Assessing the feasibility of a therapy package application on reducing psychological and physiological stress in women: A pilot study

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Glossary

ANS – Autonomic nervous system

B-C BE – Brief-cognitive behavioural education

BMI – Body mass index

BP – Blood pressure

Bpm – Beats per minute

CFS – Chronic fatigue syndrome

CNS – central nervous system

CgA – Chromogranin-A

ELISA – Enzyme-linked Immuno-sorbent assay

GOT – General osteopathic technique/therapy

GOTs – General osteopathic techniques (more than one osteopathic technique)

HPA axis – Hypothalamic pituitary adrenal axis

HPG axis – Hypothalamic Pituitary Gonadal axis

HR – Heart rate

IBD – Irritable bowel disorder

IL-6 – Interleukin 6

PNS - Peripheral nervous system

POMS – Profile of Moods State

PRECIS-2 – Pragmatic Explanatory Continuum Indicator Summary-2

PSQ – Perceived Stress Questionnaire

PSS-10 – Perceived Stress Scale-10

QOL – Quality of life

SNS - Sympathetic nervous system

sIgA – secretory immunoglobulin-A

TLP – Thoracic lymphatic pumping

TMD – Total mood disturbance

TP – Timepoint

Abstract

INTRODUCTION AND AIM: There is mounting evidence regarding the existing relationship between stress and adverse health outcomes. As stress is multidimensional, the need for a multimodal approach to manage stress is warranted. There is a high incidence of stress among women compared to men, which can be due to various factors including financial, housing, personal health and occupational stress. Despite some literature indicating the positive effect massage therapy has on stress, there is limited research demonstrating the effects general osteopathic therapy has, either with or without stress management education on reducing stress. Therefore, the aim for this project was to conduct a pilot study assessing the feasibility of a therapy package application on reducing stress in women.

METHOD: This was a pilot uncontrolled trial with mixed pragmatic and exploratory design. The therapy package comprised of ten general osteopathic techniques and ten minutes of scripted stress management education, which was applied in two sessions over two consecutive days. The effects of the therapy package were assessed by measuring pre- and post-intervention scores of self-reported perceived stress (Perceived Stress Scale-10 and Profile of Mood Scale) and salivary levels of physiological biomarkers (cortisol, secretory Immunoglobulin A and Interleukin-6). In addition, feedback from participants and challenges experienced by both participants and the researcher assessed.

RESULTS: The therapy package was easy to administer to apply to the small sample size in an osteopathic clinical setting. A total of 11 female participants (age = 33.72 years, SD = 12.8) were enrolled, with a 100% retention rate. There were no recorded dropouts and no follow ups. The early morning sessions over two consecutive days were challenging for the majority of the participants. However, all women felt “relaxed” after the therapy package with no reported adverse effects. A significant decrease was observed across all participants in the scores of Perceived Stress Scale-10 over the course of the intervention (p = < 0.03). There were also significant decreases in the subscale scores of the Profile of Mood Scale (Tense, Fatigue, Depression, Anger). Cortisol, interleukin-6 and secretory immunoglobulin-A levels did not show any specific pattern of change over the course of the intervention. Overall there was no clear relationship observed between perceived, psychological stress scores and physiological stress biomarker levels or any clear patterns of change.

CONCLUSIONS: The application of the therapy package was feasible. The overall decrease in the Perceived Stress Scale-10 scores in the majority of participants suggests that the plausibility of this therapy package (ten selected osteopathic techniques, in combination with stress management education), contributed to reducing perceived stress. However, the effect on physiological stress was not clear, the results need further investigation. Future studies may benefit by employing randomised controlled trial design, with a larger sample size. Additionally, future researchers may need to consider utilising physiological biomarkers without diurnal variation and employing a flexible time for therapy application.

KEYWORDS: stress, manual therapy, general osteopathic therapy, perceived stress, psychological stress, cortisol, IL-6, sIgA, Stress management education.

Chapter 1 - Introduction

Stress is a physiological phenomenon, fundamental for our survival. Since chronic stress has been associated with the development of several adverse health conditions, there has been a significant drive from healthcare professionals and people to manage stress.

Chronic stress can be defined as stress resulting from repeated exposure to situations, both of which lead to the release of certain stress hormones, which affect some physiological and psychological pathways (Slavich, 2016). With repeated activation of an individuals' physiological stress system, the effects accumulate over time and result in an increased risk of developing chronic health issues (Entringer, Buss, & Wadhwa, 2015). For example, high blood pressure, arrythmias, heart disease and stroke are common cardiovascular diseases that can develop as a result of chronic stress (Crestani, 2016). The other systems affected in stress include the gastrointestinal, neurological, immune and respiratory. Furthermore, psychological and mental health problems can develop such as, depression, anxiety and personality/mood conditions (Yaribeygi, Panahi, Sahraei, Johnston, & Sahebkar, 2017). This demonstrates the interdependent relationship between stress, physical health and mental health. Moreover, this highlights the multifaceted nature of stress and its effect on many systems. Dysfunction to any of these areas is likely to have an impact on ones' overall health and well-being.

Due to the multifactorial nature of stress there is a need for a multimodal approach in healthcare. Health professionals from various expertise can contribute to mitigate the effects of stress. The current literature available suggesting that people utilise osteopathic treatment, in combination with stress management and education, is limited (Dugailly et al., 2014; Lynch, Gander, Nahar, Kohls, & Walach, 2018; Maynard, Hemmings, Greenlees, Warwick-Evans, & Stanton, 1998; Romano, 1992; Ruwaard, Lange, Schrieken, Dolan, & Emmelkamp, 2012). The effects of osteopathic treatment with stress management education needs to be further explored, as stress is multidimensional. It is important for healthcare professionals to have an alternative approach for the benefit of patients and for stress management. This is based on the premise that Osteopathy is a form of manual therapy considered to take an alternative and global approach to treatment, particularly in the case of treating and managing stress, which is not considered musculoskeletal (Dugailly et al., 2014; Paulus, 2013). Our present literature review highlights the need for further studies investigating the application of such an approach to develop strategies, schemes and programmes with which to reduce this increase. The prevalence of stress in New Zealanders has increased over the last 10 years (New Zealand

Ministry of Health, 2019). Around 60% of New Zealanders report feeling stressed at least once a week, with financial, housing, personal health and occupational worries considered as primary, driving stressors (Gray, 2016). In New Zealand alone, there appears to be a plethora of published literature indicating that stress is a growing topic of interest (Djuric et al., 2008; Hellhammer, Wüst, & Kudielka, 2009; Konduru, 2011; Nedeljka, Đorđe, Maja, Dijana Popović-Grubač, & Brankica Davidović, 2016; Regehr, Glancy, & Pitts, 2013). A simple search on Pubmed indicated that from 2000, the amount of research interest on stress rose from 14,500 to 63,600 in 2019 (Pubmed, 2020). This 383% increase in stress related publications, highlights the interest regarding stress and the importance of identifying and managing stress for better health and wellbeing.

The study presented in this thesis is a pilot, uncontrolled trial with mixed pragmatic and exploratory study design. The main objective of the study was to assess the feasibility of application of our proposed therapy package in reducing stress in women. The therapy package included the application of ten general osteopathic techniques and 10-minutess of stress management education, which was accompanied with an educational pamphlet on stress. The osteopathic techniques included joint articulation and cross-fibre soft tissue massage techniques. A parallel study investigating the feasibility and effects of such an approach in a sample of men was also conducted, however those results will not be presented in this thesis.

Participants were enrolled through advertisements and posters placed around the Unitec campus and various regions within Auckland. After initial screening and inclusion into the study, participants received two session of therapy package over two consecutive days. Both perceived and physiological stress levels were measured. Scores of psychometric measures of perceived stress and mood states were collected pre- and post-intervention. The measures utilised were two well validated self-report questionnaires (Perceived Stress Scale-10 and Profile of Moods States). The physiological parameter was measured in pre- and post-intervention saliva samples, which assessed three biomarkers that represent various pathways affected by stress. This included the application of the general osteopathic techniques, deliverance of the stress management education component, administration of self- report questionnaires and the collection of the saliva samples in osteopathic clinical setting. Additionally, recommendations have been provided for researchers to conduct future studies and/or issues and challenges to be considered while desgining future studies.

The thesis is presented in a traditional style, the structure follows the described chapters. Chapter one, is the current chapter, introduction of this project and thesis. Chapter two lists the aims and objective of the study. Chapter three involves an in-depth discussion of the current literature, exploring the background around stress and related topics. Chapter four describes the Methods and Protocols utilised, this chapter is divided into two parts: Part I discusses the rationale behind the selected study design and methods, and Part II discusses the actual methods employed in this study. Chapter five discussed the results. Chapter six covers the discussion around the results and concludes this thesis with providing limitations and recommendations for future studies. Chapter six also provides conclusions that could be drawn from the outcomes of this study. Appendices provide all relevant documents, detailed protocols and procedures.

Chapter 2 - Aims and Objectives

The aim of the study was to assess the feasibility of a proposed therapy package application in reducing stress. The therapy package included a 30-minutes of gentle General Osteopathic Treatment (GOT) and 10-minutes of stress management education with the provision of a stress management pamphlet. This pilot study aims to provide recommendations for the design of a cross-sectional or larger scale study for the future.

Objectives of this study:

1. To assess the feedback from participants and challenges encountered.
2. To assess the health status in 11 women (aged 18-50 years) using health screening measures (Adapted Patient Medical History Forms, blood pressure pulse, and heart rate).
3. To determine baseline levels of selected physiological stress biomarkers (cortisol, interleukin-6 and secretory immunoglobulin) in saliva samples of women.
4. To assess baseline Perceived Stress in the decided number of females using the adapted Perceived Stress Scale (PSS-10) and the Profile of Mood Scale (POMS).
5. To undertake a cross-sectional experimental study and assess the effects of the proposed therapy package, delivered in two consecutive sessions on stress in females. Therapy package: GOT including joint articulation, harmonics, and cross-fibre techniques and stress management education.

Chapter 3 - Literature Review

Search method

Existing articles and research were retrieved via using specific keywords and search on search engines, including Google Scholar, PubMed, Cochrane Library, and ScienceDirect. Relevant articles were also identified by searching within reference lists and titles of previously retrieved articles and suggested related articles provided by online databases. This included studies published in the English language between 2005-2019. The searched keywords included: "stress", "osteopathy", "general osteopathic therapy", "perceived stress", "psychological stress", "physiological stress", "stress biomarkers", "ELISA method", "sex differences in the stress response", "psycho-education", "stress management and education", and "health and well-being".

This chapter is organised into sections defining stress, stressors, and prevalence of stress. Then it elaborates on sex differences in responding to stress and looks at the relationship between perceived and physiological stress, followed by a discussion on appropriate assessment methodologies of stress, and concludes by further exploring stress management and where to go from here. A brief concluding summary of the key points from this literature review has been provided at the end of this section.

Stress

The definition of stress has adapted and evolved for decades. Stress has been broadly defined and can be perceived by individuals in a variety of different ways (Kyrou & Tsigos, 2009; McGowan, 2001; Nast, Bolten, Meinlschmidt, & Hellhammer, 2013). However, to date, stress can best be defined as "a state that threatens or is perceived by the individual to threaten that individual's physiological and psychological equilibrium" (Weinstock, 2005). Stress is increasing significantly in today's modern world, where the demands of life threaten to disrupt an individual's homeostasis. This has resulted in people, healthcare professionals, governments and researchers trying to establish ways to best assess, treat and manage stress and to mitigate its detrimental effect.

Perceived stress

The interest surrounding stress is growing as it has been linked to the development of many adverse health events (Graignic-Philippe, Dayan, Chokron, Jacquet, & Tordjman, 2014; Joëls, Karst, & Sarabdjitsingh, 2018; Lopez et al., 2011; Slavich, 2016; Stults-Kolehmainen & Sinha, 2014). Perceived (psychological) stress is best defined as a multidimensional concept, wherethe stress response is dependent on a wide array of contributing factors. Therefore, for this thesis, "perceived stress" and "psychological stress" will be used interchangeably.

The definition of perceived (psychological) stress is “a particular relationship between the person and the environment that is appraised by the person as taxing, or exceeding his or her resources, endangering his or her well-being” (Lazarus, 1989). An individual's psychological health is negatively affected by stress, generally as a result of chronic exposure to a repetitive stressor. This is perceived as uncontrollable.

The process of a stress response begins with the initial perception of a stressor. The response to that stressor is understood and centrally controlled by the brain, where different areas are activated when the stressor is detected (Godoy, Rossignoli, Delfino-Pereira, Garcia-Cairasco, & de Lima Umeoka, 2018; Vreede, 2010). Essentially, ‘footprints’ as a result of the activation, become imprinted within the brain (Godoy et al., 2018; Graignic-Philippe et al., 2014; Serpeloni, Radtke, Hecker, & Elbert, 2016). The response that is generated after detecting a stressor generally initiates an autonomic (uncontrolled) response, or an uncontrolled action (Godoy et al., 2018).

Personality factors, mentalities, cognitive appraisal processes and personality characteristics are important in determining what an individual perceives as stressful (Terry, Lane, & Fogarty, 2003). However, perception of a stressor is the first stage of initiating a stress response, which is registered as threatening by the individual (Van Hedger, Necka, Barakzai, & Norman, 2017). Due to the subjective nature of perceived stress, the ability to identify certain stressors or stressful situations is variable and difficult to generalise. A situation can be complex, and involve a combination of factors (i.e. physical, emotional, spiritual and social), which provides context on behavioural observations. As each factor can comprise of several stressors and can be based on previous exposure or experience to that stressor, this can determine an organism's response to that stressful situation (Yaribeygi et al., 2017).

A key threat to mental wellbeing as described in a report from the Mental Health Foundation of New Zealand (2011), was that there is too much psychological stress in today's modern world. Chronic psychological stress has been linked to the development of psychological comorbidities such as depression, anxiety, and mood disorders (Godoy et al., 2018). Anxiety disorders alone appear to be 1.9–3.6-fold more common in people who experience early life stress (V. Fernandes & Oso, 2016). In New Zealand alone, there appears to be a steady increase in stress, with chronic stress presenting as a strong risk factor for developing both physical and mental illnesses (Mental Health Foundation, 2020; Mental Health Foundation of New Zealand, 2011). Currently, there is a multitude of trends in an individual's life that can be related to causing negative effects on their collective mental health. Researchers hypothesise that with increases in stress, anxiety and feelings of helplessness, simultaneous increases in common diagnosable disorders such as depression, anxiety and addictions can be observed over time (Abdel, Wafaa, & Hassan, 2017; Mental Health Foundation of New Zealand, 2011; A. Mitchell, Crane, & Kim, 2008; Oswald et al., 2006; Slavich, 2016).

There appears to be a reciprocal relationship between psychological and physiological stress, whereby studies have demonstrated that psychological stress can be a risk factor for many physiological disorders (Batista, Periera, & Vaz, 2017; Epel et al., 2018; Godoy et al., 2018; Oswald et al., 2006). Some of these disorders involve the metabolic, endocrine, gastrointestinal, neurological and cardiovascular systems (Hjortskov, Rissén, et al., 2004). Conversely, exposure to chronic stress on a physiological level has shown strong correlations to the development of several psychological conditions, such as; neuropsychiatric disorders, mood disorders and other mental health issues (Godoy et al., 2018; Novais, Monteiro, Roque, Correia-Neves, & Sousa, 2016). Therefore, evidence indicates that perceived (psychological) stress is strongly related to physiological disturbances (physiological stress), and vice versa (Djuric et al., 2008; Epel et al., 2018; Oswald et al., 2006). The two are interrelated.

Physiological stress

Behaviour integrates all biomechanical and physiological interactions of an organism in response to a stressful situation. The results of long-term, chronic stress can have serious effects on the physiological pathways of an organism. Epidemiological studies have identified chronic stress as a risk factor for the development of several disorders affecting a multitude of physiological systems (Godoy et al., 2018; Hjortskov, Rissén, et al., 2004; Yaribeygi et al., 2017). These include, but are not limited to, the immune, cardiovascular, metabolic and cognitive systems (Godoy et al., 2018; Yaribeygi et al., 2017).

With this in mind, it is important to consider the Autonomic Nervous System's (ANS) role in the stress response, because it generates the body’s response to a stressor. This system is divided into the sympathetic nervous system (SNS) and parasympathetic nervous systems (PNS). After perceiving a stressor, the acute stress response is initiated. This is controlled by the brain through structural and chemical changes (Ruiz, Neto, Schoedl, & Mello, 2007). During the acute stress response, inflammatory cytokines (i.e. interleukin-6) are released, which activate the Hypo-Thalamic-Pituitary Axis (HPA) axis (Miller, Cohen, & Ritchey, 2002). The HPA axis is essentially the central stress response system of the body, which directly controls the adrenal glands. Ultimately, with activation, the secretion of glucocorticoids and cortisol ensues (Duthie & Reynolds, 2013; Hellhammer et al., 2009; Nedeljka et al., 2016). The anti-inflammatory effects of excess cortisol then feedback and suppress the further release of cytokines (Wolkow, Aisbett, Reynolds, Ferguson, & Main, 2015).

Exposure to chronic stress causes prolonged activation of the HPA axis, which leads to the dysregulation of the HPA axis (Wolkow et al., 2015). During an acute stress response, the following physiological changes can occur: increased heart rate, vasoconstriction of blood vessels, decreased motility, widening of bronchial passages, pupillary dilation, and increased perspiration (Yaribeygi et al., 2017). Although these physiological changes are essential for survival, with chronic exposure it can have detrimental effects on one's overall health (Glover, 2011; Konduru, 2011; D. Y. Lee, Kim, & Choi, 2015).

Concerning the association of stress and physiology, firstly the human immune system has been empirically associated with chronic stress. Therefore, its capacity to respond to cortisol and reduce inflammation is affected if there are glucocorticoid abnormalities. This causes the risk of immune-related disorders such as infections, skin diseases (allergic reactions and eczema) and inflammatory conditions to develop (Jeong et al., 2006). A review by Morey et al. (2016) has indicated that the immune system can become compromised in chronic stress situations, which means an individual can be more susceptible to disease, infection and mortality (Morey, Boggero, Scott, & Segerstrom, 2016). Moreover, chronically stressed people have displayed a higher risk of developing cardiovascular disease (Marques, Silverman, & Sternberg, 2010). Chronic stress on the cardiovascular system is shown to have stimulatory effects on the ANS, which subsequently affects the function of the SNS (Baevsky & Chernikova, 2017; Hjortskov, Rissén, et al., 2004; Van Hedger et al., 2017; Yaribeygi et al., 2017). Chronic stress stimulates the ANS, which increases arterial contraction, vasodilation of skeletal muscles and overall heart rate (Baevsky & Chernikova, 2017). This can lead to myocardial infarction as a result of increased blood pressure and the increased risk of atherogenesis, coagulation, and cardiac arrhythmias (Yaribeygi et al., 2017). Additionally, the gastrointestinal (GI) system becomes affected during chronic stress, whereby the permeability of the mucus membrane and intestine is modulated by chronic stress (Abey et al., 2017; Zhu, Sun, Zhang, & Liu, 2017).

Chronic stress also has been shown to modulate stomach acid secretion, increasing the release of stomach acid, which in turn can lead to disruption of the stomach lining leading to ulceration (Yaribeygi et al., 2017). Studies have demonstrated that excess amounts of inflammatory mediators are released during repetitive stress, which further damages or inflame the GI tract (Yaribeygi et al., 2017). The development of certain GI pathologies such as Irritable Bowel Syndrome (IBS) has also been linked to psychological stress (Qin, Cheng, Tang, & Bian, 2014). Furthermore, stress has been shown to reduce cognitive function by impairing mental flexibility and reducing the ability of the pre-frontal cortex to control activity (Arnsten, Raskind, Taylor, & Connor, 2015). A study has indicated that repetitive, chronic stress dampens the pre-frontal cortex transmission, which results in reducing cognitive processes (Yuen et al., 2012). Mental fatigue, lack of concentration, irritability and overall tiredness are some examples of related symptoms (Abdel et al., 2017; Batista et al., 2017; Godoy et al., 2018).

Thus, the stress response is a complex but efficient system involving constant modulation of the Central Nervous System (CNS). After perceiving a stressor and registering it as threatening, the stress response becomes activated. This alarm reaction is the first stage of the acute stress response, known as “fight or flight”, and essential for our survival (Ranabir & Reetu, 2011). The physiological changes in response to the fight or flight response (e.g. increased heartrate, etc.) forces the body and mind to be on high alert. As the body tries to cope with these physiological changes, the cognitive system can be affected, which over time has been shown to further lead to psychological disorders and conditions (Abdel et al., 2017; Batista et al., 2017; Godoy et al., 2018). Therefore, identifying stressors may be the first step for healthcare practitioners, researchers and people to understand and alter their perception of stress, and determin how they can respond to it in a better way.

Stressors

A stressor can be best defined as any stimulus that threatens or disturbs the body’s physiological homeostasis (Bhimte, Thakur, Maurya, Balamurugan, & Singh, 2018). Stressors exist in many facets of one's life, including the physical, spiritual, psychological and social domains. Within each domain, individuals perceive these existing stressors differently. Common stressors reported in previous studies include crisis/catastrophes, academic/exams, marriage or relationships, deaths, daily hassles, domestic disputes, sport/athlete pressure, financial burdens, housing apprehension, socioeconomic status, job stress, fears for future health and ageing (Abdel et al., 2017; Di Corrado, Agostini, Bonifazi, & Perciavalle, 2014; DiPietro, 2013; Frye, Williams, Johnson, Quinn, & Fotopoulos, 2014; Golshiri, Pourabdian, Najimi, Zadeh, & Hasheminia, 2012; Islam, Wills-Herrera, & Hamilton, 2009; E.-H. Lee, 2012; Mouton, Fillion, Tawadros, & Tessier, 1989; Otsuki et al., 2004; Solivan, Xiong, Harville, & Buekens, 2016). This makes it difficult to determine and quantify the level of severity of a stressor. Furthermore, past experiences, personal characteristics, lack of support, socioeconomic status and other lifestyle factors can all influence how an individual perceives or responds to the stressor. These factors will vary from person to person, leading to distinct and individualised coping strategies, which determines an individual's susceptibility/resiliency to stress (Fliege et al., 2005; Karam et al., 2012; E.-H. Lee, 2012; Staneva, Bogossian, Pritchard, & Wittkowski, 2015).

The links between relationship status, happiness in a relationship and the latent measure of stress and well-being, for example, have been investigated in stress studies (Dush & Amato, 2005; Randall & Bodenmann, 2009). Researchers found that married individuals reported having the highest level of well-being, compared to those who infrequently dated or were single (Dush & Amato, 2005). Individuals in "happy" relationships reported feeling less stressed and having a higher level of subjective well-being than those who were in "unhappy" relationships or single. In a review by Randall et al. (2009), the authors examined the various models of stress in couples. They concluded that stress plays an increasingly harmful and important role in modern societies, particularly, its effect on the private lives of those in relationships (Randall & Bodenmann, 2009).

Additionally, the psychological impact on a partner/caregiver of an individual suffering from a terminal illness is another precursor for chronic stress (Jonathan, O'Boyle, & McDonagh, 2001). Researchers have indicated that the burden of care provided by a partner/carer can detract from their quality of life and well-being (Jonathan et al., 2001). Those caring for the incapacitated may have worseninghealth, and/or the development of depression and anxiety, impairing family and social life and increasing overall stress in the partner/caregiver. It has been suggested that a partner's quality of life can be worse than that of the chronically ill patient (Jonathan et al., 2001; Jones & Peters, 1992).

The ability to generalise "stressors" to have some consistency in clinical trials concerning contributing factors, is unachievable. Researchers (including clinicians) have endeavoured to distinguish objective stressors (financial, occupational, socioeconomic stress, etc.) from subjective stressors (ratings of distress). Beyond the factors related to the stressor itself (i.e. intensity, duration and/or predictability), there are intrinsic factors that become relevant in shaping the stress response, which can include age, sex, and genetics (Novais et al., 2016). The degree of stress that an individual may experience and how they respond to it can be influenced by one or many of these factors. Therefore, it is crucial for people, healthcare practitioners and researchers to identify and address stressors that hyperactivate the stress response. This will help determine how best to combat the increasing demands that is stress, to reduce the risk of developing future health comorbidities (Batz-Barbarich, Tay, Kuykendall, & Cheung, 2018).

Prevalence of stress

Results from the United Kingdom's Mental Health Foundations 2018 study (Mental Health Foundation, 2020), provided statistics on a sample size of 4,619 UK residents suffering from stress. Key results displayed the disparity of stress and identified stressors between age groups. The results indicated that 49% of 18-24-year-olds reported their primary stressor was feeling they had to compare themselves to others. Housing worries, financial stress and pressure to succeed are commonly reported stressors in New Zealand individuals aged between 18-24 years (New Zealand Ministry of Health, 2019). Comparatively, long-term health conditions and possibly being cared for or caring for life- partners were primary stressors recorded for 45-54-year olds (New Zealand Ministry of Health, 2019). Women have also reported having a high level of stress relating to their body image and comfort with their appearance compared to men (Mental Health Foundation, 2020).

When comparing these statistics to New Zealand, the annual update from 2018/19 from the Ministry of Health provided alarming results from the annual New Zealander's psychological distress survey. Between 2018 and 2019, psychological distress had increased in adults by 4.5% since 2011/12. Women were reported to be 1.8 times more likely to have experienced psychological distress than men in the last month. The most recent report from the New Zealand Ministry of Health (2019) regarding stress and mental health, indicated that the prevalence of stress in young adults aged 15-24 was 14.5% higher than in adults over the age of 25 (4.2%) (New Zealand Ministry of Health, 2019). Furthermore, the New Zealand Disability Survey estimated that 5% of New Zealanders are living with chronic long-term limitations in their daily life as a result of the effects of psychological impairments (Statistics New Zealand, 2013). It has also been suggested that 47% of people will experience a common mental illness in their life related to anxiety, depression or substance addiction (Mental Health Foundation of New Zealand, 2011).

Sex differences in the stress response

The differences in which male and female sexes respond to stress can be found at all stages of life and are inversely related to both the activation and organisational effects of gonadal hormones (Altemus, Sarvaiya, & Epperson, 2016). The relationship between women's age and responses to stress displays some interesting underlying mechanisms. Underlying mechanisms driving sex differences in stress responses and their relevance to disease has been reviewed in published literature (Bale & Epperson, 2015). Researchers conclude that being male or female could predict the risk of certain disease/s and resiliency.

Emotional and cognitive responses to stress have also demonstrated excessive stress levels can lead to an increased prevalence of psychopathological mood disorders such as anxiety and depression (Lewis et al., 2019; Vianna, Bauer, Dornfeld, & Chies, 2011). This has increased significantly in more recent investigations of the differences in stress responses between the sexes (Bale & Epperson, 2015). The brain's ability to continually perceive and respond appropriately to stress is important for both survival and homeostasis. Therefore, further appreciation and focus regarding 'sex differences in the stress response', may be strong predictors for disease risk. This can provide crucial evidence for prevention strategies and treating/managing women and men suffering from stress.

As literature is growing on the topic of the sex-dependent stress response, the intersect between stress and/or fear mechanisms and their modulation by gonadal hormones is becoming more relevant (Albert, 2015; Altemus et al., 2016; Godoy et al., 2018; D. Johnson & Whisman, 2013; Maeng & Milad, 2015). Women appear to be at greater risk than men to early-life adversity and hence to present with affective disorders throughout life. It has been demonstrated that women are twice as likely to experience depression and other personality/mood conditions as a result of stress, compared to men (Abdel et al., 2017; D. Johnson & Whisman, 2013). Furthermore, the ageing brain shows sex-specific changes in stress reactivity, which results from hormonal decreases among other age-related processes in different regions of the brain (Bale & Epperson, 2015). A study by Maeng and Milad (2015) investigated oestrogen levels in female rodents and found multiple fluctuations (Maeng & Milad, 2015). This caused sluggish cortisol feedback to certain pathways of the brain, as a result of delayed cortisol response to the brain. Overall, this appeared to affect the brain's ability to deal with stress. Researchers have shown that female sex hormones appear to attenuate the sympathoadrenal and HPA responsiveness (Ter Horst, De Kloet, Schächinger, & Oitzl, 2012).

Furthermore, during the menstrual cycle, hormonal dysregulation has been shown to affect the binding of cortisol and the responsivity of the HPA axis (Hellhammer et al., 2009). Menstrual cycle variation also has been strongly associated with effects on mood (Abrao, Leal, & Falcao, 2014; Kajantie & Phillips, 2006; Ossewaarde et al., 2010; Pechère-Bertschi & Burnier, 2004). For example, the dramatic changes in gonadal hormones can lead to erratic behavioural responses such as variations in how one may act or talk (Kajantie & Phillips, 2006; Ossewaarde et al., 2010). This is consistent with findings in Maeng et al. (2015) who demonstrated that fluctuations in oestrogen levels during menstruation was linked to modulating fear and stress responses in female rats (Maeng & Milad, 2015). However, human studies have produced inconsistent results for possible changes in HPA activity during the menstrual cycle.

Oral contraceptives are also shown to affect hormonal regulation and can lead to subsequent mood or psychological health conditions (Lewis et al., 2019). Although hormonal contraceptive use is regarded as beneficial for many women, there is a subset who can suffer from severe mood-related side effects (Poromaa & Segebladh, 2012). The most common side-effects include depressive and anxiety symptoms, which are typically reported as primary reasons for discontinuing use. Even though current research is inconsistent, evidence has linked some adverse effects of contraceptives to the regulation of cortisol, stress and mood in some users (Lewis et al., 2019; Mordecai et al., 2017; Pechère-Bertschi & Burnier, 2004; Poromaa & Segebladh, 2012).

Some studies have concluded that, starting from adolescence and continuing through adulthood, women are twice as likely as men to experience depression, anxiety and/or stress due to various biological, societal and/or cultural reasons (Albert, 2015; Altemus et al., 2016; Godoy et al., 2018; Johnson & Whisman, 2013). Women have also shown tendencies to ruminate more than men (Johnson & Whisman, 2013). This is of particular interest due to the associations made between rumination and the development of depression in females, which has been previously demonstrated in studies (Altemus et al., 2016; Gotlib & Joormann, 2010; Gross & Seebaß, 2014; D. Johnson & Whisman, 2013). Oestrogen levels have been shown to amplify the stress response in areas of the brain that are associated with depression, anxiety and other stress-related disorders (Ter Horst et al., 2012). This tendency for women to experience depression twice as much as men have been linked with compromised cortisol feedback effects on HPA arousal (Verma, Balhara, & Gupta, 2011).

Stress dysregulation is a common feature across neuropsychiatric diseases. Therefore, sex differences in how these pathways develop and mature could predict sex-specific periods of vulnerability to disruption and increased risk of disease across the lifespan. Various disorders could perhaps be prevented if there was a greater understanding of the impact perceived stress has on women and men. Differences in stress responses between the two sexes have been identified in the literature, both physiologically and psychologically. However, further long-term studies are required not only to understand responses and impact across the life span but also to understand the mechanism of this response, to formulate better management strategies.

Stress assessment

With the rise of chronic stress in communities today, continual assessment on how best to identify, manage and possibly treat stress is emerging to be of significant importance. Chronic stress, albeit fundamental for our survival, can have negative effects on an individual's well-being (Brinkborg, Michanek, Hesser, & Berglund, 2011; Jeong et al., 2006; Konduru, 2011; Slavich, 2016). Together, the assessment of perceived and physiological stress can provide researchers not only with a more in-depth understanding of an individual's stress, but can also highlight the close and interdependent relationship between psychological and physiological stress.

Perceived stress assessment

Subjective psychological stress assessment through self-reports can be an effective measure in assessing an individual's perceived stress and overall health when correlated and/or compared with physiological stress (Buist, Ross, Steiner, & Goldstein, 2012). However, the identification, quantification and justification of perceived, subjective stress can be challenging. With this in mind, there has been a growing interest in how to assess and categorise an individual's perception and interpretation of stressful events (Monroe, 2008). Various tools have been developed, including scores of self-report questionnaires, which aim to ascertain what events/experiences are perceived as stressful (E.-H. Lee, 2012). The psychological (perceived) stress assessment tools (self-report questionnaires) selected for this study include the Perceived Stress Scale 10 (PSS-10) and the Profile of Mood State (POMS). In the light of current literature evidence, PSS-10 and POMS are considered to be robust, validated, reliable and is commonly used, therefore these are discussed in detail in the following paragraphs.

PSS-10

One of the self-report scales that has been devised to identify an individual's perception of stress and stressors includes the PSS-10. Levenstein et al. (1993) published the “Perceived Stress Questionnaire” (PSQ) from which the PSS is derived. Authors aimed to overcome some of the difficulties concerning the definition and measurement of stress by putting the focus on the individual's subjective and emotional response to a stressor (Levenstein et al., 1993).

The PSS assesses an individual's global stress as a result of multiple factors during a specific period (Buist et al., 2012) and identifies how well an individual can change their perception to a broad range of stressors (Fliege et al., 2005; Karam et al., 2012; Solivan et al., 2016). There are three current versions of the PSS (PSS-14, PSS-10, and PSS-4), where each number indicates the number of questions used (Taylor & M., 2015). The PSS-10 is considered to be valid and reliable as a screening tool for perceived stress (Taylor, 2015). Many studies have used the PSS-10 as the screening/assessment tool in intervention-based studies assessing stress (Kalra, Einarson, Karaskov, Van Uum, & Koren, 2007; Lopez et al., 2011; A. Mitchell et al., 2008). Essentially, the PSS-10, comprised of 10 questions, categorises an individual as stressed according to the total score from the results of the questionnaire. Each item is rated on a five-point scale ranging from never [0] to almost always [4]. Positively worded items are reverse scored, and the ratings are summed, then summing across all 10 items occurs.

A study evaluating the effect of directed osteopathic manipulative therapy (OMT) on self-perceived stress, fatigue and depression in first-year osteopathic medicine was conducted (Wiegand, Bianchi, Quinn, Best, & Fotopoulos, 2015). Although authors have evaluated OMT, the techniques and philosophy behind OMT is similar to that of GOT (Paulus, 2013). Results indicated that the participants who received GOT treatment also showed decreases in self-perceived fatigue through PSS-14 scores. Overall, the authors suggested that the perceived stress measure was an effective tool in representing the level of self-perceived fatigue and stress in medical students (Wiegand et al., 2015). Furthermore, a pilot study tested the effects of cognitive behavioural stress management (CBSM) intervention on stress, quality of life (QOL), and symptoms in chronic fatigue syndrome (CFS) (Lopez et al., 2011). Researchers hypothesised that participants in the CBSM group would report improvements in perceived stress, mood, QOL, and CFS symptomatology after GOT treatment. Repeated analysis of variance revealed significant interactions in perceived stress scores, such that participants in

CBSM evidenced great improvements. To support this, a review by Lee (2012) analysed articles related to the psychometric properties of the PSS-10 (E.-H. Lee, 2012). Results indicated that 19 articles identifying the internal consistency reliability, factorial validity, and hypothesis validity of the PSS-10 were well- reported.

Despite this, the psychometric properties of the 10-item PSS are superior to those of other instruments, such as the 4-item scale. And although the PSS-14 is also widely used and considered reliable and valid, the PSS-10 is more practical and comprehensive (Lee, 2012). The author concluded that, overall, "the PSS-10 is an easy-to-use questionnaire with established acceptable psychometric properties, yet future studies should evaluate these psychometric properties in greater depth and validate the scale using diverse populations" (E.-H. Lee, 2012). In the light of current literature evidence, the PSS-10 is one of the instruments used in the study presented in this thesis. A full PSS-10 can be seen in Appendix #10.

POMS

The POMS tool is a psychometric rating scale used to examine or assess distinct and transient mood states (S. Curran, Andrykowski, & Studts, 1995; González-Ochoa, Sánchez-Rodríguez, Chavarría, Gutiérrez-Ospina, & Romo-González, 2018; P. Terry et al., 2003). The original POMS questionnaire consists of 65 items, which is impractical and too lengthy for short-term and/or complex interventional studies with time constraints. Shorter versions of the POMS (e.g. the 4-item, 30-item and 40-item) were therefore developed, which are all considered effective and well-used measures for assessing different mood states in individuals. The 40-item POMS covers all important aspects of mood states including negative emotions, such as tension, depression, fatigue, confusion, anger and positive emotions such as vigour and self-esteem-related affect (Grove, 1992; Morfeld, Petersen, Krüger-Bödeker, Von Mackensen, & Bullinger, 2007).

The POMS questionnaire assesses six mood subscales: tension-anxiety, depression, anger-hostility, vigour, fatigue, and confusion. High vigour scores reflect a good mood or emotion, and low scores in the other subscales reflect a good mood or emotion. The total mood disturbance (TMD) score is calculated by adding the five negative subscale scores (tension-anxiety, depression, anger-hostility, vigour, fatigue, and confusion) and subtracting the positive vigour subscale score (Yoshihara, Hiramoto, Sudo, & Kubo, 2011). Higher scores for the total mood disturbance score indicate a greater degree of mood disturbance. These scoring methods have been widely used in the assessment of mood states (Terry et al., 2003).

A study by Di Corrado et al., (2014) investigated the changes in mood states and salivary cortisol levels over two months of intense training in a group of elite female water polo players (Di Corrado et al., 2014). The authors in that study used an abbreviated 30-items POMS where subscales were combined to form an overall measure of the effect that was labelled as TMD. The modified subscales were useful for researchers to examine the changes in specific moods. Researchers found that TMD was useful for studies containing a small number of participants (Di Corrado et al., 2014). Another study used the POMS as a psychological measure for perceived stress and anxiety in participants receiving a GOT treatment (Dugailly et al., 2014). Following the intervention, researchers observed improvements in the psychological state in the GOT group. This group demonstrated a larger, more significant effect over the restful state for anxiety and global self-perception. Authors concluded that the use of POMS provided a good representation of the positive effect an osteopathic approach had on anxiety and global body perception. Therefore, the POMS instrument was the other self-report tool selected for the study presented in this thesis. A full POMS questionnaire can be found in Appendix 9.

Physiological stress assessment

The physiological response to stress helps an individual cope with certain demands. This is a natural and normal response that allows the body to meet challenges, thrive and succeed. However, in situations where stress is prolonged, it can alter the body's balance, harmony and overall homeostasis (Figueroa-Fankhanel, 2014). Over-time, an individual's problem-solving abilities and well-being can be limited. Continued and repetitive activation of the stress-related physiological processes damages the body leading to adrenal fatigue, burnout, maladaptation, overload and dysfunction. This further causes distress, where natural stress is no longer adaptive, but rather it contributes to a state of physical or mental pain and suffering (Yaribeygi et al., 2017). Therefore, researchers have determined ways to best evaluate stress through different biological avenues. Examples of these include through direct and/or proxy autonomic measures in biological samples such as blood, saliva, hair and urine (Figueroa-Fankhanel, 2014; Kalra et al., 2007; Van Holland, Frings-Dresen, & Sluiter, 2012). All of which can demonstrate and assess the level of stress biomarkers within the tested samples. Some of these ways will be discussed in detail below, with the justification of saliva sample emerging as the sample of choice for its non-invasive method of collection. The following paragraphs discuss appropriate biological samples and some of the biomarkers which represent pathways affected by stress (directly or indirectly).

Biological samples

Biological samples, such as serum, plasma, urine, hair, and salivary fluid are important in medical science and research as they can represent physiological systematic changes and biomarkers representing several pathways can be assessed in these samples., Each of these samples can be used to assess A multitude of different stress biomarkers (Harpole, Davis, & Espina, 2016; Hellhammer et al., 2009; Kalra et al., 2007; D. Koh & Koh, 2007; D. Y. Lee et al., 2015; Nilsonne, Lekander, Åkerstedt, Axelsson, & Ingre, 2016a; Prasad, Tyagi, & Aggarwal, 2015; Van Holland et al., 2012). The practicality and applicability of the types of biological samples mentioned will be explored further.

Firstly, blood plasma has been identified as the gold standard in representing most stress biomarkers (Peti, Locachevic, Prado, De Moraes, & Faccioli, 2018). However, this is considered to be an invasive procedure, costly, labour-intensive and typically conducted by a phlebotomist (Goldman et al., 2018). Urine samples have also been highlighted asappropriate biological samples for assessing stress biomarkers. However, urine does not represent all of the stress biomarkers correctly, all of the pathways cannot be represented correctly in urine and integrity of some of the biomarkers can also be an issue (Ivković, Božović, Račić, Popović-Grubač, & Davidović, 2015). Additionally, cortisol reactivity in human hair has been demonstrated to be an effective measure for HPA activity as it is the main central nervous system pathway that is activated under stress (Dhama et al., 2019; D. Y. Lee et al., 2015; Van Holland et al., 2012). Cortisol in hair has been suggested as an emerging and highly promising measure for the retrospective assessment of chronic stress (Van Holland et al., 2012; Wright, Hickman, & Laudenslager, 2015). However, hair cortisol can only act as a proxy to the total HPA activity in the preceding months and is not an acute marker of current HPA activity. Therefore, it can only provide researchers historic information contained in their hair samples, rather than their current HPA activity (Wright et al., 2015). Other proxy autonomic measures involve the assessment of the autonomic arousal using electrical and mechanical equipment. This can be achieved through measurements of vital signs including blood pressure, heart rate and perspiration (Figueroa-Fankhanel, 2014). Typically, these measures are used as a substitute or in combination with stress biomarkers. This type of assessment is economic in terms of finance and time, and they are relatively easy for healthcare professionals or researchers to do. When the evaluation of biomarkers such as cortisol, IL-6 and sIgA by conventional methods can be too expensive and time-consuming, proxy autonomic measures are considered appropriate alternatives or additional measures.

As the literature indicates, there is a growing need for developing additional non-invasive methods of assessments, particularly in stress research. A common option in most stress studies includes the collection of saliva samples (Marques et al., 2010), the process for which has been characterised as a non-invasive, reliable and easy to collect with relatively inexpensive biological sample collection method. Several of the stress-related biomarkers in plasma (IL-1□, IL-2, TNF-α) are now considered to be represented in saliva as well (Djuric et al., 2008; Frijhoff et al., 2015; Sjögren, Leanderson, Kristenson, & Ernerudh, 2006). Many known factors can affect saliva collection. The salivary flow rate or secretion rate can be affected by caffeine and certain foods (Hildebrandt, Tantbirojn, Augustson, & Guo, 2013). For example, coffee has been shown to suppress the saliva flow rate, which causes dehydration and diuresis. Coffee also decreases the pH of the mouth (Hildebrandt et al., 2013). This can lead to both compromised enzyme activity in immunoassays and antibody-antigen binding (Soares-Nunes, Mussavira, & Sukumaran, 2015). Although studies have indicated that the cortisol response can become elevated after caffeine intake (Lovallo, Farag, Vincent, Thomas, & Wilson, 2006), tolerance of the cortisol response has been shown to develop in individuals who consume coffee daily (Lovallo et al., 2005). Overall, collecting salivary samples is becoming a preferred biological method in stress research, for the assessment of biomarkers representing stress or being affected by the stress pathway (Prasad et al., 2015).

Biomarkers

Nowadays, interest regarding biomarkers has come from medical sciences. Biomarkers are important as they can detect pathologies, which can be crucial in the prevention of diseases and can improve quality of life (QOL) in patients (Batista et al., 2017). One way to examine the extent to which chronic stress impacts stress-related physiological mechanisms is to study the secretion patterns of associated biomarkers representing the pathways affected by the stress. Commonly assessed biomarkers in stress research include cortisol, proinflammatory cytokines, immunoglobins, catecholamines and α-amylase (Nedeljka et al., 2016; Prasad et al., 2015).
Although plasma is considered to be the gold standard in stress research, there is a growing need for utilising non-invasive methods. Researchers need to find ways to appropriate biomarkers that are similarly represented in both plasma and saliva (Batista et al., 2017).

Recently, salivary α-amylase (sAA) has emerged as a notable indicator of the ANS activity and has been demonstrated as a reliable candidate for stress research (Ali & Nater, 2020; Lambert et al., 2018). Several studies have demonstrated sAA responds to both physically and psychologically stressful conditions (Chatterton, Vogelsong, Lu, Ellman, & Hudgens, 1996;

Nater & Rohleder, 2009; Rohleder, Nater, Wolf, Ehlert, & Kirschbaum, 2004). This can be seen in Henderson et al. (2010), where researchers demonstrated the effect of rib-raising on down-regulating the SNS through significant reductions in sAA levels. Typical sAA concentrations display variable diurnal profiles from that of cortisol, which reduce within 60 minutes after waking and gradually increase throughout the day (Nater, Rohleder, Schlotz, Ehlert, & Kirschbaum, 2007). However, the number of studies in behavioural medicine that include sAA assessment remains fairly small.

Chromogranin A (CgA) is an acidic protein prohormone present in the secretory granules of different neuroendocrine tissues (Takatsuji et al., 2008). It has been recognised as a marker of psychological/perceived stress, with a diurnal profile showing higher levels at night and lower levels seen in the morning (Giampaolo, Angelica, & Antonio, 2002). However, significantly higher levels of CgA have been observed in samples collected immediately after exposure to stressors (Ng, Koh, Mok, Chia, & Lim, 2003; Takatsuji et al., 2008). Salivary lysozyme has also been identified as a prominent anti-bacterial peptide in the external secretory fluids of humans (Dhama et al., 2019). A study revealed that lysozyme is related to chronic stress (Abey et al., 2017), whilst another study exposed a negative correlation between lysozyme concentration and stress exposure (Allgrove, Gomes, Hough, & Gleeson, 2008). This was in accordance with increased susceptibility to bacterial invasion during stress.

As discussed previously under the section 'stress', the HPA axis can have a substantial influence on the immune and inflammatory pathways. The evidence within the literature suggests that assessing biomarkers, which represent these pathways, can give a more comprehensive picture of the physiological impact of stress. Cortisol, sIgA and IL-6 are representatives of HPA axis, immune and inflammatory pathways respectively (Abey et al., 2017; Ivković et al., 2015; D. Koh & Koh, 2007; Slavish, Graham-Engeland, Smyth, & Engeland, 2015). Therefore, these biomarkers are further discussed below.

Cortisol

Saliva contains free, biologically active cortisol which compares to the total amount of cortisol present in plasma or serum. Therefore, salivary cortisol is a frequently assessed biomarker in psychological stress research. Cortisol displays a controlling relationship with the functioning of the HPA axis. Excess cortisol suppresses the HPA axis during a stress response, thus, it is considered to be a good marker for chronic stress (D. Y. Lee et al., 2015). Researchers have established a strong relationship existing between different personality or mood disorders and changes in HPA activity (Batista et al., 2017; Hellhammer et al., 2009; Koh & Koh, 2007; D. Y. Lee et al., 2015). Although there is growing evidence linking the cortisol stress response and personality, the nature and the underlying mechanisms of the relationships require further clarification.

Dysregulation of stress hormones was originally thought to be a by-product of mood disorders. However, mounting evidence indicates that excess cortisol secretion is a contributor to different mood symptoms (Chennaoui et al., 2016; Di Corrado et al., 2014; Weinstock, 2005; Yoshihara et al., 2011). This happens as a result of increasing corticotropin-releasing hormone, which is expressed in the amygdala and other key brain regions (Oswald et al., 2006). A study by Oswald et al. (2006) examined the associations between personality traits and cortisol responses to stress. Results indicated that less "openness" was associated with lower cortisol responses to the challenge presented in the intervention (Oswald et al., 2006). Furthermore, blunted cortisol responses were associated with higher "neuroticism" in women. These findings indicated that a relationship exists between personality traits with blunted HPA axis responses to stress (Oswald et al., 2006).

The effects of elevated cortisol levels lead to the suppression of the normal stress response by reducing inflammation. When this happens over a long period, it can have undesired effects such as weight gain, acne, thinning skin, slowed healing, muscle weakness, severe fatigue, irritability, difficulty concentrating, headaches and high blood pressure, many of which may be implicated in psychopathologies (Joseph & Whirledge, 2017; van Hedger, Necka, Barakzai, & Norman, 2017). However, in studies assessing stress, researchers have concluded that further work needs to be carried out to determine deeper relationships between biomarker responses to stress and the variable dimensions of personality (Chennaoui et al., 2016; Di Corrado et al., 2014; Kajantie & Phillips, 2006; Oswald et al., 2006).

Cortisol has a strong diurnal profile (Ice, Katz-Stein, Himes, & Kane, 2004). Under normal circumstances, cortisol levels increase during the early morning and decrease as the day progresses, with levels lowest in the evening (Hucklebridge, Clow, & Evans, 1998). Therefore, the timing of sample collection is critical. Additionally, elevated cortisol production and the concentration of cortisol in saliva are independent of the salivary flow rate (Desai, 2014; Srinivasan, Blackburn, Mohamed, Sivagami, & Blum, 2015). With this in mind, the relationship between diurnal cortisol variation and salivary flow rate needs to be considered by researchers. A study evaluated the effects of training and competitions on mood states and salivary diurnal variation in a team of 15 elite water polo players (Di Corrado et al., 2014). Researchers concluded that after the results, flatter diurnal rhythms of cortisol appeared to play a role in determining dysfunctional mood states. Overall, cortisol has been highly recommended as a biological assessment tool in physiological stress analysis. This is primarily based on the relationship between cortisol and representation of the functioning of HPA activity (Hellhammer et al., 2009).

Interleukin-6 (IL-6)

With literature proposing that IL-6 is a major inflammatory cytokine, it captures the pathophysiology of the immune system (Sjögren et al., 2006). Therefore, IL-6 is a biomarker closely related to innate immunity, inflammation, morbidity, and mortality (Prasad et al., 2015). It can thus provide an excellent representation of the inflammatory response to psychological stress. A review on IL-6 supported this, demonstrating the biomarker exhibits the most consistent responses with acute stress (Slavish et al., 2015).

This major inflammatory cytokine plays an integral role in the mechanism linking psychological stress with an increased risk of developing cardiovascular disorders (Marques et al., 2010). Previous research investigating IL-6 with regards to psychosocial stress factors has primarily focused on plasma or serum samples (Konduru, 2011; Wolkow et al., 2015). This was highlighted in a study by Nilsonne et al. (2006) among other studies, where authors indicated that IL-6 levels can be elevated in saliva samples. This has rarely been investigated (Nilsonne et al., 2016a; Prasad et al., 2015; Sjögren et al., 2006). With the neuroendocrine system, IL-6 serves as a key messenger in its communication with this system and allows it to serve as a potent activator of the HPA axis at all levels (Willenberg, Päth, Vögeli, Scherbaum, & Bornstein, 2002). This demonstrates an existing relationship between cortisol and IL-6 during the stress response, where IL-6 can stimulate the HPA axis during inflammatory stress (Sjögren et al., 2006). This biomarker also follows a diurnal pattern, typically increasing in the early morning and decreasing towards the evening (Nilsonne, Lekander, Åkerstedt, Axelsson, & Ingre, 2016b; Sjögren et al., 2006).

Cytokine modulation has been investigated regarding its association with social and emotional behaviour (Wolkow et al., 2015). A study demonstrated the significant association between concentrations of all immune-mediators in saliva and socio-cognitive stress in students sitting academic exams (La Fratta et al., 2018). Results suggested all inflammatory markers assessed, including IL-6, could be related to different emotional responses. For example, IL-6 showed a direct association with the anger and anxiety mood states, with levels increasing throughout the intervention. This was consistent with findings in a pilot study investigating the response of IL-6 to acute stress and early-life adversity on healthy adults (Carpenter et al., 2010). Researchers found IL-6 to be an integral part of the innate inflammatory response to a physical stressor. Results from these two studies support the emerging role of using saliva as a reliable tool to measure and evaluate cytokine levels in response to stress (Carpenter et al., 2010; La Fratta et al., 2018).

Secretory Immunoglobulin A (sIgA)

Biomarkers representing immune functions, such as secretory Immunoglobulin A (sIgA), have been acknowledged as "one of the most important lines of defence against human pathogens in the mucosal immune system" (Lambert et al., 2018). Therefore, sIgA has regularly been used as a marker of immune competence. This biomarker is an important antibody in the mucosal immune system, playing a considerable role in the defence against infections of various body systems (Lambert et al., 2018). A study demonstrated that psychological conditions, such as depression, can reduce the level of sIgA and lead to decreased immunity for that individual (Golshiri et al., 2012).

Acute stress upregulates sIgA along with cortisol altering HPA axis activity (Djuric et al., 2008; Hucklebridge et al., 1998; Takatsuji et al., 2008). During high-stress situations, conditions affecting several body systems typically occurs. Emotional and cognitive systems can become impaired, which has been reflected through studies assessing sIgA levels in response to stress. Increased stress has been directly associated with a decrease in sIgA levels, producing an environment in which otherwise healthy individuals can become more susceptible to disease. This occurs as a result of the relationship between sIgA and immune competency. The type of stress sIgA reflects is one that is ongoing or chronic stress rather than acute, making it a relevant biomarker studies assessing long-term stress (Takatsuji et al., 2008).

Although the number of studies assessing the effect of GOT on stress is low, there are a handful of studies to date assessing the changes in biomarker levels in response to a GOT therapy package (Fornari, Carnevali, & Sgoifo, 2017; Otsuki et al., 2004; Rapaport, Schettler, & Bresee, 2012; Wiegand et al., 2015). A study investigated the effect of GOT on increasing sIgA levels osteopathic students under high emotional and psychological stress (Saggio, Docimo, Pilc, Norton, & Gilliar, 2011). These researchers demonstrated increased sIgA levels in more than half of the participants following GOT intervention.

(Saggio et al., 2011). Although GOT has been reported to increase lymphatic flow (Hodge et al., 2007; Knott, Tune, Stoll, & Downey, 2005), improve cardiac indices, increase peripheral circulation, and decrease sympathetic tone (Henley, Ivins, Mills, Wen, & Benjamin, 2008), this was the first study to investigate and describe its effect on sIgA levels (Saggio et al., 2011). Since then, a study by Blut (2015) aimed to determine whether a single GOT intervention, using thoracic lymphatic pumping (TLP) as a technique, would cause an immediate increase in sIgA levels in people with suppressed mucosal immune function (Blut, 2015). The researcher suggested there may be some indication for an increase in sIgA levels, but that results would be more conclusive in a larger sample. This left readers with new prospects for the effect GOT techniques, such as TLP, may have on improving immune function through the assessment of sIgA levels in the stressed population.

Today there are many commercial kits available for researchers wanting to assess salivary stress biomarkers, one of which includes the Enzyme-Linked Immunosorbent Assay (ELISA) method. There are several types of ELISA methods, including Direct, Indirect, Sandwich and either Competition or Inhibition ELISA methods. While assessing stress biomarkers from plasma or saliva samples, the Sandwich ELISA method is most frequently employed (Dorn, Lucke, Loucks, & Berga, 2007). Stress biomarkers are strong indicators of physiobiological stress processes, and literature indicates the many different ways to assess these biomarkers. However, opting for a non-invasive, easy to administer and effective measure is most optimal in studies assessing perceived and physiological stress.

Stress management

The importance of managing stress: a multimodal approach

Stress is a growing epidemic. There needs to be a multimodal approach to managing stress today. Many healthcare professionals such as psychologists, psychiatrists, hypnotherapists and/or counsellors are primary health practitioners that are within their scope to treat people suffering from psychological /or over-all stress disorders (Niks, DeJonge, Gevers, & Houtman, 2018). Currently, osteopaths and other manual therapy professionals are not within their scope to provide treatment for the psychological constraints and harm that chronic stress may cause an individual. However, they can provide gentle and rhythmic manual therapy treatment, which is aimed to reduce sympathetic drive, relax the patient and encourage homeostasis (Campbell, Winkelmann, & Walkowski, 2012; Rechberger, Biberschick, & Porthun, 2019).

Health organisations have aimed to develop and implement different stress management strategies to combat the issue that is stress. The Mental Health Foundation of New Zealand has delivered several approaches to improve mental health within all demographics in the population. They promote and organise projects, programmes, events and encourage alternative therapy/treatment modalities to address and manage stress. Their aim is to socialise messages and habits leading to mental health, to provide people with a better understanding of well-being, and where to get further help from. With increasing levels of stress being reported, particularly in the working environment, there is a need for the New Zealand government and work corporations to propose strategies to better help reduce this issue (Niks et al., 2018). Work-related stress is commonly reported, with particular attention to the increasing rise in New Zealanders across the workforce (Southern Cross Health Society & BusinessNZ, 2019). Specific strategies related to work stress have been proposed by the New Zealand government, including education on stress and coping strategies, developing schemes that improve family versus work balance, and creating organisations that promote well-being in the workforce (Burton, 2010; Southern Cross Health Society & BusinessNZ, 2019).

Diet, exercise, and healthy living have all been heavily researched to assess their correlation with stress (Lovallo et al., 2006, 2005; Slavish et al., 2015; Stults-Kolehmainen & Sinha, 2014). Most studies have indicated the strong association between poor diet, lack of exercise and living an unhealthy lifestyle with the risk of developing disease, particularly psychological stress (Lovallo et al., 2006, 2005; Slavish et al., 2015; Stults-Kolehmainen & Sinha, 2014). Dietary advice and exercise recommendations have been identified as helpful tools for self-management, aimed to reduce current or future stress. Exercise, in particular, has been shown to directly increase neurotransmitter activity, specifically endorphins, serotonin, and dopamine, which influence one's feeling of well-being (Stults-Kolehmainen & Sinha, 2014).

Body Mass Index (BMI) is a measure of the proportion of body fat to total body weight, measured by height and weight. It is a tool used to define healthy vs unhealthy weight ranges (Heart Foundation, 2019). A study by Harding et al. (2014) indicated that psychological perceived stress was positively associated with weight gain, but not weight loss (Harding et al., 2014). Furthermore, researchers concluded that healthcare practitioners need to better consider psychosocial factors as contributors to weight gain in overweight people. Practitioners should be aware of this association and adapt their treatment programmes to provide external help for individuals in need of it. Modification or application of a new diet, exercise and/or weight loss plan needs to be explained and introduced to patients of healthcare practitioners within the manual therapy field. Simultaneously, practitioners should advise these patients to seek external help to manage their psychological stress (Harding et al., 2014).

Amidst the evolving research regarding prescription medication for the management of stress, general practitioners are becoming more inclined to encourage patients to explore alternative ways to manage their stress (Bandelow et al., 2012). People suffering from chronic stress may find help through a multitude of different therapy avenues, including massage therapy, manual therapy, physical exercise, mindfulness exercises, hypnotherapy, guided therapy, meditation and other complementary/alternative therapies including osteopathy (Chiang et al., 2016; Dean & Söderlund, 2015; J. Y. Lee et al., 2014; Romano, 1992). Osteopathy may be a plausible approach in addressing and treating the issue that is stress, in the context of a manual therapy treatment plan.

Role of general osteopathic technique/therapy in stress management

Osteopathic medicine is a growing alternative practice modality that focuses on holistically treating the body as a whole, with the concept that "structure governs function" (Lavelle & Still, 2012). Particular attention is given to the body, mind, and spirit (Dugailly et al., 2014). Within the realm of osteopathy and osteopathic treatment for stress, there are only a handful of studies indicating the positive effect of GOT. It has been demonstrated that GOT can reduce stress by down-regulating the SNS, which is related to the "fight and flight" response (Fornari et al., 2017; Giles, Hensel, Pacchia, & Smith, 2013; Henderson et al., 2010; Henley et al., 2008; Kim, 2014; Nuño, Siu, Allison, Deol, Navneet, & Juster, 2019). Furthermore, studies have shown that certain GOT techniques can improve autonomic homeostasis and decrease an individual's perception of stress (Henderson et al., 2010; Henley et al., 2008; Nuño et al., 2019; Seffinger et al., 2016; Wieting et al., 2013). However, most studies conclude that further investigation with larger scale studies and better methodological design is warranted to generate conclusive results. This will allow researchers and readers to better understand GOT's role in the treatment of stress. There is a paucity of studies conducted to-date that have successfully and/or consistently assessed reliability and validity of GOT techniques and treatment protocols. However, of the studies that do exist, researchers have demonstrated potential and new perspectives with certain GOT techniques and their effects on stress (Abey et al., 2017; Henley et al., 2008; Nuño et al., 2019; Rechberger, Biberschick, & Porthun, 2019; Saggio et al., 2011).

In osteopathic practice, many manual techniques are described and commonly used by practitioners such as muscle energy techniques, active resisted muscle techniques, functional and myofascial methods or the general approach. The latter is described as GOT, which consists of using several types of body segment mobilisations for assessing the occurrence of musculoskeletal dysfunctions (Dugailly et al., 2014).

Osteopathy focuses on many different areas, including the nervous and circulatory systems, spine, viscera, cranium, thoracic and abdominal diaphragm. By working on these areas, GOT aims to increase an individual's ability to respond and adapt to various internal and external stressors (Collebrusco et al., 2018). Osteopaths base their practice on the premise that altered movement and restriction within the moving parts of the body subsequently alter the tone, contracture and elasticity or compliance of the soft tissues (Grace, Orrock, Vaughan, Blaich, & Coutts, 2016; Paulus, 2013).. This is related to and may precede changes in physiological and psychological systems (Collebrusco et al., 2018; Henley et al., 2008; Wieting et al., 2013). The techniques involved in GOT consist of a range of indirect, direct, combined, fluid and reflex-based techniques. These are applied specifically to a joint or non-specifically to a body area (Henley et al., 2008). Direct techniques apply thrust, impulse, contraction, or fascial loading to engage a restricted barrier and use an activating force to achieve tissue response and correct the somatic dysfunction (Seffinger et al., 2016). This is in contrast to indirect techniques that use fluid, balancing or reflex-based techniques such as fascial massage, soft tissue massage, postural adjustments and/or respiratory phases that do not engage the restricted barrier (Collebrusco et al., 2018).

The interest in understanding how different manual therapy modalities can reduce stress has been increasing incrementally over the decades. However, there appears to be a lack of evidence specifically looking at the effect of GOT. Other manual therapies' effects on stress, such as massage therapy, have been investigated (Field, 2010; Field, Diego, & Hernandez-Reif, 2007; Field, Hernandez-Reif, Diego, Schanberg, & Kuhn, 2005; Jane et al., 2011; Latifses, Estroff, Field, & Bush, 2005; Rapaport et al., 2012). Massage therapy has been shown to activate areas in the brain that are connected to feelings of wellbeing (Khalsa, Eberhart, Cotler, & Nahin, 2006). The skin, in particular, contains numerous sensory receptors, which various physical therapies have shown can induce positive emotion through stimulation of these receptors. Reviews show that massage therapies increase both dopamine and serotonin, neurotransmitters which activate the central nervous system and lead to a decrease in cortisol release (Field et al., 2005; Konduru, 2011).

As with massage therapy, osteopathy uses touch as the main physical interaction between patient and therapist. The few existing osteopathy studies demonstrate that certain techniques can lead to many physiological responses including increased vagal activity and decreased heart rate and blood pressure (Fornari et al., 2017; Henderson et al., 2010; Henley et al., 2008; Toro-Velasco, Arroyo-Morales, Fernández-de-las-Peñas, Cleland, & Barrero-Hernández, 2009). Studies investigating the effect of GOT on inducing states of relaxation have suggested that rhythmic body motions seen in a GOT routine can induce this relaxed state (Frye et al., 2014; Nuño et al., 2019). It could, therefore, be assumed that osteopathy may also have a positive effect, as GOT is a hands-on practice, encompassing a range of techniques using the hands to stimulate sensory receptors on the skin. However, the investigation into GOT as a therapeutic package needs to be further assessed to make such associations.

Research into the role GOT can play in stress management will continue to grow, particularly considering stress levels appear to be increasing rapidly in New Zealanders (New Zealand Ministry of Health, 2019). In studies that have assessed GOT techniques, most have indicated that certain technique can provide individuals with therapeutic benefits (Fornari et al., 2017; Frye et al., 2014; Henderson et al., 2010; Henley et al., 2008; Nuño et al., 2019). Soft tissue massage, gentle oscillation, harmonics, inhibition, muscle release, articulation and joint mobilisations are some of the techniques applied by the osteopath to most regions of the body (Hartman, 1997). Thoracic lymphatic pumping techniques (TLP) and craniosacral techniques are employed by osteopathic practitioners, yet there is a lack of evidence-based research indicating the long-term effects of the techniques (Blut, 2015; Fornari et al., 2017; Kim, 2014; Knott et al., 2005; Vreede, 2010). However, there is some literature suggesting that lymphatic drainage may modulate the neurovegetative system and decrease any congestive state by facilitating lymphatic and venous drainage (Collebrusco et al., 2018; Henley, Ivins, Mills, Wen, & Benjamin, 2008; Hodge et al., 2007; Tinning, 2014).

Overall, in the case of stress, the practitioner can take a more gentle approach to treatment, using soft and rhythmic motions to encourage a relaxed state (Rechberger et al., 2019) Specifically, rib-raising articulation, sub-occipital inhibition and cervical myofascial release have all shown positive effects on stimulating the sympathetic environment (Castro-Sánchez et al., 2011; Henderson et al., 2010; Henley et al., 2008; Wieting et al., 2013).

Most recently, a review emphasised the positive effect of rib-raising with decreasing sympathetic tone (Nuño, Siu, Allison, Deol, Navneet, & Juster, 2019). Rib-raising is a technique that can initially stimulate sympathetic efferent activity but results in a prolonged reduction in sympathetic outflow (Henderson et al., 2010). Furthermore, a study by Frye et al.

(2014) assessed the effects of GOT on lowering perceived stress in medical, dental and pharmacy students. Results demonstrated reductions in stress within the student populations after they received GOT, which included rib-raising as a technique (Frye et al., 2014). Overall, techniques involving gentle articulation from the middle thoracic segments (T4-T10) and the collateral sympathetic ganglia (celiac, upper and superior mesenteric) have been emphasised to be most effective in reducing sympathetic drive (Henderson et al., 2010). Rib head articulation is commonly employed by manual therapists, as it not only increases mobility through the thoracic cage but also stimulates the SNS through massage over the sympathetic ganglia. Literature has further suggested that suboccipital release as a GOT technique, has a supportive effect on the physiological resilience and homeostasis of stressed people (Nuño et al., 2019).

Suboccipital and myofascial release has been shown to down-regulate the SNS (Castro-Sánchez et al., 2011; Rechberger et al., 2019). The rationale for suboccipital muscle release is not complex. This technique aims to regulate parasympathetic tone by stimulation of the vagus nerve as it exits from the cranium (Wieting et al., 2013). This technique stimulates pressure receptors on the skin and muscles around sympathetic ganglia. Hyper-parasympathetic tone has been shown to cause many health problems including; bradyarrhythmia, decreased atrial contraction, decreased ventricular contractility, hypotension, and decreased coronary blood flow, which can influence gastrointestinal function (Toro-Velasco et al., 2009; Wieting et al., 2013).

With regards to cervical myofascial release, gentle massage is applied to the muscles surrounding the posterior neck (Wieting et al., 2013). In Henley et al. (2008), authors suggested the technique aims to decrease the tone of postural deep musculature, which can improve respiratory function (Henley et al., 2008). The phrenic nerve passes inferiorly down the neck to supply the diaphragm. Therefore, the breathing rate of individuals can be observed as being more relaxed and deeper, which is thought to be as a result of gentle stimulation of the nerve (Wieting et al., 2013). This was reflected in findings by Dugailly et al. (2014), where researchers assessed the effects of a 30-minute GOT treatment that included a mixture of techniques on reducing psychological stress in 34 women (Dugailly et al., 2014). The researchers found that even one session of GOT using articulation and soft tissue techniques to the upper thorax and cervical complex, had short-term effects on reducing anxiety (Dugailly et al., 2014). However, the authors concluded that further investigation was warranted to obtain more conclusive and long-term results.

Despite there being a lack of scientifically robust evidence concerning the effect of GOT in reducing stress, the current results from several studies present exciting new perspectives regarding the effect of GOT on reducing stress. Most research studies conclude that larger studies with better methodological design are necessary to generate more definitive results.

Stress management education

Stress management education is a way in which practitioners can educate their patients on how to best identify current stressors in their life and assess ways to reduce those stressors and develop coping strategies to improve quality of life and well-being.

Current literature supports the theory that stress management education, such as cognitive behavioural therapy, has an effective role in reducing the anxiety, stress, and depression and improve quality of life of an individual (Chiang et al., 2016; L. Johnson, Schwartz, & Bower, 2000; Warsi, Wang, LaValley, Avorn, & Solomon, 2004). Additional studies looked at the efficacy of stress management education on reducing work-related stress (Chiang et al., 2016; Regehr et al., 2013; Romano, 1992). These studies determined significant reductions in stress, anxiety, depressive symptoms with the implementation of stress education and management protocols (Hjortskov, Rissén, et al., 2004; Wolkow et al., 2015).

Stress management education has also been associated with improving motivation, attitudes, beliefs, and the completion of routine daily activities. Furthermore, improvements in self-esteem and an increase in active coping strategies have been shown in studies where stress management or stress education package was delivered and/or provided to participants (Brinkborg et al., 2011; J. Y. Lee et al., 2014; Leung, Chiang, Chui, Mak, & Wong, 2011; Romano, 1992; Varvogli & Darviri, 2011). Strategies to provide stress management education along with manual therapy, may be plausible approaches and work in synergy to mitigate the effect of stress. Examples of combined approaches may include manual therapy with mindfulness meditation, stress management programmes, yoga and/or group exercise. To date, there is a lack of research evidence regarding the use of a combined approach to managing stress.

Concluding summary

- Literature suggests that stress is a massive health issue being faced by communities, health systems and governments.
- Stress can significantly impact on one's health and well-being. If it is unmanaged, it can lead to many psychological and physiological disorders.
- The literature suggests that men and women respond to stress differently, with men showing a greater integration of craving and emotional stress systems. Hypertension, aggressive behaviour and drug abuse is generally observed to be higher in men. While women experiencing greater anxiety and sadness. Conditions such as chronic pain, depression and anxiety disorders are more prevalent in women. Therefore, there is a need for more tailored inventions to addresss sex-specific issues.
- Evidence suggests that there is a need for a multimodal approach to managing stress. General osteopathic therapy/treatment or manual therapy are proving to have a great scope in contributing to mitigate this health issue
- However, there is a lack of evidence in terms of general osteopathic therapy application and its effect, in addition to proper application methodologies and research designs. There is again a lack of research evidence to understand if combining stress management education while applying general osteopathic therapy would be beneficial for people suffering from stress.
- This warrants carrying out a pilot study to assess the feasibility of applying a manual therapy package and seeing its effect in reducing both psychological and physiological stress (in a target population), which can then serve as the foundation for larger, scientifically robust, clinical trials or research.

Chapter 4 - Methods and protocol

This chapter is divided into two parts. The first part will discuss the rationale behind the chosen study designs and methods, compare efficacy and effectiveness studies, and discuss the importance of implementing multimodal interventions in managing stress. The first part will conclude with a summary of the expected outcomes of our present study. The second part will discuss the methods and protocols used in this study.

Part I - Rationale for the selected study design and methods

This part will address the rationale behind the selected study designs, methods and protocols. It will also provide a discussion regarding a multimodal approach, feasibility and pilot studies, and a mixed approach of combining pragmatic and exploratory clinical trials in a clinical osteopathic setting. It will conclude with the expected outcomes of such an approach.

Efficacy versus effectiveness studies

The term efficacy, in clinical research, typically describes the performance of an intervention in an ideal or controlled environment. Efficacy studies generally focus on accuracy and substantial research methods. They tend to be conducted in larger tertiary settings and ask specific questions in a controlled environment, which typically means they have several control groups (Gartlehner, Hansen, Nissman, Lohr, & Carey, 2006). Efficacy test whether the intervention or therapy is efficacious, they focus on "how does it work" in strictly controlled settings. These studies also focus on strong research methods and the accuracy of methods, therefore, they typically have modern technical equipment and specialised researchers. This is why efficacy studies are characterised by their high internal validity, which rejects any alternative hypotheses and eliminates the prospects of bias (El-Kotob & Giangregorio, 2018).

Comparatively, effectiveness studies have a "does it work?" approach, assessing whether an intervention or therapy works in a real-life scenario (Merali & Wilson, 2017). They determine whether the tested intervention/therapy is effective in a real clinical setting. Effectiveness studies are also referred to as pragmatic interventions, which are rarely blinded but can be randomised (Leon, Davis, & Kraemer, 2011). They do not typically use placebo-controlled groups, comparisons are generally conducted with other available treatments and/or pre- and post-intervention effects on the participants. These studies have high external validity. Since these studies are generally conducted in a practical real-life setting, the findings can be generalised and the outcomes can relate to practical situations (Eldridge et al., 2016). Other measures such as health-related quality of life measures are included in effectiveness studies. Overall, this relates well with the nature of the osteopathic profession which bases the foundations of their treatment on establishing a patient-centred approach. Healthcare professionals within the manual therapy field, such as osteopaths, aim to establish and maintain concrete rapport with their patients. Therefore, interventions following a more pragmatic design may be most appropriate. This appears to be most consistent with foundational philosophies developed by the founder of osteopathy, Andrew Taylor Still, who viewed the patient as a whole, which included the body, mind and soul/spirit (Paulus, 2013).

Multimodal interventions

Multimodal therapy implies that the practitioner utilises multiple modalities such as the psychological, physical and spiritual aspects of an individual to identify and treat presenting problem/s (Bain, Keren, & Stroud, 2016). The multimodal approach is based on the seven-dimensional Multimodal Therapy Evaluation Process (Dwyer, 2000; Lazarus, 1989). Arnold Lazarus developed this process based on the fact that humans think, feel, act, sense, imagine and interact. The seven modalities represent different dimensions of personality and follow an acronym known as BASIC I.D. This stands for behaviour, affect, sensation, imagery, cognition, interpersonal relationships, and drugs (Burgener, Yang, Gilbert, & Marsh-Yant, 2008; Dwyer, 2000).

This approach of combining various treatment modalities has been and is being in trialled in many populations (children, adults and old people) presenting with complex health issues (Morton et al., 2017; Smith, 2019). This allows clinicians to test different types of therapies, techniques or treatments together, which can optimise the health outcomes for a patient (Abdel-Rahman, Elsayed, Mohamed, & Eltobgy, 2018; Bochner & Scardino, 2006; Spaggiari et al., 2014). Researchers have developed and trialled different interventions in populations with various health issues including stress, anxiety, dementia, and pain disorders (Burgener et al., 2008; Maynard et al., 1998; Morton et al., 2017; Peterson, Anderson, Bourne, Mackey, & Helfand, 2018).

The literature indicates that stress is multifactorial and multifaceted. This means that several life events and factors contribute to stress and stress affects many bodily systems and at various levels. To date, there is mounting interest from researchers wanting to explore and assess different methods to treat and manage stress. This drive emerges from the desire to improve the mental health and overall well-being of an individual. Studies have indicated that research interventions using multimodal approaches are more effective than unifocal methodologies (Beltran-Alacreu, López-de-Uralde-Villanueva, Fernández-Carnero, & La Touche, 2015; Peterson et al., 2018; Philipps, Silbermann, Morawa, Stemmler, & Erim, 2019).

A multimodal approach is advantageous to researchers, practitioners and patients regarding time efficiency and addressing multiple stressors. This approach generally includes identifying stressors, counselling, mindfulness practices, and yoga. This approach generally includes identifying stressors, counselling, mindfulness practices, and yoga. Discussions on stress management and/or education, in addition to conventional methods of treating stress, is believed to be beneficial (Giannotti, Trainito, Arioli, Rucco, & Masiero, 2014; Morton et al., 2017; Smith, 2019). Researchers/practitioners aim to provide participants with coping strategies and tools to better manage their stressors (J. Y. Lee et al., 2014; Lynch et al., 2018; Romano, 1992). Employing stress management techniques has been demonstrated to provide individuals with good results on their overall health and well-being (Varvogli & Darviri, 2011). In a study by Varvogli et al. (2011), the authors provide some examples of current evidence-based multidimensional stress management techniques. These include mindfulness meditation, stress management education, cognitive behavioural therapy, mindfulness-based stress reduction, emotional freedom techniques and guided imagery. These techniques are relatively easy to learn and implement into one's life (Varvogli & Darviri, 2011).

In conjunction with this, many epidemiological studies have determined that positive effects manual therapy, massage, therapeutic touch and other complementary and alternative therapies, can provide (Bishop et al., 2015; Hartman, 1997; Henderson et al., 2010; Henley et al., 2008; Hensel, Buchanan, Brown, Rodriguez, & Cruser, 2015; Seffinger et al., 2016). Enhanced relaxation, increased concentration and improvement of mood swings are commonly reported subjective feelings in response to the therapy application (Beltran-Alacreu et al., 2015; Bishop et al., 2015; Field et al., 2007; Hensel et al., 2015). Therefore, current literature indicates that combining stress management education with either a conventional stress treatment plan or in conjunction with manual therapy may have added advantage in reducing stress or improving over-all wellbeing. Techniques such as progressive muscle relaxation, diaphragmatic breathing and soft tissue massage in combination with stress management education have shown positive effectson reducing one's stress (Castro-Sánchez et al., 2011; Field, 2010; Lynch et al., 2018; Mental Health Foundation of New Zealand, 2011; Varvogli & Darviri, 2011). This presents promising new perspectives for combining osteopathy with stress management education as a potential therapy package for reducing stress.

In this study, we proposed to use a multimodal approach for our therapy package, which included the application of 10 selected osteopathic techniques and 10-minutes of stress management education to participants, followed by the provision of a take-home pamphlet on stress and stress management.

Types of clinical trials and study designs

Clinical trials can follow various study designs depending on their specific objectives and research questions. This next section will further elaborate on the main features of pragmatic versus exploratory studies, controlled and uncontrolled clinical trials, feasibility and pilot studies and finally mixed methodological approaches in research. Finally, this section will conclude with the discussion of the tool used to judge or determine the study type for the design of the study presented in this thesis.

Pragmatic versus exploratory designs

Exploratory studies aim to determine the best research design, collection methodology and the selection of participants being investigated in a controlled laboratory/clinical setting using several control groups (sham or placebo) (Merali & Wilson, 2017). Pragmatic research usually involves the comparison of an assessed treatment with available treatment and does not necessarily use a controlled sham group (Merali & Wilson, 2017). Pragmatic studies place a greater emphasis on naturalistic designed studies achieving a real-life setting. Essentially, they determine whether an intervention is effective and works well in every-day practice. The tested intervention or therapy application is then compared to an available and current treatment, rather than a sham. The rationale for sham-controlled groups is to limit bias, particularly concerning treatment allocation, adherence, and the assessment of subjective outcomes (Brim & Miller, 2013; Sutherland, 2007). Sham groups serve as a standard for comparison in experimental studies. They are similar in relevant characteristics to the experimental group but do not receive the experimental intervention. Therefore, no therapeutic elements of true treatment intervention are provided (Leon et al., 2011).

Controlled and uncontrolled trials

A controlled clinical trial typically involves one or more experimental treatments, at least one control treatment, a bias-free method for assigning patients to the experimental treatment, and specified outcome measures for evaluating the studied intervention (Glogowski, 2020). They compare one group, which receives the experimental intervention to a control group. This control group does not receive the experimental treatment. They can, however, receive a different intervention, which has been tested but is not the same as the experimental treatment (Li & Begg, 1994). The experimental treatment may include devices, drugs, or procedures explored for therapeutic or diagnostic effectiveness (Glogowski, 2020). Control measures can include using placebos, sham treatment, active medicines, regimens or existing historical comparisons. The randomisation of groups can be done, where participants are randomly assigned groups including an experimental or control group. This describes a randomised control trial (RCT), where participants are assigned by chance to two or more groups (Li & Begg, 1994). Various methods can be employed to randomly allocate participants including mathematical techniques, such as the use of a random numbers table or computer-generated number sequences (Glogowski, 2020).

Uncontrolled studies are referred to as observational studies, which describe the effect of an intervention or treatment in a single group of patients (Glogowski, 2020). Researchers have no control over whether subjects receive the tested experimental treatment. Participants that are given an experimental treatment are observed for some time, with no comparison against another group taking the experimental treatment or no treatment at all (Li & Begg, 1994). It is difficult to interpret results as it is unknown whether the treatment effect observed is because of the intervention, and not because of the effect of confounding variables (e.g. placebo effect) (Glogowski, 2020). However, they are useful to find out whether a treatment causes any adverse side effects or to determine therapy safety and technical information. In situations where the outcome without therapy is predictable, this type of study is most appropriate.

Essentially, in a controlled study, researchers can determine what participants receive the studied treatment that is being tested for. Alternatively, in uncontrolled studies, there is only one group. Therefore, the tested intervention/therapy is generally tested against or compared to existing therapies (Glogowski, 2020). Controlled studies are statistically stronger than uncontrolled trials and are fundamental to adequately prove causality.

Feasibility and pilot studies

Pilot and feasibility studies are the first steps before starting large randomised clinical trials. Both studies can have distinct purposes or can be combined as one initial step before initiating main clinical trials (El-Kotob & Giangregorio, 2018). A pilot study can be described as a small-scale project that evaluates the duration, potential adverse events, costs, overall feasibility and provides recommendations for future larger scale studies (Leon et al., 2011). The purpose of pilot studies is to examine the feasibility of an approach or research question, to be expanded in a larger scale study (e.g. randomised controlled trials). Pilot studies are, thus, crucial for research as they play a fundamental role in developing and/or refining research questions, studies or interventions (Moore, Carter, Nietert, & Stewart, 2011). Despite the informative nature of pilot studies, the efficacy outcomes in an intervention can determine whether a study can be published or not (Eldridge et al., 2016). (Merali & Wilson, 2017). Pilot studies, especially feasibility studies, can and need to be published to provide information on certain aspects including enrolment challenges, improvement in questionnaires, administration processes, sample collection and the feasibility of the experiment or trial settings (El-Kotob & Giangregorio, 2018). Our primary objective was to propose a therapy package comprising of osteopathic techniques and stress management education to manage stress. There is a lack of robust research in the application of a GOT combined with a stress management and education approach. Therefore, the study that is being presented in this thesis aimed to conduct a feasibility study to assess the practicality of the various aspects within the proposed intervention, by applying this approach. The various aspects assessed included the recruitment process, procedures involved, methodologies and implementation of the therapy package.

Mixed method approach

The approach of combining various study designs (mixed methods approach) in research has been defined as "the form of research allowing for a team of researchers to combine elements, to establish depth and strength of understanding and corroboration" (Schoonenboom & Johnson, 2017). Examples of both qualitative and quantitative approaches includes the use of different viewpoints, data collection, analysis and/or inference techniques (Wisdom & Creswell, 2013). As stress is a multifactorial condition affecting multiple systems and leading to the development of complex conditions, the literature has acknowledged the importance of combining pragmatic and exploratory clinical trials using a mixed methodological approach (Bronfort, Haas, Evans, Leininger, & Triano, 2010; Espí-López, López-Bueno, Vicente-Herrero, Martinez-Arnau, & Monzani, 2016; Espí López & Gómez Conesa, 2014; Leon et al., 2011; Ruwaard et al., 2012).

In recent times, applying a mixed method is more preferred in interventional clinical studies as employing a purely pragmatic or purely exploratory design, is not ideal. Mixed methodologies can contribute to research by strengthening or developing the conclusion of a study further (Schoonenboom & Johnson, 2017). An advantage of using such an approach is that it is able to reflect a participant's point of view. Additionally, by having certain aspects within a tested intervention/therapy controlled, researchers can produce mechanistic or causation answers. Alternatively, if some aspects are of pragmatic design and are uncontrolled, it allows researchers to mimic real-life settings. Essentially, by having a mixed approach, interventions are more patient-centred by providing the study participants with a voice, ensuring that the study findings are grounded in the participant's experience. Furthermore, using a mixed-methods approach can foster scholarly interaction, provide methodological flexibility and collect rich and comprehensive data (Wisdom & Creswell, 2013).

Therefore, a multimodal, mixed methodological approach of combining efficacy and effectiveness is optimal. Particularly in clinical studies assessing manual therapy, such as osteopathy. Mixed methodologies include both controlled and uncontrolled interventions within a clinical setting.

PRECIS-2 wheel

Currently, researchers use a tool called the Pragmatic-Explanatory Continuum Indicator Summary 2 (PRECIS-2) wheel (Loudon et al., 2015) (see Figure 1) to determine whether the study design is pragmatic or exploratory in nature. There are nine domains within the PRECIS-2 wheel, which visually describes whether the proposed trial is pragmatic or explanatory. Scoring of each domain can be done using a five-point Likert scale, a score of 1 indicating "very exploratory", compared to a score of 5 indicating "very pragmatic" (Loudon et al., 2015). This tool has been used in the current study to determine the nature of the study design.

Figure 1 - The Pragmatic-Explanatory Continuum Indicator Summary-2 (PRECIS-2) wheel.

Expected outcomes

This study will test the feasibility of the application of a therapy package following a mixed approach. This approach combined the use of a manual therapy and stress management education for treating women with stress. The aspects that were tested included whether the therapy package had any effects on changes within the psychological questionnaire scores (PSS-10 and POMS), and levels of stress biomarkers (cortisol, sIgA, and IL-6). With the proposed mixed therapy package, we aimed to provide researchers with recommendations to improve the study design to allow for more valid outcomes to be determined in future interventions. Recommendations will be provided for:

- The selection of times and dates for the intervention
- The application of the GOT techniques
- The deliverance and effectiveness of the stress management education aspect
- The collection of salivary samples
- The selected assessed biomarkers

Part II – Methods and protocols employed in the present study

Study design

The study being presented in this thesis was of mixed methodology, involving both exploratory and pragmatic design paradigm. The Pragmatic-Explanatory Continuum Indicator Summary 2 (PRECIS-2) wheel (Loudon et al., 2015) (see Figure 1) was used to determine the main aspects of the study design and where the design lies on this wheel. As shown in PRECIS-2 wheel (see Figure 2), some aspects in this current study were exploratory (i.e. the "eligibility" domain) and some were pragmatic (e.g. the "setting" and "organisation" domains). The aspects that were controlled included fixed therapy duration (30-minutes), fixed time of day (8 to 9.30/10 am), and treatment over two consecutive days (Tuesday and Wednesday) within 24 hours. Hence, giving the total score of 26. As discussed in part I (under PRECIS-2 section), the score of 26 suggested that our design employed a mixed, pragmatic and exploratory design paradigm, with an inclination towards being more pragmatic. This study was approved by the Unitec Research Ethics committee (UREC #2018-1066, refer to appendix A) before the commencement of the intervention.

Figure 2 - Score for our study on the PRECIS-2 Wheel. 'Setting and organisation' rated as rather pragmatic, 'eligibility' rated as very exploratory; 'recruitment', 'primary analysis' and 'flexibility: adherence' rated as rather exploratory; 'flexibility: delivery', 'follow-up' and 'primary outcome' rated as equally pragmatic and exploratory. Total score of 26.

Participant recruitment and enrolment

The student researcher organised and managed the recruitment for each participant. Participants were recruited via contacts within health professional networks and university forums or online advertising (see Appendix 2). Further advertising on social media, including Facebook and LinkedIn, was used, and posters were displayed around the Unitec campus and different Auckland areas (e.g. local supermarkets). Potential participants were asked to email their interest to an email created for this study. Recruitment took place over January, February and March 2019. Initially, 19 females expressed their interest in partaking in the study, of which seven were excluded as they did not meet the inclusion criteria or did not respond to emails (see Results Figure 1 for enrolment details). A total of 11 females were enrolled in this study (age range 21-54 years; mean age 31) (see Results Table 1 for participant characteristics). The student researcher administered an eligibility screening, the criteria for which included:

- Female
- Over the age of 18
- The ability to communicate in English
- The ability to provide informed consent
- The presence of moderate stress levels ($\geq$14 on the PSS-10) at the time of screening.

For reasons of either representing contraindications to aspects of the therapy package and/or affecting biomarker levels, the following exclusion criteria were applied:

- Presence of any pathological conditions including diabetes, Addison's disease, hypopituitarism, Cushing's syndrome, heart conditions, arthritis, any known chronic inflammatory condition and/or cancer)
- Breathing difficulties
  Hypertension (controlled by medication)
- Current musculoskeletal condition or injury
- Neurological symptoms (numbness, tingling, nausea, vomiting, sense of weakness)
- Presence of any oral disease
- Current steroidal use (not including hormone replacement therapy)
- Substance or alcohol abuse
- Pregnant or recently post-partum.

After eligibility was determined and participants agreed to participate in the study, the student researcher organised dates with the participant to begin session one of the intervention. All treatment sessions were located at Clinic 61, Unitec Institute of Technology, Mount Albert, Auckland Campus. Participants were given a participant information sheet (PIS) (see Appendix 3) and a consent form (see Appendix 4) upon arrival to session one. Participants were asked to complete these before the first session. Electronic copies of these were made and stored in a locked cabinet. To maintain participants' privacy, only the student researcher and their supervisors had access to this. Participants kept their own copy, for their own record. The study was run as part of the student researchers' research for their Masters, alongside completing clinical hours, which is a compulsory component for their studies. Therefore, the participant and student researcher then mutually agreed on a week and scheduled two consecutive study sessions between 8 am to 9:30/10 am on Tuesday and Wednesday. The participant was sent an email reminder the day before the chosen session dates.

Procedure for the proposed intervention

The objective of this study was to assess the feasibility and effectiveness of a therapy package on perceived and physiological stress in women. The effect of the therapy package was assessed by measuring pre- and post-intervention salivary stress biomarkers (cortisol, sIgA and IL-6). Two psychometric measures of perceived stress were used: the Perceived Stress Scale-10 (PSS-10) (Chaaya, Osman, Naassan, & Mahfoud, 2010) and a modified Profile of Mood Scale (POMS) (Chennaoui et al., 2016). The main steps and session outlines are discussed in the following paragraphs.

After agreeing to proceed, a general health screen was taken for each participant, which was adopted from the John Hopkins Medical School case history (see Appendix 5) (John Hopkins Medicine, n.d.). This included gathering information regarding height, weight, heart rate and blood pressure. Blood pressure was measured manually by the student researcher using an aneroid sphygmomanometer blood pressure cuff and Littmann stethoscope. Following initial health screening and PSS-10 score, eligible participants with acceptable health status and PSS-10 scores $\geq$14 were enrolled in the study. The therapy package was applied in two consecutive days, each session lasting up to 1 hour and 15 minutes. The decision to administer this therapy package over two consecutive days was consistent with a double- blinded randomised control trial (RCT) published by Rogan et al. (2019). The steps for the two mutually agreed and scheduled consecutive sessions are detailed on the next page.

Session one (1 to 1.25 hours)

1. Participants were given PIS on arrival (Appendix 3)
2. Participants then completed the Consent Form (Appendix 4)
3. Rinsing of participant's mouth with water five minutes before sample collection
4. Pre-intervention collection of saliva sample (Timepoint 1), for assessing biomarkers. Samples stored in collection chilly box for transportation and then stored in the -80°C freezer until processed.
5. Completion of stress scale questionnaires: modified POMS and PSS-10 questionnaire (Appendix 9 and 10).
6. Completion of adapted case history including blood pressure (BP) and heart rate (HR) measurement (Appendix 8).
7. Application of the 10 selected GOT techniques (Appendix 5).
8. Delivery of scripted stress management education (Appendix 6) and handing out of associated pamphlet (Appendix 7).
9. Completion of modified POMS and PSS-10 Questionnaire – post-treatment (Timepoint 2).
10. Collection of saliva samples – post-treatment (Timepoint 2).

At the end of the first session, the participants were asked routine health safety questions to ensure they were not or had not experienced any adverse effects of the treatment (e.g. questions regarding their current mood and/or whether they had any aches or pains). They were advised to contact the student researcher if they had any questions or experienced any effects from the treatment, such as feeling light-headed or headache. They were also advised to drink water throughout the day.

Session two (50-minutes to 1 hour)

Took place 24 hours after the initial session and included the following steps:

1. Rinsing of the participant's mouth with water five minutes before sample collection
2. Collection of pre-intervention saliva samples (Timepoint 3)
3. Completion of pre-intervention stress scale questionnaires (POMS and PSS-10)

4. Application of the GOT and stress management education, similar to step 8 in session one.
5. Completion of post-intervention stress scale questionnaires (POMS and PSS-10)
6. Collection of post-treatment saliva samples

After the second session, participants with remaining high-stress levels (>19) were advised to consult their general health practitioner. All participants were given the chance to ask any final questions about the study, which included the collection of participant feedback on every aspect of the therapy package (e.g. collection of saliva sample, GOT therapy, stress management package etc.). Feedback was collected verabllay in an informal semi-interview setting, by asking questions related to health, sfately, therapy application and adverse effects (including lightheadedness, fatigue, muscular pain). Feedback was colleted at the end of each session. They were also asked to leave their contact email to receive a summary of the study results. Furthermore, all participants received a $20 petrol voucher to cover travel costs.

Proposed therapy package

The therapy package composed of two components; application of general osteopathic techniques and administration of stress management education.

General osteopathic technique/therapy

Following the collection of saliva at timepoint (TP) 1, and after the participant had completed the pre-treatment stress questionnaires, the student researcher gained consent to proceed with GOT. Each participant was asked to lie supine on the treatment plinth they received the GOT routine applied to 10 different areas. These areas included the hip, sacroiliac joint, lumbar spine, thoracic spine and ribcage, glenohumeral joint, scapulothoracic joint, cervical spine, upper thoracic ribs, cervical spine and cranium region. The techniques included soft tissue massage, rhythmic joint articulation, myofascial release, gentle harmonics, muscle inhibition, and rib-raising. The student researcher individually tailored the application of each technique (see Appendix item 5).

The length of time spent on performing each technique depended on the student's perception of tissue change. This included the feeling of the joint or area "relaxing" as the technique was applied to the area. This perception of tissue change was determined by the feeling of softness of the tissues, if they became more mobile and/or if they were more pliable to stretch. Then the student researcher moved on to the next area. Seven joint articulations and three soft tissue techniques were applied to the participant for a maximum of 30 minutes. Overall, the duration of each session varied between participants (range: 50 minutes to 1.25 hours; mean 60.7, SD 6.85). The sessions were flexible depending on the participant's perceived comfort level.

Stress management education and pamphlet

The stress management and education pamphlet aimed to provide the participant with tools to help identify and manage their stress in everyday life. Stress management education can help people determine their current life stressors, identify ways to reduce these stressors and develop coping strategies to improve one's quality of health (Brigham Young University, 2012). The stress management education used for this project included a scripted discussion about tools aimed to reduce tension and promote an overall sense of wellbeing.

Stress management education was delivered after the GOT treatment. The participant was asked to slowly get up and off the treatment table at their own pace, and sit comfortably in a chair before receiving a 5-10 minute scripted session based on the Brief Cognitive-Behavioural Education Program (B-CBE) (Chiang et al., 2016) by the student researcher (see Appendix 6). The way the education script was delivered followed a pragmatic real-life setting, whereby the student researcher chose how to best deliver the information depending on the participant's mood during the session. This led to the duration of delivery varying between 5-10 minutes.

Also, a pamphlet (see Appendix 7) was given to the participant to take home and read in their own time. This contained information such as a basic and clear definition of stress, ways to identify stressors and some strategies to cope with, manage and overcome stress. These included sleep, exercise, visualisation, deep breathing, socialising, keeping a journal and creating a schedule. Participants were advised to try some of these strategies in their daily life.

Saliva sample collection

Saliva samples were collected from participants for assessment of baseline, pre- and post-intervention levels of selected biomarkers. The method is briefly discussed here, for detailed protocol is provided in Appendix #11. Passive drool method was selected for this project as it is considered to be a stress-free and easy method to administer (D. S. Q. Koh & Koh, 2007). Samples were collected between 8-9.30/10 am as the selected biomarker levels have specific diurnal profiles. A total of four saliva samples were collected from each participant at four different TPs with two samples collected during session 1 (pre-therapy, TP1 and post-therapy, TP2) and two samples collected during session 2 (pre-therapy, TP3 and post-therapy TP4).

Participants were instructed to avoid consumption of food, caffeinated beverages and over-the-counter medication for 60 minutes before both sessions. Upon arrival, participants were provided with a glass of water to rinse their mouths. This was to remove any debris, food and buccal mucosal layer residue, which could have contaminated samples. To avoid dilution of saliva with the residual water, participants waited approximately five minutes after mouth rinsing before collecting the pre-intervention saliva sample.

The student researcher placed the sterile Pasteur pipette beneath the tongue and collected 0.5-1 ml of saliva. Collection times varied between participants (5 to 10 minutes). Participants with low production of saliva were required to move their tongue back and forth to allow saliva to be accumulated sublingually (in the buccal cavity of the mouth). The student researcher recorded the time of the morning that each sample was collected with its respective TP. This was written on an adhesive label attached to the participant's cryotube. As the saliva flow rate can affect individual biomarker concentration and secretion rates (Hildebrandt et al., 2013), the duration of the collection was reordered for all participants.

All samples were aliquoted into two appropriately labelled tubes, tubes placed in cold chilly bins before transporting to -80°C freezer on site. Samples were then transported to the Spinal Cord Injury Research Unit, Centre for Brain Research, the University of Auckland to be analysed. This was completed by an experienced Research Fellow using the ELISA methods (Sigma Aldrich and Sandwich kits), which is briefly discussed below (see Appendix 12 for further details).

Psychological and physiological parameters

For assessing the effect of the applied therapy package on stress, both perceived and physiological tools were used.

Assessment of physiological stress

Although serum and blood plasma are considered the gold standard in biomarker assessment, the collection of saliva samples is less invasive. Researchers need to develop and/or explore more non-invasive methodologies to better evaluate the effect of physiological stress. Therefore, saliva samples were selected, which have been demonstrated to provide a good representation of the chosen stress biomarkers for this study (Cortisol, IL-6 and sIgA). Cortisol is a stress biomarker that represents the HPA axis, is widely used in stress research, and has a diurnal variation (Nedeljka et al., 2016). Both IL-6 and sIgA are good representative biomarkers of the inflammatory and immune systems respectively. Both of these systems are directly and indirectly affected by stress (Prasad et al., 2015; Volkmann & Weekes, 2006). The assessed salivary biomarkers can be measured using several immunoassay kits/methods currently available. The analysis of the biomarkers was completed by a Research Fellow (Spinal Cord Research Injury Unit, University of Auckland) as per the manufacturer's instructions. The Enzyme-Linked Immunosorbent Assay (ELISA) method was conducted for analysis of the biomarkers.

Enzyme-Linked Immunosorbent Assay method

The ELISA method is one of the most sensitive and reliable immunoassay methods to measure cytokines from biological samples (Cox et al., 2004). There are four main types of ELISA methods: Direct ELISA, Indirect ELISA, Competition/Inhibition ELISA and Sandwich ELISA. The Sandwich ELISA and Competition ELISA methods were used for this project.

The ELISA method specifically captures antibodies, which are coated onto a 96-well plate, which was performed as per the manufacturer's instructions (Abcam, 2019; "Human IgA, Secretory ELISA Kit," 2012). The Research Fellow performed all assays as per the respective manufacturer's instructions and analysed all biological samples at the Spinal Cord Injury Research Unit, Centre for Brain Research, University of Auckland. Cortisol was measured using ELISA competitive method (Abcam, USA), IL-6 was measured using Sandwich ELISA method (IBL International, Germany) and sIgA was measured using Sandwich ELISA method (LifeSpan BioSciences, Inc., USA). The details of the principals of these methods and the exact steps taken are provided in Appendix 12.

Assessment of psychological stress

Due to stress and stressors being multifaceted, its perception and response is dependent on the individuals past or current experiences. The PSS-10 is a widely used instrument for the assessment of perceived, subjective stress. Based on its high internal validity and the correlation with various behavioural and self-report criteria (e.g. stressful life events), it was selected for this study. The POMS is another commonly employed instrument measuring various mood states. With its briefness and multidimensionality, this tool is advantageous for psychological behavioural research. Therefore, for the validity and scientific robustness of the

PSS-10 and POMS in stress studies, these tools were selected for the study being presented in this thesis.

Perceived Stress Scale

Excellent convergent validity between different versions of the perceived stress scale-10 (PSS-10) scale has been demonstrated (A. M. Mitchell, Crane, & Kim, 2008). The scale also shows good reliability (Cronbach alpha=.78-.91), particularly when compared to alternative versions of the scale (i.e., PSS-14) (Chiu et al., 2016a; E. H. Lee, 2012). The PSS-10 instrument used for the study presented in this thesis can be seen in Appendix 10.

The PSS-10 format was modified to measure how much stress was perceived after the first session. The statement was adjusted as follows: "In the last month…" was replaced by, "In the last few days …". This made it most relevant for the short-term nature of this intervention study. Participants took around two minutes to complete the scale. Scoring was done as per the published instructions, which are described briefly in this section. Each item in the questionnaire is rated on a five-point Likert scale ranging from never (0) to almost always (4). Positively worded items were reverse-scored, for example, 0=4, 1=3, 2=2, etc. and then summing all ten items, with higher scores indicating more perceived stress. (Cohen, 1994). The items that were reverse scored for questions 4, 5, 6, 7 and 8. Although the PSS-10 does not constitute a diagnostic instrument with predetermined cut-off points for different levels of perceived stress, a score of 13 is generally considered normal (E.-H. Lee, 2012; Levenstein et al., 1993). Scores lower than 13 have been interpreted as low stress. Comparatively, scores between 14 and 19 are assumed to suggest the presence of light-moderate stress (Chaaya et al., 2010; E.-H. Lee, 2012). A score of 20 or higher is thought to indicate high stress (Chaaya et al., 2010; Levenstein et al., 1993; Solivan et al., 2016). To avoid floor effects, the present study aimed to exclude participants with initial scores of 13 or less. After calculating scores at the final TP at the end of the intervention, those with very high scores (>19) were advised to consult their general health practitioner to discuss further.

Profile of Mood Scale

The Profile of Mood Scale (POMS) is a psychometric rating scale used to assess distinct and transient mood states (Morfeld, Petersen, Krüger-Bödeker, von Mackensen, & Bullinger, 2007; P. C. Terry, Lane, & Fogarty, 2003). Because of the length and impracticality of the original 65-item version, the shorter 40-item POMS was used in this study (S. L. Curran, Andrykowski,

& Studts, 1995). The POMS instrument used for the study presented in this thesis can be seen in Appendix 9.

A total score for the 40-item POMS is calculated by adding the negative values (Tense, Fatigue, Depression, Anger and Confusion) and subtracting the total score from the positive values (Vigour and Self-Related Esteem) (Grove, 1992). Items for the negative subscale Tense included "tension", "feeling on-edge", "restlessness", "uneasiness" and "nervousness". For Fatigue subscale, the items included "worn-out" and "fatigued". For Confusion, the items include "confused" and "can't concentrate". For Depression, the items included "unhappy", "hopeless", "sad", "discouraged" and "miserable". Descriptions for the positive subscale Vigour included "lively" and "energetic". Lastly, for Esteem-Related Effect the items included "proud", "ashamed" (reversed) and "competent". The calculation for each subscale was done as per the published literature (Chennaoui et al., 2016; Dipietro, Costigan, & Sipsma, 2008; Lopez et al., 2011; Morfeld, Petersen, Krüger-Bödeker, Von Mackensen, et al., 2007; P. Terry et al., 2003).

There was a printing error by the student research which resulted in us using only the first 24-items of the original 40-item version. Because of this, we were unable to calculate a total mood disturbance (TMD) as we could not extract all subscales from the 24-item POMS. Therefore, only Tense, Fatigue, Confusion and Depression could be used as they consisted of more than four items. For the Tense subscale, the missing category was "Anxious", so we substituted this with an alternative item (with similar meaning) taken from the PSS-10, which read "How often have you felt nervous and stressed". For the Anger subscale, the missing category was "Bitter" and "Furious", we, therefore, replaced these with, "How often have you felt that things were going your way?" and, "How often have you been angered about things outside of your control?" from PSS-10. The results for the question, "How often have you felt that things were going your way?" was reversed, where [0=4, 1=3, 2=2, 3=1, 4=0] respectively. For the Depression subscale, the missing categories were "Helpless" and "Worthless". The item from PSS-10 that replaced these was, "How often have you felt that you were on top of things?" and "How often have you felt confident in your ability to handle problems in your life?". Finally, for Fatigue subscale, the missing categories were "Exhausted", "Weary" and "Bushed". Therefore, we substituted the following alternative for Exhausted: "How often have you felt that you were unable to control important things in your life?". For Weary and Bushed, we used "How often have you felt things were piling up so high you could not overcome them?" as an appropriate alternative from PSS-10.

Some of the items from the PSS-10 had similar meanings to items within the four selected POMS subscales. Therefore, these were also added to each of these subscales. As a result, we had six items for Tense subscale (Tense, Uneasy, On-edge, Restless, Nervous, “How often have you felt nervous and stressed?”). There were seven items for Depression (Sad, Unhappy, Hopeless, Discouraged, Miserable, “How often have you felt confident in your ability to handle problems in your life?” and “How often have you felt that you were on top of things?”). We had five items for Fatigue (Fatigued, Worn-out, “How often have you felt you could cope with all the things that you do?” How often have you felt that you were unable to control important things in your life?” and “How often have you felt things were piling up so high that you could not overcome them?”). Finally, there were six items for Anger (Angry, Grouchy, Annoyed, Resentful, “How often have you been angered about things outside of your control?”, and ‘In the last month how often have you felt that things were going your way?” [Reversed]) (Sayre, 2016). This questionnaire took approximately four to five minutes to complete.

Data transcription and analysis

All paper-based responses from participants were scanned to create an electronic copy stored on password-protected devices. Electronic copies were kept as a back-up, in case the hard copy data went missing somehow. The student researcher entered the data received from the PSS-10 and POMS results into a Microsoft spreadsheet in Excel, at the end of their sessions. Total scores for each questionnaire were calculated (see above section for scoring details). All demographic data and general feedback from the participants concerning the feasibility of the entire approach were recorded into the Excel spreadsheet. Attempts were also made to record any adverse events experienced by participants or observed by the researcher. The data from the assessed biomarkers were processed by a Research Fellow at the Spinal Cord Research Injury Unit laboratories, Grafton Campus, Auckland University. The final adjusted values of all biomarkers were provided in an excel spreadsheet. The student researcher then ran t-tests (paired two-tailed t-test) and normality tests on PSS-10, POMS and the biomarkers data.

Psychological stress analysis

Once completed, all psychological measures (PSS-10 and POMS questionnaires) were collected from the participants. Copies were stored in each individual's file until the end of the intervention. After this, the responses were scanned, and electronic copies were made and saved in Google Drive. Only the student researcher and their supervisors had access to this. Responses from participants from the PSS-10 and POMS were entered into Microsoft Excel.

Appropriate subscale scores were calculated within Excel and results were summed to determine totals for each questionnaire at each TP. Total scores were then imported from Microsoft Excel into SPSS for data screening for normality and descriptive statistics from both psychological parameters taken at session one (TP1 and 2) and session two (TP 3 and 4). Inferential analyses were also carried out in SPSS. Specifically, paired-sample T-tests (or the non-parametric equivalent Shapiro-Wilk) were completed between TPs for both psychological questionnaires.

Physiological analysis

All saliva samples were transported to the Spinal Cord Research Injury Unit laboratories, Grafton Campus, Auckland University. The samples were assessed and analysed by a Research Fellow at the Spinal Cord Research Injury Unit, Centre for Brain Research. To determine the change between baseline and follow-up, salivary levels of cortisol, IL-6 and sIgA were tabulated from both sessions and compared using T-tests and normality tests as necessary using SPSS software. For analysis, tabulated data was imported from Microsoft Excel spreadsheets to SPSS software where data were screened for normality and then paired-sample T-tests were administered. Graphs were created using Microsoft Excel to analyse observable differences and changes between the assessed TPs between individuals.

Assessment of correlation between studied parameters

Since this being a feasibility, pilot study with small numbers. Statistical analyses for assess correlations (Pearson's or Spearman's correlation) could not be conducted. The student researcher looked across all the parameters for any observational relationship between PSS-10/POMS and biomarkers, by assessing all graphs for any similarities in the pattern/changes seen over the course of the intervention. Finally, the student researcher looked at individual-level data for potential relationships between physiological and perceived stress markers at the individual level.

Chapter 5 – Results

Demographic characteristics of participants

Eighteen women expressed their interest in participating in the study through emails. A total of 11 were enrolled in the present study (age range: 21-58, mean = 33.72, SD = 12.8) (see Figure 3). Participants were either completing their final year in tertiary education or working, and all indicated they were experiencing moderate levels of stress. Among the various modes of advertisements used (e.g., Facebook, LinkedIn, posters around the campus and general Auckland region advertisement on the Unitec Nest Forum), the Unitec Nest Forum, a web-based staff portal, was found to be the most effective. Seven out of 11 participants were informed through this platform.

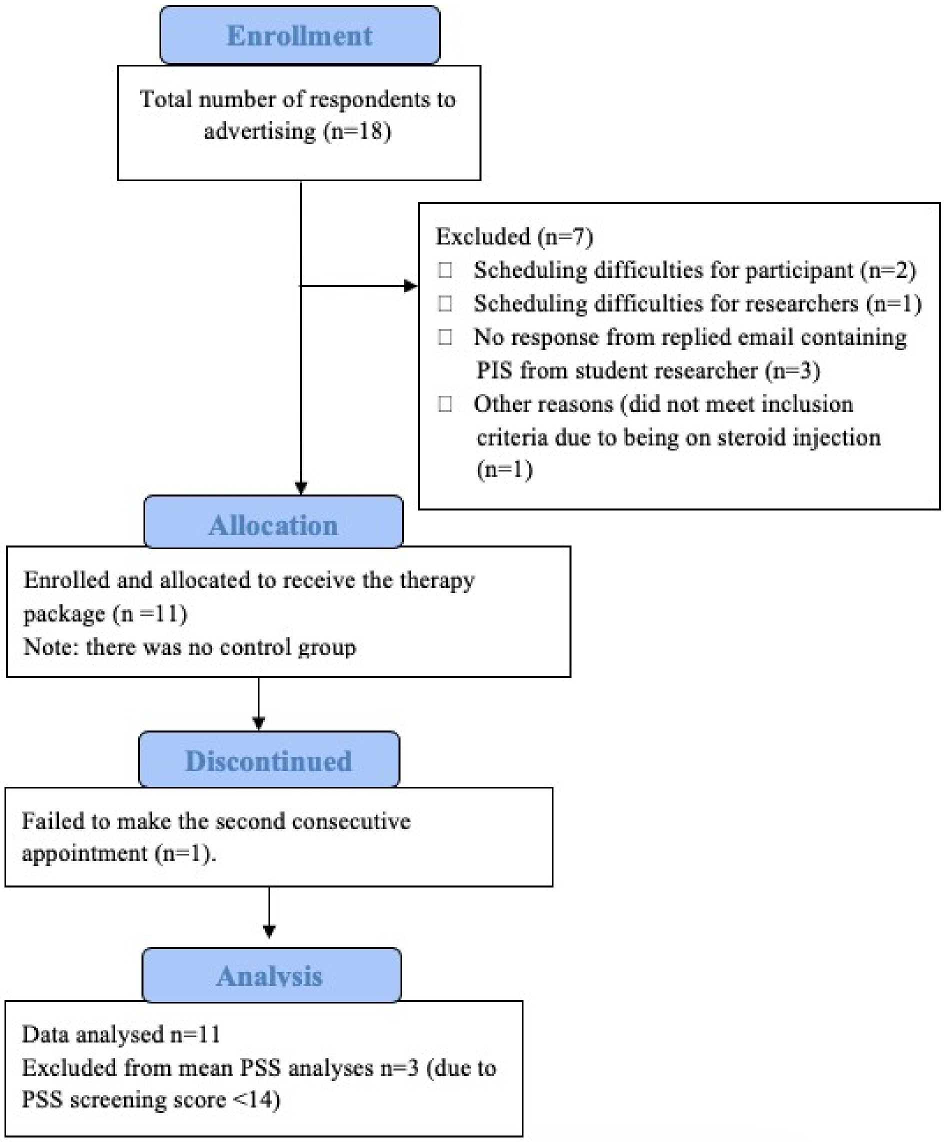

Figure 1 - Consort flowchart, displaying the flow of subjects from enrolment through analysis

For all patient demographics and baseline characteristics, see Table 1. The following is a summary of all participants enrolled referred to by Table 1.

Baseline Perceived Stress Scale (PSS-10) scores were between 9 and 29 (mean score 20.6, standard deviation 7.2, range 9 to 29). Due to a procedural error, three participants (participant #1, #2 and #4) did not meet the minimum perceived stress inclusion criterion (i.e. PSS-10 <14), but were included in the study nevertheless for ethical reasons. Enrolled participants age ranged between 20 to 60 years (mean = 33.7, SD = 12.8), of this 73% were between 20 and 35 and remaining were between 50 and 60 years. Five participants (46%) were enrolled in tertiary education, 40% of whom were currently studying for final semester exams or had important assignments due. A total of five participants indicated being affected by one or more systemic issue. Of the participants, 73% were in a relationship or married, the remaining 28% were single. Seven of the 11 participants (64%) arrived between five and 20 minutes late due to either traffic, dropping kids off at school before the intervention. This may have added to their pre-intervention anxiety. General health was good, with blood pressure (BP) ranging between 110/74mmHg – 130/87mmHg. Heart rate measurements were within the normal range (mean = 67.2bpm, SD = 13.7), of these five (46%) showed one or more systemic issue. Body Mass Index (BMI) was also measured as per the formula provided by the NZ Ministry of Health (Heart Foundation, 2019). Seven of the 11 participants' (64%) BMI were within healthy ranges (mean = 25.12kg/m^2, SD = 4.6).

Table 1 - Characteristics of the demographic

Participant ID number (#)	**Age**	**Marital status**	**Blood pressure (mmHg)**	**Heart rate (bpm)**	**Height (cm)**	**Weight (kg)**	**Body Mass Index (BMI) (kg/m²)**	**Systems Review (presence/indication of any issues)**	**General Health**	**Nervous System**	**Additional information**
1	31	S	122/82	82	168	67	23.7	N	N	N	Scored <14 in initial PSS-10 Long smoking history
2	22	R	111/69	61	158	63	25.2	N	N	N	Scored <14 in initial PSS-10 Student (academic stress) Saliva collection – required 5-7 minutes for collection. The participant felt "awkward", appeared uncomfortable during the collection
3	23	R	112/68	78	167	63	22.6	Y (Fungal infection)	N	Y (Headaches)	
4	27	Md	110/74	56	182	66	19.9	N	N	N	Scored<14 in initial PSS-10 Consumed coffee pre-intervention, low amounts of saliva, collection took 7-10 minutes, noticeably uncomfortable during the collection.
5	51	Md	118/84	79	157	51	20.7	Y (Fungal infection)	N	N	Arrived 15-20 minutes on both days.
6	58	Md	118/89	78	177	78	24.9	Y (Musculoskeletal injury)	N	N	Felt "awkward", appeared uncomfortable during the collection. Life partner with a terminal illness.
7	21	S	116/80	62	170	68	23.5	N	N	N	Student currently (academic stress).
8	28	Md	110/75	51	165	72	26.4	N	N	N	Arrived 15 minutes late on both days.
9	28	Md	120/81	41	176	70	22.6	N	N	N	
10	53	D	117/77	76	172	78	26.4	N	Y (Fatigue)	Y (Migraines)	Divorced 2 years ago. 5-7 minutes saliva collection: low amounts of saliva.
11	29	S	130/87	89	152	86	37.2	Y (Asthma – mild, controlled with Ventolin)	N	Y (Headaches)	Job stress, recently increased workload, short breaks.

Married = Md, Relationship = R, Single = S, Divorced = D
Y = Present, N = Not indicated

General feedback from participants

The GOT was applied to areas that felt most tense, which was based on the student researcher's palpation. As a result, nine participants (82%) received the GOT package to their shoulders, neck and ribs on the second day of the intervention. On average, the first session took between 1 and 1.25 hours, while the second typically lasted between 50-minutes and one hour. No participants reported any adverse effects from the GOT therapy. All of the participants commented feeling "relaxed" after treatment, two of whom said they felt "sleepy". Five participants stated they were "grateful" for the educational package on stress and stressors, two of which were "shocked" at the identification of certain stressors in their lives. All participants were impressed with the educational package delivered. From the researcher's perspective, the most commonly noted observation was the ability for the participants to relax. This was determined by body language, ease of conversation, breathing pattern changes and participants closing their eyes during treatment.

As per the feedback gathered from the participants and student researchers' experience, there were few challenges encountered by both the participants and the researcher. Challenges mentioned included the collection of the saliva samples, recruitment process and practicality of intervention dates and times, which are summarised in Tables 2 and 3.

Table 2 - Challenges encountered by participants

Category/ item	Description	Rationale for design	Challenge encountered	Feedback from participants
Time of the therapy session	8-10 am	Participants were required to attend each consecutive session at 8-9:30 am to achieve consistency and reliability with the selected salivary biomarkers, as these biomarkers show diurnal variation.	Early morning appointments on two consecutive sessions proved difficult for participants with early morning weekday commitments.	Three participants commented that they were stressed about being late for work.
Therapy sessions	Approximately 1 hr, on two consecutive days (Monday and Tuesday)	As the intervention only ran for two days, having the two sessions 24 hours apart lasting approximately 1 hour was chosen for consistency.	Arranging/organising two consecutive days were difficult for some participants in light of their daily life commitments, i.e. work. Furthermore, peak hour traffic and the clinic was located off a busy main road added to difficulties.	Four participants apologised for arriving 5-20 minutes late due to traffic.

Table 3 - Challenges encountered by the student researcher

Category/ item	Description	Reason/explanation this	Challenge encountered
Recruitment process	Errors made with initial PSS-10 scores for three participants (#1, #2 and #4)	Student researcher made an error in scoring, which led to the inclusion of three participants who scored <14.	Three participants (#1, #2, #4) were included in the study although scored <14 PSS-10. Due to PSS scores, these three participants were excluded from some of the analyses.
Therapy sessions	Feasibility regarding the timing of the sessions and consecutive days.	Timing – 8-10 am decided to avoid diurnal variation observed in the selected biomarkers. Two consecutive days of the week: The study was run as part of the student researchers' research for their Masters, alongside completing clinical hours, which is a compulsory component for their studies.	The two available dates were mid-working week, which became difficult for the student researcher to liaise with their participants. Due to the selection of the times (8-10 am) the participants encountered challenges with arriving on-time. Seven of the 11 participants arrived between 5-20 minutes late
Collection of saliva samples	Difficulty collecting saliva from participant #2, #4, and #10.	It took nine minutes to collect and fill two cryotubes with saliva for participant #5 at TP1. It took between 5-7 minutes to collect saliva from participants #4 and #10 at TP1. Participant #4 had a coffee at 6:30 am that morning.	Participants who could not produce saliva as easily appeared more stressed and agitated. Not producing enough saliva may have caused participants to feel tense, further increasing stress levels.

Timepoint = TP

Collective assessment of all parameters from all participants

The means between Timepoint 1 and Timepoint 2, Timepoint 3 and Timepoint 4, and between Timepoint 1 and Timepoint 4 were assessed. When calculating the means of PSS-10 scores for each Timepoint (TP), the data from eight participants (#3, #5, #6, #7, #8, #9, #10 and #11) were included. Analyses of POMS subscales and biomarker data included values from all eleven participants. A series of t-tests (paired two-tailed t-test) were performed to assess if there were any differences between tested Timepoints (i.e. between TP1 to TP2, TP2 to TP3 and TP1 to TP4) (see Table 4). The results from analyses of individual markers are discussed in the following paragraphs.

Table 4 - Mean differences of selected psychological and physiological stress markers at tested timepoints

	Comparison of psychological markers at timepoints (TP) 1, 2, 3 and 4	**N**	**t-statistic (t-value)**	**df**	**P-value (significant values)**	**Mean ± SD**
PSS-10	TP1 vs TP2	8	2.275	7	.057	
	TP2 vs TP3	8	2.297	7	.55	
	TP3 vs TP4	8	-.2.66	7	.798	
	TP1 vs TP4	8	2.712	7	.030*	TP1 24.38 ± 3.701 TP4: 20.13 ± 5.027
POMS - Anger	TP1 vs TP2	11	3.200	10	.009*	TP1 5.82 ± 2.32 TP2 4.36 ± 1.29
	TP2 vs TP3	11	-1.405	10	.190	
	TP3 vs TP4	11	.764	10	.194	
	TP1 vs TP4	11	.727	10	.484	
POMS- Tense	TP1 vs TP2	11	2.366	10	.040*	TP1 8.18 ± 5.55 TP2 5.55 ± 3.45
	TP2 vs TP3	11	-.711	10	.493	
	TP3 vs TP4	11	1.753	10	.110	
	TP1 vs TP4	11	2.062	10	.066	

POMS Depression	TP1 vs TP2	11	3.125	10	.011*	TP1 6.18 ± 3.95 TP2 4.00 ± 2.05
	TP2 vs TP3	11	-.820	10	.432	
	TP3 vs TP4	11	-.959	10	.360	
	TP1 vs TP4	11	1.080	10	.305	
POMS - Fatigue	TP1 vs TP2	11	-3.115	10	.011*	TP1 4.36 ± 1.91 TP2: 7.36 ± 2.50
	TP2 vs TP3	11	-.599	10	.563	
	TP3 vs TP4	11	1.982	10	.076	
	TP1 vs TP4	11	-2.007	10	.073	
Cortisol	TP1 vs TP2	11	.252	10	.806	
	TP2 vs TP3	11	.706	10	.497	
	TP3 vs TP4	11	-1.217	10	.252	
	TP1 vs TP4	11	.065	10	.949	
IL-6	TP1 vs TP2	9	.945	8	.372	
	TP2 vs TP3	8	-.944	7	.377	
	TP3 vs TP4	10	-.264	9	.798	
	TP1 vs TP4	11	-.018	10	.986	
sIgA	TP1 vs TP2	11	1.315	10	.218	
	TP2 vs TP3	11	-.002	10	.998	
	TP3 vs TP4	11	-.923	10	.378	
	TP1 vs TP4	11	.023	10	.982	

PSS-10- Perceived Stress Scale; POMS- Profile of Mood States; Physiological biomarkers cortisol; IL-6; sIgA; TP1- timepoint 1, base level before session 1; TP2 - timepoint 2, post treatment, end of session 1; TP3 - timepoint 3, base level before session 2; TP4 - timepoint 4, post treatment, end of session 2; df- degrees of freedom; SD- standard deviation.
*: significant result at $p<0.05$

PSS-10 scores: a collective assessment from all participants

We conducted two paired two-tailed t-tests for two groups. Firstly, on the group of eight participants that scored >14 in initial PSS-10 (the three participants that scored <14 were excluded). Secondly, another two paired two-tailed t-test was conducted on the whole group (n=11), no participants excluded to determine if there was a difference. There were no significant differences for PSS-10 results across the two groups between timepoint TP1 and TP2, or between TP3 and TP4. However, significant differences were observed between TP1 and TP4 for both groups, which demonstrated decreases over time ($t(1)=2.712$, $p=.030$) and ($t(1)= 2.789$, $p=.019$) respectively (see Table 4 and Figure 4 and 5).

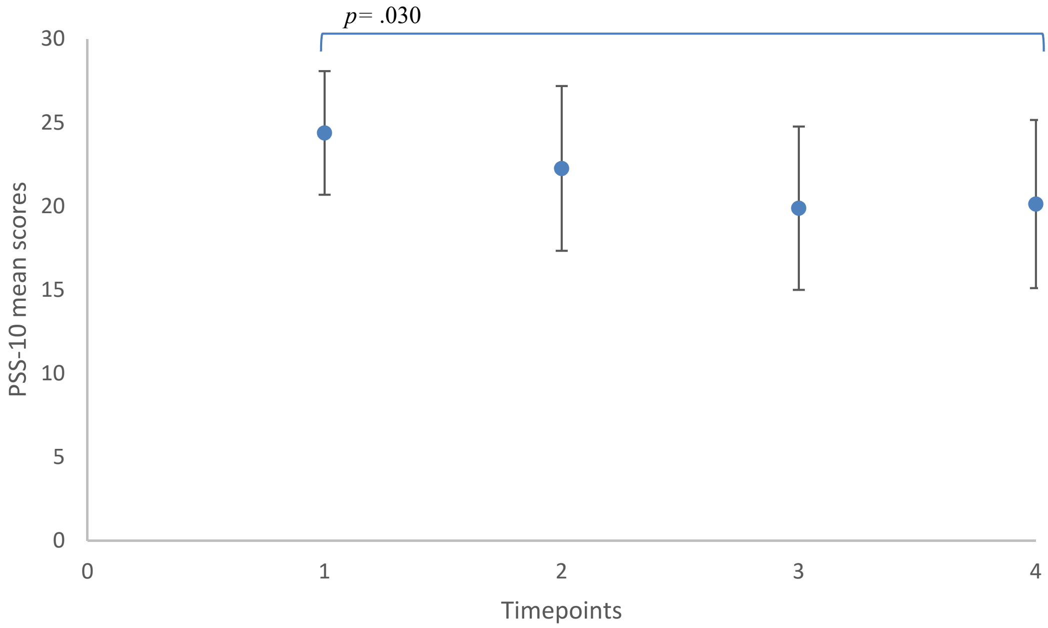

Figure 2 - Mean ± SD values for PSS-10 for eight participants that scored >14 in initial PSS-10

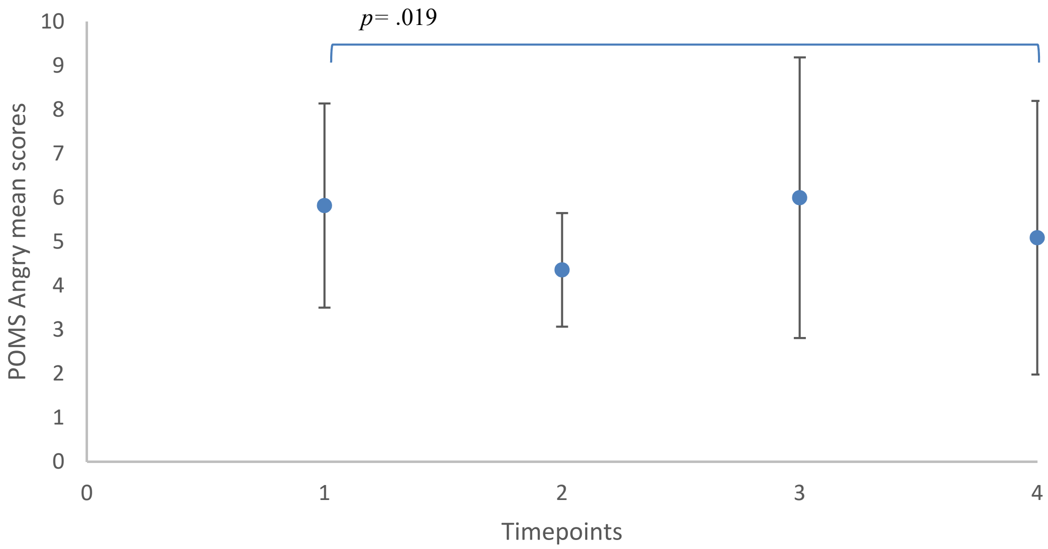

Figure 3 - Mean ± SD values for PSS-10 for all participants

Participants #1, #2 and #4 all scored ≤14 in the initial screening of PSS-10, which was below the score set in the inclusion criteria for eligibility. Therefore, two paired two-tailed t-tests were conducted, where results exhibited similar patterns of change for between TP1 and TP4. Therefore, the three that were originally excluded based on their initial ineligible PSS-10 scores were included in the overall paired two-tailed t-test (see figure 5). Inspection of the results from individual participants (see Figure 6) showed that eight of 11 participants (participants #1, #2, #3, #6, #7 #8, #9 and #11) demonstrated a gradual decrease in PSS-10 scores from TP1 to TP4. The overall average decrease was 25% for PSS-10 between these eight participants. A decrease in PSS-10 scores most commonly occurred between TP1 to TP2 and TP3 to TP4.

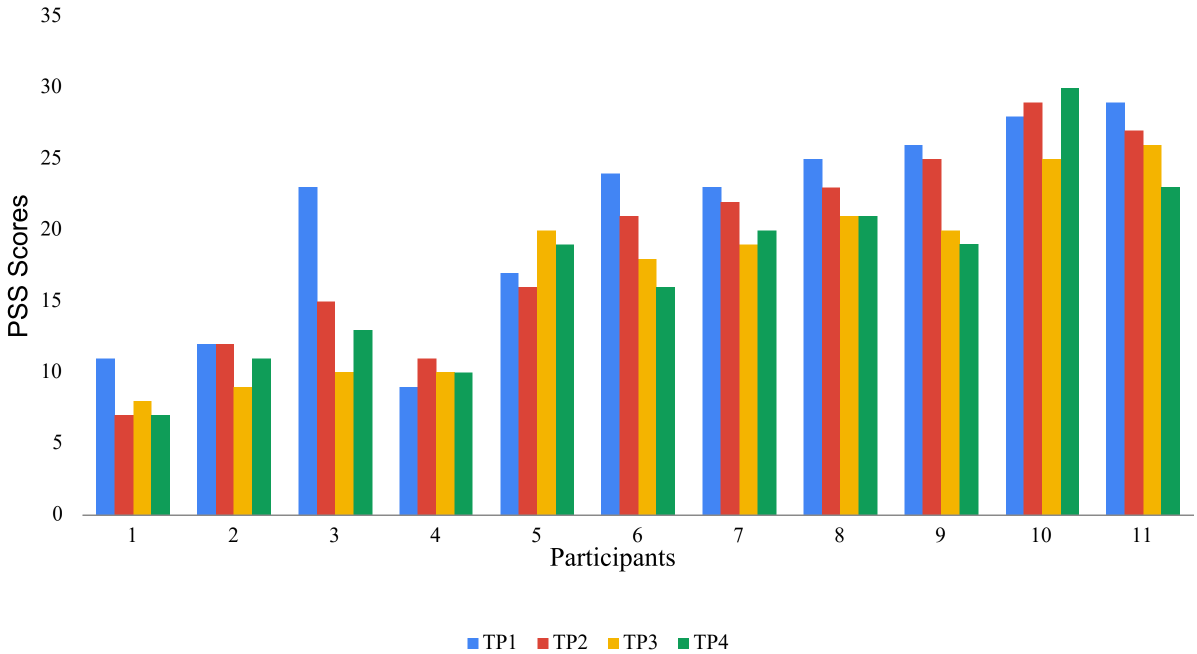

Figure 4 - Overall PSS-10 scores for all participants across all timepoints. PSS-10, Perceived Stress Scale-10; TP1 Timepoint 1, TP2, Timepoint 2; TP3, Timepoint 3; TP4, Timepoint 4.

POMS scores: a collective assessment from all participants

Due to a printing error, the full original 40-item POMS was not printed. Only 24-items were printed, which was only realised after the intervention. Therefore, the results should be viewed with caution at this stage. There was a general trend of decrease in the scores of the POMS Subscales Tense, Anger and Depression from TP1 to TP4. And a general trend of increase in POMS Fatigue. Attempts were made to assess the patterns of change in some of the subscales of POMS. As discussed in the methods section, total mood score was not calculated. The results are detailed in the following paragraphs.

POMS Subscale 'Anger'

There were no significant differences for the responses on the POMS Subscale 'Anger' across all participants between TP2 and TP3, between TP3 and TP4 and between TP1 and TP4. However, significant differences were observed showing decreases over time between TP1 and TP2, ($t(1)= 3.200$, $p=.009$) (see Figure 7 and Table 4).

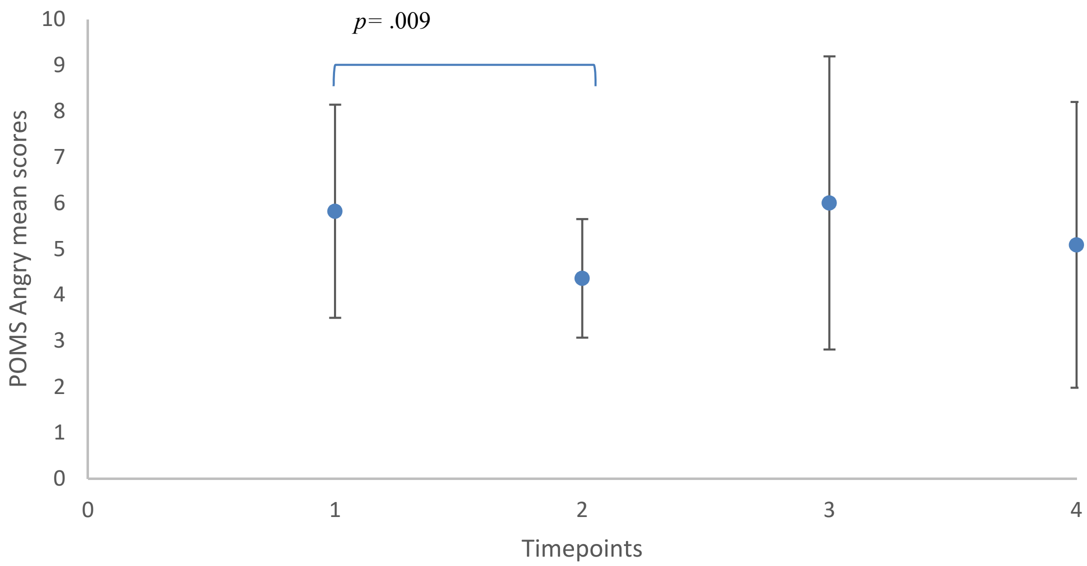

Figure 5 - Mean ± SD values for POMS Anger subscale for all participants from Timepoint 1 to Timepoint 4. SD standard deviation; POMS Anger, Profile Of Moods Anger subscale; TP1, Timepoint 1; TP2, Timepoint 2; TP3, Timepoint 3; TP4, Timepoint 4.

Inspection of the results from individual participants for the POMS Subscale 'Anger' showed that six of 11 participants (Participant #3, #4, #5, #6, #8 and #9) showed a trend of decrease in the values (ranging between 55% to 75% decrease) over the course of the intervention, from TP1 to TP4 (see Figure 8).

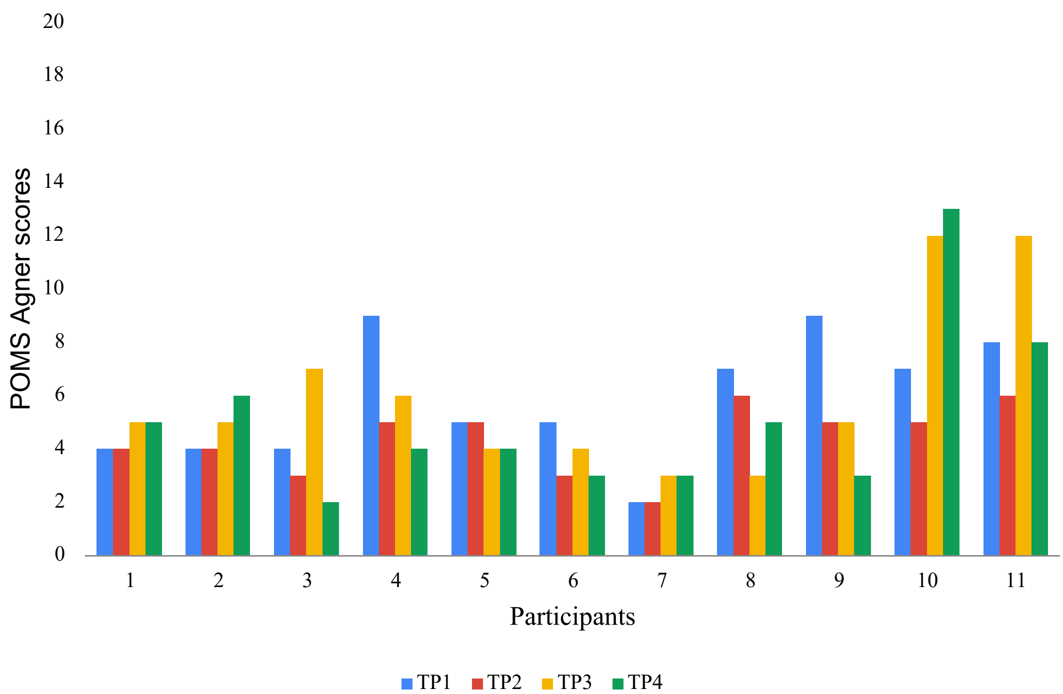

Figure 6 - Overall POMS Anger scores for all participants. POMS, Profile Of Moods State; TP1, Timepoint 1, TP2, Timepoint 2; TP3, Timepoint 3; TP4, Timepoint 4.

POMS Subscale 'Depression'

Mean scores of the POMS 'Depression' subscale from all participants showed a significant difference between TP1 and TP2, showing a decrease over time ($t(1)= 3.125$, $p=.011$). No other significant differences were observed at other timepoints (see Figure 9 and Table 4).

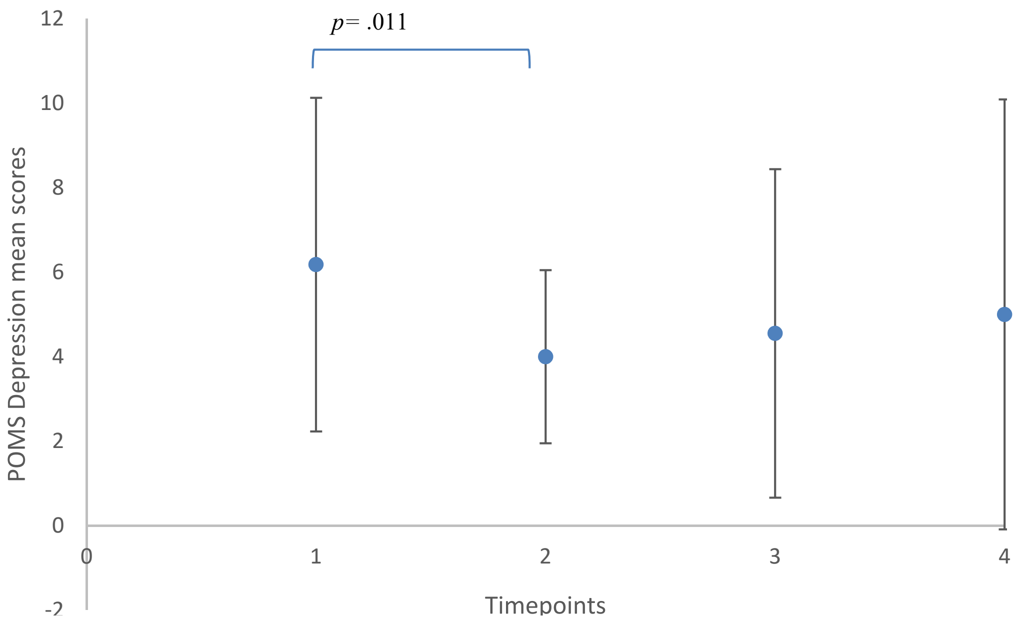

Figure 7 - Mean ± SD values for POMS Depression for all participants from Timepoint 1 to Timepoint 4. SD, Standard deviation; POMS Depression, Profile Of Moods Depression subscale; TP1, Timepoint 1; TP2, Timepoint 2; TP3, Timepoint 3; TP4, Timepoint 4

Inspection of the results from individual participants for the POMS 'Depression' subscale indicated that six participants (#2, #3, #6, #7, #9 and #11) demonstrated a trend of decrease in values at different levels (ranging between 33% to 67%) (see Figure 10). Four of the 11 participants (#4, #5, #8 and #10) showed increases (ranging from 30% to 400%) in scores from TP1 to TP4. Participant #1 did not show a noticeable change over the course of the intervention.

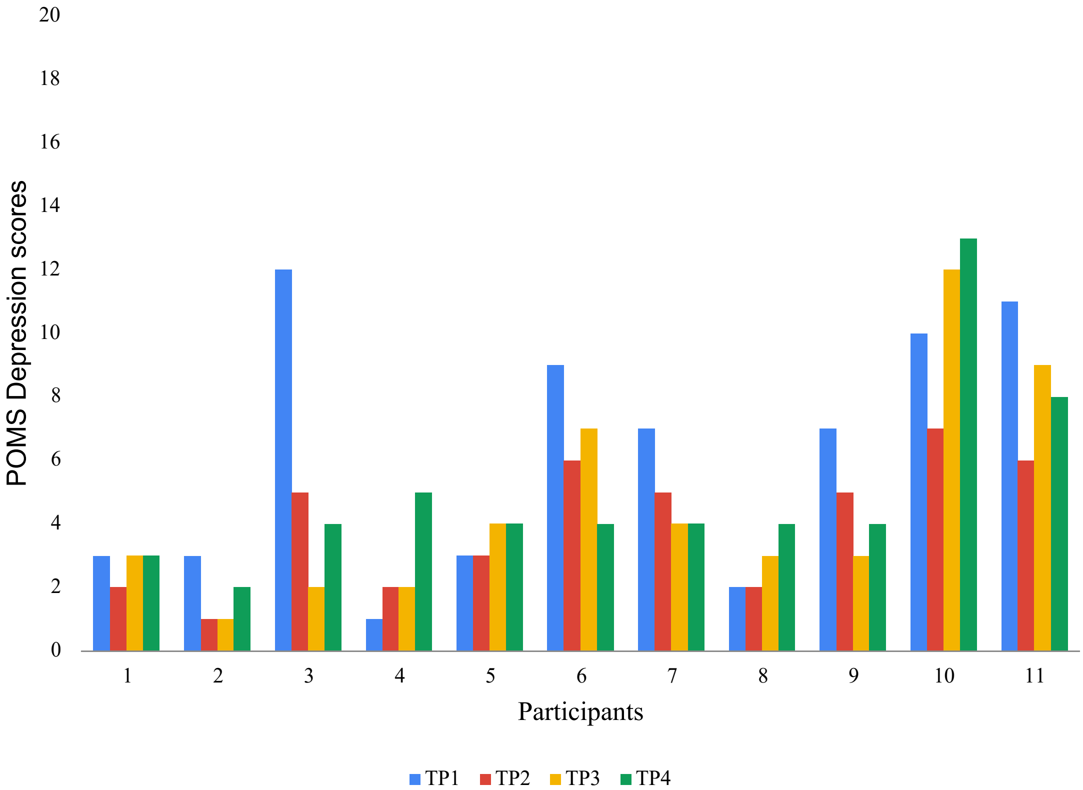

Figure 8 - Overall POMS Depression scores for all participant POMS, Profile Of Moods State; TP1, Timepoint 1, TP2, Timepoint 2; TP3, Timepoint 3; TP4, Timepoint 4.

POMS Subscale 'Fatigue'

The only significant difference observed in participants scores for POMS 'Fatigue' subscale was between TP1 and TP2, showing a decrease over time ($t(1)= -3.115$, $p=.011$) (see Figure 11 and Table 4). No other significant differences were observed at any other TP.

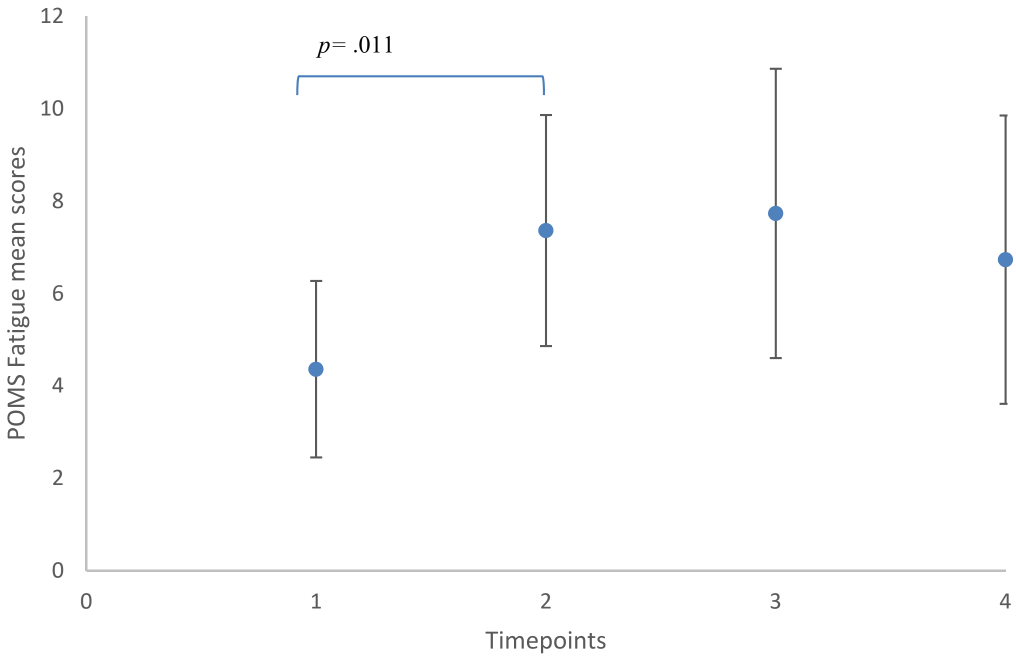

Figure 9 - Mean ± SD values for POMS Fatigue for all participants from Timepoint 1 to Timepoint 4. SD, Standard deviation; POMS, Profile of Mood States; TP1, Timepoint 1; TP2, Timepoint 2; TP3, Timepoint 3; TP4, Timepoint 4

Visual inspection of the results indicated that participants #2 and #4 demonstrated a trend of decrease in values at different levels for POMS 'Fatigue' scores. Participant #2 and #4 showed a decrease of 28% and 16% from TP1 to TP4, respectively (see Figure 12). Seven of the 11 participants displayed a trend of increase in values at different levels from TP1 to TP4. Of these participants, participant #7 showed a 600% increase and participant #10 showed a 300% increase from TP1 to TP4. Eight participants (#1, #3, #5, #6, #8, #9 and #11) rated their fatigue levels as the highest for both TP2 and TP3 out of four timepoints during the intervention. Participants #9 and 10 had scores of >10 for 'Fatigue' related items, which contrasted to remaining participants scoring <10. Participant #1 scored <5 for Fatigue-related questions for each timepoint throughout the intervention.

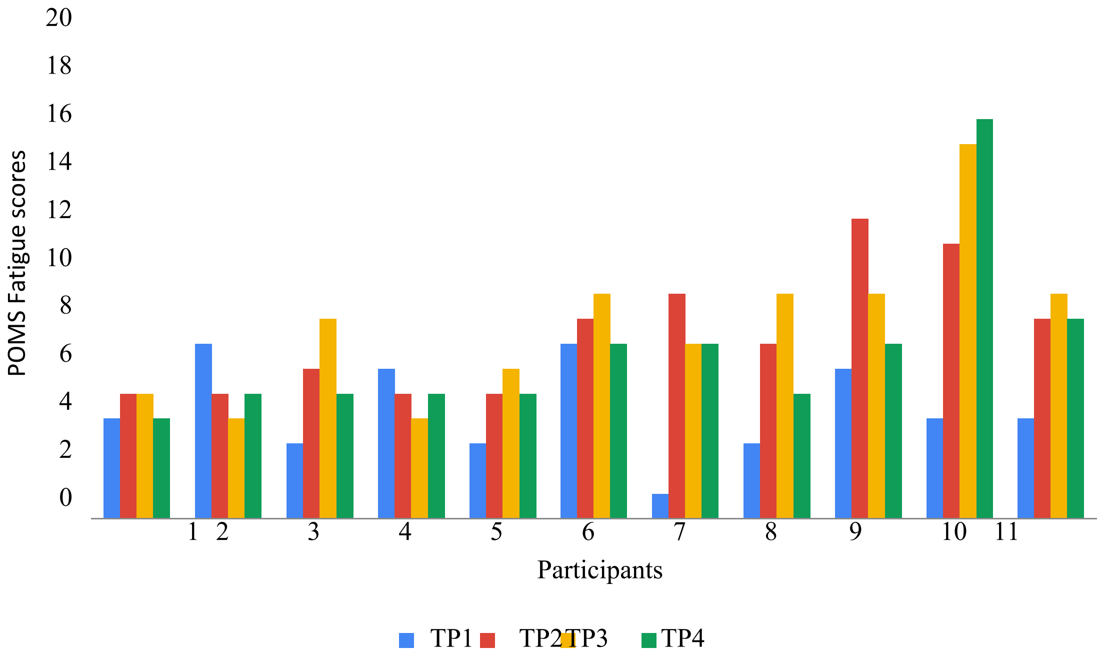

Figure 10 - Overall POMS Fatigue scores for all participants. POMS, Profile Of Moods State; TP1, Timepoint 1, TP2, Timepoint 2; TP3, Timepoint 3; TP4, Timepoint 4.

POMS Subscale 'Tense'

The only significant difference observed in participants scores for POMS 'Tense' subscale was between TP1 and TP2, showing a decrease over time ($t(1)= 2.366$, $p=.040$) (see Figure 13 and Table 4).

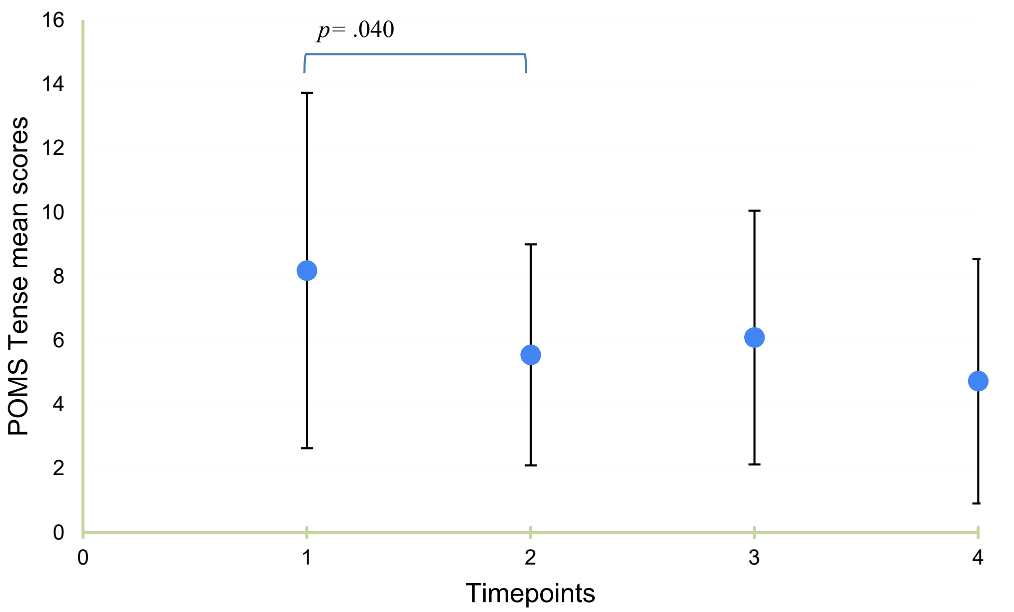

Figure 11 - Mean ± SD values for POMS Tense for all participants from Timepoint 1 to Timepoint 4. SD, standard deviation; POMS Tense, Profile Of Moods Tense subscale; TP1, Timepoint 1; TP2, Timepoint 2; TP3, Timepoint 3; TP4, Timepoint 4

Inspection of the results from individual participants for the POMS 'Tense' subscale show seven of 11 participants demonstrate a decreasing trend in values at different levels from TP1 to TP4 (see Figure 14). Participant #2 showed a 100% increase and #10 showed a 71% increase in POMS scores for the subscale 'Tense' from TP1 to TP4. Participants #4 and #5 showed little or no changes in scores over the course of the intervention. Participants #3, #6 and #9 all showed marked decreases in 'Tense' related scores between TP1 to TP2 and between TP3 to TP4, these were scores taken pre-treatment (TP1 and TP3) and post-treatment (TP2 and TP4).

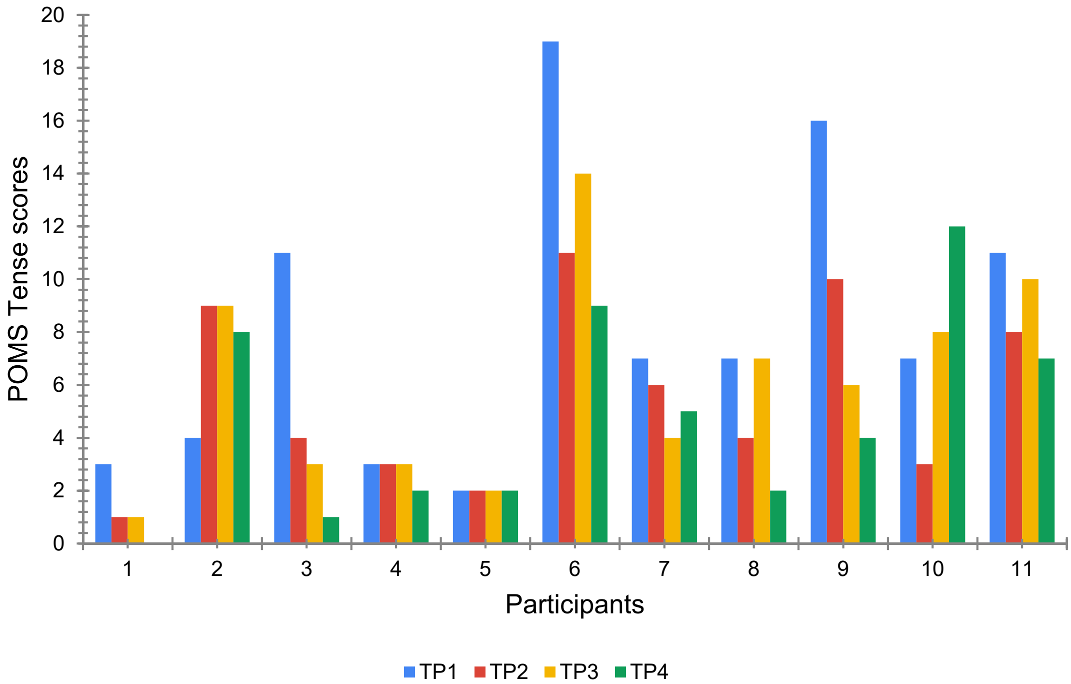

Figure 12 - Overall POMS Tense scores for all participants. POMS, Profile of Moods State; TP1, Timepoint 1, TP2, Timepoint 2; TP3, Timepoint 3; TP4, Timepoint 4.

Physiological biomarkers: a collective assessment from all participants

No significant differences were observed between levels of biomarkers (i.e., cortisol, IL-6 and Ig-A) assessed at Timepoints 1, 2, 3 or 4 (all t-values <1.4, P-values >0.260) (see Figures 15, 16, 17 and Table 4). However, an inspection of the results from individual participants reveals that cortisol levels of four individuals' (participants #2, #4, #8 and #9) showed an average decreasing trend from TP1 to TP4 (see Figure 18). Similarly, three participants (#5, #10 and #11) displayed a decrease in their IL-6 levels from TP1 to TP4 (see Figure 19). An overall decreasing trend in values can be seen from TP1 through to TP4 for sIgA levels (see Figure 20). Despite this, there was no significant trend or pattern in results observed across all participants for all assessed biomarkers.

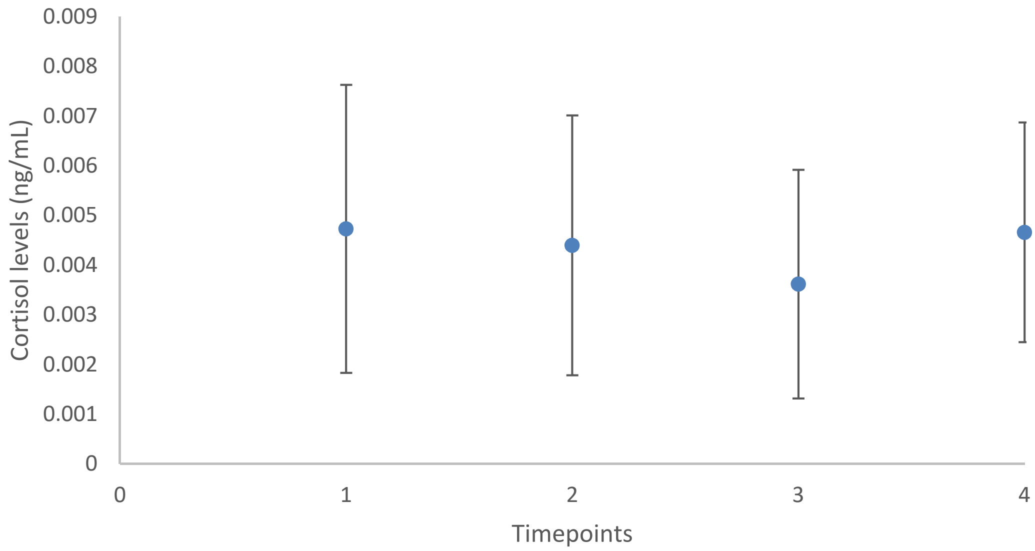

Figure 14 - Mean ± SD values for Cortisol levels for all participants from Timepoint 1 to Timepoint 4. SD, standard deviation; TP1, Timepoint 1, TP2, Timepoint 2; TP3, Timepoint 3; TP4, Timepoint 4.

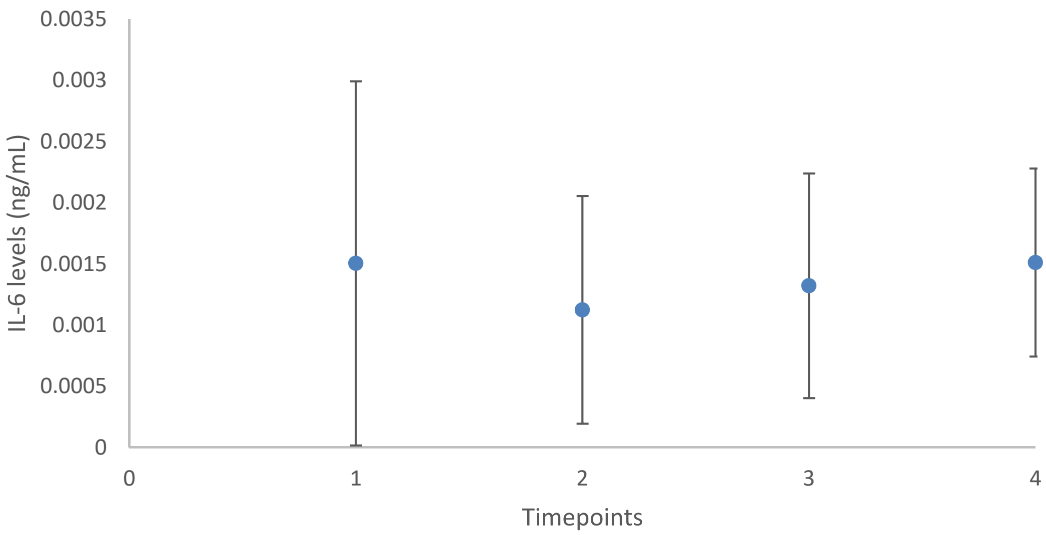

Figure 13 - Mean ± SD values for IL-6 levels for all participants from Timepoint 1 to Timepoint 4. SD, standard deviation; TP1, Timepoint 1, TP2, Timepoint 2; TP3, Timepoint 3; TP4, Timepoint 4.

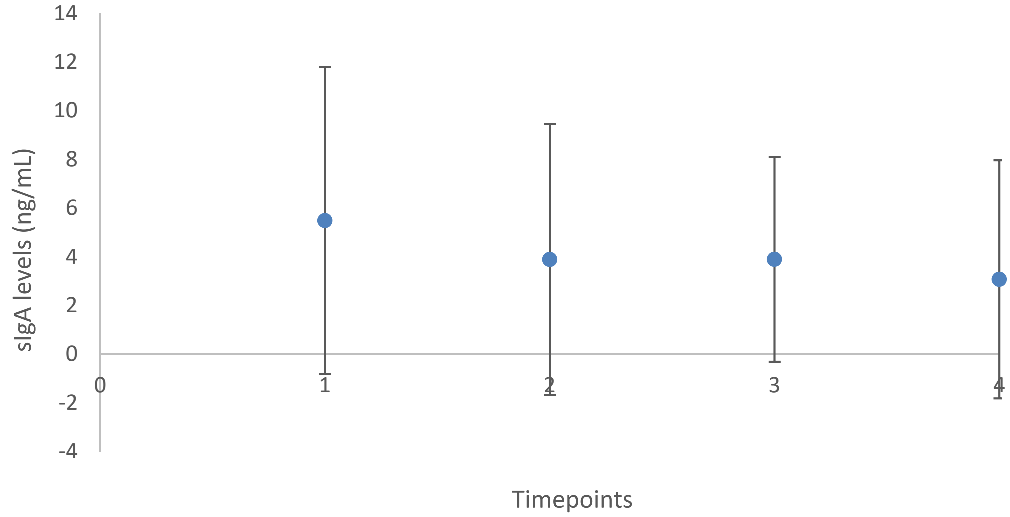

Figure 15 - Mean ± SD values for sIgA levels for all participants from Timepoint 1 to Timepoint 4. SD, standard deviation; TP1, Timepoint 1, TP2, Timepoint 2; TP3, Timepoint 3; TP4, Timepoint 4.

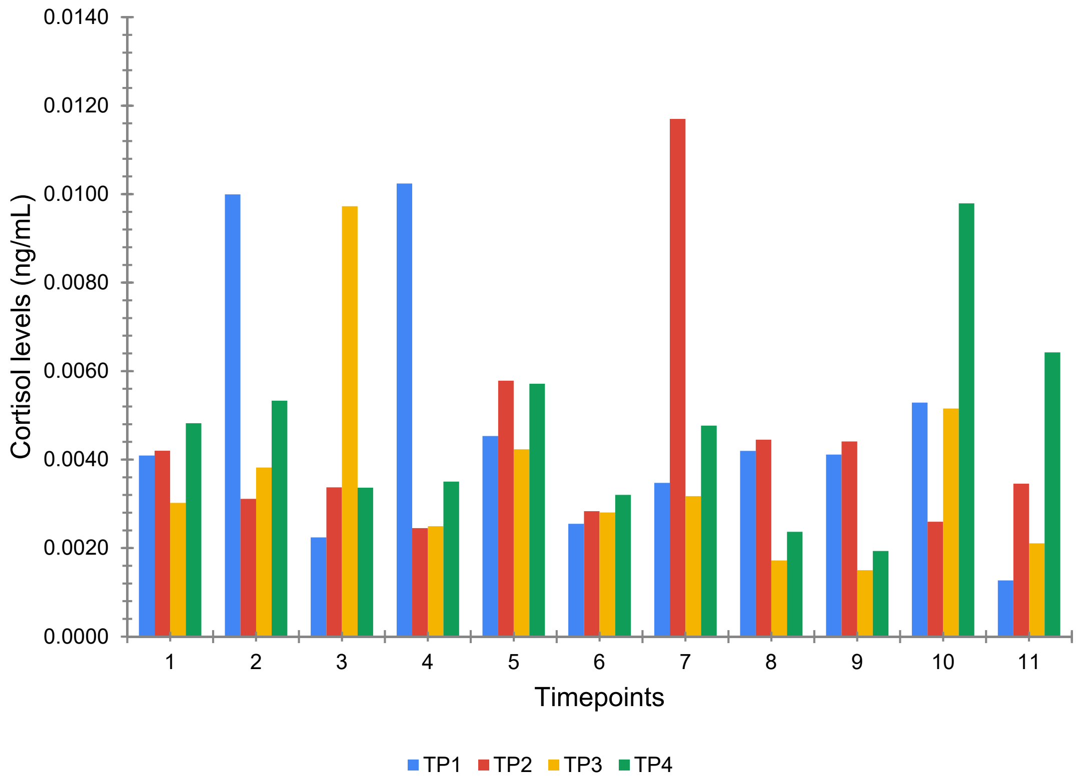

Figure 16 - Graph presenting salivary cortisol levels from individual participants across all timepoints

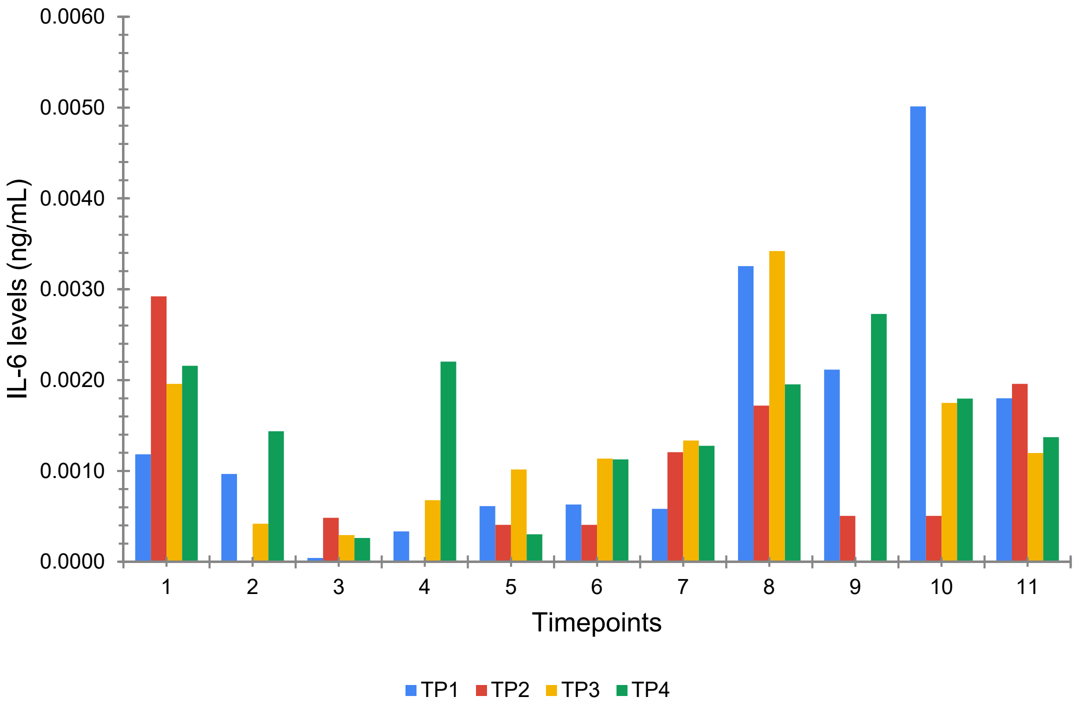

Figure 17 - Graph presenting salivary IL-6 levels from individual participants across all timepoints

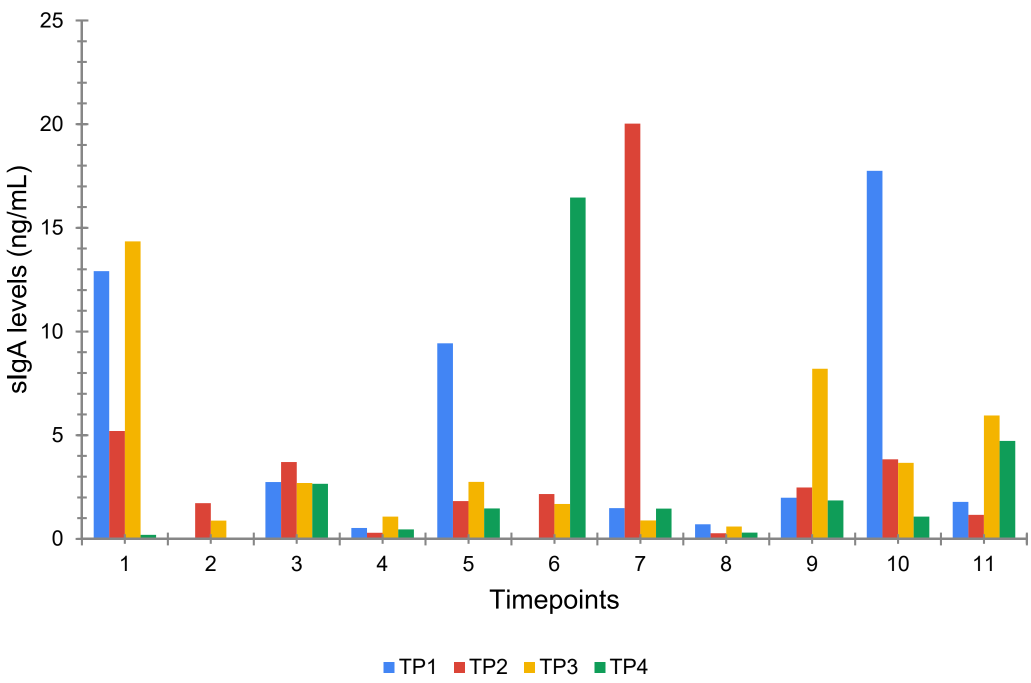

Figure 18 - Graph presenting salivary IL-6 levels from individual participants across all timepoints

Associations of psychological and physiological markers: individual results

Participant #1

Participant #1 was 31 years old, single and had normal BP (122/82 mmHg). She was one of the three participants that scored <14 in the initial PSS-10 (see Table 1). Participant #1 showed an overall 37% decrease in PSS-10 scores from TP1 to TP4. A moderate decrease of 67% was observed for POMS Tense subscale from TP1 to TP2, a 100% reduction observed overall. POMS Anger subscale scores slightly increased (from 4 to 5) between TP2 and TP3 and remained elevated for TP4. No particular patterns of change were observed in the levels of cortisol over the course of the intervention. Comparatively, a 98% decrease in sIgA levels was observed over the course of the intervention with a notable 60% decrease from TP1 to TP2 and a moderate 99% decrease from TP3 to TP4. The IL-6 levels for this participant increased after the first intervention and remained elevated after a slight drop between TP2 and TP3.

Looking at the overall picture, biomarkers did not show any relationship with psychological markers. There was no particular pattern of change over the course of the intervention. Similarly, POMS Depression and Fatigue subscales did not show any change or pattern, while the Anger subscale increased slightly. With regard to associations seen among various parameters over the course of the intervention, PSS-10 and POMS Tense showed a decreasing trend over the course of the intervention, with POMS Tense showing marked change from TP1 to TP4 (see Figure 21).

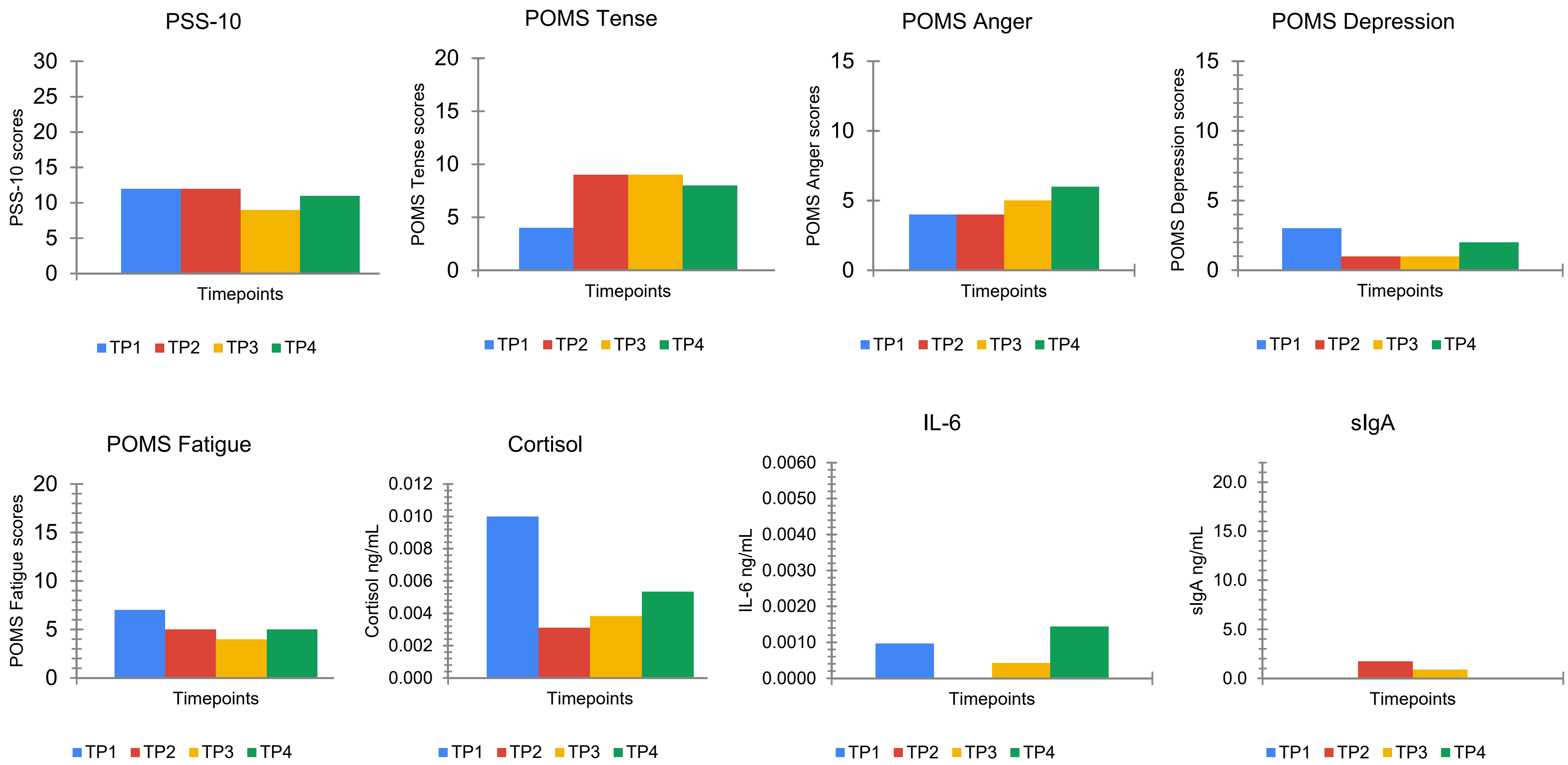

Figure 19 - Panel presenting the results from all variables for participant #1 including missing data recorded for IL-6 TP2 and sIgA TP1 and TP4. TP1, Timepoint 1; TP2, Timepoint 2; TP3, Timepoint 3; TP4, Timepoint 4; sIgA, Secretory Immunoglobin A; IL-6, Interleukin 6.

Participant #2

Participant #2 was 22 years old, in a relationship, had normal blood pressure (111/69mmHg) and a BMI of 25. 2kg/m². She was one of the three participants that scored <14 in the initial PSS-10 (Table 1). No changes were observed for participant #2 in PSS-10 scores over the course of the intervention. An increase of 100% for the POMS Tense subscale related items from TP1 to TP4 was observed for this participant. No changes were seen between TP1 and TP2 for POMS Anger subscale items. However, a 20% increase was noted from TP2 to TP4. POMS Depression related items decreased by 33% from TP1 to TP4. A decreasing trend of 29% was observed in scores for Fatigue-related questions from TP1 to TP4. Cortisol levels showed a decreasing trend of 47% from TP1 to TP4. Comparatively, IL-6 levels increased by 49% from beginning to the end of the intervention. There was not enough sample to analyse this participant's TP2 IL-6 and TP1 and TP4 sIgA samples, therefore the data recorded for these two timepoints was 0. However, a decrease of 49% was noted in sIgA levels between TP2 and TP3.

Overall, cortisol showed similar trends to POMS Depression and POMS Fatigue scores, with the two lowest scores and biomarker levels being recorded at TP2 and TP3. There was no particular pattern of change observed over the course of the therapy. Similarly, PSS-10 did not show any change. With regard to associations seen among various parameters over the course of the intervention POMS Depression, Fatigue and cortisol showed a similar pattern of an overall decrease in their scores/levels from TP1 to TP4 (see Figure 22).

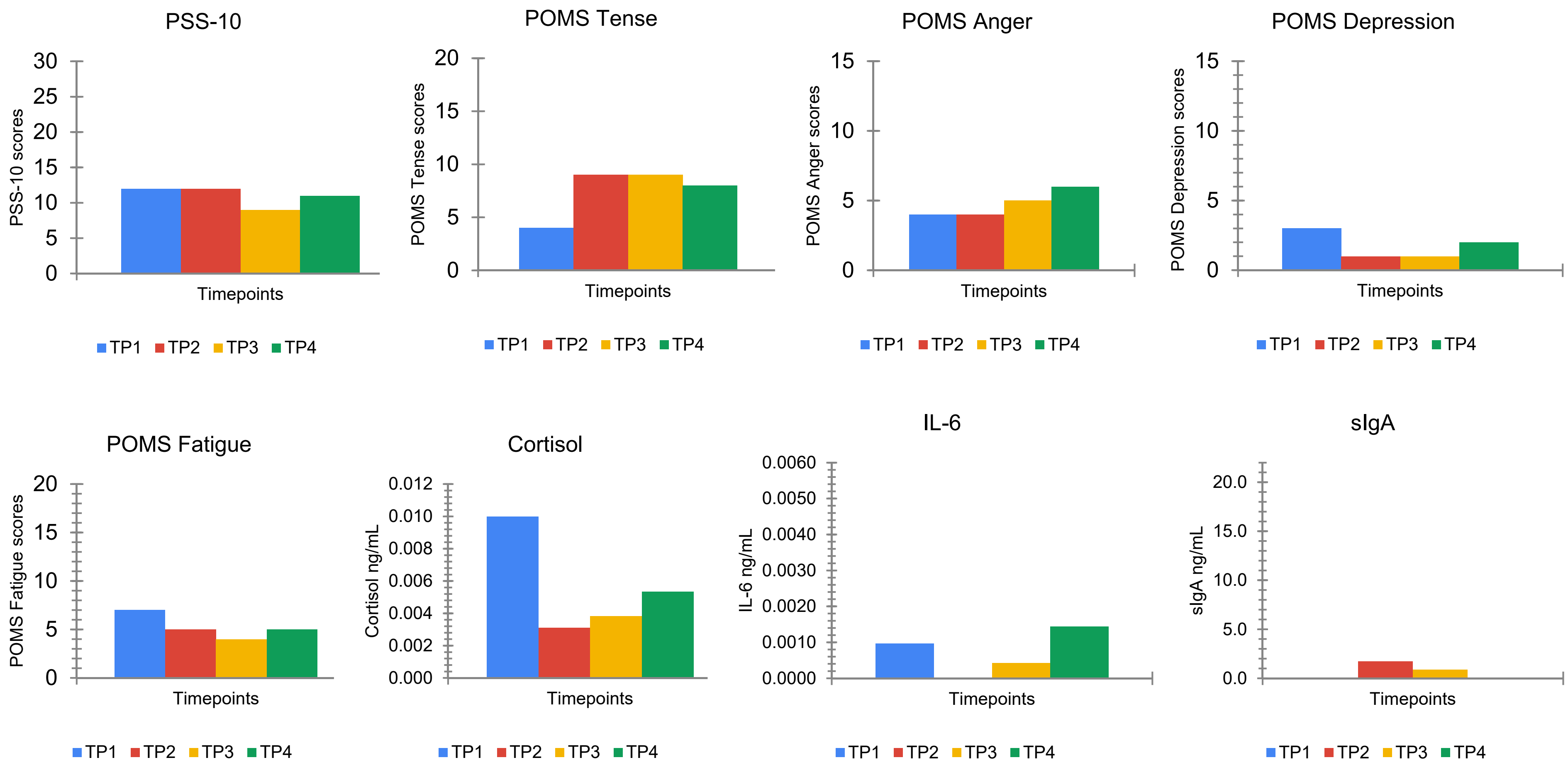

Figure 20 - Panel presenting the results from all variables for participant #2 including missing data recorded for IL-6 TP2 and sIgA TP1 and TP4. TP1, Timepoint 1; TP2, Timepoint 2; TP3, Timepoint 3; TP4, Timepoint 4; sIgA, Secretory Immunoglobin A; IL-6, Interleukin 6.

Participant #3

Participant #3 was 23 years old, in a relationship, had normal blood pressure (112/68mmHg) and a BMI of 22. 6kg/m². Participant #3 was taking non-steroidal medication for a current fungal infection and also reported suffering tension-type headaches (see Table 1). A 43% decrease in PSS-10 scores from TP1 to TP4 was observed. An overall decrease of 91% was observed from TP1 to TP4 in Tense related items, with a moderate 64% decrease between TP3 to TP4. Between TP1 to TP2 a 25% decrease was observed for Anger related items and a further 71% decrease from TP3 to TP4. Thus, an overall 50% decrease from TP1 to TP4 for Anger was shown. Depression related items decreased by 67% from TP1 to TP4. Cortisol and IL-6 levels showed an increasing trend (50% increase in cortisol; 524% increase in IL-6 from TP1 to TP4). Overall, no significant changes were observed in sIgA levels over the course of the intervention.

Overall, there were no observed relationships between the biomarkers with the psychological markers. There were no particular patterns of change observed with the biomarkers over the course of the intervention. POMS Fatigue subscale seemed to display a trend of increase in scores over the course of the intervention. With regard to associations seen amongst various parameters over the course of the intervention, PSS-10 and POMS Tense, Anger and Depression all displayed a similar pattern of an overall decrease in their scores from TP1 to TP4 (see Figure 23).

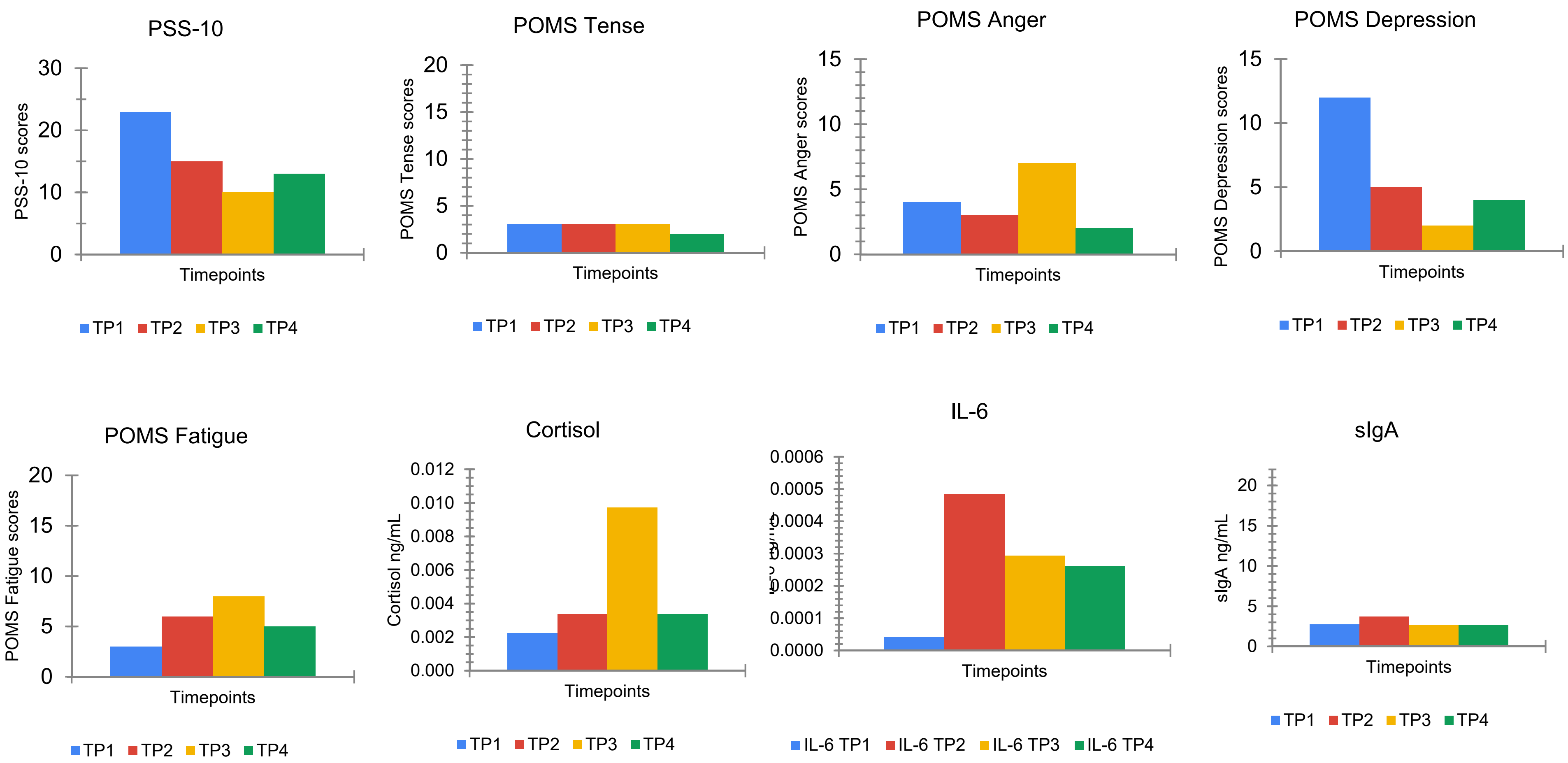

Figure 21 - Panel presenting the results from all variables for participant #3

Participant #4

Participant #4 was 27 years old, married, had normal blood pressure (110/74mmHg) and a BMI of 19.9kg/m². She was one of the three participants that scored <14 in the initial PSS-10. She also reported having consumed coffee at 6:30 am on the first day of the intervention (see Table 1). There were no obvious changes in PSS-10 scores for participant #4 over the course of the intervention. Similarly, there were no changes in Tense related items from TP1 to TP3 (score remained 3). However, a 33% decrease from TP1 and TP3 to TP4 was observed. An overall 56% decrease was noted for Anger related items from TP1 to TP4, with a 44% decrease from TP1 to TP2 and a 33% decrease from TP3 to TP4. Depression related scores increased by 400% from TP1 to TP4, whereas Fatigue decreased by 17% from TP1 to TP4. Cortisol levels appeared to decrease by 66% over the course of the intervention. Similarly, sIgA decreased by 13% from TP1 to TP4. However, IL-6 appeared to increase by 560% over the course of the intervention. Cortisol was the only biomarker to show similarities with regards to decreasing trends in POMS Tense and Anger scores from TP1 to TP4.

However, the remaining biomarkers did not show any relationship with the psychological parameters. With regard to associations seen amongst various parameters over the course of the intervention, POMS Anger and Fatigue showed a trend of decreasing scores from TP1 to TP4 (see Figure 24).

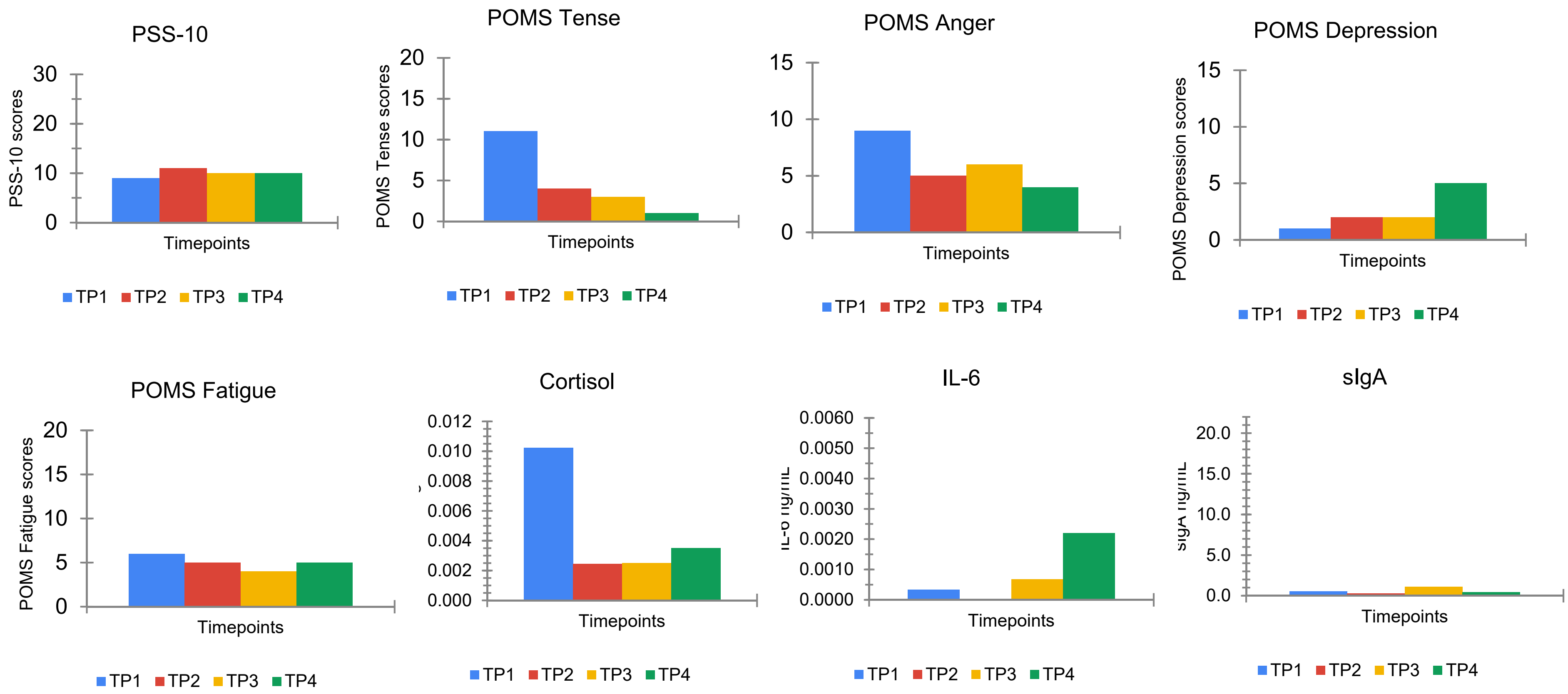

Figure 22 - Panel presenting the results from all variables for participant #4 including missing data recorded for IL-6 TP2. TP1, Timepoint 1; TP2, Timepoint 2; TP3, Timepoint 3; TP4, Timepoint 4; IL-6, Interleukin 6.

Participant #5

Participant #5 was 51 years old, married, had normal blood pressure (118/84mmHg) and a BMI of 20.7kg/m². Participant #5 was receiving non-steroidal medication for a fungal infection (see Table 1). Slight changes were observed for participant #5 in PSS-10. No changes were noted in Tense related items. A general decreasing trend was observed from TP1 to TP4 in Anger related items, with the most significant decrease occurring between TP1 and TP2 (44%) and overall between TP1 and TP4 (56%). Depression and Fatigue scores showed increases from beginning to end of the intervention (overall 33% for Depression and 67% for Fatigue respectively between TP1 and TP4). Cortisol levels appeared to increase by 26% from TP1 to TP4, whereas IL-6 levels showed a 51% decrease and an 84% decrease in sIgA levels from TP1 to TP4.

Overall, no relationship was observed between the physiological and psychological markers. With regard to associations seen among various tested markers over the course of the intervention, IL-6, sIgA biomarker levels and POMS Anger showed a similar pattern of an overall decrease in their scores/levels from TP1 to TP4 (see Figure 25).

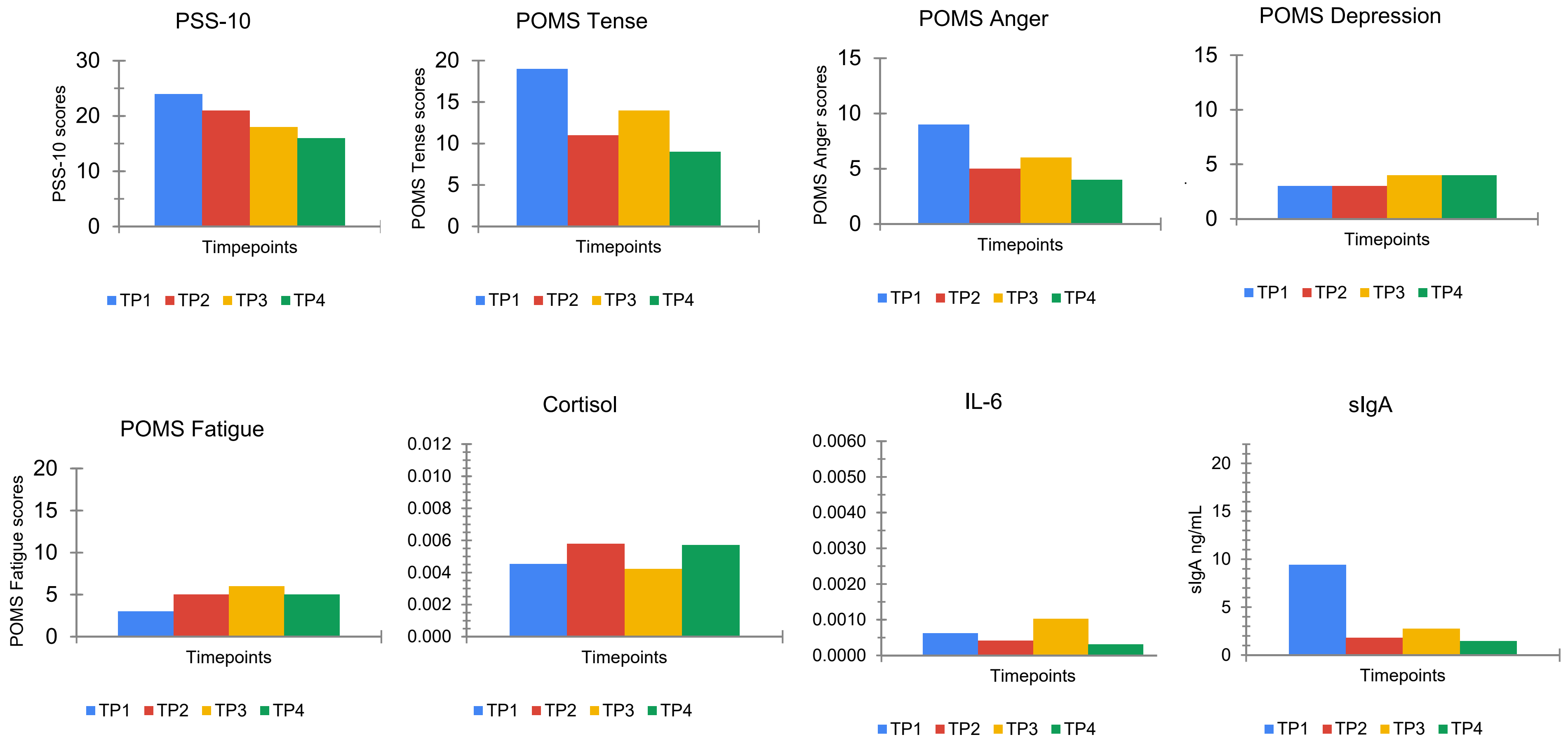

Figure 23 - Panel presenting the results from all variables for participant #5

Participant #6

Participant #6 was 58 years old, married, had normal blood pressure (118/89mmHg) and a BMI of 24.9kg/m². This participant was caring for her life partner who had a terminal illness (see Table 1). Participant #6 showed a gradual 33% decrease in PSS-10 scores from TP1 through to TP4. Upon visual analysis of all POMS subscales; Tense, Anger and Depression subscales showed general decreasing trends from beginning to end of the intervention. Tense displayed an overall 53% decrease; Anger displayed a 40% decrease and Depression displayed a decrease of 56%, all from TP1 to TP4. Fatigue scores did not display any notable changes from TP1 to TP4. Biomarker levels showed an increasing trend across all timepoints. Cortisol increased moderately by 26%, IL-6 increased by 79%. There was not enough saliva sample left to analyse participant #6's TP1, therefore the data recorded for this timepoint was 0. An increase of 878% was noted between TP3 and TP4.

Overall, no relationship was observed between the physiological and psychological markers. With regard to associations seen among various tested markers over the course of the intervention, PSS-10, POMS Tense, Anger and Depression subscales displayed patterns of decreasing scores over the course of the intervention (see Figure 26).

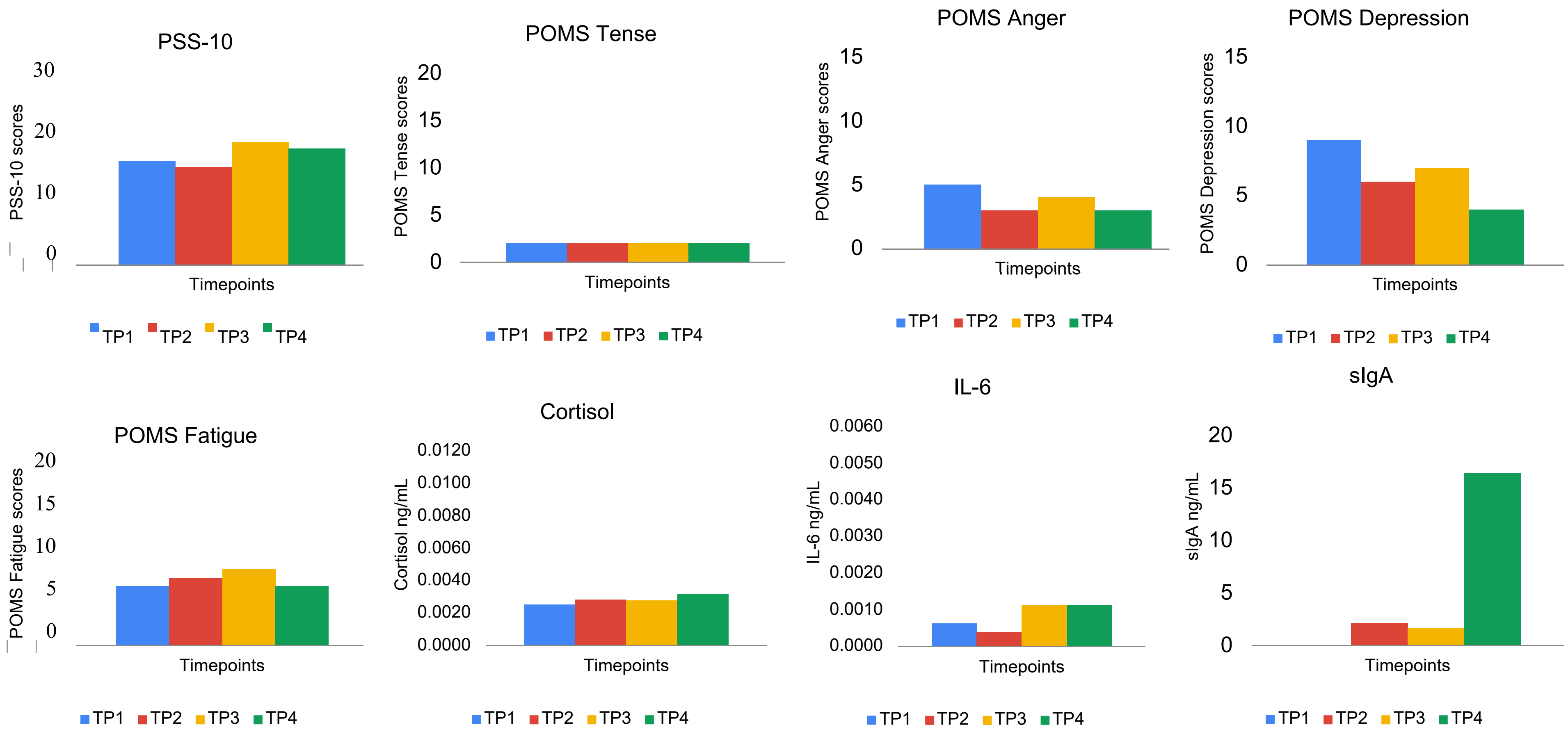

Figure 24 - Panel presenting the results from all variables for participant #6 including missing data recorded for sIgA TP1. TP1, Timepoint 1; TP2, Timepoint 2; TP3, Timepoint 3; TP4, Timepoint 4; sIgA, Secretory immunoglobulin A

Participant #7

Participant #7 was 21 years old, single, had normal blood pressure (116/80mmHg) and a BMI of 23.5kg/m². Only slight changes in PSS-10 were noted from TP1 to TP4 for PSS-10. An overall 29% decrease was observed from TP1 to TP4 for Tense related items, with a 14% decrease between TP1 to TP2 and a subsequent 14% increase from TP3 to TP4. Overall there was a 50% increase in POMS Anger related items from TP1 to TP4, with no changes in scores between TP1+TP2 and TP3+TP4, the increase occurred between TP2 and TP3. Depression related items decreased by 43% from TP1 to TP4, contrasting to the 600% increase in Fatigue subscale. Cortisol levels increased by 37% from TP1 to TP4 and IL-6 showed an increasing trend from TP1 to TP4. No changes in sIgA levels between TP1, TP3 and TP4 were observed. The recorded TP2 levels need to be further investigated as there may have been a practical error resulting in the recorded amount.

Overall, no relationship was observed between the physiological and psychological markers. With regard to associations seen amongst various parameters over the course of the intervention, PSS-10, POMS Tense and Depression all displayed a similar pattern of an overall decrease in their scores from TP1 to TP4 (see Figure 27).

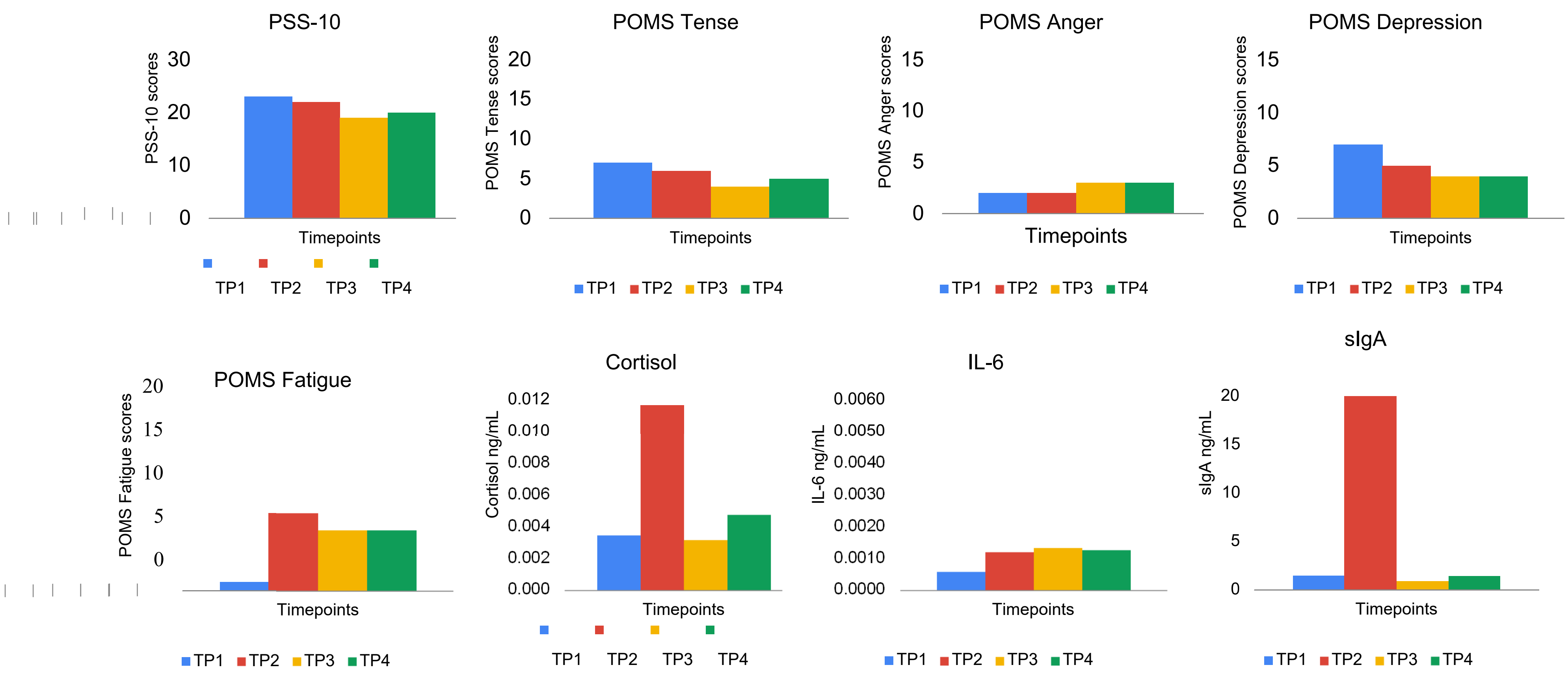

Figure 25 - Panel presenting the results from all variables for participant #7

Participant #8

Participant #8 was 28 years old, married, had normal blood pressure (110/75mmHg) and a BMI of 26.4kg/m² (see Table 1). There was a total 16% decrease in PSS-10 scores was shown, with an 8% decrease from TP1 to TP2. A 43% decrease from TP1 to TP2 and an overall 71% decrease for Tense related items were observed. The Anger subscale score decreased by 14% from TP1 to TP2 and increased by 67% from TP3 to TP4. However, an overall 29% decrease for Anger was observed from TP1 to TP4. Depression showed an increasing trend of 50% from TP1 to TP4 and Fatigue subscale also showed an increasing trend of 200% from TP1 to TP3, but an overall 67% increase from TP1 to TP4. Cortisol levels decreased by 44%, sIgA levels decreased by 67% and IL-6 levels decreased by 40% from TP1 to TP4.

Looking at the overall picture, biomarkers did not show any relationship with psychological markers. There was no particular pattern of change over the course of the intervention. POMS Depression and Fatigue subscales showed slight increases. With regard to associations seen amongst various parameters over the course of the intervention, cortisol, IL-6, sIgA, PSS-10, POMS Tense and Anger subscales all displayed a similar pattern of an overall decrease in their scores from TP1 to TP4 (see Figure 28).

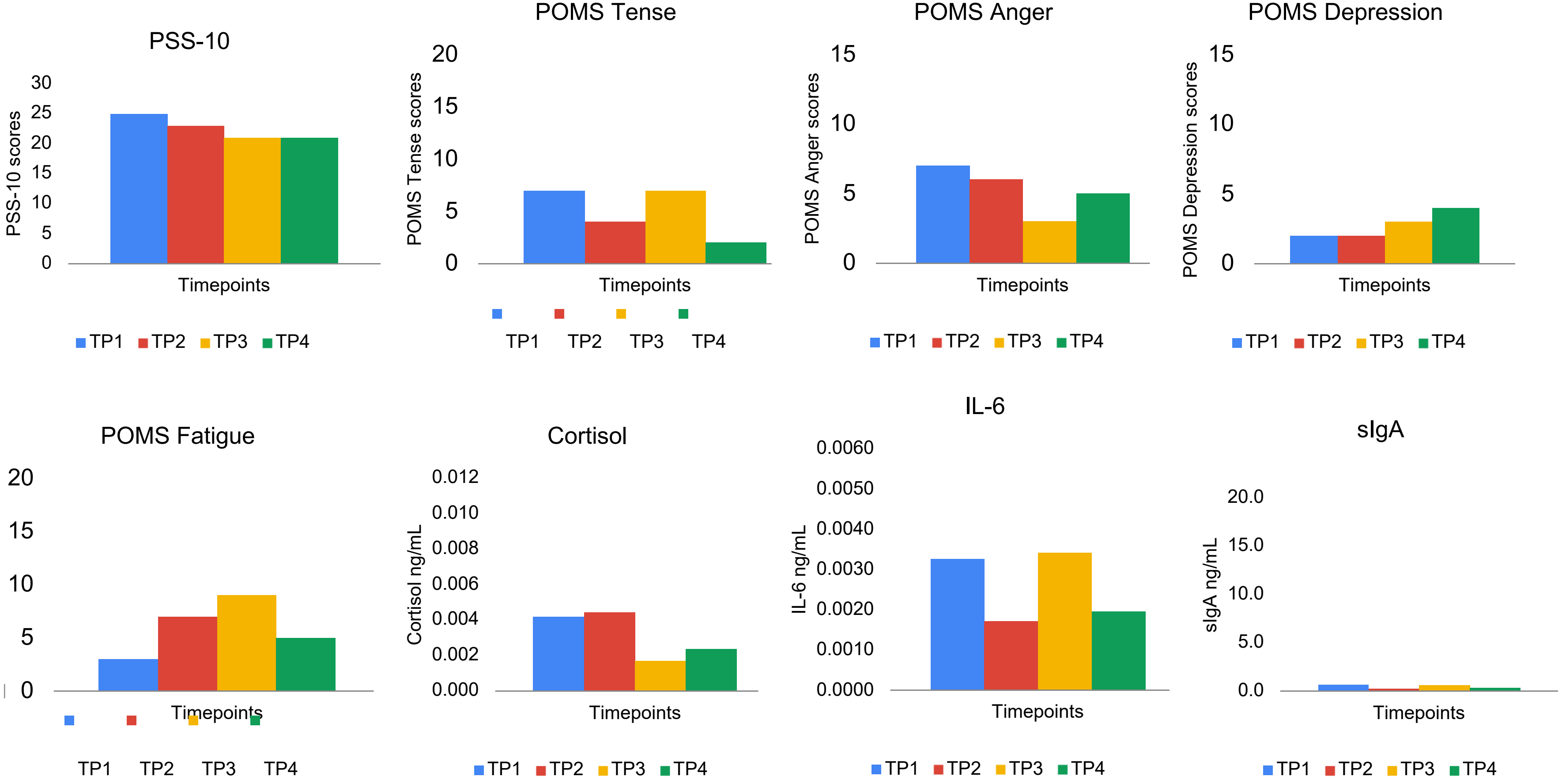

Figure 26 - Panel presenting the results from all variables for participant #8

Participant #9

Participant #9 was 28 years old, married, had normal blood pressure (120/81mmHg) and a BMI of 22.6kg/m² (see Table 1). This participant displayed a decreasing trend observed in the PSS-10 and POMS subscales excluding Fatigue, which appeared to increase over the course of the intervention. Additionally, cortisol and sIgA showed decreasing trends across the intervention. Participant #9 showed a gradual 27% decrease in PSS-10 scores from TP1 to TP4. For POMS subscales, a decreasing trend for all subscales except fatigue was noted. Tense subscale decreased by 75% over the course of the intervention with a modest 37% decrease between TP1 and TP2. Anger subscale decreased gradually by 67% from TP1 to TP4, with a 44% decrease between TP1 and TP2 and a 40% decrease between TP3 and TP4. Depression items decreased by 43%, on the contrary, Fatigue increased by 17% from TP1 to TP4. Notably, changes were observed in cortisol and sIgA, which displayed decreases by 53% and 7%, respectively from TP1 to TP4. There was not enough sample to analyse participant #9's TP3 IL-6 levels, therefore the data recorded for this timepoint was 0. However, IL-6 levels increased by 29% from TP1 to TP4.

Overall, there were no observed relationships between the biomarkers with the psychological markers. There were no particular patterns of change observed with the biomarkers over the course of the intervention. With regard to associations seen amongst various parameters over the course of the intervention, PSS-10 and POMS Tense, Anger and Depression all displayed a similar pattern of an overall decrease in their scores from TP1 to TP4 (see Figure 29).

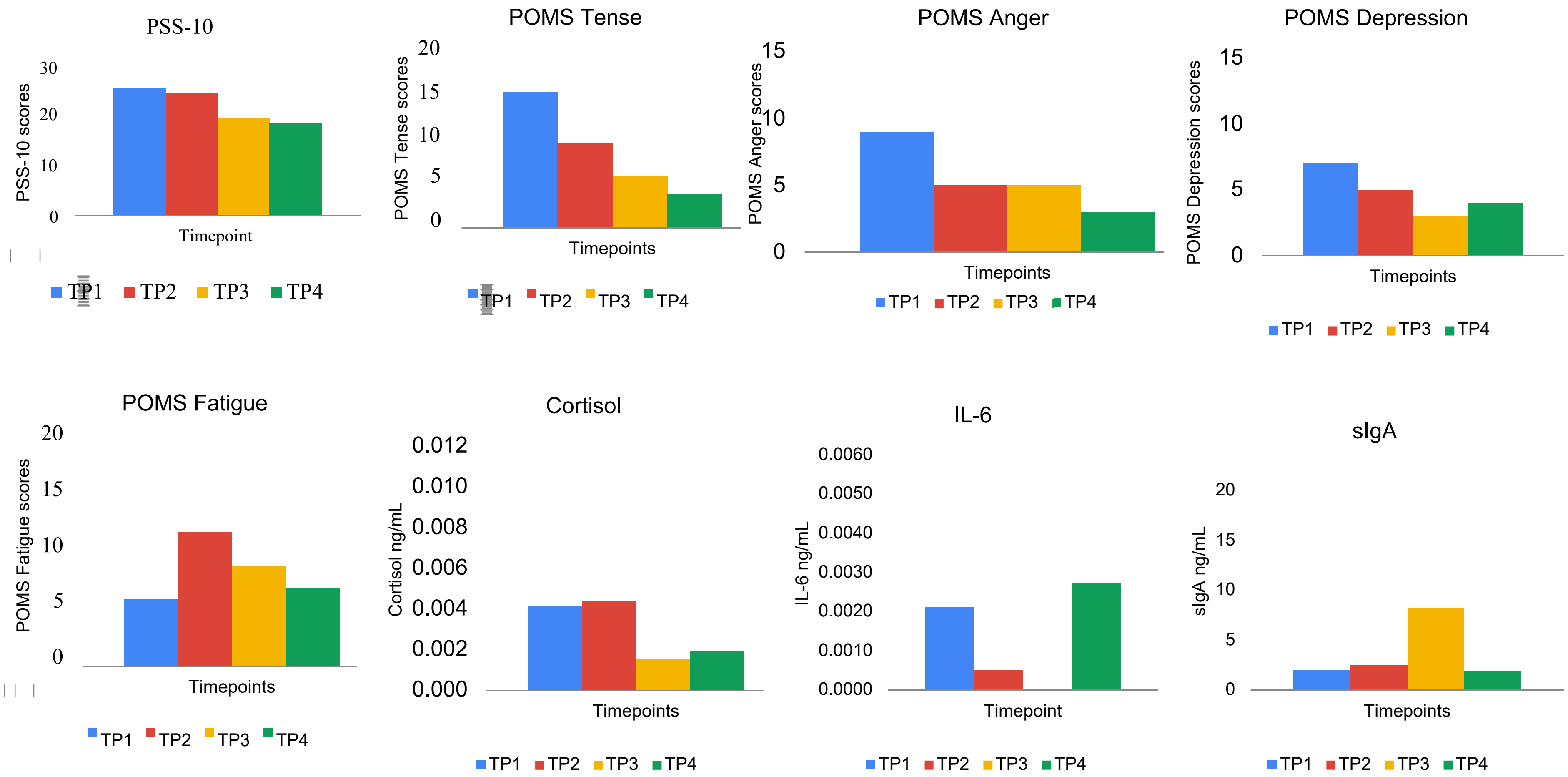

Figure 27 - Panel presenting the results from all variables for participant #9 including missing data recorded for IL-6 TP3. TP1, Timepoint 1; TP2, Timepoint 2; TP3, Timepoint 3; TP4, Timepoint 4. Missing data recorded for IL-6 TP3

Participant #10

Participant #10 was 53 years old, divorced, had normal blood pressure (117/77mmHg) and a BMI of 26.4kg/m². This participant suffered from headaches and migraines, which they said was managed with medication (see Table 1). This participant showed very little change in PSS-10 scores from TP1 to TP4, only a slight increase could be seen. There was a moderate increasing trend in this participants' Fatigue subscale from TP1 to TP4. Tense related items increased by 71% from TP1 to TP4, despite there being a 57% decrease between TP1 and TP2. An 85% increase was observed for Anger scores from TP1 to TP4, with a 29% decrease between TP1 and TP2 and an 8% increase between TP3 and TP4. For the Depression subscale, participant #10 showed a 30% increase from TP1 to TP4. However, between TP1 and TP2 a 30% decrease was noted. One of the highest overall scores for each timepoint for Fatigue was shown by this participant out of all the participants. Fatigue scores increased by 300% from TP1 to TP4. Cortisol levels increased by 46% from TP1 to TP4. In contrast, IL-6 had an overall decrease of 61%, with a 90% decrease from TP1 to TP2. sIgA also displayed a decrease of 94% from TP1 to TP4.

Looking at the overall picture, biomarkers did not show any relationship with psychological markers. There was no particular pattern of change over the course of the intervention. However, POMS Tense, Anger, Depression and Fatigue subscales showed an increasing trend over the course of the intervention. Similarly, slight increases were seen in PSS-10 scores (see Figure 30).

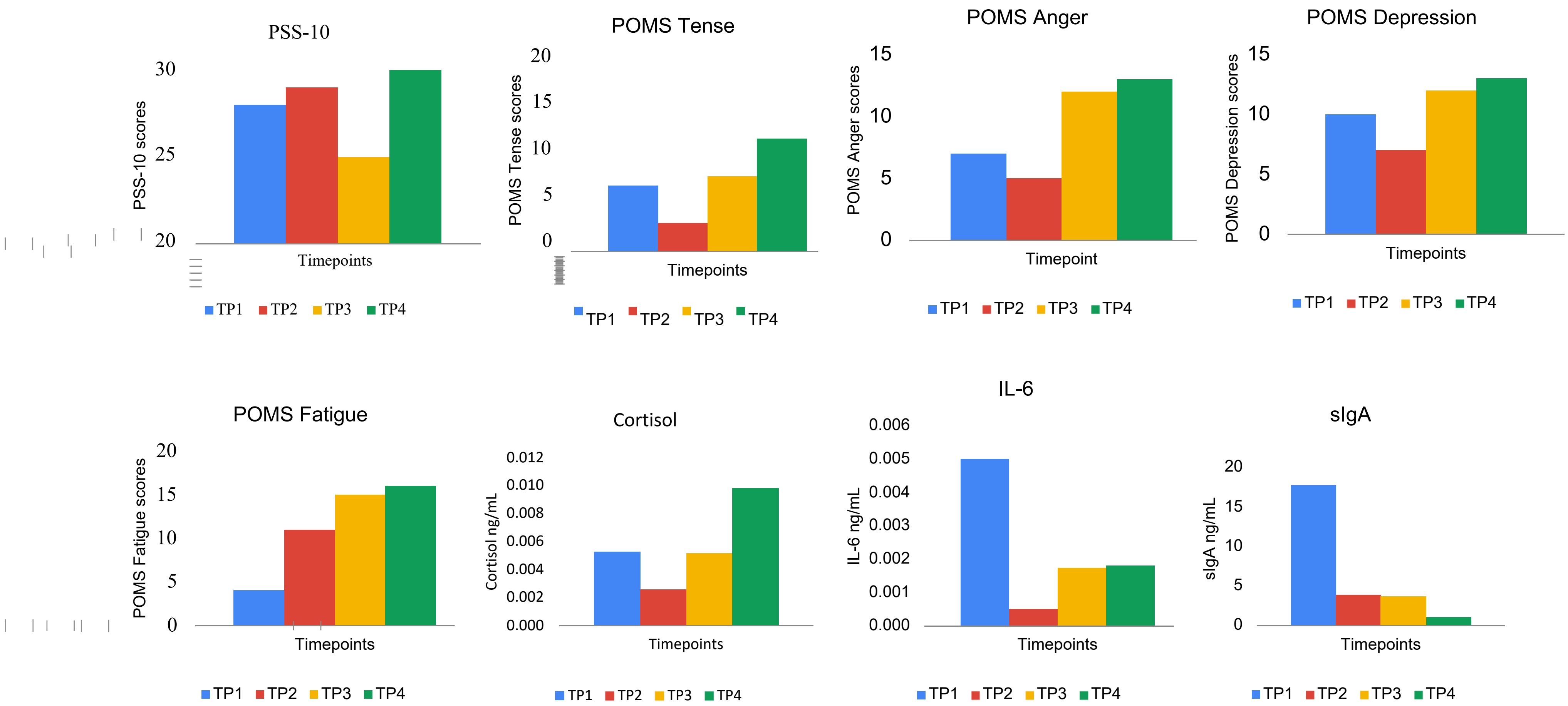

Figure 28 - Panel presenting the results from all variables for participant #10

Participant #11

Participant #11 was 29 years old, single, had high blood pressure (130/87mmHg) and a high BMI of 37.2kg/m². This participant suffered from mild exercise-induced asthma (see Table 1). This participant showed a modest 7% decrease in PSS-10 scores from TP1 to TP2, a further 12% decrease from TP3 to TP4, resulting in an overall 21% decrease from TP1 to TP4. Tense subscale showed an overall 36% decrease from TP1 to TP4. A 25% decrease in Anger subscale was observed from TP1 to TP2 and a 33% decrease between TP3 and TP4. However, no differences in scores from TP1 and TP4 were observed. Depression subscale appeared to decrease by 45% from TP1 to TP2, and an overall 27% decrease from TP1 to TP4. Fatigue increased by 100% from TP1 to TP4. A moderate change was observed in cortisol and sIgA levels from TP1 to TP4 (406% and 165% increases respectively). Whereas, IL-6 displayed a 24% decrease from TP1 to TP4.

Looking at the overall picture, biomarkers did not show any relationship with psychological markers. There was no particular pattern of change over the course of the intervention. Similarly, POMS Anger subscales did not show any change or pattern, while the Tense and Depression subscales decreased slightly. Fatigue showed slight increases from beginning to end of the intervention (see Figure 31).

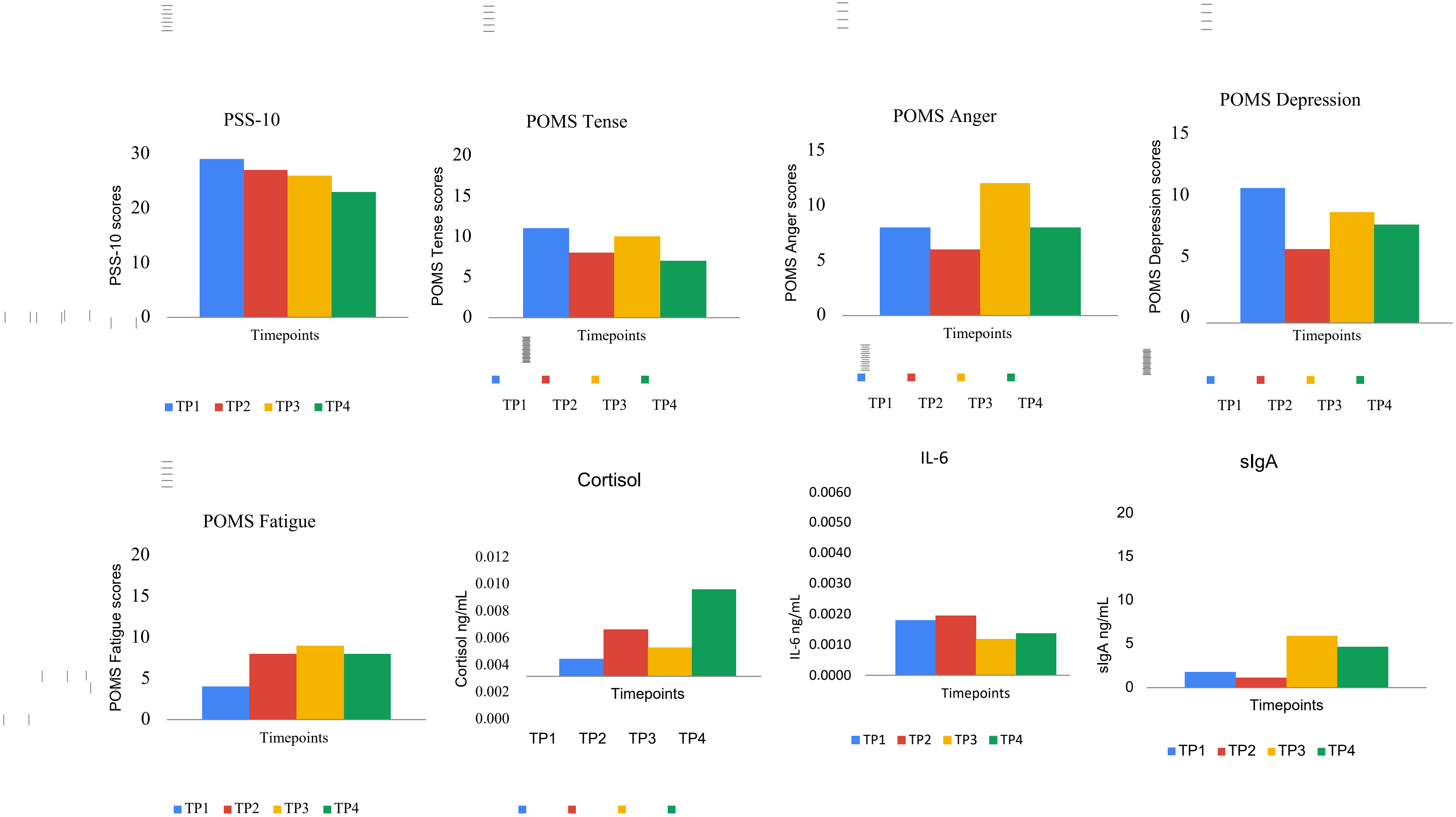

Figure 29 - Panel presenting the results from all variables for participant #11

Salient observations:

Therapy application

- No adverse effects were reported by any participants. The majority of the participants found the therapy package and techniques used "relaxing" and felt sleepy after treatment. Eight of the eleven participants commented that the education package (pamphlet) related to stress management was helpful and insightful.
- Challenges experienced included; timing of intervention, having two sessions on two consecutive days, only having two sessions in total and feasibility of timing. Eight of the eleven participants commented feeling stressed before the first and/or second session of the intervention due to stressors (traffic, running late to the session, worried about being late for work, dropping kids off at school).

Psychological and physiological parameters

- Eight out of eleven participants showed either a gradual or an overall decrease in PSS-10 scores over the course of the therapy application.
- Seven out of eleven participants showed either a gradual or an overall decrease in POMS Tense score over the course of the therapy application.
- In seven participants decreased PSS-10 scores were associated with decreased scores for POMS Tense subscale, four of which (57%) were also associated with decreased scores for POMS Anger subscale. In five participants, decreased PSS-10 scores were associated with decreased scores for POMS Anger, Tense and Depression subscales.
- The key observation for POMS Fatigue subscale was that seven out of eleven participants showed either a gradual or overall increase in POMS Fatigue subscale over the course of the therapy, despite all participants commenting feeling relaxed after the therapy package.
- With regard to observing any association or relationship between assessed markers, no clear relationship or pattern was noted between perceived and physiological markers. In four participants, decreased cortisol was associated with decreased scores for PSS-10 and POMS Tense. In three participants, IL-6 concentrations increased, which was associated with decreased POMS Anger scores. In three participants, sIgA concentrations decreased, which was associated with decreased PSS-10 scores. However, readers should be cautious of the POMS results, which are only speculative due to the use of the incomplete questionnaire.

Chapter 6 – Discussion

Stress is a multifaceted and increasing phenomenon experienced globally and at different stages in an individual's life. Due to the multifactorial nature of stress and its impact on multiple aspects of health, there needs to be a multimodal approach in managing and treating stress. There is a lack of existing research on the application of osteopathic techniques in managing stress. Therefore, the objective of this present pilot study was to assess the feasibility of applying a therapy package on stressed women. The therapy package consisted of ten general osteopathic techniques (GOT) and 10-minutes of scripted stress management education, including providing participants with an educational pamphlet on stress. The assessment of the effect of the therapy package was achieved through measurements of two parameters, collected pre- and post-intervention. Firstly, this included self-reported measures of perceived (subjective) stress and differing mood states (Perceived Stress Scale-10, PSS-10; Profile of Mood Scale, POMS). Secondly, by measuring pre- and post-GOT intervention levels of three selected stress biomarkers in saliva (cortisol; secretory Immunoglobin A, sIgA; and Interleukin-6, IL-6). Overall, the main outcomes from the study were that the application of the therapy package was feasible in an osteopathic clinical setting and that a measurable decrease in the scores for the psychological parameters was observed. However, this was not reflected in the physiological parameters of the assessed biomarkers.

This chapter will provide an in-depth discussion of the feasibility of the application of the therapy package proposed in the current study. This includes a discussion into the recruitment process and selected times for the therapy sessions. Next, the application of the GOT techniques and the feasibility of GOT protocol will be determined. Following this, the administration of psychological questionnaires will be addressed, and the suitability of the selected biomarkers will be explored. Moreover, a discussion into whether these biomarkers can provide good representations of physiological stress levels will be provided. Later, the feasibility of the sample collection, handling and transportation will be further examined. Following this will be a review regarding any inter- and/or intra-relationships between the psychological and physiological parameters of stress assessed in this study. Finally, this chapter will summarise the limitations present in this study and provide readers with recommendations for future studies.

The recruitment process for this study was feasible. All women were enrolled within two months of showing interest (between January and February). The student researcher was inundated with many women showing interest in participating. As the location of the intervention was at Clinic 61, Unitec Institute of Technology, Mount Albert, Auckland Campus, most of the participants enrolled in this intervention were either studying or employed at Unitec Campus. Therefore, the location was more convenient for the participants. Additionally, the demographics of the 11 women enrolled (age range 21-54 years; mean age 31) presented with no noticeable medical conditions or other reported health issues. All participants had reasonable levels of stress, which was identified through the initial PSS-10.

The application of the therapy package over two consecutive days was feasible. The decision to administer this therapy package over two consecutive days was consistent with a double-blinded randomised control trial (RCT) published by Rogan et al. (2019). Researchers assessed the feasibility and effectiveness of thoracic spine mobilisation on the sympathetic and parasympathetic balance in a healthy population over two consecutive days (Rogan, Taeymans, Clarys, Clijsen, & Tal-Akabi, 2019). Researchers found the application of the treatment protocol feasible. Comparatively, another RCT assessed the feasibility of mindfulness-based coping with university life (Lynch et al., 2018). Although their programme was conducted over eight weeks, researchers concluded that the programme was feasible and successfully brought students mindful awareness to their academic work, stress management and health. The study by Lynch et al. (2018) included a face-to-face follow up assessment one-week post-intervention, which our study did not include, therefore we are unable to generalise the results. However, it does highlight the potential for future studies to conduct a study over a longer period, which implements a similar stress management programme as demonstrated in Lynch et al. (2018).

The application and deliverance of the therapy package were feasible. There were no issues with the application of the selected 10 GOT techniques. All participants enjoyed the GOT therapy application, reporting feeling sleepy and relaxed. There were no adverse effects or events noted by the researcher or reported by the participants and there were no dropouts recorded throughout the intervention. The student researcher had two years of clinical experience as it was a compulsory component to complete alongside their Masters' degree. They were trained by clinical tutors and the clinical advisor for this study until they were competent with delivering these GOT techniques in a flowing routine. There was an overall decrease in the psychological self-report measures of perceived stress and all participants reporting feeling "relaxed" after the GOT sessions. This is consistent with results in a study by Fornari et al. (2017), where researchers reported the effectiveness of GOT in reducing stress from observational data and patient feedback (Fornari et al., 2017). The student researcher of the current study being discussed noted a reduction in breathing rate during the application of the GOT techniques, which was deeper and slower for all participants. This is consistent with results in a study by Emmet et al. (2018), which indicated that GOT can reduce breathing rate (Nuño et al., 2019). It was also noted in our present study that participants reported feeling relaxed after the GOT sessions, which is consistent with findings in a study by Dugailly et al. (2014). Researchers in that study assessed the effects of GOT using soft tissue techniques to reduce stress, anxiety and have an overall body relaxation effect. They found that a short term, 30-minute session was sufficient to reduce the anxiety level in participants (Wiegand et al., 2015). Even though their study needed further investigation to confirm their results and to broaden the scope, their study could be compared with the women assessed in our study, based on the sex of the group, and a similar sample size of the population assessed (n=10 per control group). From those results, it appears that a 30-minute session could be practical and effective, thus, we decided to use a 30-minute treatment. Readers need to note, that existing clinical trials assessing the effects of GOT have generally been small in sample size. Therefore, results indicating that GOT can decrease sympathetic tone is low or displays indifferent results (Henderson et al., 2010; Henley et al., 2008; Rechberger et al., 2019; Wieting et al., 2013). Small sample size limits both statistical analysis and validity, making it challenging for researchers to generalise the effects of GOT to the wider population (Fornari et al., 2017; Henley et al., 2008; Wiegand et al., 2015).

In our current study, the application of the stress education package also proved to be feasible and beneficial for the participants. It was easy for the student researcher to administer and replicate consistently for each participant as it followed a scripted education. Subjective feedback suggested that the stress management and education component was helpful and informative. Participants reported feeling more comfortable and able to manage a stressful situation when confronted by one. These positive, restorative and helpfulness effects that stress management education provides, has been well documented in stress research (Lynch et al., 2018; Romano, 1992; Ruwaard et al., 2012; Varvogli & Darviri, 2011). In addition to the scripted education in our study, participants were also provided with a pamphlet. This pamphlet was based on the Brief-Cognitive Behavioural Education programme B-CBE programme, which aims to provide psychoeducation based on cognitive-behavioural management strategies for individuals dealing with stress (Chiang et al., 2016; Substance Abuse and Mental Health Services Administration, 2012). Many positive comments were made from the participants in our study regarding B-CBE. This offered participants tools with which to better identify/manage future stressors. In a study by Leung et al. (2011), researchers utilised the (B-CBE) programme for managing stress and anxiety of family caregivers of patients in intensive care units. Similar to the current study's results, researchers in that study also demonstrated the positive effects of the B-CBE on reducing stress, anxiety and depression and overall mental health (Leung et al., 2011). This was consistent with findings in a study by Gónzalez et al. (2016), where researchers aimed to implement the B-CBE psychological intervention, which focussed on improving the ability for dental students to cope with symptoms of anxiety, stress and depression. The results indicated that the participants showed improvements in their coping skills after the intervention. However, this study had some limitations, firstly it was a non-experimental study, therefore, causation could not be attributed to the intervention and generalisations could not be formed based on the small sample size. Secondly, their study was limited to dental students only, excluding other university students and/or the wider population of stressed individuals. Despite this, the authors conclude that the results were promising in that the intervention was both useful and necessary for dentistry students psychological well-being (Gónzalez & Quezada, 2016).

The positive effect of stress management education in reducing stress, as noted in our study, was also consistent with findings in a systematic review and meta-analysis by Regehr et al. (2013). Researchers examined the effectiveness of stress management interventions aimed at reducing stress in university students and concluded that cognitive, behavioural, and mindfulness interventions are effective in reducing stress in university students (Regehr et al., 2013). Results demonstrated associations between cognitive, behavioural and mindfulness interventions with decreased symptoms of anxiety, lower levels of depression and cortisol response. The results from Regehr et al. (2013) were also consistent in another study that used a stress management and education tool, which was based on the Mindfulness-Based Stress Reduction (MBSR) programme (Lynch et al., 2018). Researchers assessed the effects of the mindfulness-based approach on decreasing levels of anxiety, depression and perceived stress in university students. Although the MBSR and B-CBE programmes are different, they focus on improving similar aspects of one's overall stress and well-being. For example, both programmes define and introduce individuals to 'stress', teaches them how to identify certain stressors (e.g. academic stress) and helps them to establish ways to manage these stressors to improve their health. Results from both Regehr et al. (2013) and Lynch et al. (2018) emphasise the positive effect of a mindfulness programme for stressed individuals and suggest that further investigation on a larger scale is needed. In all, stress and potential stressors are multifaceted, involving many systems. Therefore, researchers need to have a multimodal approach when designing any therapeutic/interventional treatment modalities. This can include discussions on stress management and education. Education and self-management should be viewed as a crucial component to improving the overall health and well-being of a stressed individual (Varvogli & Darviri, 2011). Therefore, implementation of a stress management programme may be an effective strategy to help people to treat and manage stress.

The PSS-10 and POMS self-report measures in this study being presented were feasible to administer at both pre- and post-intervention times. These tools have been commonly employed in other studies where long-term perceived stress and mood or temporal psychological states have been investigated (Chaaya et al., 2010; Chiu et al., 2016b; E.-H. Lee, 2012; Morfeld, Petersen, Krüger-Bödeker, Von Mackensen, et al., 2007; Taylor, 2015; P. Terry et al., 2003). These tools are also used widely in both clinical and basic research, thus, have high reliability and validity in terms of their application in stress or behavioural research (E.-H. Lee, 2012). A Danish consensus version of the PSS-10 indicated that the questionnaire was feasible for the use of clinical research settings. This was reflected in a review, indicating that the PSS-10 is easy to use with established psychometric properties. However, the authors concluded that future studies should evaluate the psychometric properties in greater depth and to validate the scale in wider, more diverse populations (Lee, 2012). The POMS questionnaire has also been demonstrated as a feasible and reliable measure for behavioural studies and holds good psychometric properties (Morfeld, Petersen, Krüger-Bödeker, Von Mackensen, et al., 2007). Using psychological questionnaires and documenting individual feedback is important in a formulating clinical intervention/s such as in our study. Therefore, the PSS-10 and POMS questionnaires were used for the psychological measurement of perceived stress and differing mood states in this current pilot feasibility study. No participants reported having any difficulties with interpreting questions or items in the questionnaires. Participants received the questionnaire pre- and post-intervention and completed these within five minutes. Results of the PSS-10 showed over-all decreases is the scores of perceived stress in the majority (73%) of the participants over the course of the intervention. The results from POMS Tense subscale demonstrated a decrease in score for that subscale in 64% of the participants, whilst Anger and Depression subscales showed decreasing trends in less than half

(36%) of the participants. These were encouraging results and warrants further investigation using a robust study design and a larger population.

The collection of saliva samples pre- and post-intervention was found to be feasible. Saliva sample collection is less invasive and economical. Therefore, this was the rationale for selecting saliva as the biological sample for measuring physiological stress markers in this study. Once the participant arrived at each session, they were asked to rinse their mouths with a glass of water. Rinsing the mouth would remove contaminating or interfering substances such as food or buccal mucosal layer residue. After rinsing, participants waited approximately five minutes before the first sample was collected. This was to ensure samples were clear, but not diluted. Saliva samples were taken at four timepoints (TPs), pre- and post-intervention, which took between 5-10 minutes per participant. The student researcher was a novice in the field of laboratory work, however, found the method of collecting saliva easy to administer after receiving ample training. Two participants appeared apprehensive during the collection, which may have been due to the close proximity between student researcher and participant during the collection process. All other participants appeared calm and relaxed. The samples were collected via passive drool, which is a commonly employed method in research (Dimolareva et al., 2018; A. Fernandes, Skinner, Woelfel, Carpenter, & Haggerty, 2013; D. Koh & Koh, 2007).

Although this study being presented here demonstrated the feasibility of the application of the proposed therapy package in all aspects, challenges were experienced with the execution of our study. These were experienced by both the student researcher and participants. The challenges included the fixed timing of the study over two consecutive days and the collection and transportation of saliva samples. The following paragraphs will describe these challenges in detail, with possible explanations of why these may have occurred and provide a solution where possible or applicable.

Controlled variables such as the selection of the fixed session dates and morning times (Tuesday and Wednesday) became difficult for the majority (82%) of the participants. They were invited to come to the Unitec Osteopaths' Student Clinic, (Clinic 61, Unitec Institute of Technology, Mount Albert, Auckland Campus), where the therapy intervention was being delivered. The intervention was conducted within two sessions, over two consecutive days, between 8 and 10 am. The selected times in our study were based on the diurnal variation observed in the selected biomarkers. The levels of cortisol and sIgA, for example, peak in the early hours of the morning after waking. However, the selected time coincided with peak hour traffic, which may have contributed to feelings of stress for participants trying to make their scheduled sessions. This has also been suggested in a study by Evans et al. (2002), where researchers demonstrated the association between driving, traffic, and increased levels of stress (Evans, Wener, & Phillips, 2002). Furthermore, as the majority of the participants were either students (27%) or working full-time (73%). The selected session times over two consecutive weekdays, therefore, caused some difficulties for the student researcher to liaise with participants. Other stressors such as worries for being late to work/university were other possible contributors to feeling stressed at pre-intervention times. In addition to peak-hour traffic time, both academic stress and full-time workload are other commonly reported stressors in many studies (Abdel et al., 2017; Hjortskov, Rissén, et al., 2004; Niks et al., 2018; Regehr et al., 2013).

The selected dates (Tuesday and Wednesday) were based on the student researchers' schedule as they were completing their Masters' degree, and compulsory student clinical hours at the time of this study. The selected dates were the only available dates for the student researcher. This further caused challenges, particularly for participants who were working full-time. This limited their flexibility, which may have added to work pressure in adjusting work schedules. Work-related stress has been demonstrated in previous literature to possibly increase the risk of developing poor health outcomes, depending on the work situation. In a study by Niks et al. (2018), researchers determined that effective interventions to prevent work stress and to improve health, well-being, and performance of employees are of the utmost importance (Niks et al., 2018). As there was no flexibility with the selected dates for this intervention, this would not be a problem for future full-time researchers who have more time flexibility. However, despite the challenges participants faced with timing and date selection, the retention rate of the participants enrolled in this study was 100%. This suggests that participants were interested in and enjoyed partaking in the study. This also indicates that participants were able to commit to a two-consecutive day intervention, despite the challenges reported. Furthermore, the rapport developed between the participant and student practitioner may have contributed to the positive retention rate. However, due to the small size of the group, we cannot truly comment on the justification of the retention rate. Previous studies have indicated that dropout rates are usually quite high in intervention studies, typically ranging between 15-20% (Cramer, Haller, Dobos, & Lauche, 2016).

An important aspect when collecting biological samples is transportation. To maintain the integrity of biomarkers, transportation needs to be effective and efficient. Furthermore, samples need to be stored in ideal conditions. Adequate care needs to be taken when administering these steps, therefore, practical challenges need to be considered when transporting saliva samples for future studies. Transporting the samples from a freezer located on-site to the research-grade freezer (-80ºC freezer) in another building provided a challenge for the student researcher. An associate helped to transport these samples, so the student researcher could continue the therapy package with the participant. Errors made with the execution of the transportation and handling of the biological samples may have affected the integrity and stability of biomarkers. However, it is difficult to comment on the integrity of biomarkers/samples without further investigation. The assessed biomarkers in this present study are sensitive to the external environment (Batista et al., 2017; Chiappelli, Iribarren, & Prolo, 2006; Slavish et al., 2015; Soares-Nunes et al., 2015). Cortisol, for example, needs to be stored immediately to prevent degradation or contamination (Batista et al., 2017). Therefore, recommendations for future researchers need to be made to give careful consideration regarding the collection and transportation of biological samples. Alternatively, an adequate and nearby freezer (or dry ice) is recommended to store the samples.

The effects of the proposed therapy package demonstrated positive outcomes. There were significant decreases in the overall scores of perceived stress parameters in response to the treatment. Baseline scores for the self-reported stress and mood states were reduced in the majority of the participants following two sessions of the therapy package. In our present study, there were similarities in the patterns of change observed between PSS-10 and POMS Tense, Anger and Depression subscales. For example, the majority (64%) of the participants demonstrated decreasing PSS-10 scores, which was associated with decreasing POMS Tense scores. This is consistent with the results seen in a study by Gawrysiak et al. (2016). Researchers in that study investigated the associations between distress tolerance and psychosocial changes in individuals participating in Mindfulness-Based Stress Reduction (MBSR) (Gawrysiak et al., 2016). Results demonstrated that distress tolerance, perceived stress, and mood states showed favourable changes from pre- to post-MBSR. This is consistent with the results observed in our present study, where participants reported feeling relaxed after the treatment and demonstrated decreasing PSS-10 scores. Another pilot study by Lopez et al. (2011) examined the effects of cognitive behavioural stress management on stress, quality of life, and symptoms in persons with chronic fatigue syndrome (Lopez et al., 2011). In that study, repeated measures of variance revealed a significant interaction for perceived stress and POMS-Total Mood Disturbance (TMD) scores. Although POMS-TMD could not be calculated in our study, previous research has demonstrated strong associations between POMS and PSS questionnaires in interventions aiming to improve mood states and/or reduce feelings of stress (Dipietro et al., 2008; Kennedy et al., 2010; Kokubun et al., 2018; Lopez et al., 2011). The decreases in the scores of psychological parameters noted in our study may be as a result of the effects of the therapy package, which warrants further investigation.

The combined use of both psychometric measures (PSS-10 and POMS) has been utilised in previous research (Dipietro et al., 2008; Kennedy et al., 2010; Kokubun et al., 2018; Lopez et al., 2011). Therefore, by comparing the analytical results of these two questionnaires, we can assess what kind of responses or mood states are affected by chronic stress. In a study by Newham et al. (2012), researchers indicated a decrease in the POMS Depression scores was associated with decreasing PSS-10 scores (Newham, Westwood, Aplin, & Wittkowski, 2012). This is consistent with the results for the POMS Depression and PSS-10 for three participants in our study. Another study investigated the association of fatigue and stress with grey matter volume (Kokubun et al., 2018). Researchers in that study found that grey-matter brain healthcare quotient (GM-BHQ) was lower in individuals suffering from fatigue or a combination of fatigue and stress, as measured through the POMS and the PSS-10 instruments. It has also been demonstrated that vitamin/mineral supplementation can lead to significant improvements in ratings on the PSS-10 and the 'vigour' subscale of the POMS (Kennedy et al., 2010). Although we did not document vitamin/mineral supplementation in our study, all participants reported no health issues.

Positive outcomes were also noted in response to the GOT. Soft tissue massage, cervical myofascial release, suboccipital release and rib-raising were four techniques employed in our intervention, which have been documented in previous literature (Hartman, 1997; Henderson et al., 2010; Henley et al., 2008; Nuño et al., 2019; Wieting et al., 2013). The rationale for the selection of these techniques was based on the existing literature, indicating their positive effect on the ANS and promoting overall relaxation (Giles et al., 2013; Henderson et al., 2010; Henley et al., 2008; Nuño et al., 2019; Wieting et al., 2013).

Soft tissue massage has been shown to decrease stress and anxiety and increase overall body relaxation (Field, 2010; Field et al., 2007). This is consistent with findings in a study by Saracutu et al. (2010), which indicated the effectiveness of using soft tissue massage to reduce fear avoidance, improve quality of life and general health status in people living with persistent pain (Saracutu, Rance, Davies, & Edwards, 2018). Cervical myofascial release has been demonstrated to stimulate the phrenic nerve, which provides innervation to the upper extremities and neck (Henderson et al., 2010; Henley et al., 2008; Wieting et al., 2013). It has been suggested that this technique enhances overall body relaxation as gentle rhythmic manipulation is applied to the muscles overlying the shoulders and neck (Saracutu et al., 2018). Suboccipital inhibition can assist in decreasing tension and improve ANS homeostasis by inducing a parasympathetic response (Emmet, Nuño, & Pierce-Talsma, 2018). This is consistent with findings by Wieting et al. (2013), where researchers demonstrated that suboccipital inhibition increases parasympathetic tone and heart rate variability (HRV). The vagus nerve exits from the jugular foramen, which continues to pass through the posterior neck to innervate the diaphragm. Therefore, with gentle inhibition to the suboccipital region the vagus nerve is stimulated, which decreases the activity of the diaphragm and subsequently reduces breathing rate (Emmet et al., 2018; Wieting et al., 2013). Additionally, the effects of rib-raising were investigated in a pilot study by Henderson et al. (2010) Researchers in that study aimed to determine whether this technique had any effect on the sympathetic nervous system (SNS) and could improve diaphragmatic excursion. Although rib-raising improved diaphragmatic excursion, results indicated that the HPA axis and parasympathetic activity were not altered (Henderson et al., 2010). However, in a more recent study by Weiting et al. (2013), results contradicted findings in Henderson et al. (2010), where researchers demonstrated the technique had a positive effect on downregulating the SNS. In light of the literature, it appears that the combination or individual application of these techniques (soft-tissue massage, cervical myofascial release, suboccipital inhibition and rib-raising), which were utilised in our study, may have contributed to the reduced breathing rate and overall body relaxation noted in participants of the current study (as indicated through decreasing PSS-10 scores, subjective feedback and the observed reduced breathing rate).

Although studies are demonstrating positive and promising results with the application of GOT in promoting relaxation, the main limitations of these studies were small sample size, which limited their ability to expand and justify their results (Blut, 2015; Henderson et al., 2010; Henley et al., 2008; Nuño et al., 2019; Wieting et al., 2013). For example, one study assessed the effects of a 30-minute GOT treatment on two participants (one female and one male) (Nuño et al., 2019). Despite these techniques (suboccipital release, myofascial release, rib-raising and soft-tissue massage) demonstrating positive effects such as reducing stress, they do not provide people with strategies to deal with life stressors.

The use of a multimodal approach in clinical stress research has proven advantageous to researchers as multiple stressors can be addressed (Philipps et al., 2019). Employing stress management techniques has been demonstrated to provide individuals with good results on their overall health and well-being (Varvogli & Darviri, 2011). This is consistent with findings in Wiegand et al. (2015), where researchers investigated the effect of osteopathic manipulative treatment (OMT) on self-perceived fatigue, stress, and depression in first-year medical students. Although results in that study did not determine any statistical significance in the perceived stress scores, authors concluded that with further research, OMT represents a potential modality to reduce self-perceived distress (Wiegand et al., 2015). Another study conducted by Castro-Sánchez et al. (2011), aimed to determine whether cervical-myofascial release therapy could improve pain, anxiety, quality of sleep, depression, and quality of life (QOL) in patients with fibromyalgia (Castro-Sánchez et al., 2011). The treatment caused a reduction of pain sensitivity within specific points around the body and improved QOL in patients with fibromyalgia. Despite these positive outcomes, results were only limited to those with fibromyalgia. Regardless, the literature does open new perspectives regarding osteopathy as an alternative modality in the treatment and management of stress, anxiety and quality of life. The literature also suggests that using stress management and education techniques in conjunction with alternative manual therapies can promote relaxation (Castro-Sánchez et al., 2011; Chou et al., 2017; Field, 2010; Nuño et al., 2019). While manual therapy can improve mood and reduce feelings of stress, stress management education can provide individuals with coping strategies to better manage their stressors. Therefore, combining both manual therapy and stress management education may be a potential and beneficial therapy package for reducing stress in the general population.

The psychological assessment provides researchers with perceived stress and mood ratings from individuals, which can be correlated or compared with physiological stress. The PSS-10 instrument is practical, easy to administer, time-efficient and used widely as a screening tool in stress studies (Chaaya et al., 2010; Chiu et al., 2016b; Kennedy et al., 2010; Kokubun et al., 2018; Newham et al., 2012; Wongpakaran & Wongpakaran, 2010). However, psychological assessment of perceived stress is a subjective measure, which can produce problems with quantifying, comparing, and measuring results consistently (Dohrenwend, 2006; Monroe, 2008). This was consistent with the results in Föhr et al. (2015), where researchers investigated the association between subjective perceived stress, with objective heart rate variability-based stress (Föhr et al., 2015). They acknowledged that although subjective assessment can include reporting bias, the PSS is a commonly used tool that effectively measures the extent or severity of a stressor/s effect on an individual's life. Despite its psychometric properties employing the Cronbach's alpha to determine validity, subjective stress has been criticised as it relies on individual self-reporting. Comparatively, physiological measures are considered to be more reliable as they represent objective data, which is not affected by an individual's mood, emotions, cognition or social context (Föhr et al., 2015). It is important and reasonable for future researchers to continue utilising and developing objective measures of stress, which acknowledge the psychological burden of stress. Therefore, applying a combined approach utilising both measures, is useful and necessary to establish the full picture of the impact stress has on an individual.

The literature has indicated the interdependent relationship between psychological and physiological systems. For example, mood has been demonstrated to affect certain physiological pathways and markers representing those affected pathways have been shown to be good indicators of mood. In our present study, we were unable to calculate the total mood disturbance (TMD) for POMS. This was due to a printing error, which resulted in only half of the questionnaire being printed. As this was a pilot feasibility study that had a small sample size, no statistical assessment of presumptive associations could be conducted. Instead, this present study was restricted to the assessment of visual similarities in the patterns of changes within these parameters. Moreover, we aimed to establish any possible/speculative associations from results between the psychological and physiological parameters over the course of the intervention. The visual inspection identified a group of participants showing similar patterns of change in the self-report measures with the assessed biomarkers. These included similarities between the patterns of change in the levels of IL-6, sIgA and the self-reported scores in mood subscales in POMS assessment. However, without proper statistical correlative analysis (Pearson or Spearman correlation) we can only speculate the possible relationship between the reported mood subscales and physiological parameters.

Even though there was a lack of consistent relationships observed between the self-reported stress, mood state assessments and the physiological markers of stress in the study being discussed here, the literature has indicated the importance of assessing both physiological and perceived stress together. This provides readers, researchers and healthcare professionals with an understanding of the overall picture of stress. In a recent review by Joëls et al. (2018), researchers examined how acute stress conditions change brain physiology and cognition in rodents. Results demonstrated that directly after stress, emotional behaviour was strongly associated with cognitive processing. Rodents were able to quickly and adequately respond to their stressful situation (Joëls et al., 2018). Several hours after the stressor, emotional circuits were dampened, while functions related to cognitive processing and the pre-frontal cortex became activated. These findings suggest that the organism can rationalise the stressful event and place it in the right context (Joëls et al., 2018). Furthermore, in a review by Mariotti (2015), additional systems can become affected on a physiological level during chronic stress, such as the development of cardiovascular, immunological and neural system dysfunction and/or diseases. For example, evidence indicates that during HPA axis dysfunction, excess stress hormones that are released can stimulate hematopoietic stem cell proliferation, which can increase the risk of developing various cardiovascular diseases (Mariotti, 2015). Stress-dependant neuroendocrine dysregulation has also been shown to directly damage many organs and tissues of the human body (Konduru, 2011). Moreover, biochemical pathways can be significantly impaired by chronic stress, which can lead to poor health outcomes. Researchers have searched for putative tissue alterations that are associated with chronic stress, whilst others have assessed the mechanisms of action of the main stress hormones (Ali & Nater, 2020; Arnsten et al., 2015; Crestani, 2016; Dean & Söderlund, 2015; Epel et al., 2018; Mariotti, 2015; Slavich, 2016). All of which, have determined the detrimental effect chronic stress can have on a physiological level within the human body.

In the present study, some participants displayed relationships (similar patterns of change) between self-report measures of perceived stress and IL-6 levels. This is consistent with previous literature that has demonstrated the relationship between inflammatory markers and different emotional responses (Hellhammer et al., 2009; La Fratta et al., 2018; Nedeljka et al., 2016; Prasad et al., 2015). A similar relationship is also reported in a study by Carpenter et al. (2010), where correlations were seen between IL-6 and the anger mood state. The findings in our present study also suggested a possible relationship between increasing IL-6 levels with decreasing POMS Anger ratings in less than half of the participants. A study by Rapaport et al.

(2012) indicated that massage therapy has the potential to increase the production of pro-inflammatory cytokines including IL-6. Furthermore, Field et al. (2007) demonstrated that massage therapy can influence mood states such as anger, by reducing anger levels through therapeutic relaxation of massage therapy (Field et al., 2007). Therefore, with the soft tissue massage techniques employed in our present study, this may have contributed to the increasing IL-6 levels and decreasing anger scores. This is consistent with findings in Puterman et al. (2014), who demonstrated that the relationship between anger and IL-6 stress reactivity exists. However, researchers showed some contrasting results suggesting that participants who experienced anger directly after or as a result of a stressor had significant increases in levels of IL-6. Although we did not receive any reports from any participants indicating they had experienced feelings of anger pre-intervention, the levels of IL-6 in some of the participants in our study did appear to be consistent with those in Puterman et al. (2014). Essentially, the study by Puterman et al. (2014) determined a relationship between anger mood states and IL-6, however, more research is needed to determine more conclusive results. Furthermore, the population assessed in Puterman et al. (2014) was women with low social support. Authors concluded that supportive connections could dampen the relationship of Anger to pro-inflammatory reactivity to stress, yet more research was needed to justify this.

Another observation in our present study was between sIgA concentrations and PSS-10 scores, both of which appeared to decrease in 27% of the participants. This observation is consistent with findings from a study that demonstrated the relationship between psychological conditions, such as depression, and reduced levels of sIgA (Golshiri et al., 2012). Authors concluded that with lowered levels of sIgA, the immune system can be compromised and increase the risk of developing illnesses. The patterns of change observed in our study will need further investigation and explanation.

Relationships between cortisol and the self-reported measures of perceived stress and POMS Tense was also observed in our present study. The literature has shown that different personality or mood disorders have established a strong relationship with changes in HPA function (Hellhammer et al., 2009). A study by Di Corrado et al. (2014) examined the effects of training and competitions on mood states in a team of 15 elite water polo players. The results demonstrated that flatter diurnal rhythms of cortisol appeared to play a role in determining dysfunctional mood states (Di Corrado et al., 2014). Additionally, in a study by Fornari et al. (2017), researchers explored the potential effects of GOT on the activity of the HPA axis by assessing cortisol levels. The study aimed to determine whether there was a protective potential of osteopathy for mental health. The results of Fornari et al., (2017) indicated cortisol levels were lower in participants receiving treatment. Moreover, researchers suggested that the treatment session also prevented the typical increase in cortisol levels, which was observed immediately after a brief mental challenge. Results from Fornari et al. (2017) were consistent with results in the present study, where cortisol levels decreased in 37% of the participants between TP1 and TP2, and/or between TP3 and TP4, which were the timepoints recorded post-GOT therapy. There was no psychological stress analysis assessed in Fornari et al. (2017), which did not allow for associations between subjective and objective stress to be made. However, the results in that study did suggest that the reduction in cortisol may have been attributed to the GOT application.

Overall, although the results of the biomarkers in the present study were inconclusive, they provide an opportunity for future researchers to consider assessing alternative biomarkers which are not affected by diurnal variation. Moreover, studies with larger cohorts, improved research methodologies and incorporating appropriate controls need to be conducted to determine conclusive correlations between mood, perceived stress and inflammatory biomarkers.

An additional factor that needs attention, specifically for women, is that salivary flow rate is affected during menstrual cycles. It is not clear in the literature if this flow rate, in turn, can affect cortisol levels in saliva, however, studies indicate that menstrual cycle variation may have differing effects on cortisol and mood (Abrao et al., 2014; Kajantie & Phillips, 2006; Ossewaarde et al., 2010; Pechère-Bertschi & Burnier, 2004). Therefore, menstrual cycle variations should be documented for future studies. Furthermore, oral contraceptives have been demonstrated to play a role in hormonal regulation and mood behaviour (Lewis et al., 2019; Poromaa & Segebladh, 2012). It has been demonstrated that they can cause moderate to severe mood-related side effects, in particular, depressive symptoms (Lewis et al., 2019). Although there is growing evidence linking the cortisol stress response and personality, the nature and the underlying mechanisms of these relationships require further clarification and/or investigation. Despite the inter-relationships being reported between emotional, cognitive and physiological stress in published literature (Carpenter et al., 2010; Djuric et al., 2008; Golshiri et al., 2012; Hjortskov, Garde, Ørbæk, & Hansen, 2004; Oswald et al., 2006; Otsuki et al., 2004; Van Hedger et al., 2017), we were limited in our ability to draw convincing associations between the psychological and physiological parameters from the data obtained in ourpresent study.

Although there were no clear patterns of change observed in the biomarkers of our present study, some inter-relationships between increasing sIgA and decreasing cortisol levels were seen in 55% of the participants. This was consistent with the results in a study by Volkmann et al. (2006), where researchers demonstrated the relationship between sIgA and cortisol levels with stress. Essentially, results indicated that participants with heightened cortisol and lowered sIgA levels had poorer health outcomes, compared to participants with heightened sIgA and lowered cortisol levels during academic stress (Volkmann & Weekes, 2006). These findings suggest that individual differences in basal endocrine and immune activity can predict stress-related susceptibility to poor health. Another relationship between sIgA and cortisol is the similar diurnal profiles with which they both share (Hucklebridge et al., 1998). Under normal circumstances, the literature has demonstrated that cortisol and sIgA levels are highest in the morning after waking (Pritchard, Stanton, Lord, Petocz, & Pepping, 2017).

With regard to starting the sessions early in the morning, diurnal variations seen in the selected biomarkers were the deciding factor. Based on the suggestions from the literature, biomarkers should be collected in the early morning (Evans et al., 2002; D. Koh & Koh, 2007; Nedeljka et al., 2016). In the study by Lindholm et al. (2012), researchers analysed the correlation between subjective stress, sleep, salivary cortisol, and melatonin hormones among Finnish media workers with regular daytime work (RDW) and with irregular shift work (ISW). Salivary samples were taken 60 minutes after waking compared with levels taken immediately after waking. The results indicated that the cortisol ratio was higher in the ISW group compared to the RDW group (Lindholm et al., 2012). These findings suggested that a stressful work environment, such as ISW, can increase cortisol secretion after waking. However, this was not consistent in the results in a study by Pilger et al. (2018). Results in that study indicated that midday salivary cortisol levels were superior to cortisol waking response in people experiencing burnout (Pilger et al., 2018). Despite this, results in Pritchard et al. (2017) demonstrated that salivary cortisol and sIgA levels are higher in the early morning based on their diurnal profiles. It has been indicated that more research is needed to measure cortisol and sIgA levels and to make adjustments for the diurnal patterns they exhibit. One suggestion for future studies is the consideration of assessing alternative biomarkers that do not have diurnal variation (Pritchard et al., 2017). This may provide flexibility with the selection of appropriate practical timing for the intervention. Therefore, more research may need to be conducted to find an appropriate physiological marker.

Another factor when considering the lack of consistent relationships between the two parameters assessed in this study was the small sample size. Although we received a lot of interest from women wanting to participate, this was a feasibility study and a Master's research project, therefore including a large sample size was beyond the scope of this project. Feasibility studies provide the foundations for larger studies, which would examine consistent relationships. Furthermore, some participants produced small amounts of saliva, which reduced the ability to analyse certain biomarkers. Although there were no reported health issues from participants, two of which, produced low amounts of saliva making the collection process challenging. A possible explanation for one participant was the effect caffeine has on the cortisol response. Caffeine can elevate the cortisol response and is a diuretic, known to suppress the salivary flow rate (Hildebrandt et al., 2013). Caffeine ingestion can also affect sIgA levels (Hildebrandt et al., 2013; Lovallo et al., 2006, 2005). Another cause for low production of saliva could be their high levels of stress, as recorded by their PSS-10 scores. The effect of chronic stress can ultimately reduce the salivary flow rate, which results in dry mouth syndrome (Bulthuis, Jan Jager, & Brand, 2018). Therefore, the baseline results from one participant, for example, was 28, which is relatively high.

Although serum and blood plasma are considered the gold standard saliva samples are advantageous for researchers assessing stress. Saliva collection through the passive drool method is characterised by its easy to administer and non-invasive qualities, making it an optimal choice in stress research. In normal circumstances, saliva pools within the buccal cavity of the mouth before being collected into a cryotube via a pipette (Salimetrics, 2019). With passive drool, there is an unlimited analysis potential for multi-analyte measurements. Furthermore, passive drool has been established as an easier measure for biomarkers that are dependent on the flow rate (Salimetrics, 2019). However, the practical nature of saliva sample collection was challenging for the student researcher, a novice in the field, despite having received sufficient training. Minimal production of saliva leads to low volumes collected, which was a similar issue reported in Roswith et al. (2018). Researchers expressed that patient adherence and discomfort towards saliva collection was a problem leading to insufficient amounts of the biological sample. Results in that study indicated that 1.6% (out of 5495 saliva samples) were recorded as 'missing data', due to insufficient amounts collected (Roswith et al., 2018). Thus, correct volumes of saliva must be collected to avoid having insufficient volumes of the sample resulting in missing data.

In a recent review, researchers investigated strategies to better manage risk factors during salivary sample collection and to minimize sampling errors during collection in healthy volunteers (Bhattarai, Kim, & Chae, 2018). They identified that saliva collection methods are preferred as they and inexpensive, non-invasive and easy to administer. Salivary diagnostics have even been characterised as potential substitutes for serum protein biomarkers. However, an optimal method for collecting saliva has not yet been developed. Sampling and statistical errors often occur when handling samples in various settings such as clinical trials. These errors are often accounted to subject non-adherence, collection methods, the psychological behaviour of the participants and/or sample processing (Bhattarai et al., 2018). This is consistent with the results in our study, where two participants produced low amounts of saliva and appeared non-adherent or uncomfortable during the collection. Overall, the need to assess this through non-invasive ways is important to prevent additional stress due to invasive sampling.

The assessment of stress impact on physiology is generally done through an assessment of biomarkers representing the pathways affected by stress. The literature has demonstrated that the biomarkers cortisol, sIgA and IL-6 are widely used in stress research. Therefore, they were selected for this present study. These stress biomarkers have been similarly represented in saliva and plasma. Additionally, the literature has explored the other notable biomarkers in stress research, for example, salivary α-amylase (sAA), chromogranin A (CgA) and salivary lysozyme (Giampaolo et al., 2002; D. Koh & Koh, 2007; Nater & Rohleder, 2009; Rohleder et al., 2004; Takatsuji et al., 2008). The diurnal profiles of sAA differ from cortisol, showing reductions after waking followed by higher levels as the day progresses (Nater et al., 2007). However, the number of studies in behavioural medicine that include sAA assessment remains fairly small. On the other hand, CgA as a biomarker for stress appears to be strongly associated to acute stress, with levels rising immediately after exposure to an acute stressor (Ng et al., 2003; Takatsuji et al., 2008). Therefore, these may not be appropriate for chronic, long-term stress studies.

Despite the numerous advantages of saliva, there are some practical factors that can make the collection for researchers challenging. The concentration of some of the biomarkers assessed in saliva has been reported to be lower to that of blood plasma (Jasim, Carlsson, Hedenberg-

Magnusson, Ghafouri, & Ernberg, 2018). Another factor to be mindful of is diurnal variation. As cortisol has a close association with the activity of the HPA axis, it is considered the best biomarker in stress research. Therefore, considerations need to be made with regards to the distinct diurnal profile of cortisol, particularly when collecting cortisol via saliva methods. Moreover, researchers need to be conscious of timing during the collection of biological samples. Due to the direct effect cortisol has on the HPA axis in both acute and chronic stress, researchers must determine the best ways to measure cortisol through non-invasive assessment. Cortisol in urine, for example, has been investigated in previous studies as it can be collected in a non-invasive way and in relatively large volumes (Batista et al., 2017; Harpole et al., 2016; Prasad et al., 2015).

One major difference between salivary cortisol and urinary cortisol is the secretion profile each possesses. It is known that salivary cortisol levels are highest after waking and regress as the day progresses. Whilst urinary cortisol reflects an average of the time since the previous collection. So, the first morning urine collection is generally representative of the overnight cortisol production, which is expected to be lower than a second collection. Therefore, cortisol cannot be longitudinally captured in urine and would, thus, not be a good indicator of chronic stress (Prasad et al., 2015). Alternatively, cortisol in hair samples has been demonstrated as a proxy measure to the total retrospective activity of the HPA axis over several months (Wright et al., 2015). Researchers have indicated that hair cortisol is a practical measure of long-term cortisol activity associated with chronic stress. Furthermore, collecting hair samples can be done quickly, is non-invasive and can be stored without affecting the levels of cortisol (Van Holland et al., 2012; Wright et al., 2015). Hair cortisol is a good indicator and key predictor of adverse health outcomes, which are strongly associated with future morbidities (Bautista et al., 2019; Lee et al., 2015; Wright et al., 2015). This was reflected in a study by Bautista et al. (2019), which investigated hair cortisol as a marker for chronic stress associated with hypertension. Findings suggested that elevated hair cortisol is a risk factor for hypertension based on the close relationship between excess cortisol and HPA activity. Despite this, authors concluded that although feasible, the clinical value of hair cortisol as a tool for monitoring the effect of chronic psychosocial stress or hypertension risk stratification is still uncertain (Bautista et al., 2019). Most studies assessing hair as a biological sample to study stress indicate that it can only represent the stress biomarker cortisol. Therefore, other stress-related biomarkers (e.g. sIgA and IL-6) would not be assessed in hair.

To accurately measure longitudinal chronic stress, other biomarkers, physiological and/or psychological parameters need to be measured alongside hair cortisol assessment. A recent study assessing chronic stress in adolescents through subjective measures and hair cortisol was conducted by Prado-Gascó et al. (2019). The results revealed hair cortisol concentrations and subjective measures of stress could not be related (Prado-Gascó, De La Barrera, Sancho-Castillo, De La Rubia-Ortí, & Montoya-Castilla, 2019). Although the study indicated hair cortisol as a probable measure for chronic stress, there was no relationship between perceived and objective stress (Prado-Gascó et al., 2019). The findings in a study by Xu et al. (2019) contradict this, where researchers indicate that a relationship between higher hair cortisol levels and higher tendencies of perceived stress and anxiety does exist (Xu et al., 2019). Therefore, further research needs to investigate the potential relationship between perceived stress, mood states and hair cortisol longitudinally and retrospectively to confirm or deny associations.

Alternatively, the literature has shown Heart Rate Variability (HRV) is an economical and non-invasive tool to assess the sympathetic stress response (Fornari et al., 2017). Studies assessing stress have used HRV in combination with assessing stress biomarkers, as this has been demonstrated to strengthen the results (Baevsky & Chernikova, 2017; Hjortskov, Rissén, et al., 2004; Toro-Velasco et al., 2009). Combining the assessment of stress biomarkers and HRV may provide future researchers with more conclusive physiological results and allow associations to be drawn between subjective, perceived stress and objective markers of stress. In a study by Henley et al. (2008), the effects of cervical myofascial release on the ANS was assessed through cortisol levels and HRV measurements. Authors concluded that measuring HRV in conjunction with the assessment of stress biomarkers is important when investigating stress responses and neuroimmune interactions (Henley et al., 2008). Rib-raising was another osteopathic technique employed in our present study, which has been demonstrated to decrease heart-rate and blood pressure (Henderson et al., 2010). Although we did not record HRV alongside the physiological parameters, this presents another plausible parameter to consider including in future studies.

Limitations

Overall, the present small-scale pilot study has demonstrated the feasibility of the application of all aspects of the proposed therapy package. This study aimed to test the feasibility and the effects of a therapy package on women experiencing stress. The following paragraphs will discuss the limitations experienced during this study, which should be considered when assessing the results and planning any future studies.

Sample size

Although many women showed interest in participating, it was beyond the scope of this project to include a larger sample size. Additionally, all of the enrolled participants were working or studying at Unitec Campus, therefore, the location for the intervention was convenient for participants. These two factors resulted in small sample size and a specific population for the present study, which in terms of statistical power, limited our ability to perform statistical correlative analysis. Essentially, without statistical power, we were unable to validate any changes in stress levels as a result of receiving the therapy package. This would be possible to carry out in a study with a larger sample size with the specific objectives of investigating the correlation between various factors and variables. Furthermore, with small sample sizes, the probability of the outlier effects is high. Due to this nature, we assessed and have provided results from individual participants as well.

Study design and controls

As the design of this study followed an uncontrolled and non-randomised design, there were some limitations with this approach. One part of the treatment protocol in this study involved the application of ten osteopathic techniques on each participant. This followed a semi-controlled protocol, which allowed for a mixed study design and the use of some controls. Having a mixed pragmatic and exploratory study design enabled the student researcher to tailor their treatment to suit each participant's needs. Although each participant received the 30-minutes of GOT, the duration of the techniques applied to certain areas differed between participants. The limitations of using a pragmatic approach may have affected the internal validity of the study as the duration of each technique was based on the researcher's subjective perception of tissue change. This subjective approach may be interpreted as bias (Sabini, Leo, & Moore, 2013). Regardless, this was based on osteopathic principles, which allowed the researcher to replicate what would be experienced in an osteopathic setting, thus, increasing the external validity of the study. The controlled variables in this present study (e.g. selected times of sessions and dates) was challenging to schedule with participants. In a normal osteopathic setting, a mutually agreed date and time would be discussed between practitioner and patient. Therefore, the use of strictly controlled variables may need addressing for future researchers wanting to achieve a real-world, osteopathic treatment session.

Having a mixed pragmatic and exploratory study design with the implementation of some controls is acceptable for studies such as ours. Researchers need to replicate what would be expected in a clinical osteopathic setting to establish the overall effects of the tested therapy package. However, having controlled variables is just as important as it ensures results can be generalised and compared between the assessed groups/parameters. Additionally, to note, this study also had a small population sample size, which limited the possibility of having experimental controls (sham). In our case, the effect of the intervention was assessed by looking at the pre- and post-intervention differences in the selected markers.

Time and duration of the therapy application

Considering the practical nature of the therapy package, the intervention required two consecutive days starting early in the morning. This time was decided to prevent diurnal variation seen in the selected biomarkers. However, this early morning time (8 to 10 am) proved to be challenging for the majority of participants. The participants were required to arrive at the Clinic by 8 am, which coincided with peak hour traffic. Participants may have been subject to additional stress due to this factor. This could be avoided by the investigation into other stress biomarkers with no diurnal pattern for future researchers. However, a point to be noted that while assessing the efficacy and performing controlled trials, controlling time may be necessary to achieve consistency and avoid variations. Having biomarkers without diurnal variation may make it possible to have fixed time but at times that may be more practical.

Saliva sample volume and collection time

Additionally, insufficient volumes were collected for some participants, which made results inconclusive. The collection of saliva was completed via passive drool pre- and post-treatment, a total of four timepoints for each session. Although passive drool is a standard method, researchers have also indicated that the volume of collected saliva can be low (Soares-Nunes et al., 2015). There are several issues to consider during a slow and low saliva flow rate. Firstly, the possibility of acute stress needs to be addressed. Acute stress is known to affect some of the stress biomarkers we assessed (Slavish et al., 2015). Secondly, the small amount of sample can be viscous, which may have contributed to practical difficulties with analysing biomarkers. Finally, due to the longer duration of the collection process, some samples were left at room temperature which may have affected the integrity of the biomarkers assessed.

A printing error in POMS (perceived stress tool)

There was an error with printing the initial 40-item POMS (S. Curran et al., 1995). Due to this error, only one page of the questionnaire was collected, which consisted of 24-items. Although there are some versions of the 24-item POMS used in research (Brandt et al., 2016; Morfeld, Petersen, Krüger-Bödeker, Von Mackensen, et al., 2007), it generally consists of six subscales; Tense, Anger, Fatigue, Depression, Confusion and Vigour, which contain four items in each. This compared to our 40-item version, which had seven subscales with different items in each. Due to this error, some of the subscales were missing and we could not calculate POMS-TMD. Therefore, we could not compare our findings to that in the current literature.

Recommendations

The objectives for this pilot study was to assess the feasibility of recruitment criteria, operational protocols, stress measurement tools and overall study designs. This was achieved, which provides the possibility of the intervention being replicated and used in a larger scale study such as a randomised control trial (RCT). Therefore, the next paragraphs aim to further provide recommendations for future larger studies. We aim to provide researchers with an improved approach for further investigation/s to determine the effects of this therapy package as a therapy protocol in reducing stress in females (or in the general population).

Flexibility with session timings

The overall application and deliverance of the therapy package was efficient with no adverse effects reported. Practitioners can, therefore, implement this specific protocol administered in our study, into their own clinical practice. However, the selection of early morning sessions was impractical for the majority of the participants and would not likely be replicated in a real-life setting. Furthermore, requesting that participants take two hours from work/university/other in the early morning, mid-working week, was challenging. Under normal circumstances, treatment in an osteopathic clinic is typically conducted over a few weeks, depending on the presenting complaint (Saracutu et al., 2018). It is therefore recommended for future researchers to utilise a flexible timeframe, such as scheduling sessions over days or weeks. This would be more realistic and mimic what would be experienced as a patient in an osteopathic practice.

Ensuring sufficient volumes of saliva samples are collected

Researchers may wish to adopt the use of a stimulus during the collection of the saliva samples. This may aid in increasing salivary secretion and flow rate for the participants who produce low amounts of saliva, due to the reasons explained in the discussion. Stimulated saliva is collected through mastication, citric acid use or gustatory stimulation (Soares-Nunes et al., 2015). Additionally, the restrictions of food and certain drinks (e.g. coffee) should have been better controlled as caffeine is a diuretic (Hildebrandt et al., 2013; Ice et al., 2004). Therefore, it is recommended that future researchers provide participants with an instruction/information sheet on what to avoid pre-intervention. A better explanation for these restrictions should be given at the initial screening to establish better co-operation.

Assessing additional stress hormones

It is also recommended that additional stress hormones need to be assessed in future studies measuring stress. It is widely acknowledged that cortisol affects HPA activity, which is involved in the stress response. Other stress hormones, such as the gonadal hormones also affect the HPA axis. For example, oestradiol and testosterone can have robust effects on HPA activity, which has been demonstrated in a study on male and female mice (Sanchez-Cardenas et al., 2010). It has been shown that gonadal hormones have a reciprocal inhibition effect between the hypothalamic-pituitary-gonadal (HPG) axis and the HPA axis, whereby gonadal hormones can activate and regulate HPA activity during all stages of life (Oyola & Handa, 2017). As the literature indicates, there are other systems involved in the stress response (e.g. the HPG axis). Therefore, it may be recommended for future researchers to utilise oestradiol as a surrogate measure of the HPA activity, which can provide a better representation of physiological stress in women. However, some factors do need to be considered with regards to the timing of sampling due to the diurnal variation of both cortisol and oestradiol.

Assessing biomarkers with no diurnal variation

Following on from this, it is recommended that further exploration into assessing stress biomarkers with no diurnal variation is considered by future researchers. This would allow for flexibility with timing and possibly improve recruitment for larger-scale studies. Therefore, the investigation into biomarkers with no diurnal variation would be helpful. Alternatively, other physiological stress measures would be beneficial for future researchers. For example, heart rate variability is a non-invasive and effective tool in assessing the response to stress and neuroimmune interactions (Fornari et al., 2017; Henley et al., 2008).

The exploration into other psychometric tools

Assessments that measure, compare and quantify one's perceived stress may be interpreted as bias (Sabini et al., 2013). Further investigation into determining specific personality constructs may be recommended. This could help distinguish individuals who are more susceptible to psychological stress and/or mood disturbances. There is a known psychometric evaluating test called the Trier Social Test (Kirschbaum, Pirke, & Hellhammer, 1993), which induces a psychosocial stressor in a laboratory setting to determine different stressors in individuals. It is thought to mimic similar limbic activation as the HPA axis and is much utilised in investigative studies exploring psychological stress. Other psychometric evaluative tools are also recommended to help researchers identify specific stressors relevant to diverse populations (Epel et al., 2018; Musavi, Alavi, Alimohammadi, & Hosseini, 2016). This may help to determine distinct personality foundations, relative to the stressor/s that are reported from each individual. Further investigation into how best to assess for stressors, or ways in which to categorise stressors, needs to be considered. Therefore, it is recommended that more robust tools without bias need to be explored which can better determine current or ongoing stressor/s.

Conducting larger-scale randomised controlled trials

To confidently comment on the effectiveness of the present therapy package, it is recommended to have sham and/or normal control groups, in addition to main treatment groups in future clinical trials. This will allow researchers to assess the efficacy of the treatment protocol and its therapeutic effects (Leon et al., 2011). Sham or placebo groups serve as a standard for comparison in experimental studies, limiting bias concerning treatment adherence, treatment allocation, and the assessment of subjective outcomes as a result of the tested treatment/therapy (Brim & Miller, 2013; Sutherland, 2007). Future studies should consider larger sample-sized RCTs to compare a treatment group to the sham group. This will potentially provide a deeper insight into the effectiveness of the treatment protocol proposed and tested in the present study.

Conclusion

To conclude, this present pilot feasibility study indicates that the therapy package comprising of 10 general osteopathic techniques and stress management education, was feasible to administer in an osteopathic clinical setting. All aspects of the therapy package were feasible, including the saliva sample collection. There were no adverse events reported from any participants after receiving general osteopathic treatment. Moreover, the therapy package demonstrated a measurable decrease in perceived psychological stress, which warrants further investigation. Participants reported feeling "relaxed" and benefitted from the stress management education, particularly from the pamphlet, which was based on the Brief-Cognitive Behavioural Education program. Comparatively, no specific patterns of change in physiological stress markers could be demonstrated. Furthermore, no clear relationship was noted between perceived stress, mood states and physiological stress markers. This could be due to the small sample size, which limited our ability to conduct statistical analysis. This will need to be further investigated by using larger cohorts.

The objective of the study to assess the feasibility of all aspects of the study protocol, assessing the effectiveness of the therapy package was not the main objective. Important to note was that the present study allowed the research practitioner to mimic a real-life setting. This encouraged the participant to feel relaxed, at ease and reassured by providing a relaxing environment after establishing good practitioner-participant rapport. Even though some results are speculative at this stage, the present study has the potential to contribute to the literature with regard to the management of stress. This study has successfully tested the feasibility of the proposed intervention on a group of women experiencing stress. Furthermore, we have provided readers with an insight into the positive outcomes of the present therapy package on psychological stress in women. We have highlighted the existing gap in the literature related to the effects of osteopathic treatment in managing stress. Finally, we have identified a need for cross-sectional, larger scale, longitudinal studies with better methodological design and have provided some valuable recommendations for future researchers.

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Appendices

Appendix 1 – Ethics Approval

Ethics application number: 2018-1066

Dear Victoria (Tori) Taylor and Antoine Rahier,

Your file number for this application: **2018 - 1066**
Title: **The effects of a proposed therapy package on physiological and perceived stress in females and males: A pilot study**

Your application for ethics approval has been reviewed by the Unitec Research Ethics Committee (UREC) and has been approved for the following period:

Start date: 27 November 2018
Finish date: 27 November 2019

Please note that:

1. The above dates must be referred to on the information AND consent forms given to allparticipants.

2. You must inform UREC, in advance, of any ethically-relevant deviation in the project.This may require additional approval.

You may now commence your research according to the protocols approved by UREC. We wish you every success with your project.

Yours sincerely,

Nigel Adams
Deputy Chair, UREC

cc: Asher Lewis

Appendix 2 – Advertisement

Have you been feeling stressed out recently?

Interested in helping out with a research study that looks to find out whether gentle osteopathic techniques together with stress management education can relieve your stress?

20 Volunteers Required

We are looking for volunteers (10 females and 10 males) aged 18 years or older to participate in a study on **the effects of a proposed therapy package on physiological and perceived stress in females and males: A pilot study.**

This study will involve 20 participants (10 females and 10 males) who will be required to visit Clinic 41, Unitec, on two occasions. The first visit will take about 60 minutes and is designed to assess baseline health and stress levels, followed by a 30-minutes session of gentle osteopathic techniques together with 10 minutes of stress management education. The second and final session (48-72 hours after session 1) will take around 50 minutes and will involve 30 minutes of gentle osteopathic techniques and 10 minutes of stress management education. Both sessions will commence at about 8am. A small amount of saliva sample will be collected during these sessions to measure any changes in your stress level.
You should only volunteer for the study if you are willing to commit to attend both sessions.
You should not volunteer for the study if you have diabetes, heart conditions, any known chronic inflammatory condition, cancer, breathing difficulties, presence of oral disease, any known musculoskeletal conditions and current use of steroidal medications (other than vitamins).

Please email: stressed2019@gmail.com, with your phone number.
This project has been reviewed and approved by Human Ethics Committee of Unitec Institute of Technology

Appendix 3 – Patient Information Sheet

Information for participants

Research Project Title: The effects of a proposed therapy package on physiological and perceived stress in females and males: A pilot study.

Synopsis of project:

The present study is part of a pilot study designed to examine the potential effect of a proposed therapy package on stress in a small number of women and men by measuring both perceived stress and physiological stress. This project will be carried out in two parts, by two student researchers. Part 1 will examine the effect of the proposed therapy package on females, whereas Part 2 will examine the effect of a proposed therapy package on males.

What we are doing

We want to assess the health status in ten males and ten females using health screening measures (blood pressure, pulse and adapted medical history). Additionally, we want to determine baseline levels of selected physiological stress biomarkers in saliva samples of males and females. Furthermore, we will be assessing a baseline perceived stress in ten males and females using stress questionnaires. We want to undertake a study to assess the effects of a proposed therapy package on stress, delivered in two consecutive sessions. Therapy package: general osteopathic techniques (GOT) that include gentle rhythmic techniques followed by stress management education.

What it will mean for you

- You will receive two sessions (within a week, 24 hours apart) of a proposed therapy package comprising of ten general osteopathic techniques, 10-minutess of stress management education and you will also be provided with a stress management education pamphlet.

- Between these two sessions, saliva samples will be collected, which will require you to passively drool into a tube.
- Finally, we will ask you to fill in two stress questionnaires.
- Each session will be approximately 1 hour, and will take place at Clinic 41, Unitec (you will receive a petrol voucher of $20 to cover your travel expenses).

If you agree to participate, you will be asked to sign a consent form. This does not stop you from changing your mind if you wish to withdraw from the project. However, because of our schedule, any withdrawals must be done up until the end of data collection (after session 2).
Your name and information that may identify you will be kept completely confidential. All information collected from you will be stored on a password protected file and only you, the three researchers and our supervisors will have access to this information.
Please contact us if you need more information about the project. At any time if you have any concerns about the research project you can contact our supervisor:
Our supervisors are Shamim Shaikh and Sylvia Hach, phone +64 9 892 8286 (Shamim); +64 21 673 121 (Sylvia) or email sshaikh@unitec.ac.nz or shach@unitec.ac.nz

UREC REGISTRATION NUMBER: *#2018-1066*
This study has been approved by the UNITEC Research Ethics Committee from *04/11/2018* to *16/12/2019*. If you have any complaints or reservations about the ethical conduct of this research, you may contact the Committee through the UREC Secretary (ph: 09 815-4321 ext 8551). Any issues you raise will be treated in confidence and investigated fully, and you will be informed of the outcome.

Appendix 4 – Participant Consent Form

Research Project Title: The effects of a proposed therapy package on physiological and perceived stress in females and males: A pilot study.

I have had the research project explained to me and I have read and understand the information sheet given to me.

I understand that I don't have to be part of this research project should I chose not to participate and may withdraw at any stage, without any disadvantage, up until the end of the data collection (completion of the session 2).

I understand that everything I say is confidential and none of the information I give will identify me and that the only persons who will know what I have said will be the researchers and their supervisors. I also understand that all the information that I give will be stored securely on a computer at Unitec for a period of 10 years.

I understand that I can see the finished research document.

I have had time to consider everything and I give my consent to be a part of this project.

Participant Name: ………………………………………………………………........

Participant Signature: ………………………….. *Date:* ……………………………

Project Researcher: …………………………….. *Date:* ……………………………

UREC REGISTRATION NUMBER: *#2018-1066*

This study has been approved by the UNITEC Research Ethics Committee from *04/11/2018* to *16/12/2019*. If you have any complaints or reservations about the ethical conduct of this research, you may contact the Committee through the UREC Secretary (ph: 09 815-4321 ext 8551). Any issues you raise will be treated in confidence and investigated fully, and you will be informed of the outcome.

Appendix 5 – General Osteopathic Therapy package

The following GOT techniques have been selected from the Handbook of Osteopathic Technique (Hartman, 1997).

Body region	Protocol of Technique
Hip	- The student researcher stands beside the participant, flexing the patient's leg on their thigh, then places the patient's thigh against the student researcher's abdomen. - A rhythmic circumduction movement is applied at the hip joint, whilst applying a gentle downward pressure towards the table.
Sacroiliac joint	- The student researcher stands beside the participant, flexing the patient's leg on their thigh, then places the patient's thigh against the student researcher's abdomen. Adduction and internal rotation is added with their leg. - The pads of the fingers of the palpating hand are on the sulcus of the joint (S2 level). A rhythmic and gentle circumduction movement is applied, whilst maintaining the vectors (adduction and internal rotation).
Lumbar spine	- The student researcher places her palpating onto the spinous processes of the lumbar spine. - A rhythmic circumduction movement is applied at the hip joint, whilst applying a forward force with the patient's leg, resulting in a flexion movement at the lumbar spine.
Thoracic spine/rib cage	- The student researcher stands beside the participant and holds their arm into abduction and extension, and use internal rotation to put the glenohumeral capsule on tension. - The student researcher places their thumb and thenar eminence on the intercostal space. Rocking one foot to the other, the researcher applies a careful pressure to the lower two rib heads at their joint articulation with the transverse processes of the spine. - The student researcher moves up the ribcage using the same process, applying the same pressure to all the rib heads at their joint articulation with their associated vertebral attachments.
Shoulder/ gleno-humeral joint	- The student researcher stands beside the participant and presses the participants acromion process and clavicle with a gentle force, caudally. - Different degrees of rotation and abduction can be introduced, with the circumduction movement, to focus on parts of the joint.
Scapulo-thoracic	- The student researcher stands beside the participant and abducts the patient's arm. The researcher then places both hands beneath the participant, resting the pads of their fingertips onto the medial border of the participants scapula.

	- The participants arm (lever) is placed between the student researcher's body and arm (rostal) and an anti-clockwise circumduction movement is performed, whilst applying a gentle upward pressure and stretch with the fingertips.
Cervical spine	- The student researcher stands at the head of plinth, facing the participant and places the palm of both of their hands along the occipital bone (superior nuchal line) with their fingers beside each spinous process. - The student researcher gently lifts the head of the participant, so that the head and neck are aligned. Then a gentle rhythmic circumduction movement is applied, whilst keeping both the head and neck of the participant as a unit. - A clockwise movement is used for the right side and an anticlockwise movement for the left.
Upper trapezius and cervical cross-handed stretch	1) Upper trapezius stretch: - The student researcher stands slightly to the side of the head of the table and the supports the head of the patient with his/her upper hand. - The tip of the shoulder is fixed with the other hand. Then a stretch is applied with the head hand, pulling into a combination of flexion, side-bending and rotation (away). 2) Cross-handed stretch: - The student researcher crosses their forearms, with the palm of their hands facing towards the ground. The student researcher's hands are then pressed downward, with the patient's head in their forearms. As the student researcher pushes down with their hands, they lift up with their body, through their forearms to allow a gentle flexion stretch through the patients head and neck.
Cervical erector spinae cross fibre soft tissue	- The student researcher stands at the head of plinth, facing the participant. - The practitioner fixes the participants head into the pillow with one hand applied to the forehead. Then the fingertips of the other hand are placed parallel to the spinous processes. - An upward force is applied to the orbit, whilst adding a lateral pulling force.
Suboccipital inhibition	- The student researcher stands at the head of plinth, facing the participant. - The student researcher places her fingertips under the participants head, just below the cranium. The back of the forearms is applied to the pillow with the elbows over the end of the table. - Using her arms/elbows as a lever, the practitioner will apply an upward pressure toward the orbits.

Appendix 6 – Scripted stress management education package

Stress is a normal psychological and physical reaction to the ever-increasing demands of life; however, there are both positive and negative stressors. Learning to identify and manage your stress can help you to cope with life's daily challenges. Our thoughts have a very powerful effect on our feelings and therefore on the level of stress we encounter.

Stress Awareness using the ABC model:
Most of us tend to think that challenging events occur and they automatically trigger the fight/flight response; however, this is not correct. There is another stage that happens afterthe event and influences whether the event will trigger the fight/flight response. This all comes down the role that our thoughts, beliefs, and self-talk play in triggering the fight/flight response. This is known as the **ABC model.**

A stands for Activating event: This is an event that you perceive as being stressful.
B stands for your belief: These are your underlying beliefs about the activating event.
C stands for consequences: There are choices that you make or the emotions that you based on A and B.

A does not equal C. If A=C, then we would all react the same when faced in front of a stressful event.
D= Dispute. Dispute the negative self-talk that happens because of your belief.
For example:
A= Your work colleague is late for work.
B= Your work colleague is always irresponsible and selfish.
C= You feel very angry and do not speak to your work colleague, nor you cover their work station before he arrives.

By disputing the "B", your stress level can be manipulated. You are able to choose how "A" influences you. Try to avoid negative self-talk. For example, look how disputing the belief changed the outcome:

A= Your work colleague is late for work.

B= Your work colleague must have a lot going on in his home and you can make time to cover for him while he arrives.
C= You greet your work colleague warmly and are both caught up ready to start a productive day.

Behavioural intervention - Abdominal breathing relaxation exercise

This exercise will be focussing on your breathing and guide you to focus on each stage of a breath as you breathe slowly and gently.

Let's begin. Throughout this breathing awareness exercise, breathe in this way:
Breathe into the count of four, hold for the count of 3, and breathe out to the count of 5.
It goes like this:
Breathe in...2....3....4..... hold...2...3....exhale...2....3....4. ..5...
Breathe in...2....3....4..... hold...2...3....exhale...2....3....4. ..5...
Breathe in...2....3....4..... hold...2...3....exhale...2....3....4. ..5...
Breathe in...2....3....4..... hold...2...3....exhale...2....3....4. ..5...

Continue to breathe at this slow pace.
While you are breathing slowly, I'll direct your breathing awareness to different stages of the breath. Focus all of your attention on each stage I mention.
First, notice the breath as it enters your nose. Notice each time you breathe in, the way the breath feels on your nostrils
Feel the breath as it passes through your nasal passages, and down behind your throat.
Where does the air go next? Feel each time you inhale, the breath passing down your windpipe.

Feel the breath going down.....
Feel the breath going down....
Notice where the air enters your lungs. Allow your breathing awareness to deepen the feeling of relaxation you are experiencing.
Feel the air expand your lungs with each in the breath.
Feel your lungs expand... and relax.... expand.... and relax..... expand.... and relax.....
Now notice the exhalation phase of breathing. Observe as the air leaves your lungs and begins to travel upward. Focus your attention on that moment of each breath.
Now turn your attention to the breath traveling up and out, through your mouth. Feel the breath in your throat, your mouth, and across your lips.

Notice each breath as a whole now. See how the breaths flow like waves. Fist in.... and then a pause.... and out.... and then a pause.... Notice the pauses; these rests between breaths.

Now as you relax... you can count your breaths as they continue to flow gently. Count 10 breaths.

(pause)

When you are finished counting your breaths, notice how calm and relaxed you are. See how regular your breathing has become... how calm your breathing is.

When you are ready to return to your day, you can reawaken your body and return to the present. I'll count to five. With each number, you can become more and more alert, reaching full alertness when I reach 5.

Appendix 7 – Stress management pamphlet

What is stress? Stress is a normal psychological and physical reaction to the ever-increasing demands of life; however, there are both positive and negative stressors. Learning to identify and manage your stress can help you to cope with life's daily challenges.

3 Steps for better managing your stress:

1. Establish what is stressing you the most within your life.
2. Find ways to reduce the stressor.
3. Make changes and prioritise your life suitably, with the list of stress reducers below.

Here is some options on how to reduce your stress:

From the stress reducers; pick a few to decide which works best for you.

Sleep: Getting 7-9 hours of sleep each night is fundamental in maintaining your body's overall health and wellness.

Visualisation: Try to create mental pictures of stress flowing out of your body, or of your problems, your distractions, and your everyday concerns being folded away and stashed in a padlocked chest.

Exercise: This not only releases chemicals that help you to feel happy, but it helps to break down the chemicals that raise your stress levels. A regular exercise schedule can help regulate naturally-occurring hormones.

Deep breathing: Deep breathing in through the nose and out through the mouth for a couple of minutes has shown to calm your nervous system and allow you to have a better discerning of things.

Learn to say no: You don't have to say yes to everything. If it doesn't help you achieve your goals, it really is all right to say no.

Journal: Keeping a gratitude journal or journaling about life has been shown to help boost optimism and can help to take burdens from your shoulders.

Do something you enjoy: Take a couple of minutes to unwind has shown to reduce stress quite significantly. Try taking up a new hobby, playing music, or painting nails.

Spend time with others: Although it may seem daunting at first, getting out and socializing can help reduce risk factors for stress.

Creating and sticking to a schedule will help you to prioritise those things of most importance and then add in stress reducers. Try to eliminate unnecessary stressors. Finally, make appropriate lifestyle changes for healthier living.

Appendix 8 – Patient medical case history form

Date: ______/____/______ **STRESS ASSESSMENT THROUGH PSS:**

Participant Number: ________________

DOB: _____/______/______

Marital status: **Single/ Married/ in a relationship/ any other**

Height: **Blood Pressure:**

Weight: **Heart Rate:**

SYSTEMS REVIEW

In the past month, have you had any of the following problems?

Please tick if 'Yes' or leave blank if 'No'.

GENERAL

- ❑ Recent weight gain; how much____
- ❑ Recent weight loss: how much____
- ❑ Fatigue
- ❑ Weakness
- ❑ Fever
- ❑ Night sweats
- ❑ Increasing constipation
- ❑ Heartburn

NERVOUS SYSTEM

- ❑ Headaches
- ❑ Fainting, loss of consciousness or sudden spontaneous falls (drop attacks)
- ❑ Memory loss
- ❑ Dizziness
- ❑ Dysarthria (difficulty speaking)
- ❑ Dysphagia (difficulty swallowing)
- ❑ Diplopia (Double vision)
- ❑ Numbness or tingling
- ❑ Nausea

Appendix 9 – POMS questionnaire

Name: ______________________________ Date:

Below is a list of words that describe feelings people have. Please **CIRCLE THE NUMBER THAT BEST DESCRIBES HOW YOU FEEL RIGHT NOW**.

	Not At All	A Little	Moderately	Quite a lot	Extremely
Tense	0	1	2	3	4
Angry	0	1	2	3	4
Worn Out	0	1	2	3	4
Unhappy	0	1	2	3	4
Proud	0	1	2	3	4
Lively	0	1	2	3	4
Confused	0	1	2	3	4
Sad	0	1	2	3	4
Active	0	1	2	3	4
On-edge	0	1	2	3	4
Grouchy	0	1	2	3	4
Ashamed	0	1	2	3	4
Energetic	0	1	2	3	4
Hopeless	0	1	2	3	4
Uneasy	0	1	2	3	4
Restless	0	1	2	3	4
Unable to concentrate	0	1	2	3	4
Fatigued	0	1	2	3	4
Competent	0	1	2	3	4
Annoyed	0	1	2	3	4
Discouraged	0	1	2	3	4
Resentful	0	1	2	3	4
Nervous	0	1	2	3	4
Miserable	0	1	2	3	4

	Not At All	A Little	Moderately	Quite a lot	Extremely
Confident	0	1	2	3	4
Bitter	0	1	2	3	4
Exhausted	0	1	2	3	4
Anxious	0	1	2	3	4
Helpless	0	1	2	3	4
Weary	0	1	2	3	4
Satisfied	0	1	2	3	4
Bewildered	0	1	2	3	4
Furious	0	1	2	3	4
Full of Pep	0	1	2	3	4
Worthless	0	1	2	3	4
Forgetful	0	1	2	3	4
Vigorous	0	1	2	3	4
Uncertain about things	0	1	2	3	4
Bushed	0	1	2	3	4
Embarrassed	0	1	2	3	4

THANK YOU FOR YOUR COOPERATION

PLEASE BE SURE YOU HAVE ANSWERED EVERY ITEM

Appendix 10 – PSS-10 questionnaire

Participant Number:________________ **Date: ________________**

For each statement, please tell me if you have had these thoughts or feelings: (0)= never, (1)= almost never, (2)= sometimes, (3)= fairly often, or (4)= very often.

	Never	Almost Never	Sometimes	Fairly Often	Very Often
In the past **week**, how often have you been upset because of something that happened unexpectedly?	0	1	2	3	4
In the past **week**, how often have you felt unable to control the important things in your life?	0	1	2	3	4
In the past **week**, how often have you felt nervous or stressed?	0	1	2	3	4
In the past **week**, how often have you felt confident about your ability to handle personal problems?	0	1	2	3	4
In the past **week**, how often have you felt that things were going your way?	0	1	2	3	4
In the past **week**, how often have you found that you could not cope with all the things you had to do?	0	1	2	3	4
In the past **week**, how often have you been able to control irritations in your life?	0	1	2	3	4
In the past **week**, how often have you felt that you were on top of things?	0	1	2	3	4
In the past **week**, how often have you been angry because of things that happened that were outside of your control?	0	1	2	3	4

Appendix 11 – Enzyme Linked Immunosorbent Assay Method

The ELISA method specifically captures antibodies, which were coated onto a 96-well plate, which was performed as per the manufacturer's instructions (Abcam, 2019; "Human IgA, Secretory ELISA Kit," 2012). The Research Fellow analysed all biological samples at the Spinal Cord Injury Research Unit, Centre for Brain Research, University of Auckland. Standards and samples were pipetted into wells; the target protein in the standards and samples bind to the immobilised antibody. The wells were washed, and the biotin-labelled detection antibody was added. After washing away the unbound biotinylated antibodies, Horse Radish Peroxidase conjugated streptavidin was pipetted onto the wells. A colourimetric substrate solution was added. The intensity of the colour development in the wells is proportional to the amount of target protein bound. Saliva samples were thawed at 4°C, where the samples were freeze thawed once to aliquot appropriately for use in a specific ELISA (25µL starting sample for Cortisol, 50µL starting sample for IL-6, and 100µL starting sample for sIgA). Samples were then frozen at -80°C. The appropriate set of saliva aliquots was thawed at 4°C on the day of the assaying. The samples were analysed using the ELISA kits: Cortisol ELISA kit(catalogue no. ab154996, Abcam, USA), IL-6 ELISA kit (catalogue no. BE58061, IBL International, Germany) and sIgA ELISA kit (catalogue no. LS-F4522, LifeSpan BioSciences, Inc., USA). During this process, the ELISA kits and saliva samples were brought to room temperature for 30-minutes, then duplicates containing each standard (using the diluents provided in the kits), or saliva samples were analysed according to the manufacturer's instructions.

The absorbance was read at 450nM using 72 an X-Mark Microplate Reader (*Microplate Absorbance Reader Instruction Manual*, 2012). The data generated was then processed iusing a free online tool called "My Assays", which allows the mean absorbance of each set of duplicate standards and samples to be calculated. Afterwards, the mean optical density of the zero standard was subtracted. A graph of the standard was then produced using a four parameter logistic curve to produce the most appropriate line of fit. The equation for this was then used to determine the concentration of the samples assessed. Statistical analysis was achieved using GraphPad Prism 7.03 using ANOVA.

The Sandwich immunoassay method is a powerful tool for quantifying proteins and qualifying their state of activation in complex biological samples. This method uses two antibodies, which bind to different sites on the antigen. The captured antibody, which is highly specific for the antigen (cortisol, sIgA, IL-6), is attached to a solid surface. The antigen is then added, followed by the addition of a second antibody referred to as the "detection antibody". The detection antibody binds the antigen at a different epitope than the capture antibody. As a result, the antigen is "sandwiched" between the two antibodies. The antibody binding affinity for the antigen is usually the main determinant of immunoassay sensitivity. As the antigen concentration increases, the amount of detection antibody increases, leading to a higher measured response. The standard curve of a sandwich-binding assay has a positive slope. To quantify the extent of binding, different reporters can be used (Cox et al., 2004).

The Competitive binding assay method uses both competitions of labelled and unlabelled ligand sites. Competitive binding only uses one antibody and assays are mainly used to assess smaller analytes (Wild, 2013). These assays are also used when a matched pair of antibodies to the analyte is absent. Initially, the primary antibody is added to the antigen and the antibody-antigen complexes are added to wells coated with the same antigen. The antibody is washed off after an incubation period. The higher the quantity of antigen in the sample, the more primary antibody will be bound to the sample antigen. A specific number of labelled ligand and a variable number of the unlabelled ligand are added to the antibody. As the concentration of unlabelled ligand increases, fewer labelled ligands will be able to bind to the antibody, resulting in a decreased measured response. The standard curve of the competitive binding assay is primarily negative. The total volume of saliva used was 100µL aliquots, which was separated into two separate assays, these were run in duplicates. The main advantages of this type of ELISA are its high sensitivity to an array of complex antigen mixtures. Furthermore, due to the suitability for complex samples, this method does not require the antigens to undergo purification before analysis (Osmekhina, Neubauer, Klinzing, Myllyharju, & Neubauer, 2010; Wild, 2013). For samples which were insufficient in volume to carry out all three assays, performing the cortisol assay was prioritised. Samples with less than desired volume were diluted (where necessary) and a dilution factor applied accordingly.

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Year of presentation: 2020

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