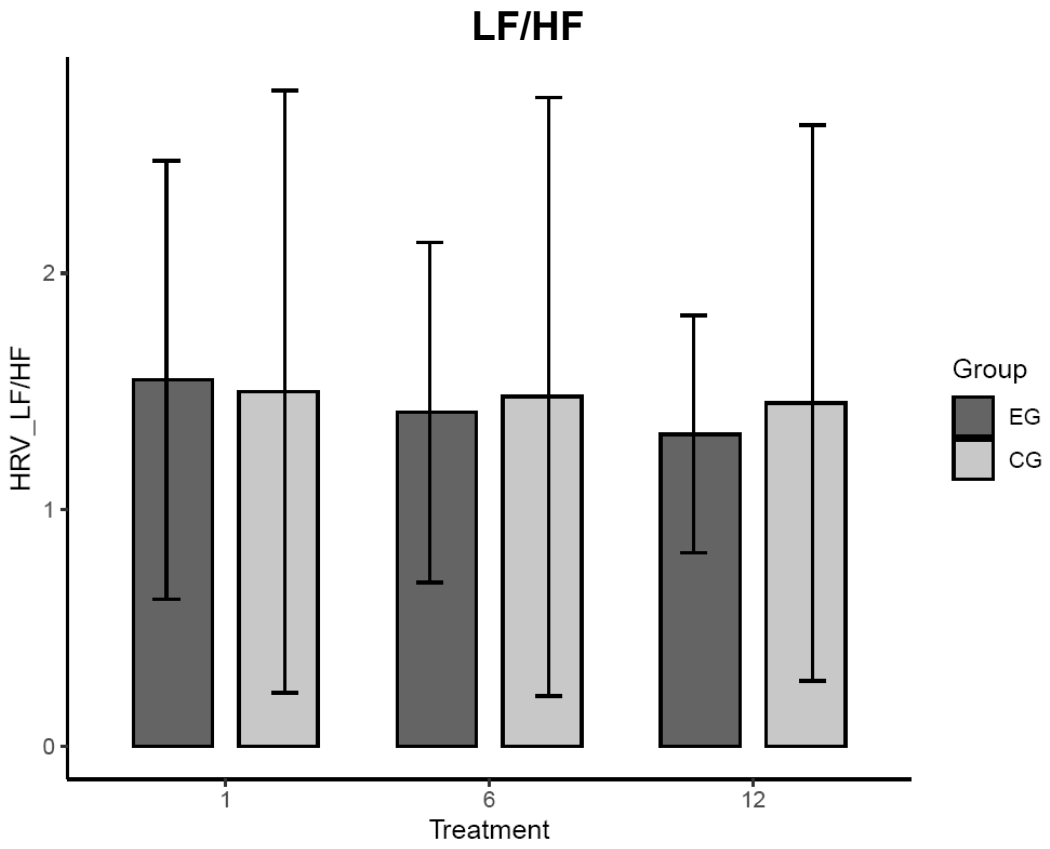


Figure 46. **Bar Graph of LF/HF change throughout treatment**

Figure 47 provides a boxplot graph that visually compares the distribution of numerical LF/HF data and skewness throughout treatment (from before 1st, after 6th and after 12th treatments), between two groups: the 'EG' (Experimental Group) and 'CG' (Active-Control Group).

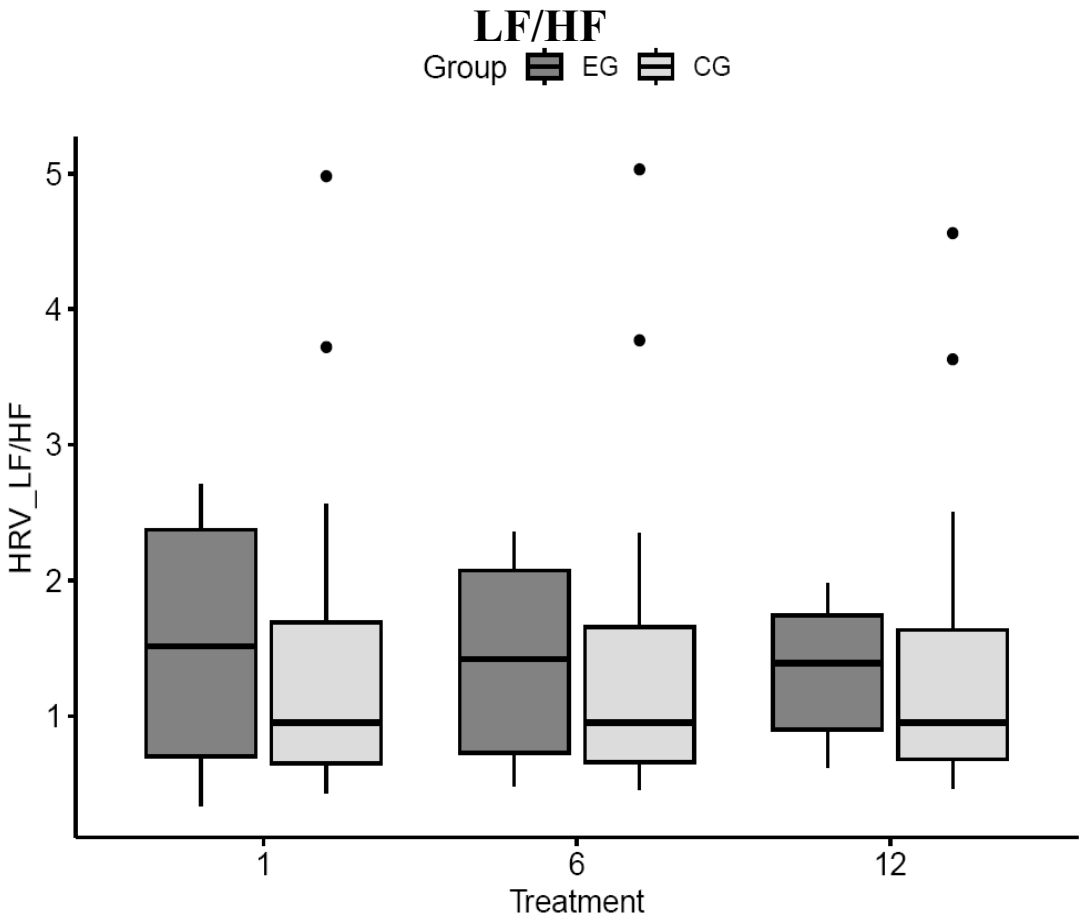


Figure 47. **Boxplot of LF/HF change throughout treatment**

- Before 1st treatments, the positions of the groups' Interquartile Range (IQR) boxes were aligned side by side, suggesting that the group's LF/HF were similar in the beginning. After 12th treatment, the position of groups' IQR boxes were still at the same level, suggesting that the groups' LF/HF changes were still not significantly different from each other at the end of trial.

3.9.2. LF/HF Difference throughout Treatment

Table 34 provides a comparison of LF/HF differences within each groups' LF/HF before and after treatment. Specifically, it compares each groups' LF/HF from before 1st week treatment with after 6th treatment, following with, comparing data between each groups' LF/HF from before 1st week treatment with after 12th treatment. Additionally, it includes *p*-values to assess the significance between the LF/HF difference before and after treatment within each group.

Table 34. **Comparison of LF/HF difference within groups**

		LF/HF	
Group	Treatment	Difference	*p*-value*
EG	6th - 1st	0.14 ± 0.27	0.080
	12th - 1st	0.23 ± 0.45	0.081
CG	6th - 1st	0.02 ± 0.08	0.776
	12th - 1st	0.05 ± 0.12	0.198

* Paired samples t-Test

- Experimental Group (EG): Between the 1st and 6th treatments, EG's LF/HF difference was 0.14 ± 0.27. Between the 1st and 12th treatments, EG's LF/HF difference increased to 0.23 ± 0.45. The *p*-values were 0.080 from before 1st and after 6th treatment and 0.081, where *p*-values were both greater than 0.05, indicating there were no statistical significant differences within EG's before and after treatment.
- Active-Control (CG): Between the 1st and 6th treatments, CG's LF/HF difference was 0.02 ± 0.08. Between the 1st and 12th treatments, CG's LF/HF difference increased slightly to 0.05 ± 0.12. *P*-values were 0.776 and 0.198, where both

p-values were greater than 0.05, indicating there were no statistical significant differences within CG's before and after treatment throughout treatments.

Table 35 provides a comparison for the LF/HF differences before and after treatments between groups. First, it compares EG's and CG's difference between LF/HF from before 1st and after 6th treatment. Second, it compares EG's and CG's difference between LF/HF from before 1st and after 12th treatment. Additionally, it includes *p*-values to assess the significance between each groups' LF/HF difference.

Table 35. **Comparison of LF/HF difference between groups**

	LF/HF			
Treatment	EG	CG	*p*-value*	Cohen's *d*
6th - 1st	0.14 ± 0.27	0.02 ± 0.08	0.586	0.580
12th - 1st	0.23 ± 0.45	0.05 ± 0.12	0.111	0.550

* Mann-Whitney U Test

- Between the 1st and 6th treatments, EG's LF/HF mean difference was 0.14 ± 0.27, and CG's LF/HF mean difference was 0.02 ± 0.08; showing EG's mean LF/HF difference was 0.12 points higher than CG's mean LF/HF difference. Between the 1st and 12th treatments, EG's LF/HF mean difference increased to 0.23 ± 0.45, and CG's LF/HF mean difference increased to 0.05 ± 0.12; showing EG's mean LF/HF difference was 0.2 points higher than CG's mean LF/HF difference.
- *p*-values: Between groups from 1st–6th treatment and from 1st–12th treatment, *p*-values were 0.586 and 0.111. Both *p*-values were greater than 0.05, indicating

there was not a statistically significant difference between the groups' LF/HF difference valves.

- Cohen's *d*: From 1st to 6th treatment, Cohen's *d* was 0.580, indicating medium effect size. Following Cohen's *d* value was 0.550 from 1st to 12th treatment, also indicating medium effect size.

Summarizing the LF/HF difference through treatment, Tables 34 compared the LF/HF differences within each groups' before and after treatment, and Table 35 compared the LF/HF differences between Experimental Group (EG) and Active-Control Group (CG). For LF/HF differences within each groups' before and after treatment, results indicated that both groups' treatments did not have statistical significance. For between the two groups' LF/HF difference throughout treatment, the groups' results were also not statistically significant, with a medium magnitude of difference. Comparing the LF/HF difference between EG and CG, EG had the slightly higher LF/HF difference at 12th treatment.

Figure 48 provides a bar graph comparing the mean LF/HF difference between two groups, the ‘EG’ (Experimental Group) and ‘CG’ (Active-Control Group), throughout the 12-week of treatments. This is a visual representation of the data displayed from Table 34 and Table 35.

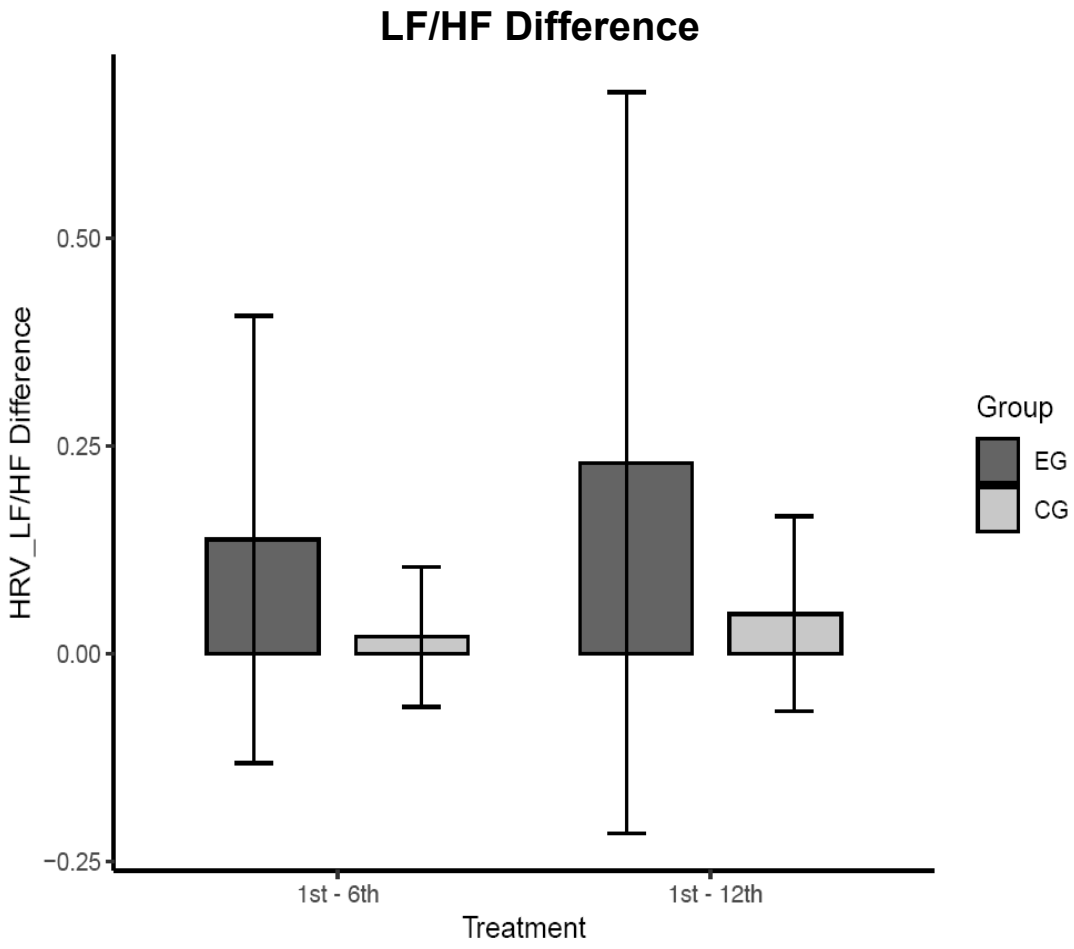


Figure 48. **Bar Graph of LF/HF Difference throughout treatment**

Figure 49 provides a boxplot graph that visually compares the skewness and distribution of numerical LF/HF difference values throughout treatment, between two groups: the 'EG' (Experimental Group) and 'CG' (Active-Control Group).

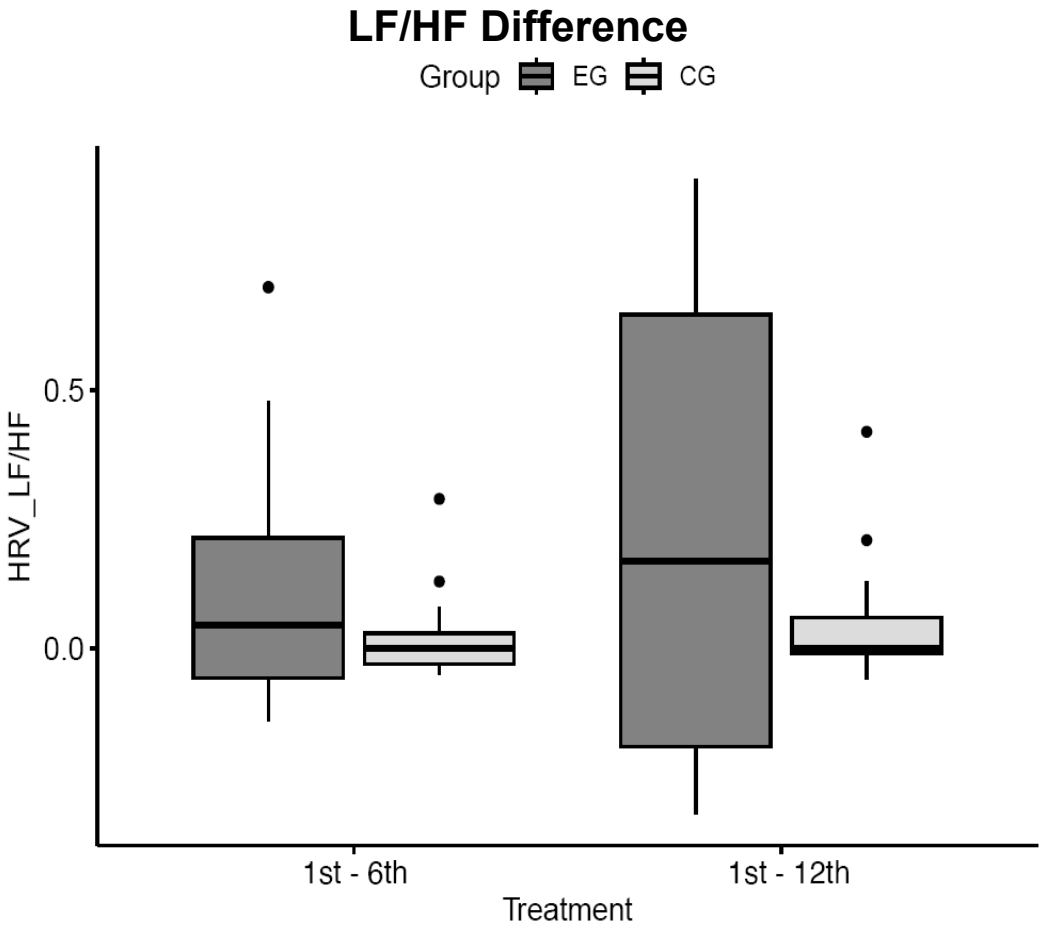


Figure 49. **Boxplot of LF/HF Difference throughout treatment**

- In both time instances, both groups' positions of the Interquartile Range (IQR) boxes were at level, suggesting that the groups' HF mean differences were actually not different from each other, after the 6th treatment and after 12th treatment.

3.10. Perceived Stress Scale (PSS) Score

PSS is measured on a scale of 0 to 40, scoring (0-13) is low stress, (14-26) is moderate stress, and (27-40) is high stress. Before any acupuncture treatment, all participants started with 'high stress' PSS scores ranging anywhere from 27 to 40.

3.10.1. PSS Change throughout Treatment

Table 36 provides a comparison of data between two groups, the 'EG' (Experimental Group) and 'CG' (Active-Control Group), regarding their PSS measurements throughout the 12-week of treatments from: before 1st treatment, after 6th treatment and after 12th treatment. Additionally, it includes *p*-values and Cohen's *d* effect size values to assess the significance and magnitude of observed change between groups.

Table 36. **Comparison of PSS change throughout treatment between groups**

	PSS			
Treatment	EG	CG	*p*-value*	Cohen's d
1st	33.1 ± 3.53	32.4 ± 3.81	0.5494	0.191
6th	27.9 ± 3.23	30.5 ± 3.12	0.0222	0.819
12th	23.2 ± 2.79	27.4 ± 3.32	0.0002	1.369

* Independent Samples t-Test / Mann-Whitney U Test

- Experimental Group (EG): Before 1st treatment, the group's mean PSS measurement was 33.1 ± 3.53, and after 6th treatment, it decreased to 27.9±3.23. After final 12th treatment, mean PSS of EG dropped even further to 23.2±2.79.

- Active-Control (CG): Before 1st treatment, the group's mean PSS measurement was 32.4 ± 3.81, and after 6th treatment, it decreased to 30.5±3.12. After final 12th treatment, mean PSS of CG dropped to 27.4±3.32.
- *p*-value*: The *p*-values in this column are indicative of whether the two groups' PSS measurements are statistically significant. From the PSS measured before 1st treatment, *p*-value was 0.5494 and is greater than 0.05, indicating that the two groups were not statistically significant from each other. From the PSS measured after 6th treatment and 12th treatment, *p*-values were 0.0222 and 0.0002 respectively, and the *p*-values here were less than 0.05, suggesting that the two groups were showing greater statistically significant results from each other as they went further into the treatment weeks.
- Cohen's *d*: Before 1st treatment, Cohen's *d* was 0.191, indicating negligible effect size. The following Cohen's *d* value was 0.819 after 6th treatment, and then 1.369 after final 12th treatment, which indicated larger effect sizes in PSS measurements between the groups as they went further though treatment weeks.

Summarizing the PSS changes through treatment, Table 36 compared the changes of PSS through the treatment weeks between EG and CG, along with their associated statistical significance (*p*-values) and effect size (Cohen's *d*). The table's results indicated that EG's and CG's PSS measurements were not statistically significant and had negligible effect size before the 1st treatment, however, after the 6th and 12th treatments, they were statistically significant and had larger effect size .

Figure 50 provides a bar graph comparing the mean PSS change between two groups, the 'EG' (Experimental Group) and 'CG' (Active-Control Group), throughout the 12-week of treatments from: before 1st treatment, after 6th and after 12th treatment. This is a visual representation of the data from Table 36.

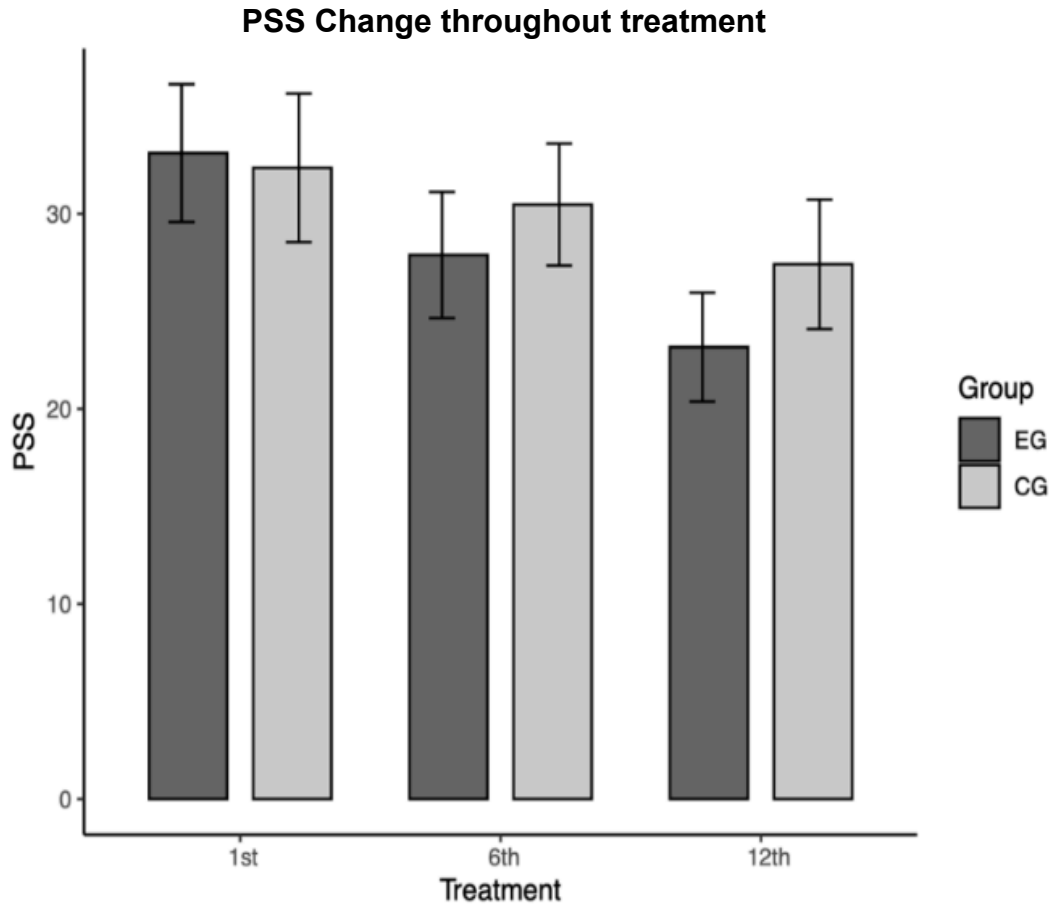


Figure 50. **Bar graph of PSS Change throughout treatment**

Figure 51 provides a boxplot graph that visually compares the distribution of numerical PSS data and skewness throughout treatment, between two groups: the 'EG' (Experimental Group) and 'CG' (Active-Control Group).

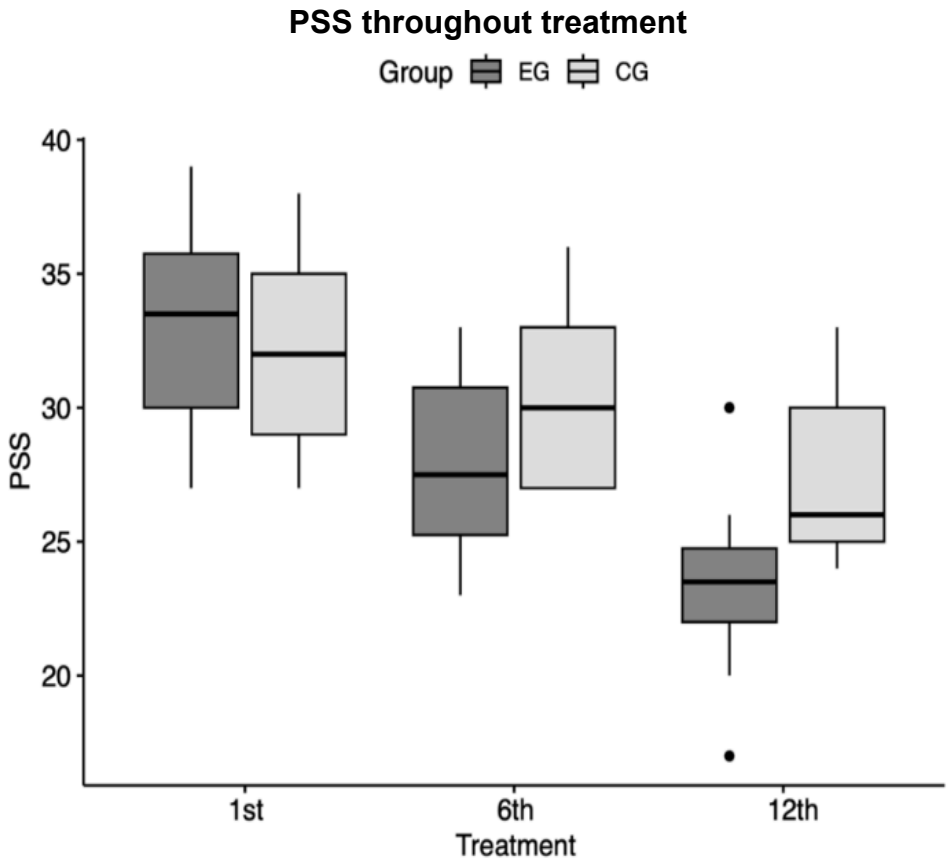


Figure 51. **Boxplot of PSS throughout treatment**

- Before 1st treatment, positions of the Interquartile Range (IQR) boxes were aligned side by side about the same level, suggesting that the group's PSS were similar from the beginning. After 12th treatment, the position of groups' IQR boxes shifted away from each other, suggesting that the group's PSS changes were different.

3.10.2. PSS Difference throughout Treatment

Table 37 provides a comparison of PSS differences within each groups' PSS before and after treatment. Specifically, it compares each groups' PSS from before 1st week treatment with after 6th treatment, following with, comparing data between each groups' PSS from before 1st week treatment with after 12th treatment. Additionally, it includes *p*-values to assess the significance between the PSS difference before and after treatment within each group.

Table 37. **PSS difference within group**

Group	Treatment	PSS	*p*-value*
EG	1st - 6th	5.2 ± 2.24	0.000
	1st - 12th	9.9 ± 3.02	0.000
CG	1st - 6th	1.9 ± 1.93	0.001
	1st - 12th	4.9 ± 2.63	0.000

* Paired Samples t-Test

- Experimental Group (EG): Between the 1st and 6th treatments, EG's PSS difference was 5.2 ± 2.24. Between the 1st and 12th treatments, EG's PSS difference increased to 9.9 ± 2.24. In both difference comparison instances, the both *p*-values were 0.000 and both less than 0.05, indicating statistical significant differences within EG's before and after treatment.
- Active-Control (CG): Between the 1st and 6th treatments, CG's PSS difference was 1.9 ± 1.93. Between the 1st and 12th treatments, CG's PSS difference increased to 4.9 ± 2.63. In both difference comparison instances, the *p*-values were less than 0.05, suggesting that there were also statistical significant differences within CG's before and after treatments.

Table 38 provides a comparison for the PSS differences before and after treatments between groups. First, it compares EG's and CG's difference between PSS from before 1st and after 6th treatment. Second, it compares EG's and CG's difference between PSS from before 1st and after 12th treatment. Additionally, it includes *p*-values to assess the significance between each groups' PSS difference.

Table 38. **Comparison of PSS difference between groups**

	PSS			
Treatment	EG	CG	*p*-value*	Cohen's *d*
6th - 1st	5.2 ± 2.24	1.9 ± 1.93	0.000	1.578
12th - 1st	9.9 ± 3.02	4.9 ± 2.63	0.000	1.766

* Mann-Whitney U Test

- Between the 1st and 6th treatments, EG's PSS mean difference was 5.2 ± 2.24, and CG's PSS mean difference was 1.9 ± 1.93; showing EG's mean PSS difference was 3.3 points higher than CG's mean PSS difference. Between the 1st and 12th treatments, EG's PSS mean difference increased to 9.9 ± 3.02, and CG's PSS mean difference increased to 4.9 ± 2.63; showing EG's mean PSS difference was 5 points higher than CG's mean PSS difference.
- In both groups' differences, *p*-values are 0.000 and both less than 0.05, indicating both groups had statistically significant mean PSS differences before and after treatments.
- Cohen's *d*: From 1st to 6th treatment, Cohen's *d* was 1.578, indicating large effect size. The following Cohen's *d* value was 1.766 from 1st to 12th treatment, also indicating even larger effect size.

Summarizing the PSS difference through treatment, Tables 37 compared the PSS differences within each groups' before and after treatment, and Table 38 compared the PSS differences between Experimental Group (EG) and Active-Control Group (CG). The results indicated that both groups' treatments have statistical significance with a large magnitude of effect for PSS difference before and after treatment, with EG treatments having higher PSS difference than CG treatments.

Figure 52 provides a bar graph comparing the PSS difference between the 'EG' (Experimental Group) and 'CG' (Active-Control Group), from their initial 1st and midpoint 6th treatments, and from initial 1st and final 12th treatments. This is a visual representation of the data from Table 37 and 38.

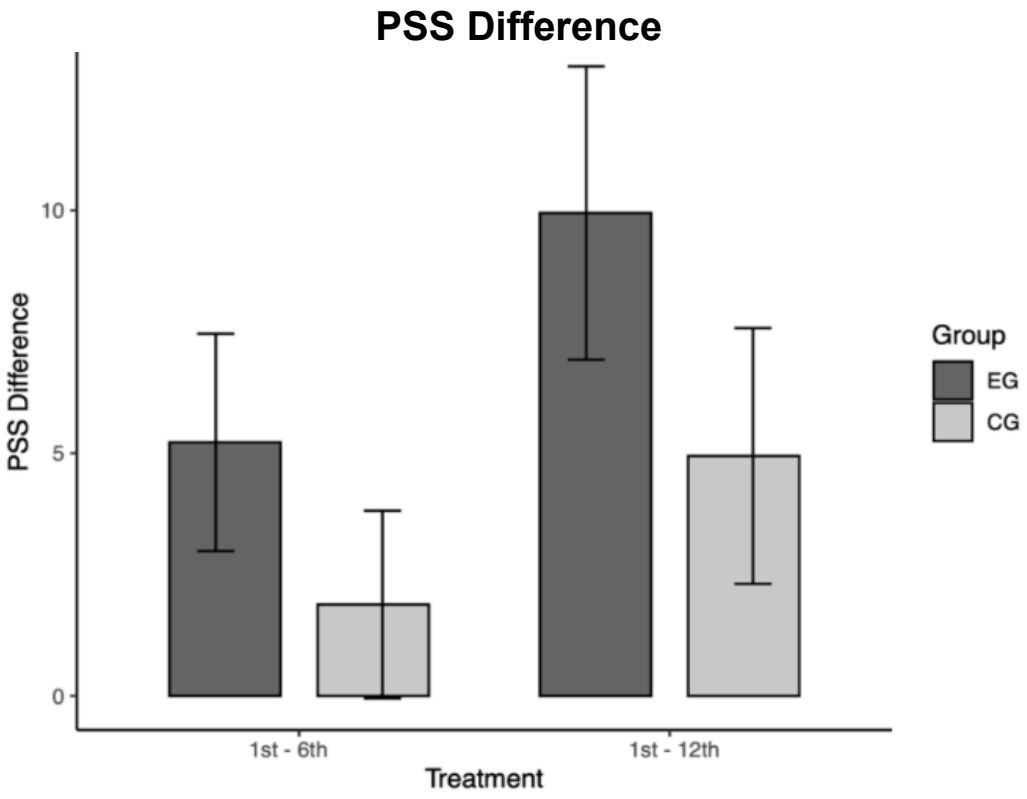


Figure 52. **Bar graph of PSS difference**

Figure 53 provides a boxplot graph that visually compares the skewness and distribution of PSS difference values, between two groups: the ‘EG’ (Experimental Group) and ‘CG’ (Active-Control Group), first (1) from their initial 1st and midpoint 6th treatments, and next (2) from initial 1st and final 12th treatments.

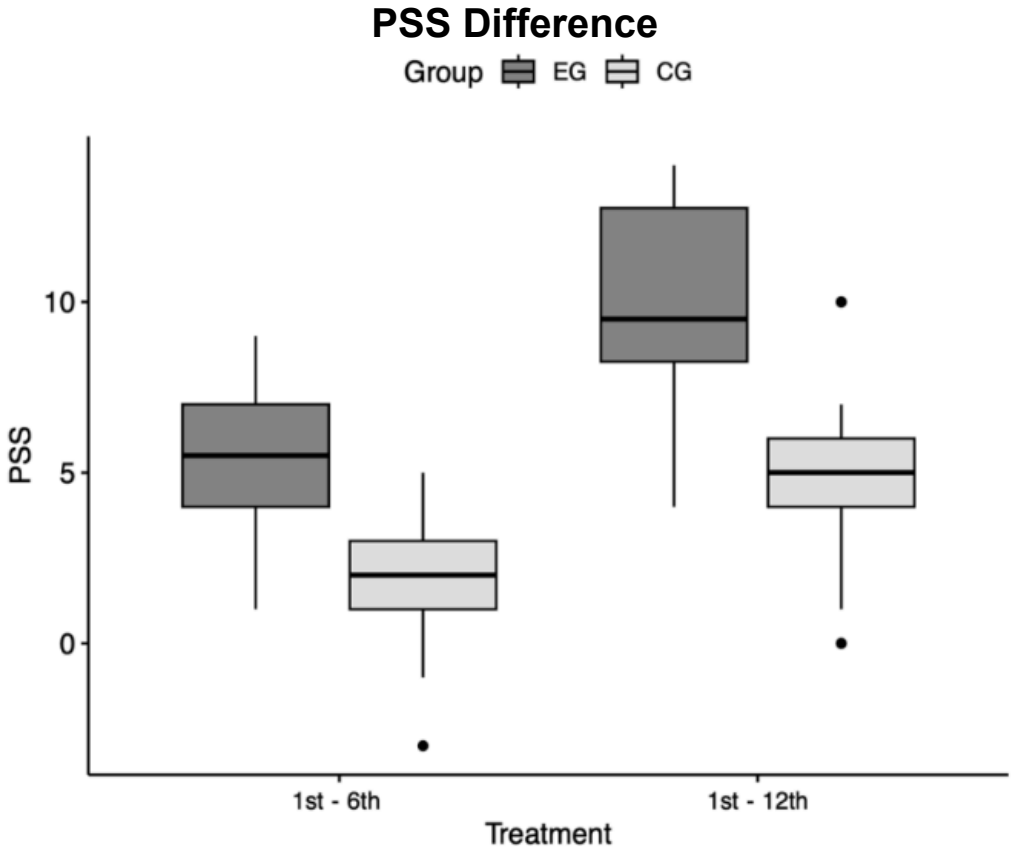


Figure 53. **Boxplot of PSS difference**

- In both time instances, both groups’ positions of the Interquartile Range (IQR) boxes were not at the same levels, suggesting that the groups’ PSS mean differences were actually different after the 6th treatment and after the 12th treatment.

3.11. Quality of Life (QOL) Score

QOL Score is measured on a scale of 0 to 100. Whereas the higher the score suggests the higher the participant's perceived their quality of life, combining aspects of physical, psychological, social and environmental quality aspects of their life.

3.11.1. QOL Change throughout Treatment

Table 39 provides a comparison of data between two groups, the 'EG' (Experimental Group) and 'CG' (Active-Control Group), regarding their QOL measurements throughout the 12-week of treatments from: before 1st treatment, after 6th treatment and after 12th treatment. Additionally, it includes *p*-values and Cohen's *d* effect size values to assess the significance and magnitude of observed change between groups.

Table 39. **Comparison of QOL change throughout treatment between groups**

	QOL			
Treatment	EG	CG	*p*-value*	Cohen's d
1st	55.3 ± 12.85	56.1 ± 11.12	0.841	0.067
6th	58.2 ± 12.42	57.2 ± 10.63	0.788	0.087
12th	61.3 ± 12.12	57.62 ± 10.70	0.348	0.322

* Independent Sample t- Test

- Experimental Group (EG): Before 1st treatment, the group's mean QOL measurement was 55.3 ± 12.85, and after 6th treatment, it increased to 58.2±12.42. After final 12th treatment, mean QOL of EG increased slightly to 61.3±12.12.

- Active-Control (CG): Before 1st treatment, the group's mean QOL measurement was 56.1 ± 11.12, and after 6th treatment, it increased to 57.2±10.63. After final 12th treatment, mean QOL of CG increased slightly to 57.62±10.70.
- *p*-value*: The *p*-values in this column are indicative of whether the two groups' QOL measurements are statistically significant or not. From the QOL measured before 1st treatment, *p*-value was 0.841 and is greater than 0.05, indicating that the two groups were not statistically significant from each other. From the QOL measured after 6th treatment and 12th treatment, *p*-values were 0.788 and 0.348 respectively, suggesting that the two groups' QOL changes were still not showing statistically significant results.
- Cohen's *d*: Before 1st treatment, Cohen's *d* was 0.316, indicating small effect size. Following the 6th treatment, Cohen's *d* value was 0.087, and then Cohen's *d* value of 0.263 after final 12th treatment, these values indicated even smaller effect sizes in QOL measurements between groups.

Summarizing the QOL changes through treatment, Table 39 compared the changes of QOL through the treatment weeks between EG and CG, along with their associated statistical significance (*p*-values) and effect size (Cohen's *d*). The table's results indicated that EG's and CG's QOL changes throughout treatment weeks were not statistically significant and had a small magnitude of effect overall.

Figure 54 provides a bar graph comparing the mean QOL change between two groups, the ‘EG’ (Experimental Group) and ‘CG’ (Active-Control Group), throughout the 12-week of treatments from: before 1st treatment, after 6th and after 12th treatment. This is a visual representation of the data from Table 39.

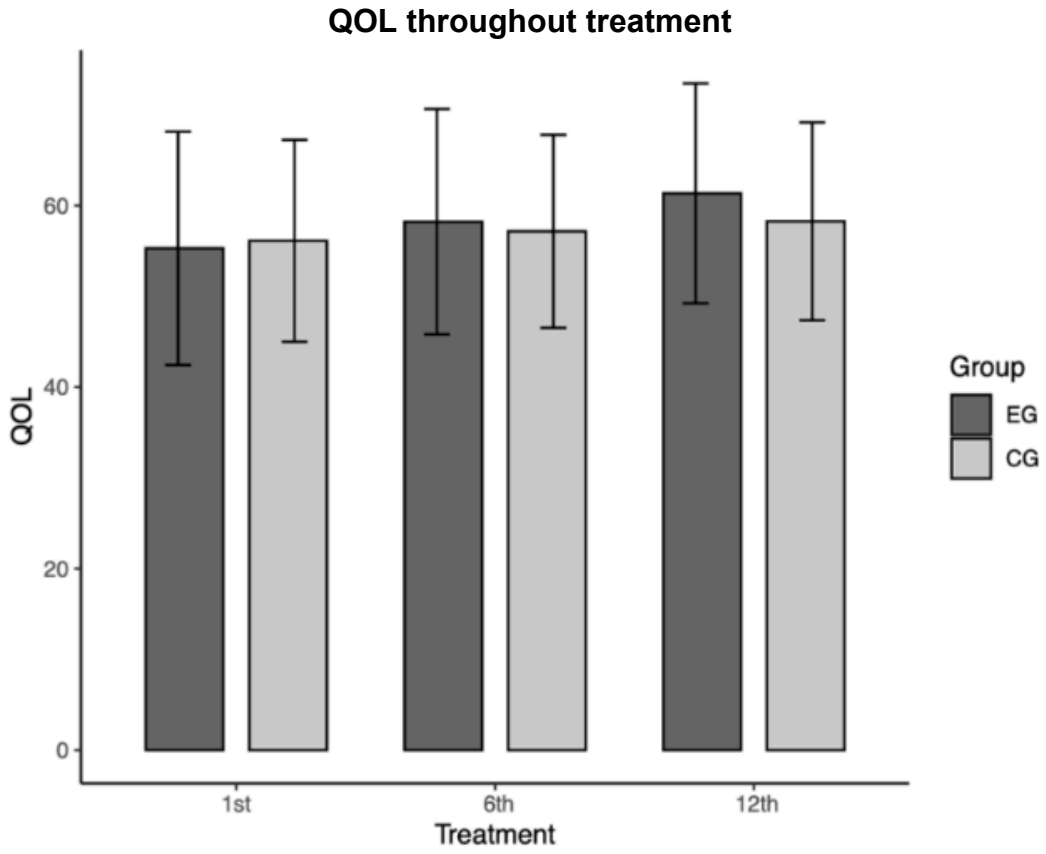


Figure 54. **Bar graph of QOL throughout treatment**

Figure 55 provides a boxplot graph that visually compares the distribution of numerical QOL data and skewness throughout treatment, between two groups: the ‘EG’ (Experimental Group) and ‘CG’ (Active-Control Group).

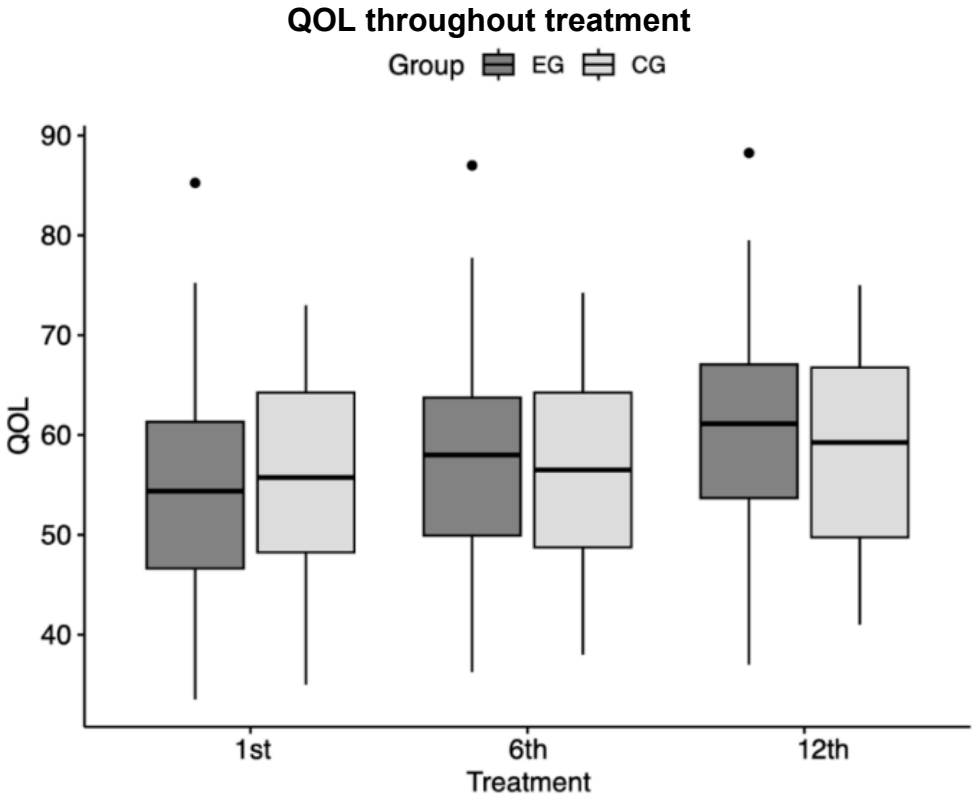


Figure 55. **Boxplot of QOL throughout treatment**

- Before 1st treatment, positions of the Interquartile Range (IQR) boxes were aligned side by side about the same level, suggesting that the group’s PSS were similar from the beginning. After 12th treatment, the position of groups’ IQR boxes were still at similar levels, suggesting that the group’s PSS changes were still not significantly different at the end of trial.

3.11.2. QOL Difference throughout Treatment

Table 40 provides a comparison of QOL difference data within each groups' QOL before and after treatment. Specifically, it compares each groups' QOL from before 1st week treatment with after 6th treatment, following with, comparing data between each groups' QOL from before 1st week treatment with after 12th treatment. Additionally, it includes *p*-values to assess the significance between the QOL difference before and after treatment within each group.

Table 40. **QOL difference within group**

QOL			
Group	Treatment	Difference	*p*-value*
EG	6th - 1st	2.9 ± 1.25	0.0002
	12th - 1st	6.1 ± 2.02	0.0000
CG	6th - 1st	1.0 ± 0.79	0.0005
	12th - 1st	1.5± 1.57	0.0019

* Paired samples t-Test / Wilcoxon Signed-rank Test

- Experimental Group (EG): Between the 1st and 6th treatments, EG's QOL difference was 2.9 ± 1.25. Between the 1st and 12th treatments, EG's QOL difference increased to 6.1 ± 2.02. In both difference comparison instances, *p*-values are both less than 0.05, indicating statistical significant differences within EG's before and after treatment.
- Active-Control (CG): Between the 1st and 6th treatments, CG's QOL difference was 1.0 ± 0.79. Between the 1st and 12th treatments, CG's QOL difference increased to 1.5± 1.57. In both difference comparison instances, the *p*-values were

less than 0.05, suggesting that there were also statistical significant differences within CG's before and after treatments.

Table 41 provides a comparison for the QOL differences before and after treatments between groups. First, it compares EG's and CG's difference between QOL from before 1st and after 6th treatment. Second, it compares EG's and CG's difference between QOL from before 1st and after 12th treatment. Additionally, it includes *p*-values to assess the significance between each groups' QOL difference.

Table 41. **Comparison of QOL difference between groups**

	QOL			
Treatment	EG	CG	*p*-value*	Cohen's *d*
6th - 1st	2.9 ± 1.25	1.0 ± 0.79	0.000	1.817
12th - 1st	6.1 ± 2.02	1.5± 1.57	0.000	2.543

* Mann-Whitney U Test

- Between the 1st and 6th treatments, EG's QOL mean difference was 2.9 ± 1.25, and CG's QOL mean difference was 1.0 ± 0.79; showing EG's mean QOL difference was 1.9 points higher than CG's mean QOL difference. Between the 1st and 12th treatments, EG's QOL mean difference increased to 6.1 ± 2.02, and CG's QOL mean difference increased to 1.5± 1.57; showing EG's mean QOL difference was 4.6 points higher than CG's mean QOL difference.
- In both groups' differences, *p*-values are 0.000 and less than 0.05, indicating groups had statistically significant mean QOL differences before and after treatments.

- Cohen's *d*: From 1st to 6th treatment, Cohen's *d* was 1.817, indicating large effect size. The following Cohen's *d* value was 2.543 from 1st to 12th treatment, also indicating even larger effect size.

Summarzing the QOL difference through treatment, Tables 40 compared the QOL differences within each groups' before and after treatment, and Table 41 compared the QOL differences between Experimental Group (EG) and Active-Control Group (CG). The results indicated that both groups' treatments had statistical significance in QOL difference before and after treatment, with EG treatments having higher QOL difference than CG treatments.

Figure 56 provided a bar graph comparing the QOL difference between the ‘EG’ (Experimental Group) and ‘CG’ (Active-Control Group), from their initial 1st and midpoint 6th treatments, and from initial 1st and final 12th treatments. This is a visual representation of the data from Table 40 and Table 41.

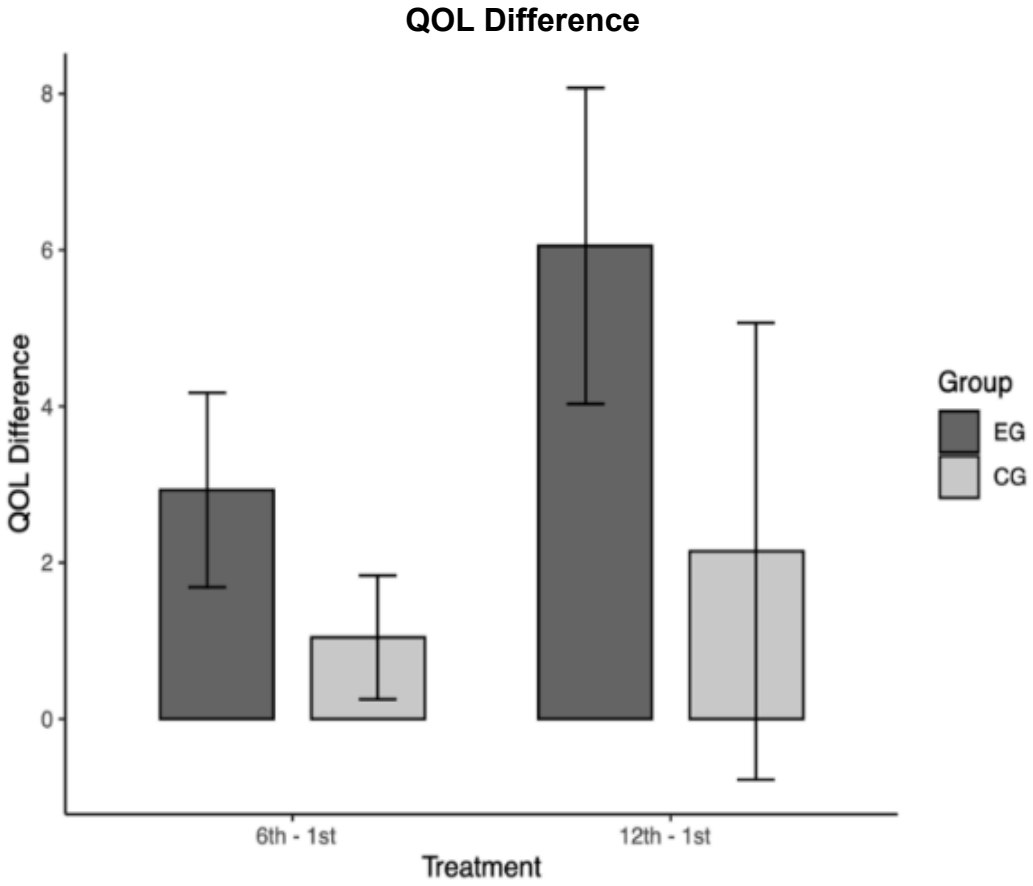


Figure 56. **Bar graph of QOL difference**

Figure 57 provides a boxplot graph that visually compares the skewness and distribution of QOL difference values, between two groups: the ‘EG’ (Experimental Group) and ‘CG’ (Active-Control Group), first (1) from their initial 1st and midpoint 6th treatments, and next (2) from initial 1st and final 12th treatments.

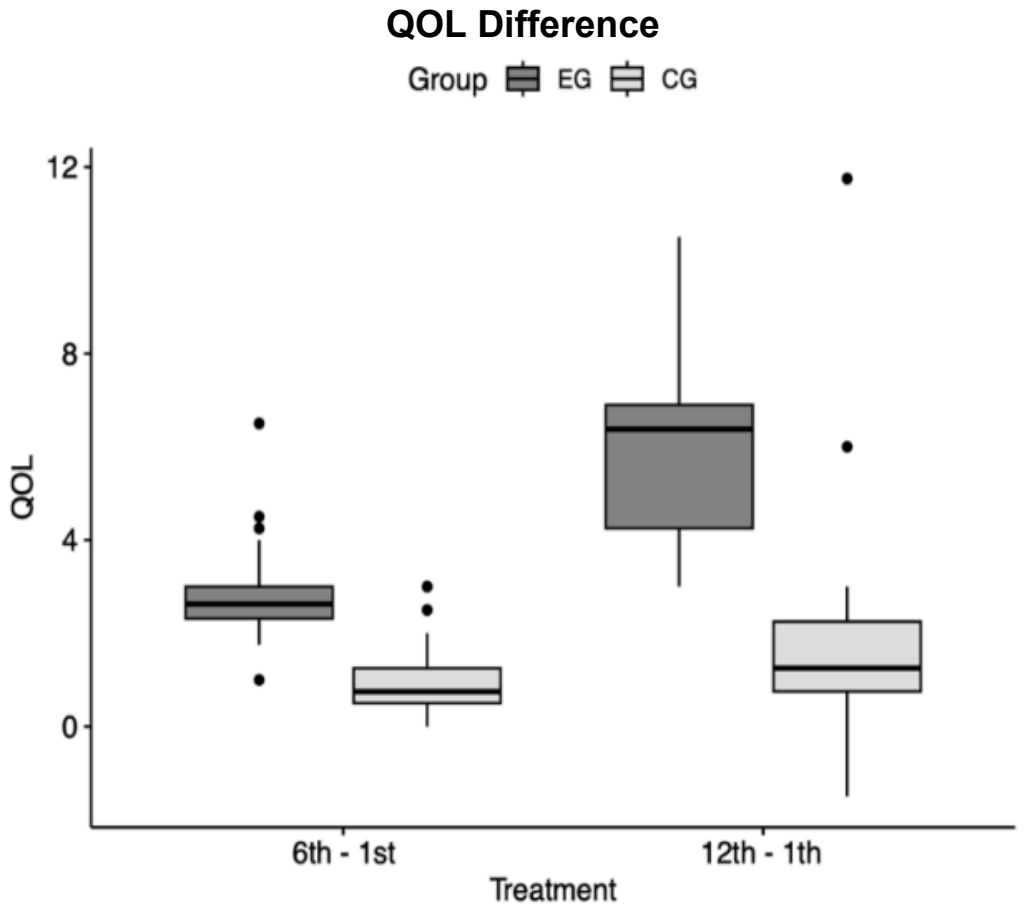


Figure 57. **Boxplot of QOL difference**

- In both time instances, both groups’ positions of the Interquartile Range (IQR) boxes were not at the same levels, suggesting the groups’ QOL mean differences were different after the 6th treatment and after the 12th treatment.

3.12. Summary of Results

Table 42 is a side-by-side categorial comparison of all measured variables, before 1st treatment with homogeneity tests and after 12th treatment with heterogeneity tests, for both groups: Experimental Group (EG) and Active-Control Group (CG).

Table 42. **Categorical Comparison of Variables throughout Treatment**

		Homogeneity Tests: Before 1st Treatment			**Heterogeneity Tests: After 12th Treatment**		
Variables	Categories	**EG** (n=18)	**CG** (n=17)	*p*-value*	**EG** (n=18)	**CG** (n=17)	*p*-value*
RMSSD	Low	12	12		2	7	
	Normal	4	5		16	10	
	High	2	0	0.353	0	0	0.042
SDNN	Low	10	6		6	4	
	Normal	7	9		12	13	
	High	1	2	0.459	0	0	0.520
pNN20	Low	7	8		0	6	
	Normal	8	4		17	8	
	High	3	5	0.392	1	3	0.006
pNN50	Low	7	9		0	5	
	Normal	8	5		17	11	
	High	3	3	0.633	1	1	0.043
LF	Low	10	8		7	7	
	Normal	7	8		11	9	
	High	1	1	0.878	0	1	0.556
HF	Low	2	4		0	3	
	Normal	11	11		18	13	
	High	5	2	0.382	0	1	0.094
LF/HF	Low	9	9		7	9	
	Normal	6	4		11	4	
	High	3	4	0.773	0	4	0.024
PSS	< 21	–	–		3	0	
	22-26	–	–		14	10	
	27-31	6	8		1	3	
	32-35	7	5		0	4	
	36-40	5	4	0.704	0	0	0.034
QOL	0-50	7	5		4	4	
	51-100	11	12	0.555	14	13	0.926

* Chi-Square Test

text = *p*-value is lesser than 0.05, indicating significant difference between groups.

Table 43 summarizes results of quantitative statistical tests throughout treatment weeks, regarding statistical significance and effect sizes for all measured variables' changes and differences, from the two groups: Experimental Group (EG) and Active Control (CG).

Table 43. **Summary of Quantitative Statistical Test Results throughout Treatment**

	Change between Groups (Before 1st treatment ➔ After 12th treatment)				**Difference within EG** (After 6th ➔ After 12th treatment)		**Difference within CG** (After 6th ➔ After 12th treatment)		**Difference between Groups** (After 6th ➔ After 12th treatment)			
Variables	*p*–value ➔ *p*–value		Cohen's *d* ➔ Cohen's *d*		*p*–value ➔ *p*–value		*p*–value ➔ *p*–value		*p*–value ➔ *p*–value		Cohen's *d* ➔ Cohen's *d*	
RMSSD	0.519	0.033	0.122^{+}	0.613**	0.003	0.001	0.012	0.000	0.001	0.001	1.013***	1.004***
SDNN	0.176	0.949	0.431*	0.023^{+}	0.001	0.000	0.982	0.009	0.005	0.000	1.014***	1.245***
pNN20	0.817	0.068	0.035^{+}	0.421*	0.130	0.058	0.385	0.549	0.014	0.008	0.415*	0.547**
pNN50	0.370	0.034	0.128^{+}	0.559**	0.046	0.026	0.261	0.021	0.058	0.008	0.502**	0.500**
LF	0.987	0.452	0.042^{+}	0.256*	0.003	0.005	0.166	0.022	0.002	0.000	0.948***	1.140***
HF	0.804	0.428	0.019^{+}	0.106^{+}	0.571	0.316	0.155	0.058	0.117	0.026	0.003^{+}	0.310*
LF/HF	0.632	0.478	0.000^{+}	0.222*	0.080	0.081	0.776	0.198	0.586	0.111	0.580**	0.550**
PSS	0.5494	0.0002	0.191^{+}	1.369***	0.000	0.000	0.001	0.000	0.000	0.000	1.578***	1.766***
QOL	0.841	0.348	0.067^{+}	0.322*	0.0002	0.0000	0.0005	0.0019	0.000	0.000	1.817***	2.543***

text = *p*-value is lesser than 0.05, indicating statistically significant difference.
$^{+}$ = Cohen's *d* is lesser than 0.2, indicating negligible effect size.
* = Cohen's *d* is greater than 0.2 and lesser than 0.5, indicating small effect size.
** = Cohen's *d* is greater than 0.5 and lesser than 0.8, indicating medium effect size.
*** = Cohen's *d* is greater than 0.8, indicating large effect size.

Table 44 summarizes final results (after 12th treatment) from all measured variables, regarding final variables' changes, differences, statistical significance and effect sizes, from the groups: Experimental Group (EG) and Active Control (CG).

Table 44. **Summary of Final Results after 12th Treatment**

	Change between Groups after 12th treatment				**Difference within EG** (Before 1st & After 12th treatment)		**Difference within CG** (Before 1st & After 12th treatment)		**Difference between Groups** after 12th treatment	
Variables:	**EG Change**	**CG Change**	***p*–value**	**Cohen's *d***	**Difference**	***p*–value**	**Difference**	***p*–value**	***p*–value**	**Cohen's *d***
RMSSD	60.6 ± 21.92	45.8 ± 26.20	0.033	0.613**	18.3 ± 15.44	0.001	6.9 ± 3.53	0.000	0.001	1.004***
SDNN	123.7 ± 31.56	123.0 ± 30.03	0.949	0.023 $^{+}$	22.2 ± 15.84	0.000	6.6 ± 7.67	0.009	0.000	1.245***
pNN20	33.4 ± 6.16	27.9 ± 17.44	0.068	0.421*	7.0 ± 14.75	0.058	0.8 ± 5.55	0.549	0.008	0.547**
pNN50	19.3 ± 8.26	13.4 ± 12.43	0.034	0.559**	6.1 ± 10.6	0.026	2.1 ± 3.41	0.021	0.008	0.500**
LF	782.1 ± 311.43	689.0 ± 408.10	0.452	0.256*	85.9 ± 92.14	0.005	9.6 ± 14.95	0.022	0.000	1.140***
HF	667.5 ± 335.26	628.4 ± 396.77	0.428	0.106 $^{+}$	38.6 ± 138.34	0.316	7.5 ± 20.09	0.058	0.026	0.310*
LF/HF	1.3 ± 0.50	1.5 ± 1.17	0.478	0.222*	0.23 ± 0.45	0.081	0.05 ± 0.12	0.198	0.111	0.550**
PSS	23.2 ± 2.79	27.4 ± 3.32	0.0002	1.369***	9.9 ± 3.02	0.000	4.9 ± 2.63	0.000	0.000	1.766***
QOL	61.3 ± 12.12	57.62 ± 10.70	0.348	0.322*	6.1 ± 2.02	0.0000	1.5± 1.57	0.0019	0.000	2.543***

text = *p*-value is lesser than 0.05, indicating statistically significant difference.

$^{+}$ = Cohen's *d* is lesser than 0.2, indicating negligible effect size.

* = Cohen's *d* is greater than 0.2 and lesser than 0.5, indicating small effect size.

** = Cohen's *d* is greater than 0.5 and lesser than 0.8, indicating medium effect size.

*** = Cohen's *d* is greater than 0.8, indicating large effect size.

IV. DISCUSSION

4.1. **Discussion Overview**

Heart Rate Variability (HRV) measures the activities of our parasympathetic (PNS) and sympathetic (SNS) nervous systems, providing physiological indicators of both health and/or abnormalities in our stress response systems. Four of the seven HRVs measured in this study have PNS implications, and the remaining three other HRVs measured have overall SNS and PNS implications. Results implications from Heart Rate Variability (HRVs) measurements will be discussed first, followed by discussion for Questionnaire Scores (PSS and QOL) implications. In addition to results implications, weakness and confounding factors in these measured variables will also be elaborated. Next, there will be commentary, regarding the acupuncture treatment limitations on improving HRVs and the potential impacts this study has on the future of TCM clinical diagnosis.

4.2. **Discussion—Result Implications**

4.2.1. **Parasympathetic HRV Variables:**

RMSSD, pNN20, pNN50 and **HF**

RMSSD, pNN20, pNN50 and HF measurements are known as the Parasympathetic HRV variables (PNS HRVs), indicating the activity of the parasympathetic nervous system (PNS) or vagal tone. Having abnormally low PNS HRVs is associated with having low vagal tone, and was the most common abnormal HRV pattern identified in this clinical trial consisting of adult participants with chronic 'toxic' stress and childhood traumas. With low vagal tone conditions, some participants (n=12 from EG and n=12 from CG) presented common symptoms of: dry eyes, dry mouth, difficulty in swallowing, migraine, high anxiety, panic, tarcardia, palpitation, difficulty falling asleep, poor appetite, low

intestinal peristalsis, constipation, slow and poor digestion, and/or urine incontinence, etc. With excessive vagal tone, or abnormally high PNS HRVs, common symptoms identified from some participants (n=3 from EG and n=5 from CG) were signs of bradycardia, diastolic hypertension, cluster headaches, fainting history, shortness of breath, numbness, fatigue, depression, anhedonia, high gastric motility, diarrhea, loose watery stools, undigested food in stool, abdominal discomfort, chronic low back pain, etc. In order to improve vagal tone or say 'improve' PNS HRVs, there are three possible treatment routes: (1) if the PNS HRV is within normal range, then maintaining the healthy PNS HRV values would be most ideal acupuncture treatment goal, (2) if the PNS HRV is abnormally low, then helping increase the PNS HRV would be the acupuncture objective, (3) if the PNS HRV is abnormally high, then decreasing the PNS HRV to normal PNS HRV ranges would be the acupuncture treatment aim.

Fortunately in both EG's and CG's acupuncture points, not only do all the acupuncture points selected are versatile in treating both excess and deficiency conditions, certain PNS HRV results also showed potential effectiveness in restoring vagal tone to normal range. The following PNS HRVs that will be discussed are RMSSD, pNN20, pNN50 and HF results from both Experimental Back Acupuncture Group (EG) and Active-Control Distal Acupuncture Group (CG).

- **RMSSD**: This study's RMSSD improvements can be shown in three ways—RMSSD change, RMSSD difference, and number of participants with normal/abnormal RMSSD results before 1st and after 12th treatment. Upon completion of experiment treatments, EG's average RMSSD increased from initial 42.3 ms to final 60.6 ms, indicating an average RMSSD difference of 18.3 ms with significant difference ($p = 0.001$). After

completion of active-control treatments, CG's average RMSSD also increased from initial 38.9 to final 45.8 ms, indicating an average RMSSD difference of 6.9 ms with significant difference ($p = 0.000$). Comparing the two groups's statistical results, the average RMSSD difference from EG is 1.004 standard deviations greater than the average RMSSD difference from CG. Implying that at least 84% of CG participants' average RMSSD difference would be below the average RMSSD difference in EG's participants. Furthermore, between the groups' RMSSD data, what is more obvious to the eye is that, in EG, the number of participants with abnormal RMSSD dropped (from n =14 to n=2) and number of participants with normal RMSSD increased (from n=4 to n=16). Compared to CG, the number of participants with abnormal RMSSD also dropped (from n =12 to n=7) and normal RMSSD increased (from n=5 to n=10). However, EG's acupuncture treatment showed greater effectiveness in terms of RMSSD change, RMSSD difference and number of participants with normal RMSSD after final treatment.

- **pNN20**: Unlike RMSSD results, both groups' pNN20 results did not show 95% confidence intervals of significance in pNN20 change and pNN20 difference before 1st and after 12th treatment. However, comparing the two groups' pNN20, there was a significant difference ($p = 0.008$) with at least medium magnitude of effect ($d = 0.547$). Looking closer, EG's average pNN20 increased from initial 26.4 % to final 33.4 %, indicating an average pNN20 difference of 7.0 %, with at least 90% confidence interval significant ($p = 0.058$). CG's average pNN20 barely showed any change with a very small pNN20 difference of only 0.8 % before 1st and after final treatments ($p = 0.549$). Comparing the two groups's statistical results, the average pNN20 difference from EG is 0.547 standard deviations greater than the average pNN20 difference from CG. Implying

that at least 66% of CG participants' average pNN20 difference would be below the average pNN20 difference in EG's participants. Furthermore, between the groups' pNN20 data, what is fairly obvious to the eye is that, in EG, the number of participants with abnormal pNN20 dropped (from n =10 to n=1) and number of participants with normal pNN20 increased (from n=8 to n=17). Compared to CG, the number of participants with abnormal pNN20 dropped (from n =13 to n=9) and normal pNN20 increased (from n=4 to n=8). Overall, EG's acupuncture treatment showed some effectiveness in improving pNN20, with at least 90% confidence interval in both pNN20 change and pNN20 difference, and with fairly obvious numbers of participants with normal pNN20 after final treatment.

- **pNN50**: Unlike pNN20's 90% confidence interval outcome, this study's pNN50 improvements can be shown with 95% confidence interval in three ways— pNN50 change, pNN50 difference, and number of participants with normal/abnormal pNN50 results before 1st and after 12th treatment. Upon completion of experimental treatments, EG's average pNN50 increased from initial 13.2 % to final 19.3 %, indicating an average pNN50 difference of 6.1 % with significant difference ($p = 0.026$). After completion of active-control treatments, CG's average pNN50 also increased from initial 11.3 % to final 13.4 %, indicating an average pNN50 difference of 2.1 % with significant difference ($p = 0.021$). Comparing the two groups's statistical results, the average pNN50 difference from EG is 0.500 standard deviations greater than the average pNN50 difference from CG. Implying that at least 66% of CG participants' average pNN50 difference would be below the average pNN50 difference in EG's participants. Furthermore, between the groups' pNN50 data, what is fairly obvious to the eye is that,

in EG, the number of participants with abnormal pNN50 dropped (from n =10 to n=1) and number of participants with normal pNN50 increased (from n=8 to n=17). Compared to CG, the number of participants with abnormal pNN50 also dropped (from n =12 to n=6) and normal pNN50 increased (from n=5 to n=11). However, EG's acupuncture treatment showed greater effectiveness in terms of pNN50 change, pNN50 difference and number of participants with normal pNN50 after final treatment.

- **HF**: From both treatment groups in this study, HF results did not show significant HF change between groups and HF difference within groups. On the contrary, while comparing the two group's HF differences, there was actually a significant difference ($p = 0.026$), with small magnitude of effect ($d = 0.310$). Comparing the two groups's statistical results, the average HF difference from EG is 0.310 standard deviations greater than the average pNN50 difference from CG. Implying that at least 58% of CG participants' average HF difference would be below the average HF difference in EG's participants. Although it is hard to differentiate between the groups' HF statistical data, what may give another perspective is looking at the number of participants with normal/abnormal before and after treatment. For instance, in EG, the abnormal HF dropped (from n =7 to n=0) and the number of participants with normal HF increased (from n=11 to n=18). Compared to CG, the number of participants with abnormal HF also dropped (from n =6 to n=4) and normal HF increased (from n=11 to n=13). Considering this, EG's acupuncture treatment showed greater effectiveness, in terms of bringing all 18 participants in its experimental back acupuncture group to normal HF ranges after 12 treatments.

In summary of the PNS HRV results, EG's experimental back acupuncture treatments helped its overall participants improve their RMSSD, pNN50 and HF measurements, suggesting EG's back acupuncture treatment has potential in regulating abnormal vagal tone as well. Moving forward, despite the optimistic outcomes in PNS HRV results, that is only examining half of the autonomic nervous system. For instance, in several participants' cases, they will not only indicate low PNS HRV, but will also show signs of high sympathetic activity. Same goes for having high PNS HRVs, in some patient's cases, they will sometimes show low sympathetic activity. Examining both the parasympathetic and sympathetic activities is very crucial for understanding patients' overall health of their autonomic nervous system or stress response system.

4.2.2. **Sympathovagal HRV Variables**

SDNN and **LF**

SDNN and LF measurements are known as Sympathovagal HRV variables (SV HRVs), indicating the overall activity of the sympathetic nervous system (SNS) and parasympathetic nervous system (PNS / vagal tone). While SV HRVs is a physiological indicator of the overall autonomic nervous system activities, these SV HRV measurements can be primarily helpful in identifying sympathetic nervous system health and/or abnormalities, and even more helpful when examined along with PNS HRVs. Having high SV HRVs is associated with having a high sympathetic tone, being in the frequent state of 'fight or flight' mode. High sympathetic tone condition symptoms often overlapped with the symptoms that occur in the participants with low vagal tone (n=12 from EG and n=12 from CG). In addition to the low vagal tone signs mentioned in the

previous section, participants with especially high sympathetic tone (n=2 from EG and n=3 from CG) reported to have frequent mood swings, irritability, chronic migraines, systolic hypertension, neck and shoulder pain, etc. On the other hand, having abnormally low SV HRVs is associated with low sympathetic tone, and coincidently, participants' (n=10 from EG and n=8 from CG) symptoms tend to overlap with high vagal tone symptoms mentioned in the previous section, with additional signs of cold extremities, swelling, brain fog, weight gain and/or whole body pain. In order to improve sympathetic tone or say 'improve' SV HRVs, there are three possible treatment routes: (1) if the SV HRV is within normal range, then maintaining the healthy SV HRV measurements would be most ideal treatment objective, (2) if the SV HRV is abnormally low, then helping increase the SV HRV would be the goal, (3) if the SV HRV is abnormally high, then decreasing the SV HRV to normal SV HRV ranges would be the acupunctures' aim.

The following SV HRVs that will be discussed are SDNN and LF outcome measurements, from both Experimental Back Acupuncture Group (EG) and Active-Control Distal Acupuncture Group (CG).

- **SDNN**: Both groups' SDNN changes throughout treatments and the number of participants with normal SDNN after final treatment were not significant, however, the groups' SDNN improvements can still be shown in terms of SDNN difference before 1st and after 12th treatment. Upon completion of 12 weekly treatments, EG's average SDNN increased from initial 101.4 ms to final 123.7 ms, indicating an average SDNN difference of 22.3 ms with significant difference ($p = 0.000$). CG's average SDNN difference was small at 6.6 ms ($p = 0.009$). Comparing the two groups, the average SDNN difference from EG is 1.245 standard deviations greater than the average SDNN difference from

CG. Implying that at least 88% of CG participants' average SDNN difference would be below the average SDNN difference in EG's participants.

- **LF**: Although the change between groups's LF was insignificant (p=0.452), the Average LF difference from EG was actually 1.140 standard deviations greater than the average SDNN difference from CG. Implying that at least 84% of CG participants' average SDNN difference would be below the average LF difference in EG's participants.

Summarizing SV HRV results, both groups' acupuncture treatments did improve participants' SDNN and LF, suggesting this study's treatment were both effective in regulating abnormal sympathetic tone.

4.2.3. **Predominance or Balance of Autonomic Nervous System**

LF/HF Ratio

Moving forward, now that both PNS HRVs and SV HRVs have been reviewed, it is important to note that PNS HRVs and SV HRVs have physiological indicators for PNS and SNS, but they cannot determine the overall balance or predominance of the autonomic nervous system (ANS). The only HRV variable measured in this study that fairly examined the balance or predominances in the ANS, was the LF/HF ratio. LF/HF ratio (also referred as LF/HF from the previous chapters) indicates either predominance of PNS, balanced PNS and SNS, or predominance of SNS. LF/HF below 1 is considered abnormally low and suggests there is PNS predominance and/or underactive SNS; LF/HF between 1-2 is normal range and indicates a balanced ANS; and LF/HF above 2 is abnormally high and indicates there is SNS predominance and/or underactive PNS.

- **LF/HF:** This study's LF/HF improvements can only be shown in one way—the number of participants with 'normal/abnormal' LF/HF results before 1st and after 12th treatment. Final quantitative statistical test results, from LF/HF changes and differences, are inconclusive due to weaknesses in the small sample size and study design, which will be discussed further in Section 4.3. Moving on, in EG, the number of participants with abnormal LF/HF dropped (from n =12 to n=7) and the number of participants with normal LF/HF increased (from n=6 to n=11). Compared to CG, the number of participants with abnormal LF/HF and normal LF/HF had no changes at the completion of the trial. All in all, EG's acupuncture treatment showed effectiveness in improving the balance of the autonomic nervous system, in terms of the number of participants with normal LF/HF after final treatment.

4.2.4. Perceived Stress Scale (PSS)

- **PSS:** Perceived Stress Scale Score is measured on a scale of 0 to 40, scoring (0-13) is low stress, (14-26) is moderate stress, and (27-40) is high stress. Before any acupuncture treatment, all participants started with 'high stress' PSS scores ranging anywhere from 27 to 40. This clinical trial's perceived stress reduction can be shown in three ways—PSS change, PSS difference, and number of participants with normal/abnormal PSS results before 1st and after 12th treatment. Upon completion of experiment treatments, EG's average PSS decreased from initial 33.1 ms to final 23.2 ms, indicating an average PSS difference of 9.9 with significant difference (p = 0.000). After completion of active-control treatments, CG's average PSS also decreased from initial 32.4 to final 27.4

ms, indicating an average PSS difference of 4.9 with significant difference ($p = 0.000$). Comparing the two groups's statistical results, the average PSS difference from EG is 1.766 standard deviations greater than the average PSS difference from CG. Implying that at least 95% of CG participants' average PSS difference would be below the average PSS difference in EG's participants. Furthermore, between the groups' PSS data, what is more obvious to the eye is that, in EG, the number of participants with high PSS dropped (from n =18 to n=1), finishing the trial with an increased number of participants with moderate to low PSS (n=17). Compared to CG, the number of participants with high PSS dropped (from n =17 to n=7), finishing the trial with an increased number of participants with moderate PSS (n=10). While CG's acupuncture treatment allowed some of its participants to decrease their PSS, overall, EG's acupuncture treatment helped more of its participants lower their PSS to more moderate/low stress levels.

4.2.5. Quality of Life (QOL) Scores

- **QOL:** Both acupuncture groups' QOL results did not show significant QOL change, but did show some significant QOL difference before 1st and after 12th treatment. Comparing the two groups' QOL, there was a significant difference ($p = 0.000$) with a very large magnitude of effect ($d = 2.543$). Looking closer, EG's average QOL increased from initial 55.3 % to final 61.3 %, indicating an average QOL difference of 6.1 %, with significant ($p = 0.000$). CG's average QOL barely showed any change with a very small QOL difference of only 1.5 % before 1st and after final treatments ($p = 0.0019$). Comparing the two groups's statistical results, the average QOL difference from EG is 2.543 standard deviations greater than the average QOL difference from CG. Implying that at least 99% of CG participants' average QOL difference would be below the average

QOL difference in EG's participants. Furthermore, EG's number of participants with QOL Score (0-50%) dropped (from n =7 to n=4) and the number of participants with QOL Score (51-100%) increased slightly (from n=11 to n=14). CG's number of participants with QOL Score (0-50%) dropped for 1 participant (from n =5 to n=4), and the number of participants with QOL Score (51-100%) increased by the same 1 participant (from n=12 to n=13). Compared to CG, EG's acupuncture treatment showed to have a large magnitude of effect on its QOL Score difference, however, EG's improvements in overall QOL Score was insignificant and negligible. Therefore, both EG's and CG's acupuncture treatments were not effective in improving Quality of Life Score for the study's participants. However, these insignificant QOL results may not be because of the acupuncture treatments, but also due to disadvantages in using QOL questions for acupuncture research, refer next Section 4.3.

4.3. Discussion— Weakness in Measured Variables

4.3.1. Small Sample Sizes

As indicated by the long standard deviations bars on the bar graphs, and the long whiskers from box plot graphs, they suggest there was a wide distribution of HRV measurements amongst the small sample size of participants in Experimental Group 'EG' (n=18) and Active-Control Group 'CG' (n=17). Unexpectedly, EG's overall standard deviations were drastically higher compared to CG's, leading to insignificant differences and/or inconclusive implications for some measured variables. For example, the average HF difference within EG (38.6 ± 138.34) was higher than the average HF difference within CG (7.5 ± 20.09), but the p-value was actually lower in CG (p=0.058) compared to EG (p=0.316); this could be due to the small sample size and wide standard deviation in average HF Difference within EG. Similarly, this wide standard deviation issue also happened in other average HRVs' Differences within EG—like RMSSD, pNN20 and pNN50. Increasing the sample size may resolve this wide distribution issue, but what may have a bigger impact is improving the study design.

4.3.2. Issues in the Study Design

Regardless of any health conditions and treatments, it is common for adults of older ages to have lower HRV values than the adults of younger ages. Even though this clinical trial enrolled an even distribution of participants of different ages from ages 18 to 80 in both EG and CG from the beginning, it did not take into account each participants' initial HRV readings will vary immensely, causing the high standard deviations, long

whiskers (in box plots) and some insignificant Average HRV changes and Average HRV differences in both groups throughout the treatment weeks.

Not only does this issue affect the HRV data distribution, but both groups' average HRV changes, *p*-values and effect size calculations could also be impacted by individual participants' HRV treatment objectives. In general HRV principles, if the person has abnormally low HRVs, the goal of treatment would be to increase their HRVs and it would be ideal for this person to have a positive HRV data trend throughout treatment. While, if the person has abnormally high HRVs, the treatment goal would be to help lower their HRVs to normal HRV range and it would be ideal for this person to have a negative HRV data trend throughout treatments. To eliminate this issue for the future, the investigators should apply a block randomization process, sorting individuals into similar initial HRV measurements groups before treatment. In order for this to be possible, the investigators should coordinate separate initial HRV screening appointments for the participants before further randomization into experimental or control groups.

Another factor to consider is the multiple types of HRVs that will be measured for any HRV intervention study. For example, participant A and participant B may have similar RMSSD measurements from the initial screening, however, depending on their health conditions, their SDNN measurements may be very different, potentially resulting in widely distributed and inconclusive SDNN data. Understanding this explains what happened in results for SDNN change between groups, where *p*-value increased and effect size decreased after 12th treatment. In this scenario, future investigators will need to be more specific in their participants' inclusion criterias. For instance, a solution may

be to screen and enroll participants with similar initial HRVs for all HRV variables being examined, before randomization into experimental and control treatment groups.

These solutions may not be feasible for investigators and participants, due to time, availability and financial limitations, however, if initial HRV screenings and sorting can be applied before treatments, this can vastly help with for all HRV data analysis and interpretations for advancing acupuncture research or any clinical trials involving HRV variables.

4.3.3. **Confounding Variables in Measuring HRVs**

The possibility of confounding factors occurring while measuring HRVs was inevitable and problematic in some participants' HRV data collected. As proactive as the investigator was in giving participants clear guidelines to follow prior to their appointments, there were still uncontrollable variables that may have easily fluctuated HRV measurements, such as participants sleeping late, drinking alcohol the night before appointment, or drinking coffee, eating a large meal an hour before appointment time. Due to the nature of unplanned situations in life, occasionally, participants could not attend their weekly scheduled appointment time and had to reschedule another time of the day. The HRV measurements from the morning time can differ from HRV measurements from the evening time. Measuring the frequency-domain types of HRVs, such as the LF, HF and LF/HF HRVs, were especially sensitive to several of these confounding variables. Furthermore, if the participant(s) had to postpone too long and too frequently during the trial, they would have to withdraw. Consequently, this would not only be a HRV confounding factor issue, but also a problem in maintaining sufficient sample sizes in both treatment groups overall.

4.3.4. **Disadvantages of Using QOL Questions**

The specific Quality of Life Questionnaire used in this study is called the World Health Organization Quality of Life - Brief Version (WHOQOL-BREF). This questionnaire examines the participants' perspective of four categories in their life—physical health, psychological health, social health, and environmental health; each category is equally weighted into their QOL Score. This study's acupuncture treatments showed potential in improving participants' perspective on their physical health and psychological health, but the acupuncture treatments did not significantly change their ratings on their social health and environmental health. Acupuncture has its limitations, for instance, acupuncture cannot directly change the participant's housing situation that is factored into their environmental health perspective or 25% of their QOL score. Consequently, this contributed to the insignificant differences and/or inconclusiveness from the QOL data collected in this study. Hence, it would be more appropriate to measure and evaluate Perceived Stress Scale (PSS) Score for studies on treating participants' chronic stress.

4.4. Discussion— Strengths and Limitations

4.4.1. Effects of EG's & CG's Acupuncture on Parasympathetic HRVs

Parasympathetic HRVs (PNS HRVs) were the RMSSD, pNN50, pNN20 and HF data collected in this study. After 12th treatment, EG's average PNS HRVs differences were greater than CG's. Both EG and CG showed to have at least 95% confidence interval (CI) of significant differences in all PNS HRV variables after 12th treatment, except for pNN20 with marginal significance of at least 90% CI, and for HF with no significance. While RMSSD, pNN20 and pNN50 are time-domain HRV variables indicating the primarily parasympathetic activities from circulatory and respiratory systems; HF is a frequency-domain HRV variable indicating primary parasympathetic activity from gastrointestinal, endocrine, urinary and immune systems. Perhaps, both EG's and CG's acupuncture treatments were effective for regulating PNS activities of the heart and lungs after 12 weekly treatments, but for regulating PNS activities of the spleen, kidneys and liver may have limitations or may take longer time and more treatments to start seeing improvements.

4.4.2. Effects of EG's & CG's Acupuncture on Sympathovagal HRVs

Sympathovagal HRVs (SV HRVs) were the SDNN and LF data collected in this study. After 6th treatment, only EG showed to have at least 95% confidence interval (CI) of significance difference in both SV HRVs measured, whereas CG did not. After 12th treatment, both EG and CG showed to have at least 95% confidence interval (CI) of significance difference in both SV HRVs measured. After 6th and after 12th treatments, EG's average SV HRVs differences were greater than CG's. Overall, both EG's and CG's

acupuncture treatments showed effectiveness in regulating sympathovagal activity after 12 weeks of treatment, with EG showing quicker results in improving SV HRVs.

4.4.3. **Advantages of EG Acupuncture on Autonomic Nervous System**

The premise behind these selected EG acupuncture points integrated perspectives from biomedical science's Autonomic Nervous Systems (ANS) physio anatomy and Traditional Chinese Medicine (TCM) Yin Yang Theory. When the sympathovagal (SNS & PNS) nervous systems become unbalanced and Yin Yang become disharmonized, the body's functional capabilities in regulating mental and physical stresses, hormone systems, immunity, blood circulation, respiration, digestion, sleep are all disrupted. When under excessive stress or toxic stress, the SNS becomes overactive, and PNS becomes overpowered or underactive. Referring back to Figures 4 & 5 in Chapter 1, the ANS is mapped out on the posterior spine, where the SNS chain roots from thoracic vertebrae 1 (T1) to lumbar vertebrae 2 (L2), and the PNS chain roots from sacrum (S2 to S4). By bilaterally acupuncturing Urinary Bladder (UB) channel points from T1 to L2, in theory, may help sedate or tonify the dysregulated SNS & PNS, and allow better regulation between SNS and PNS, reducing a person's overall stress. Acupuncturing Urinary Bladder (UB) channel points from T1 to L2 may also help relieve the muscular and internal tension held in the back shoulders when under excessive stress or flight/fight response. Therefore, this could reduce the qi and blood stagnation and improving qi and blood circulation locally, allowing for better fluid nourishment and circulation from the corresponding organs and UB-channel acupuncture points at T3 level (Lung / UB13 & UB42), at T5 level (Heart / UB15 & UB44), at T10 level (Liver / UB18 & UB47), at T12 level (Spleen / UB20 & UB49), and at L2 level (Kidney / UB23 & UB52). In TCM, the

more lateral UB channel points (or UB channel second line points) correspond with the Five Psychic Aspects points that help strengthen mental and emotional capacity. Although in theory, UB channel points along the level of S2 to S4 may help regulate the parasympathetic nervous system, for this pilot trial, only points along the levels of T2 to L2 vertebraes were examined. In future full-scale studies, UB channel acupuncture points from T1 to L2 and points from S2 to S4 may be examined and compared.

4.4.4. **Acupuncture's Limitations on ACEs and AAHCs**

ACEs: In both acupuncture treatment groups' participants that have 5 or less Adverse Childhood Experiences (n=10 in EG and n=12 in CG), participants showed significant improvements in their HRV measurements after 6th treatments. For participants with ACEs Score higher than 5 (n=8 in EG and n=5 in CG), EG's acupuncture treatments appeared to have improved EG participants' HRVs after 12th treatment, while CG's acupuncture treatments did not significantly change their participants' HRVs.

AAHCs: Both groups' participants that have one ACE-Associated Health Condition (AAHC) showed to improve their HRVs more quickly after 6th treatment (n=4 in EG and n=3 in CG). Compared to participants that have greater than one AAHCs, they showed later improvements in their HRVs after 12th treatment (n=14 in EG and n=14 in EG).

4.5. Discussion— Potential Impact and Future Direction

From this pilot clinical trial alone, there is not only evidence indicating more effectiveness from EG's acupuncture protocol of Back-shu and Psychic Aspect points, in improving heart rate variability (HRVs) and reducing stress (PSS) in adults with toxic stress and ACEs, but there is also potential impact of using HRV measurements for supplementing Traditional Chinese Medicine (TCM) differential diagnosis. Note that more participants and studies are needed to confirm this potential TCM diagnostic tool, hence, this is still a working theory, and this is not yet intended for clinical diagnosis.

Moving on, the HRV data, vital signs and symptoms observed from the participants (n=35) in this study, suggest that HRVs may be related to TCM's Yin Yang Theory of Mutual Consumption. For instance, when the patients have signs of Yang Deficiency and/or Yin Excess: they may have tendency for abnormally low SV HRVs (SDNN and LF), and/or abnormally high PNS HRVs (RMSSD, pNN20, pNN50 and HF), and/or abnormally low LF/HF (ratio less than 1). Vice versa, when the patients have signs of Yin Deficiency and/or Yang Excess: they may have tendency for abnormally low PNS HRVs (RMSSD, pNN20, pNN50, HF), and/or abnormally high SV HRVs (SDNN, LF), and/or abnormally high LF/HF (ratio greater than 2). When the patients have signs of balance of Yin and Yang: most of their HRVs will tend to be in normal or healthy ranges. More research and participants are needed to confirm this working theory.

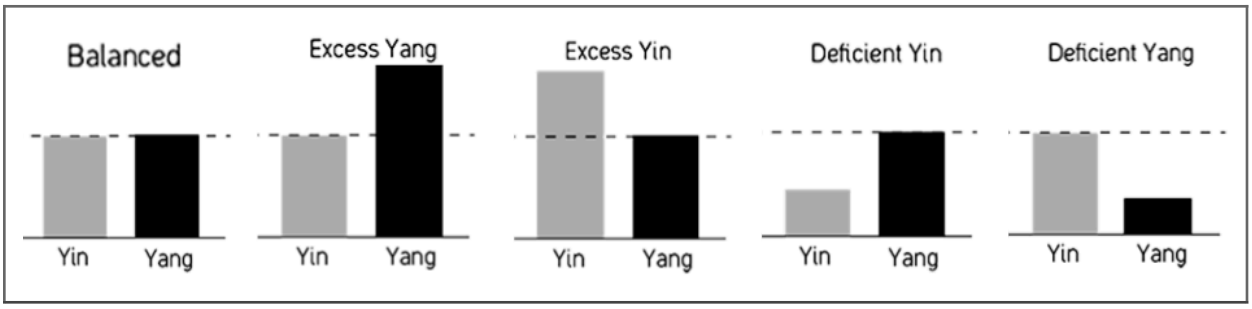


Figure 58. **Yin Yang Theory of Mutual Consumption**

As of March 2024, the investigator continues to conduct ACE screenings from all new patients and collect HRV data from returning patients bimonthly. Regarding future research studies, the next step would be conducting a HRV diagnosis analysis cross-sectional study for each of the five Yin Yang Mutual Consumption patterns. Before conducting any future randomized control trials (RCTs), cross-sectional studies would be essential to observe and record the potential correlation between HRV variables and Yin Yang theory patterns in more participants. Each cross-sectional study can run one after the other, or run simultaneously if there are multiple dedicated acupuncturists/investigators, sufficient sample sizes of participants and economical resources available. In order to establish more validity of HRV data analysis in potential TCM clinical diagnosis, the study design solutions mentioned in previous Section 4.3, regarding inclusion, exclusion criterias and initial HRV screenings are important to implement. Since HRV analysis is well-known amongst western medicine practitioners, such as neurologists, cardiologists and psychologists, the potential for integrated TCM healthcare and the future collaborations in these HRV diagnostic analysis cross-sectional studies could be possible.

Although HRV analysis can be conducted on various patients with a wide range of health conditions, the investigator will continue to focus on studying and treating underrepresented population with toxic stress and ACEs. As an acupuncturist and a mental health advocate, the investigator recognizes the importance of treating both the root causes and symptomatic manifestations, especially in chronic health conditions that stem from untreated childhood traumas (ACEs) and/or chronic stress— the health epidemic of the 21st century.

V. CONCLUSION

This pilot randomized single blind active-controlled trial examined the comparative effectiveness of experimental–back acupuncture to the active-control–traditional distal acupuncture, in helping improve the Heart Rate Variability (HRVs) for adults ages 18-80 with chronic 'toxic' stress, Adverse Childhood Experiences (ACEs) and ACE-Associated Health Conditions (AAHCs).

Prior to treatment, all adult participants met the inclusion criterias of an Adverse Childhood Experiences (ACEs) Score of 1 or higher, and a Perceived Stress Score (PSS) of 27 or higher. The investigator enrolled 35 qualified participants (total n = 35), and then blinded and randomly assigned participants to either the experimental group 'EG' (n =18) or active-control 'CG' group (n = 17). During 12-week acupuncture trial, all participants's HRVs (RMSSD, SDNN, pNN20, pNN50, LF, HF and LF/HF) were recorded weekly, while Perceived Stress Scale (PSS) Scores and Quality of Life (WHOQOL-BREF) Scores were collected three times (before 1st, after 6th and after 12th treatments).

As a result, the HRV final variables after 12th treatment indicated: RMSSD for EG was 60.6 ± 21.92 with a difference of 18.3 ± 15.44 (p = 0.001), and for CG was 45.8 ± 26.20 with a difference of 6.9 ± 3.53 (p = 0.000); comparing the two groups' difference was significant (p = 0.001). SDNN for EG was 123.7 ± 31.56 with a difference of 22.2 ± 15.84 (p = 0.000), and for CG was 123.0 ± 30.03 with a difference of 6.6 ± 7.67 (p = 0.009); comparing the two groups' difference was significant (p = 0.000). pNN20 for EG was 33.4 ± 6.16 with a difference of 7.0 ± 14.75 (p = 0.058), and for CG was 27.9 ± 17.44 with a difference of 0.8 ± 5.55 (p = 0.549); comparing the two groups' difference was significant (p = 0.008). pNN50 for EG was 19.3 ± 8.26 with a difference of 6.1 ± 10.6 (p = 0.026), and for CG was 13.4 ± 12.43 with a difference

of 2.1 ± 3.41 (p = 0.021); comparing the two groups' difference was significant (p = 0.008). LF for EG was 782.1 ± 311.43 with a difference of 85.9 ± 92.14 (p = 0.005), and for CG was 689.0 ± 408.10 with a difference of 9.6 ± 14.95 (p = 0.022); comparing the two groups' difference was significant (p = 0.000). HF for EG was 667.5 ± 335.26 with a difference of 38.6 ± 138.34 (p = 0.316), and for CG was 628.4 ± 396.77 with a difference of 7.5 ± 20.09 (p = 0.058); comparing the two groups' difference was significant (p = 0.026). LF/HF for EG was 1.3 ± 0.50 with a difference of 0.23 ± 0.45 (p = 0.081), and for CG was 1.5 ± 1.17 with a difference of 0.05 ± 0.12 (p = 0.198); comparing the two groups' difference was no significant (p = 0.111).

Additionally, questionnaire scores after 12th treatment revealed: PSS for EG was 23.2 ± 2.79 with a difference of 9.9 ± 3.02 (p = 0.000), and for CG was 27.4 ± 3.32 with a difference of 4.9 ± 2.63 (p = 0.000); comparing the two groups' difference was significant (p = 0.000). QOL for EG was 61.3 ± 12.12 with a difference of 6.1 ± 2.02 (p = 0.000), and for CG was 57.62 ± 10.70 with a difference of 1.5± 1.57 (p = 0.0019); comparing the two groups' difference was significant (p = 0.000).

Upon completion of the final acupuncture treatments, there was significant difference in the change between groups for variables— RMSSD, pNN50 and PSS. Although there was not significant change between groups for variables— SDNN, pNN20, LF, HF, LF/HF and QOL, there were definitely significant differences between the groups for all measured variables except for LF/HF. Within EG, the differences were significant for RMSSD, SDNN, pNN50, LF, PSS and QOL as early as after 6th treatment and after 12th treatment. Within CG, the differences were significant for RMSSD, SDNN, pNN50, LF, PSS and QOL only after 12th treatment. The measured variables pNN20, HF and LF/HF were all not significantly different, for the changes between groups, for differences within EG and for differences within CG.

In conclusion, CG's traditional distal acupuncture treatments were effective in improving HRVs (RMSSD, SDNN, pNN50, LF), reducing stress (PSS), and making a difference in quality of life (QOL) only after the 12th treatment. Yet overall, EG's experimental back acupuncture was more effective and more efficient in improving HRVs (RMSSD, SDNN, pNN50, LF), reducing stress (PSS), and making a difference in quality of life (QOL) from early-on throughout treatments. Although more research is needed beyond this pilot clinical trial, nevertheless, EG's experimental back acupuncture treatment, consisting of Back-shu and Psychic Aspect acupuncture points, shows great potential and effectiveness as an acupuncture treatment protocol for toxic stress and ACEs-Associated Health Conditions (AAHCs).

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APPENDICES

Appendix A: Consent Documents

Informed Consent Form

You are invited to participate in a research study about "Comparative Effectiveness of Experimental Acupuncture to Traditional Acupuncture for Improving Heart-Rate-Variability (HRV) in Adults with Toxic Stress and ACE-Associated Health Conditions (AAHCs): A Pilot Randomized Single-Blinded Active-Controlled Trial".

Total goal of this research study is to compare the effectiveness of Experimental Acupuncture point combination with Traditional Acupuncture point combination in improving Heart Rate Variability (HRV) for adults with Toxic Stress and ACE-Associated Health Conditions (AAHCs), in other words, for adults with chronic stress.

The study design is a pilot randomized single blind active-controlled trial that applies Experimental Acupuncture point combination to the Experimental Group (EG) participants, and compares its effectiveness with established Traditional Acupuncture point combination for treating chronic stress to the Active-Control Group (CG) participants, on a weekly basis for 12-weeks. Adult participants, ages 18–80, who scored a 1 or higher on Adverse Childhood Experiences (ACE) Screening and scored a 27 or higher on Perceived Stress Scale (PSS), are qualified to participate. All participants's Heart Rate Variability (HRVs) are recorded weekly for 12-weeks, while Perceived Stress Scale (PSS) Scores and Quality of Life (WHOQOL-BREF) Scores are collected three times during the 12-week trial.

This study is being conducted by researcher and licensed acupuncturist:
Jennifer So, L.Ac.

Your participation in this research is entirely voluntary. It is your choice whether to participate or not. Whether you choose to participate or not, all the services you receive from this acupuncturist will continue and nothing will change. If you choose not to participate in this research project, you will be offered the treatment that is routinely offered by this

acupuncturist. You may change your mind later and stop participating even if you agreed earlier.

Participating in this study may not benefit you directly, but it will help to enrich the knowledge on Acupuncture and Asian Medicine.

By Participating in this research it is possible that you will be at greater risk than you would otherwise be.

There is, for example, a risk that your condition will not get better and that the new acupuncture treatment doesn't work even as well as the old one. If, however, the acupuncture treatment is not working, the acupuncturist will give the acupuncture treatment routinely offered to make you more comfortable. While the possibility of this happening is very low, you should still be aware of the possibility. Please also see and complete another separate form called "Acupuncture Informed Consent to Treat Form" if you consent to participating in this study.

You should be aware that your Heart Rate Variability (HRV) data will be measured by an electrocardiogram (ECG) recording device that follows the Food Drug Administration (FDA)'s Investigational Device Exemption (IDE) regulations. Please note that the ECG recording device, being used in this clinical trial, is an investigational device in order to collect safety and effectiveness data. This ECG recording device, being used in this clinical trial, follows the IDE regulations and is exempted from Code of Federal Regulations Title 21 (21 CFR 812) because it is: (1) safe and noninvasive, (2) does not require an invasive sampling procedure that presents any significant risk, (3) does not by design or intention introduce energy into a human subject, and (4) is not used as a diagnostic procedure without confirmation by another medically established diagnostic product or procedure. While the possibility of adverse effects happening from using this ECG recording device is extremely low, you should still be aware of risk. Potential risk(s) from using the ECG recording device are not from the ECG device itself, but possibly from the ECG device's adhesive attachments—three small 3M electrode/patches adhered to your skin during ECG recordings. Side effects from the patches may be temporary skin dryness/sensitivity and/or loss of hairs on wrists and ankle when patches get removed. Note, by signing this Informed

Consent Form, you are completely aware of the risks, you have given permission for the researcher to attach this ECG recording device on you to collect data for this clinical trial.

If at any moment pre-trial or during trial, you feel uncomfortable, you need to inform the researcher immediately, and you have the right to withdraw from this trial.

The information you will share with us if you participate in this study will be kept completely confidential to the full extent of the law. The information that the researcher collects from this research project will be kept confidential. Information about you that will be collected during the clinical trial will be put away and no-one but the researcher will be able to see it. Any information about you will have a Participant ID# on it instead of your name. Only the researcher will know what your Participant ID#. It will not be shared with or given to anyone except Jennifer So, L.Ac.

If you have any questions about this study, please contact Jennifer So, L.Ac. at jennifer.so.org@gmail.com. If you have any questions or concerns regarding your rights as a subject in this study, you may contact the Chair of the South Baylo University Institutional Review Board (IRB) at 213-738-0712.

YOU WILL BE GIVEN A COPY OF THIS FORM WHETHER OR NOT YOU AGREE TO PARTICIPATE.

Certificate of Consent:

I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction. I consent voluntarily to participate as a participant in this research.

_____	_____
Name of Participant (Print)	Name of Witness (Print)
_____	_____
Signature of Participant	Signature of Witness
_____	_____
Date: Day/Month/Year	Date: Day/Month/Year

Statement by the researcher/person taking consent:

I have accurately explained the information sheet to the potential participant. I confirm that the participant was given an opportunity to ask questions about the study, and all the questions asked by the participant have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

A copy of this ICF has been provided to the participant

Jennifer So

_____

Print Name Researcher (Print)

_____

Signature of Researcher

31th of August 2023

_____

Date: Day/Month/Year

Acupuncture Informed Consent to Treat Form:

ACUPUNCTURE INFORMED CONSENT TO TREAT

I understand that I am the decision maker for my health care. Part of this office's role is to provide me with information to assist me in making informed choices. This process is often referred to as "informed consent" and involves my understanding and agreement regarding the care recommended, the benefits and risks associated with the care, alternatives, and the potential effect on my health if I choose not to receive the care. Acupuncture is not intended to substitute for diagnosis or treatment by medical doctors or to be used as an alternative to necessary medical care. It is expected that you are under the care of a primary care physician or medical specialist, that pregnant patients are being managed by an appropriate healthcare professional, and that patients seeking adjunctive cancer support are under the care of an oncologist.

I hereby request and consent to the performance of acupuncture treatments and other procedures within the scope of the practice of acupuncture on me (or on the patient named below, for whom I am legally responsible) by the acupuncturist indicated below and/or other licensed acupuncturists who now or in the future treat me while employed by, working or associated with, or serving as back-up for the acupuncturist named below, including those working at the clinic or office listed below or any other office or clinic, whether signatories to this form or not.

I understand that methods of treatment may include, but are not limited to, acupuncture, moxibustion, cupping, electrical stimulation, Tui-Na (Chinese massage), Chinese herbal medicine, and nutritional counseling. I understand that the herbs may need to be prepared and the teas consumed according to the instructions provided orally and in writing. The herbs may have an unpleasant smell or taste. I will immediately notify a member of the clinical staff of any unanticipated or unpleasant effects associated with the consumption of the herbs.

I appreciate that it is not possible to consider every possible complication to care. I have been informed that acupuncture is a generally safe method of treatment, but, as with all types of healthcare interventions, there are some risks to care, including, but not limited to: bruising; numbness or tingling near the needling sites that may last a few days; and dizziness or fainting. Burns and/or scarring are a potential risk of moxibustion and cupping, or when treatment involves the use of heat lamps. Bruising is a common side effect of cupping. Unusual risks of acupuncture include nerve damage and organ puncture, including lung puncture (pneumothorax). Infection is another possible risk, although the clinic uses sterile disposable needles and maintains a clean and safe environment.

I understand that while this document describes the major risks of treatment, other side effects and risks may occur. The herbs and nutritional supplements (which are from plant, animal, and mineral sources) that have been recommended are traditionally considered safe in the practice of Chinese Medicine, although some may be toxic in large doses. I understand that some herbs may be inappropriate during pregnancy. I will notify a clinical staff member who is caring for me if I am, or become, pregnant or if I am nursing. Should I become pregnant, I will discontinue all herbs and supplements until I have consulted and received advice from my acupuncturist and/or obstetrician. Some possible side effects of taking herbs are: nausea; gas; stomachache; vomiting; liver or kidney damage; headache; diarrhea; rashes; hives; and tingling of the tongue.

While I do not expect the clinical staff to be able to anticipate and explain all possible risks and complications of treatment, I wish to rely on the clinical staff to exercise judgment during the course of treatment which the clinical staff thinks at the time, based upon the facts then known, is in my best interest. I understand that, as with all healthcare approaches, results are not guaranteed, and there is no promise to cure.

I understand that I must inform, and continue to fully inform, this office of any medical history, family history, medications, and/or supplements being taken currently (prescription and over-the-counter). I understand the clinical and administrative staff may review my patient records and lab reports, but all my records will be kept confidential and will not be released without my written consent.

I understand that there are treatment options available for my condition other than acupuncture procedures. These options may include, but are not limited to: self-administered care, over-the-counter pain relievers, physical measures and rest, medical care with prescription drugs, physical therapy, bracing, injections, and surgery. Lastly, I understand that I have the right to a second opinion and to secure other options about my circumstances and healthcare as I see fit.

By voluntarily signing below, I confirm that I have read, or have had read to me, the above consent to treatment, have been told about the risks and benefits of acupuncture and other procedures, and have had an opportunity to ask questions. I agree with the current or future recommendations for care. I intend this consent form to cover the entire course of treatment for my present condition and for any future condition(s) for which I seek treatment.

PATIENT NAME:

ACUPUNCTURIST NAME:

(Date)

PATIENT SIGNATURE **X**

(Or Patient Representative) (Indicate relationship if signing for patient)

A2015

Appendix B: Questionnaires

Adverse Childhood Experience (ACEs) Questionnaire

WHAT'S MY ACE SCORE?

Prior to your eighteenth birthday:

1. Did a parent or other adult in the household often…
   Swear at you, insult you, put you down, or humiliate you?
   or
   Act in a way that made you afraid you might be physically hurt?

   Yes **No** **If yes enter 1 _____**

2. Did a parent or other adult in the household often…
   Push, grab, slap, or throw something at you?
   or
   Ever hit you so hard that you had marks or were injured?

   Yes **No** **If yes enter 1 _____**

3. Did an adult or person at least five years older than you ever…
   Touch or fondle you or have you touch their body in a sexual way?
   or
   Attempt to actually have oral, anal, or vaginal intercourse with you?

   Yes **No** **If yes enter 1 _____**

4. Did you often feel that…
   No one in your family loved you or thought you were important or special?
   or
   Your family didn't look out for each other, feel close to each other, or support each other?

   Yes **No** **If yes enter 1 _____**

5. Did you often feel that…
   You didn't have enough to eat, had to wear dirty clothes, and had no one to protect you?
   or
   Your parents were too drunk or high to take care of you or take you to the doctor if you needed it?

   Yes **No** **If yes enter 1 _____**

6. Were your parents ever separated or divorced?

   Yes **No** **If yes enter 1 _____**

7. Was your mother or stepmother…
   Often pushed, grabbed, slapped, or had something thrown at her? or Sometimes or often kicked, bitten, hit with a fist, or hit with something hard?
   or
   ever repeatedly hit over at least a few minutes or threatened with a gun or knife?

   Yes **No** **If yes enter 1 _____**

8. Did you live with anyone who was a problem drinker or alcoholic or who used street drugs?

   Yes **No** **If yes enter 1 _____**

9. Was a household member depressed or mentally ill, or did a household member attempt suicide?

   Yes **No** **If yes enter 1 _____**

10. Did a household member go to prison?

    Yes **No** **If yes enter 1 _____**

Now add up your "Yes" answers: _____ This is your ACE Score.

Perceived Stress Scale (PSS) Questionnaire

WHAT'S MY PSS SCORE ?

For each question choose from the following alternatives:

0 - never **1** - almost never **2** - sometimes **3** - fairly often **4** - very often

☐	I. In the last month, how often have you been upset because of something that happened unexpectedly?
☐	2. In the last month, how often have you felt that you were unable to control the important things in your life?
☐	3. In the last month, how often have you felt nervous and stressed?
☐	4. In the last month, how often have you felt confident about your ability to handle your personal problems?
☐	5. In the last month, how often have you felt that things were going your way?
☐	6. In the last month, how often have you found that you could not cope with all the things that you had to do?
☐	7. In the last month, how often have you been able to control irritations in your life?
☐	8. In the last month, how often have you felt that you were on top of things?
☐	9. In the last month, how often have you been angered because of things that happened that were outside of your control?
☐	10. In the last month, how often have you felt difficulties were piling up so high that you could not overcome them?

You can determine your PSS score by following these directions:

- First, reverse your scores for questions 4, 5, 7, and 8.

On these 4 questions, change the scores like this: 0 = 4, 1 = 3, 2 = 2, 3 = 1, 4 = 0.

- Now add up your scores for each item to get a total.

My total PSS score is ☐ .

* Individual scores on the PSS can range from 0 to 40 with higher scores indicating higher perceived stress.

► Scores ranging from 0-13 would be considered low stress.

► Scores ranging from 14-26 would be considered moderate stress.

► Scores ranging from 27-40 would be considered high perceived stress.

Quality of Life (QOL) Questionnaire —

"World Health Organization Quality of Life - Brief Version (WHOQOL-BREF)"

WHAT'S MY QOL SCORE?

Instructions: This assessment asks how you feel about your quality of life, health, or other areas of your life. Please answer all the questions. If you are unsure about which response to give to a question, please choose the one that appears most appropriate. This can often be your first response.

Please keep in mind your standards, hopes, pleasures and concerns. We ask that you think about your life in the last two weeks.

1. How would you rate your quality of life?

⬜Very Poor (1)	⬜Poor (2)	⬜ Neither Poor nor good (3)	⬜Good (4)	⬜Very Good (5)

2. How satisfied are you with your health?

⬜Very Dissatisfied (1)	⬜Dissatisfied (2)	⬜ Neither Satisfied nor Dissatisfied (3)	⬜Satisfied (4)	⬜Very Satisfied (5)

The following questions ask about how much you have experienced certain things in the last two weeks.

3. To what extent do you feel that physical pain prevents you from doing what you need to do?

⬜Not at all (5)	⬜A little (4)	⬜ A moderate amount (3)	⬜Very much (2)	⬜An extreme amount (1)

4. How much do you need any medical treatment to function in your daily life?

⬜Not at all (5)	⬜A little (4)	⬜ A moderate amount (3)	⬜Very much (2)	⬜An extreme amount (1)

5. How much do you enjoy life?

⬜Not at all (5)	⬜A little (4)	⬜ A moderate amount (3)	⬜Very much (2)	⬜An extreme amount (1)

6. To what extent do you feel your life is meaningful?

⬜Not at all (5)	⬜A little (4)	⬜ A moderate amount (3)	⬜Very much (2)	⬜An extreme amount (1)

7. How well are you able to concentrate?

⬜Not at all (5)	⬜A little (4)	⬜ A moderate amount (3)	⬜Very much (2)	⬜An extreme amount (1)

8. How safe do you feel in your daily life?

⬜Not at all (5)	⬜A little (4)	⬜ A moderate amount (3)	⬜Very much (2)	⬜An extreme amount (1)

9. How healthy is your physical environment?

⬜Not at all (5)	⬜A little (4)	⬜ A moderate amount (3)	⬜Very much (2)	⬜An extreme amount (1)

The following questions ask were able to do certain things in the last two weeks.

10. Do you have enough energy for everyday life?

⬜Not at all (1)	⬜A little (2)	⬜ Moderately (3)	⬜Mostly (4)	⬜Completely (5)

11. Are you able to accept your bodily appearance?

⬜Not at all (1)	⬜A little (2)	⬜ Moderately (3)	⬜Mostly (4)	⬜Completely (5)

12. Do you have enough money to meet your needs?

⬜Not at all (1)	⬜A little (2)	⬜ Moderately (3)	⬜Mostly (4)	⬜Completely (5)

13. How available to you is the information that you need in your day-to-day life?

⬜Not at all (1)	⬜A little (2)	⬜ Moderately (3)	⬜Mostly (4)	⬜Completely (5)

14. To what extent do you have the opportunity for leisure activities?

⬜Not at all (1)	⬜A little (2)	⬜ Moderately (3)	⬜Mostly (4)	⬜Completely (5)

15. How well are you able to get around?

⬜Very Poor (1)	⬜Poor (2)	⬜ Neither Poor nor good (3)	⬜Good (4)	⬜Very Good (5)

The following questions ask you to say how good or satisfied you have felt about various aspects of your life over the last two weeks.

16. How satisfied are you with your sleep?

⬜ Very Dissatisfied (1)	⬜ Dissatisfied (2)	⬜ Neither Satisfied nor Dissatisfied (3)	⬜ Satisfied (4)	⬜ Very Satisfied (5)

17. How satisfied are you with your ability to perform your daily living activities?

⬜ Very Dissatisfied (1)	⬜ Dissatisfied (2)	⬜ Neither Satisfied nor Dissatisfied (3)	⬜ Satisfied (4)	⬜ Very Satisfied (5)

18. How satisfied are you with your capacity for work?

⬜ Very Dissatisfied (1)	⬜ Dissatisfied (2)	⬜ Neither Satisfied nor Dissatisfied (3)	⬜ Satisfied (4)	⬜ Very Satisfied (5)

19. How satisfied are you with yourself?

⬜ Very Dissatisfied (1)	⬜ Dissatisfied (2)	⬜ Neither Satisfied nor Dissatisfied (3)	⬜ Satisfied (4)	⬜ Very Satisfied (5)

20. How satisfied are you with your personal relationships?

⬜ Very Dissatisfied (1)	⬜ Dissatisfied (2)	⬜ Neither Satisfied nor Dissatisfied (3)	⬜ Satisfied (4)	⬜ Very Satisfied (5)

21. How satisfied are you with your sex life?

⬜ Very Dissatisfied (1)	⬜ Dissatisfied (2)	⬜ Neither Satisfied nor Dissatisfied (3)	⬜ Satisfied (4)	⬜ Very Satisfied (5)

22. How satisfied are you with the support you get from your friends?

⬜ Very Dissatisfied (1)	⬜ Dissatisfied (2)	⬜ Neither Satisfied nor Dissatisfied (3)	⬜ Satisfied (4)	⬜ Very Satisfied (5)

23. How satisfied are you with the conditions of your living place?

⬜Very Dissatisfied (1)	⬜Dissatisfied (2)	⬜ Neither Satisfied nor Dissatisfied (3)	⬜Satisfied (4)	⬜Very Satisfied (5)

24. How satisfied are you with your access to health services?

⬜Very Dissatisfied (1)	⬜Dissatisfied (2)	⬜ Neither Satisfied nor Dissatisfied (3)	⬜Satisfied (4)	⬜Very Satisfied (5)

25. How satisfied are you with your transport?

⬜Very Dissatisfied (1)	⬜Dissatisfied (2)	⬜ Neither Satisfied nor Dissatisfied (3)	⬜Satisfied (4)	⬜Very Satisfied (5)

Following question refers to how often you have felt or experienced certain things in the last 2 weeks.

26. How often do you have negative feelings such as blue mood, despair, anxiety, depression?

⬜Never (5)	⬜Seldom (4)	⬜ Quite Often (3)	⬜Very Often (2)	⬜Always (1)

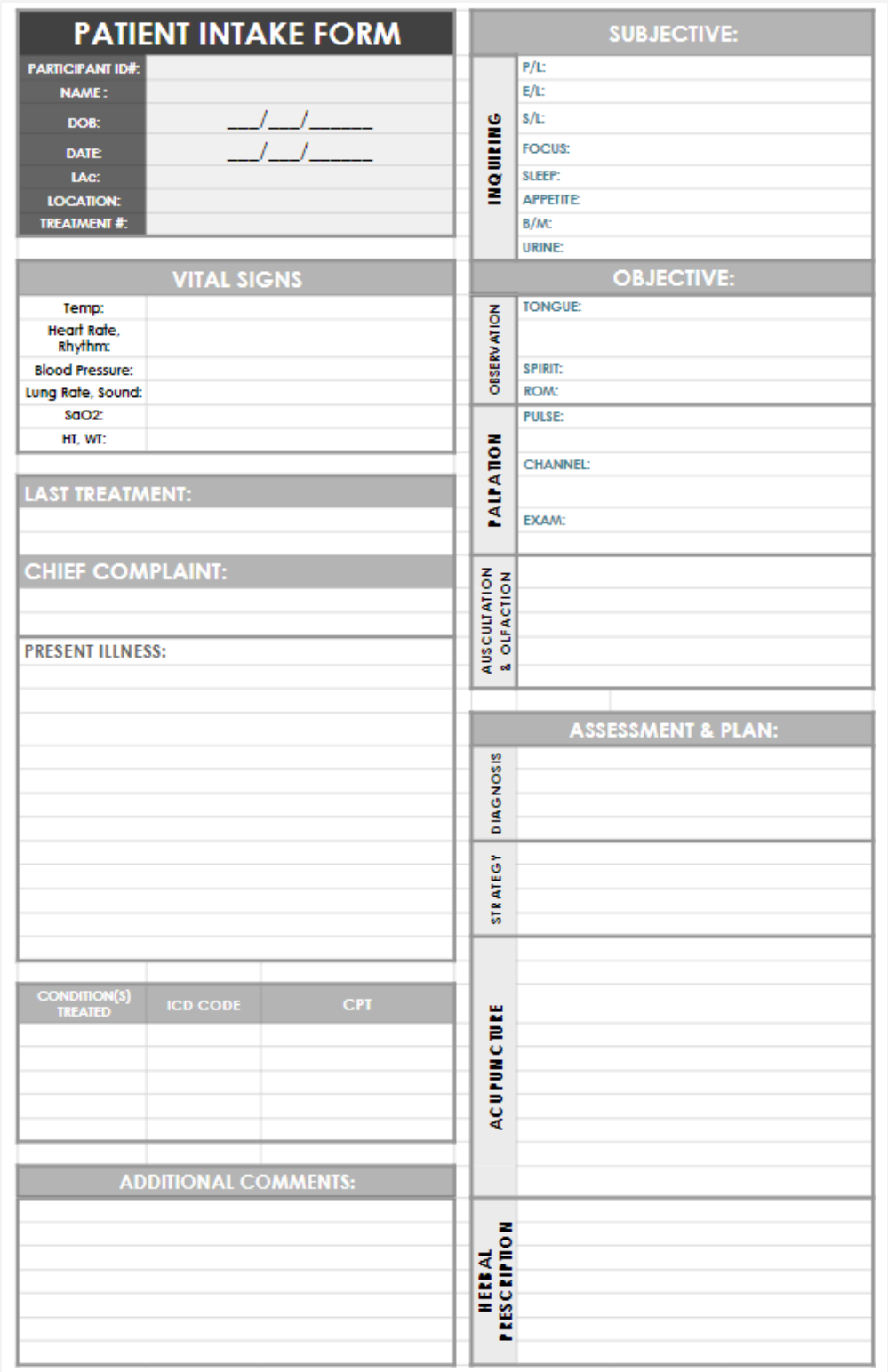
*The WHOQOL-BREF is designed to measure a person's perception of their quality of life, defined by the WHO as "individuals' perceptions of their position in life in the context of the culture and value systems in which they live and in relation to their goals, expectations, standards and concerns".

This WHOQOL-BREF Questionnaire can be completed and scored easily at https://neurotoolkit.com/whoqol-bref/, for it provides an automatic calculation software for overall QOL Score Percentage, as well as score percentages for individual categories.

The WHOQOL-BREF measures quality of life across 4 domains (Physical Health, Psychological, Social Relationships, and Environment). The measure is calculated by summing the point values for the questions corresponding to each domain and then transforming the scores to a 0-100 point interval, or alternatively, a 4-20 point interval. The first two questions of the WHOQOL-BREF do not correspond to a domain, but are meant to provide a global assessment of quality of life. Higher scores in each of the domains correspond to greater perceived quality of life.

Appendix C: Case Report Forms 'CRF'

Patient Intake Form



PATIENT INTAKE FORM

PARTICIPANT ID#:	
NAME :	
DOB:	___/___/_____
DATE:	___/___/_____
LAc:	
LOCATION:	
TREATMENT #:	

VITAL SIGNS

Temp:	
Heart Rate, Rhythm:	
Blood Pressure:	
Lung Rate, Sound:	
SaO2:	
HT, WT:	

LAST TREATMENT:

CHIEF COMPLAINT:

PRESENT ILLNESS:

CONDITION(S) TREATED	ICD CODE	CPT

ADDITIONAL COMMENTS:

SUBJECTIVE:

INQUIRING
- P/L:
- E/L:
- S/L:
- FOCUS:
- SLEEP:
- APPETITE:
- B/M:
- URINE:

OBJECTIVE:

OBSERVATION
- TONGUE:
- SPIRIT:
- ROM:

PALPATION
- PULSE:
- CHANNEL:
- EXAM:

AUSCULTATION & OLFACTION

ASSESSMENT & PLAN:

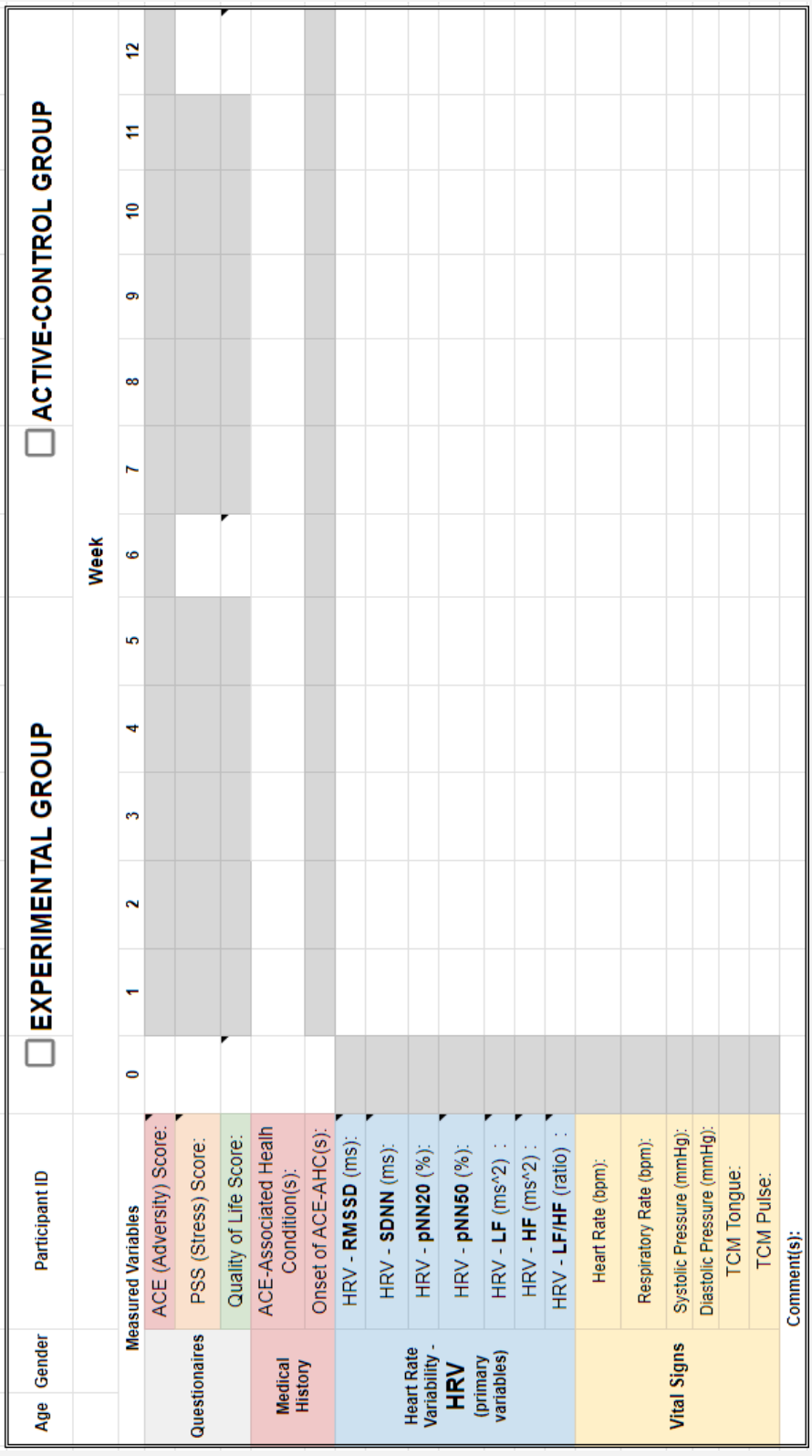
DIAGNOSIS

STRATEGY

ACUPUNCTURE

HERBAL PRESCRIPTION

Participant's Data Collection Form



Thank You