University of Southern Queensland

Exploring the Effects of Acceptance and Commitment Therapy (ACT) on Biomarkers of Stress in Breast Cancer Survivors

Bachelor of Science (Honours)

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Date: 9th November 2018

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Abstract

Breast cancer is the most commonly diagnosed cancer in Australian women, with advances in detection and treatment of breast cancer there is a current overall five-year survival rate of approximately 90%. For some individual breast cancer survivors, survival is associated with psychological and physiological stressors that can negatively affect well-being and quality of life as well as have a further adverse impact on long-term survival. These stressors can affect the body's physiological stress response system, causing allostatic load (AL) and dysregulations that may be associated with symptoms such as fatigue, depression, anxiety, hypertension, and inflammation. AL is a multisystem approach to measure the cumulative negative effects that stress has on the body's systems and overall health. Psychological and physiological stress is also associated with accelerated cellular aging and chromosome instability caused by telomere attrition. These cellular deficits can lead to an increased risk for the development of other diseases such as cardiovascular disease, diabetes and cancer recurrence.

Recent research has provided evidence that mindfulness-based psychological interventions that have been developed to meet the needs of breast cancer survivors can improve their psychological well-being and quality of life. Furthermore, other research has demonstrated that these interventions can lead to a reduction in physiological stress biomarkers. Physiological stress biomarkers indicate the activity of the stress response system, therefore a reduction subsequent to participation in a psychological intervention may indicate clinical health benefits.

The aim of this pilot study was to examine the effects of Acceptance and Commitment Therapy (ACT), a psychological treatment that incorporates acceptance and mindfulness strategies, on physiological and molecular biomarkers of stress and AL in breast cancer survivors. Breast cancer survivors, who had completed primary breast cancer treatment in the previous two years, were randomly allocated into three groups. Two groups attended ninety minutes of either a group-based ACT (Group 1) or Breast Cancer Education (BCE) program (Group 2) for six weeks; these two groups then crossed-over to the other

intervention for a further six weeks. The third group (Group 3) was waitlisted for the first six weeks and then attended the ACT program for the subsequent six weeks. Physiological and molecular biomarkers of stress: heart rate, blood pressure, fasting blood glucose, telomere length, interleukin-6, cortisol and salivary alpha-amylase, were assessed before and after each intervention or wait-list, with a fourth measurement time point at six months after completion of the interventions. Six of these biomarkers were used to calculate the change in AL for each participant in response to the ACT intervention.

The study identified a significant reduction in cumulative allostatic load in Group 3 participants following the ACT intervention, but AL was increased in Groups 1 and 2. The study did not identify a statistically significant reduction in any individual biomarker from pre- to post-6 week ACT intervention for any group however, a reduction in blood pressure in Group 1 participants after 12 weeks (ACT and BCE) was evident. These results suggest that the current ACT intervention could drive improvements in AL, but the effects are highly variable. It may be that a longer intervention is required before a change in physiological and molecular biomarkers becomes measurable. Alternatively, it is plausible that the majority of participants in this study were not under substantial stress at the beginning of the trial and therefore their stress biomarkers were not elevated and had no potential to improve. A future trial could specifically recruit participants with elevated stress biomarkers.

Keywords

Allostasis, allostatic load, acceptance and commitment therapy, stress biomarkers, relative telomere length, salivary cortisol, salivary alpha-amylase, cytokines, interleukin-6.

Declaration

I hereby certify that the experimental work, results, analyses, discussion and conclusions reported in this dissertation are entirely my own effort, except where otherwise acknowledged, I also certify this work is original and has not been previously submitted.

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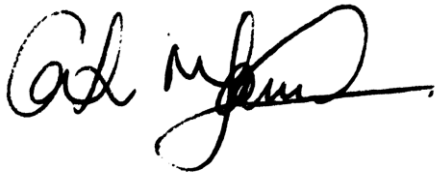

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Endorsement

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List of Abbreviations

ACT	acceptance and commitment therapy
ACTH	adrenocorticotropin hormone
AL	allostatic load
ALI	allostatic load index
CNS	central nervous system
CRH	corticotropic releasing hormone
CVD	cardiovascular disease
FCR	fear of cancer recurrence
HPA axis	hypothalamic-pituitary-adrenal axis
IL-1β	interleukin-1β
IL-1ra	interleukin-1 receptor antagonist
IL-6	interleukin-6
MBI	mindfulness-based intervention
MBSR	mindfulness-based stress reduction
PBMC	peripheral blood mononuclear cells
PSNS	parasympathetic nervous system
PVN	paraventricular nucleus
QoL	quality of life
RTL	relative telomere length
sAA	salivary alpha amylase
SNS	sympathetic nervous system
sTNF-RII	soluble tumour necrosis factor receptor type 2
TNF	tumour necrosis factor

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Chapter 1: Introduction and literature review

1.1 Breast cancer survivorship

Breast cancer is the most commonly diagnosed cancer in Australian women and was the second most commonly diagnosed cancer in Australia in 2014 (Australian Institute of Health and Welfare (AIHW) 2017). There were 16,753 new cases of breast cancer in Australia in 2014 (140 men and 16,613 women), with one in eight Australian women diagnosed with breast cancer by the age of 85 years (AIHW 2017). Due to advances in cancer treatments and increased rates of early detection, the overall five-year survival rate for breast cancer has increased to 90% (AIHW 2017). It is predicted that there will be 18,235 new breast cancer cases in 2018 (AIHW 2017) and therefore, over 16,000 new breast cancer survivors in 2018.

Despite the high survival rates, breast cancer is a stressful experience (McGregor & Antoni 2009), with breast cancer diagnosis, treatment and survivorship found to be associated with a complex array of stressors, all of which can impact survivor's wellbeing and quality of life (QoL). The extent to which these stressors are experienced by the individual breast cancer survivor will vary due to individual characteristics such as age, stage of cancer, treatment modality, social support and coping ability (Boyes et al. 2009).

The early or extended survivorship period is defined as the period from completion of primary treatments (including surgery, radiotherapy and chemotherapy), to up to five years after completion of treatment (Boyes et al. 2009). This period of survivorship is a vulnerable phase in which survivors may face multiple physiological and psychological issues (Cheng et al. 2014).

Physiological issues may be treatment-related, including persistent and/or late emerging symptoms such as fatigue, sleep disturbance, physical function decline, pain, lymphoedema, peripheral neuropathy (Alfano et al. 2016; Cheng et al. 2016), vasomotor complaints including hot flushes and cold sweats (Fallowfield & Jenkins 2015), sexual dysfunction, early onset menopause and infertility (Boyes et al. 2009). Although not life threatening, these

consequences of treatment may compromise daily activities and ability to return to pre-cancer life, potentially negatively affecting individual survivors overall sense of well-being and QoL (Cheng et al. 2016).

Breast cancer survivors often report experiencing psychological symptoms such as emotional distress and mood disturbance including depression and anxiety (Matchim et al. 2011; Starkweather et al. 2013). These psychological issues may continue from the stress experienced during diagnosis and treatment of breast cancer or they may arise due to new issues in the survivorship period. Breast cancer survivors may experience anxiety about the transition from active treatment and their return to a 'new normal' lifestyle (Boyes et al. 2009). Breast cancer survivors may also experience stress associated with survivorship guilt, loneliness, concerns about burdening loved ones and uncertainty about the future. A common psychological issue in breast cancer survivors is fear of cancer recurrence (FCR) and, it is reported that half of all cancer survivors experience FRC (Rouleau et al. 2015). There are many potential sources of increased stress in breast cancer survivors, and increased stress can be associated with lower QoL (Custers et al. 2017).

1.2 The human stress response, allostasis and allostatic load

The human body responds to stressors (physiological and/or psychological) by activating the physiological stress response system. The physiological stress response is complex and tightly regulated. It involves multiple systems and pathways that interact to produce physiological adaptations that improve the chance of survival when faced with stressors (Smith & Vale 2006). The initial responses are referred to as 'fight or flight' responses and include increased awareness and cognition, increased heart and respiratory rates, as well as the inhibition of general functions such as feeding, digestion, reproduction and growth (Smith & Vale 2006). Once the stressor is removed, the stress response systems attempt to return to basal functioning states, thus restoring balance and homeostasis (Sturmberg et al. 2017).

The major systems involved in the stress response include the sympathetic nervous system (SNS), the hypothalamic-pituitary-adrenal (HPA) axis, and the immune system. These systems are mediated by a complex network of bidirectional feedback signals (Figure 1), in which activation or suppression of one component of the system stimulates or suppresses other components of the system (Glaser & Kiecolt-Glaser 2005; Reiche et al. 2004).

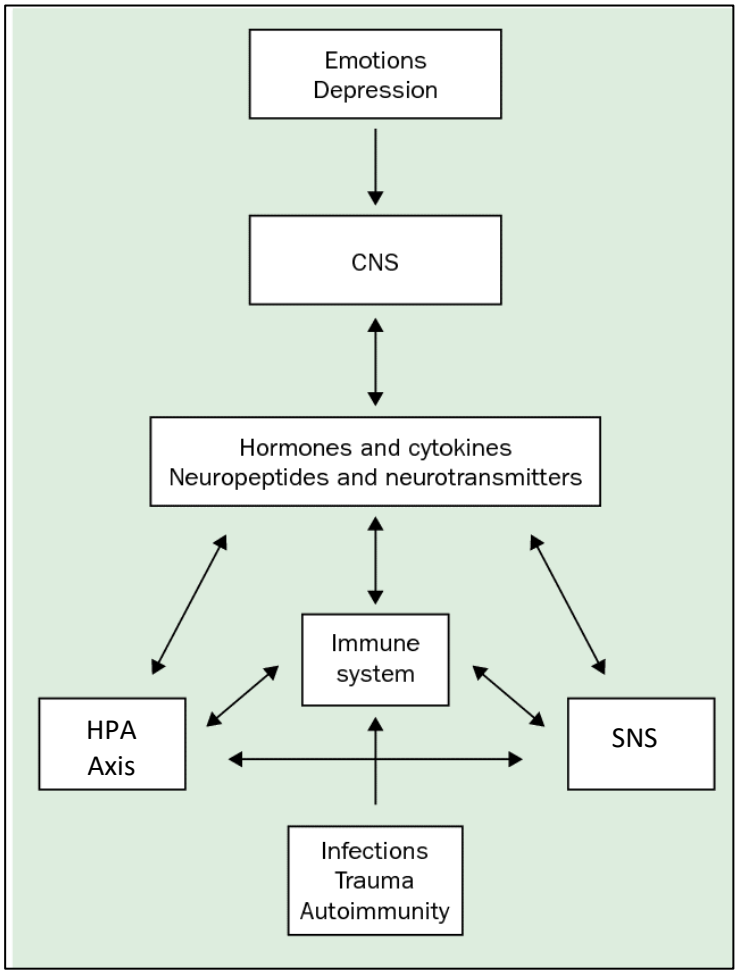

Figure 1: Stress response system network of bidirectional communication between the SNS, HPA axis and immune systems. Modified from Reiche et al. 2004

Optimal functioning of the stress response system after exposure to a stressor requires successful coordination and adaptation of all three major systems to maintain healthy functioning and homeostasis (Glaser & Kiecolt-Glaser 2005; Sturmberg et al. 2017). The dynamic and adaptive regulatory process that maintains homeostasis during exposure to stressors is defined as allostasis (McEwen 2000; McEwen 2017; McEwen & Gianaros 2010;

Streeter et al. 2012). Stress response and allostasis are protective in the short-term, but long-term activation and adaptation of the stress response systems results in allostatic load/overload (AL), potentially causing cumulative 'wear and tear' on the body's systems (Figure 2) (McEwen 2000). AL and the associated wear and tear, damages components of the stress response systems leading to dysregulations that can result in either inadequate or overactive activity (Gallo et al. 2014; Wan et al. 2016). Dysregulations can lead to hypertension, fatigue, anxiety, depression, sleep disturbance, cognitive functional decline (Sturmberg et al. 2017) and ultimately increased cellular aging and the risk of disease (Epel 2009; McEwen 2017).

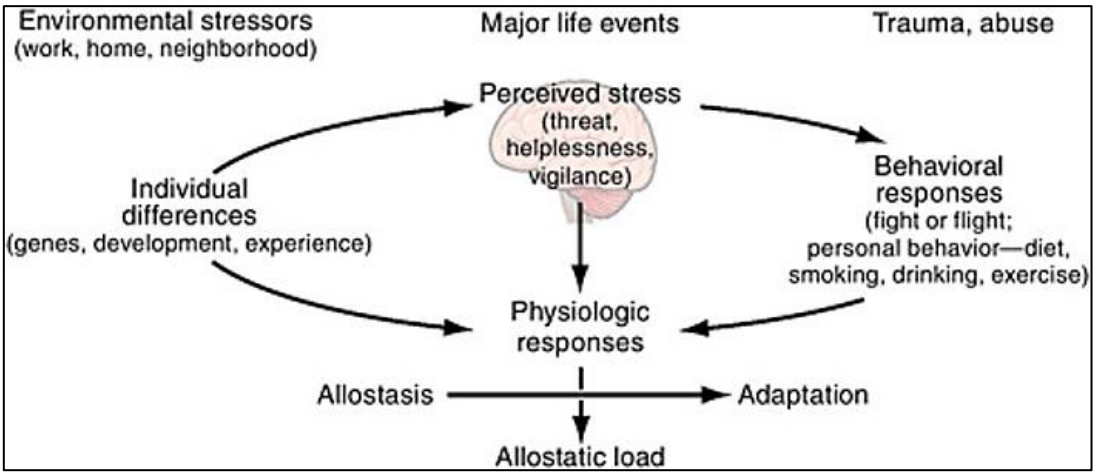

Figure 2: Model of allostasis and allostatic load (McEwen 2000).

1.2.1 The sympathetic nervous system (SNS)

The brain, via the central nervous system (CNS), perceives psychological and physiological stressors (Streeter et al. 2012), and controls how the body responds to the stressor. Interpretation of the stressor as a threat results in the suppression of the calming, parasympathetic nervous system (PSNS) and stimulation of the activating, sympathetic nervous system (SNS). Stimulation of the SNS occurs immediately following the stressor and results in the rapid release of catecholamines (noradrenaline and adrenaline), from the adrenal medulla (Ali & Pruessner 2012; Sturmberg et al. 2017), inducing the initial fight or flight responses (Hulett & Armer 2016). These responses include elevated heart rate,

increased blood pressure, and increased respiratory rate (Ali & Pruessner 2012), all of which aim to promote survival in the instance of a threat. Increased SNS activity is beneficial in the short term for survival, but sustained high SNS activity may incur negative effects such as anxiety, hypertension, inflammation and cardiovascular disease (QoL) (Raison & Miller 2003; Streeter et al. 2012).

1.2.2 The hypothalamic-pituitary-adrenal (HPA) axis

The HPA axis is the major endocrine axis that regulates circulating concentrations of the primary stress mediator, cortisol. Cortisol, is a hormone secreted from the adrenal cortex via multiple negative feedback loops (Figure 3) (Spiga et al. 2011). Circulatory cortisol concentrations follow diurnal circadian rhythms of secretion, with a peak blood concentration in the morning (half an hour to an hour after waking), followed by a steady decline over the course of the day (Matousek et al. 2011; O'Leary et al. 2016). Under basal non-stressed conditions, cortisol functions to maintain blood glucose concentrations, thus providing energy for the actively functioning brain and muscular system (Hannibal & Bishop 2014). In addition to the diurnal rhythm, the activity of the HPA axis increases as a response to stress to promote survival and avoid danger (Hannibal & Bishop 2014).

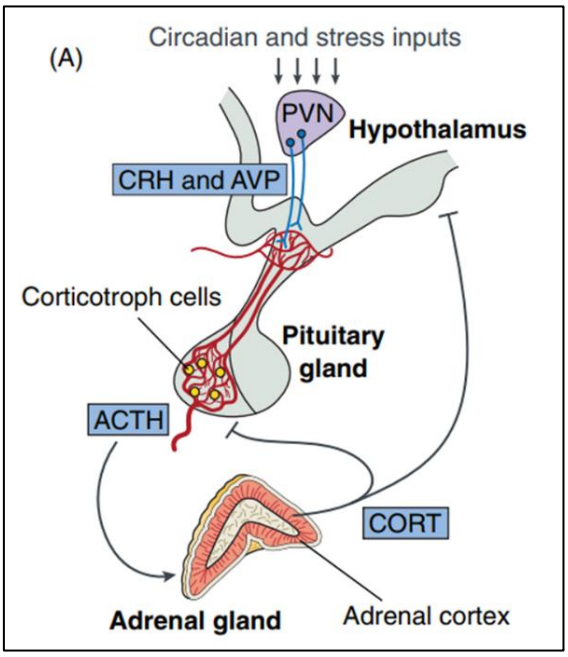

Figure 3: Hypothalamic-pituitary-adrenal (HPA) axis (Spiga et al. 2011).

The perception of a stressor (physical or psychological), activates neural pathways that induce the release of corticotropin releasing hormone (CRH) from the paraventricular nucleus (PVN) in the hypothalamus (Figure 3). CRH is carried by the portal system to the anterior pituitary gland, stimulating the release of adrenocorticotropin hormone (ACTH) into the circulatory system. ACTH binds to receptors in the adrenal cortex, stimulating the production and secretion of cortisol (O'Leary et al. 2016; Ryan et al. 2007; Streeter et al. 2012). Systemic cortisol concentration rises, to nearly double the basal concentration, approximately 15 minutes after the onset of stress and can remain elevated for several hours (Hannibal & Bishop 2014). The increased concentration of cortisol mobilises stored glucose to provide the extra energy needed to maintain the flight or fight responses. Elevated cortisol also conserves energy usage through suppressing non-vital organ systems thus increasing the energy available for the stress response (Hannibal & Bishop 2014).

Elevated concentrations of cortisol regulate the stress response by negative feedback loops to both the hypothalamus and the pituitary gland, decreasing the production of both CRH and ACTH (Spiga et al. 2011). These negative feedback mechanisms adapt to the elevated concentrations of cortisol (allostasis) in order to maintaining homeostasis within the HPA axis. Once the stressor is removed, the decreased neural stimulation as well as the negative feedback loops return the HPA axis and cortisol concentration to homeostatic functioning states (Spiga et al. 2011).

Chronic stress, either continuous or repetitive, can cause chronic activation or reactivation of the HPA axis, and continuous high concentrations or repeated surges of cortisol. This can lead to AL in the HPA axis and can result in HPA axis dysregulation and dysfunction (Hannibal & Bishop 2014; O'Leary et al. 2016). The resultant cortisol dysregulation may vary due to individual characteristics such as age, sex, genetics, type of stressor, individual perception of stress and coping abilities (Sturmberg et al. 2017).

Dysregulations in the HPA axis and cortisol functioning have been found to be associated with symptoms such as fatigue (Jain 2012) and sleep disturbances (Ryan et al. 2007). Cortisol plays important roles in the regulation of both the SNS and immune systems and as such dysregulation in the HPA axis may affect these systems and result in symptoms such as anxiety, hypertension and inflammation and therefore increase the risk of disease and reduce QoL (Sturmberg et al. 2017).

1.2.3 The immune system

The immune system is also an extremely complex system, principally functioning as the human body's defence against threats, including pathogens (such as bacteria, virus and fungi) and tissue injury (Luecken & Compas 2002). The immune system is comprised of organs, vessel systems, individual cells, and signalling proteins. The primary immune organs include the bone marrow and the thymus, where immune system cells such as lymphocytes are produced and matured. The secondary immune organs include the lymph nodes, tonsils, and the spleen; these are the locations in which the lymphocytes function to defend against pathogens.

There are two arms of the immune system, innate and adaptive, the initial immune response to stressors occurs by the activation of the innate immune system. This component of the immune system is a non-specific, first line of defence that relies on a number of different types of immune cells including monocytes, macrophages, and neutrophils (Seruga et al. 2008). These immune cells are constantly in circulation and become activated when surface receptors come in contact with a range of molecules including pathological agents and endogenous molecules (Seruga et al. 2008). Activation of receptors on immune cells leads to the production of cytokines, which facilitate the inflammatory response (Seruga et al. 2008). The inflammatory response attempts to protect and defend the body against any threat to its integrity and homeostasis (Halaris 2015). This occurs by complex interactions between the activated cells and signalling molecules, resulting in the mobilisation of more immune cells, with a goal to control and eliminate the pathological agent and to enable tissue repair and regeneration (Halaris 2015). While the inflammatory response in the short-term is beneficial

and protective, longer-term or excessive activation can cause AL and dysregulations that can result in tissue damage and induce inflammatory associated diseases such as atherosclerosis, diabetes, neurodegeneration and cancer (Halaris 2015; Irwin & Cole 2011).

Cytokines, mediators of the immune system, are small soluble glycoproteins that have multiple functions including the promotion of growth, differentiation and activation of immune cells (Seruga et al. 2008). Cytokines can either have pro- or anti-inflammatory activity depending on the microenvironment. Under homeostatic conditions the inflammatory response is regulated by multiple active mechanisms that maintain a balance between pro- and anti-inflammatory cytokines (Seruga et al. 2008), when the balance is not maintained chronic immune activation and inflammation can occur.

The immune system is not only activated by pathological agents but may also be activated by psychological stress via the HPA axis and the SNS (Bower et al. 2014; Irwin & Cole 2011). Stress activates the HPA axis and SNS, and the primary mediators of these systems (cortisol and catecholamines), then alter the function of immune organs and cells (Maier & Watkins 1998). The complex interaction between these three systems involves bidirectional feedback (Figure 4) (Glaser & Kiecolt-Glaser 2005), and the resulting neuro-immune circuits coordinates the immune response with the fight or flight response maximising the overall chance of survival (Irwin & Cole 2011). Chronic stress can dysregulate the immune response by affecting the interplay between the three major stress response systems (Glaser & Kiecolt-Glaser 2005).

The SNS regulates the immune system via the release of adrenaline from the adrenal medulla into systemic circulation. Immune cells have receptors for adrenaline, and so circulating adrenaline activates immune cells to increase production of proinflammatory cytokines (Irwin & Cole 2011). The SNS also directly innervates immune sites such as the lymph nodes, thus directly delivering noradrenaline and further increasing the production of proinflammatory cytokines (Glaser & Kiecolt-Glaser 2005; Irwin & Cole 2011). Chronic stress and continued activation of the SNS results in increased production of proinflammatory cytokines.

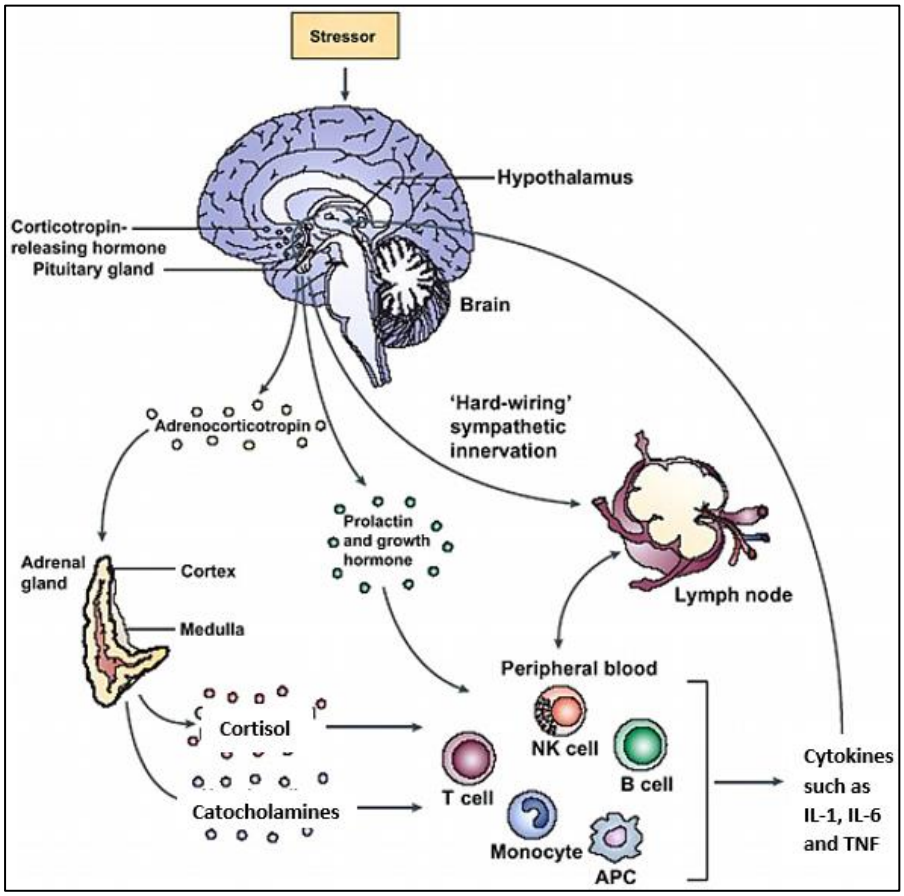

Figure 4: The stress response system. Activation of immune system via SNS and HPA axis. Bidirectional communication between immune system, SNS and HPA axis. Modified from Glaser & Kiecolt-Glaser 2005.

In addition to its role as a stress hormone, cortisol is a potent anti-inflammatory hormone, functioning to supress the production of proinflammatory cytokines (Irwin & Cole 2011). Cortisol also assists in returning the inflammatory response to homeostasis after cessation of a stressor (Raison & Miller 2003). Chronic stress and the associated dysregulation in the HPA axis and cortisol function, may result in increased production of proinflammatory cytokines and tissue inflammation (Seruga et al. 2008).

The bidirectional relationship between the immune system and both the SNS and the HPA axis means that the SNS and HPA axis influence the immune system and the immune system also has effects on these systems (Raison & Miller 2003). The increased production of proinflammatory cytokines in response to stress triggers a cluster of behavioural symptoms known as 'cytokine induced sickness behaviour' (McGregor & Antoni 2009). Symptoms include fatigue, sleep disturbance, reduced cognition and depression (Bower et al. 2011; Halaris 2015; Seruga et al. 2008). This cluster of symptoms is found to be associated with poor QoL in breast cancer survivors (Bower et al. 2011), and has also been linked to the early onset of age-related diseases and conditions including CVD, osteoporosis, arthritis, diabetes, frailty and functional decline and some cancers (Bower et al. 2014; Glaser & Kiecolt-Glaser 2005).

1.3 Biomarkers of stress

The finding that psychological stress is associated with alterations in the SNS, HPA axis and immune systems has led to the use of biological variables within these systems as possible biomarkers of stress and therefore QoL. Biomarkers are predominantly measured in blood samples, but recently saliva has been investigated as an alternative and less invasive source of biomarkers (Lipschitz et al. 2013).

1.3.1 Cortisol

Cortisol has been extensively studied and utilised as a biomarker of stress, anxiety and depression (Levine et al. 2007; O'Leary et al. 2016). Cortisol is measured in blood, urine and saliva. In blood, cortisol exists in two forms, bound and free (Levine et al. 2007). The bound form is described as the non-active form, with approximately 95% of cortisol being bound to proteins such as albumin and corticosteroid-binding globulin (Bozovic et al. 2013). The remaining 5% is the free, biologically active form (Bozovic et al. 2013). Measuring blood cortisol concentrations has been the most commonly used method in assessing cortisol but this method measures both free and bound forms. The stress from the procedure itself can

induce a stress response and therefore alter cortisol concentration, giving false results (Bozovic et al. 2013). Due to these shortcomings there is increased interest in the utilisation of salivary cortisol as a surrogate measure of free cortisol (Levine et al. 2007). Cortisol enters saliva via diffusion through the salivary glands, with the concentration of cortisol in saliva accounting for 70% of the free non-bound blood cortisol (Bozovic et al. 2013). The measurement of salivary cortisol is a minimally invasive method, therefore should not induce a stress response.

1.3.2 Salivary alpha-amylase

Methods for assessing SNS activity vary and include cardiovascular measures such as heart rate, blood pressure and urinary catecholamine concentrations (Ali & Pruessner 2012). Salivary alpha-amylase (sAA) concentration is also associated with SNS activity and has been used as a biomarker for the SNS component of the body's stress response (Ali & Pruessner 2012; Nater & Rohleder 2009; Wan et al. 2016). SAA is a digestive enzyme, secreted by the oral cavity that functions to assist in the breakdown of starch molecules from food. While sAA is not a direct by-product of the SNS, it has been found that physical and psychological stress conditions are associated with elevated concentrations of sAA (Ali & Pruessner 2012). sAA concentrations have also been found to be positively associated with overall changes in noradrenaline in response to stress (Ali & Pruessner 2012). Stress hormones of the SNS, catecholamines, are not found in saliva in reliable qualities but the association between SNS activity and sAA concentrations has led to the use of sAA as a minimally invasive surrogate measure of SNS activity (Holt-Lunstad et al. 2008; Lipschitz et al. 2013).

1.3.3 Proinflammatory cytokines

The similarities between cytokine-induced sickness behaviour and symptoms experienced by breast cancer survivors led to the investigation of mediators of the immune system as possible inflammatory biomarkers of stress (Miaskowski & Aouizerat 2012). Serum cytokines as well as downstream markers of cytokines are the most common inflammatory proteins that have been investigated (Miaskowski & Aouizerat 2012). The current method for measuring

inflammatory biomarkers is via blood specimens (Slavish et al. 2015) and further investigation is needed to identify possible inflammatory biomarkers in non-invasive samples such as saliva.

Stress is associated with immune activation and the release of proinflammatory cytokines, such as tumour necrosis factor (TNF), interleukin-6 (IL-6), and interleukin-1β (IL-1β) (Glaser & Kiecolt-Glaser 2005). These cytokines have been found to be elevated in breast cancer survivors with persistent fatigue (Barsevick et al. 2010). Concentrations of IL-1β and IL-6 have also been found to be positively associated with depressive symptoms (Law et al. 2016; Miaskowski & Aouizerat 2012; Slavish et al. 2015). Downstream markers of proinflammatory cytokines have also been measured as stress biomarkers as they are hypothesised to reflect cytokine activity (Bower et al. 2014). Higher concentrations of soluble TNF receptor type II (sTNF-RII), a marker of TNF activity, and IL-1 receptor antagonist (IL-1Ra), a marker of IL-1β activity have been identified in fatigued individuals compared to non-fatigued individuals (Miaskowski & Aouizerat 2012). The links between concentrations of proinflammatory cytokines, TNF, IL-1β and IL-6, and stress-related symptoms such as fatigue and depression has identified that these cytokines may be possible biomarkers of stress.

1.3.4 Telomere length

Chromosomal telomere length in peripheral blood mononuclear cells (PBMC) is an emerging biomarker of stress, aging and QoL. Telomeres are non-coding DNA sequences, consisting of a simple repeat sequence (TTAGG) that caps the ends of chromosomes (Epel et al. 2006). They promote chromosomal stability by preventing the loss of genetic information and protecting chromosomes from fusing together during cellular division; these processes can lead to cancer processes (Starkweather et al. 2013).

Regulation of telomere length is a complex interplay between the mechanisms of telomere attrition and elongation (Nersisyan 2016). Telomeres naturally shorten with each cell division, resulting in a gradual decline of telomere length over time, with older cells tending to have shorter telomeres compared to younger cells (Epel 2009; Starkweather et al. 2013). Telomere shortening is therefore a marker of cellular aging (Epel 2009). Critically short telomeres signal

the cell to stop dividing and enter cellular senescence (Biegler et al. 2012). Telomere shortening and cellular senescence is thought to be an indicator of bodily aging (Epel et al. 2009). Short telomeres in PBMCs have been found to be associated with increased risk factors for age-related diseases, such as, atherosclerosis, hypertension, obesity, diabetes and cancer (Biegler et al. 2012; Epel et al. 2009; Epel 2009). Telomere length may also predict cancer survival and cancer recurrence, as well as early mortality (Biegler et al. 2012; Duggan et al. 2014; Erdmann et al. 2017).

The primary mechanism of telomere length maintenance and elongation, is dependent on the expression of telomerase, an enzyme that functions by adding telomeric DNA to shortened telomeres (Epel 2009; Nersisyan 2016). Telomerase promotes cell longevity by slowing telomere shortening and therefore stopping the activation of senescence. Thus, the interaction between short telomeres and telomerase appears to affect the risk of cell death and therefore bodily aging (Epel et al. 2009).

Long-term exposure to stress and the resultant AL appears to be associated with impaired telomere maintenance having effects on both telomere length and telomerase activity (Law et al. 2016; Tomiyama et al. 2012). While the exact mechanisms in which stress affects telomere length are complicated and not fully understood, it has been identified that increases in stress response mediators such as cortisol, adrenaline, IL-6 and TNF are associated with accelerated telomere shortening and dampened telomerase activity in PBMCs (Epel 2009; Law et al. 2016; Tomiyama et al. 2012). The identification of the links between stress and telomere length has led to the measurement of telomere length in PBMCs as a biomarker of stress.

1.3.5 Allostatic load as a cumulative measure of stress

As the stress response and allostasis affects many physiological systems leading to AL, it is postulated that a cumulative, multisystem AL measure may have greater value in the assessment of stress and the associated biological burden than individual biomarkers. Extensive research into the human body's physiological responses to stress has linked

individual regulatory systems to health risks, yet little attention has been paid to the co-occurrence of physiological dysregulations across multiple systems (Seeman et al. 2010). The measure of AL by a composite score or index aims to address this limitation. AL has been proposed as a conceptualisation of cumulative biological burden that could signal early signs of dysregulations across multiple physiological systems (Seeman et al. 2001).

Allostasis and AL is proposed to occur in three stages; beginning with the initial, primary stage which is associated with acute stress and primary mediators of multiple physiological systems (McEwen 2003). Continued activation of primary mediators leads to primary effects (anxiety, sleep problems and mood changes). Primary effects and continued response to primary mediators can lead to secondary outcomes or dysregulations in multiple systems including the metabolic, cardiovascular and immune systems. The final stage is associated with tertiary outcomes including CVD, depression, cognitive decline, fatigue, cellular aging, cancer and ultimately mortality (Juster et al. 2010; Mauss et al. 2015).

The Allostatic Load Index (ALI), first developed in 1997 by Seeman and colleagues, aimed to provide an assessment of the cumulative multisystem AL associated with stress and ageing. There have been many different approaches to developing an ALI to measure allostatic load, with all approaches aiming to create a composite score based on indicators (biomarkers) from multiple systems associated with mediators, effects and outcomes of allostasis and AL (Beckie 2012). The initial ALI measure utilised 10 biomarkers (Seeman et al. 1997). Four of the biomarkers were primary mediators of AL that are secreted by the adrenal glands: cortisol, adrenalin, noradrenalin and dehydroepiandrosterone sulphate (DHEAS) (Figure 5). The remaining six biomarkers were measures of secondary outcomes of allostatic load: systolic and diastolic blood pressure, waist-hip ratio, high-density lipoprotein, total cholesterol and glycosylated haemoglobin (Figure 5). These original 10 measures were chosen by Seeman et al. (1997) as they covered both primary mediators and secondary outcomes of allostatic load, they also included multiple systems associated with allostasis. This measure of allostatic load has formed the foundation for many subsequent studies utilising an ALI but there is a large variation in the specific methods used to calculate ALI among these published studies. These variations in ALI calculation include the difference in the number and range of biomarkers

utilised (Beckie 2012; Juster et al. 2010). Read and Grundy (2012) summarised the different biomarkers that have been incorporated into ALI calculations (Figure 5).

Stress mediation	System	Biomarker
Primary mediators	Neuroendocrine	Epinepherine, norepinephrerine, dopamine, cortisol, dehydroepiandrosterone (DHEAS), aldosterone
Secondary outcomes	Immune	Interleukin-6, tumor necrosis factor-alpha, c-reactive protein (CRP), insulin-like growth factor-1 (IGF-1)
	Metabolic	HDL and LDL cholesterole, triglycerides, glucosylated hemoglobin, glucose insulin, albumin, creatinine, homocysteine
	Cardiovascular and respiratory	Systolic blood pressure, diastolic blood pressure, peak expiratory flow, heart rate/pulse
	Anthropometric	Waist-to-hip ratio, body mass index (BMI)
⬇	⬇	⬇
Tertiary outcomes	Poor subjective health, disability, cognitive decline, cellular aging, diseases, death	

Figure 5: Biomarkers used in the calculation of allostatic load (Read & Grundy 2012).

The statistical methods and approaches used to create composite ALIs have also varied among past studies. The original ALI used a count base method in which the sample distribution for each biomarker was divided into quartiles and participants with values that fell within the highest quartile were given a score of '1' or '0' for those not in the highest quartile (Seeman et al. 1997). The sum of the scores for all biomarkers resulted in the individual participant's ALI (0 – 10) (Seeman et al. 1997). Another statistical method used to measure AL is the z-score method, which is a summary measure that represents the sum of an individual's z-scores for each biomarker based on the sample's distribution of biomarker values (Juster et al. 2010; von Känel et al. 2003). This method allows the weight of each biomarker to be considered differently depending on its deviation from the mean.

The utilisation of a composite measure of AL has increased over the last two decades with the majority of studies using ALIs in relation to health and cognitive outcomes throughout the lifespan (Juster et al. 2010) or for comparing the AL of a stress or diseased cohort to a healthy cohort (Glover et al. 2006). The MacArthur Successful Aging Study was the first study that utilised an ALI as a cumulative measure of biological burden. This longitudinal study used ALI as a predictor of disease and health deterioration in the elderly (Seeman et al. 2001). This study revealed that those with higher AL showed greater declines in cognitive and physical functioning and had higher risks of CVD and all-cause mortality (Seeman et al. 2001). The results of the MacArthur Study and subsequent research has provided evidence for ALIs that utilise a multisystem measure, to potentially predict a range of health outcomes (Juster et al. 2010). Composite measures of allostatic load have been widely used in investigative studies, with the majority of research that incorporates an ALI, utilising a baseline ALI as a longitudinal predictive index in relation to health and cognitive outcomes throughout the lifespan of the cohorts (Juster et al. 2010). Although there are few studies that assess the change in AL to date, Juster et al. (2010) suggests that the next stage in AL research could be to assess the efficacy of interventions in reducing AL.

1.4 Psychological interventions and stress biomarkers in breast cancer survivors

The escalating number of survivors and the increased awareness of the psychological distress experienced by many breast cancer survivors has identified the need to investigate psychological interventions that may improve the wellbeing and QoL of breast cancer survivors. Although it has been identified that breast cancer survivors are vulnerable to increased stress and stress-related symptoms, the investigation of psychological interventions in this population is still limited (Haydon et al. 2018; Meneses et al. 2007).

Commonly, the effectiveness or efficacy of psychological interventions is measured by the analysis of participant self-assessed surveys, including validated psychological instruments or interviews that measure QoL, depression, anxiety and fatigue. Due to the increased understanding of the effects of psychological stress on physiological stress response systems, investigations into biomarkers of stress in psychological interventions has become a developing area of research (O'Leary et al. 2016), particularly in the breast cancer survivor population (Haydon et al. 2018) indicating that this area of research in breast cancer survivors is still in its infancy (O'Leary et al. 2016).

1.4.1 Mindfulness-based psychological interventions and stress biomarkers

Although past research has reported on the benefits of psychological interventions in improving QoL for breast cancer survivors, there is a limited number of studies that assess the effects on stress biomarkers. The majority of research literature exploring the effects of psychological interventions on stress biomarkers in breast cancer survivors utilises mindfulness-based interventions (MBIs). Mindfulness is defined as paying attention to the present moment, with non-judgemental acceptance of experience (Carlson 2016). The objective of mindfulness practices is to enhance the quality of being mindful in everyday life (Carlson 2016). The practice of mindfulness may improve emotion regulation, cognitive and behavioural flexibility and as such is thought to have beneficial effects on stress response systems (Epel et al. 2009). Investigations in MBIs in breast cancer survivors have assessed biological outcomes such as heart rate and blood pressure as well as biomarkers such as, cortisol, proinflammatory cytokines, and more recently telomere length and sAA.

Mindfulness-based stress reduction (MBSR) is the most commonly studied MBI in breast cancer survivors as it has been shown to improve QoL and reduce symptoms of fatigue, depression, anxiety and fear of recurrence (Carlson et al. 2004; Johns et al. 2015; Lengacher et al. 2014; Matousek et al. 2011). In addition to improving psychological symptoms and QoL, evidence is beginning to indicate that participation in MBIs such as MBSR, may be associated with alterations in biomarkers of stress (Rouleau et al. 2015).

A MBSR intervention in breast cancer post-treatment outpatients by Carlson et al. (2004) found alterations in morning cortisol concentrations. The alterations varied (increased or decreased), depending on individual pre-intervention cortisol concentrations, suggesting that MBSR has effects on cortisol concentrations but the response depends on the individual participant's initial concentrations (Carlson et al. 2004). Cortisol concentrations were also investigated by Matousek et al. (2011) in a MBSR study in breast cancer survivors. The study identified that breast cancer survivors had perturbed cortisol diurnal rhythm concentrations pre-intervention and that MBSR participation returned cortisol concentrations towards expected diurnal rhythm concentrations (Matousek et al. 2011). These two studies suggested that participation in a MBSR intervention influences the HPA axis in breast cancer survivors, as evidenced by alterations in cortisol concentrations following the interventions.

A one-year follow-up assessment of cortisol and proinflammatory cytokines after a MBSR intervention in breast cancer survivors identified that the improved cortisol concentrations resulting from the intervention were maintained after one-year and that there was also a continuing decrease in the concentration of TNF after the intervention (Carlson et al. 2007). They also identified that participation in MBSR had positive effects on blood pressure and heart rate (Carlson et al. 2007). This evidence indicates that participation in an MBI could result in long-term physiological benefits, but further longitudinal studies are required to support this concept.

Evidence is beginning to emerge that stress-reducing interventions may improve telomere length and telomerase activity (Lengacher et al. 2014). It has been reported that the psychological process of mindfulness disrupts the stress response pathways thus decreasing the physiological stress response and therefore promoting telomere maintenance (Lengacher et al. 2014). There are limited studies in breast cancer survivors that assess the effects of a MBI on telomere length and telomerase activity. A study by Lengacher et al. (2014) utilised MBSR in breast cancer survivors, and identified increased telomerase activity in PBMCs, but no changes in telomere length over the six-week intervention. This indicates that alterations in telomere length may not be evident in such short periods and so should be measured at longer follow-up time points. It is suggested that changes in telomere length in response to a

psychological intervention may take at least one year (Lengacher et al. 2014). Carlson et al. (2015) also studied the effects of MBSR on telomere length in breast cancer survivors. This study identified that telomere length was maintained over a three month intervention period in participants compared to the control condition in which it was demonstrated that telomere length decreased (Carlson et al. 2015). This result suggests that MBIs such as MBSR may slow the attrition of telomeres in breast cancer survivors experiencing psychological stress.

The effects of two mind-body interventions (mind-body bridging and mindfulness meditation) on sAA concentrations were investigated by Lipschitz et al. (2013) in a population of both male and female cancer survivors. Reductions in waking sAA concentrations post-intervention in the mind-body bridging participants were evident, suggesting positive influences of the intervention on SNS activity in cancer survivors (Lipschitz et al. 2013). The investigation of the effects of MBIs on sAA is extremely limited with the study by Lipschitz et al. (2013) being the only study in cancer survivors.

Bower et al. (2015) investigated the effects of a mindfulness meditation intervention based on mindful awareness practices, in young breast cancer survivors. Circulating biomarkers of inflammation assessed included IL-6, sTNF-RII. It was found that the intervention led to improvements in fatigue and sleep disturbance, but IL-6 was the only biomarker that decreased in concentration over the course of the intervention (Bower et al. 2015). While, there is emerging evidence that MBIs have positive effects on biomarkers of stress more studies are needed to further support the current evidence.

1.4.2 Acceptance and commitment therapy (ACT) and stress biomarkers

Acceptance and Commitment Therapy (ACT) is a psychological intervention that incorporates acceptance and mindfulness-based strategies (Graham et al. 2016) and has been shown to be effective in a diverse range of clinical conditions (Öst 2014). ACT doesn't aim to reduce physiological symptoms but rather improve psychological flexibility and therefore the ability to cope with symptoms (Harris 2006). The goal of ACT is to create a rich and meaningful life, while accepting the pain that inevitably accompanies life (Harris 2006). While ACT has not

been previously investigated in breast cancer survivors it has been found to have a positive effect on mood and QoL as a one on one intervention, in a cohort of breast cancer patients (Feros et al. 2013). ACT has also been found to be an effective intervention in participant cohorts with depression and anxiety (Feros et al. 2013), and as many breast cancer survivors experience both depression and anxiety it is hypothesised that a group-based ACT intervention may be effective in improving QoL and reducing levels of stress biomarkers in breast cancer survivors.

1.5 Knowledge gaps

The analysis of stress biomarkers is increasingly being used as a measure of efficacy for psychological interventions, indicating that the intervention may not only have effects on psychological health, but also on physiological health (Kryza 2011).

The evidence from MBIs in breast cancer survivors indicates that psychological interventions that incorporate mindfulness may be beneficial in dealing with psychological and physical stressors related to cancer diagnosis, treatment and survivorship, such as fatigue, depression, anxiety and fear of recurrence (Carlson 2016). There is emerging evidence that MBIs in the breast cancer survivor population may have an effect on stress biomarkers but it still remains unclear whether these biological changes translate to clinically important health benefits (Rouleau et al. 2015).

The use of ACT in the cancer survivorship space is in its infancy however there is emerging evidence of its effectiveness in improving psychological wellbeing and quality of life when delivered in individual therapy sessions. The effectiveness of group-based delivery of ACT in improving quality of life in breast cancer survivors has not yet been reported. It is also unknown whether an ACT intervention can affect physiological or molecular biomarkers of stress in cancer survivors.

1.6 Project hypothesis and aims

The overall project hypothesis was that a six-week program of ACT delivered in a group-based setting will lead to a reduction in physiological stress in breast cancer survivors. This reduced stress will be measured as alterations in the individual physiological and molecular biomarkers of stress, inflammation and chromosomal stability. It is also hypothesised that the ACT intervention will reduce participants' cumulative AL.

The project investigated whether ACT is beneficial in reducing physiological and molecular stress biomarkers by analysing pre- and post-intervention measures of:

1. Resting heart rate, blood pressure and fasting blood glucose;
2. Relative telomere length in PMBCs using DNA extraction and real-time polymerase chain reaction;
3. Inflammatory cytokine IL-6 using enzyme-linked immunosorbent assay (ELISA);
4. Stress hormones including salivary cortisol and salivary alpha amylase using ELISA.

This project also aimed to develop an AL change calculation that could be used to assess the change in AL that incorporates biomarkers of stress from multiple systems, including cardiovascular, metabolic, endocrine and immune system biomarkers to determine if a six-week program of group-based ACT is effective in reducing cumulative physiological stress in breast cancer survivors.

Chapter 2: Materials and Methods

2.1 Study design

The study was a randomised control crossover trial with three Groups (1, 2 and 3) and a cross over between the two interventions as outlined in Figure 6. Group 1 attended weekly 90-minute group-based Acceptance and Commitment Therapy (ACT) sessions for six weeks followed by 90-minute group-based breast cancer education (BCE) sessions for six weeks. Group 2 attended 90-minute group-based BCE sessions followed by 90-minute group-based ACT sessions. Finally, Group 3 were waitlisted and did not attend an intervention for the first six weeks and then attended 90-minute group-based ACT sessions in the following six weeks. The study had Human Research Ethics Committee approval from both the Darling Downs Hospital and Health Service (HREC/17/QTDD/51) and the University of Southern Queensland (USQ H17REA184). The trial also had approval from the St Andrew's Hospital Governance Committee to be conducted in the Hospital's group activity rooms. The study was registered by the Australia and New Zealand Clinical Trials Group (Registration number ACTRN12617001322325), http://www.ANZCTR.org.au/ACTRN12617001322325.aspx.

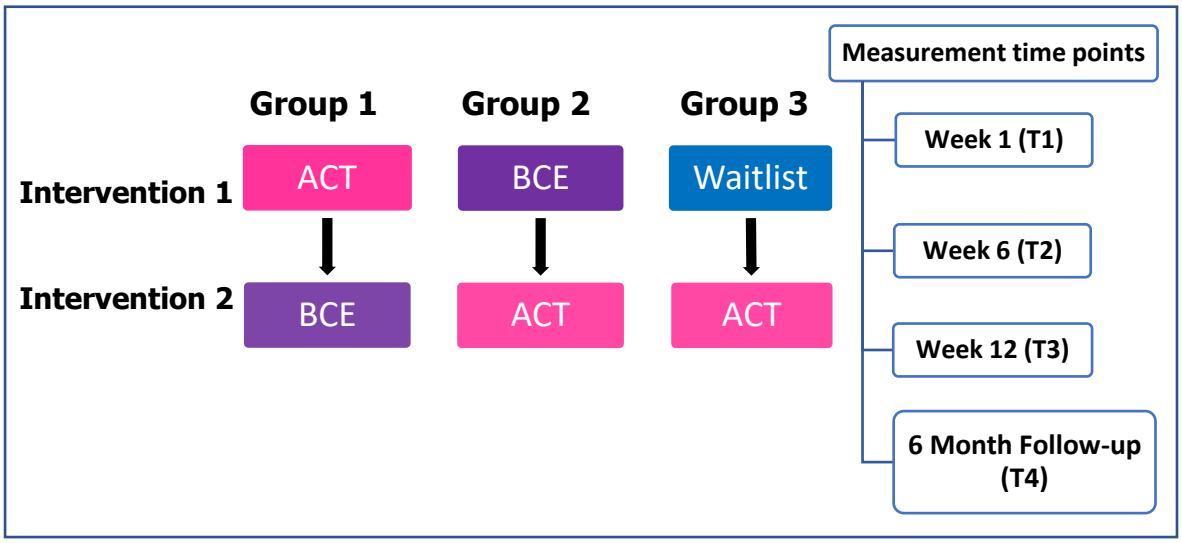

Figure 6: Diagram of the trial design and biomarker measurement time points.

2.2 Participants

Potential participants were invited by the Blush Breast Care Nurse from St Andrew's Hospital to attend an initial screening interview conducted by a clinical psychologist (USQ PhD student May Chi). Participants provided informed consent and completed an information questionnaire prior to their screening interview. Participants were informed of their eligibility at the completion of their screening interview. Eligible participants were female (older than 18 years old) who had completed all primary treatments (surgery, neoadjuvant or adjuvant chemotherapy and radiation therapy) for early (stage 1 – 3) breast cancer at least three months and no longer than two years previously. Participants were ineligible if they were currently undergoing treatment with a psychiatrist or mental health therapist and if they had a history of prior mood disorder diagnosed before their cancer diagnosis. A total of 24 individuals were enrolled in the trial.

2.3 Randomisation

Randomisation was conducted using Microsoft Excel, once 24 eligible participants were identified. Participants were randomised into one of three Groups: 1, 2 or 3, with a 1:1:1 ratio. The intervention commenced within two weeks of group allocation.

2.4 Interventions

2.4.1 Acceptance and commitment therapy

The group-based ACT intervention protocol was based on the individual session protocol utilised in the Feros et al. (2013) study, developed by Joseph Ciarrochi and John Blackledge (University of Wollongong, New South Wales). The protocol was adapted from nine, 60-minute, individual sessions to six, 90-minute group sessions. Two ACT trained psychologists facilitated the group sessions over six consecutive weeks. The protocol consisted of a

facilitator manual and individual participant work books. The work book provided structure for the four modules targeting the ACT processes of increasing effective action, orientation, mindfulness, self-context and formal value clarification and commitment.

2.4.2 Breast cancer education

The breast cancer education (BCE) intervention was utilised as a control intervention, to control for the effect of social interaction amongst breast cancer survivors (without ACT). The BCE intervention consisted of six, weekly group information sessions of 90-minutes in duration. Guest speakers who were experts in their respective topics facilitated the BCE sessions. Each speaker provided information or activities to improve the self-care of breast cancer survivors. Topics included yoga and meditation, sexuality after treatment, exercise and osteoporosis, diet and nutrition, and mindfulness. Sessions were conducted at the same time and at the same venue (St Andrew's Hospital) as the ACT sessions.

2.5 Physiological and molecular biomarkers

2.5.1 Demographics and clinical characteristics

Demographic and clinical information were obtained in the initial questionnaire (Appendix A) at the time of study enrolment, including participant's age, breast cancer clinical characteristics, treatment modalities and time since diagnosis.

2.5.2 Physiological measures

Physiological measures were assessed at four measurement time points during the study. The first measurement time point (T1) was pre-intervention 1 and was measured the morning before the Group 1 and 2 sessions began. The subsequent measurement time points were at week six (T2 - post-intervention 1/pre-intervention 2), week twelve (T3 - post-intervention 2) and at six months after completion of the second intervention (T4 - six month follow-up) (Figure 6). Group 3 participants were also required to attend all measurement time points on

the same days as participants from the other two Groups. Physiological measures included resting heart rate (bpm) and blood pressure (mmHg). Resting heart rate was measured using a fingertip pulse oximeter model SB100 (Rossmax Innotek Corp., Taipan, Tiawan) and blood pressure was measured using a Welch Allyn blood pressure cuff, stethoscope and sphygmomanometer (Hill-Rom, New York, USA).

2.5.3 Molecular biomarkers

Fasting blood specimens were collected at the same four time points as physiological measures (T1, T2, T3 and T4), by qualified phlebotomist, Edward Bliss. Blood was collected into two vacutainer tubes: one 4.5ml plasma separator tube (PST) II lithium heparin tubes and one 4ml ethylenediamine tetraacetic acid (EDTA) tube (Becton-Dickinson, Plymouth, United Kingdom). The vacutainer tubes were mixed well and then stored in a cooler until processing in the USQ laboratory. Blood collection tubes were centrifuged at 3,000 revolutions per minute (rpm) for 10 minutes to separate blood components. Plasma was removed from each tube and aliquoted into 2.5 mL microcentrifuge tubes, labelled with participant identification number, collection date, time point and stored at -80°C until molecular biomarker analysis could be performed on all specimens at one time. Buffy coats, containing peripheral blood mononuclear cells, were removed from the EDTA vacutainer tubes and stored at -80°C until DNA extraction could be performed. Fasting blood glucose was measured using an Accu-Chek Performa (Roche, Mannheim, Germany), following the collection of blood samples before venous clotting at the same venepuncture site to minimise participant discomfort.

Participants were provided with Salivettes (Sarstedt, Nübnbrecht, Germany) at study enrolment and were instructed to collect saliva upon waking, before getting out of bed, on the morning of each collection time point (T1, T2, T3 and T4) for the duration of the study (see Appendix B). Salivettes were stored at -20°C until processing in the USQ laboratory.

2.5.4 DNA extraction

Total genomic DNA was extracted from buffy coats using an Invitrogen Pure Link® Genomic DNA Mini Kit (Life Technologies Corp., Carlsbad, CA, USA). The manufacturer's protocol for human blood specimens was followed. To each specimen of 200 µL of buffy coat, 20 µL of Proteinase K and 20 µL of RNAse A was added. The mixtures were briefly vortexed and then incubated at room temperature for two minutes. 200 µL of lysis/binding buffer was then added to each tube, they were briefly vortexed and incubated at 55°C for 10 minutes. Approximately 640 µL of specimen lysates were transferred to spin columns with collection tubes and centrifuged for one minute at 10,000 rpm. Collection tubes and contents were discarded, and new collection tubes were added to the spin columns. 500 µL of wash buffer 1 was added to each spin column (containing the DNA specimens), followed by centrifugation at 10,000 rpm for one minute. Collection tubes and contents were again discarded, and new collection tubes were placed beneath the spin columns. 500 µL of wash buffer 2 was added to the spin columns, followed by centrifugation at maximum speed for three minutes. Collection tubes and contents were discarded, and spin columns placed in 1.5 mL microcentrifuge tubes. 25 µL of elution buffer was added to each spin column and each was incubated for one minute at room temperature and then centrifuged at maximum speed for one minute to remove the extracted genomic DNA from the spin column into the microcentrifuge tube. Extracted genomic DNA was then stored at -80°C until quantification and purity was assessed using a nanospectrophotometer (7122V2.4.2) (Implen, München, Germany).

2.5.5 Relative telomere length

The relative telomere length (RTL) of each genomic DNA sample was measured using a monochrome multiplex quantitative real-time polymerase chain reaction (MMqPCR) technique utilising protocols from both (Cawthon 2009) and (Hsieh et al. 2016). Genomic DNA from each buffy coat specimen was quantified and diluted in deionised water to 20 ng/mL. All specimens were assessed in triplicate reactions in one 384-well PCR plate. Each reaction was run using 2 µL of sample genomic DNA and 13µL of master mix. The master mix for each

reaction contained 8 µL of SsoAdvanced™ Universal SYBR® Green Supermix (Biorad, Hercules, CA, USA), 1 µl of 5M Betaine, 2 µL deionised water, 0.5 µL of each DNA oligonucleotide primer (Telg, Telc, Albd, Albu, see Table 1) (Integrated DNA Technologies, Republic of Singapore).

Table 1: Oligonucleotide primer sequences

Telg: 5'- ACA CTA AGG TTT GGG TTT GGG TTT GGG TTT GGG TTA GTG T - 3'
Telc: 5'- TGT TAG GTA TCC CTA TCC CTA TCC CTA TCC CTA TCC CTA ACA - 3'
Albd; 5'- GCC CGG CCC GCC GCG CCC GTC CCG CCG GAA AAG CAT GGT CGC CTG TT - 3'
Albu: 5'- CGG CGG CGG GCG GCG CGG GCT GGG CGG AAA TGC TGC ACA GAA TCC TTG - 3'

The MMqPCR was run using a C1000 Touch Thermal Cycler CFX384 real-time PCR system (Biorad, Hercules, CA, USA). The thermal cycling profile was programmed as follows: **Stage 1** - 15 minutes at 95°C. **Stage 2** - 15 seconds at 94°C, 15 seconds at 49°C, (2 cycles). **Stage 3** - 15 seconds at 94°C, 10 seconds at 62°C, 15 seconds at 74°C (with fluorescent signal acquisition), 10 seconds at 84°C, 15 seconds at 88°C (with fluorescent signal acquisition), (25 cycles). **Stage 4** - melt curve analysis (72°C - 95°C) signal acquisition at 0.5°C intervals with 30 second holds.

Signals acquired from the melt curve analysis confirmed there were two melting peaks and therefore two different amplicons. Two cycle quantification (Cq) values were obtained for each reaction, Cq (T) for the telomere primer amplicon and Cq (S) for the single copy gene alb primer amplicon. Reaction Cq triplicates were assessed for precision and Cq values that varied by more than 0.5 Cq values were removed from the analysis. Both Cq averages were calculated for each DNA sample for the calculation of RTL via the comparative delta-delta Cq method:

$$\boldsymbol{RTL} = \mathbf{2}^{-(\Delta\Delta \boldsymbol{Cq})}$$

$$= \mathbf{2}^{-\left(\boldsymbol{Sample\ Cq\ (T)} - \boldsymbol{Sample\ Cq\ (S)}\right) - \left(\boldsymbol{Reference\ Cq\ (T)} - \boldsymbol{Reference\ Cq\ (S)}\right)}$$

This method used the same control sample as the reference for all DNA samples. The difference between the telomere amplicon and the single copy gene amplicon was calculated for each sample and the reference sample to determine the telomere length of the sample relative to the reference telomere length, resulting in the RTL for each specimen.

2.5.6 Interleukin-6

Interleukin-6 (IL-6) concentrations for each plasma specimen separated from the EDTA blood tubes were quantified using a sandwich enzyme-linked immunosorbent assay (ELISA) kit, Human IL-6 ELISA MAX™ Deluxe Set (Biolegend, San Diego, CA, USA). Individual specimens were assayed in duplicate on the same 96-well ELISA plate. A total of two plates were used, both plates were run on the same day under the same conditions. A control sample was used as a quality control on both plates to allow for adjustment for inter-plate variance. All reagents and samples were prepared as outlined in the manufacturer's protocol. The ELISA was performed by initially adding 100 µL of capture antibody to all wells in both ELISA plates. Plates were then sealed and incubated at 4°C overnight. After 16-18 hours incubation to coat the wells with the primary capture antibody, the plates were then washed four times with wash buffer. The plates were then blocked by adding 200 µL of assay diluent A to each well, sealed and incubate at room temperature for one hour with shaking on a plate shaker. Plates were again washed four times with wash buffer. 100 µL of each specimen, standard or control was then added to the appropriate wells, plates were then sealed and incubated at room temperature for two hours with shaking on a plate shaker. Both plates were washed a further four times with wash buffer and 100 µL of avidin-horseradish peroxidase solution was added to each well. Plates were then sealed and incubated at room temperature for 30 minutes. A final wash was performed five times with wash buffer and 100 µL of freshly mixed TMB substrate solution was added to each well and incubated in the dark for thirty-minutes to produce a blue colour in proportion to the concentration of the IL-6 present in the specimen. 100 µL of stop solution was then added to each well turning positive wells from blue to yellow. The absorbance of each well at 450 nm was then read on a Fluostar Omega 96-well ELISA plate reader (BMG Labtech, Offerburg, Germany). Average absorbance values were calculated for each individual specimen, standard and control. Control absorbancies from both ELISA

plates were used to adjust and correct for inter-plate variance. Standard values were used to create standard curves for each plate and the IL-6 concentrations of each individual specimen were calculated from the standard curves.

2.5.7 Salivary cortisol

Cortisol concentrations in saliva samples collected from the Salivettes were measured using a competitive ELISA kit (Enzo Life Sciences, Farmingdale, NY, USA). All individual specimens were prepared by firstly centrifuging Salivettes at 3,000 rpm for five minutes and removing saliva specimens from the collecting tubes. Each specimen was then diluted to a 1:4 ratio in assay buffer as per the manufacturer's protocol for human saliva. All specimens were assayed in duplicate with all specimens for the same individual participant measured on the same 96-well plate. Two plates were used in total and both plates were assayed on the same day under the same conditions. 100 µL of assay buffer, standard or specimen was added to the appropriate wells (as determined by random allocation on a plate layout sheet). 50 µL of blue conjugate was then added to each well, except the total activity and blank wells. 50 µL of yellow antibody was added to each well, except the blank, total activity and non-specific binding wells. Plates were then sealed and incubated at room temperature on a plate shaker for two hours. After incubation, all wells were washed three times with wash solution. Wells were then emptied and 5 µL of blue conjugate was added to the total activity wells and 200 µL of the pNpp substrate solution was then added to all wells. Plates were again sealed and incubated at room temperature without shaking for one hour. The reactions were then stopped by adding 50 µL of stop solution to each well. The absorbance at 405 nm of each well was then read immediately using a Fluostar Omega 96-well ELISA plate reader (BMG Labtech, Offerburg, Germany). Cortisol concentrations for each individual saliva specimen were then determined using the same standard curve methods as described for IL-6.

2.5.8 Salivary alpha-amylase

Concentrations of alpha-amylase in saliva samples were measured from the same saliva samples as cortisol concentrations using a liquid phase enzymatic assay kit (IBL international, Hamburg, Germany). All reagent, standards and specimens were prepared as per the

manufacture's guidelines, with specimens being diluted in dilution buffer to a factor of 1:301. All specimens were assessed in duplicate, with all specimens for the same individual participants being analysed on the same 96-well ELISA plate. Two plates were used in total, both plates were assayed on the same day under the same conditions. A quality control specimen was assayed on both plates. 10 µL of each control, standard and specimen were added to appropriate wells. 200 µL of substrate solution was then added to each well and plates were gently shaken and incubated at room temperature for three minutes and the absorbance at 405 nm was promptly measured (measurement 1) using a Fluostar Omega 96-well ELISA plate reader (BMG Labtech, Offerburg, Germany). The plates were incubated for a further five minutes and absorbance at 405 nm was again measured (measurement 2). Concentrations of sAA were then calculated by determining the change in absorbances from measurement 1 to measurement 2 and then using the same standard curve methods as described previously for IL-6.

2.5.8 Statistical analysis

Data analysis was performed using Statistical Package for Social Scientists (SPSS) version 25 (IBM, Armonk, NY, USA). One-way ANOVA and parametric paired and independent t-tests were used for various forms of analyses and are described in more detail later in this section. Where the parametric tests produced significant ($p < 0.05$) results the analysis was repeated using the non-parametric equivalent test (i.e. Wilcoxon-Signed Ranks test or Mann-Whitney U test). The parametric t-test is a more powerful test unless the assumption of normality is violated. Given the small sample sizes in this study, tests of normality would be inconclusive and therefore the non-parametric test results were used as an alternate method of checking that conclusions drawn from statistically significant parametric analyses were not subject to inflated Type I error rates. In all cases the nonparametric test produced similar significant results as the parametric tests and the parametric test results are therefore reported.

Baseline descriptive statistics were calculated for four demographic variables (mean and SD) and four clinical characteristic variables (percentage frequencies and n). A one-way ANOVA was performed for each of the four demographic variables (age, time since diagnosis, weight

and BMI) to determine if there were any significant mean differences among the three groups of participants. Within each biomarker variable the by-group cell counts included at least some cells with zero frequencies or expected counts < 5, and therefore no statistical analysis (such as, χ^2 tests) were applied.

Group-by-time boxplots were created to descriptively illustrate the distributions of values observed for each of the eight biomarkers. Pair-wise dependent samples t-tests ($\alpha = 0.05$) among the different measurement time points were performed for each group separately. Due to the large number of missing values (and therefore a decrease in sample sizes from those used in the descriptive analyses) only T1, T2 and T3 were analysed for heart rate, systolic and diastolic blood pressure, blood glucose, salivary cortisol, alpha-amylase and IL-6. Only T1 and T4 were analysed for RTL as changes in RTL require longer than 6 or 12 weeks to become evident. For each biomarker variable, group-by-time bar plots showing the mean ± 2 SEM were used to illustrate the results of the dependent samples t-tests. Due to the lack of a washout period between intervention 1 and intervention 2 it could not be assumed that the effects of intervention 1 had not subsequently influenced the data recorded at T3.

To investigate any effects of the ACT intervention on a larger sample size than available within each group, the measurement time points corresponding to pre- and post-ACT intervention for all participants were combined. T1 and T2 were the respective pre- and post-ACT measurements for Group 1, and T2 and T3 were the respective pre- and post-ACT measurements for Group 2 and Group 3. The pre- and post-ACT data for each variable was then analysed using pair-wise dependent t-tests with a significance level of $\alpha = 0.05$. Pearson correlation coefficient (r) between pre- and post-ACT measures for each of the biomarkers was calculated, as well as the effect size using Cohen's D statistic for paired samples: $D = \frac{t}{\sqrt{n}}$ (Lakens 2013).

An estimate of change in cumulative allostatic load (AL) was calculated for each participant using the change between pre- and post-ACT measurements averaged over six biomarkers of allostasis: heart rate, systolic and diastolic blood pressure, fasting blood glucose, IL-6 and cortisol. Only those participants with pre and post measures for at least five of these six

variables were included in the AL calculations. The change in each biomarker from pre- to post-ACT intervention measurements was first calculated for each participant and then these difference values were normalised by calculating z-scores. The mean and standard deviation of the six normalised difference variables were then calculated to give an estimate of the change in AL for each participant associated with the ACT intervention. Z-scores were used instead of raw differences to ensure that each variable contributed equally to the calculation of the mean change in AL. The mean change in allostatic load ± 1 SD was plotted for each participant (n = 15) for visual and descriptive comparison. A one-sample t-test was used to test if the mean change in allostatic load among the 15 participants was significantly different to zero ($\alpha = 0.05$). In addition, a one-way ANOVA was used to determine if the mean change in allostatic load was different among the three Groups of participants and subsequent post-hoc Least Significant Difference (LSD) pairwise tests were used to identify individual significant group difference ($\alpha = 0.05$).

Chapter 3: Results

3.1 Participants

Twenty-four participants were initially enrolled in the trial; however, two participants withdrew before measurement time point 1 (T1). A further two participants withdrew within the first six weeks of the trial due to ill health for one participant and an unexpected time commitment for the second participant. Data for these two participants are not included in final data analysis. Not all participants provided full data on each measure at each time point for a range of reasons including not attending the collection time appointment, forgetting to collect their saliva specimen at the correct time or an inability to obtain blood samples. The latter is a common side effect of chemotherapy treatment as veins collapse or become scarred due to repeated venepuncture for drug delivery and blood tests (Piredda et al. 2017).

3.2 Demographics and clinical characteristics

Baseline demographic data and clinical characteristics are reported in Table 2. The number of participants (n) at baseline for Groups 1, 2 and 3 were 7, 6 and 7 respectively. Participants were a mean age of 59.3 (SD = 4.5), 50.5 (SD = 3.8) and 58.3 (SD = 10.4) years old for Groups 1, 2 and 3 respectively. Average time since breast cancer diagnosis ranged from 28.29 (SD = 15.9) months for Group 1 to 35.86 (SD = 21.2) months for Group 3. No significant differences ($p > 0.05$) were found between Groups for the four demographic variables: Age, Time since diagnosis, Weight and BMI. Four participants within each group had oestrogen positive breast cancer, and one participant from Group 3 reported progesterone positive breast cancer (Table 2). One participant in each group indicated hormone receptor classification as unknown, and the remaining participants (n = 2, 1 and 1, for Groups 1, 2 and 3 respectively) reported hormone receptor negative breast cancer. Four participants from Group 1 reported human epidermal growth-factor receptor 2 (HER-2) positive cancer, with another two participants reporting HER-2 negative and one participant reporting unknown classification. No participants from Groups 2 or 3 reported HER-2 positive. However, unknown HER-2 classification was reported at n = 2 and n = 3, for Groups 2 and 3 respectively. HER-2 was

reported as negative for n = 4 participants, for both Group 2 and 3. Over half of all participants reported receiving both radiotherapy and chemotherapy treatments. Between 28 % (Group 1) and 67 % (Group 2) of participants were receiving continuing hormone therapy. Group 3 participants had the highest mean BMI, 31.43 (SD = 9.3), this was not significantly different to Group 1 or Group 2.

Table 2: Baseline descriptive statistics for demographic and clinical characteristics of participants within each group. Relevant mean and standard deviation (SD) or percentage frequencies (%) and number (n) of participants are given. No mean significant differences ($p > 0.05$) were found among groups for any of the four demographic variables.

	Group 1 (n = 7)	Group 2 (n = 6)	Group 3 (n = 7)
Age (years), mean (SD)	57.86 (7.48)	56.33 (9.65)	54.71 (6.63)
Time since diagnosis (months), mean (SD)	28.29 (15.91)	31.00 (17.85)	35.86 (21.24)
Weight (kg), mean (SD)	76.59 (13.72)	82.75 (17.34)	83.79(21.56)
BMI, mean (SD)	28.25 (4.27)	27.67 (5.62)	31.43 (9.34)
Breast affected, % (n)			
Both	14.29 (1)	16.70 (1)	28.57 (2)
Left	42.86 (3)	33.33 (2)	42.86 (3)
Right	42.86 (3)	50.00 (3)	28.57 (2)
Hormone receptor type, % (n)			
Oestrogen positive	57.14 (4)	66.66 (4)	57.14 (4)
Progesterone positive	0.00 (0)	0.00 (0)	14.29 (1)
Receptor negative	28.57 (2)	16.70 (1)	14.29 (1)
Unknown	14.29 (1)	16.70 (1)	14.29 (1)
HER2 receptor positive, % (n)			
Yes	57.14 (4)	0.00 (0)	0.00 (0)
No	28.57 (2)	66.66 (4)	57.14 (4)
Unknown	14.29 (1)	33.33 (2)	42.86 (3)
Treatment modality, % (n)			
Radiotherapy	57.14 (4)	87.50 (6)	87.71 (6)
Chemotherapy	87.71 (6)	75.00 (6)	71.43 (5)
Hormone therapy – past	28.57 (2)	0.00(0)	42.82 (3)
- current	28.57 (2)	66.66 (4)	42.82 (3)
Ovarian treatments	14.29 (1)	25.00 (2)	0.00 (0)
Other	14.29 (1)	0.00 (0)	0.00 (0)

3.3 Physiological and molecular biomarkers

3.3.1 Heart rate

The heart rates for participants in Group 1 had similar distribution ranges for measurement time points T1, T2 and T3 (T1: 61 – 79 bpm, T2: 62 – 79 bpm, T3: 64 – 79 bpm) with a larger range at the six-month follow-up measurement time point, T4 (62 – 87 bpm) (Figure 6). Group 2 had larger ranges of distribution of heart rates for measurement time points T1, T2 and T4 (T1: 55 – 90 bpm, T2: 55 – 82 bpm, T4: 65 - 107 bpm). Group 3's range in heart rates across measurement time points was largest at T3 (56 – 82 bpm). Group 3 had an outlier at three measurement time points (T1: 85 bpm, T2: 86 bpm, T4: 88 bpm), and this was the same participant in each case. The outliers were within the heart rate ranges of other two groups (Figure 6).

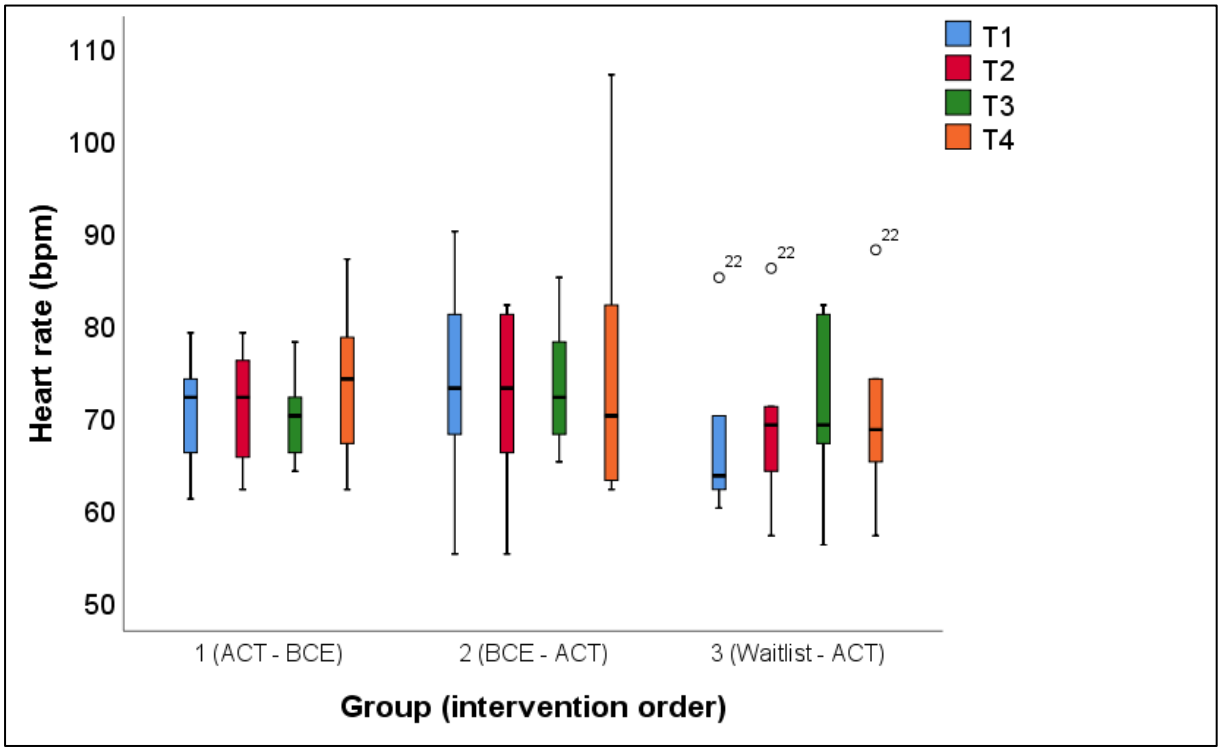

Figure 6: Heart rate (bpm) for all four measurement time points for each of the three groups. Samples sizes for measurement time points (T1, T2, T3, T4) were: Group 1 (n = 7, 7, 6, 7); Group 2 (n = 5, 6, 6, 5); Group 3 (n = 6, 5, 5, 6).

Mean heart rate did not vary significantly across time points for any group ($p > 0.05$) (Figure 7). Group 1 (n = 6) had a relatively consistent mean heart rate around 70.0 bpm at each measurement time point, with a small range of SEMs (2.02 bpm at T3 to 3.02 bpm at T2). There was only limited variation in the mean heart rates for Group 2 (n = 5, 69.8 ± 4.59 bpm at T2 to 73.4 ± 5.9 bpm at T1). Group 3 (n = 3) also had similar mean heart rates across measurement time points but with larger SEMs ranging from 7.5 bpm at T4 to 8.4 bpm at T3.

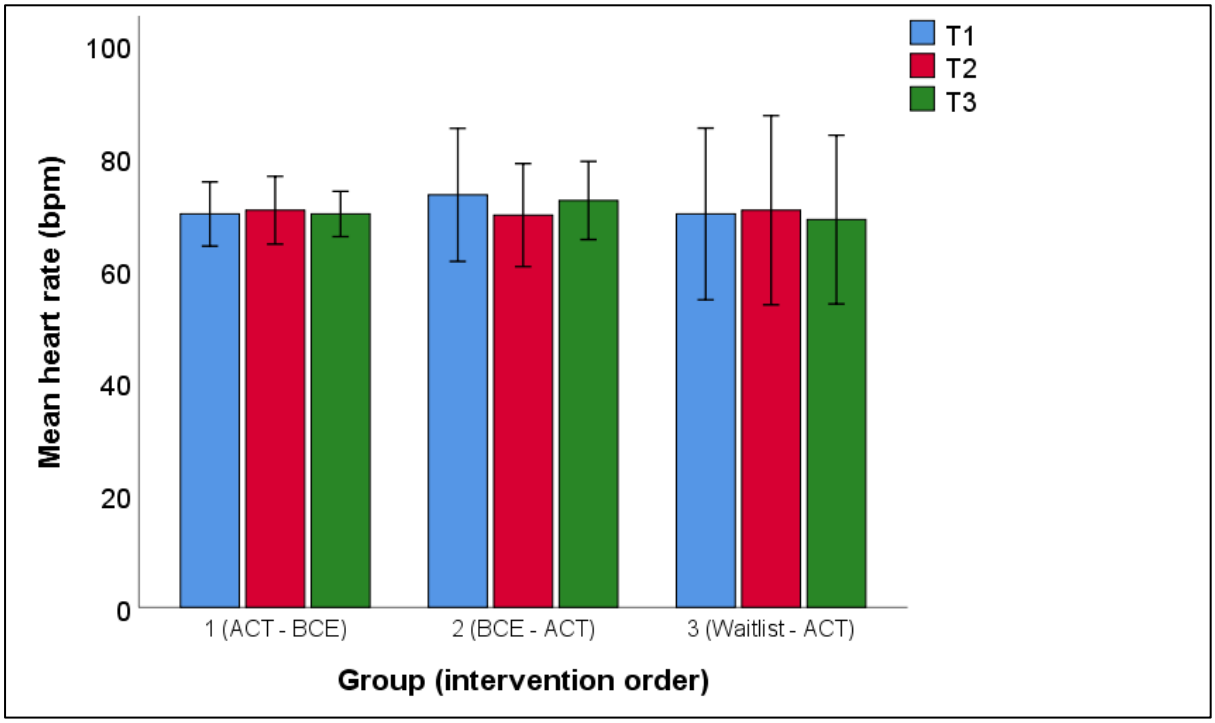

Figure 7: Mean heart rate (bpm) (± 2 SEM) for each Group at T1, T2 and T3. Only participants with measurements at all three time points were included in these samples for analysis: Group 1 (n = 6), Group 2 (n = 5), Group 3 (n = 3).

3.3.2 Systolic Blood Pressure

Measures of systolic blood pressure showed a relatively wide range across all measurement time points for each group (Figure 8), with the largest range in Group 2 at T2 (92 – 138 mmHg) and the smallest range also in Group 2 at T1 (121 – 140 mmHg). In the distribution of systolic blood pressure, the range for Group 1 was similar for T1, T2 and T3 but suggested a decreasing trend over these three measurement time points (T1: 119 – 143 mmHg, T3: 100 – 128 mmHg). Group 2's systolic blood pressure had a relatively small range at T1 (121 – 140 mmHg) and larger ranges at the later three measurement time points. The Group 3 distributions of systolic blood pressure also suggested a decreasing trend from T1 to T4. The maximum systolic blood pressure for all three groups was at T1 (143 mmHg, 140 mmHg and 143 mmHg for Groups 1, 2 and 3 respectively) (Figure 8).

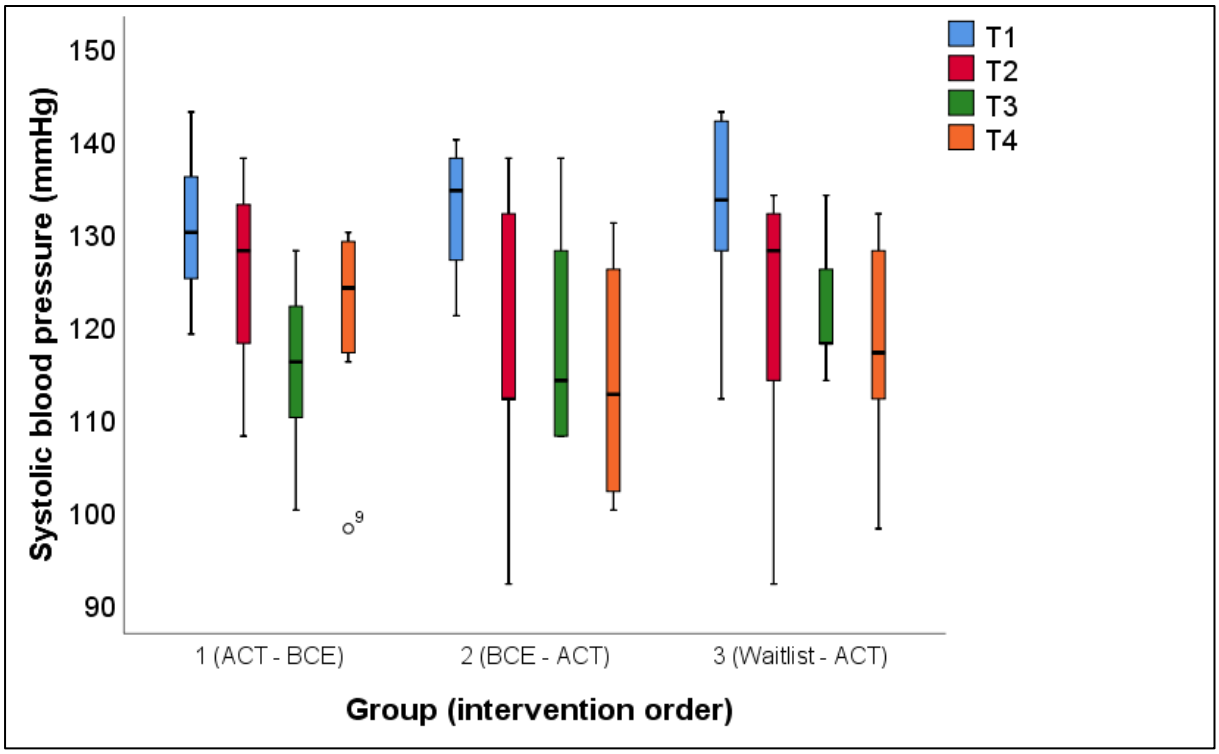

Figure 8: Systolic blood pressure (mmHg) for all four measurement time points for each of the Groups. Samples sizes for the four measurement time points (T1, T2, T3, T4) were: Group 1 (n = 7, 7, 6, 7), Group 2 (n = 4, 5, 5, 4), Group 3 (n = 6, 5, 5, 6).

Pair-wise analysis of mean systolic blood pressure among T1, T2 and T3 (Figure 9) for each group, indicated that the mean systolic blood pressure for Group 1 decreased significantly (p = 0.010, n = 6), from T1 to T3 (130 ± 3.8 mmHg to 115 ± 4.0 mmHg). The mean systolic blood pressure for Group 2 (n = 4) or Group 3 (n = 3) did not demonstrate any significant differences (p > 0.05) among any of the three measurement time points (Figure 9).

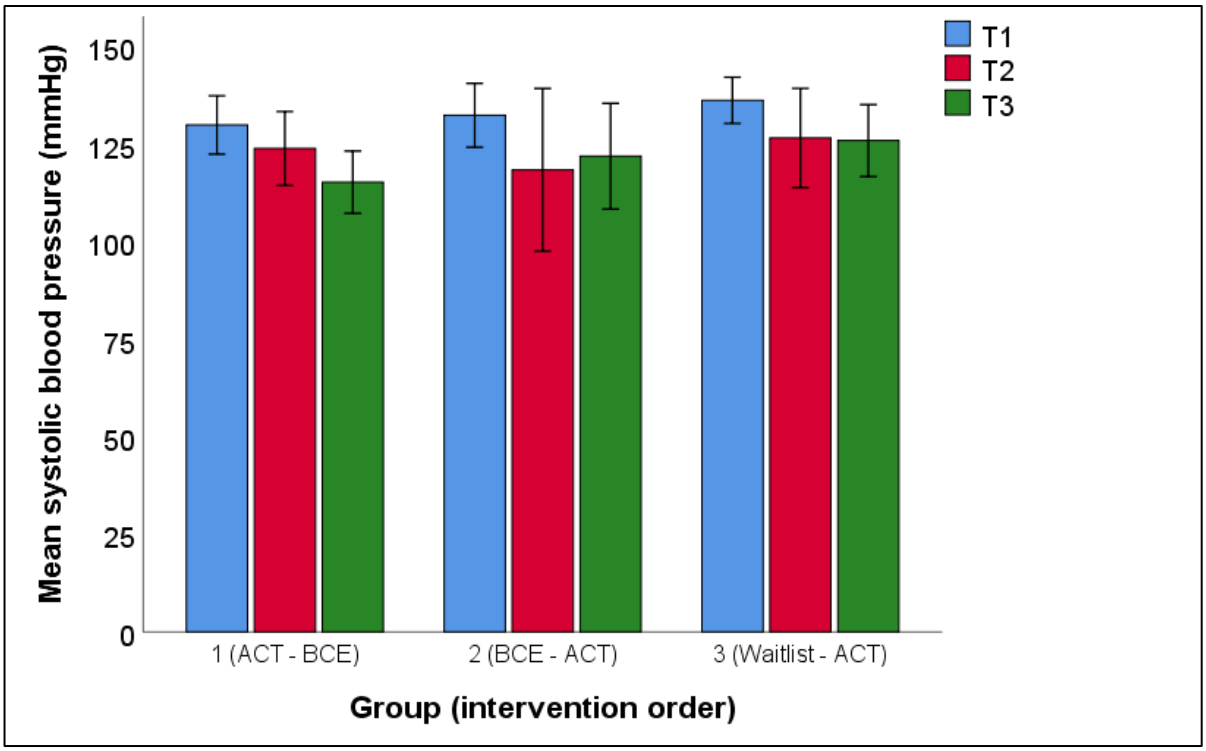

Figure 9: Mean systolic blood pressure (mmHg) (± 2 SEM) for each Group at T1, T2 and T3. Only participants with measurements at all three time points were included in these samples for analysis: Group 1 (n = 6), Group 2 (n = 4), Group 3 (n = 3). Means within a group with significant differences are indicated with notations, different notations represent significant differences, p < 0.05. No notation indicates no significant differences between the measurement time points for the group.

3.3.3 Diastolic blood pressure

Diastolic blood pressure (Figure 10) had distributions with varying ranges between Groups at T1. Group 1's range for diastolic blood pressure increased from T1 to T2 and then decreased again at T3 (68 mmHg to 78 mmHg, n = 6). The distribution at T4 for Group 1 (n = 7) was strongly influenced by two outliers, one higher and the other lower (75mmHg and 88mmHg). These outliers fell within the ranges of diastolic blood pressure at other measurement timepoints for Group 1. The range of diastolic blood pressure for both Group 2 and Group 3 were similar at all four measurement time points, however both also indicated a decrease in both maximum and minimums from T1 to T2. The maximum diastolic blood pressure for Group 2 decreased from 96 mmHg at T1 to 88 mmHg at T2 and stayed at similar values at T3 and T4 (Figure 10).

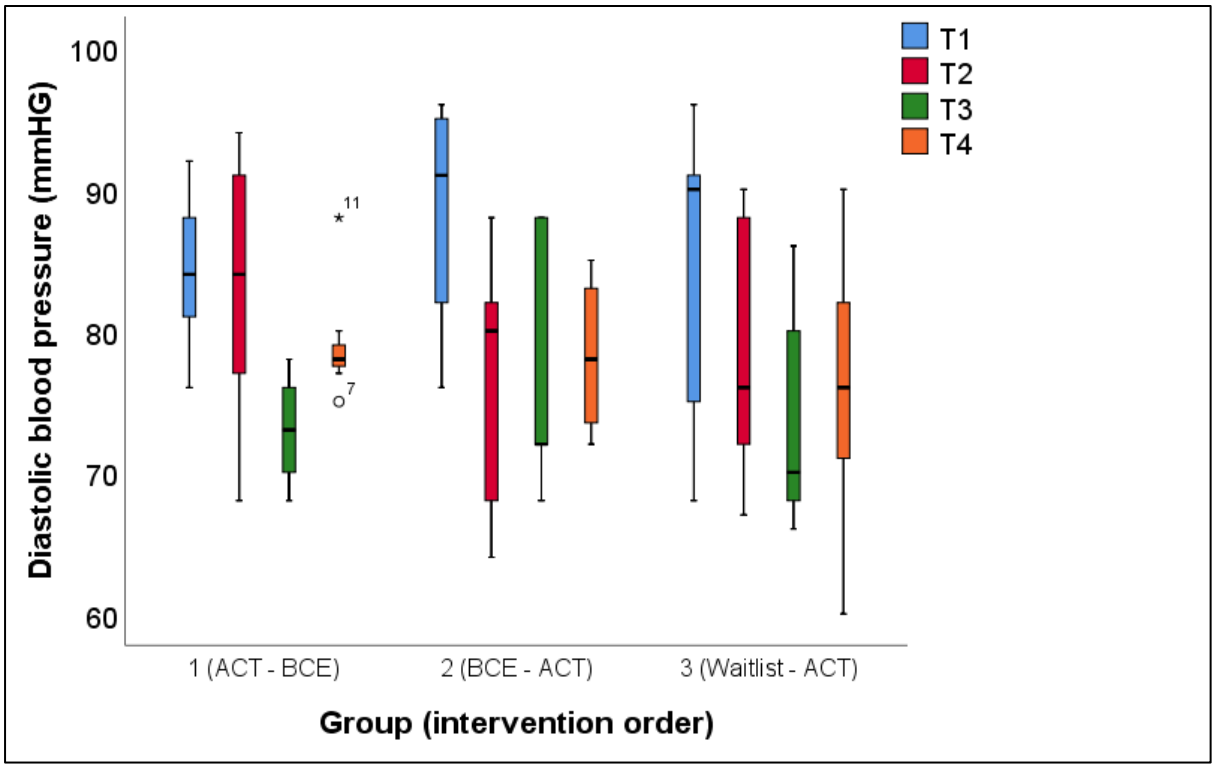

Figure 10: Diastolic blood pressure (mmHg) for all four measurement time points for each of the three groups. Sample sizes for (T1, T2, T3, T4) were: Group 1 (n = 7, 7, 6, 7), Group 2 (n = 4, 5, 5, 4), Group 3 (n = 6, 5, 5, 6).

Pair-wise analysis of mean diastolic blood pressure among times (T1, T2 and T3) for each group separately (Figure 11), identified that the mean diastolic blood pressure for Group 1 (n = 6) decreased significantly (p = 0.006) from 84.8 ± 2.4mmHg at T1 to 73.0 ± 1.7 mmHg at T3. The mean diastolic blood pressure for Group 2 (n = 4) and Group 3 (n = 3) were highest at T1 (Group 2: 88.5 ± 4.5 mmHg, Group 3: 92.0 ± 2.0 mmHg), and appeared to decrease across measurement time points, however, there were no significant differences (p > 0.05) within each group.

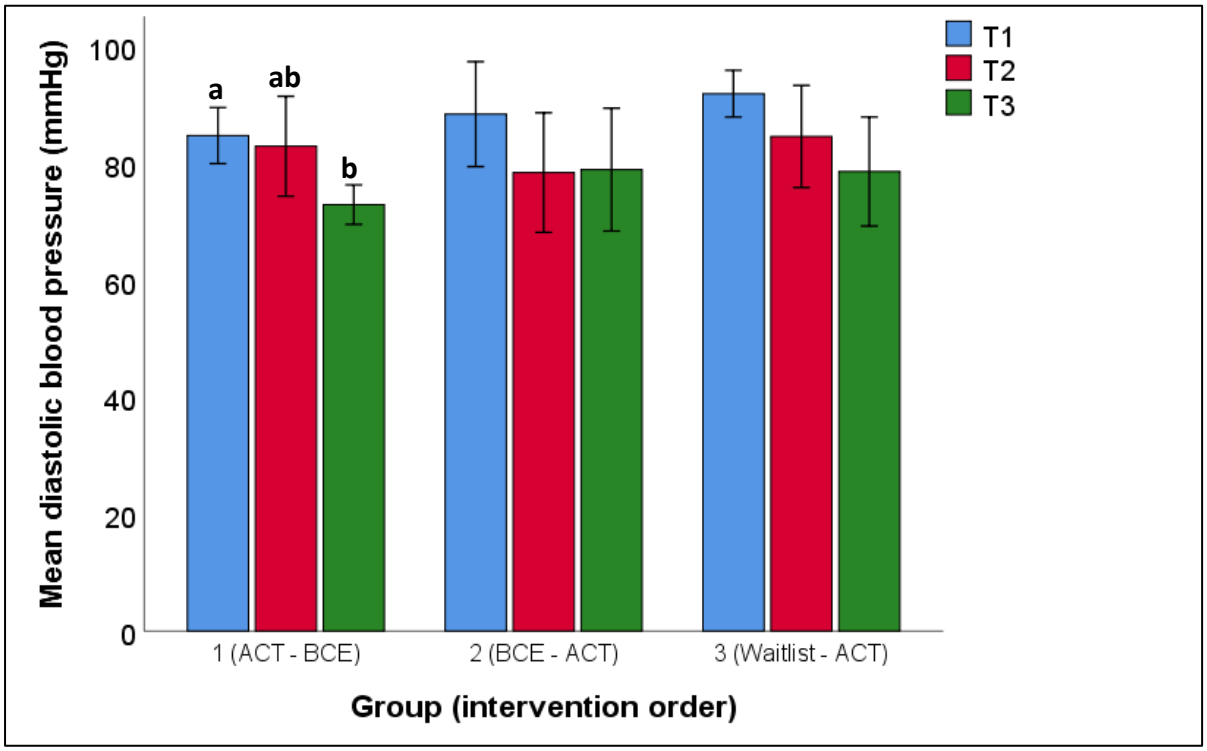

Figure 11: Mean diastolic blood pressure (mmHg) (± 2 SEM) for each group at T1, T2 and T3. Only participants with measurements at all three time points were included in these samples for analysis: Group 1 (n = 6), Group 2 (n = 4), Group 3 (n = 3). Means within a group with significant differences are indicated with notations, different notations represent significant differences, p < 0.05. No notation indicates no significant differences between the measurement time points for the group.

3.3.4 Blood Glucose

Blood glucose had a wide range for Group 1 across all measurement time points, increasing from T1 to T2 (Figure 12). The minimum blood glucose for Group 1 was between 4.1 mmol/L at T1 and 4.8 mmol/L at T3 and T4. The highest maximum blood glucose for Group 1 was at T2 (6.6 mmol/L). Group 2's blood glucose had smaller distribution ranges, with the lowest minimum at T1 (4.3 mmol/L) and the highest maximum of 6.3 mmol/L at T3 (excluding and outlier of 7.3 mmol/L at T2). The ranges of blood glucose for Group 3 varied across measurement time points, with one outlier at T1 recording one of the lowest blood glucose values in the study (4.3 mmol/L). The ranges for all three time points were consistently smaller for Group 2 and Group 3 than the ranges for Group 1.

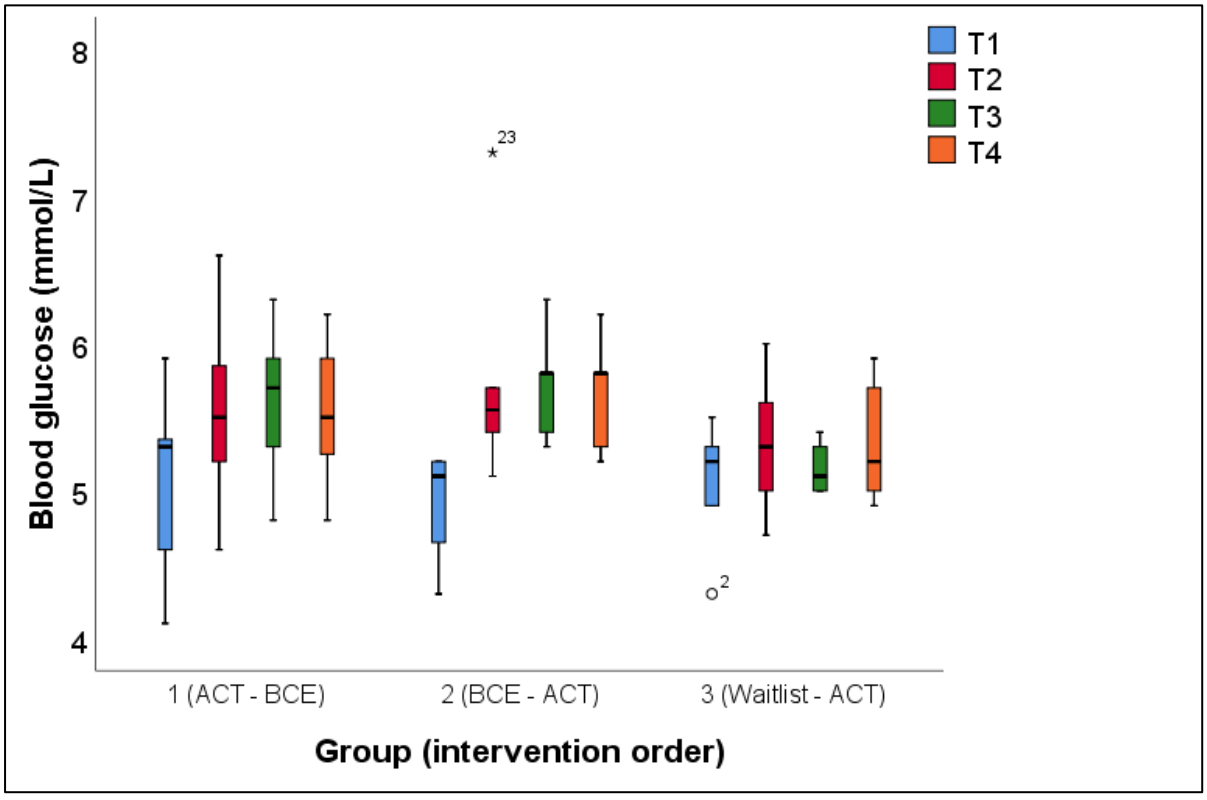

Figure 12: Fasting blood glucose (mmol/L) for all four measurement time points for each of the three groups. Samples sizes for (T1, T2, T3 and T4) were: Group 1 (n = 7, 7, 6, 7), Group 2 (n = 4, 5, 5, 4), Group 3 (n = 6, 5, 5, 6).

Analysis of mean fasting blood glucose for measurement time points T1, T2 and T3 by pair-wise dependent samples t-tests, identified significant increases in mean blood glucose within Group 1 and Group 2 (Figure 13). Blood glucose in Group 1 (n = 6) significantly increased from 4.98 ± 0.27 mmol/L at T1 to 5.55 ± 0.30 mmol/L at T2 (p = 0.023). At T3 the mean of 5.62 ± 0.22 mmol/L was significantly higher than T1 (p = 0.034) but not significantly different to T2 (p > 0.05). Group 2 (n = 4) significantly increased from 4.93 ± 0.21 mmol/L at T1 to 5.70 ± 0.23 mmol/L at T3 (p = 0.018). There was no significant difference in mean blood glucose for Group 3 (n = 3) across all three measurement time points, with the means ranging from 5.10 ± 0.10 mmol/L at T1 to 5.30 ± 0.17 mmol/L at T2.

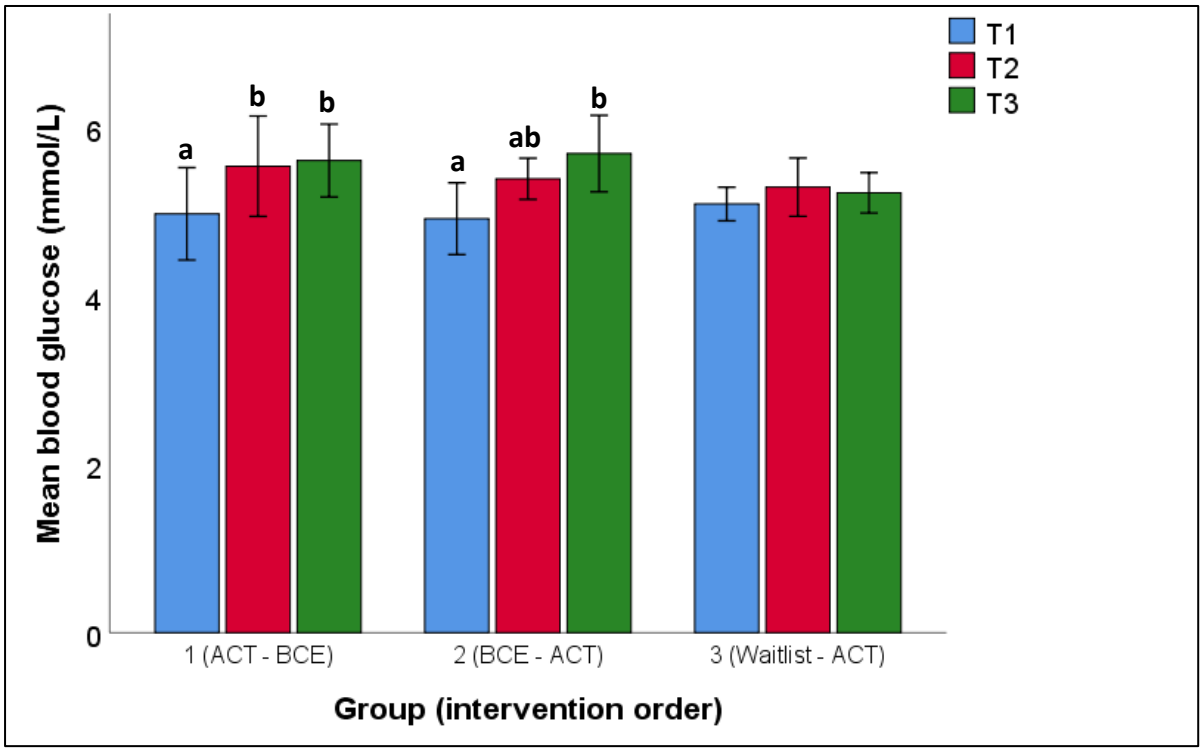

Figure 13: Mean fasting blood glucose (mmol/L) (± 2 SEM) for each group at T1, T2 and T3. Only participants with measurements at all three time points were included in these samples for analysis: Group 1 (n = 6), Group 2 (n = 4), Group 3 (n = 3). Means within a group with significant differences are indicated with notations, different notations represent significant differences, p < 0.05. No notation indicates no significant differences between the measurement time points for the group.

3.3.5 Relative telomere length

Relative telomere length (RTL) had varying distribution ranges across measurement time points in each Group (Figure 14). Group 1's range of distribution decreased from T1 to T3 with a similar trend for Group 2. Group 1 and Group 2 had wide ranges of distribution of RTL at T4 (Group 1: 0.54 to 2.76, Group 2: 0.57 to 3.36). The range of distribution for Group 3 at T4 was strongly influenced by two outliers, one lower (0.27) and the other higher (2.82) than other measures.

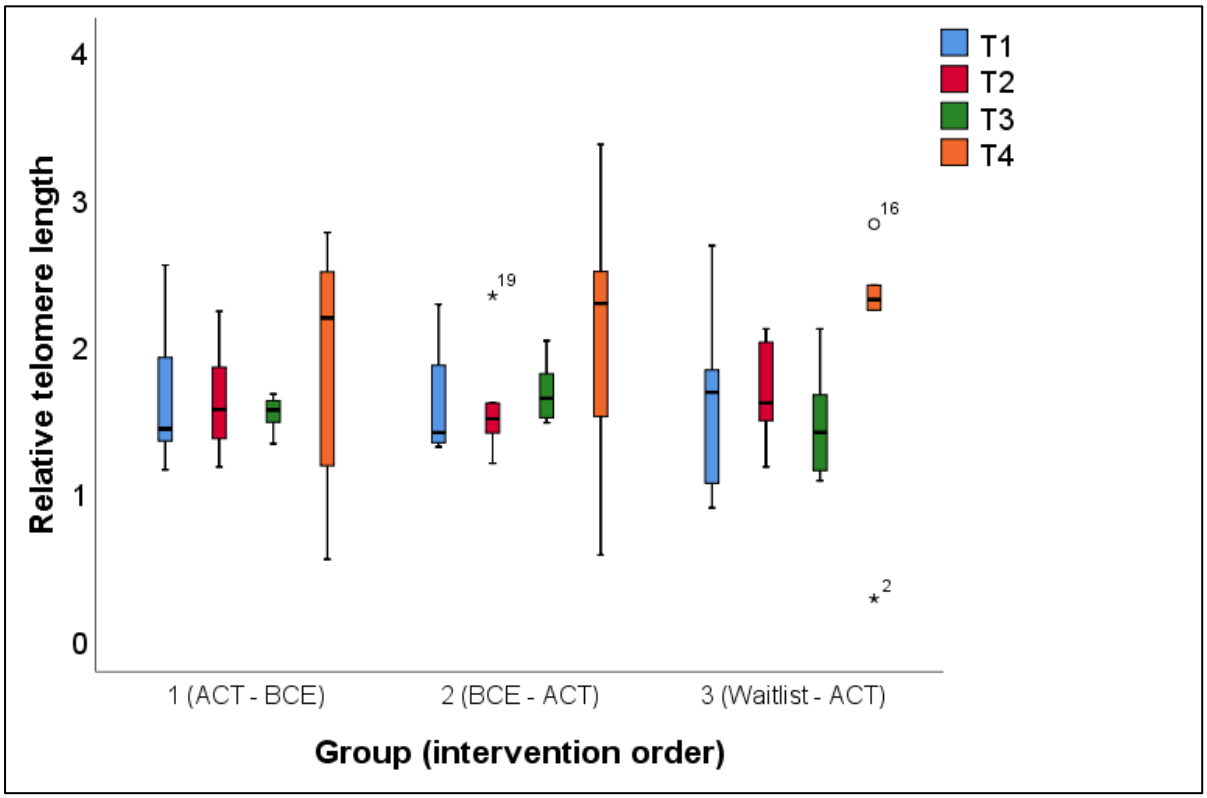

Figure 14: Relative telomere length for all four measurement time points for each of the three groups. Samples sizes for measurement time points (T1, T2, T3, T4) were: Group 1 (n = 6, 6, 5, 5), Group 2 (n = 4, 5, 5, 5), Group 3 (n = 6, 5, 5, 5).

Mean RTL appeared to increase slightly from T1 to T4 for each group, but these changes were not statistically significant ($p > 0.05$) (Figure 15). Group 1 (n = 5) increased from 1.69 ± 0.25 to 1.83 ± 0.41. Group 2 (n = 4) increased from 1.59 ± 0.23 to 1.72 ± 0.44, and Group 3 (n = 5) increased from 1.75 ± 0.28 to 2.00 ± 0.45.

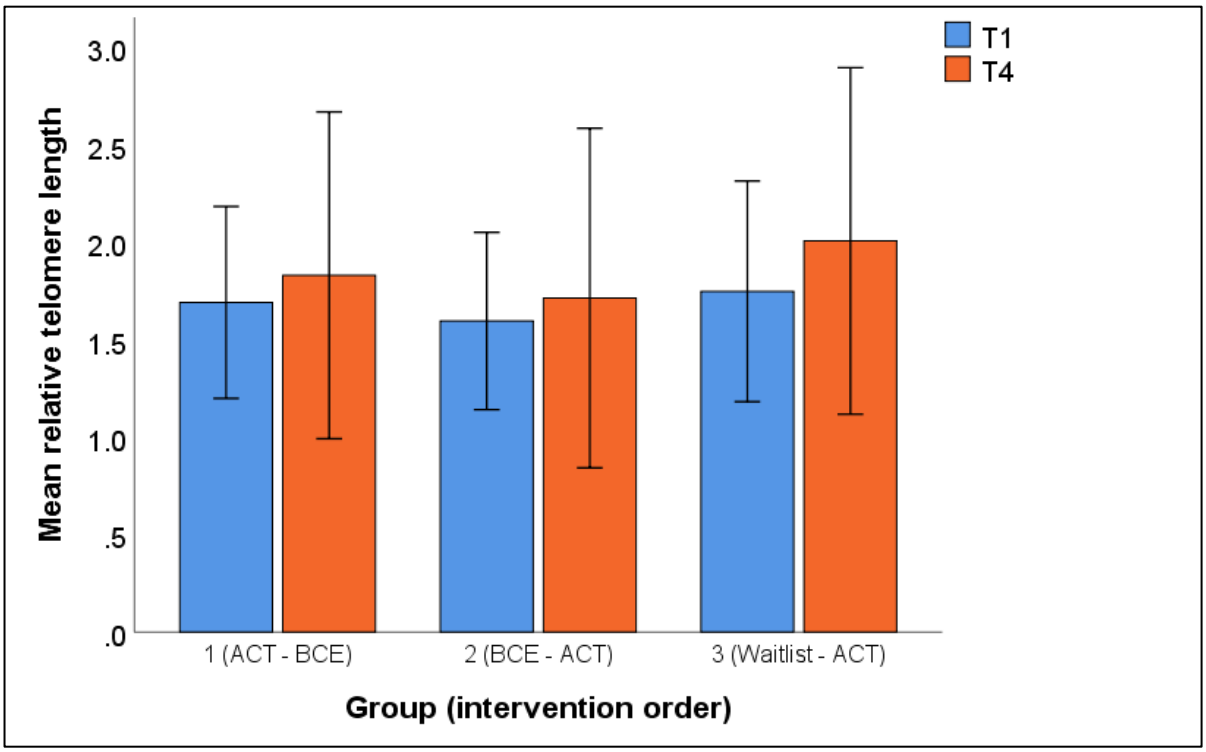

Figure 15: Mean relative telomere length (± 2 SEM) for each group at T1 and T4. Only participants with measurements at both time points were included in these samples for analysis: Group 1 (n = 5), Group 2 (n = 4), Group 3 (n = 5).

3.3.6 Interleukin-6

Interleukin-6 (IL-6) had varying ranges across measurement time points for each group (Figure 16). Distributions of IL-6 appeared to increase in range from T1 to T2 for all groups, with the highest concentration of IL-6 for each group across all four measurement time points occurring at T2 (Group 1: 8.48 pg/mL, Group 2: 7.31 pg/mL, Group 3: 12.60 pg/mL). Group 3 had outliers (above and below the distributions) that strongly influenced the distribution of IL-6 at T3 and T4, however these outliers fell within the ranges observed for Group 1 and Group 2.

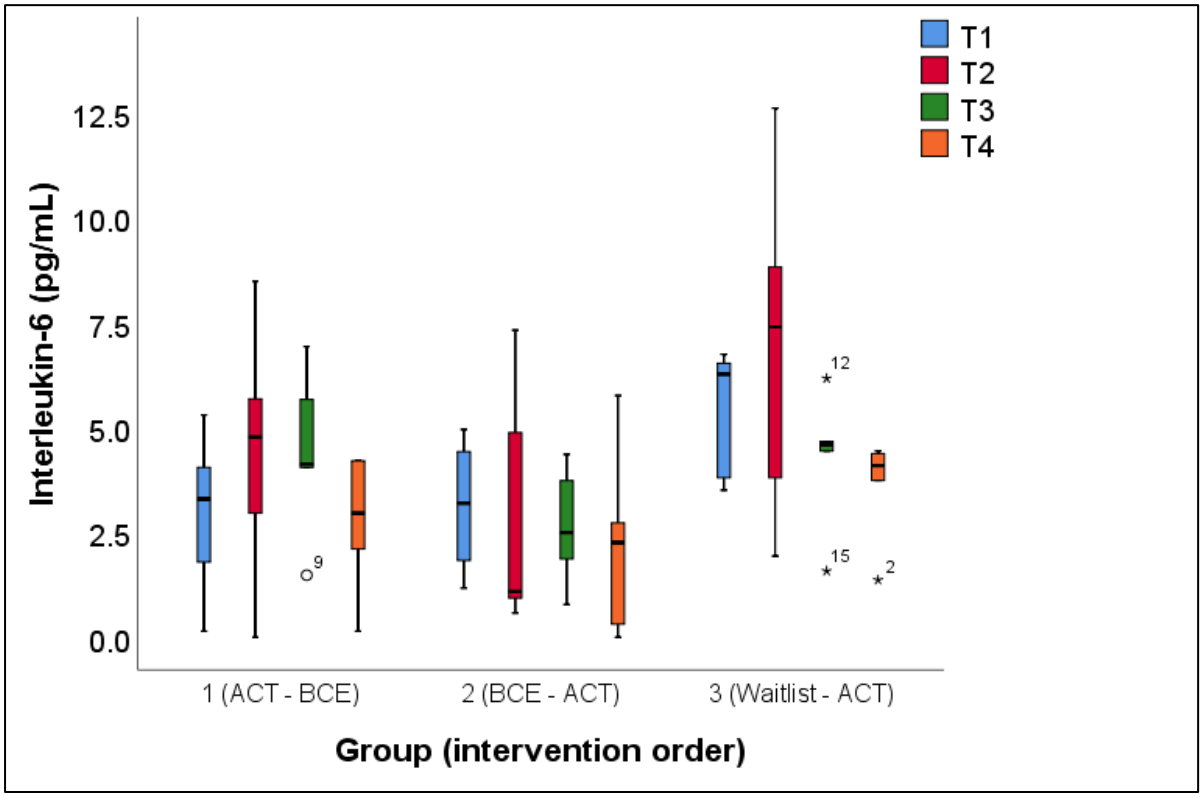

Figure 16: Interleukin-6 (pg/mL) for all four measurement time points for each of the three groups. Samples sizes for measurement time points (T1, T2, T3, T4) were: Group 1 (n = 6, 6, 5, 5), Group 2 (n = 4, 5, 5, 5), Group 3 (n = 6, 5, 5, 5).

Mean IL-6 appeared to increase from T1 to T2 for all groups (Figure 17), but there were no significant differences across measurement time points within each group ($p > 0.05$). Group 1's (n = 5) mean IL-6 increased from 3.54 ± 0.58 pg/mL at T1 to 4.48 ± 1.42 at T2 and stayed at a similar mean concentration for T3. There was little variation in mean IL-6 concentrations across measurement time points for Group 2 (n = 4). Group 3's (n = 3) mean IL-6 increased from 5.34 ± 0.92 at T1 to 9.60 ± 1.55 at T2 and then decreased to a mean concentration at T3, similar to the Group's T1 mean Il-6. Group 3 had the highest mean IL-6 concentrations compared to the other groups at all measurement time points. There appeared to be changes in the mean IL-6 for Group 3, but these were not significant due to large variance and small sample size (n = 3).

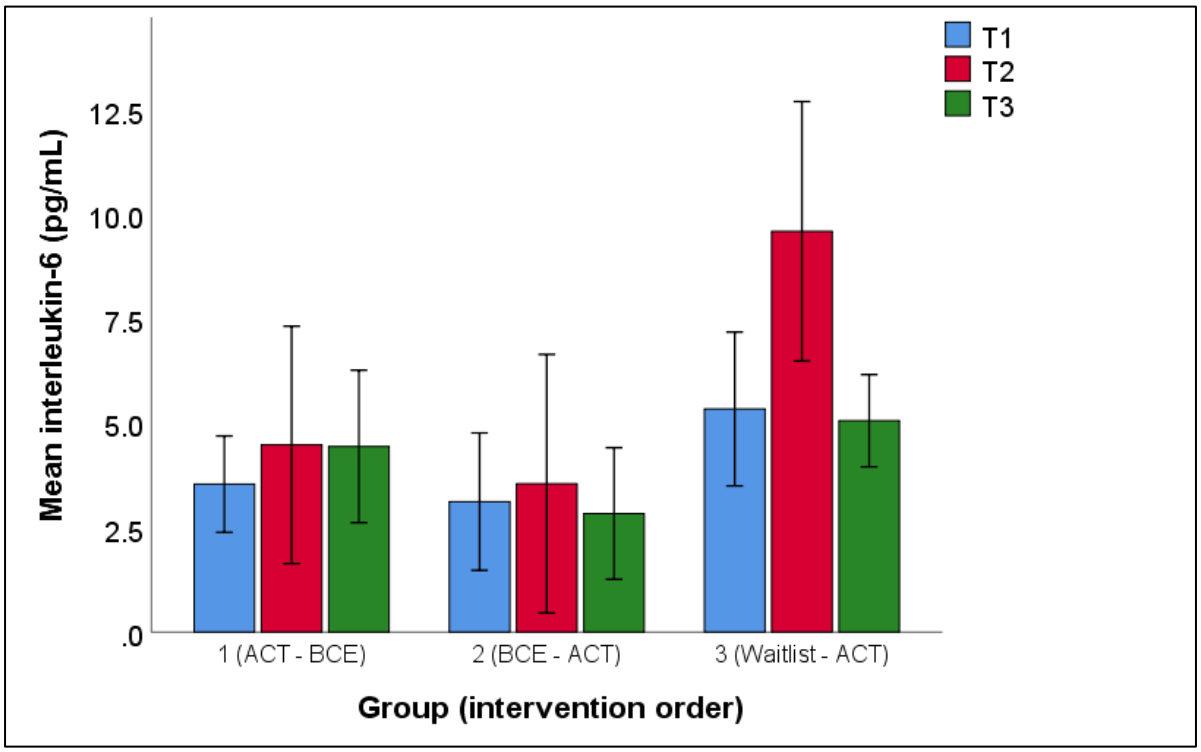

Figure 17: Mean interleukin-6 (pg/mL) (± 2 SEM) for each group at T1, T2 and T3. Only participants with measurements at all three time points were included in these samples for analysis: Group 1 (n = 5), Group 2 (n = 4), Group 3 (n = 3).

3.3.7 Cortisol

Salivary cortisol concentration distribution ranges varied among measurement time points for each group (Figure 18). Group 1 had wider ranges of cortisol distribution at T1 and T2 compared to T3 and T4 but higher outliers at both T3 and T4 (T3: 13.85 ng/mL, T4: 27.35 ng/mL). Group 2 had similar ranges of cortisol concentrations at T1, T2 and T3 and had a narrower distribution range T4 (4.93 ng/mL to 7.32 ng/mL). Group 3 had relatively narrow distributions ranges of cortisol concentration at both T1 and T2. Group 3 also had an outlier at T1 and T4 (T1: 14.95 ng/mL, T4: 21.65 ng/mL).

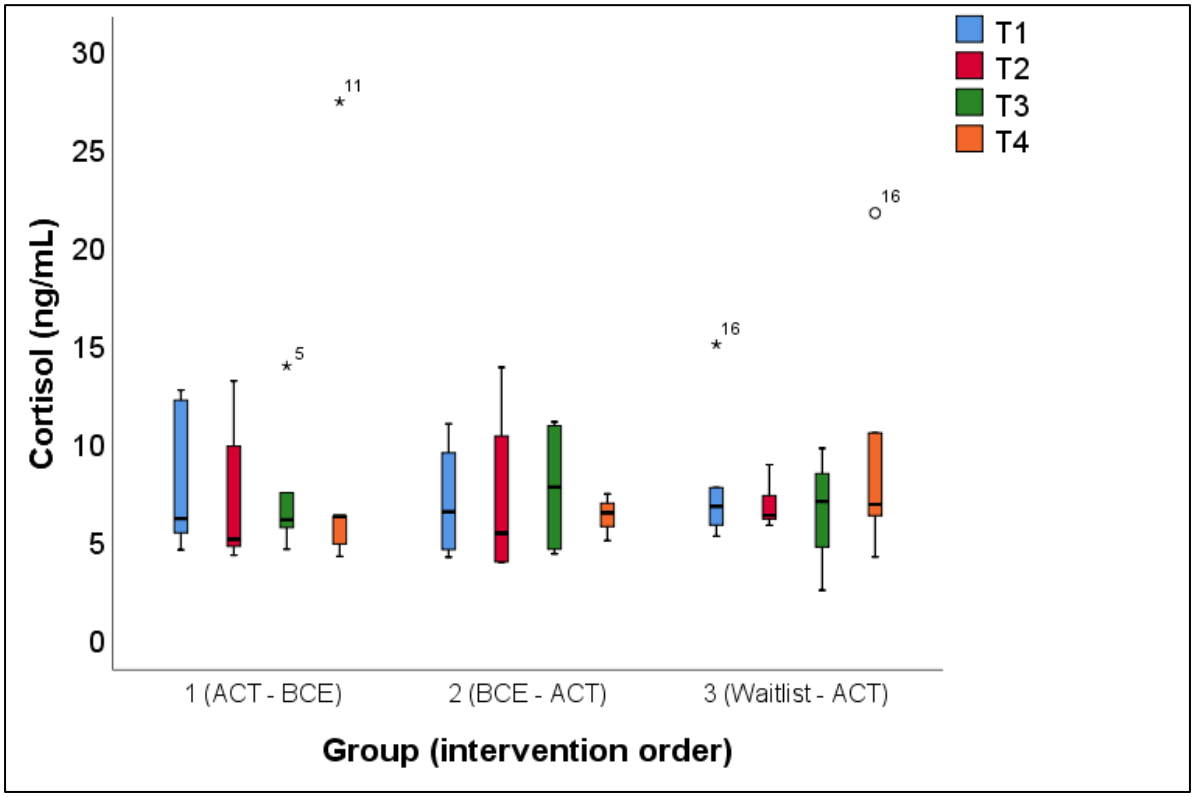

Figure 18: Cortisol (ng/mL) for all four measurement time points for each of the three groups. Samples sizes for measurement time points (T1, T2, T3 and T4) were: Group 1 (n = 5, 6, 5, 5), Group 2 (n = 4, 4, 4, 3), Group 3 (n = 5, 5, 4, 6).

Mean cortisol concentrations across measurement time points (T1, T2 and T3) for Group 1 and Group 2 separately had no significant differences among time points ($p > 0.05$) (Figure 19). The mean concentration of cortisol for Group 1 (n = 4) deceased slightly from T1 to T2 (6.98 ± 1.73 ng/mL to 5.81 ± 1.32 ng/mL) and then increased to 7.83 ± 2.09 ng/mL at T3. Group 2's (n = 4) mean cortisol concentration had similar values across all three measurement time points. Group 3's mean cortisol concentrations indicated a significant increase ($p = 0.038$, n = 3) from T1 to T2 (5.85 ± 0.44 ng/mL to 7.42 ± 0.75 ng/mL). The mean cortisol concentration for Group 3 appeared to decrease at T3 (6.28 ± 2.11 ng/mL) but this was not significant compared to T2 ($p > 0.05$).

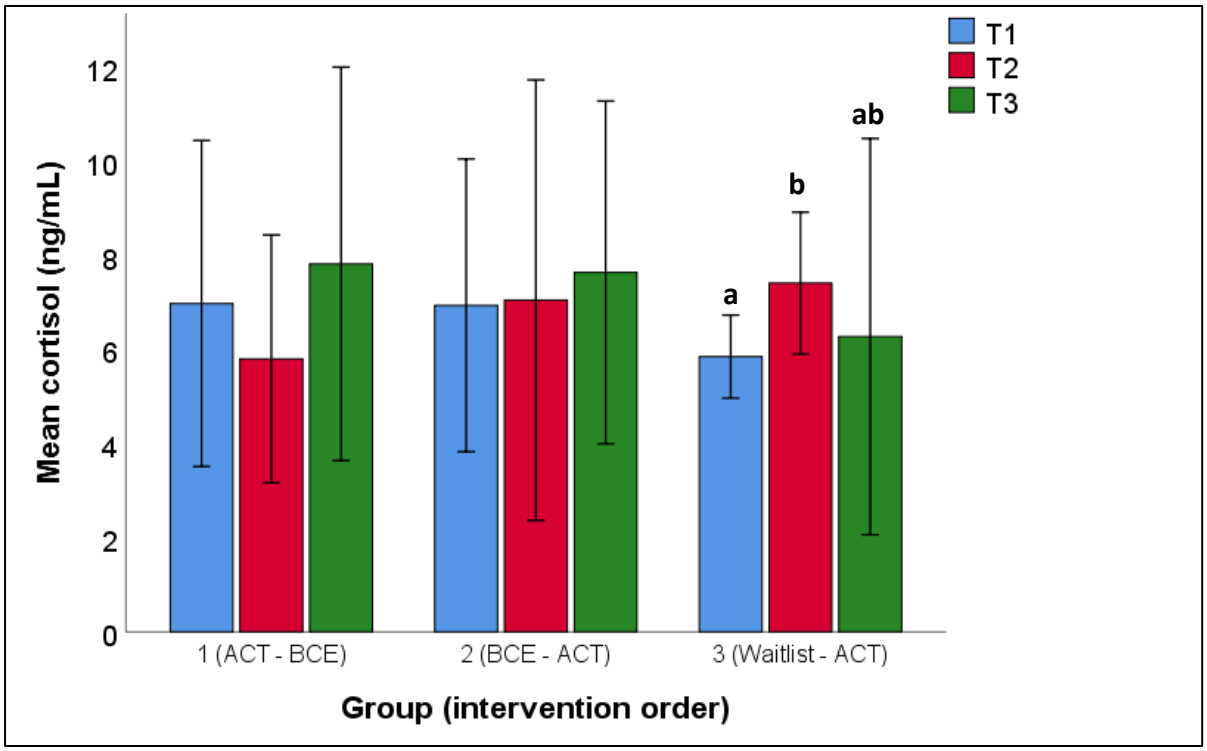

Figure 19: Mean cortisol concentration (ng/mL) (± 2 SEM) for each group at T1, T2 and T3. Only participants with measurements at all three time points were included in these samples for analysis: Group 1 (n = 4), Group 2 (n = 4), Group 3 (n = 3). Means within a group with significant differences are indicated with notations, different notations represent significant differences, $p < 0.05$. No notation indicates no significant differences between the measurement time points for the group.

3.3.8 Salivary alpha-amylase

Concentrations of salivary alpha-amylase (sAA) had wide distributions across all measurement time points for each group (Figure 20). The was an outlier (same individual participant) at all measurement time points in Group 1, with all but the T1 outlier (490.45 U/mL) falling within the ranges for sAA concentrations of Group 2 and Group 3. Group 2's and Group 3's ranges of sAA concentrations appeared to increase from T1 to T2. Group 2's range narrowed from T2 to T4, with the smallest range at T4 (23.94 U/mL to 206.05 U/mL). Group 3's range increased across all measurement time points with T4 having the widest range (7.18 U/mL to 377.76 U/mL).

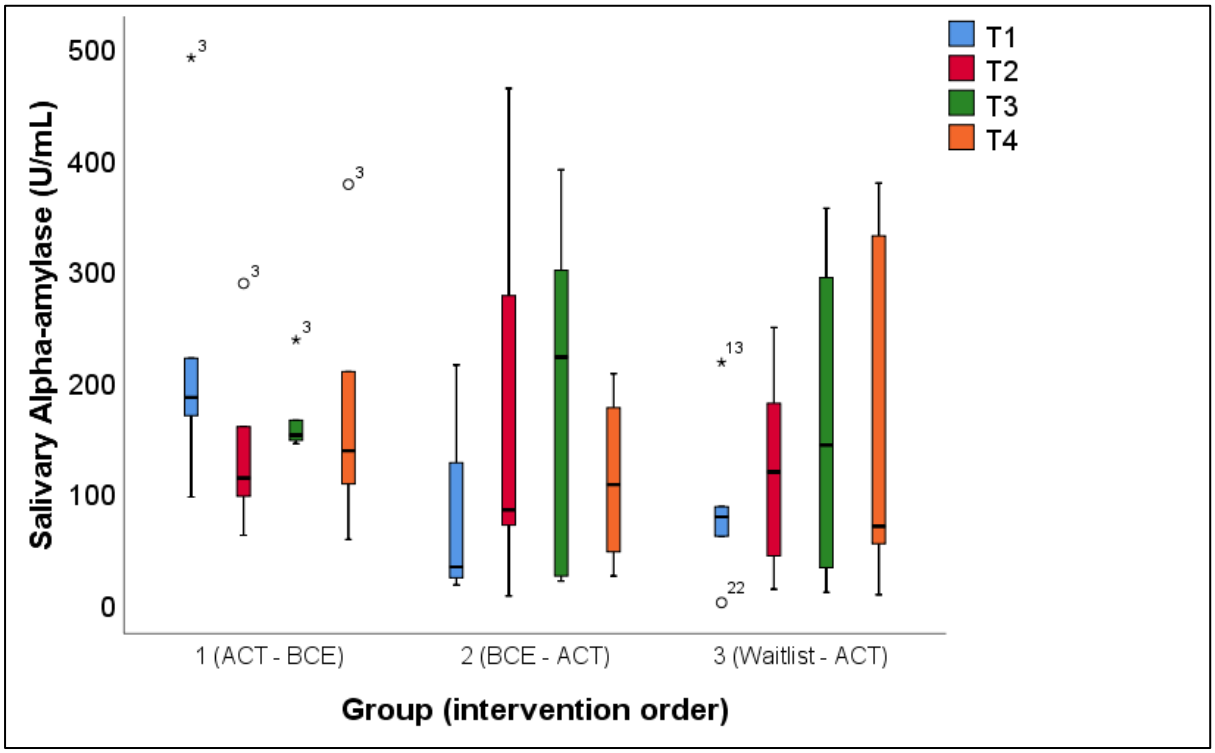

Figure 20: Salivary alpha-amylase (U/mL) for all four measurement time points for each group. Samples sizes for measurement time points (T1, T2, T3 and T4) were: Group 1 (n = 6, 6, 5, 5), Group 2 (n = 5, 5, 5, 4), Group 3 (n = 6, 5, 4, 6).

Mean sAA concentrations did not vary significantly ($p > 0.05$) between measurement time points T1, T2 and T3 for each group (Figure 21). The mean concentration of sAA at T1 for Group 1 appeared to be higher compared to the other groups, however this mean was also associated with a relatively large SEM. The mean sAA concentration of Group 1 (n = 5) appeared to decrease from T1 to T2 (224.64 ± 68.60 U/mL to 153.15 ± 35.15 U/mL), whereas the mean sAA concentration for Group 2 (n = 5) appeared to increase from T1 to T2 (82.03 ± 38.53 U/mL to 179.96 ± 83.98 U/mL). Group 3's (n = 4) mean sAA concentration increased from 86.94 ± 45.86 U/mL at T1 to 161.98 ± 80.08 U/mL. There were large variances in sAA for each group at all measurement time points as indicated by the large SEMs (Figure 21).

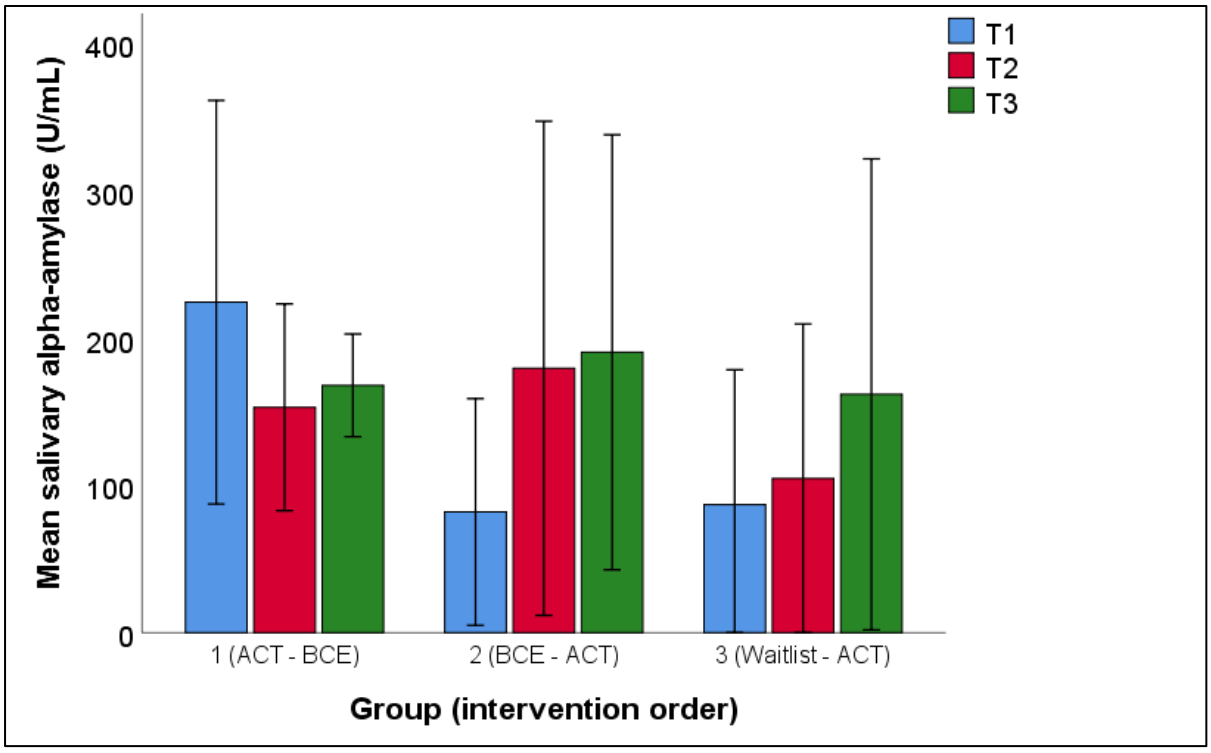

Figure 21: Mean salivary alpha-amylase (U/mL) (± 2 SEM) for each group at T1, T2 and T3. Only participants with measurement at all three time points were included in these samples for analysis: Group 1 (n = 5), Group 2 (n = 5), Group 3 (n = 4).

3.3.9 Physiological and molecular biomarkers for pre- and post-ACT intervention

There were no significant differences between mean values for pre- and post-ACT intervention samples (regardless of group) for any of the eight measured biomarkers (p > 0.05) (Table 3). The effect size (D) of ACT on heart rate was negative and small, indicating a slightly higher but not significantly different post-ACT intervention measurement (D = -0.04). Pearson correlation analysis identified a significant positive correlation (r = 0.67, p = 0.003) between mean heart rates from the pre- and post-ACT intervention samples (n = 17), indicating a strong positive linear relationship between both heart rate measurements. There was a decrease in mean systolic blood pressure from pre- to post-ACT intervention measures, with a positive and moderate effect (D = 0.33). The correlation between the two measures was significant (r = 0.76, p = 0.001), indicating a moderate-strong positive linear relationship (Table 3). Similarly, there were moderate-strong, positive linear relationships between pre- and post-ACT diastolic blood pressure (r = 0.63, p = 0.009), RTL (r = 0.72, p = 0.002), cortisol (r = 0.81, p = 0.001) and sAA (r = 0.63, p = 0.013). The correlation coefficients for blood glucose (r = -0.402) and IL-6 (r = 0.512) were not significant (p > 0.05) (Table 3).

Table 3: Physiological and molecular biomarkers pre- and post-ACT intervention for all groups combined. Only participants with measurements at both time points were included in samples for analysis for each individual biomarker. Significant correlations between pre- and post-ACT measurements for an individual biomarker are identified in bold.

		Time Point		Pair-wise Significance	Effect Size	Pearson Correlation	Correlation Significance
		Pre-ACT	Post-ACT	*P*	*D*	*r*	*P*
Heart Rate (bpm)	N	17	17				
	Mean (SD)	70.88 (8.67)	71.18 (7.67)	0.859	-0.04	**0.670**	**0.003**
Systolic Blood Pressure (mmHg)	N	16	16				
	Mean (SD)	125.50 (13.05)	122.75 (10.88)	0.213	0.33	**0.765**	**0.001**
Diastolic Blood Pressure (mmHg)	N	16	16				
	Mean (SD)	81.13 (8.19)	79.50 (9.48)	0.410	0.21	**0.631**	**0.009**
Blood Glucose (mmol/L)	N	17	17				
	Mean (SD)	5.32 (0.70)	5.31 (0.99)	0.973	0.08	-0.402	0.110
Relative Telomere Length	N	15	15				
	Mean (SD)	1.64 (0.44)	1.61 (0.35)	0.701	0.10	**0.728**	**0.002**
Interleukin-6 (pg/mL)	N	15	15				
	Mean (SD)	4.23 (3.55)	3.74 (2.29)	0.555	0.16	0.512	0.050
Cortisol (ng/mL)	N	13	13				
	Mean (SD)	7.47 (0.93)	7.14 (0.92)	0.580	0.22	**0.805**	**0.001**
Salivary Alpha-Amylase (U/mL)	N	15	15				
	Mean (SD)	177.41 (146.96)	161.84 (127.00)	0.623	0.13	**0.625**	**0.013**

3.4 Allostatic load index

The mean change in allostatic load for the 15 trial participants with a complete dataset (Figure 22) showed each participant's average change across the six biomarkers included in the allostatic load calculation (see Methods section 2.6.8). Group 3 participants all had a mean positive change in allostatic load, indicating that their normalised (z-score) post-ACT values were, on average, lower than their normalised pre-ACT values. However, for each of these participants the standard deviations representing the variation across the six biomarkers, indicated that, for at least some of the biomarkers, an increase in values from pre- to post-ACT time points occurred (that is, a negative change in allostatic load). Participant 22 from Group 3 had the highest mean change allostatic load (0.55, SD = 0.96) indicating that they recorded the greatest average reduction in biomarker values after the ACT intervention.

Eight of the participants (from Group 1 and Group 2) had a negative mean change in allostatic load indicating that post-ACT biomarker values were, on average, higher than pre-ACT values for these participants. The largest negative mean change in allostatic load was calculated for participant 19 (-0.76, SD = 1.08).

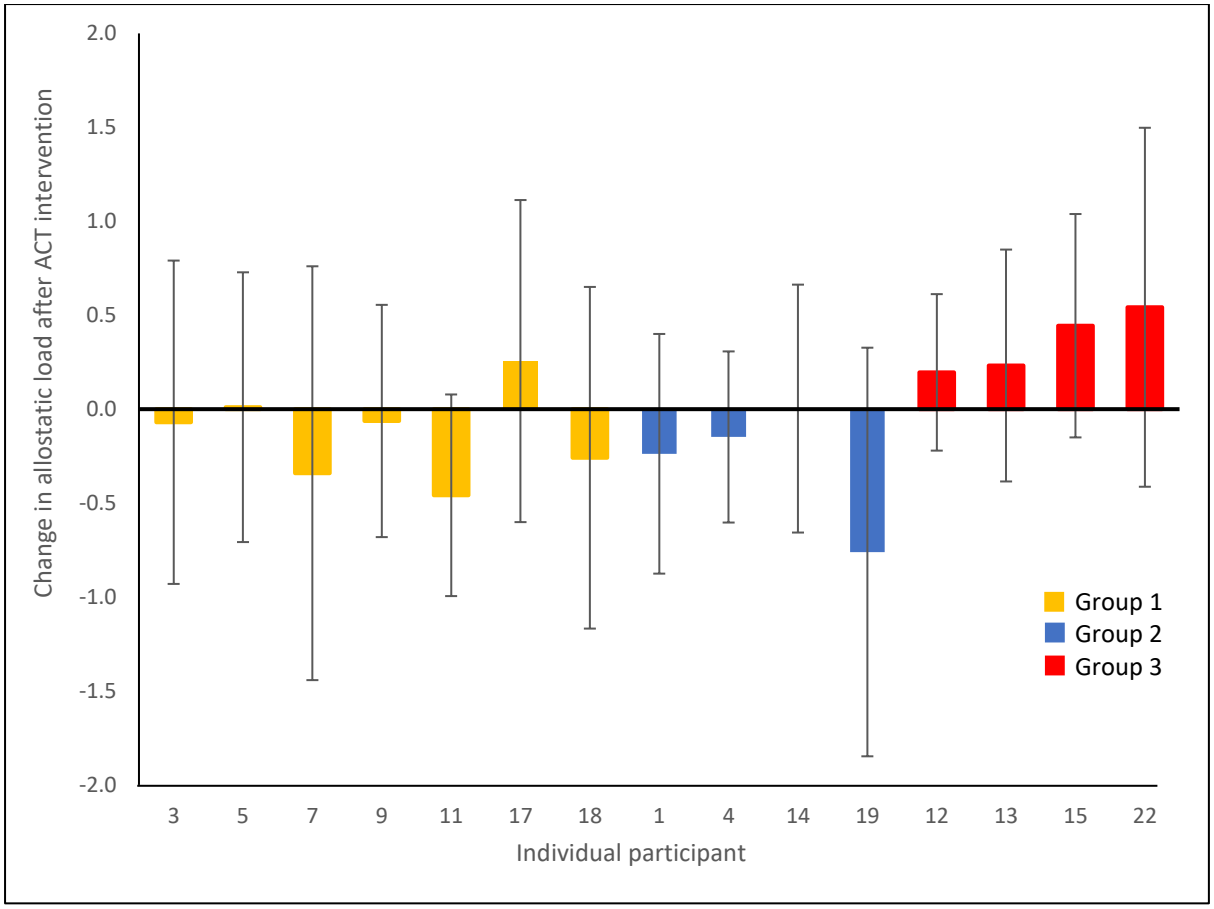

Figure 22: Change in allostatic load for individual participants representing the average change in values for pre- and post-ACT intervention biomarker measures (positive values indicate a positive effect i.e. an average decrease in AL. Mean ± SD of the standardised (z-score) change in six biological variables is given for each individual. Group 1: (n = 7), Group 2: (n = 4), Group 3: (n = 4).

The one-sample t-test analysis indicated that the mean change in allostatic load (n = 15) was not significantly different to zero (p > 0.05). However, the one-way ANOVA analysis did indicate a difference between at least two of the Group means (p = 0.009) and subsequent post-hoc pair-wise tests showed that the mean (± SEM) change in allostatic load for Group 3 (0.35 ± 0.08, n = 4) was significantly higher (decrease in AL) than for Group 1's (0.13 ± 0.09, n = 7, p = 0.01) or Group 2's (0.29 ± 0.17, n = 4, p = 0.004) (Figure 23).

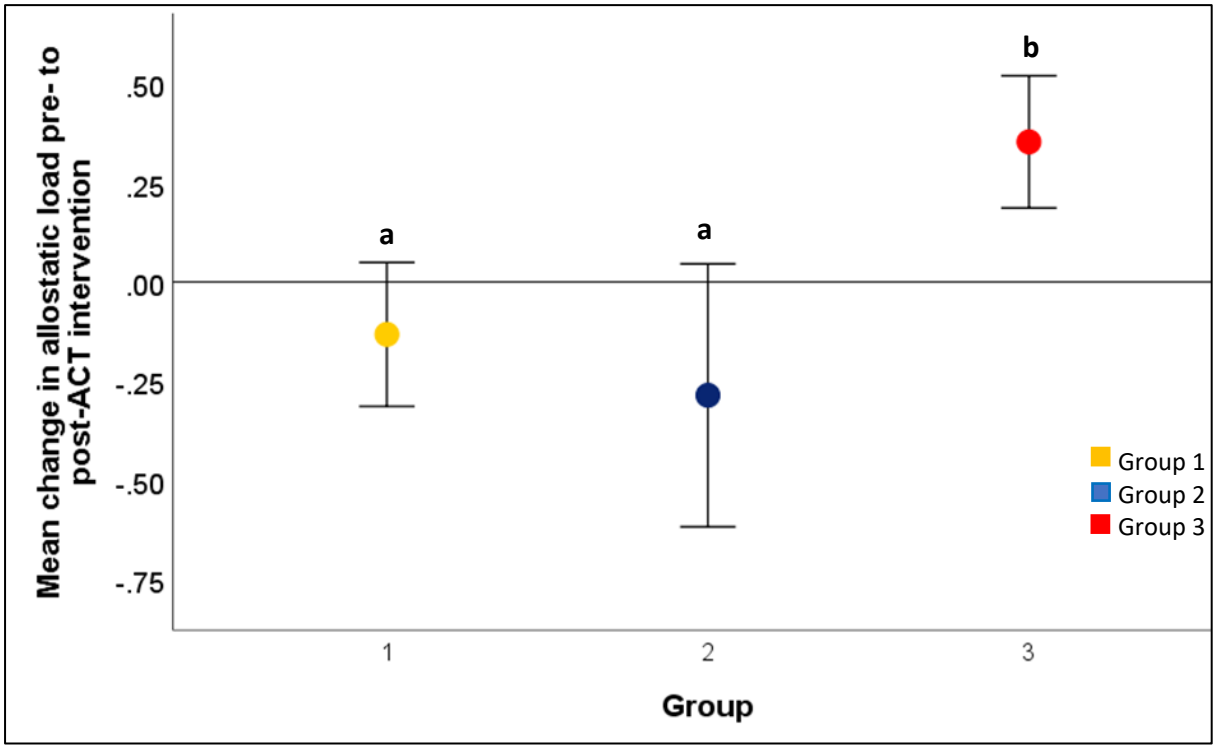

Figure 23: Mean (± 2 SEM) change in allostatic load pre- to post-ACT intervention for each group. Only participants with measurements for both time points for at least five of the six biomarkers were included in samples for analysis: Group 1 (n = 7), Group 2 (n = 4), Group 3 (n = 4). Significant difference between groups are indicated by notations, different notations indicate significant differences.

Chapter 4: Discussion

This pilot study investigated the effects of a six-week group-based Acceptance and Commitment Therapy (ACT) intervention on physiological and molecular biomarkers of stress in breast cancer survivors using a randomised, controlled clinical trial protocol. Physiological measures included resting heart rate and blood pressure and molecular biomarkers assessed included fasting blood glucose levels, relative telomere length (RTL) in peripheral blood leukocytes, plasma interleukin 6 (IL-6), salivary cortisol, and salivary alpha-amylase. The changes in six of these biomarkers were then used to calculate the cumulative change in allostatic load (AL) for participants following the ACT intervention. The primary hypothesis of the study was that individual biomarkers of stress would be reduced in participants following the ACT intervention and this was not supported by the study's findings. The secondary hypothesis was that cumulative AL would reduce following the ACT intervention. The findings indicated that allostatic load was significantly reduced in Group 3 participants who attended the ACT therapy following a six-week wait but was increased in Groups 1 and 2 participants.

The aggressive and prolonged therapies that have led to the increased survival rates from breast cancer can also cause adverse side effects, including impairment of cardiovascular functions such as elevated heart rate (Caro-Moran et al. 2016) and long term elevated heart rate is associated with cardiovascular disease and all-cause mortality (Anker et al. 2016). The study by Caro-Moran et al. (2016) investigating the resting heart rate of breast cancer survivors (n = 22) compared to healthy age-matched controls (50 ± 7.68 years) found that the breast cancer survivors had significantly higher resting heart rates (79.29 ± 11.19 bpm) compared to healthy controls (68.27 ± 7.6 bpm). Several other studies have reported that higher resting heart rate is associated with poorer prognosis in breast cancer survivors (Lee et al. 2016; Lee et al. 2018). Resting heart rate of the participants in this study did not change in response to the ACT intervention. This may be because the mean resting heart rate before the intervention (70.88 ± 8.67 bpm) was in the clinically healthy range and therefore not likely to improve. While the mean age of participants in the Caro-Moran et al. (2016) study was on average eight years lower than the participants in the current study, participants in this study had resting heart rates comparable to the healthy controls and not the breast cancer

survivors. This suggests that the population of breast cancer survivors in the current study have, on average, healthy resting heart rates.

Elevated blood pressure (hypertension) is defined as systolic greater than 140 mmHg and/or diastolic lower than 90 mmHg, and is a reversible risk factor for cardiovascular disease, heart failure and stroke (Carlson et al. 2007; Weber et al. 2014). Breast cancer patients who have undergone chemotherapy and radiotherapy are at a greater risk of having damaged heart muscle making them more susceptible to future cardiovascular disease (Carlson et al. 2007). Thus, it is important for breast cancer survivors to maintain optimal blood pressure in an effort to reduce the risk of cardiovascular disease. There is substantial evidence in the literature that stress-reducing interventions can lead to decreases in blood pressure (Cohen et al. 2015). While a select few participants in this study had hypertension at varying measurement time points the average systolic and diastolic blood pressures for all participants pre-ACT intervention were not elevated but rather in the normal range. There was a small decrease in both systolic and diastolic blood pressure post-ACT intervention with the mean blood pressures trending towards optimal ranges of less than 120 mmHg systolic and 80 mmHg diastolic (Weber et al. 2014). Participants in Group 1 of the study did show a decrease in both systolic and diastolic blood pressure from the initial measure to post-intervention 2 (following 12 weeks of intervention), however it is unclear if this decrease is due to the combination of the two interventions or if the effects of ACT are not evident until after a longer time period such as 12 weeks. A study by Carlson et al. (2007) utilising mindfulness-based stress reduction (MBSR) reported a decrease in blood pressure following an eight-week intervention protocol, suggesting that a longer protocol or time between measurements in blood pressure may be needed before effects are evident.

Several studies have demonstrated that elevated levels of fasting blood glucose are associated with a poorer prognosis after breast cancer (Contiero et al. 2013; Muti et al. 2002; Sieri et al. 2012). Elevated fasting blood glucose is an indicator of diabetes and research has revealed a link between breast cancer and the development of diabetes with the risk of diabetes increasing two years after diagnosis (Lipscombe et al. 2013). This increase in risk is particularly high in patients who have had adjuvant treatment including chemotherapy,

radiation and hormonal therapy (Juanjuan et al. 2015; Lipscombe et al. 2013). This study identified significant increases in the fasting blood glucose concentrations for Group 1 following the ACT intervention and in Group 2 after completion of both interventions. These increases in fasting blood glucose may be associated with participants' breast cancer treatment (chemotherapy, radiation and hormonal therapy) as all of the participants had at least one form of adjuvant treatment.

Previous research has explored the effects of psychotherapy interventions on reducing fasting blood glucose, most commonly in relation to obese and overweight individuals and those with diabetes (Raja-Khan et al. 2017). Raja-Khan et al (2017) observed a significant decrease in blood glucose after eight weeks of a MBSR program however, this decrease was even greater after 16 weeks of the program. When all participant data were combined for pre- and post-ACT intervention in the current study, there were no significant changes in fasting blood glucose over the six-week ACT intervention suggesting ACT had no effect on fasting blood glucose over this time period. It may be that a longer ACT intervention such as a 16 week program is required to illicit a positive effect.

Relative telomere length (RTL) is a measure of chromosomal stability with reduced telomere length in peripheral blood mononuclear cells (PMBCs) associated with accelerated cellular ageing (Epel 2009). Although RTL decreases with ageing, previous intervention studies have demonstrated that psychotherapies such as MBSR can lead to an increase in RTL or at least a decrease in the rate of telomere attrition (Daubenmier et al. 2012; Lengacher et al. 2014). In this study, RTL did not significantly change from pre- to post-ACT intervention for all participants combined. This result was expected as measurable changes in telomere length would not be evident over short periods of time such as the study's six-week ACT intervention (Svenson et al. 2011). It has been suggested that stable alterations to telomere length are only evident after one year (Lengacher et al. 2014). At the six-month follow up, the mean RTL of all three groups increased from the initial measurement but there were no statistically significant differences suggesting that changes are perhaps occurring but may not be significantly different at six months. Telomere length is expected to decrease with age (Epel 2009) however an increase may be a consequence of other factors such as recovery after

cancer treatments. The current literature on the effects of cancer therapies on telomere length in PBMCs are contradictory with some reporting significant shortening (Engelhardt et al. 1998; Schröder et al. 2001) or no changes (Sanoff et al. 2014). A more recent investigation into the effects of chemotherapy on telomere length identified that treatment duration is linked to increased telomere shortening and that telomeres then lengthen in correlation to time after treatment (Benitez-Buelga et al. 2015). This suggests a recovery phase in which telomeres return to their original length (Benitez-Buelga et al. 2015) which may explain our study's findings. A longitudinal study by Scuric et al. (2017) examined the differences in telomere length between breast cancer survivors who had received chemotherapy and/or radiotherapy and survivors who had surgery alone and reported no difference in telomere length between the groups three to six years after the completion of treatment (Scuric et al. 2017). The majority of participants in the current study had either chemotherapy and/or radiotherapy so this makes it difficult to extrapolate the effect of the ACT intervention on telomere length especially in such a small sample size.

Interleukin 6 (IL-6) is a biomarker of inflammation and has been previously shown to be decreased following psychotherapy interventions aimed at reducing stress in participants affected by illnesses other than cancer (Gallegos et al. 2015; Pullen et al. 2008) however a study by Bower et al. (2014) found no significant change in IL-6 in response to a yoga-based mindfulness intervention in fatigued breast cancer survivors. The current study supported Bower et al. (2014)'s finding as it did not identify significant changes in IL-6 concentrations in response to ACT. The IL-6 concentrations of participants enrolled in the Bower et al (2014) study were lower than those found in this study's participants however, this could be due to the effects of fatigue. The current study did not explicitly recruit participants who were experiencing fatigue, so this could account for the difference. The Pullen et al (2018) study reported a significant reduction in IL-6 concentrations in heart failure patients in response to the yoga intervention. The participants in the yoga trial had initial IL-6 concentrations similar to the average participant IL-6 concentrations in the current study however, the yoga trial only identified a significant decrease in IL-6 after a two month treatment period. This suggests that alterations in IL-6 from a mindfulness-based intervention may only be evident after a longer intervention.

Salivary cortisol is one of the most common molecular stress biomarkers that is measured in psychotherapy interventions. While the ACT intervention did not have a significant effect on waking salivary cortisol concentrations, a strong correlation was evident between cortisol concentration pre- and post-ACT intervention, indicating that measuring cortisol once in the morning resulted in consistent cortisol concentrations for individual participants. The average cortisol concentrations in the current study (7.47 ± 0.93 ng/mL, pre-ACT to 7.14 ± 0.92 ng/mL, post-ACT) were comparable to a study by Carlson et al. (2004), in which cancer patients had an average morning cortisol concentration of 6.34 (2.77) ng/mL (n = 31). The Carlson et al. (2004) study indicated that individual participants with higher average daily cortisol concentrations trend to a decrease in cortisol following the intervention whereas participants with lower average daily cortisol concentrations trend to an increase following the intervention (Carlson et al. 2004). This suggests that the effects of a mindfulness-based intervention (MBI) on cortisol concentrations depends on the individual's pre-intervention concentrations. Rather than use a single measurement on one day, the effects of stress on the hypothalamic-pituitary-adrenal (HPA) axis and cortisol concentrations may be better assessed by determining the diurnal cortisol rhythm or response to stress which would involve taking multiple cortisol measurements across the day. A more recent MBI study by Carlson et al. (2015) in distressed breast cancer survivors utilised the multiple cortisol measurement approach. It identified that participants had regular diurnal cortisol rhythms following the intervention compared to the control group, again indicating that multiple cortisol measurements throughout the day rather than a single measurement would be a more effective approach to measuring cortisol changes in response to a MBI (Carlson et al. 2015).

Salivary alpha-amylase (sAA), a biomarker of sympathetic nervous system (SNS) activity, was previously shown to be reduced in cancer survivors following a MBI (Lipschitz et al. 2013). The current study did not find alterations in sAA concentrations in response to the ACT intervention. The sAA concentration changes in the current study (177.4 (146.9) U/mL pre-ACT to 161.8 (127.0) U/mL) were higher than the Lipchitz et al. (2013) study (25 – 60 U/mL) and there was also a large variance in the current study compared to the Lipchitz et al. (2013) study. The large variance in sAA concentrations measured by the current study, indicated variance between individuals. This variance is potentially due to multiple factors that

influence the production of sAA including genetics, epigenetics and saliva collection technique (Beltzer et al. 2010; Sobas et al. 2016). Although the current study did not find significant alterations in sAA concentrations in response to the ACT intervention, similar to the trends in cortisol there was a strong correlation between pre- and post-ACT sAA.

Extensive research into the physiological responses to stress has linked individual physiological systems to health risks, yet little attention has been paid to the co-occurrence of physiological dysregulations across multiple systems (Seeman et al. 2010), the measure of cumulative allostatic load (AL) has aimed to address this limitation. Utilising a composite measure of AL may also potentially measure early pre-clinical signs of dysregulations across multiple systems (Seeman et al. 2010). The majority of research that incorporates an AL index utilise AL as a longitudinal predictive index in relation to health and cognitive outcomes throughout the lifespan, there are limited studies that assess the change in AL in response to an intervention. A study by Carlsson et al. (2017) determined the change in AL in response to the stress of workplace reorganisation (n = 359). This study used a measure of AL based on the original Seeman et al. (2001) ALI, with 13 biomarkers from four different systems (cardiovascular, metabolic, neuroendocrine and immune) and identified an increase in AL over the two-year study period (Carlsson et al. 2017). Ideally, an ALI would be calculated using biomarkers from a wide range of both primary mediators of stress responses and secondary outcome biomarkers. The current study explored the change in AL as measured by six biomarkers from four different systems. The biomarkers included: systolic and diastolic blood pressure, heart rate, fasting blood glucose, IL-6 and salivary cortisol. The ACT intervention resulted in varying effects on the AL for individuals with some having increased and others decreased ALs. As a cohort (n = 15) there was not a significant change in AL following the ACT intervention, but it was identified that Group 3 participants (n = 4) had a statistically significant positive change in allostatic load compared to both Group 1 (n = 7) and Group 2 (n = 4). The ACT intervention appeared to reduce the cumulative biological burden in the participants in Group 3, as measured by the change in AL. Due to circumstances beyond the control of the study team, the facilitator of Group 3's ACT program was a different to Group 1 and Group 2 who had the same facilitator. As Group 3 was the only group to demonstrate a significant and positive change in AL in response to the ACT intervention, the effect of the

facilitator is plausible. Previous studies have revealed a significant impact of therapist attributes and adherence to treatment manuals on patient outcomes in MBI and cognitive behavioural therapy interventions (Snippe et al. 2018; Zickgraf et al. 2016).

The most obvious limitation of this study was the small sample size. This limited the power to discover statistically significant differences however, as a pilot study, this was expected. The other limitation was the number of confounding variables such as the variability in types and combinations of cancer treatments (chemotherapy, radiation and hormone therapy) undertaken by the participants, the contribution of lifestyle changes over the time of the study (such as diet and exercise) and the use of two different ACT facilitators. Furthermore, the inclusion of a wash-out period, such as a week of no intervention, could add strength and facilitate a clearer understanding of whether the six-week ACT program had an effect on the biomarkers.

Chapter 5: Conclusions and Future Directions

In conclusion, this pilot study revealed that a six-week group-based ACT program could drive an improvement in AL but that this effect is highly variable. As the ACT intervention did not result in a consistent and significant change in physiological or molecular biomarkers, we can speculate that the participants in the study were not experiencing enough distress or the individual stress response systems were not dysregulated and therefore had no potential to improve.

Improvements to a future trial could include a larger sample size, longer ACT intervention periods and a wash-out period between each intervention. In addition, recruiting participants with elevated physiological and molecular biomarkers could also be incorporated into the trial design. Finally, expanding the AL calculation by including additional biomarkers may further increase the value of this measure.

In summary, although this pilot study did not demonstrate a significant improvement in physiological or molecular biomarkers of stress and AL in response to the group-based ACT intervention in all participants, it did demonstrate a significant improvement for some. The study also established a successful biomarker testing protocol in the study cohort.

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Appendices

Appendix A

Initial demographics Questionnaire

Demographics

Progress

The following are questions about your basic demographic details.

First Name

Surname

Date of birth

Postcode

Weight (kg)

Height (cm)

Gender

- ◯ Female
- ◯ Male
- ◯ Prefer not to answer

Is your primary language English?

- ◯ Yes
- ◯ No

Do you require support for reading and/or comprehension?

- ◯ Yes
- ◯ No

Please indicate your highest level of completed education

- ◯ Primary school
- ◯ Secondary school
- ◯ Certificate I, II, III, or IV
- ◯ Diploma
- ◯ Bachelor's Degree
- ◯ Postgraduate Degree

Please indicate your relationship status

- ◯ Partnered
- ◯ Not Partnered

Please indicate the number of people you live with

Please indicate the number of dependents you have

Demographics (cont.)

Progress

Now there will be a few pages to fill out about your treatment and treatment history.

When were you diagnosed with breast cancer? (Month/Year)

Which breast(s) was affected?

- ◯ Left
- ◯ Right
- ◯ Both

Was your breast cancer hormone receptor positive?

- ◯ Yes, oestrogen positive (ER)
- ◯ Yes, progesterone positive (PR)
- ◯ No, hormone receptor negative
- ◯ Don't know

Was your breast cancer HER2 receptor positive?

- ◯ Yes
- ◯ No
- ◯ Don't know

Demographics (cont.)

Progress

Please skip over the questions that do not apply.

Are you currently undergoing medical intervention for any conditions (other than cancer related)?

- ◯ Yes
- ◯ No

If you are currently undergoing medical intervention for other conditions, please specify

Have you had a hysterectomy?

- ◯ Yes
- ◯ No

Have you had both ovaries removed?

- ◯ Yes
- ◯ No

Have you had a menstrual period in the past 12 months?

- ◯ Yes
- ◯ No

Have you had a menstrual period in the past 3 months?

- ◯ Yes
- ◯ No

What age were you at menopause or when your ovaries were removed?

Were you at menopause prior to breast cancer treatment?

- ◯ Yes
- ◯ No

What age were you at menarche (your first menstrual period)?

Have you ever been pregnant?

- ◯ Yes
- ◯ No

How many pregnancies?

What age were you at your first pregnancy?

What age were you at your last pregnancy?

Have you ever taken birth control pills?

◯ Yes ◯ No

If yes, for how many years did you take birth control pills?

Have you ever taken any form of oestrogen or hormone replacement therapy (HRT) for menopause?

◯ Yes ◯ No

If yes, for how many years?

During the past month, what was your usual bedtime?

During the past month, how long (minutes) did it usually take you to fall asleep?

During the past month, what time did you usually get up in the morning?

During the past month, how many hours of actual sleep did you get? (May be different to number of hours spent in bed)

During the past month, how would you rate your sleep quality overall?

◯ very good ◯ fairly bad

◯ fairly good ◯ very bad

During the past month, how often did you have a day nap?

◯ over 5 days/week ◯ 1-2 days/week

◯ 3-4 days/week

Treatment

Please indicate whether you are currently receiving, or have received the following treatments for cancer.	Progress: Current	Past	Not applicable
Breast surgery	◯	◯	◯
Surgery to the armpit	◯	◯	◯
Breast prostheses	◯	◯	◯
Breast reconstruction	◯	◯	◯
Radiotherapy	◯	◯	◯
Chemotherapy	◯	◯	◯
Hormonal therapies	◯	◯	◯
Ovarian treatments	◯	◯	◯
Other therapy	◯	◯	◯

Appendix B

Hygienic collection of Saliva using the Salivette Cortisol device (Sarstedt 51.1534.500)

- The night before your Saturday morning session at the Hospital, label the Salivette Cortisol device with your date of birth.
- As soon as you wake up on Saturday morning and before you get out of bed, remove the blue lid of Salivette Cortisol device to expose the swab material.
- Place the cotton swab directly into mouth without touching with fingers.
- Gently chew swab for about 60 seconds to produce saliva.
- Then replace the swab directly into labelled tube (again, do not touch swab with fingers).
- Replace the blue cap and seal securely.
- Write the time and date of collection on the tube.
- Place Salivette Cortisol device upright into the container provided and bring to your Saturday morning session at the Hospital.

Appendix C

Table 4: Physiological and molecular biomarkers for each group at all four measurement time points. Mean and standard deviation calculated using all individual results at each time point are included.

		Group 1				Group 2				Group 3			
		Time Point				Time Point				Time Point			
		1	2	3	4	1	2	3	4	1	2	3	4
Heart Rate (bpm)	N	7	7	6	7	5	6	6	5	6	5	5	6
	Mean (SD)	70.29 (6.42)	70.86 (6.77)	70.00 (4.94)	73.43 (8.87)	73.40 (13.24)	71.67 (10.25)	73.33 (7.31)	76.80 (18.67)	67.33 (9.33)	69.40 (10.74)	71.00 (10.79)	70.17 (10.42)
	Minimum	61	62	64	62	55	55	65	62	60	57	56	57
	Maximum	79	79	78	87	90	82	85	107	85	86	82	88
Systolic Blood Pressure (mmHg)	N	7	7	6	7	4	5	5	4	6	5	5	6
	Mean (SD)	130.57 (8.52)	125.14 (11.01)	115.33 (9.77)	120.57 (11.41)	133.50 (8.18)	117.20 (18.31)	119.20 (13.31)	114.00 (14.54)	132.00 (11.37)	120.00 (17.50)	122.00 (8.00)	117.33 (12.50)
	Minimum	119	108	100	98	121	92	108	100	112	92	114	98
	Maximum	143	138	128	130	140	138	138	131	143	134	134	132
Diastolic Blood Pressure (mmHg)	N	7	7	6	7	4	5	5	4	6	5	5	6
	Mean (SD)	84.29 (5.56)	83.14 (9.58)	73.00 (4.15)	79.14 (4.18)	88.50 (9.00)	76.40 (10.04)	77.60 (9.63)	78.25 (5.85)	85.00 (10.92)	78.60 (10.04)	74.00 (8.60)	75.83 (10.13)
	Minimum	76	68	68	75	76	64	68	72	68	67	66	60
	Maximum	92	94	78	88	96	88	88	85	96	90	86	90
Blood Glucose (mmol/L)	N	7	7	6	7	4	6	5	5	6	5	5	6
	Mean (SD)	5.03 (0.63)	5.54 (0.67)	5.62 (0.53)	5.54 (0.52)	4.93 (0.43)	5.77 (0.78)	5.72 (0.40)	5.66 (0.41)	5.07 (0.42)	5.32 (0.51)	5.16 (0.18)	5.32 (0.40)
	Minimum	4.1	4.6	4.8	4.8	4.3	5.1	5.3	5.2	4.3	4.7	5.0	4.9
	Maximum	5.9	6.6	6.3	6.2	5.2	7.3	6.3	6.2	5.5	6.0	5.4	5.9

Table 4 Continued: Physiological and molecular biomarkers for each group at all four measurement time points. Mean and standard deviation calculated using all individual results at each time point are included.

		Group 1				Group 2				Group 3			
		Time Point				Time Point				Time Point			
		1	2	3	4	1	2	3	4	1	2	3	4
Relative Telomere Length	N	6	6	5	5	4	5	5	5	6	5	5	5
	Mean (SD)	1.64 (0.51)	1.62 (0.37)	1.53 (0.13)	1.83 (0.94)	1.60 (0.46)	1.61 (0.43)	1.69 (0.23)	2.04 (1.05)	1.63 (0.63)	1.67 (0.39)	1.48 (0.42)	2.01 (1.00)
	Minimum	1.15	1.17	1.33	0.54	1.31	1.19	1.47	0.57	0.89	1.17	1.08	0.27
	Maximum	2.54	2.23	1.67	2.76	2.28	2.33	2.03	3.36	2.67	2.13	2.13	2.82
Interleukin 6 (pg/mL)	N	6	6	5	5	4	5	5	5	6	5	5	5
	Mean (SD)	2.98 (1.81)	4.44 (2.85)	4.45 (2.04)	2.72 (1.69)	3.12 (1.65)	2.96 (3.00)	2.64 (1.43)	2.21 (2.31)	5.52 (1.46)	6.91 (4.20)	4.29 (1.67)	3.60 (1.28)
	Minimum	0.14	0.00	1.48	0.14	1.17	0.57	0.78	0.00	3.50	1.94	1.58	1.36
	Maximum	5.29	8.48	6.92	4.20	4.95	7.31	4.36	5.76	6.74	12.60	6.17	4.43
Cortisol (ng/mL)	N	5	6	5	5	4	4	4	3	5	5	4	6
	Mean (SD)	8.11 (3.92)	6.94 (3.64)	7.46 (3.72)	9.72 (9.90)	6.94 (3.11)	7.06 (4.68)	7.64 (3.65)	6.20 (1.20)	8.03 (3.98)	6.80 (1.26)	6.47 (3.01)	9.32 (6.38)
	Minimum	4.47	4.19	4.49	4.12	4.09	3.84	4.25	4.93	5.16	5.72	2.39	4.09
	Maximum	12.62	13.08	13.85	27.35	10.89	13.78	11.00	7.32	14.95	8.81	9.64	21.65
Salivary Alpha Amylase (U/mL)	N	6	6	5	5	5	5	5	4	6	5	4	6
	Mean (SD)	223.88 (137.22)	137.76 (79.80)	168.12 (39.08)	176.96 (124.26)	82.03 (86.17)	179.69 (187.79)	190.61 (165.38)	110.55 (80.93)	86.12 (71.07)	119.75 (96.88)	161.98 (160.16)	150.91 (159.58)
	Minimum	95.04	60.69	143.12	56.83	15.96	6.05	19.35	23.94	0.00	12.10	9.23	7.18
	Maximum	490.45	287.31	236.52	376.55	213.79	462.88	389.58	206.05	216.21	247.65	355.02	377.76

Appendix D

Table 5: Physiological and molecular biomarkers for each group at time points T1, T2 and T3. Mean and SEM are calculated for each biomarker and each group separately to include individuals with all three time point measurements. Bold values indicate significant difference ($p < 0.05$) between time points within one group. Time points with different superscripts within a biomarker for one group identify significance ($p < 0.05$) between time points. No superscripts indicate no significant differences ($p > 0.05$).

		Group 1 (ACT - BCE)			Group 2 (BCE - ACT)			Group 3 (Waitlist - ACT)		
		Time Point			Time Point			Time Point		
		1	2	3	1	2	3	1	2	3
Heart Rate (bpm)	N	6	6	6	5	5	5	3	3	3
	Mean (SEM)	70.00 (2.85)	70.67 (3.02)	70.00 (2.02)	73.40 (5.92)	69.80 (4.59)	72.40 (3.47)	70.00 (7.64)	70.67 (8.41)	69.00 (7.51)
Systolic Blood Pressure (mmHg)	N	6	6	6	4	4	4	3	3	3
	Mean (SEM)	**130.00^{a} (3.75)**	124.00^{ab} (4.73)	**115.33^{b} (3.99)**	132.50 (4.09)	118.50 (10.44)	122.00 (6.78)	136.33 (2.96)	126.67 (6.36)	126.00 (4.62)
Diastolic Blood Pressure (mmHg)	N	6	6	6	4	4	4	3	3	3
	Mean (SEM)	**84.83^{a} (2.40)**	83.00^{ab} (4.28)	**73.00^{b} (1.69)**	88.50 (4.50)	78.50 (5.12)	79.00 (5.26)	92.00 (2.00)	84.67 (4.37)	78.67 (4.67)
Blood Glucose (mmol/L)	N	6	6	6	4	4	4	3	3	3
	Mean (SEM)	**4.98^{a} (0.27)**	**5.55^{b} (0.30)**	**5.62^{b} (0.22)**	**4.93^{a} (0.21)**	5.40^{ab} (0.12)	**5.70^{b} (0.23)**	5.10 (0.10)	5.30 (0.17)	5.23 (0.12)
Relative Telomere Length	N	5	5	5	4	4	4	3	3	3
	Mean (SEM)	1.58 (0.25)	1.50 (0.11)	1.53 (0.06)	1.60 (0.23)	1.63 (0.25)	1.74 (0.11)	1.26 (0.28)	1.56 (0.25)	1.29 (0.18)
Interleukin-6 (pg/mL)	N	5	5	5	4	4	4	3	3	3
	Mean (SEM)	3.54 (0.58)	4.48 (1.42)	4.45 (0.91)	3.12 (0.82)	3.55 (1.55)	2.83 (0.79)	5.34 (0.92)	9.60 (1.55)	5.06 (0.55)
Cortisol (ng/mL)	N	4	4	4	4	4	4	3	3	3
	Mean (SEM)	6.98 (1.73)	5.81 (1.32)	7.83 (2.09)	6.94 (1.56)	7.06 (2.34)	7.64 (1.83)	**5.85^{a} (0.44)**	**7.42^{b} (0.75)**	6.28^{ab} (2.11)
Salivary Alpha Amylase (U/mL)	N	5	5	5	5	5	5	4	4	4
	Mean (SEM)	224.65 (68.60)	153.18 (35.15)	168.12 (17.48)	82.03 (38.53)	179.69 (83.98)	190.61 (73.96)	86.94 (45.86)	104.84 (52.52)	161.98 (80.08)