THE PSYCHONEUROIMMUNOLOGY OF WOMEN EXPERIENCING STRESSFUL LIFE EVENTS:

TESTING THE OXIDATIVE MODEL

A thesis submitted for the Degree of

DOCTOR OF PHILOSOPHY

THE UNIVERSITY of ADELAIDE

by

Jodie Merle Oliver-Baxter

B. A. (Hons)

School of Psychology

The University of Adelaide

May, 2011

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Jodie Oliver-Baxter and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968.

I also give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library catalogue, the Australasian Digital Theses Program (ADTP) and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

Signed..... Date.....

ABSTRACT

It has been acknowledged that psychological stress can impact on one's health. A definitive link between psychological state, immune suppression, and disease has yet to be established. A possible mechanism has been termed The Oxidative Model. This refers to the oxidative imbalance of cells associated with antioxidant status and psychological distress. The aim of this dissertation was to use this theoretical model to establish an evidence basis for future interventions in vulnerable populations. For cancer patients the post-treatment period has been identified as psychologically challenging. In addition bio-psycho-immunological models remain underexplored in post-treatment breast cancer samples to date.

Two longitudinal studies were employed. The first, an observational study of a sample of women (N=17) concluding treatment (chemotherapy and/or radiotherapy) for early stage (I-III) breast cancer at the Royal Adelaide Hospital, South Australia. The second study tested the benefits of antioxidants during prolonged stress using an 8-week RCT. A sample of general population women (N=60) reporting mild to severe psychological distress was recruited. Psychological parameters measured included Psychological Distress, Defense Styles, Loneliness, Anger Expression, Psychological Adjustment, the Impact of Events Scale (IES-R), and State-Trait Depression, Curiosity, Anxiety, and Anger. Biochemical parameters included 5'-ectonucleotidase (NT), homocysteine (HCY), tissue ascorbate (VIT C), c-reactive protein (CRP), cholesterol (CHOL), folate (FOLATE), Vitamin B12 (VIT B12), and inflammatory cytokines (IL-1 β, IL-5, IL-6, IFN-γ, TNF-α, TNF-β, and IL-10).

Findings from study 1 indicated severe psychological distress was experienced for a subset of breast cancer patients post-treatment. Fluctuating levels of psychological distress, anger, anxiety, and curiosity were observed across the 20-weeks. A pro-oxidant state was evident during this period. Pro-inflammatory measures were low and relatively stable. Associations between psychological measures and biomarkers supported Oxidative Model relationships. The second study revealed improved pro-oxidant and pro-inflammatory biomarkers favoured the multivitamin supplemented group. Collectively both studies reveal the influence of demographic and health behaviours on bio-psycho-social measures central to the Oxidative Model propositions. This thesis brings out the case for exploring complementary interventions, like multivitamin use, in the post-treatment period for those patients experiencing distress.

ACKNOWLEDGEMENTS

Sincere thanks to my supervisors Professor Deborah Turnbull, Professor Ian Olver, and Dr Hayley Whitford. It has not been the most straightforward of candidatures, but you have each been contributed uniquely across the course of this project. You patience and persistence has not gone unnoticed.

For those who assisted me with collection of biomarkers- Breast Care Nurse, Judy Isiello, and staff at the Women's Health Service at the Royal Adelaide Hospital, and Louise Turnbridge at the Nursing School, at UniSA. This project wouldn't have been possible with out the facilities, your skills, can-do attitude, and wonderful bedside manners.

To dear Dr Ainsley Chalmers, for coming out of retirement to lend his biochemical expertise & to Flinders Medical Centre for finding space for him to work his magic. Thank you also to the Immunology Department at the Women's and Children's Hospital for their technical expertise for cytokine assays, specifically Trish, Kathy, and of course Professor Toni Ferrante, and Pete Pascoe and staff at SA pathology.

To all the women who took part in this research. Their contribution of valuable data as well as sharing their personal journeys allowed this dissertation to take shape.

To my family and friends- especially Ben and Fletcher, and our baby in waiting I appreciate your patience and understanding-

'Life is what happens when you are doing a PhD'!

Table of Contents

APPENDICES

List of Tables

List of Figures

Structure of the Dissertation

This dissertation is dedicated to the Psychoneuroimmunology of women. It comprises one longitudinal observational investigation, followed by a randomised controlled trial of women experiencing stressful life events. Due to the multidisciplinary nature of the topics studied, a thorough introduction to each study will be provided in the respective chapters. A brief overview of the chapters follows:

Chapter 1 focuses on briefly describing the paradigm of Psychoneuroimmunology. This chapter provides a review of the immune system prior to the presentation of a theoretical model- The Oxidative Model- in Chapter 2. Chapter 1 is not intended as a comprehensive description of the field of immunology but rather a review of the literature important to the Psychoneuroimmunology framework as it exists currently. It involves a brief introduction to Psychoneuroimmunology, identifying general trends, the conceptual, methodological, and design challenges for research in this area. It also discusses proposed Models of immune change during stress.

Chapter 2 introduces one specific PNI model - The Oxidative Model. This chapter provides a detailed review and critique of the literature applying to this Model to date. This chapter encompasses a detail of the pro-oxidant and pro-inflammatory biomarkers employed, followed by a thorough critique of the previous research which has employed this theoretical Model. This chapter sets the background for designing studies for this dissertation based on previous research findings and limitations.

Chapter 3 introduces a population for which The Oxidative Model has yet to be applied: women treated for early stage breast cancer. This section focuses on the first

6-months following the conclusion of active treatment. The focus of this review is specific to literature on psychosocial implications during this period. It attempts to highlight both the disparities and similarities of this period with psychological constructs utilized in The Oxidative Model. These constructs include both positive and negative, and include a spectrum of constructs which incorporate distress, anxiety, depression, anger, curiosity, post traumatic stress disorder (PTSD), coping styles, and social needs. The aim is to clarify this period as one which has chronic stress characteristics like previous Oxidative Model research.

This chapter also presents a review of psychoneuroimmunological studies undertaken on breast cancer patients' once active treatment has ceased. It aims to provide the framework for the proposed research questions. This section comprises both psychosocial and psychoneuroimmunological research in order to align this research with The Oxidative Model literature to date.

Chapter 4 outlines the thesis rationale in the context of the literature reviews provided in the previous chapters. Principal research questions are proposed.

Chapter 5 describes Study 1, an observational study of breast cancer patients. It involves the measurement, longitudinally, of psychological and biochemical markers that have been associated with chronic stress. The associations between psychological and biochemical variables are explored and discussed in view of psychoneuroimmunological findings from previous Oxidative Model research. In addition trends, in psychological constructs like distress, anxiety, depression, anger, curiosity, PTSD and coping are investigated. Pro-oxidant and pro-inflammatory biomarkers are assessed, based on propositions of a bio-psycho-immunological model

relating chronic stress to a pro-oxidant and pro-inflammatory internal state; The Oxidative Model (Blake-Mortimer, Winefield, & Chalmers, 1996).This chapter provides a generic methodology section which describes data collection and laboratory assay techniques used across both studies in this dissertation.

Results from Study 1, explore both cross-sectional and longitudinal data. Limitations of this study are discussed, including sample size, inter-individual variability, and heterogeneity. The heterogeneous nature of the sample was highlighted by several areas of disparity. This included how stressful individuals found the post-treatment period, demographic differences, varied treatment regimes prior to the study and diversity in individuals' health behaviours.

This level of diversity was a major challenge for drawing conclusions for this study. Several health behaviours and demographic variables were identified as confounders. The Oxidative Model proposes vitamins, specifically those with antioxidant properties, to alleviate the negative impact of chronic stress on pro-oxidant and pro-inflammatory biomarkers. This was partially supported by findings from study 1; regular vitamin-taking by patients was identified as a confounder for only one Oxidative Model biomarker, HCY. In light of this relationship and based on gaps in past Oxidative Model literature, a randomised controlled trial (RCT) to further assess the influence of vitamin-taking during chronic stress was proposed in a larger and more homogeneous sample.

Chapter 6 is based on previous Oxidative Model research and the findings and limitations from Study 1 of this dissertation. A new direction away from the oncology population was taken with regard to the sample utilized. In order to attain a more

homogenous sample with less 'nuisance' variables (i.e. treatment confounders), a sample of healthy women screened to be experiencing chronic stress were recruited from the general population. Eligible participants were randomised to either an Active or Placebo Group. The Active Group was the intervention group and consisted of an 8-week course of multivitamins targeted to be beneficial during periods of stress. Conversely, those allocated to the Placebo Group received a placebo; an identical capsule with non-active ingredients. The data collection methodology outlined in Chapter 3 was adhered to. Pre- to post-intervention changes, plus group comparisons are discussed. Correlational analyses were also employed to clarify psychoneuroimmune associations. Partial support was observed for Oxidative Model mechanisms.

In Chapter 7, major conclusions from Study 1 and Study 2 are reviewed. Strengths and Limitations of the dissertation are presented. Future directions for The Oxidative Model are discussed.

Chapter 1

An Introduction to Psychoneuroimmunology

1.0 Overview

The aim of the current chapter is to provide the reader with an overview of the theoretical framework associated with Psychoneuroimmunology. In order to achieve this a brief overview of the immune system will be presented in order to orientate the reader to terminology and concepts employed throughout this thesis. Although this section attempts to introduce concepts and terms of immunology, this is not a comprehensive description of the field of immunology but sufficient to set the context for the psychological work. It is provided as a background to aid in the discussion of psycho-neuro-immunological (PNI) connections at a later stage in this dissertation. This will be followed by an overview of methodological and conceptual issues associated with PNI.

1.1 Psychoneuroimmunology

Principles explored in this dissertation fall under a broad research area. PNI is the study of the interaction between the nervous system, the endocrine system, and the immune system and how psychological stressors modulate these interactions (Glaser, 2007; Maier, Watkins, & Fleshner, 1994). It explores biological, psychological, and behavioural pathways by which the mind influences physical health(Martin, 1997). The influence of personality characteristics on cancer development and prognosis is perhaps one of the earliest PNI research questions. As far back as the second century

AD, Greek physician Galen proposed that women who developed cancer had more melancholic traits as opposed to sanguine characteristics (Sali, 1997). This was perhaps one of the first written accounts linking psychological characteristics to disease. The past three decades have seen an increase in evidenced-based research which highlights the existence of links between psychological stress and illness (Herbert & Cohen, 1993; Segerstrom & Miller, 2004). These observed links range from psychological well-being associated with simple allergic responses, susceptibility to herpes and influenza vaccines, through to more complex relationships such as cancer development, prognosis and progression, wound healing, coronary heart disease, and human immunodeficiency virus (HIV) progression documented through comprehensive reviews.

The diversity of populations' studied in addition to the varied biochemical, endocrine, neurological and immune components explored requires some background in order to define the body's systems which underpin this research area.

1.2 The immune response

This section provides an overview of the physiology of the immune system in a simple manner. It is recognized that the immune system comprises nervous, endocrine, and immune components. For this thesis the focus is on elements of the immune system but touch on both nervous and endocrine components. However for amore comprehensive explanation the reader is advised to refer to sources such as Rabin's (1999), *Stress, Immune Function and Health: The Connection*.

At the outset it is important to clarify that immune responses are highly redundant and interdependent, thus defining the system is challenging (Segerstrom & Miller, 2004). For this review it is useful to distinguish between innate/natural and acquired/adaptive immune responses (Anderson et al., 1998; Rabin, 1999; Thornton, Anderson, Crespin, & Carson, 2007) as they are central to the focus of this thesis.

The function and efficacy of the immune system is to distinguish between self and non-self, to protect an organism from foreign or invading organisms (e.g., protection from bacteria, viruses, protozoa, fungi, worms) or from the development of cancerous cells (Coico, Sunshine, & Benjamini, 2003). For this review we will use the term *antigen* to encompass all of the aforementioned terms. An antigen is a molecule which stimulates an immune response. Different types of immune cells (T-cells and B-cells) involved in an immune response are spread throughout the body forming a complex defense system. The immune system can broadly be divided into two elements, innate and acquired immunity (Sarafino, 1998). These are not isolated systems; they overlap and complement each other.

1.2.1 Innate versus acquired.

Innate immunity is the first-line of defense against invading antigen. It involves a general non-specific response (Peakman & Vergani, 1997). The generalised response of innate immunity is inflammation. It can be thought of as three different but interconnected systems: physical barriers (i.e., skin), extracellular secreted products (i.e., substances for bacteria breakdown in mucus, acidic environment of the stomach), and cellular components (i.e., phagocytic cells like macrophages and neutrophils that engulf and destroy invading antigen with which they come in contact with). The innate

response is characterized by being rapid and antigen non-specific (Coico et al., 2003). The process of inflammation is a protective response against injury and infection.

Innate immune responses are largely pro-inflammatory because the immune cells which characterize this response (phagocytes) release cytokines which are communication molecules with a range of functions, including fever initiation, inflammation, and wound healing. In addition further contribution to the inflammatory process is by the release of oxygen radicals. These are toxic substances. This type of immunity is generalised.

In contrast to the generalised innate immunity, acquired immunity is a well-coordinated response to a specific antigen (Martin, 1997). This type of immunity is present at birth but is weak, and strengthens as part of the bodies' developmental process. Acquired immunity is considered adaptive because firstly it is acquired from previous exposure or experience and secondly it increases in intensity with repeated exposure to antigen, and hence has memory (Coico et al., 2003).

Acquired immunity complements the innate immune response because it is specific, and facilitates a response to a specific immunological threat (Maier et al., 1994; Martin, 1997). As such, it is mediated by three types of lymphocytes: T-helper cells (e.g., $CD4^{+}$), T-cytotoxic cells (e.g., $CD8^{+}$), and B-cells. The T-helper cells produce cytokines thereby promoting a cascade of other immune processes; T-cytotoxic cells lyse compromised cells; and B-cells produce antibodies to counteract bacterial and viral infections.

It is important to reiterate that the innate and acquired immune responses are not mutually exclusive, but intertwined. For instance, a rapid innate response activates acquired immunity whilst defending the body until the acquired response is generated.

1.2.2 Cell-mediated and humoral immune responses.

Acquired immunity can be further divided into two branches of immune response, cell-mediated and humoral responses. Cell-mediated immune responses focus on attacking antigens present inside cells (i.e., virus infected cells) and are coordinated by a subset of T-helper cells, called T_H1 cells. T_H1 cells release particular cytokines, e.g., Interferon-gamma (IFNγ) or Tumor necrosis factor-beta (TNF-β), which activate T-cytotoxic cells and NK cells (Coico et al., 2003; Rabin, 1999).

The humoral immune response is mediated by serum antibodies, proteins secreted by B cells and involves several cytokines (IL-1, IL-4, IL- 5, IL-10, IL-13) and Tumor necrosis factor-alpha (TNF-α). This response is coordinated by the T_H2 cells and directed against extracellular antigens such as bacteria and parasites. T_H2 cells suppress intracellular defense reactions (Maier et al., 1994). It is important to recognize that the cytokine activity promoted by one T_H cell type inhibits the other.

1.2.3 Hypothalamic-pituitary-adrenal (HPA) axis.

There are two main biological systems that mediate the stress response, the sympathetic nervous system (SNS) and the Hypothalamic-Pituitary-Adrenal (HPA) axis (Figure 1).

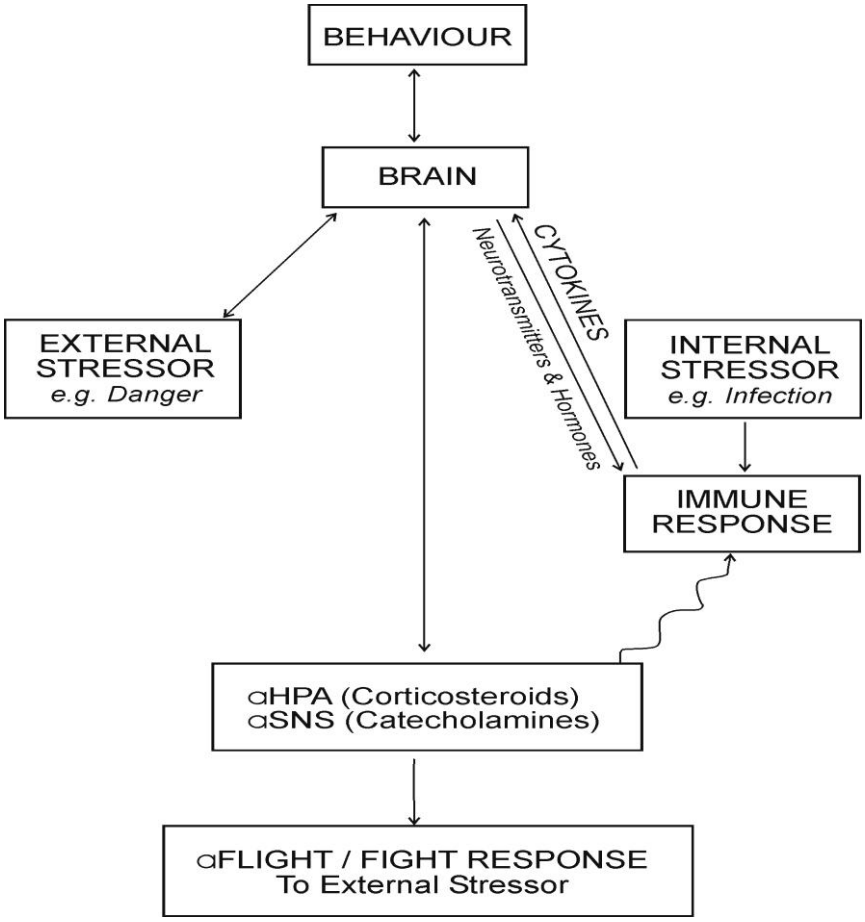


Figure 1: Nervous system and Immune System Interaction reproduced with permission from author (Blake-Mortimer et al., 1996)

This figure indicates that during a response to a stressor the nervous system is activated with the intent to avoid the stressor. The hypothalamus, which maintains the body's homeostasis, sends messages by both electric (neurotransmitters) and endocrine (hormones) pathways to the pituitary gland. These messages activate the sympathetic nervous system; this occurs rapidly and is responsible for the automatic and unconscious regulation of bodily functions. For example, during a stress reaction heart rate, blood pressure, and pupil dilation initially decline but then rapidly increase (Sapolsky, 2004). These are considered measures of an immediate physical response to a stressor. Concurrently the hypothalamus is activated and subsequently releases corticotrophin-releasing hormone (CRH) triggering the pituitary gland to secrete

adrenocorticotrophic hormone (ACTH) into the bloodstream. Once in the blood stream this causes the adrenal glands to release adrenalin and cortisol (Martin, 1997; Sarafino, 1998). These hormones stimulate increases in blood pressure, blood sugar levels, and have an immunosuppressive action. These are important processes which supply energy for the body to escape the stressor.

Similarly, with infection or stress, the immune system releases cytokines - chemical messengers. These stimulate the release of CRH from the hypothalamus. In turn ACTH is released from the pituitary gland triggering the release of corticosteroids from the adrenal glands. The release of corticosteroid from the adrenal gland results in the down regulation of the immune response (Barnes et al., 1993; Blake-Mortimer et al., 1996; Sternberg, Chrousos, Wilder, & Gold, 1992). Once cortisol levels rise, CRH release is shut down, in this way normalizing the HPA axis from its hyper-excitable state. This pattern of activation and suppression maintains the body's internal environment; this is known as homeostasis. The aim of this feedback mechanism is to decrease variability and maintain constancy in the system (Carlson & Chamberlain, 2005).

1.2.4 Bi-directional communication.

Although the immune system has been the focus of the above review, the body's regulatory systems pertinent to a discussion on PNI, namely the central nervous system, the endocrine system, and the immune system have all been implicated in regulation of the body's homeostasis. Rather than a top down hierarchy for regulation, the systems interface continuously and information flows in both directions (Martin, 1997) and can be likened to a feedback loop. The basis of PNI rests on the

interconnectedness of these three systems in the body and specifically the regulation of the immune response by the brain. The brain has two ways to control the peripheral organs and processes. The autonomic nervous system is composed of sympathetic and parasympathetic branches. These are responsible for not only innervating visceral organs like the heart and stomach (Sarafino, 1998), but also immune organs like the thymus, bone marrow, and spleen. The brain can also communicate with peripheral organs by releasing factors that in turn cause endocrine glands to secrete hormones (i.e., like cortisol) into circulation (Maier et al., 1994); this can be described as communication via chemical or endocrine pathways.

The presence of stress has often been defined by the existence of high levels of these hormones in the blood (Maier et al., 1994). T- and B-cells have receptors for many hormones, including these stress hormones. The immune system also communicates with the brain using cytokines as messengers. For example, during an infection cytokines released by white blood cells tell the brain to increase body temperature. Catecholamines, glucocorticoid hormones, and cytokines are considered the principle messengers responsible for bi-directional communication between the central nervous system (CNS) and the immune system (Maes et al., 1998).

Psychoneuroimmunologists direct their attention to understanding the mechanisms of stressor-induced alterations of immune system function and subsequent health alterations. This review of the immune system has introduced terms and concepts central to discussion of PNI research. It is apparent that the immune system is complex and that stressor-induced immune alteration involves many components of health and can affect many different cells and tissues. These

cells, organs, and systems are often independent of the immune system's alterations. However the body's systems are all interdependent and work in an integrated manner. Proper function of the total system is a highly variable process. This review highlights the interconnectedness of the body's systems in maintaining the body's homeostasis ; that is the body's internal balance or equilibrium.

1.3 Challenges to PNI research

The physiological response to a stressor has been outlined. This review highlighted the complexity of the body's systems, in particular the immune system. The perception of stress is also complex. It is widely acknowledged that the perception of an event as stressful initiates both physiological and behavioral responses. Mechanisms underlying this response require further exploration. Research in this area is growing but there are several limitations to the research to date. These challenges will be discussed in order to familiarise the reader to the conceptual and methodological challenges.

1.3.1 Conceptual difficulties: the stress definition.

Defining and measuring stress is complex. 'Stress' although widely used in everyday language has many different meanings. Stress has been defined as a state or feelings experienced when a person perceives the demands of a situation to surpass their available resources (Sarafino, 1998). Further to this definition it has come to represent a non-specific response of the body to any demand (Selye, 1956), thereby incorporating the physiological response as well as cognitive processes by which an event is perceived and appraised by an individual.

There are several significant difficulties around the concept of stress. The initial difficulty with defining stress is that the term has been applied to include actual events, as well as to an individual's reaction to an event (Keller, Schleifer, Bartlett, Shiflett, & Rameshwar, 2000) or a perceived event. To remedy this, it has been suggested that a definition of stress requires three key elements: the event (or stressor), the appraisal (weighing up of demands vs. available resources), and finally the undesired response of the individual resulting from their perception of the stressor (Lazarus & Folkman, 1984).

Secondly, Keller and colleagues (2000) also point out that a self-report of stress may not always correspond with observational accounts. This may be due to lacking sensitive psychometric measures and/or the desire to portray a positive state of mental health.

Thirdly, although the aforementioned definitions are quite general and broad, the everyday language around 'stress' is commonly negative. There is little recognition that there is some degree of stress in almost every aspect of our lives. However like Lazarus' definition (Lazarus & Folkman, 1984) suggests, it is only when an excess of demands outweighs the available resources that this becomes negative, or distressing. The optimal amount of stress which helps to promote wellbeing has been termed, Eustress. This term first coined by Hans Selye (1975). Unlike distress this has both innervating and motivating qualities, and actually improves performance. It has been suggested that Eustress is characterized by short-term and not a severe level of stress. Some stressors cause both good and bad stress e.g. exercise is considered a good stressor, but overtraining when your body is weak and fatigued can cause injury and

increase illness susceptibility. However negative distress can be both acute and chronic in duration. The characteristics of stress require further exploration; no distinct boundaries exist between positive and negative, acute and chronic.

1.3.2 Categorizing stress: course and duration.

Research suggests that subjective measures of self-reported stress do not conclusively associate with immune change (Segerstrom & Miller, 2004).Rather information about the quality of a stress scenario has been identified as important to the interpretation of PNI research. The use of taxonomies for categorizing stress has been proposed. PNI research has attempted to define and categorise 'stress' according to the two key characteristics – duration and course (Herbert & Cohen 1993). This has largely led to a dichotomous approach which includes acute and chronic stress. However there have been attempts to further refine the definition in order to distil the findings of many studies which have found differing physiological mechanisms (Herbert & Cohen, 1993; Segerstrom, 2004). The taxonomy of stressors referred to in this dissertation is based on alternative characteristics than those two proposed above; duration and course OR discrete vs. continuous.

Categories have been established to broadly define stress type in order to assess immune response to different stressors. This is imperative as stress experiences have been shown to elicit different physiological responses (Segerstrom & Miller, 2004). Stressor classification (Elliot & Eisdorfer, 1982) includes five categories of stressors. Acute time-limited stressors include laboratory challenges, i.e., public speaking or mental arithmetic. Brief naturalistic stressors are when a person confronts a real-life short term challenge, i.e., academic examinations. Stressful event sequences

identify stress that is based on a focal event and a related series of challenges, i.e., bereavement or natural disaster. Chronic stressors are identified as stressors that pervade a person's life forcing one to restructure their role or identity, i.e., caring for a spouse with dementia. Chronic stressors are, by definition, very stable with no clear idea when the challenge will come to an end (Segerstrom & Miller, 2004). Distant stressors are identified as traumatic experiences that have occurred in the past yet still influence cognitive and emotional thinking, thus still impacting on immunological markers, i.e., experiencing combat, or childhood sexual assault.

1.3.3 Stress response measures as an additional, objective measure of stress.

Developments in laboratory techniques and biomedical sciences have allowed for the assessment of physiological components of a stress response. These responses include nervous, endocrine, and immune system components. The stress response is a basic survival response (fight or flight). It is important to reiterate how important the stress response is for the human body to deal with situations perceived as threatening, regardless of whether a threat is of actual or perceived physical harm or psychological distress. The difficulty in defining and measuring stress psychometrically has been discussed. The advantage of contemporaneously measuring a physiological response to stress is that it is an autonomic. Thus measuring self-report and physiologic measures potentially provides more detailed information and clarification of how stress-inducing a situation actually is. Furthermore it allows for individual differences to be explored. For example the meta-analyses by Segerstrom and colleagues (2004) suggest an individual experiencing chronic stress may have a different reaction to that of someone experiencing a short burst of an acute stressor (i.e., examination). In short,

assessing physiological measures has the potential to improve assessment of the experience and impact of stress.

1.3.4 Methodological limitations: samples, measures, design.

There are several broad limitations that have been identified in the PNI literature. Firstly a majority of studies recruit young, healthy samples. For example in a recent meta-analysis of over 300 empirical studies less than 20% of all participants across studies were over 55 years of age(Segerstrom & Miller, 2004). Similarly only a small proportion comprised samples drawn from populations experiencing disease (HIV/AIDS, arthritis, cancer, and asthma). This is despite research suggesting that both age and health status is a source of vulnerability to functional immune changes (Boss, Thompson, Spielberg, Pichler, & Seegmiller, 1980). More pronounced decreases in Natural Killer cell activity (involved in cell-mediated defense) and proliferation of T-cells were observed in older samples from this meta-analysis suggesting a potential decrease in acquired immunity (Segerstrom & Miller, 2004). Therefore more research on PNI in populations with health problems and older populations is required.

PNI research relies on the measurement of the immune system. The complexity and redundancy of elements of an immune response mean that the assessment itself becomes a limitation. The measurement of immune function in PNI research is based on enumerative counts of immune measures (total or %) OR the functional ability of these markers (i.e., ability to respond to foreign antigen). The normal range for various biochemical and immune markers are relatively wide, reflecting the adaptive capacity of the body to cope with minor changes sufficiently; thus it is unlikely that small changes will have clinical significance (Segerstrom & Miller, 2004). A key challenge

facing PNI is to find a sensitive and relatively stable measure of immunity during psychological distress that would represent a clinically meaningful change. For instance NK cell cytotoxicity, a commonly measured marker of cell-mediated immune response, is a relatively volatile measure with one study reporting only a 25% stable variance over a 1-week period(G. E. Miller, Cohen, & Herbert, 1999). However improved reliability can be achived as a result of repeated testing of immune parameters across a period of time. In this case the magnitude of obtained relationships between NK cell cytotoxicity and other reliably measured variables (i.e., cancer outcomes) improves two-fold (Segerstrom, 2003).

There are a few key research directions to consider based on these challenges alone. Firstly, repeated assessments of subjects over a period of time is recommended. In addition it would be wise to assess different categories of stress separately and identify immune measures relevant and reliable for these specific stressors. In addition assessment of psychological constructs like emotion states and traits would add contextual information of an individuals' vulnerability to different types of stressors.

1.3.5 Proposed models linking stress and immunity.

Although the last 30 years has seen an exponential increase in PNI research and published articles, the evidence to date is intriguing, mixed, and largely inconclusive. While it is beyond the scope of this dissertation to review all literature linking stress and immune changes, the following section will present the main models to date. These are presented to provide a framework for the subsequent model proposed in Chapter 2.

Originally it was suggested that stress suppressed the entire immune response (Selye, 1975); this was termed a Global Suppression Model. However based on the notion that the immune response is adaptive, more recent research has challenged this assumption. Proposed models have taken into account the impact of both the duration and course of a stressor.

The Biphasic Model proposes that acute stressors should actually cause a redistribution of immune cells to trigger a quick and efficient immune response. This model suggests that the immune response under acute stress is enhanced and under chronic stress is suppressed (Dhabhar & McEwen, 1997; Seeman, McEwen, Rowe, & Singer, 2001). Since its inception, this model was altered to take into account the adaptive reduction in energy expenditure. Rather than shifting all immune cells during stress there is a proposed shift toward increased activation of the innate immune response and a reduction of the acquired or specific processes. The principle underlying this is that a heightened innate response is better suited to managing potential life threatening complications (i.e., injury, fleeing) as they are subject to fewer inhibitory constraints, require less energy, and importantly take less time to unfold (Dopp, Miller, Myers, & Fahey, 2000; Sapolsky, 2004).

This model has evidenced support more recently (Segerstrom & Miller, 2004) with acute stressors (lasting minutes) being associated with potentially adaptive up-regulation of some parameters of innate immunity (increased NK cell counts) and little change in acquired immune measures (T-lymphocytes and B-cells). Similarly brief naturalistic stressors, such as academic examinations, evidenced a shift from a T_H1 (cell-mediated), whilst maintaining a T_H2 immune response (humoral immunity).

However chronic stressors were associated with overall suppression, including innate and acquired immune responses as well as T_H1 (e.g., T-cell proliferative responses) and T_H2 (e.g., antibody response to vaccine) processes (Segerstrom & Miller, 2004). Although this model accounts for differences across stressors it does not address the link between chronic stress and disease outcomes associated with inadequate immunity (e.g., neoplastic diseases like cancer) or disease outcomes associated with excess immune response (e.g., autoimmune or allergic responses). To resolve this contradiction the Cytokine Shift Model was proposed.

The Cytokine Shift Model suggests that chronic stress has a simultaneous enhancement and suppression of the immune response (Marshall et al., 1998). It does this by altering patterns of cytokine secretion. T_H1 cytokines, which activate cell mediated immunity responsible for defense against infection and some types of neoplastic disease, are suppressed. Contemporaneous enhancement of the T_H2 response activates humoral immunity which is responsible for allergy type responses and autoimmune diseases. It is proposed that this shift can occur by the release of stress hormones such as cortisol (Chiappelli, Manfrini, Franceschi, Cossarizza, & Black, 1994). This model attempts to remedy the link between stress-related immune change and stress-related disease outcomes.

1.3.6 Proposed models linking stress, health, and chronic disease.

Presented above are the predominant models of stress and immunity which have provided the framework for research in the PNI area. It is evident that these models have evolved with increased evidenced-based research. The Cytokine Shift Model begins to address the association of chronic stress with health and chronic

disease. However the focus is predominantly on cytokine secretion patterns. A framework which addresses stress-related immune change and stress-related disease outcomes in more detail is termed Allostatic Load (McEwen, 1998a, 1998b). Allostasis is the process whereby an organism maintains physiological stability by changing parameters of its internal state to match environmental demands (Juster, McEwen, & Lupien, 2010), in short, where stability is maintained through change. This is an autonomic response and an extension of the homeostasis concept.

Homeostatic systems are essential for life (e.g., temperature, glucose levels, oxygen saturation) whereas the mechanisms that maintain the overall systems in balance is known as allostasis. Allostasis represents the adaptation processes of the body's complex physiological systems to physical, physiological, psychosocial, and environmental challenges or stress (Logan & Barksdale, 2008). Biomarkers of allostasis include immune measure, neuroendocrine, cardiovascular, and metabolic biomarkers. If components of any of these systems are out of balance (i.e., due to chronic stress) an allostatic state results. Some examples of allostatic states include chronic hypertension, flattened cortisol rhythms in major depression, and sustained elevation of inflammatory cytokines accompanied by low cortisol in chronic fatigue syndrome (McEwen, 2005). Allostatic states have the capacity to cause wear and tear on regulatory systems throughout the brain and body, further exacerbating allostatic imbalances.

Uniquely, this model emphasises the protective as well as the damaging effects of stress on the body's attempts to cope with the challenges known as stressors. Every system of the body responds to acute stress with allostasis leading to adaptation, but

when acute responses are over-activated usually over a period of time allostatic overload results. Frequent or chronic challenge produces dysregulation of major physiological systems including the HPA axis, the nervous and immune systems. The burden of allostatic overload has been found to impact on secretion of stress hormones (like adrenalin and cortisol), and cardiovascular measures (e.g., hypertension, atherosclerosis, stroke, etc) as well as immune system changes discussed in the section above.

The Allostatic Load framework is discussed here because it is most relevant to the model proposed in Chapter 2, The Oxidative Model. In addition, Allostatic Load better addresses the negative impact of stress on an individual. This model also integrates the burden of chronic stress and associated personal health behaviours like diet, smoking, and alcohol intake.

1.4 Summary

The psychoneuroimmunology framework has been introduced. In addition this chapter has reviewed the immune system and stress response in order to orientate the reader to the Psychoneuroimmunology approach. Critically this contains simplistic definitions of what is an intricate system. However this review highlights the complex patterns and bi-directional relationships which comprise the body's systems which are pertinent to this dissertation. Proposed models of Psychoneuroimmunology were presented, ending with a focus on the Allostatic Load concept. Several conceptual and methodological issues have been explored; these include defining stress, course and duration of stressors as well as methodological challenges highlighting why findings in this area remain tenuous. For the scope of this dissertation we will narrow the focus

to one particular model which attempts to explore the mechanism underpinning stress-related immune changes and stress-related disease outcomes- The Oxidative Model.

Chapter 2

The Oxidative Model

2.0 Overview

This chapter will introduce the psychoneuroimmune framework to be tested in this dissertation: The Oxidative Model. This will include an overview of the Model, introduction to the biomarkers, and research supporting the Model's assertions. This will be followed by a more detailed critique of the literature on The Oxidative Model to date. The aim is to provide a thorough review of the current research. Published studies are the focus; however unpublished dissertations are also examined. Conceptual and methodological challenges will also be discussed.

2.1 The Rationale of The Oxidative Model

The Oxidative Model proposes that prolonged maladaptive emotion states such as stress, anxiety, depression, and anger are associated with increased cellular oxidative stress, susceptibility to infection, and predisposition to cardiovascular disease (CVD). The Model suggests the experience of chronic stress disrupts the homeostatic mechanisms between the immune system and the hypothalamic-pituitary-adrenal axis (HPA), resulting in compromised immune functioning. During an experience of stress (whether infection or stress or depression), the immune system releases cytokines which stimulate the activation of the HPA axis. This results in the release of corticosteroids from the adrenal glands. An increase of corticosteroids is commonly associated with a down-regulation of immunity (Katzung, Masters, & Trevor, 1992).

Blake-Mortimer, Winefield and Chalmers (1996) propose a system by which a down-regulation of the acquired immune response is observed contemporaneously with an up-regulation of innate immunity resulting from chronic stress. As outlined in Chapter 1, chronic stress has been identified as stress that pervades a person's life, forcing one to restructure their role or identity. These stressors, are by definition, very stable with no clear idea when the challenge will come to an end.

The Oxidative Model linking chronics stress with immune dysregulation proposes two biochemical pathways illustrated in Figure 2. The first pathway illustrated in the bottom loop of Figure 2 suggests that during chronic stress prolonged release of cortisol results in excess neutrophils activation. Neutrophils generate oxygen free radicals. These are highly reactive and unstable molecules used by the immune system as a way to attack and kill pathogens. When functioning in regular circumstances neutrophils are beneficial, and levels fluctuate as required. However during a chronic stress response an excess of these free radicals deplete (oxidize) vital antioxidant stores. This in turn can damage biological molecules and key cellular components leading to oxidative stress. Oxidative stress has been linked with decreased 5-'ectonucleotidase (NT). NT is an ectoenzyme on the surface of lymphocytes which controls maturation of immune cells, specifically lymphocytes. A decrease in NT levels compromises lymphocyte maturation (Blake-Mortimer et al., 1996; Blake-Mortimer, Winefield, & Chalmers, 1998b). It is this process which interferes with acquired immunity.

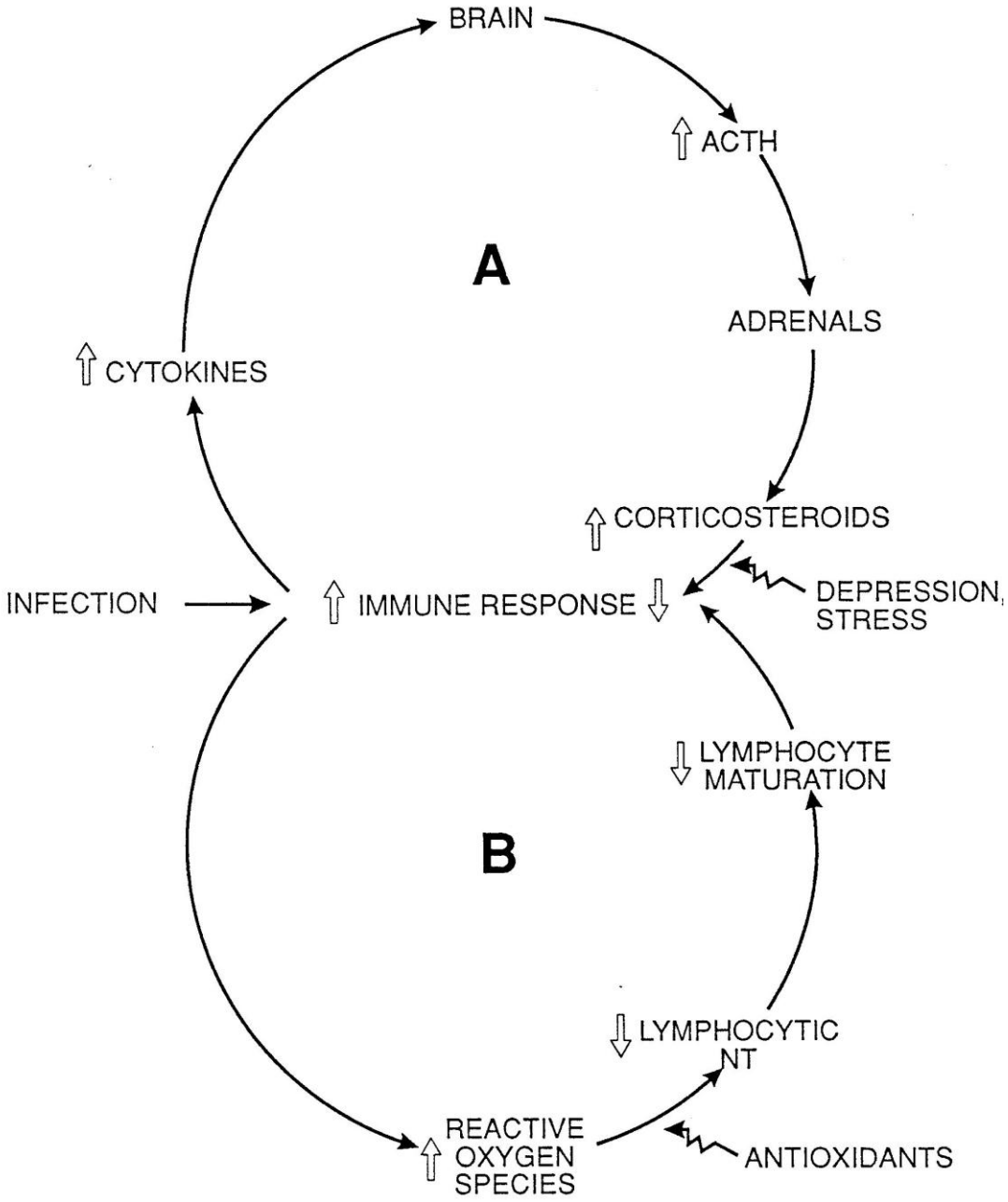


Figure 2: The Oxidative Model reproduced with permission from author (Blake-Mortimer et al., 1996)

The second biochemical pathway illustrated in the top loop of Figure 1 implies that lymphocyte counts are directly affected by the sustained release of cortisol. The lymphoid tissue becomes desensitized to the effects of the corticosteroids and stress responses remain activated. The result is a lack of suppression of the innate immune response. Although these responses are adaptive during acute stress, in the case of chronic stress they lead to overstimulation of the HPA axis, and repeated activation of allostatic responses as is indicated in Figure 1. These two pathways exist simultaneously.

The repeated activation leads to interconnected systems producing byproducts in order to compensate for dysfunction in other systems (i.e., decreased acquired immune responses compensated with increased innate immune response). This process in the Allostatic Load literature has been coined the 'domino effect' (Juster et al., 2010). Aligned with this, interconnected pro-oxidant biomarkers have been incorporated, as illustrated below in Figure 3. For instance during chronic stress increased homocysteine (HCY) levels have been observed (Hapuarachchi, Chalmers, Winefield, & Blake-Mortimer, 2003). HCY is recognized as a clinical measure for assessing risk factor for atherosclerosis. Due to their role in synthesizing HCY, FOLATE and Vitamin B 12 (VITB12) have been included in this Model.

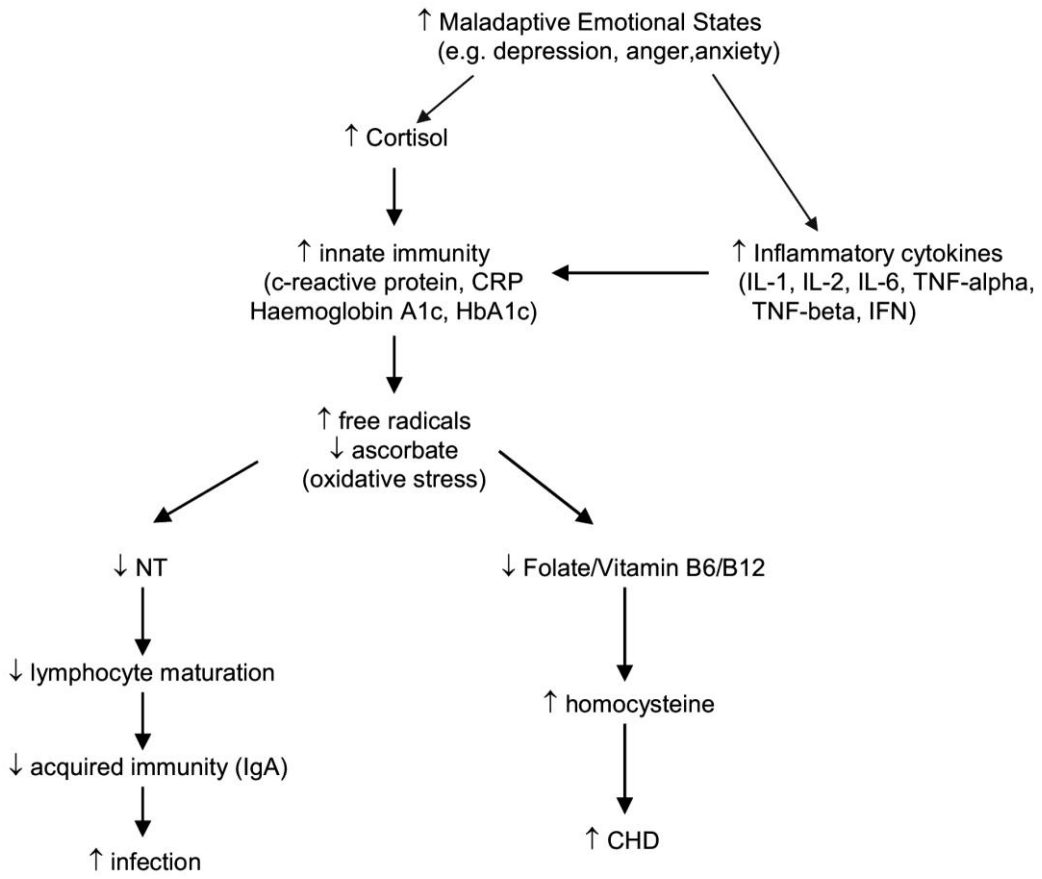


Figure 3: Possible pathways of an increased susceptibility to infections and cardiovascular disease (with permission from Blake-Mortimer, 2004)

This 'domino effect' described above also involves pro-inflammatory processes. As discussed in Chapter 1, inflammation is an important part of an innate immune response. C-reactive protein (CRP) is a measure of systemic inflammation or pro-inflammatory processes and has been implicated in The Oxidative Model (Hapuarachchi et al., 2003). Inflammatory cytokines (IL-1, IL-2, IL-6, TNF-α, TNF-β, and IFN) were more recently incorporated into the Model because they play a well-defined role in immune responses and inflammatory processes. Cortisol release triggers shifts in cytokine secretion. Specifically T_H1 cytokines, which activate cellular immunity-important in screening for neoplastic disease and infections, are suppressed. Concurrently T_H2 cytokines, which activate humoral immunity and are pro-

inflammatory, are enhanced, exacerbating allergy and various autoimmune diseases (Coico et al., 2003).

2.2 Biochemical markers implicated in The Oxidative Model

The following section will present each individual biomarker presented in The Oxidative Model introduced above. The markers are broadly divided into immune activation, as part of pro-inflammatory processes and pro-oxidant markers that indicate immune changes resulting from oxidative stress. The relationship of the biomarkers to chronic stress will be also discussed. Novel biomarkers will also be explored. A brief description of biochemical markers referred to in this section is presented in Table 1.

Table 1: Oxidative Model Biomarkers: Definitions, Functions and Expected Change during Chronic Stress

Biochemical Marker	Definition	Function	Expected Change
5-'ectonucleotidase (NT)	Ecto-enzyme on the external surface of lymphocytes	Regulator of lymphocyte maturation (Bastian et al., 1984)	Lowered
Tissue Ascorbate (VIT C)	Level of Vitamin C in the cells of the body	An essential antioxidant in human metabolism (Chalmers, Winefield, & Blake-Mortimer, 2003)	Lowered
Homocysteine (HCY)	A sulphur-containing amino acid occurring naturally in the body	A risk factor for cardiovascular incidence. Associated with ageing, Folate and Vitamin B deficiencies (Friso, Jacques, Wilson, Rosenberg, & Selhub, 2001)	Elevated
Vitamin B12 (VITB12) & Folate (FOLATE)	Essential nutrients required for healthy cellular function	Act as cofactors in the breakdown of HCY by specific enzymes (Friso et al., 2001)	Lowered
C-Reactive Protein (CRP)	An acute-phase protein produced by the liver during an innate inflammatory	Binds receptors on micro-organisms enabling complement-mediated lysis and phagocytosis (Goldsby, Kindt, &	Elevated

2.2.1 Pro-oxidant markers

2.2.1.1 5'-ectonucleotidase (NT).

NT is essential for the maturation of lymphocytes. Lower levels indicate a lowered acquired immune response; full review of the immunology specific to this biomarker are detailed in several studies (Bastian et al., 1984; Boss et al., 1980; Chalmers, Hare, Woolley, & Frazer, 1990). It is the central biochemical marker of The Oxidative Model.

Psychoneuroimmune studies employing this Model have observed NT levels to be lowered during periods of chronic psychological stress in both clinical and non-clinical samples. These examples of chronic stress include recently diagnosed HIV patients (Chalmers & Hare, 1990), clinically depressed patients, self-reported stress over an academic year for students (Blake-Mortimer et al., 1996) (Blake-Mortimer et al., 1998b; Jolly, 2004; Le, 2004; Oliver, 2004), university staff reporting occupational strain (Hapuarachchi et al., 2003), victims of crime (Pfitzer, 2008), and returned servicemen (Vietnam Veterans)(Jolly, 2004).

Blake-Mortimer and colleagues (1996; 1998) identified an association between chronic stress and increased oxidative stress as indicated by lowered NT. Students undertaking an intensive 12-month Honours Psychology course were found to have lowered NT levels (33%) prior to final examinations and thesis submissions compared with NT levels earlier in the same academic year ($p < .05$, $N = 47$). Depletion of NT, as a result of psychological stress, was evidenced to occur over a period of 1-3 months (Blake-Mortimer et al., 1996). This suggests NT is a marker of chronic stress rather than acute stress due to the length of time students experienced the ongoing stressor. A subsequent study of occupational strain in university employees observed that workers who reported severe psychological stress in the past 2-weeks, as measured by the GHQ-12 (1978) concurrently evidenced lower levels of NT (36%), $r = -.49$, $p < .01$, $N = 43$ (Hapuarachchi et al., 2003).

2.2.1.2 Tissue ascorbate (VIT C).

VIT C has been included in this Model due to its antoxidant (AO) properties and subsequent protective role for the NT biomarker. As described in Chapter 1, there are

millions of processes occurring at all times within the human body. These processes often require oxygen. As a byproduct, oxygen radicals (free radicals) are generated. For example, monocytes and neutrophils generate free radicals as part of an innate immune response. Free radical generation has been observed to be increased in depression (Joyce, Hawes, Mulder, Sellman, Wilson, Boswell, 1992; Maes, et al. 1993). Excess oxygen radical generation can cause cell damage and lead to chronic disease.

In-vitro and in-vivo studies of these biomarkers indicate the VIT C is depleted prior to NT (Blake-Mortimer et al., 1998b). The in-vitro studies suggested that VIT C prevented oxidation by free radicals. Outside the laboratory this effect has been observed in both clinical and non-clinical samples. For instance clinically depressed patients not taking a supplement comprised of high levels of VIT C had significantly lower NT levels (50%) compared to patients consuming a high VIT C supplement ($p < .05$, $N = 32$). Patients taking the high VIT C supplement had NT values twofold higher than the non-supplemented patients. These levels reflect NT activity within a normal range (Blake-Mortimer et al., 1996). In-vitro studies further observed VIT C levels to be depleted prior to NT levels, suggesting the protective effect of this AO (Blake-Mortimer 1998).

These findings provide suggestive evidence that AO supplements like VIT C might protect NT from oxidative damage during periods of ongoing stress, like major depression. Qualifying whether major depression can be defined as a chronic stress scenario has its challenges. However recent research has suggested a reciprocal relationship between stress and depression (Liu & Alloy, 2010). Specifically that depression plays a role in generating the very stresses that place individuals at heightened risk for future depression.

Similar patterns of VIT C and NT have been observed in a more recent study examining occupational stress in university staff. This study found that those reported to be regular vitamin takers had normal levels of NT relative to those who were not taking supplements (p = .03, N = 43; Hapuarachchi et al., 2003). In addition other PNI research has indicated that stress may be a significant factor in the pathogenesis of metabolic disorders and subsequently nutritional intervention or pharmacological agents targeted at moderating stress should be investigated (Seematter, Binnert, Martin, & Tappy, 2004).

2.2.1.3 Homocysteine (HCY).

HCY is an indicator of pro-oxidant state is implicated in the Model. The aforementioned study of occupational stress in university staff explored this biomarker. Happuarachchi and colleagues (2003) grouped together all regular (3-5 times per week) vitamin takers. A significant difference was found between high and low vitamin-consuming groups on HCY levels and several stress parameters. Specifically, the high vitamin-consuming group had significantly lower levels of HCY than the low vitamin-consuming employees (p = .04, N = 43). In addition psychological distress and absenteeism were lower in the regular vitamin-consuming group. Increased distress symptoms (r = .31, p = < .05, N = 43) and absenteeism (r = .29, p < .05, N = 43) were also positively correlated with HCY levels.

Additional support for the association between heightened emotion states and HCY has been reported. Specifically, increased stress and anger expression emotion states have been found to be associated with higher HCY levels (Oliver, 2004; Stoney, 1999; Stoney & Engebretson, 2000). HCY levels are associated with atherosclerosis, risk

of coronary heart disease, stroke and peripheral vascular diseases (Ross, 1999). Homocysteine has also been implicated in senile dementia, Alzheimer's disease (Minagawa, Watanabe, Akatsu, Adachi, Ohtsuka, et al, 2009), osteoporosis (Tyagi, Vacek, Fleming, Vacek, and Tyagi 2011), recurrent miscarriage, and pregnancy complications (Dasarathy, Gruca, Bennett, Parimi, Duenas, Marczewski, et al 2009).

2.2.1.4 Folate (FOLATE) and Vitamin B12 (VITB12).

The breakdown of HCY relies on B vitamins and FOLATE. It is considered that levels of these nutrients are influential to The Oxidative Model. FOLATE is also known as folic acid and is sometimes referred to as Vitamin B9. For this dissertation it is referred to as FOLATE. Critically the study by Happuarachchi and colleagues (2003) encompassed a wide variety of vitamins, some AO and others vitamin B rich supplements. These findings provide partial support for AO and vitamin B rich supplementation contributing to the prevention of oxidative stress associated with chronic psychological stress. Specifically, as outlined above, those regularly taking vitamin supplements had improved NT (p = .03, N = 43) and HCY (p = .04, N = 43).

2.2.2 Pro-inflammatory markers.

The role of HCY in The Oxidative Model has been previously outlined above. HCY is recognized as a risk factor for cardiovascular diseases (CVD). Several other measures incorporated into The Oxidative Model have also been linked to increased risk of CVD. These predominantly measure inflammation. In contrast to the immune changes associated with pro-oxidant measures, pro-inflammatory processes influence immune activation via alternative pathways. Pro-inflammatory measures C-reactive

protein (CRP) and inflammatory cytokines have been explored in the context of The Oxidative Model.

2.2.2.1 C-reactive protein (CRP).

CRP is a marker of systemic inflammation. As outlined in Chapter1, the inflammatory response is closely intertwined with oxidative processes like the release of oxygen radicals by immune cells. CRP is also a predictor of CVD risk for both men and women (Ridker, Buring, Shih, Matias, & Hennekens, 1998). For these reasons it was included in the Model. Supporting evidence has also been established. Happuarachchi and colleagues (2003) identified significant mean differences for CRP levels between university staff experiencing normal and severe levels of self-reported distress experienced in the past 2-weeks($p \leq .05$, $N = 43$), as measured by the GHQ-12 (1978) Higher levels of CRP were observed in the severe stress group.

Further exploration of pro-inflammatory processes and The Oxidative Model have observed CRP levels to be significantly higher in a sample of victims of crime (VOC) with a clinical diagnosis of PTSD than levels observed in age-matched controls ($p < .05$, $N = 58$, $d = .36$; Pfitzer, 2008). Pro-inflammatory processes have been suggested by the high levels of CRP and the inflammatory cytokine, TNF-α in the post traumatic stress literature (Melamed, Shirom, Toker, Berliner, & Shapira, 2004; R. G. Miller, Sutherland, Hitchinson, & Alexander, 2001). Further afield, in a large population based study of adults aged 17 to 39 years ($N = 458$), an association between depression and CRP levels was observed (Danner, Kasl, Abramson, & Vaccarino, 2003). Men with a history of a major depressive episode were about twice as likely to have elevated CRP compared with men without a history of depression: 24.0% vs. 12.6%, (un adjusted

odds ratio, 2.17; 95% CI, 1.81–4.00). However these results were not reflected in women. This suggests that there may be gender-specific differences for PNI biomarkers. In short, CRP has been included in this Model because of the evidence linking this pro-inflammatory indicator with psychological stress, depression, and trauma.

2.2.3 Novel biomarkers.

Given the interconnectedness of the body's systems additional biomarkers have been explored in the context of The Oxidative Model with inconclusive findings. The following biomarkers have not been directly implicated in The Oxidative Model to date. Evidence is presented here to confirm that these novel markers have the potential to add to The Oxidative Model as a gauge of either pro-oxidant or pro-inflammatory processes. It will also contribute to the overall picture of an individual's health status.

2.2.3.1 Cytokines.

Pro-inflammatory cytokines are novel to The Oxidative Model literature, with only one study to date employing these measures (Pfitzer, 2008). Table 2 provides an outline of the functions of cytokines. As discussed earlier, the most recent Oxidative Model research employed victims of crime (VOC) with a clinical diagnosis of PTSD (Pfitzer, 2008). Exploration of cytokine patterns showed TNF-α ($p < .05$, $N = 58$, $d = .19$) and IFN-γ ($p < .05$, $N = 58$, $d = .41$) levels were significantly higher in the VOC group compared to healthy non-stressed controls. This suggests heightened inflammatory processes.

This finding reflects similar patterns found in other clinical samples, where inflammatory cytokines have been associated with psychiatric disorders like major depression and schizophrenia (Myint, Leonard, Steinbusch, & Kim, 2005). Furthermore, several studies have identified stress to be associated with increased inflammatory cytokines. One such study of academic examination stress and cytokine production observed those experiencing psychological stress (*N* = 38) to exhibit increased production of TNF-α ($p < .00$), IL-6 ($p = .05$), IL-1 ($p = .01$), IFN-γ ($p < .00$), and IL-10 ($p < .00$; Maes et al., 1998). This suggests that a T_H1-like response was induced, i.e., an inflammatory response. Similarly a separate study (Paik, Toh, Lee, Kim, & Lee, 2000) of academic examination stress (*N* = 42) observed increased levels of IL-1 β ($p < .00$), Il-6 ($p < .00$), and IL-10 ($p < .00$), and decreased IFN- γ ($p = .04$). No change was observed for TNF- α. These findings also suggest an increased T_H1 or cell-mediated immune response.

In contrast Kang and Fox (2001) found a decrease in T_H1 and an up-regulation of T_H2 cytokines (IL-2) during academic stress (*N* = 24), represented a decrease in inflammatory processes. There are several possible reasons for the discrepancy between these findings. These include disparities between sampling times, assay techniques, and psychological scales employed.

There is evidence of changes in cytokine secretion patterns in response to psychological stress in clinical and non-clinical samples. However contradictory findings have been reported. These measures require further investigation because of their importance for homeostatic mechanisms and immune response. The measurement of cytokines provides an additional marker of inflammation (Peakman &

Vergani, 1997). The Oxidative Model literature to date suggests prolonged stress to be associated with increased inflammatory cytokines, but this requires further investigation.

2.2.3.2 Cholesterol (CHOL).

Cholesterol levels have been explored in many of The Oxidative Model studies. This measure is largely included as a general measure of well-being and cardiovascular risk. Patterns of change for the biomarker during periods of chronic stress remain unclear. Additionally the association of this biomarker with psychological status remains undetermined, and requires further exploration.

Table 2: Cytokine production, function and expected change during periods of chronic stress

Cytokine	Produced by	Function	Reference	Expected Change
Interleukin-1 (IL-1-β)	Monocytes	Induces fever, stimulates acute-phase protein synthesis, promotes proliferation of helper T-cells	(Song et al., 2004)	Elevated
Interleukin-2 (IL-2)	Helper T cells	Promotes T-cell proliferation	(Coico et al., 2003)	Elevated
Interleukin-6 (IL-6)	T-cells, Macrophages	T-cell activation, stimulates antibody secretion, blood cell production, stimulates acute-phase protein synthesis (CRP production)	(Murtaugh, Baarsch, Scamurra, & Lin, 1996)	Elevated
Tumour Necrosis Factor-β (TNF-β)	T cells	Induces oxidative stress; targets tumour and inflammatory cells, enhances phagocytosis, and necrosis of tumor cells	(Coico et al., 2003)	Elevated
Tumour Necrosis Factor-α (TNF- α)	Macrophages, Mast cells	Induces cytokine secretion associated with chronic inflammation, induces fever and septic shock, targets tumour and inflammatory cells	(Coico et al., 2003; Goldsby et al., 2000)	Elevated
Interferon-γ (IFN-γ)	Helper T cells, Macrophages	Activates NK cells and macrophages, targets uninfected cells to inhibit viral replication	(Coico et al., 2003)	Elevated
Interleukin-10 (IL-10)	Macrophages, B cells, and some T cells	An anti-inflammatory cytokine, capable of inhibiting synthesis of pro-inflammatory cytokines like IFN- γ and TNF-α,	(Coico et al., 2003)	Lowered

2.3 A critical review of The Oxidative Model literature

So far the Oxidative Model has been presented with a glimpse of the supporting evidence for the separate biomarkers currently included in the Model. In addition some other novel markers that could improve or extend the Model have been discussed. This section will discuss the literature related to the Model in greater depth in relation to specific dissertation aims. Literature is presented in chronological order to give a picture of how the Model has evolved.

2.3.1 Recently diagnosed HIV positive patients.

NT was first identified of interest as a marker of lymphocyte maturation in an immunodeficient population. As lymphocytes (white blood cells) are key cells in an immune response it was considered very important in this immune-compromised population. In the early 1980's researchers confirmed that NT was a marker of lymphocyte differentiation/maturation (Bastian, Ruedi, MacPherson, Golembesky, O'Connor and Thompson, 1984). Following this, it was observed that recently diagnosed HIV positive men (*n* = 12) had 40-50% lower NT levels (Chalmers, Hare Woolley and Fraser, 1990). In this recently diagnosed population, lowered NT levels were initially considered part of the course of the acquired immunodeficiency syndrome (AIDS). This was thought to be a result from the specific damage to the immune system caused by the human immunodeficiency virus (HIV). On further in-vitro investigation of the impact of HIV virus on lymphocytes it was discovered that the virus was not directly responsible for the initial decrease in NT. It was hypothesised that perhaps it was the stressor associated with a of HIV positive diagnosis which was

linked to this initial decline in NT. This was the beginnings of exploring the connection between stress and NT.

2.3.2 Academic stress.

Blake-Mortimer, Winefield and Chalmers (1996) suggested that there may be a psychoneuroimmunological factor which would explain the depleted NT in the group of recetly diagnosed HIV positive patients. They initially observed and compared levels of NT in three groups; (1) low stress controls (*n* = 10), (2) high stress group (*n* = 18) and (3) Honours Psychology students (*n* = 21). These groups were defined based on screening scores on the POMS Tension-Anxiety and Depression-Dejection scales. The Honours psychology course is considered a most stressful academic challenge as it encompasses a research/thesis component, formal coursework, mid-year and end-of-year examinations in a short, competitive period of time (less than one year). This group's stress level was considered to vary across the academic year. Higher reported levels of stress were based on the Profile of Mood scores (POMS; McNair, Lorr, & Droppleman, 1971). Identification of high stress 'cases' were based on Tension-anxiety or Depression-dejection subscale scores being above 20 and participants self-report of experiencing 'extremely' stressful events for one month or longer. Based on definitions of stress, discussed in Chapter 1 (Segerstrom & Miller, 2004), this sample covers several categories including: brief naturalistic, stressful event sequence and chronic stress scenarios.

This was an observational study. Data were collected across three time points throughout the year, identified as one high (due to academic examinations) with two low stress periods either side. Findings indicated that Group 3, the Honours students,

had a 33% lowering of their NT values during the high stress period of study ($p < .05$, $n = 21$). NT levels rose significantly post-stress to within 20% of their initial low stress levels ($p < .05$, $n = 21$). A similar pattern was found for Group 2, the high stress group, with significant depletion observed between initial low to high stress period ($p < .05$, $n = 18$), and significant improvement at post-stress assessment ($p < .05$, $n = 18$). Group 1, the low stress controls, did not differ significantly over three testing times. Correlations were performed using only the Honours and high stress data in order to explore the specific relationships between aspects of mood and NT. This potentially removed some of the variability as low stress controls should have had 'normal' NT and psychological stress levels than the other two groups. This possibly confounds these findings.

2.3.3 Major depression

To further explore the relevance of these biomarkers as a potential objective marker of psychological distress, Blake-Mortimer and colleagues (1996) conducted another study to explore the relationship between NT and antioxidant (AO) supplementation . Two groups diagnosed with major depression disorder (MDD) were compared. Group 1, (n = 9) were inpatients placed on a high dose AO supplement (e.g., 1-4 g VIT C per day). Group 2 ($n = 23$) consisted of a mixture of hospitalized and community-dwelling patients not on the high AO supplement. Group 3 was a control group of non-clinical subjects ($n = 28$) with Trait Anxiety scores less than 35 on the State Trait Anxiety Inventory (STAI; Spielberger, Gorsuch, Luschene, Vagg & Jacobs, 1983) and less than 10 on the Beck Depression Inventory (BDI; Beck & Steer, 1993). The low stress cohort was assessed only once. This was an observational study and the

clinician attending these patients elected to use high dose AO supplementation as part of their routine therapeutic management. It was not specified as to whether Group 3 (the control group) was supplemented with any AO.

Findings reported compared NT levels between groups. There was a significant difference between Group 3 and Group 2 ($p < .05$, $n = 33$), and also between Group 2 and Group 1 ($p < .05$, $n = 32$). Depressed patients taking the high AO supplement had NT values twofold higher than the non-supplemented depressed group. When supplements increased, NT levels increased. However it is important to note there was no suggestion as to whether supplementation made any difference to depression or stress levels.

From these findings it was proposed that NT appeared to be a reliable marker for depressed patients and that the mechanism for reduction of NT appeared to be mediated via oxygen radicals, as participants taking supplements had normal NT values. However there was no random allocation to groups so we cannot rule out that there were other differences contributing to the significant findings. This is supported by the initial comparisons of groups which highlighted that a significantly higher proportion of the MDD patients smoked. The oxidative impact of smoking (Lesgards et al., 2002) poses a threat to the overall reliability of the findings. There were no attempts to control statistically for this or any other possible confounders in the analyses.

There was also a difference in education levels between MDD groups, with those taking supplements having completed a higher level of education. It is possible that this could be associated with more health awareness and possibly other health

behaviours (i.e., diet, exercise) not assessed. These could influence these findings. Furthermore across-subject variation could be a hindrance in this study design as individual differences, unrelated to supplementation, of NT levels cannot be ruled out. Future research would employ repeated measures designs to remedy this problem.

Blake-Mortimer and colleagues (1998) continued to further evaluate NT and the influence of vitamin supplement taking. Again this was an observational study (N = 72), this time comparing five groups' NT levels as well as VIT C stores. VIT C is an AO and has a protective role against oxidative stress. Group 1 were those with MDD taking supplements rich in AO (n = 9), Group 2, MDD not taking supplements (n = 18), Group 3, controls taking supplements rich in AO (n = 9), Group 4, controls not taking supplements (n = 15), and Group 5 were the academic Honours students (n = 21). It was unclear whether these were the same MDD participants from the previous paper (Blake-Mortimer et al., 1996). Findings for NT levels indicated a significant difference between the five groups. This could be pin-pointed to Group 2, the un-supplemented MDD group ($p < .05$, N = 72), which had NT levels significantly lower than all the other groups. Similarly this group had much lower VIT C stores compared to the other groups ($p < .05$, N = 72). Notably there was not a great difference between control groups. NT and VIT C had a significant positive relationship ($r = .27$, $p < .05$, n = 53). However there are some significant challenges to these findings.

Firstly, the un-supplemented MDD groups were significantly older than the students and control groups; this is considered important as decreased NT has been associated with increased age (Boss et al., 1980). Secondly, as mentioned in the previous study description, the MDD groups had a significantly higher proportion of

smokers than the other groups. Smoking is known to decrease whole blood resistance against free radical aggression (Lesgards et al., 2002), or put simply increasing oxidative stress. Thirdly, the MDD groups were generally less educated, as well as taking psychotropic drugs. Age, smoking, education, and medication use are all potential confounding variables as they all have potential to impact physiological measures. There was no attempt to control for any covariates in the analyses. There was no clearly defined timeframe (weeks, months, or years) to describe the length of time of supplement intake.

2.3.4 In-vitro studies on The Oxidative Model.

Further research by Blake-Mortimer, Winefield, and Chalmers (1998) focused on case studies and in-vitro studies of NT and potentially protective AO commonly found in multivitamin supplements. The in-vitro studies suggested that VIT C at physiological levels prevented oxidation by free radicals. This strengthens the evidence that VIT C plays a protective role. This was followed by a case-study (1998) design comparing blood AO capacity from two participants, a 32-year-old female taking AO and a 57-year-old male recently widowed and not taking AO. Findings suggested that VIT C was required to protect NT from free radical damage.

Methodologically this case study design satisfies biochemical analysis. However although standardised psychological tests were employed to assess depression and mood scores, because of the differences between the two individuals (i.e., male vs. female, young vs. old, and the use of subjective accounts of lifestyle, such as a widower 4 years standing, neither ate healthily nor took supplements) findings remain questionable. Broadly the results suggest that taking AO like VIT C protected NT from

oxidative stress during periods of chronic psychological stress. This finding is cautiously accepted but leaves many questions unanswered. A larger RCT incorporating a placebo an active supplement and placebo group would be the best way to evaluate this relationship further.

2.3.5 Animal studies.

Blake-Mortimer and colleagues (1998) considered whether these mechanisms could be replicated in an animal model of depression. A learned-helplessness Model of depression was tested in rats as the next evolution of The Oxidative Model. It was proposed that NT levels, VIT C levels, and immune response to foreign antigen (sheep red blood cells) would be significantly reduced in the group experiencing 'uncontrollable shocks' when compared with a 'no shocks' group and 'control over shocks' group.

There were two levels of 'uncontrollable shocks', acute (100 shocks in one day) and chronic (20 shocks a day over five days). No significant difference was observed for immune response to foreign antigen or NT levels, although following their shock-treatment means were lower for both acute and chronic groups. Groups receiving shocks had a significantly elevated antibody response ($p > .05$, $N = 46$) when compared with no shock groups and VIT C stores were significantly different for acute and chronic shock conditions. Specifically chronic shock rats had lower VIT C stores. Chronic shock mean VIT C levels were much lower regardless of escapability/control over shocks.

These findings suggest that chronic stress rather than acute stress depletes VIT C stores. However there was no observed difference between NT levels. It was

suggested that this is due to rats being able to synthesize their own VIT C stores, unlike humans, that need to derive VIT C from dietary intake. These findings also suggested that the stressor enhanced the immune response, although variability in timing between antigen and stressor administration poses added variability. Learned helplessness as a Model of depression is rife with controversy and makes it difficult to locate this research within the human Oxidative Model framework. This study poses some potential links to the previous Oxidative Model literature but actually raises more questions than it answers.

2.3.5 Occupational stress.

A more recent study of The Oxidative Model by Hapuarachichi and colleagues (2003) further explored this Model in a human sample. A population of academic and general staff employed by two South Australian Universities (*N* = 43, 23 men and 20 women) were recruited to assess the impact of occupational stress. A subset of participants in this study (*n* = 24) reported taking vitamins (B-vitamins supplements and VIT C) three to five times per week. These were called the 'high AO group and the remainder of participants, not taking any vitamins, were labeled the 'low AO group (*n* =19). The GHQ-12 (1978) scores enabled classification into three categories - normal, mildly stressed, and severely stressed. Derived from binary coding (0, 0, 1, 1) this score is considered a measure of severity or 'caseness' for the identification of cases at risk of psychological breakdown. Measures of occupational strain, burnout, and perceived stress were also included in this study. Significant mean differences were found between 'normals' and 'severely stressed' for NT and CRP (both $p < .05$, $n = 35$), although no significant difference was found between the mild to severe stress groups.

A significant difference was found between high and low AO groups on NT ($p < .03$, $N = 43$), CRP ($p < .04$, $N = 43$), HCY ($p < .04$, $N = 43$) and four stress parameters - perceived stress ($p < .04$), work burnout ($p < .02$), personal burnout ($p < .02$), and occupational strain ($p < .01$). This corroborates a pro-oxidant and pro-inflammatory state associated with increased stress.

As presented above, the high AO group had higher levels of NT and lower levels of CRP and HCY. These findings provide support for the Oxidative Model which proposes a link between stress, immune changes, and CVD. It also poses the notion that vitamin supplementation contributes to the prevention of oxidative stress caused by psychological stress. This is interpreted cautiously for two reasons. Firstly, sample size may have compromised power, increasing the likelihood of Type II error, as effect sizes were not reported. Secondly, there may have been other confounding health behaviours not substantially evaluated in statistical analyses (age, diet, medication, smoking, alcohol use, physical exercise, etc). It was an observational study grouping together an assortment of vitamins with different compositions and there is no discussion of the length of time used. It is possible that the health behaviours of supplement-takers might add to their wellbeing. Recent work in the field of PNI has indicated that stress may be a significant factor in the pathogenesis of metabolic disorders, and subsequently nutritional intervention or pharmacological agents targeted at moderating stress should be investigated (Seematter et al., 2004).

2.3.6 Unpublished dissertations employing The Oxidative Model.

There are several unpublished dissertations on The Oxidative Model in stress groups. Several have relied on student samples of acute academic stress and two are

focused on more chronic stress conditions, including returned servicemen (Vietnam veterans) with a history of PTSD and victims of crime (VOC).

2.3.6.1 Academic examination stress.

The first of three studies employing student samples evaluated The Oxidative Model in first-year University students (N = 23) undergoing academic examination (Arthurson, 2003). This was a repeated measures design. Measures proposed in The Oxidative Model were collected a month prior to examinations and then again during exam week, either the morning of, or the morning before an exam. The hypothesis was that biomarkers indicative of oxidative stress would be observed during the high stress period (Time 2) as opposed to data collected 4-weeks prior to examinations (Time 1). This was considered an acute stress scenario with an obvious external and predictable stressor. According to Elliot's stressor classification system academic examinations are categorized as brief naturalistic stressors. Brief naturalistic stressors are best described as situations where a person confronts a real-life short term challenge. Psychological measures included were the STAI (Speilberger, et al, 1983), the State-Trait Anger Expression Scale (STAXI: Speilberger, 1988), the Perceived Stress Scale PSS; Cohen, Karmack, Mermelstein, 1983), and the UCLA Loneliness scale (UCLA: Russell & Cutrona, 1988).

This was the first of The Oxidative Model literature to attempt to control for covariates specifically health and lifestyle behaviours which may impact on psychological and physiological measures. After investigation, the variables statistically controlled for included sex, age, exercise, smoking, alcohol intake, medication use, and vitamin use. Major results indicated that state anxiety, perceived stress, and state

anger varied between low stress and high stress periods. No differences were found for NT, VIT C, CRP, and HCY. However other measures explored in this particular study including Haemoglobin (HbA1c-increased), salivary –Iga (S-IgA decreased), and Peripheral Benzodiazepine Receptor (PBR increased) varied across the two time points.

The most noteworthy correlations were between PBR and anxiety and anger measures. However high VIT C levels were associated with high distress and perceived stress and internalized anger (anger control- in), which does not concur with the Models proposed oxidative state during periods of sustained stress. However given that this was a brief-naturalistic stress scenario. The Oxidative Model proposes that sustained or chronic stress is associated with increased oxidative stress as indicated by depleted levels of biomarkers like VIT C. Given the short period between low and high stress periods (4-weeks); depletion of AO stores may not have taken place. NT levels were not depleted significantly either and as VIT C plays a protective role according to the Model this fits the theoretical framework. However it remains counterintuitive that distress, perceived stress, and anger be associated with high AO levels (i.e., VIT C).

2.3.6.2 Academic stress.

Leading on from previously critiqued studies of Honours students (Blake-Mortimer et al., 1996), and the study of acute stress in first-year students by Arthurson (2003), an intervention study was conducted using a multivitamin supplement across a high stress period of coursework and examinations for a group of Honours students (N = 21; Oliver, 2004). Unlike the previous study of first-year students, this post-graduate year, although naturalistic, is not over a brief period. With regard to defining the stressor, this sample covered both the brief naturalistic stressor classification but also

identified elements of a stressful event sequence (Elliot, 1982). This sequence is based on a focal event, in this case examinations, and a related series of challenges, i.e., several dissertation deadlines including oral presentations.

This was a repeated measures study, which collected pre- and post-intervention biochemical and psychological data. This was a single-blinded study where participants and biochemists were blind to the randomization schedule. Participants were assigned to either an Active Group or a Placebo Group. The Active Group was given a 3-week course of multivitamins and the Placebo Group identical capsules comprised of non-active ingredients. Similar psychological measures were used previously by Arthurson (2003). Two additional scales from Spielberger were included State and Trait curiosity (1983) in order to assess the frequency and intensity of the positive emotion curiosity and the Lifestyle Defense Mechanism Inventory (Spielberger & Reheiser). The latter explores dispositional amounts of rational, non-emotional thought processes and behaviour, and efforts to achieve harmonious interpersonal relationships. Perceived stress was not recorded. The hypotheses were that those assigned to the Active Group would have improved psychological, pro-oxidant, and pro-inflammatory measures compared to the Placebo Group.

Covariates were not assessed or controlled in this study, thus the findings should be interpreted with caution. As predicted by The Oxidative Model, NT was found to decrease across the 3-week course of data collection for all participants, as stress levels increased with impending academic examinations. The Active Group showed a greater decrease in NT levels than the Placebo Group. This result does not support the hypothesis. On further exploration it was discovered that this finding was

influenced an failed randomization with more participants with severe psychological distress according to Goldberg's GHQ-12 (≥ 4) allocated to the Active Group than the Placebo Group NT was not found to be associated with any psychological variables of distress, anxiety, or depression. Increased levels of VIT C and VITB12 in the treatment group was taken as a sound indicator of compliance, however multivitamin containers/capsules were not recollected at the end of the trial. In addition it was a relatively short trial 2 to 2 ½ weeks which may not have left time for an effect on significant markers to be observable.

Another prospective study employing The Oxidative Model biomarkers as objective health measures was conducted by an Honours student (Le, 2004). This study was conducted in a general population sample (*N* = 28) and explored the health benefits of a 10-week course of yoga (*n* = 14) vs. relaxation (*n* = 10). Participants were screened for eligibility based on whether they had a score of ≥2 on the GHQ-12 (1978). Stressors were considered to be constant and persistent and included work strain, study, and caring for a sick or disabled family member. Assessments were conducted pre- and post-intervention. All biochemical analysts were blinded to group allocation and psychological scores. Randomisation was stratified by age and generated by a researcher not involved in the trial. Post intervention there was extreme attrition in the relaxation group (*n* = 3). Subsequent analyses focused on the yoga group changes pre- and post-intervention.

It was hypothesized that yoga would reduce both negative psychological strain and improve measures of health status. Assessment tools included measures of psychological distress (GHQ-12; Goldberg, 1992), perceived stress (PSS; Cohen,

Karmack, Mermelstein, 1983), state and trait anxiety, anger, depression and curiosity (Spielberger, 1995) , anger expression (Spielberger, 1988),and loneliness (Russel and Cutrona, 1988). Contemporaneously, NT, VIT C, HCY, FOLATE, VITB12, Cholesterol, Haemoglobin A1c and inflammatory cytokines (TNF-α, TNF-β, IFN-γ, IL-2, IL-1β, and IL-6) were assessed.

Results indicated that S-anxiety, psychological distress and perceived distress all decreased between pre and post- intervention. All evidenced large effect sizes (Cohen's d >1), and S-anxiety reached significance (p = .04). Comparisons between pre and post- intervention biomarkers showed improved (increased) NT, VIT C and VIT B12 levels. Large effects were observed but all failed to reach significance. For inflammatory cytokines, haemoglobin A1c, and cholesterol mean decreases were observed. However subsequent large effect sizes failed to reach significance. A contrary finding, increased HCY post-intervention was observed. This suggests pro-oxidant stress increased after the 10-week yoga intervention. Although the magnitude of the increase was large (d = 1.6), it did not reach significance.

The small sample size, problems with participant withdrawals and incomplete biochemical datasets, meant the effect of time (pre and post- intervention) and group (yoga vs. relaxation) could not be undertaken. This left questions around interaction effects. In addition the lack of a sufficient control group, which did not take part in either intervention makes interpretation of findings tenuous. There was no exploration and/or subsequent control for the influence of possible covariates like health behavior and lifestyle influence, which could account for some of the unexpected results (i.e. increased HCY). The stressors experienced by participants could be divided into acute,

brief naturalistic (work strain and academic strain) chronic stress (carer role) scenarios based on Elliot's classifications (1982).

2.3.6.3 Post traumatic stress and The Oxidative Model.

Post traumatic stress has also been explored in the context of the Oxidative Model. One such study by Jolly (2004) attempted to compare the acute stress (*n* = 25) experience of first-year university students undergoing examinations, with a PTSD group comprised of returned servicemen with current or past PTSD diagnose (*n* = 16). The underlying notion that stress can be thought of as points along a continuum with the two groups mentioned above representative of two positions on this continuum. University students represent an acute stressor in contrast to a traumatic experience(s) that occurred in the past yet still influence cognitive and emotional thinking- a distant stressor (Elliot & Eisdorfer, 1982).Specifically, this study hypothesized that the PTSD Group would have higher levels of oxidative stress (decreased NT and VIT C), inflammation (increased HCY and CRP) and lowered IgA, a marker of the acquired immune response than the Student Group.

At the outset, this observational study identified the PTSD Group to have a higher frequency of pro-inflammatory conditions (cardiovascular disease, arthritis, and diabetes). Although these conditions may have been attributable to the mean ages of the two groups, this was not explored further. The PTSD Group also reported a higher rate of alcohol and tobacco use. These differences were not controlled in further analyses. Moderate-to-large effect sizes were found for group differences on CRP, VIT C, NT, IgA, and HCY. Again, these results reflect only partial support for The Oxidative Model. As expected, NT levels for the PTSD Group were significantly lower than the

Student Group, and levels were notably lower than the healthy reference range. Furthermore lowered levels of VIT C were observed in both groups, with the PTSD Group Traumatic Stress group having markedly lower levels. This suggests exam stress may be associated with oxidative stress as previously discussed (Arthurson, 2003; Oliver, 2004). An anomaly was observed with IgA levels higher in the PTSD Group than the Student Group.

Critically this study comprised a single observation of participants' biochemical measures. This does not allow for the influence of inter-individual differences on variables. In addition the composition of co-morbid depression, anxiety and both current and past PTSD diagnoses in the PTSD Group may have contributed to contrary findings. In addition no psychological measures were collected other than a structured clinical interview of the PTSD group. This makes it difficult to ascertain levels of stress during the study. The influence of confounding variables (i.e., demographic, health and behaviour) were not explored or controlled for in statistical analyses. Therefore these findings should be interpreted with caution and they only provide partial support of The Oxidative Model (Jolly, 2004).

The most recent research on The Oxidative Model is also from a post traumatic stress perspective, this time using a sample of victims of crime (VOC; Pfitzer, 2008). Again it was an observational study of VOC (n = 27) and a control group drawn from the general population (n = 31) with no history of a major traumatic experience and with mild-to-normal stress levels (≤ 3) according to the GHQ-12 (1978).The hypothesis was that VOC would experience greater psychological distress and aversive emotions

(anxiety, anger, depression and loneliness) than the control group. Additionally VOC will evidence higher pro-inflammatory, pro-oxidant measures than the control group.

The VOC sample had to be exposed to or to have witnessed a crime at least three months prior to the initial assessment. The exposure to crime ranged from 1 to 60 years prior to taking part in this study (*M* = 13.88, *SD* = 17.10). Both groups had a high proportion of female participants (>77%). The groups were not significantly different on age, gender, parenthood, level of education, or tobacco use. These need to be interpreted cautiously as the small sample size inflates the likelihood of Type II error, i.e. believing the groups do not differ when in fact they do. VOC were significantly lower on frequency of alcohol consumption and participation in all levels of physical activity. It was indicated that the VOC group had a higher intake of medication for cardiovascular conditions, painkillers/anti-inflammatory medication, and antidepressants. However this was not significant. Self reported health problems (i.e., cardiovascular disease, chronic pain, and recurrent infections) were significantly higher in the VOC group. The demographic and health behavior disparities present between the two groups were not controlled for in subsequent statistical analyses.

Results indicated that the groups differed significantly across the majority of psychological measures. VOC evidenced more psychological distress (GHQ-12), and aversive emotions like S/T-depression, S/T-anxiety, and S/T-anger as measured by the State-Trait Personality Inventory (STPI) and the Beck Depression Inventory Second Edition (BDI-II; Beck, Steer & Brown, 1996). Furthermore, VOC experienced increased loneliness (UCLA) and decreased S- and T-curiosity. All these observed differences were large in magnitude and reached significance ($p > .001$). In the context of The

Oxidative Model biomarkers, a moderate magnitude of difference was observed between the groups for in CRP levels and this reached significance. Moderate differences were also observed for VIT C and FOLATE although these failed to reach significance. This is perhaps due to the inflated Type II error as a result of small sample size.

Interestingly this study also measured an assortment of cytokines. Of the cytokines measured TNF-α was higher in the VOC group although only a small to moderate effect size was recorded. This cytokine is implicated in pro-inflammatory processes. Significant changes in other markers implicated in The Oxidative Model (like NT and HCY) could be not be ascertained, meaning the results of the study do not provide support for an oxygen radical pathway in VOC. However a pro-inflammatory process is implicated by the high levels of CRP and TNF-α, as has been found in several other post traumatic stress studies (Melamed et al., 2004; R. G. Miller et al., 2001).

Correlation matrices were undertaken on both VOC group and controls separately and then as a combined sample. As a combined sample exploration of associations revealed (N = 58) CRP to be positively correlated with S/T-depression (r = .36 and .36 respectively, p < .01), psychological distress (r = .36, p < .01), state and trait-anxiety (r = .36 and .35 respectively, p < .01), state-anger (r = .39, p < .01), and negatively correlated with state and trait-curiosity (r = -.23 and -.26 respectively, p < .01). FOLATE was also inversely related to increased psychological distress in this VOC group (r = -.26, p < .01), suggesting depletion of nutrients during periods of distress. These findings are subject to scrutiny as mass correlation matrices are prone to Type 1 error.

2.4 Limitations

The Oxidative Model has been proposed and supporting evidence presented. It is evident from this review that the Model relies heavily on a few specific articles, namely Blake-Mortimer (1996, 1998) and Happuarachchi (2003). Several unpublished dissertations also provide support for the Model. (Arthurson, 2003; Jolly, 2004; Le, 2004; Oliver, 2004; Pfitzer, 2008) but it is often only partial support. It is important at the outset of this dissertation to reiterate limitations and weaknesses in the research thus far.

Oxidative Model research has employed several research designs. Multiple assessments, specifically three assessment points over a 10-month period, were utilized in the foundational research (Blake-Mortimer, 1996). Similarly for the consecutive case-control study (Blake-Mortimer et al., 1998b) assessments occurred on three occasions but at 6-week intervals, encompassing a four and a half month overall period. In subsequent research, pre and post assessments occurred at 3-week (Oliver, 2004), 4-week (Arthurson, 2003), and 10-week intervals (Le, 2004). Furthermore, several recent studies rely on a single cohort observed only once (Jolly, 2004; Pfitzer, 2008)(Happuarachci, 2003).

Observational studies which are limited to assessment at a single time-point risk being overly influenced by inter-individual differences on both biochemical and psychological variables. Blake-Mortimer and colleagues' (1996) original study was the only one to assess three time points. This study allowed for biochemical and psychological trends to be observed. Future research using this model should endeavor to follow this design, with at least two assessment points.

Like much of the PNI literature, The Oxidative Model is resource intensive. The undertaking of recruiting a stressed sample, followed by collecting blood samples plus facilitating biochemical assays make attaining adequate sample sizes a challenge. As a result the use of small samples increases the possibility that non-significant results may be due to insufficient power. For example, several unpublished dissertations presented provided only partial support for The Oxidative Model. This could be that the relationships between variables are not apparent or that the sample size influenced power to detect these relationships. A further drawback is that early Oxidative Model studies (Blake-Mortimer, 1996 and 1998) do not report effect sizes. Therefore these significant results based on *p* values need to be interpreted with caution as the magnitude of change is unknown.

The largest sample employed in Oxidative Model literature up until now has been 72 participants (Blake-Mortimer, Winefield, & Chalmers, 1998a). This comprised five separate subgroups containing low stress controls, as well as those experiencing academic stress, and depressed patients. These are extremely disparate stress experiences. Ideally a single homogenous stress sample would be used in future Oxidative Model studies.

As has been discussed previously the research to date using this Model includes a broad variety of 'stressed' samples. This suggests this Model is relevant, and can be applied, across a number of populations; potentially a strength. However all studies to date have employed mixed samples on demographics (i.e., age and gender) and health-behaviour variables (tobacco and alcohol use, exercise, diet, sleep, etc) which potentially confounding findings. Throughout The Oxidative Model literature, health

behaviours, like those aforementioned, were not always assessed. This is despite potentially accounting for variance in biomarkers central to this Model and subsequent immune function. For example it is documented that NT levels decline with age (Boss et al., 1980) and in general, oxidative processes increase (Gil et al., 2006).

In the study of depressed patients by Blake-Mortimer and colleagues (1998) patient groups, considered high stress were significantly older than the student and low stress controls. Similarly a significantly higher proportion of depressed patients smoked compared to controls. Considering the potential influence of ageing processes and the known oxidative impact of smoking (Lesgards et al., 2002), this suggests there are two plausible alternate reasons, other than stress, for lowered NT observed in this group.

Furthermore education levels between groups were significantly different, with the vitamin-supplemented group having completed a higher level of education. There was no random allocation to vitamin-taking group. It is plausible that higher education could be an indicator of increased health awareness. This knowledge could affect other health behaviours mentioned (i.e., diet, exercise, alcohol consumption) which was not assessed. The main methodological criticism is that covariates are haphazardly measured with non-standardised assessment tools across The Oxidative Model literature. Only on one occasion were confounders including sex, age, exercise, smoking, alcohol intake, medication use, and vitamin use controlled for in subsequent analyses (Arthurson, 2003).

A key mechanism described by the Model to ameliorate the detriment al effects of chronic stress is vitamin intake or more specifically AO intake. AO intake has

been described but statistically unaccounted for across the foundational Oxidative Model studies (1996 & 1998) except for the in-vitro study case study. This was a case-study design (N = 2; Blake-Mortimer et al., 1998a) comparing blood AO capacity of a young female taking AO-rich supplements and an older male not taking any supplements. Methodologically this satisfies biochemical analysis. However the influence of demographic variables like age (i.e., old vs. young), gender (i.e., male vs. female) and health behaviors (i.e., diet, exercise, medication use) remain underexplored. To date covariate assessment by Arthurson (2003) did not employ standardized psychometric tools. However the influence of confounding variables (like health behaviours) becomes more difficult to control in small, cross-sectional cohorts, which comprise the majority of the most recent Oxidative Model studies (Jolly, 2004; Pfitzer,2008; Happuarachchi, 2004).

The Model speculates on the potential benefits of vitamin supplementation during times of ongoing psychological distress. However vitamin supplementation remains to be sufficiently assessed or controlled for (e.g., statistical control for confounders). The argument has predominantly been that any differences between groups were naturally occurring reflecting 'real' lifestyle factors (Stevens, 2002), however many of the behavioral and demographic variables described potentially contribute to immune enhancement (i.e., exercise) or suppression (i.e., tobacco use). This requires examination. The use of well-chosen covariates could assist in reducing the confounding influence of group differences. However identifying covariates requires careful consideration and a sound understanding of the previous research which has been conducted on this Model. As Arthurson (2003) has been the only study

of The Oxidative Model literature to explore covariates there is a need to build on this preliminary research. Covariates must be measured prior to any treatments, and ideally should be continuous variables measured reliably.

The use of healthy 'unstressed' controls with 'stressed' clinical samples (depression, PTSD) further confounds the experience of stress with an individual's clinical status. In research on stress-immune relationships, group differences on health behaviours between stressed and non-stressed samples have not been reliably linked to changes in immunology (Anderson, Kiegolt-Glaser, & Glaser, 1994). The Oxidative Model studies which use healthy controls and clinically 'stressed' samples describe significant inconsistencies across groups for health behaviours (smoking, alcohol, exercise, diet, etc)(Blake-Mortimer 1998; Jolly, 2004, Pfitzer, 2008). Again these authors argue that these differences were naturally occurring reflecting 'real' lifestyle factors. However the influence on biochemical as well as psychological states has largely been overlooked. Psychological stress can certainly influence the frequency of both positive and negative health behaviours. This requires further exploration. The omission of health-behaviour variables is a weakness in The Oxidative Model literature. Health behaviours have the potential to account for changes in psychological, pro-oxidant, and pro-inflammatory processes proposed by the Model.

Several studies base support for the Model on correlational analyses alone. There are a number of issues associated with this. Firstly, correlation coefficients only provide an indication of the linear relationship between variables, they do not imply causality. Secondly, statistical outliers can have a significant impact on correlation coefficients especially in small samples like those detailed in The Oxidative Model

research. Additionally, in such an integrated system like the human body it is important to consider a third possible variable (confounders) that influence both observed variables. Lastly, statistical significance and clinical significance are quite separate matters. In summary , although correlation analysis has yielded interesting findings future research should aim to use more confirmatory techniques to explore causality.

In the previous chapter the definition of a stressor was discussed. To reiterate according to a meta-analysis (Segerstrom & Miller, 2004), different stressors elicit different patterns of immune change(s). The Oxidative Model is based on a chronic stress scenario. However the nature of the stress samples utilizing this Model included academic examinations and thesis submissions, clinically diagnosed major depression, occupational stress, and PTSD. In the context of the stress taxonomy described in the previous chapter (Elliot & Eisdorfer, 1982), these studies represent both-

(1) stressful event sequences, where the identified stress is fixed on a focal event and a related series of challenges,

(2) chronic stressors, where a stressor(s) pervades one's life forcing a restructuring of one's role or identity, and

(3) distant stressors, identified as traumatic experiences that have occurred in the past yet still influence cognitive and emotional thinking.

Therefore it is plausible that inconsistent findings throughout The Oxidative Model literature might be explained due to differing stressors.

Defining the experience of stress prompts consideration and critique of psychometric scales use. Across The Oxidative Model literature measures employed to assess the experience of stress have been diverse. The original studies by Blake-Mortimer and colleagues (1996) employed the Profile of Mood State (POMS) Scale (McNair et al., 1971) to determine eligibility for the study. In addition eligible participants reported experiencing 'extremely' stressful events for longer than a month in duration. Events identified included bereavements, diagnosis/possible diagnosis of serious illness, and assault. Both a high POMS score and a longer-than-a-month stressor were required for allocation to the high stress groups. Having experienced a month-long-stressor but not scoring high on the POMS meant no allocation to the high stress group. The accrued sample were defined as experiencing a chronic stress scenario However in the context of the stress taxonomy (Elliot & Eisdorfer, 1982) these experiences bridged both stressful event sequences and chronic stress definitions. The impact of the different stressors on immune changes has been discussed and requires careful consideration for future Oxidative Model research.

Other psychometric scales utilized have included both the original Beck's Depression Inventory (BDI; Beck & Steer, 1993) in MDD sample (Blake-Mortimer, 1996) and the revised second edition (BDI-II; Beck, Steer & Brown, 1996) by Pfitzer (2008). Other than identifying a diagnosis of MDD, critically this study did not give reference to the duration since the clinical diagnosis. Six patients were classified into MDD, single episode and melancholia, and 26 were classified into MDD, recurrent episode and melancholia. Twenty-five were inpatients and six were outpatients at the time of assessment, one was not specified. Controls were determined by a low (< 10) score on

the BDI. Groups were compared on BDI and T-anxiety scores as measured by the STAI. Similarly the in-vitro case study (*N* = 2) included in the same paper (Blake-Mortimer et al., 1998b) measured chronic stress using POMS, BDI, and STAI scores but also did not give any reference to duration of stress experience.

The most recently published study (Hapuarachchi et al., 2003) assessed stress using the 12-item General Health Questionnaire (GHQ-12; Goldberg, 1978). However this study did not define whether participants had been experiencing stress for a specific duration other than the two-week period defined by the GHQ-12. The assessment of stressor duration and scales used to define the stressor do not appear to reflect a chronic stress scenario. The GHQ-12 was useful as it delineated between two stress levels (normal-mild and severe) for psychological and biochemical variables. Happuarachchi and colleagues (2003) also measured subjective stress, specifically using the Perceived Stress Scale (PSS; Cohen & Williamson, 1983) in their occupational stress study. This tool assesses the degree to which situations in one's life are appraised as stressful. Findings suggested that increased PSS scores were significantly associated with lowered NT, increased CRP and HCY for this sample; this indicates a pro-oxidant and pro-inflammatory state. Since then the PSS has not evidence conclusive findings across several unpublished Oxidative Model dissertations (Arthurson, 2003; Le, 2004; Oliver, 2004). This has also been reflected in broader findings (Segerstrom & Miller, 2004). It is likely that this is due to different types of stress. The utility of this measure for The Oxidative Model requires further consideration.

Post traumatic stress studies of The Oxidative Model varied in their criteria for stress. Although a past or present PTSD diagnosis was considered a chronic stressor compared to academic examinations, Jolly (2004) did not attempt to assess psychological well-being or stress levels. The most recent research on The Oxidative Model in VOC (Pfitzer, 2008) required participants to have been exposed to or to have witnessed a crime at least three months prior to the initial assessment. In addition psychological distress as well as other indicators of distress (i.e., anxiety, depression, anger, and loneliness) was measured.

It is clear that a variety of scales have been used to assess samples identified as experiencing chronic stress. Like much PNI research the constructs around stress are not well-defined. However The Oxidative Model proposes to overcome some of the methodological difficulties inherent within the acute/chronic stress dichotomy by defining a stress experience using tools with psychometric properties to indicate and/or identify psychological distress and well-being. Identifying groups of 'stressed' participants for large scale studies remains a challenge as does finding reliable, yet sensitive, measures to assess the experience of stress. Future Oxidative Model research should endeavor to do this.

2.5 Strengths

The Model has been studied across a broad range of stress scenarios, suggesting it has potential wide-ranging application. Secondly, The Oxidative Model approach relies on an individual's patterns of emotional distress and maladaptive emotions as opposed to a subjective stress measure. Thirdly, research to date has informed reliable measures of psychological distress. For example the GHQ-12 is the

only measure used consistently across the majority of studies. It appears to be reliable in defining stress across several samples including academic, occupational stress, and trauma groups.

2.6 Summary

The Oxidative Model provides a novel approach and suggestive findings linking psychological stress with measures of inflammation and oxidation. The Model proposes a means to ameliorate the detrimental effects of stress on the body, via simple vitamin supplementation. More research is required to increase the quantity and importantly the quality of the evidence base. The criticisms of The Oxidative Model literature to date are largely criticisms of the design and methodology, as opposed to theoretical criticisms. These limitations can be remedied and the Model has the potential to be refined to improve its applicability.

Future research would focus on including

(1) samples of same sex participants or comparisons of genders,

(2) samples of stressors with equivalent duration and course,

(3) the employment of relevant psychometric measures with proven sensitivity and specificity,

(4) longitudinal design, to see trends and also allowing to control for inter-individual variability, and

(5) the exploration of health behaviours as potential covariates.

It is unclear from the literature whether interventions should be aimed toward simply reducing the negative physiological effects associated with chronic stress OR whether there is a concurrent clinical impact on decreasing psychological stress and improving well-being. With this in mind the employment of The Oxidative Model is proposed in a novel population.

Chapter 3

Breast Cancer Patients in the Post-Active Treatment Period

3.0 Overview

In the previous chapters broad PNI findings have been discussed, specifically, chronic stress is associated with immune changes via pro-oxidant and pro-inflammatory pathways. Conceptual and methodological difficulties associated with this type of research have been discussed. The Oxidative Model linking chronic stress, pro-oxidant, pro-inflammatory, and subsequent immune dysfunction has been proposed. Uniquely the Model also proposes a means to ameliorate the physical impact of ongoing stress. The Oxidative Model has the potential to be applied in a disease setting, as restoring individuals to complete health following a physiological challenge is an important consideration.

The Oxidative Model has yet to be applied in a cancer population, an experience commonly associated with sustained distress, depression, anxiety, and trauma. The psychological challenges associated with an experience of early stage breast cancer will be the focus of this chapter. The treatment for early stage (I-III) breast cancer is will be identified as a sustained stressor. The cessation of active treatment is a time of psychological stress, as this chapter will demonstrate. This review will present evidenced-based research on the psychological experience of during this period. It is important to keep in mind the cancer experience is a unique and individual journey for each patient. However this section should leave the reader clear as to the reasons why this sample was selected as a chronic stress scenario in which to test The Oxidative Model. In short, the aim of this chapter is to review

literature around stress for early stage breast cancer patients once they cease active treatment.

3.1 Breast Cancer Incidence & Survival

Breast cancer is one of the most common invasive cancers diagnosed in females in Australia, representing over a quarter (28%) of all reported cancer cases for women in 2006(AIHW, 2009). Breast cancer is second only to lung cancer in being the most common cause of cancer death among Australian women, with over 12,567 newly diagnosed cases per year, and subsequently over 2680 deaths per year(AIHW, 2009). Similar trends are apparent across a majority of Western countries making breast cancer the most widespread cancer experienced by women (Olver, 1998). Australian women have a 1 in 9 lifetime risk of developing breast cancer. Over 70% of all cases of breast cancer are stages I-III *(AIHW, 2006).

In recent years outcomes after a diagnosis of breast cancer have improved significantly. For example, 5-year survival for women diagnosed with breast cancer in 1998 to 2004 was 88% compared with 73% for women diagnosed between 1982 to 1986. Improved survival has also been observed worldwide in countries including Canada, the United Kingdom, and the United States of America. Despite improved survival rates, women who have had breast cancer have an elevated mortality risk

* Staging is a convenient way of allowing comparisons of cancers with similar extent and prognostic importance. One of the simplest means for staging cancer divides the disease into three categories; localized, cancer is confined to a particular organ, regional means the cancer has spread beyond the organ of origin, and distant spread indicates that there is metastatic spread to distant locations in the body. Another staging system employs Roman numerals; for this system stages I-II generally represents localized diagnoses, stage III for regional, and stage IV for distant/metastatic.

even decades after diagnosis (Brenner & Hakulinen, 2004), suggesting a long term impact of the disease-course.

3.2 Linking Oncology and Psychoneuroimmunology

Research on the impact of psychosocial factors on the development and/or progression of cancer is extensive (Cohen & Herbert, 1996) (Bovbjerg & Valdimarsdottir, 1998; Kiegolt-Glaser & Glaser, 1999). With early detection and treatment advances there are an increasing number of breast cancer survivors and numbers are expected to grow worldwide. The ongoing health and quality of life of this population will continue to be an important area of health research (Armes et al., 2009). Over the past 40 years psychological research around cancer has been aimed toward preventing and/or reducing the psychological and behavioral burdens and improving quality of life of cancer patients (Anderson et al., 1994). This field is broadly known as psycho-oncology (Holland, 2002). It broadly encompasses:

> the emotional responses of patients at all stages of disease, as well as their families and caretakers (psychosocial); and the psychological, social and behavioral factors that may influence cancer morbidity and mortality (psychobiological) (Holland, 1992, p.1)

The inclusion of the psychobiological aspects parallels this research area with PNI research with additional challenges. At the outset it is important to clarify these difficulties in studying psychobiological/PNI factors in cancer populations.

Firstly there are obvious differences in the biology of tumours and subsequent treatment for different cancers (i.e., type, site, stage, etc). Secondly, assessing and

controlling for the influence of health behaviours (i.e., treatment, medication, compliance, sleep, diet, etc) within individuals as well as across mixed cancer samples is difficult (Anderson et al., 1994). Thirdly, the stage of the disease has been identified as having a marked difference on psychological coping styles employed (Luecken & Compas, 2002). Lastly the assessment of different time points (i.e., prevention, pre- vs. post-diagnosis, surgery, and adjuvant treatment), not surprisingly, reveals conflicting findings. With these limitations in mind this dissertation focuses on a specific post-adjuvant treatment period.

Psycho-oncology research encompasses the whole gamete of phases of cancer including prevention, detection, diagnosis, active treatment, palliative care, as well as end of life, short and long term survival. Survival or survivorship commonly refers to those who are living with varying levels of health and well-being after a diagnosis (Feuerstein, 2007). The time immediately following active treatment fits into this period. Despite this, in many ways post-treatment is an arbitrary term which can extend from days to decades following treatment for cancer. For this review the 12-months following the cessation of primary treatment for early stage breast cancer (i.e., after the completion of surgery, radiotherapy, and/or chemotherapy) is the focus. For comprehensiveness some research presented in the following section will encompasses longitudinal designs which include treatment periods. These are only included if the immediate post-treatment period was inclusive of this 12-month period. After treatment there is no detectable cancer left so any symptoms are not due to cancer unless it reoccurs.

3.3 Sources of stress post-treatment.

Diagnosis and subsequent treatment for breast cancer are considered objective and negative events. Negative events do not always generate stress and an altered quality of life. However there is evidence of ongoing distress accompanying cancer-related events (Lebel, Rosberger, Edgar, & Devins, 2007) (Anderson et al., 1994). The stress associated with cancer-related events does not simply conclude at the end of active treatment (Figley, 1978) (Andrykowski & Cordova, 1998) as was previously thought. There is a new set of challenges which arise at this time.

Following a diagnosis and the subsequent completion of surgery and adjuvant treatment, the post-treatment period has its own set of unique stressors. This period has been associated with distress due to the impact of residual treatment effects including toxicity (Cella et al., 2006) (Thornton, Carson, Shapiro, Farrar, & Anderson, 2008), fatigue, hair loss, early menopause symptoms, lympho-edeama, and decreased libido (Arora et al., 2001; Costanzo et al., 2007). It is important to note that some of these physical effects (i.e. libido) also have psychological components. Furthermore intertwined with the physical impact, psychosocial influences including financial, occupational, and interpersonal difficulties have been observed, at this time (Sammarco, 2001).

The post-treatment period, spanning months to years, has been marked by illness uncertainty (Mishel, 1988) and fear of disease recurrence (Kornblith et al., 2003) and uncertainty of the future (Lebel et al., 2007). The potential for illness uncertainty as a stressor among this population is considered substantial and it is possible that this can worsen when treatment ends due to diminishing contact with oncology staff and

health professionals. These concepts have recently been categorized under a broader operational definition- 'unmet needs'.

A multicentre, prospective, longitudinal study found a third of cancer patients (*N* = 1425) identified fear of recurrence as their number one unmet need immediately following the cessation of treatment (Armes, et al., 2009). Even more concerning is that for the majority (60%) this fear remained unresolved 6-months later (*n* = 1152). Contact with oncology staff and involvement in active treatment has been identified as a 'safety net' and seen by patients as a form of 'active coping' (Deshields et al., 2005) which is lost once active treatment concludes.

The cessation of active treatment marks the end of an often intensive treatment regime, and the end of close monitoring and regular contact with hospital staff. This complex array of physical, psychological, and social challenges makes the post-treatment period one likely to be marked by psychological adjustment. This period has the potential to be a time where women treated for early stage breast cancer experience chronic stress. To reiterate, chronic stressors are those that pervade a person's life, forcing one to restructure their role or identity (Elliot & Eisdorfer, 1982). By definition these stressors are ongoing. It is this period of change which is the focus for this dissertation in order to align with findings generated from Oxidative Model studies.

3.4 Literature review guidelines

There is much anecdotal evidence for psychological distress during the immediate post-treatment period as evidenced by published personal accounts (McKinley, 2000; Mullan, 1985; Schnipper, 2001; Tierney & McKinley, 2002) as well as numerous online blogs regarding this topic. Empirical evidence of distress in the 12-months following active treatment is mixed. Current literature exploring psychological well-being in the early post-treatment period for women with early stage breast cancer is the focus of this review. This is in order to compare this population, during this period, with the psychological states reviewed in the preceding Oxidative Model chapter. At the outset several stipulations have been enforced for this review.

Firstly, although other psycho-oncology reviews often go outside of one area (i.e., to include other cancers), this review will include studies which have employed samples of breast cancer patients, stages I to III predominantly. In the absence of research specifically on early stage breast cancer samples, on the odd occasion findings will be drawn from mixed stage breast cancer samples (I – IV). The aim of this guideline is to enable a review of literature on the psychological experience of women with a similar type, stage, and treatment stage to come to the forefront.

Secondly, post-treatment studies are the focus for this dissertation. This is due to the unique set of stressors that arise at this time (i.e., unmet needs). In addition any kind of 'immunology' or physiological marker research, like The Oxidative Model, becomes increasingly difficult to do unless adjuvant treatments like chemotherapy or radiation has finished and had time to settle. For thoroughness, longitudinal studies spanning the treatment period (i.e., surgery, chemotherapy, and radiation) which also

incorporate the early post-treatment period (i.e., 1 to 12 months following active treatment) as an assessment point are included.

The post treatment period has been chosen as a potential period when restoring the body's internal inflammatory and oxidative balance would be beneficial for patient's health. The Oxidative Model proposed in Chapter 2 suggests a means to potentially ameliorate detrimental effects of stress on the body. Although it was first observed in a disease setting (i.e., newly diagnosed HIV positive patients; Chalmers, et al. 1990) it has yet to be studied in a cancer setting.

Thirdly, as will be come apparent in the following review of literature, there is no universal definition for psychological stress experienced by women in the post-treatment period (Potter, 2007). This is not unique to psycho-oncology research, but is common across all aspects of PNI literature. One definition which has been used to describe cancer specific distress in the psycho-oncology literature is as

> an affective cognitive and behavioural response to a crisis-precipitating event perceived as threatening, manifested by anxiety and depressive symptoms. (Potter 2007, p 239)

However, divergent conceptual and operational definitions have been employed across many studies. These studies have used numerous standardized and investigator-designed instruments to explore these constructs. Therefore this review of post-treatment research will be presented chronologically and will specify how the authors for each study have conceptualized and quantified stress (i.e., psychological distress, anxiety, depression, trauma, coping styles, etc). In addition some brief

definitions for the predominant areas incorporated in the distress literature will be presented.

Whilst this provides a framework for understanding distress it does not cover all the psychosocial elements which come under the umbrella of distress. The manifestations of distress in the post-treatment period are complex and at the same time undeniable, as will become apparent from the following review. Distress is often considered a more acceptable term than "depression" or "anxiety," since it can describe feelings ranging from the normal distress that follows a diagnosis of cancer to more serious levels that may reflect true depression or serious anxiety.

3.5 Evidence of psychological distress post-treatment.

Post-treatment rates of clinical anxiety and depression were initially explored in a longitudinal study of early stage (I-II) breast cancer patients (*N* = 269). The aim of this research was to assess the clinical incidence and psychiatric morbidity associated with diagnosis, surgery and adjuvant treatment for early stage breast cancer, specifically whether surgery type (lumpectomy vs. mastectomy) influenced clinical rates of anxiety and depression. The findings were published across two papers (Fallowfield, Hall, Maguire, & Baum, 1990; Fallowfield, Hall, Maguire, Baum, & A'Hern, 1994).

Assessment of anxiety and depression for this study was via a semi-structured interview which took place in the patient's home, called the Present State Examination (PSE; Wing, Cooper, & Satorius, 1974). In addition self-report questionnaires were completed including the Hospital and Anxiety Depression Scale (HADS)(Zigmond & Snaith, 1983), the Spielberger State/Trait Anxiety Inventory (STAI: Spielberger, 1989)

and the Rotterdam Symptom Checklist (RSCL)(De Haes, Van Knippenberg, & Nejit, 1991). These self-report questionnaires were mailed out to patients.

Psychological measures were assessed on five separate occasions including the post-operative period (within 3 weeks of surgery), during treatment at 3 months, and in the post-treatment period at 1, 2 and 3 years. It was unclear what the predominant stage breast cancer was for this sample. However all women underwent surgery with over half having mastectomy surgery (57%) as opposed to lumpectomy. Adjuvant cytotoxic chemotherapy was given to only 20 (7%) of the 269 women. Postmenopausal women, irrespective of surgical treatment, received adjuvant Tamoxifen(85%). Participants ranged in age from 20 to 75 years (*M* = 56, SD = 11), and the majority were married (71%).

Findings from this longitudinal study identified that for a subset of patients clinical rates of morbid anxiety and depression were evident following surgery and adjuvant treatment. Pertinent to the current dissertation, anxiety disorders were identified not only 3-months post- surgery (49.6 %) but in the post active treatment period. Specifically at the 1-year post-surgery assessment, 27% of the women initially experiencing morbid anxiety at 3-month assessment remained in this state. Similarly, at one year post-surgery, 18% of patients identified as depressed 2-weeks following surgery remained clinically depressed (Fallowfield et al., 1990) and at three years this proportion was 11.9% (Fallowfield et al., 1994). There was no significant difference between anxiety and depression for women who had undergone lumpectomy vs. mastectomy.

It is evident that for a subset of women with early stage breast cancer clinical anxiety and depression is experienced and remains present across surgery, treatment, and well into the post-treatment period. The Oxidative Model has been applied to a sample of people with a clinical diagnosis of major depression. This research has shown them to be vulnerable to oxidative and inflammatory stress (Blake-Mortimer et al., 1996) in contrast to non-depressed participants. It is likely that breast cancer patients described in this current study could experience similar patterns of physiological well-being in the period when active treatment ceases.

Another paradigm which has been used to assess the stress experienced by cancer patients is the post traumatic stress disorder (PTSD) framework. PTSD is typically associated with trauma such as violent crimes, rape, and combat experience; it is characterized by the re-experiencing of an extremely traumatic event accompanied by symptoms of increased arousal, intrusive thoughts, and by avoidance of stimuli associated with the trauma (DSM-IV: APA, 1994) Since 1994 the Diagnosis of life-threatening illness was included in the Diagnostic and Statistical Manual –IV (DSM-IV) criteria for traumatic stressor exposure for posttraumatic stress disorder. Symptoms can include general restlessness, insomnia, aggressiveness and depression, dissociation with reality, emotional detachment and nightmares.

One of the first studies following the inclusion of life-threatening illness to the diagnostic criteria undertaken in a sample of breast cancer patients post treatment was by a research team in Kentucky, US (Cordova et al., 1995). This study recruited women (*N* = 55) in the 6 to 60 months post-treatment (*M* = 30.5, *SD* = 16) with stage I to III breast cancer who had undergone surgery, chemotherapy or radiotherapy. This

study was an examination of the frequency and correlates of PTSD-like symptoms following diagnosis and treatment.

At assessment participants completed the self-report battery including the Medical Outcomes Study 20-Item Short-Form General Health Survey (MOS-20; Stewart, Hays, & Ware, 1988) which assesses quality of life, the Impact of Events Scale (IES; (Horowitz, Wilner, & Alvarez, 1979)) and the PTSD-Checklist - Civilian version (PCL-C; (Weathers, Huska, & Keane, 1991)). The IES is one of the most widely used self-report measures to assess the impact of a 'distressing event'. It provides an overall score as well as subscales for the experience of intrusive thoughts (e.g., 'pictures about it popped into my mind') and avoidance (e.g. 'I tried not to talk about it'). It is commonly used in PTSD literature. Unlike the IES, responses on the PCL-C can be used to identify respondents likely to merit a formal diagnosis of PTSD. Participants ranged in age from 35 to 84 years (M = 55.5, SD = 9.7) and were predominantly married (60%), Caucasian (>90%), college educated (38 %), and from middle class income homes. Over half (62%) were diagnosed with stage I breast cancer (62%).

Findings for this study identified 5% to 10% of women 6 to 60 months post-treatment were likely to merit a formal DSM-VI diagnosis of PTSD. Correlates associated with PTSD-like symptoms included age (r = -.34, p < .05), lower income (r = -.34, p < .05) and to a lesser extent, lower levels of education (r = -.25, p < .05). Nineteen participants (35%) indicated they experienced physical reactions when something reminded them of cancer treatment or their experience with cancer. The most common reactions were nausea *(n* = 13), heart palpitations *(n* = 8), and general feelings of panic (n = 7). Prominent triggers of these physical reactions were being near

or in the hospital in which they underwent treatment *(n* = 7), thoughts about chemotherapy (*n* = 6), and thoughts of recurrence (*n* = 5).

A small cross-sectional cohort design, findings were limited and potentially influenced by inter-individual differences. In addition there was a great deal of discrepancy between patients as to the cessation of treatment prior to assessment for this study (6 – 60 months). The addition of face to face diagnostic interviews would strengthen this research rather than relying solely on self-report measures as the basis for a clinical diagnoses of depression or anxiety.

Shortly after this study, another project exploring the prevalence of PTSD for early stage (I-II) breast cancer patients within the 4-12 month post-treatment was published (B.L. Green et al., 1996). This time frame was specifically chosen to ensure that women were not still experiencing the acute effects of treatment, yet was close enough in time to diagnosis to recall reactions to illness and therapy. With a similar focus to Cordova and colleagues (1995), this study was designed to determine the extent to which women reporting significant distress would meet diagnostic criteria for PTSD. In addition, individual characteristics to predict the development of cancer-related PTSD were investigated.

A comprehensive battery of tests was completed by participants, including: Trauma History Questionnaire (THQ: (B. L. Green, 1996)), the IES, Brief Symptom Inventory (BSI: (Derogatis & Spencer, 1982)), and the Stressful Illness Experiences (SIE) developed by the investigators to assess a full range of experiences across the course of cancer diagnosis and treatment that might be stressful and be targets for intrusive thinking. Specific events were generated from our clinical experience with these

patients (e.g., discovery of a mass or lump herself, having to decide between lumpectomy vs. mastectomy), with items being added following pilot testing. The women rated each of the 30 experiences on a scale ranging from 1 (not at all stressful) to 5 (extremely stressful) or not applicable. Following this self-report assessment a Structured Clinical Interview for DSM-III-R (SCID: (Spitzer, Williams, & Gibbon, 1990)) was conducted to establish past and current PTSD for cancer and non-cancer stressor events.

The mean age of the sample was 53.40 (*SD* = 9.66, range: 26–75). Participants were largely Caucasian (66%), married (58%) or living with a partner (19%), employed at least part-time (67%), and college educated (64%). Household income for the sample was high (57% >$60,000 US). The women were on average 6.5 months (*SD* undefined) post-treatment. The majority had .been undergone mastectomy (19%) or lumpectomy with adjuvant chemotherapy (24%), or lumpectomy with radiation (38%). Fifty-two percent were taking Tamoxifen at the time of the study.

Findings from this study suggest a clinical diagnosis of cancer-related PTSD was relatively rare (< 2%), although the experience of PTSD symptoms were common, with 36% of women reported having experienced at least one symptom of intrusion since their diagnosis (20% currently). Among the other clinical diagnoses observed in this sample, the most common was major depression (28%). The diagnoses of depression more often preceded the cancer, with only 4% of women having their first MDD episode following their diagnoses with cancer. The rate of current diagnosis of depression was 12%. Both cancer-related and non-cancer PTSD were co-morbid with depression.

The rates of current PTSD are consistent, although slightly lower than Cordova's findings (1995). Authors suggest this is due to the use of self-report measures tending to overestimate diagnoses. Self-report measures remove the opportunity for an interviewer to assess severity and/or the clinical significance of a symptom. IES levels were comparable to Cordova and colleagues' findings. The length of time since diagnosis and the type of treatment (i.e., surgery, chemotherapy, radiation) did not relate to PTSD symptoms. Younger women were at greater risk for PTSD symptoms than older women according to the IES subscales intrusion (r = –0.29, p < 0.000) and avoidance (r = -0.16, p < 0.05).

The experience of psychological and symptom distress in the post treatment period has also been explored at the cessation of active treatment for early stage breast cancer patients (Mast, 1998). Symptom distress refers to the number and severity of physical symptoms experienced by patients. It was assessed in this study by the administration of the Symptom Distress Scales (SDS; (Holmes, 1989)) to a cross-section of women aged 29 to 90 years of age (M = 60.0, SD = 12.9) with early stage breast cancer(I-II). Variables associated with illness uncertainty and emotional distress were also explored. This study hypothesized that symptom distress, 1-6 years post-treatment, would be associated with more illness uncertainty. Distress was assessed using several scales including: the Uncertainty in Illness Scale, (UIS; Mischel & Epstein, 1990), Fear of Recurrence Questionnaire (FRQ; Northouse, 1981) and the Profile of Mood States (POMS; McNair, 1971). Positive life changes in response to chronic illness uncertainty were also explored using a tool constructed by the authors of this study, the Growth through Uncertainty Scale (GTUS; Mischel & Fleury, 1994).

This sample (*N* = 109), recruited from a single site, had a mean age of 60 years (SD = 12.9), were married (63%), Caucasian (97%), high school educated (90%), involved in full (32%) or part-time (16%) work, and from households with an income of ≥US$30,000 (47%). Women in this sample underwent surgery alone or surgery combined with chemotherapy or radiation therapy; rates were not specified. Nearly half of the women (46%) were taking Tamoxifen medication at the time of this study. Tamoxifen is a hormone treatment designed to interfere with cases where hormone-stimulated (i.e., oestrogen) growth of breast cancer occurs (Olver, 1998).This treatment is commonly used in post-menopausal women. Tamoxifen is often taken for several years following adjuvant therapy.

Findings showed that heightened symptom distress in the post-treatment period was associated with greater illness uncertainty (r = .42, p = .001, N = 109). Illness uncertainty is a concept which incorporates stress appraisal and coping (Lazarus & Folkman, 1984) and occurs when illness outcomes are unpredictable and information or cues are inadequate or inconsistent. It appears the experience of ongoing side-effects in the post-treatment period exacerbate psychological strain. In support of this assumption, greater symptom distress, illness uncertainty, and fear of disease recurrence accounted for 48% of observed variance in emotional distress scores. This study also revealed that age was negatively correlated with fear of recurrence. Authors suggest that is due to older women having faced more adversity and challenges to their mortality across their lifespan than younger women.

Of interest, in the context of The Oxidative Model, having a concurrent illness (other than cancer) post-treatment worsened the experience of uncertainty and

emotional adjustment. This suggests that resuming 'good' health is vital in this period. Although these findings provide some insight into the cancer-stressor post-treatment as a cohort there was a great deal of discrepancy between the age of and the length of time participants were post-treatment, spanning 1-6 years after the initial diagnoses. In addition, it was unclear what treatment regimes patients (i.e., chemotherapy and/or radiation) had been through which could contribute to different types of symptom distress and emotional distress. This makes it difficult to ascertain specific points during the post-treatment period which were more distressing than others. This study would be improved using a longitudinal design.

In 1999, Wenzel, Fairclough, and Brady further explored age-related differences that had been raised by previous research (Cordova et al., 1995) (Green et al,. 1996) by exploring quality of life, symptoms of depression, and PTSD in the two-months following the completion of treatment for early stage breast cancer. The objective of this study was explicitly to compare younger (< 50 years) and older (> 50 years) patients' quality of life following recent completion of active treatment.

Quality of life was quantified in this study by assessment of The Functional Assessment of Cancer Therapy - Breast (FACT-B; (Brady, Cella, & Mo, 1997)). Depression was assessed using the Centre for Epidemiological Studies - Depression scale (CES-D; (Radloff, 1977)), cancer-specific distress (IES), and Sexual Functioning and Body Image Scales developed specifically for this study. Participants (*N* = 304) were recruited from 21 sites across the US, over half were over 50 years of age (53%). The majority of the sample comprised women who were Caucasian (90%), married (> 67%) and college educated (>40%).

Findings uncovered differences between younger (< 50 years) and older groups of statistical significance with respect to global QOL (p = 0.02), emotional well-being, breast carcinoma specific concerns –IES (p = 0.02), symptoms of depression (p = 0.04), and disease specific intrusive thoughts (p = 0.01). In contrast data suggests that age alone does not predict sexual dysfunction. No participants were identified as meeting the full PTSD criteria. Although this was a large sample collected across multiple sites, this is a cross-sectional snapshot which limits the power, and is at risk of being influenced by inter-individual differences. Despite this, findings from this current study serve to underscore the importance of recognizing "at risk" populations and targeting QOL interventions toward those populations(Wenzel, Fairclough, & Brady, 1999).

More invasive or painful treatments have also been suggested as a possible reason for increased psychological distress. In previous studies in this review the majority of diagnoses have been early in the diagnostic staging with relatively good prognoses. However incidence of PTSD at a minimum of 100 days post-treatment for a specific intensive chemotherapy regime: autologous bone marrow transplant (BMT) has been investigated (Mundy et al., 2000). Women having undergone autologous BMT (n = 17) were compared to women who had not (n = 20). Participants in this study were diagnosed with stages II to IV breast cancer, predominantly married (76%), college educated (62%), all Caucasian, and with a mean age of 42 years (SD = 7.6).

PTSD, MDD, and generalized anxiety disorder were assessed at 3, 6 and 12-months after the cessation of treatment. Assessment was by self-report measure (POMS)(McNair et al., 1971), as well as a semi-structured clinical interview using the modules of PTSD, major depressive disorder, generalized anxiety disorder and

dysthymia (SCID; (First, Spitzer, Gibbon, & Williams, 1995)). Participants were asked to recall retrospectively how they felt on learning of the diagnosis of cancer, when receiving subsequent treatment (i.e., surgery, chemotherapy, or radiation), on learning of disease recurrence, when receiving subsequent treatment, and during hospitalization for BMT, 3, 6 and 12-months after their last treatment.

At the outset there were inequities between groups. The BMT group was significantly younger (t (35) = -2.13, $p < 0.05$), had more advanced disease at diagnosis ($\chi^2 = 6.09$, $p < 0.05$), and less time had lapsed since their last treatment (t (35) = -4.56, $p < 0.001$), 10.4 months compared to the non-BMT group's 43.3 months. However the time since diagnosis with breast cancer was not significantly different. Similarly the rate of PTSD was not statistically different between the two groups. Despite this, as an overall sample, PTSD were observed (24.3%), occurring at some stage over the entire diagnosis, treatment and post-treatment period.

Critically all assessment of PTSD was done retrospectively. The study findings would be have been stronger if done prospectively. In addition only parts of the SCID module were employed so other psychopathology like sub-threshold symptoms or other psychopathologies remain unknown. On the basis of PTSD the most psychologically difficult period in this study was identified as at the time of the initial diagnosis. The invasive BMT procedure was not associated with developing PTSD.

Bleiker, Pouwer, van der Ploeg, Leer & Ader (2000) conducted a prospective study to investigate the frequency of and predictors for psychological distress in early stage(Stage I-II) breast cancer patients post-treatment(Bleiker, Pouwer, van der Ploeg, Leer, & Ader, 2000). Distress relating to the cancer experience was quantified by

assessment using the IES (Horowitz et al., 1979). In addition a Dutch adapted version of the Social Readjustment Rating Scales (SRRS; Holmes & Rahe, 1967) was used to assess whether participants had experienced specific life-events in the ten years prior to breast cancer diagnosis. The Self Assessment Questionnaire - Nijmeen (SAQ-N)(Van der Ploeg, Defares, & Spielberger, 1980) was employed to assess trait characteristics (i.e., anxiety, anger, depression, rationality, anti-emotionality, and understanding). The Social Experiences Checklist (SEC; (van Oostrom, Tijhuis, De Haes, Tempelaar, & Kromhout, 1995)) was used to measure perceived social support.

Participants in this study (*N* = 170) ranged in age from 29 to 75 years (*M* = 51.9, *SD* = 10.5), predominantly reported low or intermediate education (45- 31%), and were married (81%). Clinically the majority of participants were diagnosed with stage II breast cancer (58%). Subsequently the majority of patients were treated with breast conserving therapy (lumpectomy); some underwent chemotherapy (30%) and/or hormone therapy (26%). Baseline assessment occurred 2-months post-surgery and then follow-up occurred 19-months post-surgery. Scores on each IES subscale (Intrusion and Avoidance) equal to or above 20 were considered by the authors as strong indicators of a stress response syndrome and termed psychological distress. This was how psychological distress was defined for this study.

Baseline assessment revealed 30% of participants reported moderate levels of Intrusive thoughts and Avoidance as measured by the IES. At follow-up, 16% of patients reported high levels of Intrusive thoughts, while only 8% had high Avoidance scores. Notably 60% of patients who scored poorly on Intrusions at baseline also

scored poorly on this subscale at the follow-up assessment. For the Avoidance scale this was 20%.

Exploration of psychological, demographic, and biomedical variables reported at baseline revealed that health complaints (β = .29, partial correlation = .0.22) and T-anxiety (β = .22, partial correlation = 0.22) were the best predictors of psychological distress in the post-treatment period. These two variables explained 19% of intrusion at the post-treatment assessment. Trait anxiety is defined in terms of anxiety proneness as reflected in the frequency that anxiety states have been manifested in the past and the probability that feelings of state-anxiety (i.e., feelings of tension, apprehension, nervousness and worry, with associated activation of the autonomic nervous system) will be experienced in the future (Spielberger & Reheiser, 2009).

The finding that health complaints were predictive of psychological distress was in line with findings discussed in the previous study (Mast, 1998) which identified concurrent illness (other than cancer) to worsen the experience of uncertainty and emotional adjustment post-treatment. This reiterates the importance of the post-treatment period as a time to be aware of psychological, social and physiological health for early stage breast cancer patients. A theoretical framework encompassing all these elements is The Oxidative Model. However the inclusion of this study assumes the sample is post-treatment but it is actually unclear what percentage of patients were post-treatment by the authors use of –'19-months post surgery' description to define the sample.

A longitudinal-designed study has been used to explore distress over a more narrow timeframe, specifically in the immediate 6-months following active treatment

(Deshields et al., 2005). The sample (*N* = 94) comprised a group of women with early stage (I-III) breast cancer who had completed surgical, chemotherapy, and radiation treatment. For this study distress was quantified by a battery of self-report measures including measurement of depressed mood as measured by the CES-D, anxiety via the STAI, and quality of life measured using the FACT-B.

Women ranged in age from 28 to 87 years (*M* = 55.4, *SD* = 11.3), were predominantly Caucasian (71%) , married (57%), not employed (42%), and had completed more than 12 years of formal education (84%). Sixty three percent reported no children living at home and overall participants were evenly distributed across income levels. Patients were assessed on five separate occasions by telephone; on the last day of radiation treatment, 2-weeks later, prior to their first medical follow-up appointment (4-6 weeks post treatment), and subsequently at 3 and 6-months post-treatment.

At the outset it was hypothesised that breast cancer patients would demonstrate increased distress across the post-treatment period with an increase observed in anticipation of their first medical follow-up (4-6 weeks post-treatment). Findings indicated the mean depression scores were significantly higher ($t = 3.16$, $p < .001$: $M = 12.9$, $SD = 11.0$) at initial assessment when compared with normative adult data ($M = 9.25$). Immediately following treatment one third of participants depression scores exceeded the cut-off (>16) for clinically significant symptoms of distress. Anxiety and quality of life were comparable to normative scores. Mean depression scores decreased significantly over the 6-month period ($\beta = 0.51$, $t = 2.55$, $p = .011$) with the greatest decrease observed between the first and second assessment points, at the

end of radiation treatment and two weeks later. Quality of life scores showed significant improvement (β = 1.92, p <0.001) over time; however there was no significant change in anxiety across time.

Despite the decrease in depression scores over time, approximately 25% of women scored above the clinical cut-off at each time point, reflecting depressive symptoms had not been resolved. In The Oxidative Model literature the sample diagnosed with clinical depression was observed to be associated with deleterious oxidative and inflammatory marker levels in comparison to a healthy non-depressed sample. For cancer patients the post-treatment period is critical for physical and mental recuperation and healing after intensive treatment regimes. These findings suggest that those not experiencing symptoms of clinical depression psychological recovery occurs rapidly in the 6-months following cessation of active treatment. It is plausible that the influence of ongoing psychological strain, for a subset of women, like depressive symptoms, may have a detrimental physiological impact which requires exploration.

Similar mixed findings, regarding the experience of psychological distress, were observed in a recent longitudinal design study exploring distress across the treatment/post treatment period (Costanzo et al., 2007). This study assessed distress in a sample of (N = 89) stage 0-III breast cancer patients-midway through adjuvant chemotherapy or radiation therapy- and then subsequent assessments by mail at 3-weeks and 3-months post-treatment.

Distress in this study was quantified using the following battery of standardized measures: the CES-D, assessment of general anxiety via the Primary Care Evaluation of

Mental Disorders Patient Health Questionnaire (PRIME-MD; Spitzer, Kroenke, & Williams, 1999), cancer-related anxiety using the IES, Concerns About Cancer Recurrence Scale (CARS; (Vickerberg, 2003)) to assess worry about cancer recurrence, cancer-related symptoms via the Memorial Symptom Assessment Scale (MSAS; Portenoy et al, 1994), and health related quality of life using the Medical Outcomes Study Short-Form 36 Version 2.0 (Ware, Snow, & Kosinski, 2000). Four of the original eight scales were used including physical functioning, role–physical (role limitations due to physical problems), bodily pain, and vitality scales.

Sources of distress were identified based on a 12-item list compiled from data drawn from interviews with the patients. Participants ranged in age from 32 to 89 years (*M* = 55.0, *SD* unspecified) and were predominantly Caucasian (93%), married (73%), high-school educated (68%), and from mid-to-high income households (34%, > US$70,000). The majority of patients were stage I and II (> 80%). All participants received adjuvant chemotherapy or radiation therapy with over half (58%) receiving both types.

Findings suggested that for the majority the treatment and post-treatment period was not a time of disrupted psychological adjustment, with observed levels similar to normative samples. There was no statistically significant change observed for depression, general anxiety, or cancer-specific anxiety (IES). Intrusion was the only distress variable that changed significantly declining from baseline to 3-months post-treatment ($F(2,144) = 3.48$, $p = 0.034$). Despite the majority of patients showing little evidence for disrupted psychological adjustment, there was a subset of participants whose depression scores continued to exceed clinically significant cut off scores at

baseline (19.3%). This pattern continued 3-weeks post-treatment (22.1%) and remained 3-months post-treatment (17.4%).

Mean IES scores suggested a moderate stress response was sustained for a number of women from baseline, to 3-weeks post-treatment, and 3-months post-treatment. Memorial Symptom Assessment Scale symptom scores changed significantly over time, F (2,124) = 8.98, p = 0.001, improving from baseline to 3-weeks post-treatment, and then remained steady. With respect to SF-36 quality of life domains, there were significant improvements in physical functioning, F (2,148) = 4.57, p = 0.012; role–physical, F (2,149) = 12.02, p = 0.001; and vitality, F (2,155) = 3.48, p = 0.033 over the 3 assessment points.

Predictors of post-treatment distress were investigated across three assessment points using mixed models analyses. Women with a history of anxiety showed a different trajectory of depression to others in this sample; their depressive symptoms decreased slightly from mid-treatment to 3-weeks post-treatment, and then increased steeply from 3-weeks to 3-months post-treatment, F (2,143) = 3.52, p = 0.032 and F (2,143) = 3.50, p = 0.033, respectively. Like previously mentioned findings (Cordova et al., 1995; B.L. Green et al., 1996; Mast, 1998; Wenzel et al., 1999) age was identified as an influential variable, with younger women experiencing greater distress on all measures F (1, 72) = 9.62, p = 0.003, intrusion, F (1, 76) = 12.12, p = 0.001, and recurrence worry, F (1, 82) = 19.67, p = 0.001.

Interviews with patients revealed two salient sources of distress following treatment. The first was around dealing with residual side-effects and physical problems and the second the fear of disease recurrence, reflecting similar findings to

Mast (1998). Two more minor sources of distress included trying to get back to 'normal' or attempting to create a 'new normal'. The additional feeling that one had lost a 'safety net' related to having regular contact with health-care providers was also a source of distress. This parallels the 'unmet needs' literature (Armes, et al., 2009).

Social supports were not assessed through standardized measures, but were raised as a source of distress (i.e., not getting the assistance or emotional support from family and friends). This requires further exploration. The timing of the baseline assessment (mid-treatment) was less than ideal it varied based on individual treatment schedules As a result the assessments varied considerably on length of time since diagnosis and prior to the end of treatment.

This concludes the review specific to the experience of distress in the post active treatment period. It is apparent that there are many manifestations that characterize psychological distress during this period. This makes the review process complex, but at the same time compelling. The next step is to incorporate research of PNI mechanisms in this population, post-treatment.

3.6 Psychoneuroimmunology and Breast Cancer in the Post-Treatment Period

It is evident from the previous review of psycho-oncology literature that the cancer journey in the post-treatment period remains a period interspersed with distress- as defined by anxiety, depression, trauma, fear, and uncertainty as well as emotional and symptom distress. For this population, once active treatment has ceased, psychological challenges remain influential.

As outlined in Chapter1, psychoneuroimmunological research in healthy samples suggests stress responses are accompanied by changes in a broad spectrum of immune measures. In particular, chronic stressors have been indentified as having a detrimental impact on immunity (Herbert & Cohen, 1993; Segerstrom & Miller, 2004). A frequent promise of PNI research has been that it would lead to the amelioration of disease via the course of immune change (Schleifer, 2007). Psychological responses to cancer have often been proposed as potential prognostic factors influencing survival, cancer outcomes, and quality of life. However, causal mechanisms remain undefined and research in the area is conflicting.

The breast cancer PNI literature highlights a range of diverse approaches and research designs. For instance there are: retrospective studies where patients are asked to recollect stressors and psychological factors from before their diagnosis (Ginzburg, Wrensch, Rice, Farren, & Spiegel, 2008); quasi-prospective studies which examine suspected breast cancer patients prior to diagnosis (Ollonen, Lehtonen, & Eskelinen, 2005), and large prospective epidemiological research of healthy samples that go on to develop cancer (Cohen & Herbert, 1996). A detailed review of all PNI research in breast cancer samples is beyond the scope of this dissertation. The next step in the review process is to shift the scope towards specific constructs (i.e., immune, oxidative, and inflammatory measures) for this population following the cessation of active treatment. Biochemical measures similar to those employed in The Oxidative Model (outlined in Chapter 2) where possible will be included.

3.7 Immune measures

There is a vast array of design approaches to PNI in cancer patients; similarly the immunological measures assessed are equally varied. However one immune measure which features prominently throughout the cancer literature is natural killer cells (NK cells). NK cells are a type of cytotoxic lymphocyte. NK cells are of particular interest for cancer research due to their role in immune surveillance against tumours (Kiegolt-Glaser, McGuire, Robles, & Glaser, 2002). In addition subsets of white blood cells (i.e., like lymphocytes) and proliferative responses (i.e., reactions of immune cells to antigen) are often secondary measures to NK cell research.

Research into the role of NK cells and other white blood cells is commonly quantified in two ways: enumerative counts of these cells in plasma samples, and/or functional assessment of cell cytotoxicity (i.e., assessed by exposing cells to virus infected cells in-vitro). Cell collection methods used in The Oxidative Model research do not incorporate NK cells, rather these methods focus mainly on lymphocyte numbers (T and B cells), a specific type of white blood cells. Although not assessed in The Oxidative Model, NK cells are part of an innate immune response, playing an early role in detecting virus-infected or cancer cells whilst the acquired immune response is generating cytotoxic T lymphocytes (Coico et al., 2003). The following section will review PNI research encompassing immune measures like NK cells and lymphocytes and any other related immune measures.

One study which meets the criteria for this review explored the relationship between social support and immune variables during and immediately after treatment for breast cancer (Lekander, Furst, Rotstein, Blomgren, & Fredrikson, 1996). The

rationale being that since chemotherapy has been identified as having negative effects on the immune system; social support could buffer stress and modify immunity. The aim was to examine the relationship between social support and immune variables during and after treatment for breast cancer. Between October 1988 and July 1992, participating patients had undergone surgery followed by adjuvant chemotherapy (*N* = 38). The stage of disease diagnoses was unspecified. Women in this sample ranged in age from 27 to 68 years (*M* = 49, *SD* unspecified).

Two cohorts were assessed; one group consisted of women during adjuvant treatment. Adjuvant chemotherapy consisted of six courses of CMF (100mg cyclophosphamide/m2 p.o. days 1-14, 40mg i.v. methotrexate/m2 days 1 and 8 and 600 mg i.v. 5-fluorouracil/m2 days 1 and 8), and was given to patients with positive nodes in the axilla or breast tumours exceeding 30mm. Each course was repeated every 28th day. Participation occurred with their 4th or 5th course of chemotherapy.

The second group consisted of women 3-months after cessation of this chemotherapy protocol. Blood samples were collected from patients and assayed for lymphocyte, granulocyte, and monocyte numbers. Patients completed the abbreviated version of the Interview Schedule for Social Interaction (ISSI)(Henderson, Duncan-Jones, & Byrne, 1980) to assess the perceived quantity and quality of social support. This was by self-report and completed in the patient's homes. Groups did not differ significantly on age; however it was unclear whether groups differed significantly on disease stage, surgery type, ethnicity, marital status, education, and socioeconomic status.

Results suggested that patients receiving chemotherapy had significantly different leukocyte numbers; specifically the group still receiving treatment had lower levels overall levels (p = .05) and significantly different leukocyte subsets. These patterns observed in subsets were lower granulocyte (p = .05), but higher monocyte counts (p = .01). Lower granulocyte counts are an expected side-effect of chemotherapy. However there was no difference with respect to social support. Furthermore age was ruled out as influencing immune parameters. For the group receiving chemotherapy no significant trends were identified for social support and immune measures.

For the post-treatment group, the subscale Perceived Attachment of the ISSI was significantly and positively related to the total number of white blood cells (β = 0.50, R^2 = .25, p <0.10). It was also related to the composition of white blood cell groups. Specifically, attachment was positively associated with total numbers of white blood cells (β = -0.56, R^2 = .32, p <0.10) and granulocytes, (β = 0.58, R^2 = .33, p <0.10) but a negative relationship was observed between attachment and lymphocyte percentages.

There a few key points which these findings highlight. The first is that chemotherapy has an impact on immune cells. Although this would seem intuitive, whether psychological well-being can in fact buffer these effects during treatment seems unlikely. However the findings support the assumption that social support and interpersonal relationships are related to immune measures in the post-treatment period. It was unclear what the marital, work, and education status of these groups was. These could potentially contribute to social support levels; however this is not

conclusive and requires additional research. In addition the time of blood collection was not specified. Diurnal fluctuations can contribute to changes in biomarker levels.

Statistically these findings were based on correlations thus making it difficult to discern causality. In addition these were two separate cohorts and there may be distinct inter-individual differences on immune measures which remain unknown. Importantly this study identified social support as a potential buffer relevant in the post-treatment period and that during chemotherapy treatment these effects may be overridden by the biological factors (i.e., chemotherapy killing the immature dividing granulocytes) that impinge on the formation of blood cellular components like immune cells.

A more recent prospective study, meeting the sample and treatment phase criteria for this review, explored lymphocyte number and function, NK cell activity, plasma cortisol, prolactin, and 8-year survival. Osbourne and colleagues (2004) concurrently measured immunological and psychosocial parameters in the 4-weeks following the completion of adjuvant treatment to estimate whether they were predictive of breast cancer outcomes 8 years later. Women with stage I-III breast cancer (*N* = 62) entered the study if they were free of infections and if they had completed chemotherapy treatment more than 4-weeks earlier. In addition a brief semi-structured interview and the HADS were administered. The Duke-UNC functional social support scale (DUFSS; Broadhead, Gehlbach, de Gruy, & Kaplan, 1988) was administered, along with the Mental Adjustment to Cancer (MAC) scale (Watson, et al. 1988).

The mean age at diagnosis was 56 years (range 27 – 75 years). Two thirds were interviewed 5 to 9-months after diagnosis and the remaining one third 9 to 17-months following diagnosis. The majority of patients had stage I (41%) or stage II disease (54%), and had been treated with mastectomy (59%). Only 10% had received chemotherapy. Ethnicity, marital status, education, and socioeconomic status were unspecified.

The overall findings from this study suggest that survival at 5 years was predicted by only one immunological measure, lowered NK cell activity, and one psychosocial measure, Fighting Spirit (from the MAC scale), minimizing the illness. Authors discuss that this immune finding is counter intuitive, as NK cell activity is considered important for immuno-surveillance, thus preventing the spread or development of further metastases (Ader, 1991, Garsen & Goodkin, 1999). This finding would suggest that there is little survival benefit associated with NK cell activity. It is worth commenting that all other lymphocyte counts evidenced higher levels in the survival group. This would suggest greater immune activation for the survival group in the 4-week period following adjuvant treatment, although none reached significance.

The small sample size compromised power in the study possibly leading to Type II errors. Multiple significance testing may also have increased Type I errors. Furthermore the authors acknowledge that there is the potential for immunological rebound. This term describes an acute increase in immune parameters above baseline which can occur in response to recent administration of adjuvant treatments including chemotherapy, radiotherapy, and preventative treatments like Tamoxifen. Authors argue that it would be unlikely for this type of rebound 4-weeks post-treatment

(Osborne et al., 2004a), however it is possible. This type of rebound is common across PNI research undertaken in cancer samples during treatment and post-treatment as is the case here. This presents an additional confounding variable and makes interpretation of the impact of psychological states on immune measures complex.

NK cells and proliferative responses have also been explored in another study of early stage breast cancer patients encompassing the post-treatment period (Thornton et al., 2007). Specifically this research focused on individual trajectories of stress and immunity across treatment. It is included in this review because post-treatment assessment was incorporated. Women with early stage breast cancer (*N* = 113) were assessed at 4, 8, 12, and 18 month intervals following diagnosis and surgery. This sample comprised 30 to 75 year old women (*M* = 51.2, *SD* = 10.8) with 6 to 22 years of education (*M* = 14.3, *SD* = 2.6) and a median household income (US $50,000). Most were employed (69%), had a spouse or partner (71%), and were Caucasian (90%). The majority had stage II disease (92%) and all had undergone radical mastectomy with most receiving adjuvant treatment (chemotherapy, 85%; radiation, 52%; hormonal therapy, 80%).

Assessment included both psychological and immunological measures. Psychological assessment included a measure of subjective stress (Perceived Stress Scale)(Golden-Kruetz, Browne, Frierson, & Anderson, 2004) and emotional distress (POMS; McNair, 1971). Immune status was assessed by both enumerative measures (NK and lymphocyte counts) and functionality (i.e., NK cell cytotoxicity and T-cell blastogenesis) measured by in-vitro exposure to antigen. The trajectory of each individual's experience of stress (subjective and emotional) was tracked along the

treatment/post-treatment continuum. Latent growth curve analysis methods were employed testing the longitudinal relationships between stress and immune measures.

On preliminary observation NK cells showed a linear trend for improvement but T-cell blastogenesis (i.e., performance in-vitro) was more inconsistent. The statistical techniques employed for this study controlled for the influence of treatment (surgery and chemotherapy), which was identified as influencing T-cell blastogenesis. Results suggested that there were no significant influences of socio-demographic, prognostic, treatment, and inter-individual variables on variation in immune cells. Participants, who at baseline had higher levels of subjective stress, evidenced poorer in-vitro proliferative responses as measured by T-cell blastogenesis ($p < .05$). Similarly, the relationship between psychological distress and blastogenesis was also negative, but not significant ($p = .14$).

This suggests perceived stress and distress to be associated with evidence of poorer immune response to challenge from antigen or viruses. Importantly on further exploration authors discovered that for the participants whose stress levels declined rapidly there was a corresponding rapid improvement in NK cell count at subsequent assessments but not improvement observed for blastogenesis. On the other hand slower reductions in stress did not correspond to improvements in NK cell counts. This finding can be interpreted as the duration of stress having a more pronounced effect as opposed to a short burst of stress which is resolved; this finding is supported by a recent meta-analysis (Segerstrom & Miller, 2004).

The findings also suggest stressor appraisal (perceived stress) to be more indicative of immune function than emotional distress (i.e., depression, anxiety, stress)

and this is likely to be due to individual variability in personal/social factors like coping styles (repression, defensiveness) and trait characteristics which weren't assessed in this study. The authors suggest that stressor appraisal and associated immune changes may in fact not be linear and this requires future consideration.

Overall this was a very thorough and comprehensive study which assessed demographic variables, stage of disease, hormone receptor expression, number of positive lymph nodes, extent of surgery and type of adjuvant treatment as relevant disease/treatment variables. It was unclear as to whether blood collection was undertaken at the same time at each assessment in order to minimize diurnal variation. In addition the treatment and post-treatment period were evaluated with the assumption that stress decreases over the 18-month period. Critically, from our previous review of the psycho-oncology post-treatment literature, this is not always the case.

Research which incorporates immune and psychological measures in samples of women with early stage breast cancer post-treatment has been reviewed. It is important to clarify that there are two key limitations to assessing NK cells. Firstly, the number NK cells evidence a high degree of inter-individual variation. Studies, like Thornton and colleagues (2007), which measure longitudinal data rather than one-off measures will have some control over this variability. Secondly, NK cell cytotoxicity is a relatively volatile measure with some assays showing only 25% stable variance over a 1-week interval compared with over 53% for enumerative counts and a similar 42-53% for proliferative measures (G. E. Miller et al., 1999). For these reasons the

interpretation of their role in PNI research remains difficult. The review of these three key studies highlights the lack of focus on the immediate post-treatment period.

3.8 Pro-inflammatory processes

Activation of an innate immune response is entwined with inflammatory responses. Measures of inflammation include immune cell numbers components (i.e., like white blood cells) as well as acute phase proteins like C-RP and inflammatory cytokines. Inflammation is common among cancer patients (Shankar et al., 2006). This can be due the nature of the tumour and/or the body's heightened immune response to a tumor. In addition inflammation can result from surgical, chemotherapy, or radiation treatments (A. H. Miller, Ancoli-Israel, Bower, Capuron, & Irwin, 2008). Inflammation is considered a cancer promoting factor (Balkwill & Mantovani, 2001; Coussens & Werb, 2002).

The relationship between psychological states and inflammation (i.e., CRP, inflammatory cytokines) has been discussed in the context of The Oxidative Model in Chapter 2. To reiterate, severe distress has been associated with increased inflammation in healthy adult samples experiencing occupational strain (Hapuarachchi et al., 2003). A clinical diagnosis of PTSD has also been associated with increased inflammation (Pfitzer, 2008).

Psychosocial influences on inflammation have been explored in a prospective design which measured changes in social activity in early stage breast cancer (Marucha, Crespin, Shelby, & Anderson, 2005). This sample was drawn from a larger randomised controlled trial of a psychological intervention (Anderson et al., 2004). This

study recruited women with stage I/II breast cancer 5-weeks post-surgery (17-81 days) and again 12-months later when most (90%) had finished chemotherapy treatment. This is a limitation as it is likely that chemotherapy was impacting on immune markers for 10% of this sample.

Inflammation was assessed by measuring serum inflammatory cytokine levels, specifically IL-6, IL-1β, and TNF-α. As outlined in Chapter 1 and 2, cytokines are important immune factors in coordinating the immune response. TNF-α is a potent pro- inflammatory cytokine. Psychosocial measures assessed included family, social and leisure activities (Katz Social Adjustment Scale; Katz & Lyerly, 1963) as well as partner satisfaction (the Dyadic Adjustment Scale: DAS; (Spanier, 1976)). Serum cytokine levels and physiological status were assessed by nurses reporting patients functional status (Karnofsky Performance Status, KPS; Karnofsky & Burchenal, 1949) and symptoms and side-effects of cancer treatment toxicity (Southwest Oncology Group criteria: SWOG)(Moinpour et al., 1989).

The mean age of women recruited for this pilot study was 51.07 years (*SD* = 10.89). The majority of the sample were Caucasian (95%), received over 14 years of education (*SD* = 2.75), had a spouse or equivalent, and were from households with incomes over US$60,000. Patients were predominantly diagnosed with stage II breast cancer (92%) underwent mastectomy surgery, (43%), radiation (51%), chemotherapy (85%); and hormonal therapy (80%). Hierarchical multiple regression analyses revealed that change in social activity explained 9.4 % variance in 12-month TNF-α levels ($p < .05$) and. For patients with partners (*n* = 29) both change in social activities and change

in partner satisfaction explained a significant ($p < .05$) increment of variance (17.2 %) in 12-month TNF- α levels.

These findings suggest that breast cancer patients who increased their leisure, home, and social activities with friends and family exhibited a stronger TNF-α response. Notably TNF-α production between baseline and 12-month follow-up revealed very little change. Change in health as assessed by KPS change scores was, as expected, a significant predictor of 12-month TNF-α in this model ($p < .05$). IL-6 and Il-1β were also assessed but were uncorrelated with social variables. This pilot data suggest a possible relationship between positive changes in social functioning and TNF-α. The authors speculate that if social disruption can activate a biological stress response which impairs TNF-α production then the reverse might also hold true; there is potential for increasing quality and frequency of social relations to ameliorate biological stress during cancer as well as post-treatment. Parallel with this research, the Oxidative Model proposes links between inflammatory cytokines and measures of psychological trauma (Pfitzer, 2009), as discussed in Chapter 2.

A recent psychosocial intervention in a sample of breast cancer patients (N = 45) experiencing depression, observed that the alleviation of depressive symptoms was associated with a concurrent decrease in inflammation. This study was a secondary analysis of a larger randomised controlled trial of a psychological intervention (Anderson et al., 2004). This study recruited stage II and III breast cancer patients 17-81 days post-surgery and followed them over a 12-month period.

Patients in the intervention arm were involved in 26 weekly, psychologist-led group sessions over a 12-month period. The comparison group was involved in

assessments only. Depressive symptoms (CES-D Iowa short form)(Radloff, 1977), mood and fatigue (POMS)(McNair et al., 1971), and quality of life related to pain (Bodily pain subscale (SF-36)(Ware et al., 2000) were assessed at 4, 8, and 12-months post-surgery as were health behaviours implicated in inflammation such as diet, exercise, and smoking. Inflammation was assessed by white blood cell count (WBC), a nonspecific biomarker of inflammation. At 4-months the majority were in treatment. At 8-months, only 10 % remained in treatment and at 12-months 100 % had completed treatment.

Mixed-effects modeling was used to test the effects of the study arm (intervention vs. control), time (linear change in months), time 2 (quadratic change in months), and the study arm x time and study arm x time2 interactions. At the outset there was no significant difference between depressive symptoms or inflammation between the groups. However, depression recovery rates were significantly faster for the intervention participants as measured by the CES-D (p = .04) and POMS depressed mood (p = .02). Similarly improvements in reported pain were significantly faster for patients in the intervention arm (p = .04). Rapid improvement was observed to occur in the early months and then stabilize (p = .02). The measure of inflammation WBC showed a similar pattern; the intervention arm evidenced statistically significant reductions for WBC (p = .005) and neutrophils (p = .006) counts over time.

Further exploration to test the causal pathways for this reduction in both depression and inflammation was undertaken. The following data was selected: baseline data provided values for control, the 8-month assessment depressive symptom mediators, and 12-month assessment for inflammatory markers to remove impact of cancer treatments on biomarkers. A significant indirect effect of study arm

on WBC via depressive symptoms was observed (p = .006). Furthermore the effect of study arm on 8-month depressive symptoms (p = .003) and subsequent effect of 8-month depressive symptoms on 12-month WBC (p < .001) were both statistically significant. In short psychological processes were identified as being the mediating factor in inflammatory processes regardless of changes in health behaviours like diet and smoking.

The largest reductions in depressive symptoms were observed at the 8-month assessment; given this corresponded with most of the sample having completed active treatment (90 %) this may not solely be due to the intervention but rather the cessation of chemotherapy. However this piece of research provides incentive to further explore and understand interventions which interrupt inflammatory processes. These interventions could have long-term health and quality of life benefits.

The findings presented in this section are both exploratory. Yet they reveal plausible mechanisms linking improvement in psychological variables with physiological improvement, specifically decreased inflammation. In line with this a recent review has identified that the activation of an innate immune response (like inflammation) may contribute to the development of behavioural alterations in both medically ill and medically healthy individuals (A. H. Miller et al., 2008). This review details inflammation-induced behaviours- specifically depression, fatigue, sleep disturbance, and cognitive function. These behaviours are commonly evidenced in cancer patients and prompt the authors to suggest that behaviour be a vital sign as an indicator of immune activation, inflammation, and central nervous system function for cancer patients across the disease encounter.

3.9 Pro-oxidant processes

As detailed in Chapter 2, pro-oxidant processes are related to the immune response in many ways. One of the functions of components of an innate immune response (i.e., neutrophil) is to produce oxygen reactive species (also known as free-radicals) as part of their normal function in combating infections and injury. This response is also responsible for inflammatory pathways. With regard to cancer risk, excessive free-radicals can damage critical cellular macromolecules including DNA. Oxidative damage that remains unrepaired can result in mutations and transformation of cells to a cancerous state, hence the importance of oxidative stress for cancer patients.

Patients diagnosed and receiving treatment for cancer often use complementary or alternative medicines (CAM). However rates of use are mixed depending on the definitions employed. Investigators (Burstein, Gelber, Guadagnoli, & Weeks, 1999) studied 480 patients with newly diagnosed early-stage breast cancer and found that 28 percent of them began to use alternative medical therapies as an adjunct to conventional therapy. Pertinent to The Oxidative Model, CAM use includes antioxidant supplementation (e.g., Vitamin C, etc). This is often to alleviate treatment toxicities and to improve long-term outcomes. The rationale being antioxidant supplementation during chemotherapy is to compensate for treatment or cancer induced antioxidant depletion. Up until recently evidence of depletion was limited. However a review (Ladas et al., 2004) of observational studies (*N* = 31) on antioxidant levels supports the hypothesis that chemotherapy lowers total antioxidant status (TAS).

However inconclusive findings were reported for vitamin C, vitamin E, selenium, and β-carotene. Furthermore it was suggested that cancer cells use antioxidant vitamins more efficiently than healthy cells, thereby depleting plasma antioxidant levels. Despite this, no specific studies were identified looking at the AO status of women treated for early stage breast cancer in the post active treatment period.

3.10 Summary

There is little doubt that the experience of cancer diagnosis and treatment is a potential stressor. This review suggests that sustained psychological strain following the cessation of active treatment is evident, although findings are complex and often mixed depending on the methodology (i.e. prospective vs. retrospective) and design (i.e. cohorts versus longitudinal) of studies. One of the main complexities of research in this population, in this period, is that manifestations of distress have been quantified by a variety of constructs including emotional distress, anxiety, depression, PTSD (both full and sub-threshold), fear of recurrence, as well as symptom distress. In line with the many constructs utilized, different psychometric tools have been employed which further makes comparing and contrasting studies difficult.

In addition to research focusing just on psychological distress at this time, PNI research in this population has been explored. There are only few studies which explore this narrow (1- 12-months) post-treatment period. There are none that explore the same set of biomarkers like those implicated in The Oxidative Model. However those like The Oxidative Model, which attempt to explore oxidative and inflammatory processes in the post-treatment timeframe, suggest psychological

distress to be an influential factor in immune, inflammatory and oxidative mechanisms. It is also worth noting that women who experience poor health or illness other than cancer in this post-treatment period experience more emotional distress. This suggests that this is a time when both psychological and physiological recovery and recuperation is vital.

The Oxidative Model provides a framework to assess psychological stress and immune changes based on pro-oxidant and pro-inflammatory markers. It has yielded suggestive findings in several populations including occupational strain, academic stress, major depressive disorders, and trauma samples. It is yet to be applied to a cancer population. Given the evidence of psychological adjustment and immune, inflammatory and oxidative challenges in this population, it is feasible that the application of this Model could enhance understanding of these mechanisms and provide pathways (i.e. interventions) to remedy the impact of ongoing psychological challenge for women in this fragile period of recovery.

Chapter 4

Principal Research Aims

4.0 Overview

Chapter 1 introduced the area of psychoneuroimmunology (PNI) and provided an outline and description of physiological components and processes to guide the reader for the presentation of a PNI model. Chapter 2 proposed a theoretical model linking chronic stress with pro-oxidant and pro-inflammatory processes and a subsequent depleted immune system. Subsequent to this, Chapter 3 outlined a novel population which remains unexplored in the context of the OM.

This novel population is women diagnosed and treated for early stage (I-III) breast cancer in the 6-months following cessation of active treatment. This period has been identified as having both psychological and physiological challenges. The Oxidative Model provides a theoretical framework whereby the impact and interaction of both physiological and psychological challenges can be explored. The application of this Model to a post-treatment breast cancer population aims to better understand and find novel ways to improve patient well-being and if necessary implement future interventions.

4.1 Gaps in The Oxidative Model literature

Based on the previous review of literature, several points have been raised. To summarise The Oxidative Model has evidenced some support for identifying oxidative and inflammatory burden associated with stress across several studies. These samples encompass: academic examination stress (Arthurson, 2003; Oliver, 2004), occupational

strain (Hapuarachchi et al., 2003), post-traumatic stress experienced by Vietnam veterans (Jolly, 2004), victims of crime (Pfitzer, 2008) as well as clinically depressed patients (Blake-Mortimer et al., 1996). This suggests the model has potential for broad application across stress scenarios and relevance in clinical samples. However several limitations have been indentified.

The first limitation of Oxidative Model studies to date is the use of heterogeneous stress scenarios within samples with regard to timeframe and duration. For example, Blake-Mortimer and colleagues (1996 & 1998) did not identify the length of time since diagnosis of a sample of patients with MDD. Similarly Happuarachchi (2003) did not specify the length of time occupational strain was experienced in a sample of academic staff and Pfitzer (2008) had a sample of victims of crime (VOC) with the time since the initial crime was committed ranging from one to sixty years. Heterogeneous sampling was apparent also with regard to the use of mixed samples of men and women in all Oxidative Model literature to date. The use of these mixed samples blurs the influence gender has been implied to have on stress responses (Taylor et al., 2000)and also the impact of hormones on biomarkers. For example, the case-control study (Blake-Mortimer et al., 1998b) compared an older, recently widowed, male with a young and healthy female.

Secondly, past research designs have largely employed cross-sectional cohorts (Blake-Mortimer et al., 1996; Hapuarachchi et al., 2003; Pfitzer, 2008), that doesn't allow assessment of directional trends for psychological or biochemical measures. Thirdly, several significant findings rely solely on correlational analyses limiting causal conclusions (Hapuarachchi et al., 2003). Fourthly, for all Oxidative Model research,

with the exception of Arthurson (2003), the influence of demographic and health behaviour on biomarkers have often been mentioned but not controlled in statistical analyses.

General population studies illustrate the potential for the influence of confounding variables. Such studies conclude that demographic and health behavior variables like exercise, vitamin consumption, fruit, and vegetable intake have a positive influence on biomarkers indicative of oxidative processes (Lesgards et al., 2002). On the other hand, aging (Boss et al., 1980)and increased tobacco use have been identified to have a detrimental effect. In an oncology setting these variables become all the more influential as distressed cancer patients experience a range of changes in health behaviours. Like general population samples, exercise has been identified as having a positive impact for cancer patients' psychological well-being (Vardy, 2009). However cancer patients often experience disruption to appetite and sleep patterns and have been observed to self-medicate with alcohol, tobacco and drugs (Anderson et al., 1994). The influence of drugs, prescription (i.e. for specific treatment of the disease) or non-prescription, is likely to have an influence on oxidative and inflammatory biomarkers not to mention psychological states. Identifying influential variables relevant to the samples being studied is a real challenge in the area of PNI and not specific to the Oxidative Model. It requires consideration and further exploration.

As previously stated, The Oxidative Model has the potential for clinical application across stress scenarios. In addition it proposes a novel means of reducing the pro-oxidant, pro-inflammatory, immune depletive impact of chronic stress through

behavioural interventions (Le, 2004) and/or nutritional interventions (Blake-Mortimer et al., 1998a; Hapuarachchi et al., 2003) like vitamin supplementation. The Model incorporates pro-oxidant as well as pro-inflammatory pathways. The most frequently tested pathway and hence most reliable is for biomarkers NT and VIT C. These biomarkers have been tested across all studies reviewed in Chapter 2. Less frequently assessed are the pro-oxidant pathways incorporating HCY, VITB12 and FOLATE. Inflammatory pathways incorporating cytokines (IL-1, IL-2, IL-5, TNF-α, TNF-β and IFN) are not assessed in all Oxidative Model literature. This dissertation aims to increase and improve the evidence base for both the established and the less well-defined biochemical pathways in the Model.

4.2 Applying the Oxidative Model to a Breast Cancer Sample

The post-treatment period is a potential period where patients are experiencing chronic stress. The review of women in the 12-months following the cessation of active treatment for early stage breast cancer revealed this period as one marked by elements associated with stress. Specifically sources of distress were identified in the 'unmet needs' literature. These included the experience of emotional distress and uncertainty (Mast, 1998), fear of disease recurrence (Kornblith et al., 2003), as well as fear and uncertainly about the future especially whilst moving away from the supportive hospital network and attempting to resume 'normal' lives, (Lebel et al., 2007). The experience of residual treatment effects, (Cella et al., 2006), symptom distress (Mast, 1998), as well as financial, occupational and interpersonal difficulties (Sammarco, 2001)were also identified as contributing to psychological stress following the cessation of treatment.

In line with the taxonomy of stressors outlined in Chapter 1, (Elliot & Eisdorfer, 1982)it is likely that cessation of active treatment for early stage breast cancer can best align with chronic stressors or a stressful event sequence. The former category is identified as involving stressors that pervade a person's life forcing one to restructure their role or identity. These stressors are, by definition, very stable with no clear idea when the challenge will come to an end. The stressful event sequence category could also be used to describe this period - given the challenge active treatment has only just ceased, but an unknown post-treatment period ensues. The stressor in this instance is based on a focal event (i.e. cancer diagnosis) and a related series of challenges (i.e., treatment regime and post-treatment monitoring).

With these two categories of stress in mind as potential categories for describing the post- treatment experience, it is not surprising that mixed findings of psychological stress in this period have been observed. There is certainly evidence for clinical rates of anxiety and depression for a subset of patients in this period (Fallowfield et al., 1990; Fallowfield et al., 1994), (Bleiker et al., 2000). Distress post-treatment has been quantified using the PTSD criteria (Cordova et al., 1995; B.L. Green et al., 1996; Mundy et al., 2000; Wenzel et al., 1999). However the clinical rates observed across these studies vary from zero incidence to up to 1 in 4 (24.3%). Critically, many of these studies employ varied time frames post-treatment (i.e., 2-weeks through to 60-months). This may explain the disparity between observed rates. In addition the standardized measures employed and techniques (interviews vs. self report) used also contribute to reported rates of clinical and sub-threshold symptoms.

It is important that patients resume good physical and psychological health in the post-treatment period. Research combining psychological and physiological measures in breast cancer patients in the post-treatment period is scarce. The review in this dissertation identified a cross-sectional study comparing two groups during treatment with those post-treatment (Lekander et al., 1996), assessment post-treatment for long term survival studies at 8-years (Osborne et al., 2004a), and prospective longitudinal studies which encompass assessments across both the treatment and post-treatment (Marucha et al., 2005; Thornton et al., 2007). There is no single study which employs measures outlined by The Oxidative Model during the 6-months following active treatment.

The Oxidative Model has the potential to prove useful in this population as it proposes a framework of physiological change associated with stress, thus reflecting the body's adaptive ability. The previous review revealed those women who experienced more health complaints (Bleiker et al., 2000) and illness other than cancer (Mast, 1998) in the post-treatment period also reported more psychological distress. This is a potentially vulnerable period. The application of the Model will allow insight into the PNI post-treatment journey and theoretical ideas regarding immune dysfunction can be tested.

For instance, if a pro-oxidant state is evident resulting in immune dysfunction (as it is theoretically suggested), intervention studies (both psychological and/or nutritional) aimed at decreasing inflammation and improving immune function following treatment may be accurately designed and tested (in latter studies). In essence, the more quickly patients can resume good levels of physiological well-being

post-treatment, the less risk of further illness such as infection. There is a need to develop the evidence base in this population prior to proposing interventions

The influence of confounding variables has been raised as a criticism of The Oxidative Model. There is a clear lack of understanding of the influence of fundamental demographic (i.e. age) and health behaviours (i.e. alcohol, tobacco use, exercise) in The Oxidative Model literature to date. Employing single sex samples to remedy one of the key criticisms is one suggestion. This would be possible using a sample of women diagnosed with early stage breast cancer. However an oncology sample poses even more complex confounding variables (i.e. cancer medications). The scope of this project was narrowed to incorporate only the post-treatment timeframe for the following reasons.

Firstly, applying this Model post-treatment theoretically avoids the biological influence of cancer influencing biomarkers, unlike other PNI research which incorporates the treatment and post treatment periods (Lekander et al., 1996; Marucha et al., 2005; Thornton et al., 2007). Secondly, the review of Oxidative Model literature identified the need for a confirmatory analysis to test the role of interventions (like vitamins use) on pro-oxidant and pro-inflammatory states during chronic stress. In order to avoid contraindications with treatment regimes, the post active treatment period seemed a favorable time for a future intervention.

Secondly, the post-treatment period avoids the additional influence of cancer treatments on Oxidative biomarkers including surgery, chemotherapy, and radiation. Based on what is known about the regeneration of the Oxidative Model's central biomarkers NT and VIT C (6-8 weeks; Blake-Mortimer et al., 1998) a buffer

incorporating this regeneration period was identified- patients at least 4-weeks after the cessation of active treatment were considered to meet this requirement. Following recommendations from previous research in breast cancer patients (Osborne et al., 2004a) a 4-week post-treatment gap prior to testing immune cell number and function was established in order to avoid 'immunological rebound'.

To summarise, the aim of this dissertation is to firstly test The Oxidative Model framework in a sample of breast cancer patients' post active treatment. Primarily the intent is to investigate the naturalistic relationship of stress and pro-oxidant and pro-inflammatory processes following treatment for cancer. This research will expand knowledge on the applicability of the Oxidative Model in this theoretically depleted population. It will explore the influence of demographic and health behaviors on measures implicit to the Model. The interaction of psychological states and biomarkers in this period will also be explored. Lastly, gaining evidence in order to test beneficial interventions for this population during this period is central to this dissertation.

4.3 Design

At the outset an observational study of women who have completed active treatment for early stage (I – III) breast cancer will be undertaken. Cessation of active treatment for the purpose of this research is defined as the completion of adjuvant chemotherapy and radiation therapy at least 4-weeks prior to first assessment.

An observational design is proposed given the previous literature review. This is for the following reasons. Firstly, this is a novel sample; biomarkers central to The Oxidative Model have not been tested in this population. Secondly, more evidence is

required on the less examined pro-inflammatory pathways in the Model. Thirdly, the research exploring psychological distress during the post-treatment period has observed mixed patterns and rates of distress, anxiety, depression, and trauma. The post-treatment timeframe represents a period where psychological well-being can improve or where additional psychological challenges arise, or both. Lastly there are few studies which explore PNI measures during the 6-months following active treatment.

A longitudinal design will be utilized for this study. This study will incorporate three repeated measures assessments over the 6-month post-treatment period in order to attempt to control inter-individual differences. This has been a key criticism of previous Oxidative Model research. A further criticism of Oxidative Model research to date is the lack of interest in the influence of confounding demographic (i.e. age) and health behaviours (i.e. smoking, exercise, vitamin use) on both psychological and biochemical measures implicated in this Model. Specific attention will be paid to the assessment of influential confounding variables using standardized assessment tools. If any are identified these will be included in statistical analysis.

The research design will involve a preliminary semi-structured clinical interview taking the modules of anxiety, depression, and PTSD from the Mini International Neuropsychiatric Interview (MINI; (D. V. Sheehan et al., 1998)). Following this psychological data, in the form of a battery of questionnaires plus immune, oxidative and inflammatory markers by way of blood samples will be collected. Due to the resources required for data collection (blood collection facilities and a registered nurse) at the outset a single hospital site is proposed for recruitment.

Selection of the battery of scales for this study was based on previous research on The Oxidative Model (Blake-Mortimer et al., 1996, 1998b; Hapuarachchi et al., 2003) and on findings from breast cancer literature review. The following psychological variables will be assessed using standardised measures utilized in previous Oxidative Model as well as post-treatment breast cancer patient samples. Psychological distress (Hapuarachchi et al., 2003; Oliver, 2004; Pfitzer, 2008) will be assessed by the General Health Questionnaire- Short Form (GHQ-12; (Goldberg, 1978a)). This measure will assist identification of sustained stress like that defined by Elliot, et al. (1982). In addition stress will be conceptualized using Spielberger's State-Trait Personality Inventory to assess measures of anxiety, depression, anger, curiosity and anger expression (STAXI-2; (Spielberger, 2003)). These measures has been employed extensively in Oxidative Model research (Arthurson, 2003; Hapuarachchi et al., 2003; Le, 2004; Oliver, 2004; Pfitzer, 2008) as well as breast cancer studies (Costanzo et al., 2007; Deshields et al., 2005; Fallowfield et al., 1990; Fallowfield et al., 1994).

Social support was often assessed in the PNI studies reviewed in this dissertation (Lekander et al., 1996; Marucha et al., 2005). In keeping with The Oxidative Model application, social needs will be assessed using the revised UCLA Loneliness Scale (Russel, Peplau, & Cutrona, 1980; Russel, Peplau, & Ferguson, 1978). The UCLA loneliness scale is a unidimensional emotional response to the unfulfilled wishes of social contact and has been used in two unpublished pieces of Oxidative Model research (Arthurson, 2003; Oliver, 2004).

In addition to measures regularly utilized in The Oxidative Model literature measures specific to the cancer experience will be included. This will add to the

evidence base for The Oxidative Model framework within a cancer sample. The 40-item Mental Adjustment to Cancer (MAC; (Watson et al., 1989)) scale and the Lifestyle Defense Mechanism Inventory (LDMI)(Spielberger & Reheiser, 2002)will be used to assess psychological coping. Both measures have been employed in cancer samples. In addition the LDMI has been explored within The Oxidative Model literature (Oliver, 2004).

The revised Impact of Events Scale (IES-R; (Weiss & Marmar, 1997)) will be utilized. The IES measure has been used to identify significant distress resulting from cancer diagnosis across much of the psycho-oncology literature (Bleiker et al., 2000; Cordova & Andrykowski, 2003; B.L. Green et al., 1996; Wenzel et al., 1999). It was considered that this revised measure will provide a measure of cancer-specific distress. The IES measure has frequently been used in the post-treatment literature reviewed in chapter 3, but is yet to be explored within The Oxidative Model paradigm.

These measures, in addition to the pro-oxidant biomarkers (NT, VIT C, HCY, FOLATE, VIT B12, and CHOL) and the less explored pro-inflammatory measures (CRP, IL-1, IL-2, IL-5, TNF-β, TNF- α, IFN-γ) will be assessed. Repeated collection of both psychological and biochemical data will occur at 8-week intervals to explore change across this period. In sum these data will be collected at 4-weeks, 12-weeks and 20-weeks following active treatment. These data will provide a detailed picture of the post-treatment period with regard to demographic, treatment, and health behaviours, as well as a description of psychological constructs, oxidative, and inflammatory measures based on The Oxidative Model. Study 1 will inform whether there is

potential for an intervention to be employed in this population. An intervention based on The Oxidative Model will be explored further in Chapter 6.

4.4 Research Questions

1. Four-weeks post-treatment for early-stage (I- III) breast cancer-

    a. Are women experiencing psychological distress?

    b. Are women experiencing physiological states indicative of increased oxidative stress, pro-inflammatory processes, and subsequent immune depletion as proposed by The Oxidative Model?

    c. Is increased psychological distress associated with pro-oxidant and pro-inflammatory markers proposed by The Oxidative Model?

2. Between 4-20 weeks post-treatment for early-stage (I- III) breast cancer-

    a. Are there influential variables (e.g. demographic, treatment, health behaviours) which impact on psychological distress, oxidative and inflammatory processes in this period?

    b. Do psychological distress levels change for women over time in the post-active treatment period after controlling for influential demographic, treatment and health variables?

    c. Do physiological measures indicative of increased oxidative stress, pro-inflammatory processes, and subsequent immune depletion change during this period, after controlling for influential demographic, treatment and health variables?

3. Is recruitment of patients in the post-treatment setting, through the participating hospital feasible for future intervention studies?

4.5 Hypotheses

1. Four-weeks post-treatment for early-stage (I- III) breast cancer is their evidence of psychological stress as measured by-
    a. Psychological distress
    b. S/T-anxiety, S/T -depression, S/T -anger and S/T –curiosity
    c. Loneliness
    d. Psychological responses- Fighting Spirit, Helpless/Hopeless, Avoidant, Anxious Preoccupation and Fatalistic, Rationality/Emotional Defensiveness and Need for Harmony
    e. Post-traumatic stress symptoms
2. Four-weeks post-treatment for early-stage (I- III) breast cancer is there evidence of pro-oxidant stress as assessed by-
        i. Lower NT
        ii. Low VIT C
        iii. High HCY
        iv. Low FOLATE & VIT B12
        v. High CHOL

b. Four-weeks post-treatment for early-stage (I- III) breast cancer is there evidence of increased pro-inflammatory processes as measured by-

   i. High CRP

   ii. Increased pro-inflammatory cytokines (IL-1, IL-2, IL-5, TNF-β, TNF-α, IFN-γ)

3. Four-weeks post-treatment for early-stage (I- III) breast cancer are pro-oxidant measures associated with increased distress, depression, anxiety, anger, loneliness, negative coping responses and cancer-specific stress?

   a. Decreased NT, VIT C, FOLATE & VIT B12 will be associated with increased distress, anxiety, depression, anger, loneliness, decreased curiosity, negative coping responses and cancer-specific stress.

   b. Increased HCY will be associated with increased distress, anxiety, depression, anger, loneliness, negative coping responses and cancer specific stress.

4. Four-weeks post-treatment for early-stage (I- III) breast cancer are pro-inflammatory measures associated with higher levels of psychological distress and dysfunctional emotion states-

   a. Increased CRP will be associated with increased distress, anxiety, depression, anger, loneliness, negative psychological responses, and trauma

b. Increased inflammatory cytokines will be associated with increased distress, anxiety, depression, anger, loneliness, negative coping responses and post-traumatic stress

c. Decreased anti-inflammatory cytokines will be associated with increased distress, anxiety, depression, anger, loneliness, worsening psychological responses, and trauma

5. Longitudinally, what is the psychological pattern across the 5-month post-treatment period for early-stage (I- III) breast cancer patients? The psychological experience will be assessed three times measuring-

   a. Psychological distress

   b. S/T-anxiety, S/T -depression, S/T -anger and S/T –curiosity

   c. Loneliness

   d. Psychological responses- Fighting Spirit, Helpless/Hopeless, Avoidant, Anxious Preoccupation and Fatalistic, Rationality/Emotional Defensiveness and Need for Harmony

   e. Cancer-specific stress

6. Longitudinally, across a 5-month post-treatment period for early-stage (I- III) breast cancer patients, do pro-oxidant states improve as assessed by-

   a. Increased NT

   b. Increased VIT C

c. Decreased HCY

d. Increased FOLATE & VIT B12

e. Decreased CHOL

7. Longitudinally, across a 5-month post-treatment period for early-stage (I- III) breast cancer patients, do pro-inflammatory processes improve as assessed by-

a. Decreased CRP

b. Decreased inflammatory cytokines (IL-1, IL-2, IL-5, TNF-β, TNF- α, IFN-γ)

c. Increased anti-inflammatory cytokine (IL-10)

Chapter 5

The Psychoneuroimmunology of Breast Cancer Patients Post-treatment

An Observational Study

5.0 Overview

Based on review of the literature and the refining of principal research aims outlined in the previous chapters, this empirical study will focus on the chronic stress scenario of breast cancer patients during the post-adjuvant treatment period. This is a period which has been explored only in a handful of studies (Lekander et al., 1996; Marucha et al., 2005; Osborne et al., 2004a; Thornton et al., 2007; Thornton, Anderson, Schuler, & Carson, 2009). However these studies have highlighted the potential for increased stress, immune dysfunction, and increased inflammation during this period.

A longitudinal, observational design over a 16-week post-treatment period to assess patients' psychological, pro-oxidant, pro-inflammatory, and immunological function was proposed. A theoretical framework- The Oxidative Model-was employed, which assesses a specific set of measures previously shown to link psychological stress pro-oxidant, pro-inflammatory, and immunological function (Blake-Mortimer, et al. 1996, 1998; Happuarachchi, et al. 2003). In addition, due to gaps identified in the literature reviews, confounding factors like demographic and health behaviour variables were investigated. Little is known about The Oxidative Model markers in the post-adjuvant treatment period following a cancer diagnosis. Findings from this research will inform the need for future examination of potentially beneficial interventions based on The Oxidative Model.

5.1 Method

5.1.1 Site.

This study was conducted at the Royal Adelaide Hospital (RAH) Cancer Centre, Adelaide, South Australia from 2006 through to 2008. Due to the focus on the post-treatment period, the Breast Care Nurse at the Women's Health Service informed all eligible women near the conclusion of their adjuvant treatment (chemotherapy and/or radiation therapy) about this study. Once interest in project participation was confirmed, the researcher contacted each patient by telephone. This study was approved by the RAH Research Ethics Committee.

5.1.2 Inclusion criteria.

Women aged between 18 and 65 years, treated for early stage breast cancer (stages I to III), were eligible for this study, and was limited to adults not children. To be included in this sample it was required that patients had completed adjuvant treatment for a primary breast cancer diagnosis, such as chemotherapy and/or radiation therapy, at least 4-weeks prior to baseline assessment.

Women who had completed adjuvant chemotherapy but were scheduled to have 3-weekly sessions of Trastuzumab were eligible for this study. Trastuzumab is a monoclonal antibody, commonly known as Herceptin, which is given for a year intravenously every 3-weeks. It does not have same type of toxicities as conventional chemotherapy and is usually better tolerated so can be taken over the long term without it being as stressful an experience as taking chemotherapy. About 25% breast cancer patients have the target for this drug and are eligible to receive it

These women were 'post-treatment' for chemotherapy and/or radiation therapy but continued to come to the hospital every 3-weeks for Trastuzumab for a 12-month period. Due to the exploratory nature of this study, it was considered that any arising influences for those women undertaking a regime of Trastuzumab could be statistically controlled for in later analyses.

Patients were required to be able to speak and read English fluently as patients were required to give written informed consent to procedures which involved serial testing including blood as well as complete self-report psychological questionnaires.

5.1.3 Exclusion criteria.

Apart from requiring the specific diagnosis and subsequent treatment for early stage breast cancer, it was required that patients could only enter the study if they were free from infections. Further to this, people suffering from chronic conditions (i.e., severe heart disease or diabetes), autoimmune and inflammatory diseases (e.g., rheumatoid arthritis, Addison's disease, Cushing's disease, Lupus Erythematosus), or taking immunosuppressive medication (e.g., Cortisone) were excluded from participation. People taking blood thinning agents such as Warfarin were excluded to avoid adverse consequences of blood taking.

To further ensure that women taking part in this study did not suffer from other health conditions, a full blood examination (FBE) was conducted at each assessment point. It was planned that any participants with abnormal blood results would be excluded and referred to their medical clinician; this was not necessary for any women taking part in this study. In addition to physical health assessments,

participants were screened for the presence of a current psychological diagnosis of severe depression, anxiety, and/or PTSD. This identification procedure was in place to allow participants to be referred to hospital counseling services if required, although they remained eligible for this study. The identification procedure for psychotic symptoms involved participants undertaking a structured interview, specifically the MINI International Neuropsychiatric Interview (MINI)(D. V. Sheehan et al., 1998).

5.1.4 Withdrawal criteria.

Participants had the right to refuse to continue with the study at any time. Furthermore if distress scores warranted further psychological intervention the researcher was able to refer participants to the RAH Cancer Centre psychologist, although they remained eligible for this study.

5.1.5 Design.

This study was an observational, longitudinal within-subjects design, utilising a consecutive sample of breast cancer patients. Participants were recruited consecutively into the study using only the above inclusion and exclusion criteria in order to ensure, as far as possible, patients reflected the population from which they were drawn. In total, there were three repeated assessments, over the first 6-months post-treatment. The first of these assessments commenced 4-weeks post-adjuvant treatment (baseline), the second assessment occurred 8-weeks later or 12-weeks post-treatment (Time 2), and the last assessment occurred another 8-weeks later or 20-weeks post-treatment (Time 3). See Table 3 for average times for assessment.

Table 3: Average Number of Days Post-Treatment for Assessment Points for Participants

Assessment Time Point		Days Post-treatment M (SD)
Baseline	4-weeks	34.57 (9.15)
Time 1	12-weeks	62.33 (7.20)
Time 2	20-weeks	120.43 (13.58)

5.1.6 Flow of patients.

Flow of participants through the study is presented in Figure 4. Pre-baseline data were collected for 16 patients with one participant unwilling to complete demographic and trait assessment. This patient was un-contactable on several occasions and subsequently counted as a withdrawal from the study.

Baseline biochemical and psychological state measures were collected for 16 participants. All 16 participants' blood samples were successfully assayed. In addition all psychological scales were complete in full at this time.

At Time 2, 15 participants completed the biochemical and psychological measures as one participant, previously mentioned, was not contactable. Two participants' NT and VIT C assays were unable to be successfully performed due to blood collection difficulties. Assays on the remaining 13 blood samples were successfully completed. All 15 participants completed psychological state measures

At Time 3, 15 participants completed the psychological measures. However only 14 provided data for the biochemical assays, due to one participant being unable

to make it in to the hospital to provide a blood sample. All 14 assays were successfully performed.

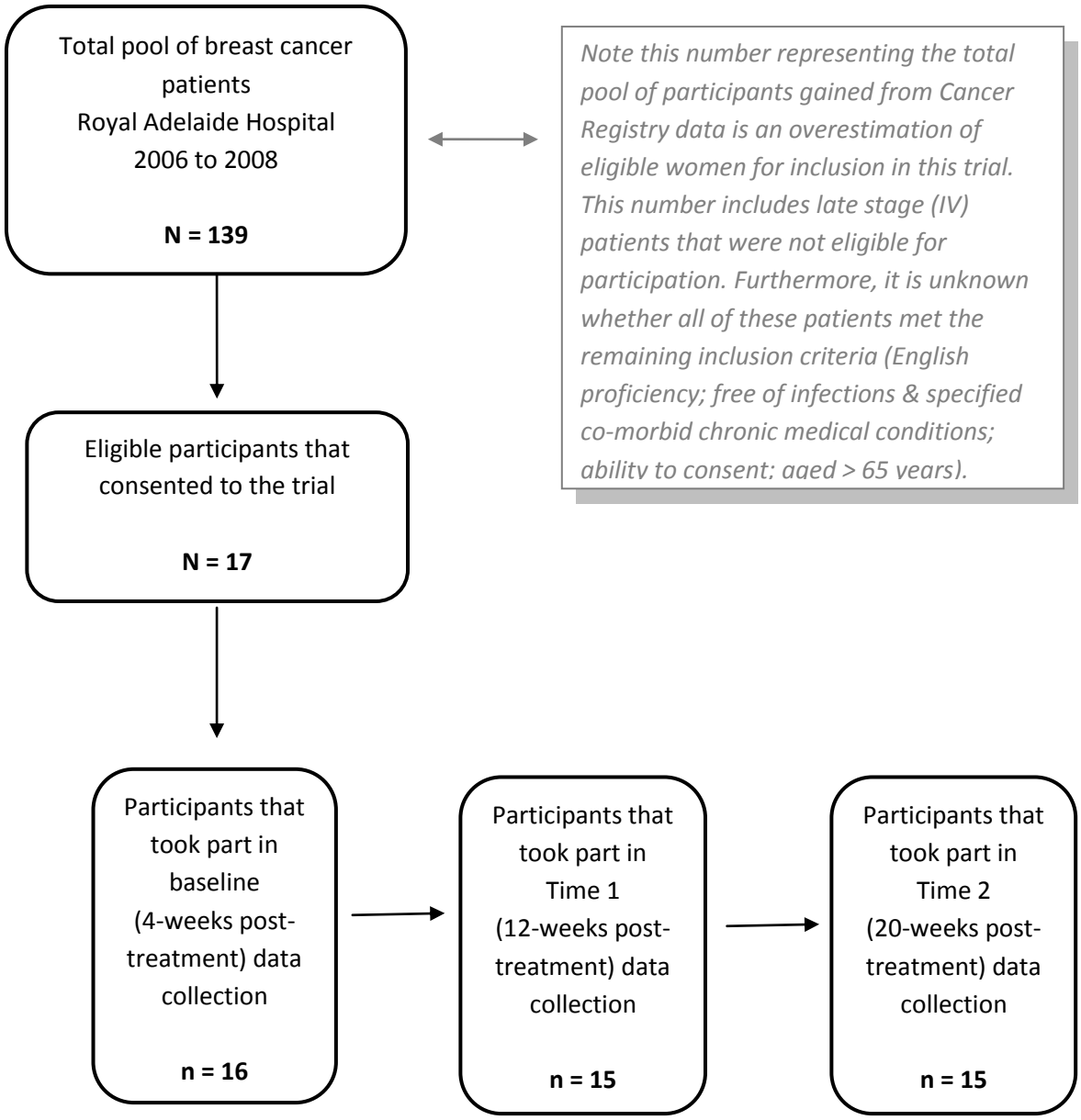


Figure 4: Flow of patient consent and participation throughout the observational study

5.1.7 Data collection procedure.

On initial contact, participants were informed of the objectives of the study and of possible adverse effects that might occur because of participation in the study (i.e., a slight risk of bruising associated with blood taking), in the form of a Patient Information Sheet (Appendix A). Often patients were contacted prior to their last scheduled adjuvant therapy so thereby were given sufficient time (4-weeks) to consider the information, ask questions, and seek advice prior to being asked whether they wished to participate in the study. Participants were asked to complete a consent form once they had decided to participate.

Contact by the researcher (following initial contact by the Breast Care Nurse) was initially by telephone with additional information mailed to the participant. The researcher obtaining consent made a conscientious effort to be fully satisfied that the participant had truly understood the nature of participation in the study to which consent was given. Apart from the researcher and Breast Care Nurse at the Women's Health Service, the identity of participants was concealed. Preservation of the confidentiality of patients taking part in this study was maintained at all times.

5.1.8 Pre-baseline assessments.

Following recruitment and consent, participants were given a self-report battery of questionnaires (Appendix), comprised of two parts. The first part included demographic and health behavior information. The second part incorporated assessment of psychological trait characteristics.

5.1.8.1 Demographic and treatment assessment.

Part one of the pre-baseline questionnaire targeted specific information on patients' disease and treatment, including date of diagnosis, chemotherapy regimen, stage of cancer, and concomitant medication. For accuracy it was further cross referenced with information from the Breast Care Nurse at the Women's Health Service, RAH. It also included demographics such as age, marital status, employment, etc. This battery assessed participants' health behaviours specifically: tobacco use, alcohol use, physical activity levels, dietary requirements and vitamin use.

For collection of data on alcohol use, researchers adapted questions from the WHO Alcohol Use Disorders Identification Test (AUDIT (Babor, Higgins-Biddle, Saunders, & Monteiro, 2001).) For physical activity measurement the International Physical Activities Questionnaire (IPAQ short form: International Physical Activities Questionnaire Committee, 2004) was employed. Both the IPAQ and AUDIT are outlined in detail below. Smoking behavior was assessed from an adapted version of another measure (West, 2004).

5.1.8.1.1 International physical activities questionnaire- short form.

The IPAQ short form, short-last-7-days-self-administered format is for use with young and middle-aged adults (15-69 years). IPAQ assesses physical activity undertaken across a number of domains. These include leisure time, domestic and gardening activities, work-related, and transport related activity. Additionally the IPAQ short form asks about three specific types of activity, including walking, moderate-intensity activities, and vigorous intensity activities. These are assessed on their

frequency (days per week) and overall duration (time per day). Both categorical and continuous indicators of physical activity are possible from the IPAQ short form. This measure was included in this study as a means to control for possible physiological differences arising from different health behaviours engaged in by participants. Regular physical activity has been linked to enhanced blood resistance to oxidative stress (Lesgards et al., 2002).

Three levels of physical activity are suggested for categorizing populations. These proposed levels take into account the total physical activity of all domains of this scale. These categories include 'health enhancing physical activity' (HEPA); a high activity category. This identifies people who exceed the minimum public health physical activity recommendations, and are accumulating enough for a healthy lifestyle. This cut-off point equates to at least one-and-a-half to two hours of total activity per day. Higher levels of participation can provide greater health benefits, although there is no consensus on the exact amount of activity for maximal benefit.

Subsequent to this category there is the 'minimally active group'. The minimum pattern of activity to be classified as 'sufficiently active' is any one of the following three criteria, (a) three or more days of vigorous activity of at least 20 minutes per day, (b) five or more days of moderate-intensity activity or walking of at least 30 minutes per day, or (c) five or more days of any combination of walking, moderate-intensity, or vigorous intensity activities. 'Inactive' is the lowest level of physical activity, with those who don't meet criteria for the first two categories considered insufficiently active according to this scale.

5.1.8.1.2 The alcohol use disorders identification test.

The AUDIT was developed as a tool to screen excessive drinking behaviour and to assist practitioners in identifying people who would benefit from reducing or ceasing drinking. There are many forms of excessive drinking that cause substantial risk to an individual. Alcohol can deplete the body of nutrients that are important during periods of stress such as FOLATE and VIT B12 (Laufer et al., 2004), for this reason it was included in this study. The AUDIT was developed as an international measure; it has good reliability as an epidemiological tool across populations and can also be used to compare samples. For many patients it is unnecessary to administer the complete AUDIT, (incorporating three sections: hazardous, harmful use, and dependence) because they drink infrequently, moderately, or abstain entirely from alcohol. For this sample only the hazardous drinking domain was applied. This is a pattern of alcohol consumption that increases the risk of harmful consequences for the user or others. Despite the absence of any current disorder in the individual user, hazardous drinking patterns are of public health significance. The domains covered for this pattern of alcohol use include 'frequency of drinking', 'typical quantity', and 'frequency of heavy drinking'.

5.1.8.2 Psychological assessment.

The second part of the pre-baseline battery of questionnaires incorporated several standardised psychological measures of trait characteristics including Spielberger's Trait Personality Inventory (STPI; Spielberger, 1996), the Lifestyle Defense Mechanism Scale (LDMS; Spielberger & Reheiser, 2002), and the revised T-anger Expression Inventory (STAXI-2; Spielberger, 2003). These measures assess dispositional characteristics which are considered to be stable across time.

5.1.8.2.1 State-trait anger expression inventory.

The State-Trait Anger Expression Inventory (STAXI-2; Spielberger, 1999) assesses the intensity of state and T-anger and additionally two anger expression and two anger control constructs. In this dissertation anger expression and control were assessed using this scale, as state and T-anger were measured by the aforementioned STPI. The anger expression construct includes two subscales: (1) 'anger-in' which relates to the suppression of anger and the tendency to direct anger towards oneself, and (2)'anger-out', which assesses the tendency to direct anger outward towards the environment. Additionally there is the anger control scale construct including (1) a 'control-out' subscale assessing the frequency of efforts to suppress any outward expression of anger, and (2) 'control-in' which measures the frequency of efforts to control the suppression of angry feelings. The 32 items are rated on a 4-point rating scale including (1) 'almost never', (2) 'sometimes', (3) 'often', to (4) 'almost always'. Reliability coefficients have been shown to range from .73 to .93 for the subscales (Spielberger, 1999).

As with other trait measures, anger expression assessments were only assessed at pre-baseline, as these are considered stable across time.

5.1.8.2.2 Lifestyle defense mechanism inventory.

The Lifestyle defense mechanism inventory (LDMI)(Spielberger & Reheiser, 2002) assesses two constructs, firstly individual differences in the frequency that a person engages in rational, non-emotional thought processes and behaviours, and secondly in the frequency of efforts to maintain harmonious interpersonal

relationships with family and friends. Respondents are asked to report how they generally react by rating themselves on 4-point frequency scales including (1) 'almost never', (2) 'sometimes', (3) 'often', to (4) 'almost always'.

The LDMI is comprised of two 12-item subscales: Rationality/Emotional Defensiveness (R/ED) and Need for Harmony (N/H). The subscales were derived from prospective epidemiological studies (Grossarth-Maticek, 1980) of heart disease and cancer. Higher scores on a scale of Rationality/Emotional Defensiveness have been found to be associated with a greater risk of the development of heart disease or cancer; similarly higher scores on the Need for Harmony subscale were found to be associated with a greater risk for the development of cancer. More recently the LDMI has also been applied to breast cancer samples (Fernandez-Ballesteros, Ruiz, & Grade, 1998); similar negative patterns of emotional suppression, inhibition, and denial were identified. This research identified breast cancer patients to have a higher tendency to sacrifice their own needs in an attempt to maintain harmonious interpersonal relationships. The LDMI constructs are considered to be stable traits, thus they were assessed only at the pre-baseline stage of this research.

5.1.8.2.3 State-trait personality inventory.

The State-Trait Personality Inventory (STPI; Spielberger, 1996) includes scales from both Spielberger's State-Trait Anxiety Inventory (STAI; Spielberger, 1983), and State-Trait Anger Scale (STAS; Spielberger, 1983). In addition two scales addressing state and T-depression and curiosity were included. Thus the six subscales include: state and T-depression, anger, anxiety and curiosity. Each subscale comprises 10 items,

including 8 items with reversed scores. Each subscale measures the intensity of emotion experienced, with scores ranging from 10-40.

In the assessment of state measures, participants are asked to indicate on a 4-point rating scale how they feel at that particular moment. Ratings include (1) 'not at all', (2) 'somewhat', (3) 'moderately', and (4) 'almost always'. Trait measures are assessed by asking respondents to rate how they generally feel. Reliability coefficients assessing consistency have been recorded at .92 for anxiety, .93 for anger, .95 for curiosity, and .87-.93 for the depression subscale (Spielberger, 1996).

To reiterate, for the purpose of this research trait characteristics were assessed only at pre-baseline assessment as these are considered stable traits over time. However the scales comprising state characteristics were measured at each of the three data collection point across the study (baseline, Time 1, and Time 2). Further measures used only across the three assessments are discussed below.

5.1.9 Repeated assessments (Baseline, Time 1, and Time 2).

Once informed consent and the aforementioned demographics and trait data were obtained by mail out, participants were scheduled to attend the Women's Health Service at the RAH. At the initial visit a brief structured interview was undertaken in order to screen patients for clinical anxiety, depression, and PTSD.

This verbal screening was added to ensure that patients' participation in this research project was suitable. The relevant sections of the Mini International Neuropsychiatric Interview (MINI; Sheehan, 1998) were utilized (D. V. Sheehan et al., 1998). The MINI is a short, structured diagnostic interview that was developed for

Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) and the International Diagnosis of Disease (ICD-10) for psychiatric disorders. With an administration time of approximately 15 minutes, it is the structured psychiatric interview of choice for psychiatric evaluation and outcome tracking across clinical psychopharmacology trials and epidemiological studies.

The MINI has been validated against the much longer Structured Clinical Interview for DSM diagnoses (SCID-P) in English and French and against the Composite International Diagnostic Interview for ICD-10 (CIDI) in English, French, and Arabic. It has also been validated against expert opinion in a large sample in four European countries (France, United Kingdom, Italy, and Spain)(Lecrubier et al., 1997; D. Sheehan et al., 1997). If during the screening process the researcher became aware of increased emotional distress or had concerns about a patient's mental health, they were subsequently referred to the RAH Cancer Centre psychologist. Subsequent appointments at the Women's Health Service were arranged with the participants to occur at intervals of approximately 12-weeks (Time 1 assessment) and 20-weeks (Time 2 assessment) post-adjuvant treatment.

At each of the three visits, current psychological well-being was assessed using several standardized self-report measures detailed below; these included the 12-item General Health Questionnaire (GHQ-12: Goldberg, 1978), state measures from the State-Trait Personality Inventory (STPI: (Spielberger, 1996))as detailed in the previous section above, the Impact of Events Scale- revised version (IES-R:(Horowitz et al., 1979)), the Mental Adjustment to Cancer scale (MAC:(Watson et al., 1989)), and the UCLA Loneliness Scale (UCLA:(Russel et al., 1980)).

5.1.9.1.1 General health questionnaire-short form.

The GHQ has been used in numerous studies of breast cancer patients (Dean & Surtees, 1989). The GHQ-12 (GHQ-12, Goldberg, 1992) is a shortened version of the well validated GHQ-60 (Goldberg, 1978b). It is a self-report instrument developed to detect non-psychotic psychiatric disorders in a general community setting without making any judgment as to the causal relationship. For the purpose of the current study the GHQ-12 was assessed at three time points (baseline, Time 2, and Time 3). Internal consistency (Cronbach's α) for the GHQ-12 has been published to be between .82 and .90 {Goldberg, 1988 #221).

It is possible to score the GHQ-12 in two ways; a binary coding system (categorical) or a Likert-scale rating (continuous), ranging from 0-3, i.e., (0) 'not at all/better than usual', (1) 'no more than usual', (2) 'rather more than usual', to (3) 'much more than usual'. This continuous form of scoring allows a comparison of the degree of stress experienced, as the distribution of scores is expected to be less skewed than the aforementioned categorical scoring method (Johnston, Wright, & Weinmann, 1995). In the current cancer sample both Likert-scale rating were reported. In addition clinical cut-off scores were considered. These use the binary scoring system. This gives rise to scores indicating the extent/ presence of symptoms of distress.

The GHQ-12 has been recommended as a reliable, sensitive, and appropriate self-report questionnaire to use with cancer samples as it is able to detect those at risk of psychological distress. Its employment in this population is for three main reasons. Firstly the GHQ-12 avoids asking questions with somatic items which could be

attributable to disease or treatment; secondly this measure asks patients to evaluate their present psychological state relative to their normal functioning which allows for their responses to be in context to their general functioning; and thirdly the GHQ-12 is simple to administer and score and as a result is not to taxing on patients (Hall, A'Hern, & Fallowfield, 1999).

5.1.9.1.2 The Revised UCLA loneliness scale.

The revised UCLA Loneliness Scale (Version 3: Russel, Peplau, and Cutrona, 1980) measures loneliness as a unidimensional emotional response to unfulfilled wishes of social contact. It comprises twenty items assessed on a 4-point rating scale including (1) 'never', (2) 'rarely', (3) 'sometimes', to (4) 'always'. Whether the UCLA scale is a state or trait measure is unclear as it does not specify a time frame for respondents. However 2-month and 7-month test re-test correlations (.73 and .62 respectively) suggest a substantial trait element (Shaver & Brennan, 1991). Despite this probability, it was decided for the purpose of the current study to assess loneliness at all three time points alongside biomarker measurement (baseline, Time1, and Time 2).

5.1.9.1.3 Impact of events scale-revised version.

The original Impact of Events Scale (IES; Horowitz, et al. 1979) is one of the most widely used trauma self-report measures. It provides an overall score as well as subscales for the experience of intrusive thoughts (e.g., 'pictures about it popped into my mind') and avoidance (e.g. 'I tried not to talk about it'). The revised version of this scale (IES-R) comprises 22 items. The scale was revised in 1997. At this time revisions to the original scale were made based on DSM-IV criteria for PTSD by the addition of a

hyper-arousal subscale (e.g., 'I felt watchful and on guard') and one additional intrusive thoughts item. These modifications brought its content and format closer to current PTSD diagnostic criteria. However it is still considered a non-DSM-correspondent measure (Gurevich, Devins, & Rodin, 2002) meaning it cannot be used to confirm a clinical diagnosis of PTSD. The presence of symptoms is rated from (0) 'not at all' to (5) 'extremely'.

The authors recommend using mean scores instead of raw sums for each of the subscales, and total score. Only the total score for the IES-R was used in this study. Finally it is important to note that participants were asked to specifically consider their experience with breast cancer when responding to the IES-R as has been done in other research (Cordova, Andrykowski, Kenady, McGrath, Sloan, & Redd, 1995). The authors (Weiss & Marmar, 1997) report a high internal consistency for the three subscales with α coefficient between .84 and .92. The IES-R is targeted towards the assessment of symptom change in a defined sample.

5.1.9.1.4 Mental adjustment to cancer scale

The Mental Adjustment to Cancer scale (MAC; Watson, et al. 1988) is a 40-item measure developed to assess cognitive and behavioural response to the diagnosis of cancer. It was developed in response to the high level of psychological morbidity associated with cancer and additionally the possible impact that cancer coping styles may have on patients' subsequent length of survival. The scale comprises five subscales: the Fighting Spirit (FS) subscale includes items like ' I firmly believe I will get better' it is comprised of sixteen items; the Helpless/Hopeless (HH) subscale includes items like, 'I feel that there is nothing I can do to help myself' comprised of 6 items; the

Anxious Preoccupation (AP) subscale, i.e., 'I worry about the cancer returning or getting worse' is comprised of 9 items; the Fatalistic (F) scale, i.e., 'I've left it all to my doctors' is comprised of 8 items; and lastly the Avoidance (A) scale contains 1 item, 'I don't really believe I had cancer'.

This scale is self-administered; responses are scored on a 4-point scale including (1) 'definitely does not apply to me', (2) 'does not apply to me', (3) 'applies to me', to (4) 'definitely applies to me'. Internal consistency has been assessed to range from .65 to .84 {Watson, et al. 1989), satisfactory but lower scores are associated with subscales with less items (Anxious Preoccupation, and Fatalism). Reliability cannot be assessed for the Avoidance subscale as it is only comprised of a single item. In addition similar reliability coefficients were published from more recent research in a breast cancer population. The scale had significant associations {Osborne, Elsworth, Kissane, Burke, & Hopper, 1999) with anxiety and depression subscales of the Hospital Anxiety and Depression Scale (HADS), indicating concurrent validity.

5.1.9.1.5 Reliability.

Table 4 shows each scale and its corresponding reliability relative to the sample in this study. The measures employed are well-validated scales and for this sample the internal reliability of these scales was considered satisfactory.

Table 4: Psychological Measures Assessed and their Reliability Coefficients for the Current Study

Instrument	Measures	Scales	Reliability
General Health Questionnaire(GHQ-12)	Psychological distress	GHQ*	.83
State Trait Personality Inventory (STPI)	Personality style	S- Anxiety*	.88
		S- Curiosity*	.91
		S- Anger*	.90
		S- Depression*	.87
		T-Anxiety	.72
		T-Curiosity	.85
		T-Anger	.63
		T-Depression	.86
		Anger Expression Out	.72
		Anger Control In	.80
		Anger Expression In	.64
		Anger Index	.75
Lifestyle Defense Mechanisms Scale (LDMS)	Control and suppression of emotions	Rationality & Emotional Defensiveness	.62
		Need for Harmony	.78
UCLA Loneliness	Experience of social isolation	Loneliness*	.92

Note. Reliability reported is based on Cronbach's alpha for the current sample (N = 17); *indicates multiple assessments of this measure

Table 4 cont'd: Psychological Measures Assessed and their Reliability Coefficients for the Current Study

Instrument	Measures	Scales	Reliability
Impact of Events Scale – Revised (IES-R)	A scale of current subjective distress, related to a specific event. Assesses post traumatic stress-type symptoms	Total Impact of Events	.93
		Intrusion subscale	.86
		Avoidance subscale	.87
		Hyperarousal subscale	.78
Mental Adjustment to Cancer Scale (MAC)	A measure of cognitive and behavioural responses to the diagnosis of cancer	Anxious Preoccupation	.72
		Helpless/Hopeless	.69
		Fighting Spirit	.73
		Fatalistic	.62
		Avoidance	n/a

Note. Reliability reported is based on Cronbach's alpha for the current sample (N = 17); *indicates multiple assessments of this measure

5.1.9.2 Biochemical assessments.

Biochemical parameters outlined in Table 5 were collected for the current study and are based on The Oxidative Model described in Chapter 2. Biochemical parameters were assessed from blood samples attained from participants. The following materials (Table 5) were required (per participant) for collection of biochemical markers for this study.

Table 5: Materials Required for Blood Collection for Each Participant at Each Assessment

Vacuette for blood collection	Measures	Abbreviation
1 x 4ml K3E EDTA vacuette	Homocysteine	HCY
1 x 4ml K3E EDTA vacuette	Full blood examination	FBE
1 x 9ml Lithium Heparin vacuette	C-reactive protein	CRP
1 x 8ml serum Sep. Clot Activator vacuette	Vitamin B12	VIT B
	Folate	FOLATE
1 x 9ml Lithium Heparin vacuette	5-‘ectonucleotidase	NT
	Tissue ascorbate	VIT C
	Cholesterol	CHOL
1 x 8ml serum Sep. Clot Activator vacuette	Interleukin 1 beta	IL-1β
	Interleukin- 5	IL-5
	Interleukin- 6	IL-6
	Interferon- gamma	IFN-γ
	Tumor necrosis factor- alpha	TNF-α
	Tumour necrosis factor- beta	TNF-β
	Interleukin- 10	IL-10

5.1.9.2.1 Blood collection procedure.

At each assessment (baseline, Time 1, and Time 2) blood samples (approximately 40mls) were drawn from each participant by a Registered Nurse (RN) via peripheral venipuncture using the evacuated system and vacuette tubes. Each blood collection tube was coded for confidentiality and identification purposes. The initial assessment procedure took approximately 1 hour, and subsequent assessments 30 minutes. For each participant the whole process took 2 hours in total over the entire study.

One EDTA vacuette for HCY assay were immediately placed at 4 degrees Celsius, on ice, following collection. EDTA describes what the interior of these vacuettes is coated with and this substance binds calcium ions thereby blocking the coagulation cascade. They were centrifuged within 1.5 – 2 hours of collection. Along with HCY, blood samples for Full Blood Examination (FBE), NT, and VIT C were also centrifuged. Serum samples to be used for Interleukin analysis were stored at –20 degrees Celsius. FBE and HCY were analysed by IMVS (formerly Southpath Pathology) at Flinders Medical Centre (FMC). NT and VIT C were analysed by Dr Chalmers (at FMC) using the procedure described in Chalmers and Hare (1990). Interleukins were analysed by Professor Ferrante's laboratory at the Women's and Children's Hospital (WCH). The detection limit of the interleukin assay technique (ELISA) is 16.5pg/ml. All assays were performed using routine clinical diagnostic methodologies. Coding of samples allowed for the samples to be double blinded; biochemists were blind to (unaware of) the nature of the participant and had no access to psychological scores.

5.1.9.2.2 Biochemical assay techniques.

FBE, HCY, FOLATE, VIT B12, CHOL, and sensitive CRP were analysed by Southpath Pathology at FMC. NT and VIT C were analysed by Dr Chalmers (at FMC) using the procedure described in Chalmers and Hare (1990). All assays were performed using routine clinical diagnostic methodologies.

Measurement of serum cytokines (IL-1 β, IL-5, IFN-γ, TNF-α, TNF-β, and IL-10) was done by the Immunology Department at the WCH. These cytokines in serum samples were measured by fluorescent cytokine capturing beads with the assistance of the Becton Dickinson (BD) Bead Array (CBA) Flex Set System (BD, California). As dilution of serum samples is required for the BD array system this equates to 40pg/ml. Results below this limit may not be accurate. The detectable limits 10pg/ml- curve flat below this.

5.1.10 Statistical analysis.

Data were analysed using SPSS version 17. Data were initially screened for missing data, outliers, normality, heterogeneity, and skewness before descriptive statistics were presented for demographic, psychological, and immunological baseline assessments. Further screening was done in order to meet assumptions for within-subject repeated measures analyses of variance (ANOVA) techniques.

Hypotheses 1a, 1b, and 1c (section 4.5) were explored using Descriptive Statistics for pre-baseline and baseline data. For hypotheses 1d and 1e correlational analyses of baseline associations between demographic, psychological, and immunological variables were explored. Due to the sample size correlations of .4 or

higher, indicative of moderate- to- large effect sizes were focused on. For the sample (N = 17), *r* of .40 with an alpha level of .05, would achieve the power to detect moderate to large effects on 20% of the time {Rosenthal, 1991 #458}. These analyses attempted to answer the directional hypotheses proposed by The Oxidative Model literature to date.

Hypotheses 2a, 2b, and 2c were explored with a series of within-subject repeated measure ANOVAs to assess any changes over time across the three assessment periods (baseline, Time 1, and Time 2). Prior to the ANOVA analyses, preliminary correlation matrices of psychological, demographic, and health behaviour variables at baseline were performed in order to elucidate any covariates which needed to be accounted for. If covariates were identified within-subjects repeated measure analysis of covariance (ANCOVA) techniques were utilized.

5.1.10.1 Sample size.

It was proposed that a sample size of 30 would have adequate power to detect moderate changes over three time points, within subjects (80 % power, two-sided tests, $\alpha = .05$; Stevens, 2002). Initially it was anticipated that 30 patients would also be attainable over a 12-month period given current cases (990) of breast cancer in South Australian women identified between 1998 and 2002 (AIHW, 2006)

However due to extremely slow accrual, recruitment was extended over a two-year period. Based on Cancer Registry data obtained from the RAH Cancer Centre, during the 2-year study period from 2006 to 2008, the estimated number of women meeting eligibility criteria to take part in this study was 139 (see Figure 3). Note, this

number is a conservative estimate of eligible patients for this study, as all stages of breast cancer are included in this number (stages I to IV). Data collection for this study took place from 2006 through to 2008 the accrual of the current sample (N = 17), although small was considered to represent a sizeable proportion (> 12%) of patients in light of other eligibility criteria (i.e., English fluency, free of infections, < 65 years of age, voluntary participation, etc).

Given the sample accrued for the current study (N = 17), analyses of effect sizes were conducted to determine the magnitude of associations rather than simply relying on null hypothesis significance testing, and exact p values are always shown (J. Cohen, 1988). Bonferroni adjustments were not employed for this study as the threat of Type II error was the main concern. Adding these adjustments would have further increased the Type II error (Perneger, 1998).

Although effect sizes are not reliable without improved power, they allow some insight into the magnitude of change rather than simply relying on null hypothesis significance testing and making Type II errors (Perneger, 1998; Field, 2009). Partial eta squared scores (partial η^2) of .01, .06, and .14 represent small, moderate, and large effect sizes, respectively (J. Cohen, 1988). Due to previously mentioned recruitment difficulties (slow patient accrual), and project time constrains only 17 participants were attained. With this sample size only large effects will be seen as significant.

5.2 Results

5.2.1 Data Screening.

5.2.1.1 Normality.

Normality was assessed via histograms, Q-Q plots, and measures of skewness (Pallant, 2007). Several variables showed variations in normality. These were investigated further. The psychological variables of S-anger, anger expression in, and the IES-R scale were all negatively skewed. There is a great deal of discrepancy between studies on the experience of trauma in breast cancer populations with some indicating high incidence (Andrykowski, Cordova, McGrath, Sloan, & Kenady, 2000)and others very low (Mundy et al., 2000). For this study few individuals exhibited high scores on these variables, therefore skewing score distributions. More specifically, the IES-R scale histogram indicated a bimodal distribution, rather than a continuum of high versus low trauma scores. Normality was also explored further for biochemical variables. VIT C, HCY, and CRP were negatively skewed. Cytokines were also explored. Although histograms indicated a bimodal distribution, this was expected based on the pattern of inflammatory response.

5.2.1.2 Outliers.

Variables were further checked by conversion to standardized scores (z-scores) to determine any scores above 3.29. These would be considered outliers (Tabachnick & Fidell, 2007). Only HYC and CRP had an influential outlier. These outlier scores on HCY and CRP scores were represented by one single case, so her scores on these two

biomarkers were excluded from further statistical analyses. The remaining scores were within normal distributions and therefore included to improve power.

Despite skewness, no transformations were deemed necessary for the remaining variables. This was for a number of reasons. Firstly, as repeated measures ANCOVAs were employed, any transformation would have had to be across all time points to achieve repeated score comparisons, possibly forcing transformations of 'normally' distributed variables. Secondly, in order to keep results interpretable data transformations are not universally recommended, as interpretation of analyses using transformed variables can be more difficult (Tabachnick & Fidell, 2007). This is especially important when comparing scores to normative means or ranges of a variable.

Finally, although it is argued that analyses can be misleading if based on non-normal distributions, it is really only of particular significance if they are non normal in very different ways (i.e., skewed in different directions)(Tabachnick & Fidell, 2007). As this was not the case with this data set transformations were not undertaken. Although ANCOVAs are considered reasonably robust when dealing with non normal distributions, the inclusion of non normally distributed variables should be noted as a caution when interpreting results (Pallant, 2007).

5.2.1.3 Attrition.

Refer to Figure 3, section 5.1.6, of the method section for flow of participants through the study. An attrition analysis was not attempted due to there being only one

drop out across the three assessments. This patient dropped out prior to baseline assessment due to worsening health.

5.2.2 Descriptives.

Due to the low sample size of this observational study, a detailed description of the cohort recruited is required. This is presented across the following section.

5.2.2.1 Demographic information.

The participants were comprised of women ranging from 33 to 65 years of age with an average age of about 49 years (see Table 6). Over 80% of women were married or living in a defacto relationship, and three quarters had had children. Two thirds of women in this sample were involved in the workforce which is reflective of the age range (18-65 years). Of those involved in formal paid work, the type of work engaged in was predominantly professional or white collar. Home duties made up for the majority of women not engaged in formal paid work.

Table 6: Participant Demographic Information (N = 17)

Participant Characteristics		n	%
Age in years, M (SD)		49.47	(10.24)
Marital Status	Married/Defacto	14	82.35%
	Divorced/Separated	2	11.76%
	Single/Never married	1	5.88%
Children		13	76.74%
Occupation	Professionals	7	41.18%
	White Collar Workers	4	23.53%
	Home duties	5	29.41%
	Disability Pensioner	1	5.88%

5.2.2.2 Treatment information.

Diagnostic and treatment reports (Table 7) revealed that this sample comprised women predominantly diagnosed with stage II breast cancer. In this sample over three quarters of women had undergone mastectomy surgery, adjuvant chemotherapy, and completed radiation therapy. Only about a quarter were not undertaking any secondary treatment. Secondary or preventative ongoing treatments were predominantly identified as hormonal (i.e., Tamoxifen) or Trastuzumab or both. Of this group only two women underwent surgery plus radiation therapy without adjuvant chemotherapy. Thus adjuvant treatment for this small group was heterogeneous despite attempts to include a homogenous sample.

Table 7: Diagnostic and Treatment Information (N = 17)

Treatment Information		n	%
Breast Cancer Stage	I	3	17.65%
	II	12	70.59%
	III	2	11.76%
Surgery Type	Lumpectomy	4	23.53%
	Mastectomy	13	76.47%
Chemotherapy		15	88.24%
	Standard	7	46.67%
	Taxane-based	8	47.06%
Radiation therapy		15	88.24%
Ongoing Treatment	None	4	23.53%
	Hormone	8	47.06%
	Trastuzumab	3	17.65%
	Trastuzumab + Hormone	2	11.76%

Note. Hormone Treatment refers to oral tamoxifen medication.

On further exploration of specific treatment regimes, it became evident that there was indeed variability with regard to type, frequency, and duration of chemotherapy (Table 8). For example, post-surgery the duration of adjuvant treatment varied from a month through to 6-months. Hence the time between first diagnosis of breast cancer and baseline assessment for this study varied from 4-months to 1 year, (M = 8.44 months, SD = 2.16).

Table 8: Patient Treatment Regimes

ID	ACx4	Doxcetaxel x 3	Doxcetaxel x 4	FEC 100 x 6	FEC x 3	Paclitaxel x 4	Trastuzumab	Radiation	No. Treatments
1				✓			✓		2
2	✓								1
3	✓					✓			2
4		✓			✓				2
5				✓					1
6		✓			✓				2
7				✓					1
8								✓	1
9								✓	1
10	✓								1
11	✓		✓				✓		3
12				✓					1
13				✓					1
14		✓			✓				2
15	✓	✓					✓		3
16	✓		✓				✓		3
17	✓		✓				✓		3

Note. Abbreviations: AC = Doxorubicin and Cyclophosphamide, FEC100 = 5Fluorouracil, Epirubicin and Cyclophosphamide,

5.2.2.3 Health behaviour information.

Health behaviours have been identified as influencing both psychological, biochemical, and immune parameters in previous Oxidative Model research. For this study, participant health behaviours were collected pre-baseline (Table 9).

In summary, according to the AUDIT, hazardous alcohol use was relatively low in this sample and only three participants smoked tobacco. With regard to exercise, over half of the sample was identified as being physically inactive 4-weeks post-treatment. This was not surprising given the side-effects often associated with chemotherapy; however six participants remained minimally active at this time.

Medication use was mixed for this sample. Not surprisingly cancer specific medications were common (i.e., Trastuzumab and Tamoxifen). In addition cardiovascular drugs (i.e., Karvezide, Norvasc) were frequently used. Four women in this sample were not currently taking any medication at this time. The average number of medications used was 1.5 per participant with the highest total number of medication used by an individual patient being six medications.

Table 9: Baseline Participant Health Characteristics (N = 17)

Participant Characteristics		n	%
Alcohol Use, M (SD)	range 0-12	2.93	(2.23)
Tobacco Use		3	17.65%
Physical Activity (PA)	Inactive	10	58.82%
	Minimally Active	6	35.29%
	Health Enhancing	0	0.00%
Current Medication Use	None	4	23.53%
	Psychotropic	2	11.76%
	Respiratory	2	11.76%
	Cardiovascular	4	23.53%
	Analgesics	1	5.98%
	Osteoarthritis	1	5.88%
	Trastuzumab	5	29.41%
	Endocrine/Hormonal	4	23.53%

Other health behaviours of interest in this observational study included the use of a variety of vitamin/nutrient supplements (Table 10). Collection of this information comes under what is broadly termed complementary and alternative medicine (CAM). Nearly two thirds of this sample identified themselves as taking some form of diet and/or nutritional supplement. This level of use reflects other findings of supplement use in breast cancer samples (Lengacher et al., 2002).

The list of supplements attained from participants was in turn categorized into those with antioxidant properties and those without. This was done due to the influence of antioxidants on biomarkers implicated in The Oxidative Model. Nearly half taking supplements (41%) were taking a supplement high in antioxidant properties. Of those taking supplements with antioxidants properties most were likely to be taking

more than two in total, with the highest being seven. Antioxidant intake is implicit to The Oxidative Model and has been associated with decreased pro-oxidant state and improved immunity, specifically improved NT (higher) and HCY (lowered) levels, in healthy samples during periods of sustained stress (Blake-Mortimer et al., 1996, 1998b; Hapuarachchi et al., 2003).

Table 10: Participant Dietary Supplement Use at Baseline

Participant ID	Antioxidant (AO) Use	Other Supplements	Total number of high AO supplements
#6	Glucosamine, Thompson's Immunofort, Super B, Complex, Coenzyme Q-10, Livertonic, Digestone		7
#7	Vitamin C, Vitamin B Complex , Vitamin E, Fish oil	Calcium, Iron, Zinc	4
#9	Indole 3 carbinol, Selenium, Vitamin D, Flaxseed oil, Kelp, Broccoli sprout powder, Herbal tonic, Vitamin C		8
#10	Fish oil		1
#14	Berroca performance, Cenovis women's multivitamin	Sandra Cabot's quickloss	2
#15	Fish oil		1
#16	Nature's Own multivitamin	Nature's Own calcium plus	1

5.2.3 Hypothesis 1a - Women will experience poor psychological well-being 4-weeks post treatment.

A principal aim of this observational study was to ascertain the post-treatment psychological well-being for women treated for early stage breast cancer. In order to do this firstly the sample are described and discussed. This section aimed to identify how comparable or representative this sample were to other research undertaken in similar samples under similar circumstances. This psychological picture is based on data attained pre-baseline as well as at baseline assessment. It includes both state and trait psychological measures, as well as cancer-specific measures.

5.2.3.1 High distress scores as measured by the GHQ-12, 4-weeks post-treatment (i).

Psychological distress was assessed using the GHQ-12. Using the Likert scoring method, scores can range from 0 to 36, with higher scores indicating higher distress. Distress scores for this sample at baseline assessment were not unusually high (M = 9.89, SD = 6.08). To further explore distress levels the GHQ-12 cut-off scores were considered. These use a binary scoring system. This gives rise to scores indicating the extent/ presence of symptoms of distress. Score 0-1 indicate 'normal stress', 2-3 'mild stress', and scores ≥ 4 'severe stress'. For this sample the majority (n = 13) could be categorized into the normal category and no participants in the mildly stressed category. However three (17.65%) women were experiencing severe stress at baseline assessment. This provides only partial support for the hypothesis.

5.2.3.2 High S-anxiety, S-depression, S-anger, and low scores for S-curiosity 4-weeks post-treatment (ii).

At baseline it became apparent that this group of women was psychologically comparable to studies of healthy women of a similar age range, with regard to the dispositional characteristics of T-anxiety, T-depression, T-anger, T-curiosity, and Anger Expression (Table 11 & 12). This suggests that these women were not unusual in their personality profiles. At baseline, state characteristics (S-anxiety, S-depression, S-curiosity, and S-anger) for this sample were also comparable to standardized norms. However T-anxiety and S-anger for this sample was slightly lower than normative reference ranges. In contrast S-depression scores were slightly higher.

Table 11: Comparisons between Current and Normative Samples for State-Trait Anxiety, Depression, Curiosity and Anger (STPI)

			Normative Samples		Current Sample (N = 17)
Psychological measure		Possible Range	N	M (SD)	M (SD)
State-Trait Personality Inventory	T-anxiety	10 - 40	133	19.38 (5.65)	17.33 (4.17)
	T-depression	10 - 40	171	18.52 (5.88)	17.67 (4.22)
	T-anger	10 - 40	133	19.14 (4.97)	18.67 (4.53)
	T-curiosity	10 - 40	133	29.30 (6.25)	29.20 (6.06)
	S-anxiety	10 - 40	133	19.06 (5.75)	18.25 (7.07)
	S-depression	10 - 40	171	14.79 (5.05)	16.50 (5.55)
	S-anger	10 - 40	133	14.24 (5.75)	11.63 (2.80)
	S-curiosity	10 - 40	133	26.17 (5.45)	27.88 (7.85)

Note. Normative data based on normal female college sample , age unspecified (Spielberger, 1996)

Table 12: Comparisons between Current Sample and Normative Samples for Revised State-Trait Anger Expression Inventory (STAXI-Revised)

Psychological measure		Possible Range	Normative Sample N	Normative Sample M (SD)	Current Sample (N = 17) M (SD)
State Trait Anger Expression Index	AX-in	8 - 32	952	15.69 (4.38)	15.13 (3.09)
	AX-out	8 - 32	952	14.79 (3.78)	14.40 (3.38)
	AC-in	8 - 32	952	23.28 (5.82)	24.07 (4.38)
	AC-out	8 - 32	952	23.28 (5.82)	24.27 (4.01)
	AX-index	48 - 96	952	32.04 (13.06)	29.20 (8.26)

Note. Normative data based on normal female sample age unspecified (Spielberger, 2003)

Abbreviations: anger expression out (AX-out), anger expression in (AX-in), anger control out (AC-out), anger control in (AC-in), anger expression index (AX-index)

5.2.3.3 Loneliness levels 4-weeks post-treatment (iii).

Scores can range from a possible 20-80 on the UCLA Loneliness Scale; higher scores indicate an discrepancy between the amount of social contact in contrast to desired levels of contact, from the participants perspective. At baseline, mean scores (M = 35.56, SD = 9.77) suggested that this sample were not dissatisfied with their level of social contact when compared with a mixed sample (N = 240) of middle-aged men and women (M = 36.30, SD = 2.80; Steptoe, Owen, Kunz-Ebrecht, & Brydon, 2004). This supports the hypothesis that women post-treatment were not experiencing high levels of loneliness.

5.2.3.4 Poorer psychological adjustment styles 4-weeks post-treatment (iv).

Trait characteristics for psychological defense mechanisms were somewhat lower when compared with normative data from a breast cancer sample (Table 13).

Lower scores observed for the current study suggest less use of psychological defense mechanisms to repress or deny painful thoughts, memories, or feelings. Mean and standard deviation scores obtained from the current sample lie between the two normative data from healthy female samples without a breast cancer diagnosis, and those with a diagnosis of cancer. This provides only partial support for the hypothesis that women post-treatment will experience poorer psychological response mechanism profiles.

Table 13: Comparisons between Current Sample and Normative Samples from Healthy and Breast Cancer Samples of Psychological Defense Mechanisms (LDMS)

		Healthy[1]		Breast Cancer[2]		Current Sample N = 17
Psychological measure	Possible Range	N	M (SD)	N	M (SD)	M (SD)
R/ED	12 - 48	585	34.13 (5.52)	132	44.20 (5.02)	36.07 (3.75)
N/H	12 - 48	577	35.60 (5.74)	132	45.59 (3.59)	38.73 (4.38)

Note. Abbreviations: R/ED = Rationality & Emotional defensiveness, N/H = Need for Harmony

[1]Normative breast cancer means and standard deviations based on data collected for a sample of women approximately 12-months after treatment for breast cancer (Fernandez- Ballesteros, Ruiz, & Grade, 1998)

[2]Normative healthy sample data (N = 577) based on female sample, age unspecified (Spielberger, 2002)

Scores attained on the Mental Adjustment to Cancer (MAC) scale (Table 14), indicated that this sample's FS response scores were comparable to other literature of predominantly early stage breast cancer patients in an Australian setting (Whitford, Olver, & Peterson, 2008). However the literature suggests that these scores were recorded close to diagnoses as opposed to post-treatment like the current sample. Similarly, scores for the HH and AP coping responses were comparable with studies of

similar samples. The F coping response was lower in comparison with the normative sample. This does not provide support for the hypothesis that women post-treatment were experiencing psychological adjustment difficulties.

Table 14: Comparisons between Current Sample with Normative Samples for Mental Adjustment to Cancer (MAC)

Psychological Measure		Possible Range	Norm N	Norm M (SD)	Current Sample n = 16 M (SD)
Mental adjustment to cancer	FS	16 - 64	838	51.71 (6.85)	52.44 (5.82)
	HH	6 - 24	882	9.51 (3.03)	9.19 (3.37)
	AP	9 - 36	851	22.01 (4.13)	22.94 (5.17)
	F	8 - 32	831	18.37 (3.64)	16.63 (4.30)
	A	1 - 4	915	n/a	1.63 (0.81)

Note. Normative data (Whitford et al., 2008), mixed cancer sample, predominantly breast, urological and lung cancer, age unspecified

Abbreviations: Fighting Spirit (FS), Helplesss-Hopeless (HH), Anxious Preoccupation (AP), Fatalistic (F), Avoidant (A)

In some cases FS and HH responses have been amalgamated as a psychometric analysis show them to form a bipolar scale. Hence Watson et al. (1989) propose the use of cut-off scores to distinguish clinical from 'cases' from 'non-cases'. In this instance 'cases' are defined as scoring 47 or less on the Fighting Spirit subscale in combination with a score of 12 or more on the Helpless/Hopeless subscale. Only one participant met this criterion at baseline.

Women will experience high levels of trauma associated with the cancer experience 4-weeks post treatment (v).

The IES-R was employed to assess the experience of trauma, specifically PTSD symptoms associated with the experience of breast cancer. There are no 'cutoff' points for the IES-R unlike previous versions (Weiss & Marmar, 1997). Rather the measure is intended to give an assessment of symptomatic status over the past 7 days with respect to the three domains of PTSD symptoms. Raw scores of the IES-R show wide standard deviations observed for this scale (M = 17.44, SD = 16.98). This suggests that there exists a discrepancy between scores attained from individuals in this sample- a bimodal pattern either low or high. As a general rule scores > 25 are considered high. In the current sample four (25%) participants evidenced scores > 25. Possible range of scores is 0 – 88. The highest recorded score was 56.

The availability of normative data for early stage breast cancer patients in the post-treatment period employing the revised IES scale is limited. Mean scores for the IES-R scale were calculated (M = .2.55, SD = 0.79, n = 16) in order to allow comparison to IES-R scores for a sample of rectal cancer patients (N = 80) shortly following surgery (M = 1.22, SD = 0.4). In light if this comparison, cancer-related stress 4-weeks post-treatment appears to be moderate. However it is remains apparent that this is experienced by a minority. For the majority in the current sample post-traumatic stress symptoms were not present 4-weeks post-treatment. This does not support the current hypothesis that 4-weeks post-treatment is a period of heightened trauma.

5.2.4 Hypothesis 1b - Increased pro-oxidant mechanisms 4-weeks post-treatment.

A pro-oxidant state is described by The Oxidative Model as low levels of NT, VIT C, FOLATE, VIT B12, and concurrent high levels of HCY.

Baseline scores for women in this sample on pro-oxidant measures were within reference ranges proposed for normal samples (Table 15). However levels of NT, the marker of lymphocyte maturation, were on the low end of the clinical reference range. Concurrently lower than normative reference ranges for VIT C, plus HCY levels at the higher end of their respective reference ranges for normative data. These findings suggest a pro-oxidant state. Concurrently FOLATE and VIT B12 fell within normal clinical ranges suggesting that these nutrient levels were sufficient. This only provides partial support for this hypothesis, based on the NT, VIT C, and HCY levels observed.

Table 15: Comparisons between the Current Sample and Normal Reference Ranges on Pro-Oxidant Biomarker Levels

Biomarkers	Normal Range	Current Sample n = 16 M (SD)
NT	0.4-1.4 nmol/h/µgDNA	0.4 (0.15)
VIT C	50-150 pg/ugDNA	30.25 (13.51)
HCY	3-13 umol/L	8.91 (2.56)
FOLATE	5-45 nmol/L	24.27 (9.41)
VIT B12	140-700 pmol/L	371.50 (101.19)
CHOL	<5.5 mmol/L	5.07 (0.77)

Note. Biomarker Abbreviations: 5' –ectonucleotidase (NT), tissue ascorbate (VIT C), homocysteine (HCY), Vitamin B12 (VIT B12), Folate (FOLATE), Cholesterol (CHOL)

I

5.2.5 Hypothesis 1c - Increased pro-inflammatory mechanisms 4-weeks post-treatment.

A pro-inflammatory state evidenced by high CRP and pro-inflammatory cytokines (IL-1β, IL-5, TNF-β, TNF- α, IFN-γ), and lowered anti-inflammatory cytokines (IL-10) was not observed in this sample (Table 16). In the context of normal clinical reference ranges, baseline levels for pro-inflammatory cytokines for this sample of women 4-weeks post treatment suggest a low level of inflammation. CRP levels were moderate. This does not provide complete support for this hypothesis.

Table 16: Comparisons between the Current and Normal Reference Ranges for Inflammatory Measure Levels

Biomarkers	Normal Range	Current Sample n = 16 M (SD)
CRP	<6 mg/L	1.77 (2.05)
IL-1 β	<426pg/ml	2.95 (4.30)
TNF-β	<439pg/ml	0.70 (1.77)
TNF- α	<479pg/ml	2.06 (2.47)
IFN-γ	<365pg/ml	1.44 (2.38)
IL-5	<44pg/ml	2.03 (3.72)
IL-10	<44pg/ml	3.41 (3.17)

Note. Biomarker Abbreviations: C-reactive protein (CRP), Interferon (IFN-γ), Tumour necrosis factor-α (TNF-α), Interleukin-1 (IL-1β), Interleukin-5 (IL-5), Interleukin-10 (IL-10), Tumour necrosis factor- β (TNF-β)

5.2.6 Hypothesis 1d and 1e - pro-oxidant and pro-inflammatory measures will be associated with higher levels of distress and poorer psychological well-being post-treatment.

A principal aim of this observational study was to explore the relationships between psychological, pro-oxidant, and pro-inflammatory measures, 4-weeks post-treatment. This was based on the findings from previous Oxidative Model research associating several measures of psychological distress and emotional dysfunction with an increased pro-oxidant and pro-inflammatory state (Blake-Mortimer et al., 1996, 1998b; Hapuarachchi et al., 2003).

Relationships between psychological distress (GHQ) and Oxidative Model biomarkers were investigated using Pearson product-moment correlations. In addition exploration of psychological measures and cytokines was undertaken. These analyses were undertaken on data collected at pre-baseline and baseline assessment.

In addition to state or current psychological measures, exploration of associations between trait psychological measures, pro-oxidant, and pro-inflammatory states was undertaken. These analyses were done in order to develop a picture of the influence of personality characteristics on biomarkers implicated in The Oxidative Model. Additionally associations between biomarkers were explored to allow development of an understanding of biomarker interactions occurring in this 4-weeks post-treatment period.

5.2.6.1 Pro-oxidant measures will be associated with higher levels of distress, and poorer psychological well-being (i, ii, iii).

On exploration of relationships between psychological state measures, cancer coping styles (as measured by the MAC scale), and trauma or PTSD symptoms (as measured by the IES-R) with pro-oxidant biomarkers, the decision to focus only large

associations (>.40), indicative of large effect sizes, was made in order to attempt to balance Type I and Type II error (Perneger, 198). Several significant relationships were also observed (Table 17).

Table 17: Associations between Pro-Oxidant Biomarkers and Measures of Psychological State Assessed at Baseline (n = 16)

Variable	Pro-oxidant biomarkers									
	NT		VIT C		HCY*		VIT B12		FOLATE	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*
Distress	-.38	.15	.06	.82	.13	.65	-.36	.18	.28	.30
S-depression	**-.68**	**.00**	-.05	.85	.11	.69	-.38	.14	-.12	.67
S-anxiety	**-.42**	.11	-.08	.76	-.01	.97	**-.46**	.07	.20	.45
S-curiosity	**.69**	**.00**	-.06	.82	.01	.99	**.53**	**.03**	-.01	.96
S-anger	-.20	.45	.08	.76	.05	.86	**-.65**	**.01**	.06	.84
Loneliness	-.34	.19	**.41**	.12	**.42**	.12	-.17	.52	-.18	.50
IES-R	**-.47**	.07	.19	.48	.13	.64	-.32	.23	.17	.53
FS	.16	.54	-.28	.29	-.01	.96	**.42**	.10	.09	.73
HH	**-.43**	.09	.02	.95	-.06	.83	**-.47**	.07	.24	.36
AP	-.30	.26	-.10	.70	.15	.58	**-.47**	.07	.23	.38
F	-.03	.91	.23	.40	.12	.67	-.23	.38	-.05	.85
A	-.39	.14	.17	.52	**.50**	.06	-.18	.50	-.19	.48

Note. **Biomarker Abbreviations:** 5' –ectonucleotidase (NT), tissue ascorbate (VIT C), homocysteine (HCY), Vitamin B^{12} (VIT B12), Folate (FOLATE); **Psychological Abbreviations:** Impact of Events- revised version (IES-R), Fighting Spirit (FS), Helplesss-Hopeless (HH), Anxious Preoccupation (AP), Fatalistic (F), Avoidant (A)

*n = 15

As hypothesized, the central biomarker of The Oxidative Model, NT, indicated moderate- to- large negative associations with several measures of current psychological stress (Table 17) , specifically S-depression, S-anxiety, trauma, and HH coping responses. In contrast NT evidenced one positive association with S-curiosity. This means improved NT levels were associated with this state. These associations were all large however only associations with S-depression and S-curiosity reported significance, suggesting these are the only two which could be reported with confidence.

VIT B 12 levels evidenced negative associations with S-anxiety, S-anger, Helpless/Hopeless, and Anxious Preoccupation coping responses. This follows what the Model predicts that VIT B12 levels are depleted at times of high stress and/or anger In contrast higher Fighting Spirit and S-curiosity scores were associated with higher VIT B12. These findings mean that negative emotion states and coping responses were more pro-oxidant than positive emotion states and more active coping responses in this sample post-treatment. Despite these large effect sizes, only S-anger and S-curiosity reached significance.

Loneliness was associated with increased oxidative stress as measures by increased HCY but in contrast it was also associated with increased VIT C levels. In the context of the Model, higher VIT C means greater availability of antioxidants to ameliorate oxidative stress. Neither reached statistical significance and thus should be interpreted with caution.

Similar patterns were observed for trait measures of stress (Table 18). T-anxiety, T- depression, AX-index and AX-out all evidenced moderate- to- large negatively associated with NT levels. This means these trait characteristics are associated with pro-oxidant states for this sample. In contrast positive trait characteristic, T-curiosity had a very large positive association with NT, suggesting that this trait characteristic to be associated with improved oxidative stress within the body. This provides support for the Oxidative Model, however only T-depression and T-curiosity reached significance.

Increased VIT B12 levels were associated with higher T-curiosity scores. In contrast T-depression and T-anxiety associated with lower levels of VIT B12. This suggests depletion to be associated with these trait characteristics. Depleted FOLATE levels were also associated with several measures of heightened anger expression traits, AX-in, AC-in, AC-out. However the overall experience of anger (AX-index), was associated with increased FOLATE levels, which is contradictory to the findings mentioned previously. Furthermore measures of anger suppression/control (AC-in, AC-out) and overall anger (AX-index) were associated with heightened pro-oxidant states also, specifically higher HCY levels. T-anxiety also evidenced moderate- to- large associations with HCY. These associations provide support for The Oxidative Model, and the current hypothesis.

Table 18: Associations between Pro-Oxidant Biomarkers and Trait Psychological Measures (n = 15)

Variable	Pro-oxidant biomarkers									
	NT		VIT C		HCY*		VIT B12		FOLATE	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*
T-depression	**-.56**	**.04**	.14	.61	.20	.50	**-.49**	.06	-.02	.95
T-anxiety	**-.46**	.10	.40	.14	**.51**	.06	**-.46**	.09	-.32	.25
T-curiosity	**.77**	**.00**	.08	.78	.07	.81	**.55**	**.04**	.07	.81
T-anger	-.17	.55	.16	.58	-.04	.90	-.27	.33	.25	.37
R/ED	.25	.40	.35	.20	.10	.73	-.14	.60	-.29	.30
N/H	-.18	.53	-.07	.80	.13	.65	-.20	.49	-.30	.27
AX-out	**-.40**	.16	-.11	.70	-.21	.47	-.15	.60	.35	.21
AX-in	-.16	.60	.19	.50	.38	.18	.14	.62	**-.49**	.07
AC-out	.27	.34	.07	.80	**.61**	**.02**	-.13	.64	**-.50**	.06
AC-in	.38	.18	.06	.82	**.56**	**.04**	-.06	.83	**-65**	**.01**
AX-index	**-.54**	**.05**	-0.4	.88	**.54**	**.05**	.09	.76	**.55**	**.03**

Note. **Biomarker Abbreviations:** 5' –ectonucleotidase (NT), tissue ascorbate (VIT C), homocysteine (HCY), Vitamin B^{12} (VIT B12), Folate (FOLATE) **Psychological Abbreviations:** Rationality and Emotional Defensiveness (R/ED), Need for Harmony (N/H), anger expression out (AX-out), anger expression in (AX-in), anger control out (ACon-out), anger control in (ACon-in), anger expression index (AX-index)

* n = 14

5.2.6.2 Pro-inflammatory measures will be associated with higher levels of psychological distress and dysfunctional emotion states (i, ii, iii).

As hypothesized, the several markers of inflammation indicated moderate- to-large negative associations with several measures of psychological stress (Table 19) Psychological distress levels showed moderate- to- large, non-significant, associations with both increased CHOL and IL-1β. CHOL a marker of increased CVD risk paired with inflammation support the current hypothesis. Counter intuitively higher C-RP levels had a large negative association with loneliness levels, which reached significance. This suggests that unmet social needs actually decreased inflammation. This finding does not support the current hypothesis.

Psychological responses specific to cancer indicated that Helpless/Hopeless scores had a large association with heightened CHOL and also with the pro-inflammatory cytokine, IL-1β, which reached significance. Helpless/Hopeless coping is a depressive coping style in response to a cancer diagnosis. Higher CHOL levels were also related to higher scores on the Fatalist coping responses, showing a large effect, which reached significance. This suggests inflammation with more maladaptive psychological responses in this sample.

IL-1β showed many associations with psychological measures. Psychological distress, S-anxiety, S-anger and Helpless/Hopeless all showed moderate- to- large positive associations with IL-1β. At the same time Fighting Spirit had a moderate association with IL-1β levels. These patterns provide support for the current hypothesis.

Table 19: Associations between Pro-Inflammatory Measures and Measures of Psychological State Assessed at Baseline (n = 16)

Variable	Pro-inflammatory markers															
	C-RP*		CHOL		IFN-γ		TNF-α		IL-1β		IL-5		TNF-β*		IL-10	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*
Distress	.12	.68	**.44**	.09	.17	.53	.37	.16	**.45**	.08	.13	.62	-.23	.39	.27	.31
S-depression	-.13	.64	.37	.28	-.15	.59	.32	.23	.29	.28	.03	.91	-.23	.39	.33	.22
S-anxiety	-.13	.65	.18	.50	-.01	.99	.27	.32	**.40**	.13	.20	.46	-.14	.61	.23	.40
S-curiosity	.13	.65	-.22	.42	.09	.74	-.23	.40	-.30	.27	-.12	.66	.14	.60	.28	.29
S-anger	.00	.99	.29	.28	.17	.54	.25	.34	**.48**	.06	.24	.37	.01	.98	.15	.58
Loneliness	**-.55**	**.04**	-.09	.75	**-.44**	.09	.29	.27	-.11	.67	-.10	.70	-.29	.27	-.04	.89
IES-R	.02	.95	.24	.34	.05	.87	.16	.57	.22	.41	-.03	.90	-.35	.19	.10	.72
FS	.17	.55	-.20	.47	-.03	.90	**-.45**	.08	**-.44**	.09	-.30	.26	-.32	.23	-.37	.15
HH	-.07	.82	**.49**	.06	.18	.52	.37	.16	**.58**	**.02**	.30	.27	-.01	.98	.39	.13
AP	.24	.40	.02	.96	.10	.72	.17	.53	.33	.22	.02	.93	-.23	.39	.17	.54
F	.10	.74	**.62**	**.01**	-.15	.57	.06	.83	.08	.78	-.21	.44	-.37	.16	-.10	.77
A	-.04	.89	-.03	.91	-.26	.33	.11	.67	-.03	.92	-.24	.37	-.29	.28	-.04	.88

Note. **Biomarker Abbreviations:** C-reactive protein (CRP), Cholesterol (CHOL), **Cytokine Abbreviations:** Interferon (IFN), Tumour necrosis factor-α (TNF-α), Interleukin-1 (IL-1B), Interleukin-5 (IL-5), Interleukin-10 (IL-10), Tumour necrosis factor- β (TNF-β); **Psychological Abbreviations:** Impact of Events (IES-R)

*n = 15

Similar patterns were observed for trait characteristics and pro-inflammatory measures (Table 20).

Trait measures of anger evidenced several moderate- to- large associations with inflammatory measures. Increased CRP showed a moderate- to- large association with increased T-anger and AX-out. In contrast AC-out traits were associated with lower CRP levels, suggesting the control of anger expression to decrease inflammation. In line with this finding other measures of anger expression evidenced several moderate- to- large associations with pro-inflammatory cytokines. However there was often a disparity between anger expression and anger suppression traits. For instance increased TNF-α levels were associated with lower AC-in and AC-out, but higher AX-index scores, which reached significance.

Anger control lowered inflammation, but on the other hand anger expression traits were inflammatory. This was based on the moderate- to- large associations of AX-out with increased CRP, IFN-γ, and IL-1β. AC-out traits suppress inflammation as observed by moderate- to- large associations with lower CRP, TNF-α, and IL-5. These opposite patterns reflect the importance of not just anger but that the expression style may have an influence on inflammatory mechanisms. Further support of this is the assessment of IL-10 an anti-inflammatory cytokine levels. AC-in had a moderate negative association with IL-10. In contrast AX-index and AX-out had moderate positive association. This suggests that anger expression may also have anti-inflammatory qualities. In addition to expression versus control there is also the direction of anger expression, outwards (out) or towards the self (in).

Need for Harmony had a moderate association with TNF-β, similarly T-anxiety had a moderate- to- large association that reached significance. These patterns, described above, support the current hypothesis that psychological stress is associated with inflammation.

Table 20: Associations between Pro-Inflammatory Measures and Trait Psychological Measures (n = 15)

Variable	Pro-inflammatory markers															
	CRP*		CHOL		IFN-γ		TNF-α		IL-1β		IL-5		TNF-β		IL-10	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*
T-depression	.26	.37	.32	.25	.11	.70	.26	.35	.32	.24	-.05	.87	-.33	.23	.35	.20
T-anxiety	-.21	.48	.06	.83	-.33	.23	.13	.66	-.17	.55	-.30	.28	**-.54**	**.04**	-.06	.83
T-curiosity	-.05	.86	-.32	.25	.00	.99	-.15	.60	-.14	.61	-.02	.95	.35	.20	-.31	.27
T-anger	**.50**	.07	.39	.15	.26	.36	.27	.33	**.41**	.13	.02	.95	-.19	.49	.16	.58
R/ED	.16	.58	.30	.27	.09	.75	.29	.30	.05	.86	-.02	.94	-.15	.60	-.29	.30
N/H	-.07	.81	.21	.46	-.19	.51	-.03	.92	-.16	.58	-.09	.76	**-.40**	.14	-.31	.26
AX-out	**.56**	**.04**	.21	.46	**.51**	**.05**	.35	.21	**.62**	**.01**	.21	.46	.04	.90	**.47**	.08
AX-in	-.13	.66	**-.46**	.09	**-.63**	**.01**	-.23	.40	**-.64**	**.01**	**-.66**	**.01**	**-.47**	.08	-.27	.34
AC-out	**-.50**	.86	.06	.84	-.35	.21	**-.40**	.14	-.27	.34	**-.42**	.12	-.21	.46	-.34	.21
AC-in	-.13	.66	.12	.67	.30	.28	**-.50**	.06	**-.45**	.09	**-.49**	.07	-.09	.76	**-.43**	.11
AX-index	.26	.40	-.05	.86	.30	.28	**.51**	**.05**	.38	.16	.30	.28	-.01	.96	**.49**	.07

Note. **Biomarker Abbreviations:** C-reactive protein (CRP), Cholesterol (CHOL), **Cytokine Abbreviations:** Interferon (IFN), Tumour necrosis factor-α (TNF-α), Interleukin-1 (IL-1B), Interleukin-5 (IL-5), Interleukin-10 (IL-10), Tumour necrosis factor- β (TNF-β); **Psychological Abbreviations:** Rationality and Emotional Defensiveness (R/ED), Need for Harmony (N/H), anger expression out (AX-out), anger expression in (AX-in), anger control out (AC-out), anger control in (AC-in), anger expression index (AX-index)

5.2.7 Inferential statistics.

A principal aim of this observational study was to explore patterns of psychological and physiological well-being over a five-month post-treatment period. Prior to the proposed repeated measures ANOVAs, exploration of potential 'nuisance' variables (covariates) was considered.

5.2.7.1 Covariate exploration.

One of the aims of this observational study was to explore the influence of covariates on measures employed by The Oxidative Model. In the literature review of PNI, cancer patients, and The Oxidative Model, the notion of health behaviours influencing physical and psychological well-being was explored. A critique of The Oxidative Model highlighted the lack of research sufficient design and/or statistical control for the influence of health behaviours on biomarkers central to this Model. The influence of these variables on psychological well-being as well as immune and pro-oxidant measures requires consideration.

Before conducting the principal analyses, data were checked for the contribution of covariates that could potentially be related to psychological stress, pro-oxidant, or pro-inflammatory outcomes, or both. The variables examined were measures of demographic information (i.e., age), treatment regimes, medication use, health behaviours (i.e., exercise), and other behavioural measures (i.e., smoking, alcohol intake, vitamin intake, etc) that have been shown to be associated with psychological wellbeing, biomarkers, and

immune measures in similar studies (Lesgards et al., 2002) (Boss et al., 1980; Hapuarachchi et al., 2003) (Thornton et al., 2007).

The relationships between these variables and each of the psychological, pro-oxidant, and pro-inflammatory outcome variables were examined. However several issues required consideration. Firstly significant associations among medication variables were discovered. Covariates were only included if it was considered they contributed uniquely to the reduction in error variance. Therefore for the covariates with high correlations (i.e., psychotropic, analgesic, and osteoarthritis medication use) on further exploration they were combined into one variable (i.e., Psych/Analg/Osteo).

Secondly, due to the small sample size, in order to maintain as much power as possible, covariates were added to an ANCOVA only if they were indicated to influence the dependent variable with a correlation of $r \geq .40$. This indicates a moderate-to-large effect according to Cohen (1988). This is an arbitrary cut-off score, but it was considered that adding variables with too small an effect would compromise power.

Thirdly, each outcome variable of interest had an independent ANCOVA run separately to allow for the inclusion of influential covariates for that specific variable. This was done to minimize any further power loss by adding all covariates to all ANCOVAS. As a result, each of the longitudinal analyses was discussed separately to allow for discussion of covariates specific to that analysis.

5.2.7.1.1 Covariates influencing psychological well-being.

It was anticipated that health behaviours, lifestyle choices, and medication use could influence psychological well-being across the post-treatment period. Subsequently a correlation matrix (Appendix D) was performed to explore these relationships to show which variables need to be later added as covariates in repeated measure ANCOVAs. Alcohol use was the only health behaviour measured which showed a large association with both S-depression (r = .66, p = .01, n = 15) and A coping responses (r = .59, p = .02, n = 15). With regard to medication use, a large negative correlation was identified for S-depression and the use of endocrine medication (r = -.52, p = .05, n = 15).

Large positive associations were found between FS coping responses and immunomodulator medications (r = 57, p = .03, n = 15) and cardiovascular medications (r = .59, p = .02, n = 15). The use of these two medications was significantly correlated but further exploration, using partial correlation techniques, confirmed that although some variance was shared both variables contributed unique variance. Thus it was considered that they were not accounting for the same error variance from the dependent variable, Both were used as covariates for the FS variable as they would have incremental validity.

Treatment regimens, specifically whether patients had undergone chemotherapy (yes = 1, no = 0), radiation therapy (yes = 1, no = 0), and surgery (lumpectomy = 0, mastectomy = 1) were also explored in relation to their impact on dependent variables. Having undergone chemotherapy was associated with large associations with increased

psychological distress (r = 58, p = .03, n = 16) and decreased S-anger (r = -.61, p = .01, n = 16) scores at baseline assessment.

5.2.7.1.2 Covariates influencing pro-oxidant measures.

Based on previous research (Thornton et al., 2007) it was anticipated that certain immune cell trajectories could be obscured by cancer treatments. However on exploration of the potential influence of covariates on biomarkers, only medication use and vitamin use were identified as influential. Specifically pro-oxidant markers evidenced three moderate-to-large associations. FOLATE levels and cardiovascular medication use evidenced a large negative association (r =-.55, p = .03, n = 15), NT a large positive correlation with respiratory medication use (r = .57, p = .03, n = 15) and HCY had a large negative association with vitamin use (r = -.67, p = .01, n = 14).

5.2.7.1.3 Covariates influencing pro-inflammatory measures.

On exploration of potential influences of health variables on cytokines (Appendix D), the following were identified. At baseline large negative correlations were found between age and cytokines- IL-5 (r = -.59, p = .02, n = 15) and TNF-β (r =-.65, p = .01, n = 15). Similarly, both IL-5 and TNF-β cytokines were evidenced large positive associations with endocrine medication use (IL-5: r =.53, p = .04, n = 15; TNF-β: r = .71, p = .01, n = 15) respectively. Thus for further analyses of these cytokines age and endocrine medicine use were applied as covariates.

5.2.8 Hypothesis 2a – Women's psychological well-being will improve over a 20-week post-treatment period.

Repeated measures ANOVAs were employed to determine whether psychological variables changed across time (4-weeks, 12-weeks, and 20-weeks post-treatment). Descriptive findings of means and standard deviations at each time point across the study are presented (Table 21). In addition F tests are presented (Table 22). Following these tables, each variable will be presented and discussed separately with regard to observed change over time. Headings for each variable assessed reflect the hypothesised direction of change proposed in the Principal Aims chapter (see Chapter 5).

Table 21: Psychological Variables: Means and Standard Deviations Across Time (n = 16)

Variable	Range	Baseline M (SD)	Time 1 M (SD)	Time 2 M (SD)
Psychological Distress	0 - 36	9.87 (6.08)	7.07 (3.26)	9.07 (3.92)
S-anxiety	10 - 40	18.00 (7.24)	15.73 (3.86)	16.07 (4.94)
S-curiosity	10 - 40	28.53 (7.65)	30.87 (5.01)	29.47 (5.91)
S-depression	10 - 40	16.13 (5.54)	13.33 (3.04)	15.00 (3.80)
S-anger	10 - 40	11.43 (2.71)	10.14 (0.36)	12.29 (4.48)
Loneliness	20 - 80	35.13 (9.96)	34.40 (8.33)	34.53 (9.24)
FS	16 - 64	52.44 (5.82)	52.47 (5.78)	52.80(5.35)
HH	6 - 24	8.87 (3.23)	7.40 (2.10)	7.80 (1.61)
AP	9 - 36	23.00 (5.35)	21.93 (4.48)	22.33 (3.46)
F	8 - 32	16.60 (4.45)	16.93 (4.10)	15.80 (2.91)
A	1 - 4	1.60 (0.83)	1.67 (0.82)	1.60 (0.83)
IES-R	0 - 88	17.44 (16.98)	14.67 (9.96)	16.13 (15.61)

Note. Abbreviations- Fighting Spirit (FS), Helpless-Hopeless (HH), Anxious Preoccupation (AP), Fatalistic (F), Avoidant (A) Impact of Events- revised version (IES-R)

Table 22: Analyses of Variance (ANOVA) Change in Psychological Measures over a 20-week Post-Treatment Period

Variable	n	F	p	Partial η^2
Psychological Distress*	15	4.21	.04	.25
S-anxiety	15	0.91	.41	.06
S-curiosity	15	0.90	.42	.06
S-depression*	15	0.05	.91	.00
S-anger*	15	2.71	.12	.18
Loneliness	15	0.14	.81	.01
FS	15	0.24	.79	.02
HH	15	1.44	.25	.09
AP	15	0.78	.43	.05
F	15	1.34	.28	.09
A*	15	0.81	.44	.06
IES-R	15	0.47	.59	.03

Note. Omnibus F tests reported

Partial η^2 = magnitude of change, .01 = small, .06 = moderate, and .14 = large;

* indicates that Analysis of Covariance performed

5.2.8.1 Decreased psychological distress over the post-treatment period (i).

To date literature on whether psychological distress improves or worsens post-treatment period is mixed. The current study of psychological distress assessed using the GHQ-12. Results indicated that there was a statistically significant change across time for psychological distress scores (Table 22); the magnitude of this change was large. Mean distress levels at baseline (4-weeks post-treatment) were not considered to be clinically severe. Subsequently the trend across time was for scores to further improve between 4-12 weeks (baseline to T1), followed by worsening between 12- 20 weeks (T1 to T2) (Figure 5).

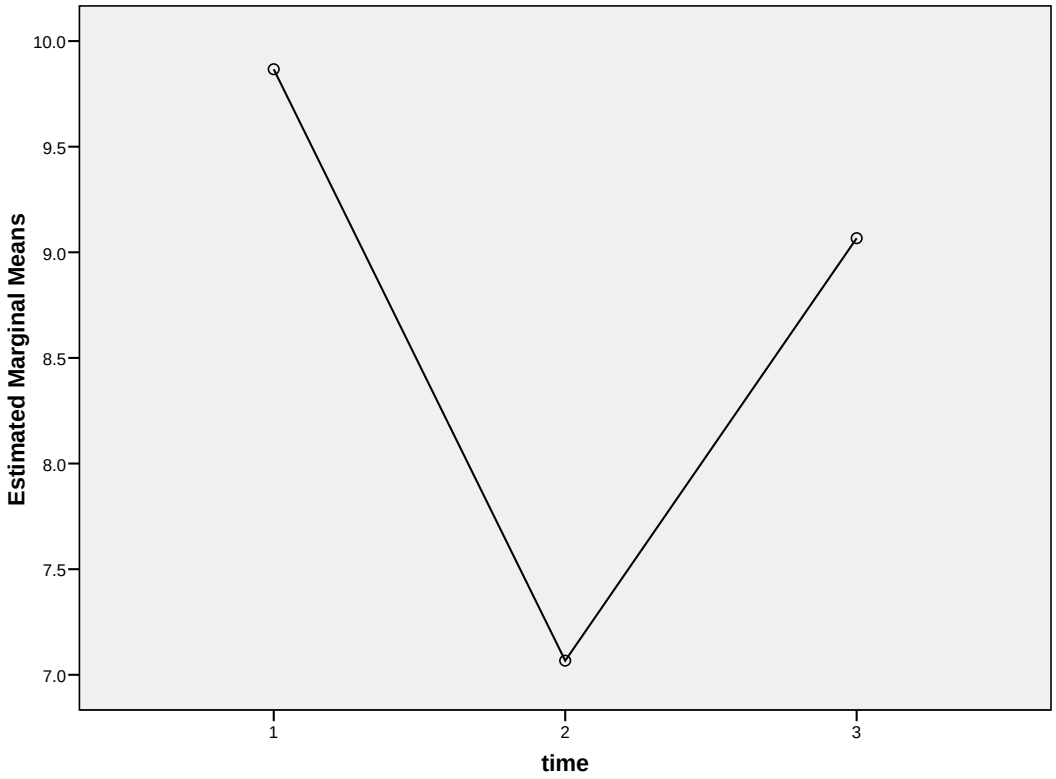


Figure 5: Psychological distress (GHQ-12) scores for early stage breast cancer patients at 4-weeks (baseline), 12-weeks (T1), and 20-weeks (T2) post-treatment

From the initial covariate exploration, chemotherapy was identified as a confounding variable and included in the analyses accordingly. Addition of this variable to the analysis indicated that having undergone chemotherapy treatment did not uniquely, significantly adjust psychological distress scores, $F\ (1.66, 11) = 3.25$, $p = .07$, partial $\eta^2 = .20$. However, this result was approaching significance and this covariate evidenced a large effect.

RCIs were calculated to determine reliable positive, negative, and no change without measurement error (Table 23). These results indicate that there was little reliable

change observed across 4-12 and 12-20 weeks post-treatment. Only improvement in distress scores was observed between 4-12 weeks post-treatment. This occurred for 20% of the sample. In contrast worsening in distress scores was observed between 12-20 weeks post-treatment for over a quarter of participants. Between 4-20 weeks improvement was observed for two participants. Equal numbers experienced worsening distress.

These findings provide partial support for the hypothesis that psychological distress will improve over the post-treatment period observed. Yet the patterns of improvement and worsening for individuals in this study were not simply linear.

Table 23: Reliable Change Indices (RCIs) For Psychological Distress (GHQ-12) Scores From 4-12 Weeks, 12 -20 Weeks, and 4-20 Weeks Post-Treatment

	4-12 weeks	12-20 weeks	4-20 weeks
Positive reliable	3 (20.00%)	0 (0.00%)	2 (13.33%)
Negative reliable	0 (0.00%)	4 (26.67%)	2 (13.33%)
No reliable change	12 (80.00%)	11 (73.33%)	11 (73.33%)

Note. Positive reliable change refers to distress scores improving during the time period; negative reliable change refers to distress scores worsening

5.2.8.2 Decreased S-anxiety, S-depression, S-anger, and Increased S-curiosity over the post-treatment period (ii).

S-anxiety levels were explored. It was anticipated that scores would decreases over the post-treatment period. Scores indicated that there was not a statistically significant change across time (Table 22); however moderate effect sizes were found. Figure 6

depicts a decrease in S-anxiety between 4-12 weeks (baseline to T1), with a slight increase/worsening in S-anxiety levels between 12-20 weeks (T1 to T2). No covariates were identified for this variable.

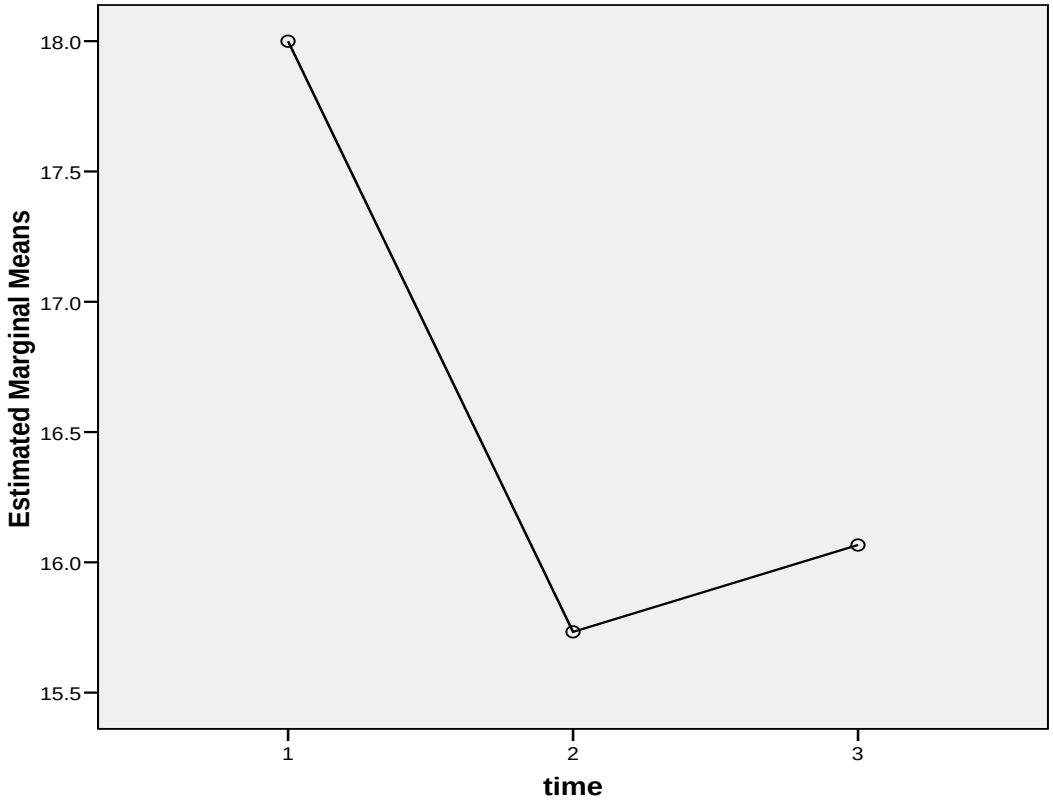


Figure 6: S-anxiety experienced by early stage breast cancer patients at 4- weeks (baseline), 12-weeks (T1), and 20-weeks (T2) post-treatment

The slight rebound between 12- 20 weeks was further explored by calculation of RCIs (Table 24).

Table 24: Reliable Change Indices (RCIs) For S-Anxiety (STPI) Scores From 4 -12 Weeks, 12 -20 Weeks and 4- 20 Weeks Post-Treatment

	4-12 weeks	12-20 weeks	4- 20 weeks
Positive reliable	3 (20.00%)	4 (26.67%)	3 (20.00%)
Negative reliable	0 (0.00%)	3 (20.00%)	1 (6.67 %)
No reliable change	12 (80.00%)	8 (53.33%)	11 (73.33%)

Note. Positive reliable change refers to S-anxiety scores improving during the time period; negative reliable change refers to S-anxiety scores worsening

These results indicate that there was little reliable change observed across 4 to 12 and 4 -20 weeks post-treatment. When improvement in S-anxiety scores was observed this occurred between 4-12 weeks post-treatment for 20%, and 12-20 weeks for 26% of this sample. In contrast worsening in S-anxiety scores was observed between 12-20 weeks post-treatment for 20% of participants. This suggests trajectories for individuals differed.

On the other hand exploration of S-curiosity (Table 21 and 22) anticipated improvement would be observed in the post-treatment period. Yet there was no statistically significant change identified across time, however again, a moderate effect was found. The trend was for an increase in S-curiosity scores between 4- 12 weeks (baseline to T1), followed by a slight decrease between 12-20 weeks (T1 to T2) (Figure 7). No covariates were identified for S-curiosity.

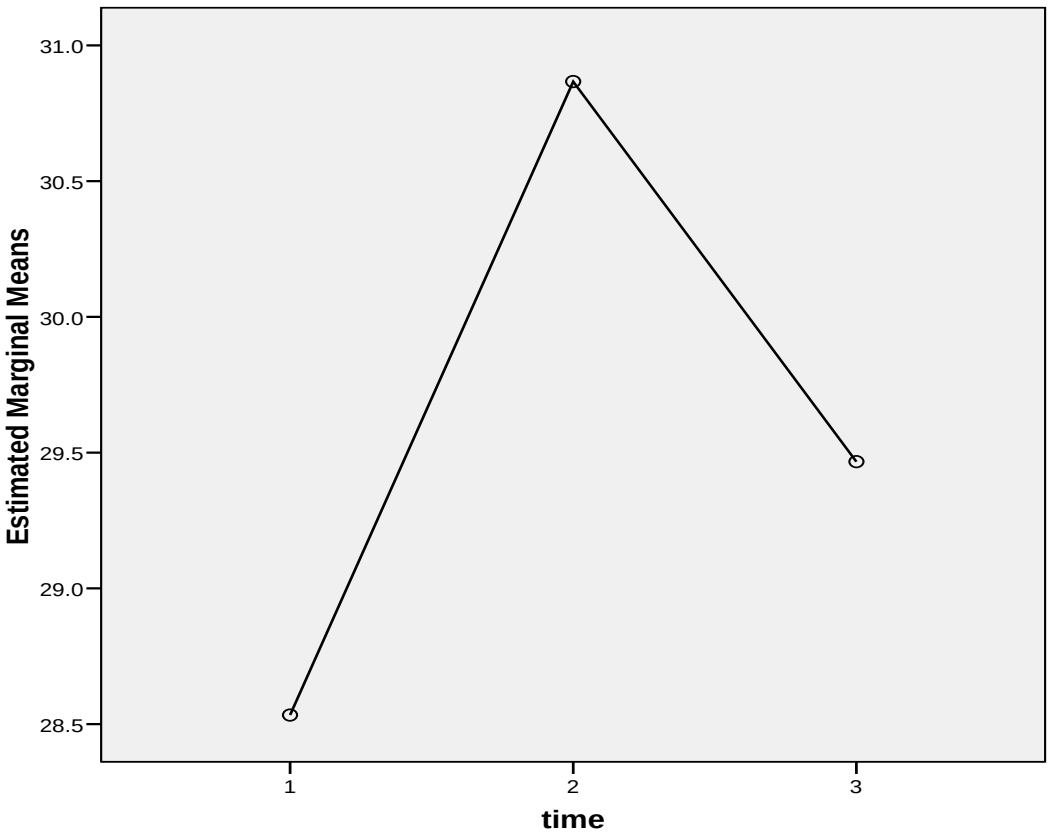


Figure 7 : S-curiosity experienced by early stage breast cancer patients at 4-weeks (baseline), 12-weeks (T1) and 20-weeks (T2) post-treatment

RCIs were calculated (Table 25) and results indicate that there was little reliable change observed across 4-12 weeks post-treatment. Worsening in S-curiosity scores was observed for only one participant between 4-12 weeks post-treatment. However between 12-20 weeks scores worsened for over 30% of the sample. In contrast improvement in S-curiosity scores was observed for 4 participants between 4-20 weeks post-treatment.

Table 25: Reliable Change Indices (RCIs) For S-Curiosity (STPI) Scores From 4-12-Weeks, 12-20 Weeks and 4-20-Weeks Post-Treatment

	4-12 weeks	12-20 weeks	4-20 weeks
Positive reliable	2 (13.33%)	2 (13.33%)	4 (26.67%)
Negative reliable	1 (6.67%)	5 (33.33%)	2 (13.33%)
No reliable change	12 (80.00%)	8 (53.33%)	9 (60.00%)

Note. Positive reliable change refers to S-curiosity scores improving during the time period; negative reliable change refers to S-curiosity scores worsening

S-depression scores across the post-treatment period indicated that there was no significant change across time for this variable; furthermore the magnitude of change was less than .01, the lowest possible reference point for a small effect size (see Table 22). This suggests that no change was evident in S-depression over time (Figure 8).

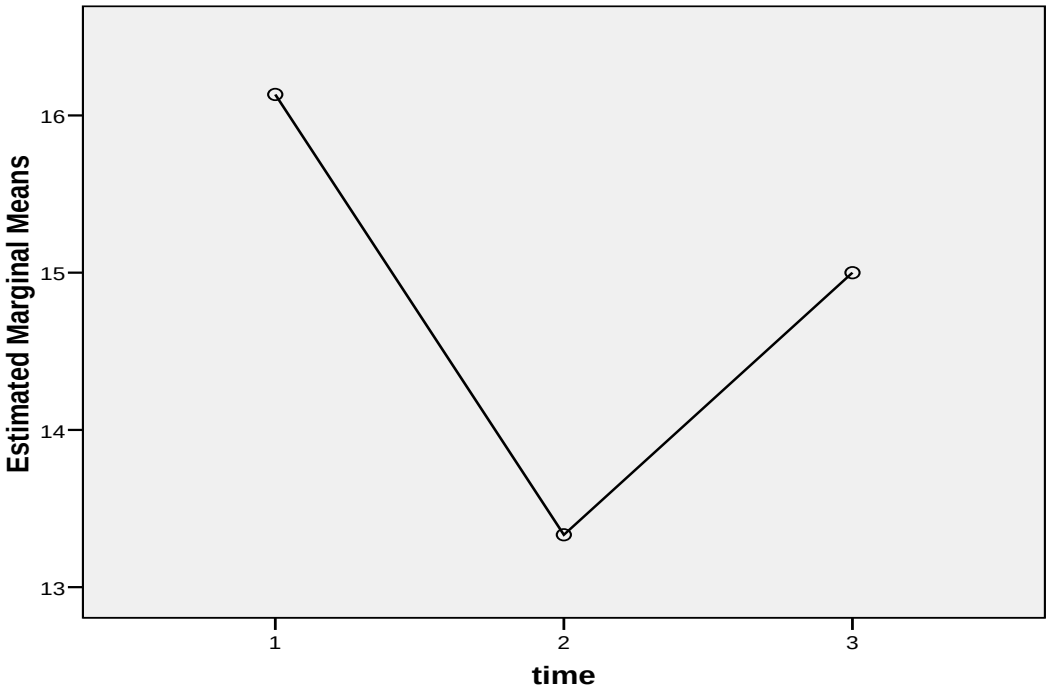


Figure 8: S-depression experienced by early stage breast cancer patients at 4-weeks (baseline), 12-weeks (T1), and 20-weeks (T2) post-treatment

Calculation of RCIs (Table 26) indicated little reliable change observed across 4-12 and 12-20 weeks post-treatment. When reliable improvement in S-depression scores was observed this predominantly occurred between 4-12-weeks post-treatment (20% of the sample). In contrast worsening in S-depression scores was observed between 12-20 weeks post-treatment for over a quarter of participants. Between 4-20 weeks improvement was observed for 4 participants and 2 participants experienced worsening S-depression.

Table 26: Reliable Change Indices (RCIs) For S-Depression (STPI) Scores From 4-12 Weeks, 12-20 Weeks and 4-20-Weeks Post-Treatment

	4-12 weeks	12-20 weeks	4-20 weeks
Positive reliable	5 (33.33%)	1 (6.67%)	4 (26.67%)
Negative reliable	0 (0.00%)	4 (26.67%)	2 (13.33%)
No reliable change	10 (66.67%)	10 (66.67%)	9 (60.00%)

Note. Positive reliable change refers to S-depression scores improving during the time period; negative reliable change refers to S-depression scores worsening

Covariates included in the S-depression repeated measures analysis, based on univariate investigations, were endocrine medication use and hazardous alcohol use. Results of this analysis indicated (1) that use of endocrine medication did not uniquely, significantly adjust S-depression scores, $F(1.48, 11) = 1.85$, $p = .19$, partial $\eta^2 = .13$, however (2) alcohol use did uniquely, significantly adjust scores, $F(1.48, 11) = 7.58$, $p = .01$, partial $\eta^2 = .39$. Both covariates evidenced large (or near large) effects.

Exploration of S-anger scores in the current sample observed on the whole very low scores across all assessments. S-anger scores across 4-weeks (baseline), 12-weeks (T1), and 20-weeks (T2) post-treatment, reflected no statistically significant change (Figure 9). However a large effect size was observed.

Having undergone chemotherapy was identified as a potential covariate, but did not appear to contribute unique, significant adjustment to S-anger scores. However, a very large effect size was apparent $F (1.26, 11) = 3.10$, $p = .09$, partial $\eta^2 = .21$.

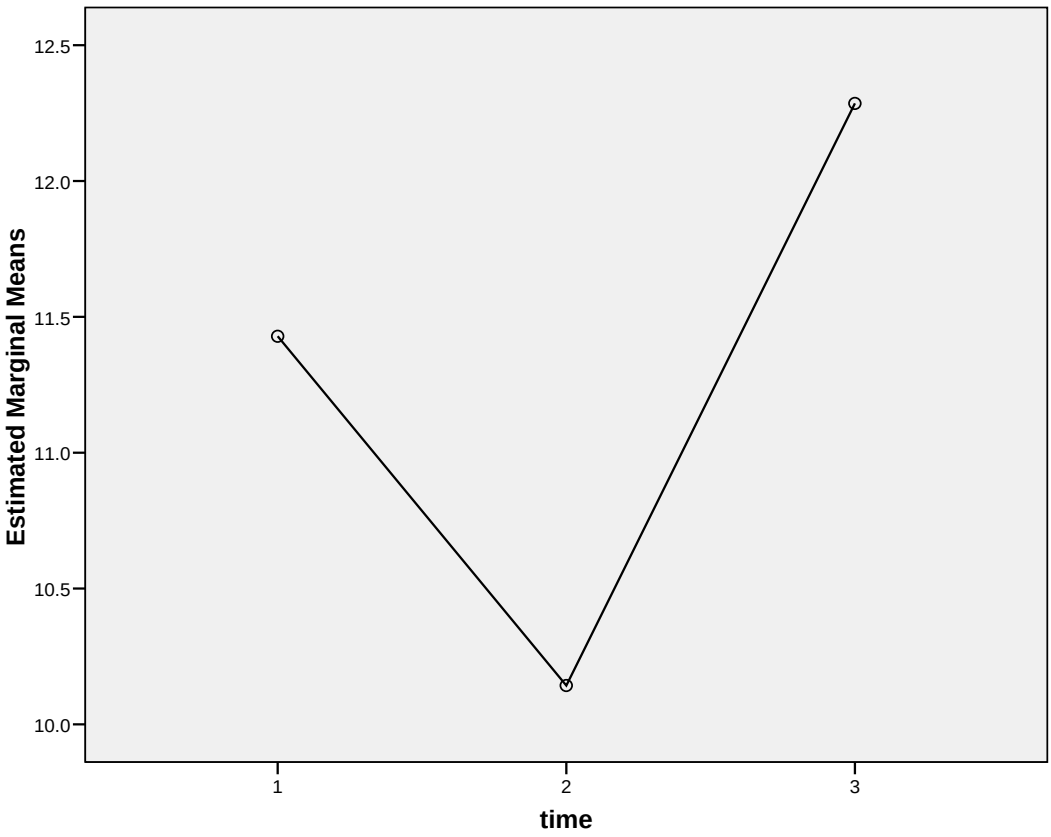


Figure 9: S-anger experienced by early stage breast cancer patients at 4-weeks (baseline), 12-weeks (T1), and 20-weeks (T2) post-treatment

RCIs were calculated (Table 27) and these results indicate that there was little reliable change observed across 4 to 12 post-treatment, with only 2 participants showing improvement. Minimal improvement in S-anger scores was observed 12 to 20 (T1 to T2), and 4 -20 weeks (baseline to T2) post-treatment. In contrast worsening in S-anger scores was observed between 12 to 20 weeks post-treatment (T1 to T2), for 40% of participants. This finding does not support the hypothesis that S-anger would decrease over time.

Table 27: Reliable Change Indices (RCIs) For S-Anger (STPI) Scores From 4-12 Weeks, 12-20 Weeks and 4-20 Weeks Post-Treatment

	4-12 weeks	12-20 weeks	4-20 weeks
Positive reliable	2 (13.33%)	1 (6.67%)	1 (6.67%)
Negative reliable	0 (0.00%)	6 (40.00%)	2 (13.33%)
No reliable change	13 (86.67%)	8 (53.33%)	12 (80.00%)

Note. Positive reliable change refers to S-anger scores improving during the time period; negative reliable change refers to S-anger scores worsening

5.2.8.3 Loneliness over the post-treatment period (iii).

Loneliness was explored using the UCLA- loneliness scale, version 3. Higher scores indicated an increased experience of loneliness. A directional (improvement or worsening) hypothesis for this variable was not specified, as literature in Chapter 3 observed both increases and decreases in social needs in the post-treatment period. Yet the observed changes in self reported loneliness levels, 4-weeks (baseline), 12-weeks (T1), and 20-weeks (T2) post-treatment, were not significant (Table 21 & 22). In support of this finding only a small effect size was reported $F (1.5, 11) = 1.4$, $p = .81$, partial $\eta^2 = .01$. No covariates were identified for this variable. In short, loneliness levels varied between individuals however remained fairly constant across this post-treatment period (Figure 10).

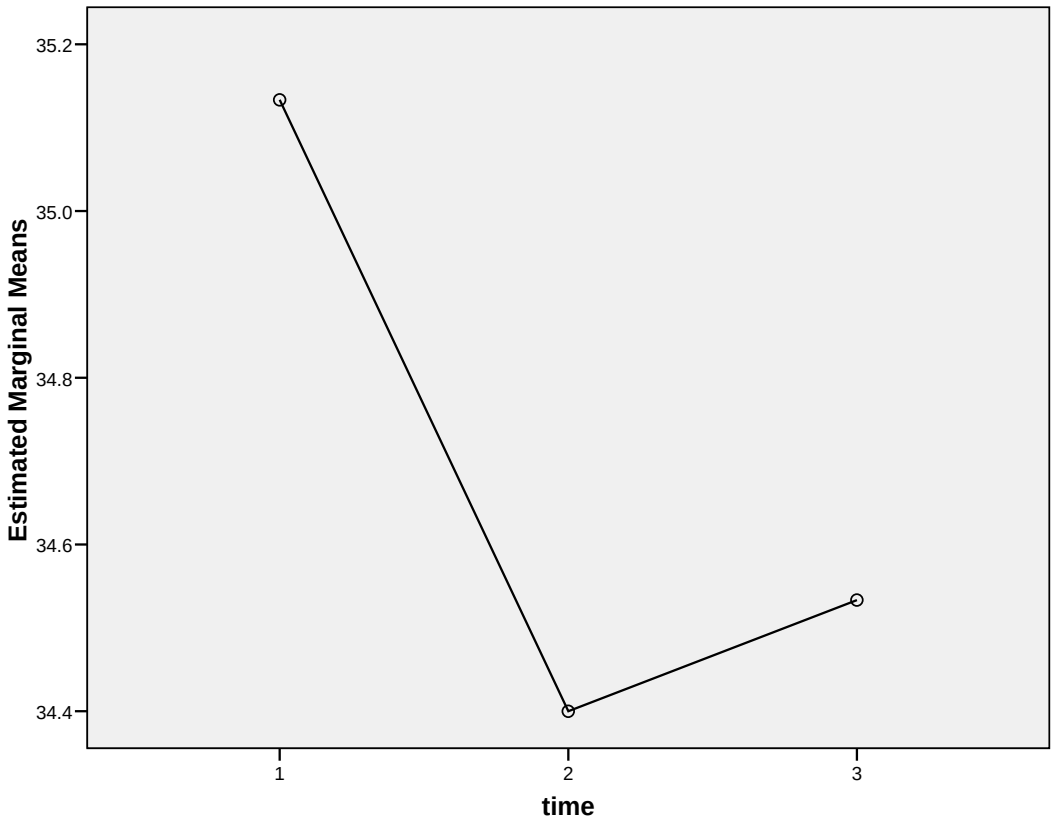


Figure 10: Loneliness experienced by early stage breast cancer patients at 4-weeks (baseline), 12-weeks (T1), and 20-weeks (T2) post-treatment

RCIs were calculated to determine positive, negative, and no change without measurement error (Table 28). These results indicate that there was very little reliable change observed across post –treatment assessments. When reliable improvement in distress scores was observed this only occurred between 4-12 weeks (baseline to T1) for only one participant, and for 2 participants between 12-20 weeks (T1 to T2). In contrast worsening loneliness scores was observed between 12- 20 weeks (T1 to T2) post-treatment. Between 4-20 week assessments, reliable improvement and worsening was observed for 2 participants respectively. These findings provide little evidence of

improvement/worsening, but rather inter-individual differences in social needs over this period.

Table 28: Reliable Change Indices (RCIs) For Loneliness (UCLA) Scores From 4-12 Weeks, 12-20 Weeks, and 4-20 Weeks Post-Treatment

	4-12 weeks	12-20 weeks	4-20 weeks
Positive reliable	1 (6.67%)	2 (13.33%)	2 (13.33%)
Negative reliable	0 (0.00%)	2 (13.33%)	2 (13.33%)
No reliable change	14 (93.33%)	11 (73.33%)	11 (73.33%)

Note. Positive reliable change refers to Loneliness scores improving during the time period; negative reliable change refers to Loneliness scores worsening

5.2.8.4 Mental adjustment to cancer over the post-treatment period (iv).

Psychological responses associated with the experience of cancer were explored also at 4-weeks (baseline), 12-weeks (T1), and 20-weeks (T2) post-treatment. This was done using the Mental Adjustment to Cancer (MAC) scale, comprised of five subscales. The results for each subscale are presented in two separate sections, defined by the expected direction of change in these coping styles (i.e., improvement or worsening). The relevance of covariates for specific variables will be discussed following each variable within each section.

5.2.8.4.1 Increased Fighting Spirit response.

It was anticipated that FS scores would increase over time. Observed scores for FS did not evidence statistically significant change between (baseline), 4-12 weeks, (T1) 12-20

weeks, and (T2) 4-20- weeks. Yet a moderate effect size was reported (Table 22). Graphical depiction (Figure 11) suggests there was a trend for a decrease between 4-12 weeks (baseline to T1), with an increase observed between 12-20 weeks (T1 to T2).

Two covariates were identified as contributing to Fighting Spirit scores. Both related to the intake of medications, specifically the taking of Immunomodulator medications [F (1.14, 11) =.91, p = .37, partial η^2 = .08] and cardiovascular medications [F (1.14, 11) =.02, p = .92, partial η^2 = .00]. Neither contributed unique significant adjustment to the Fighting Spirit variable, accordingly small effect sizes were observed for cardiovascular medication.

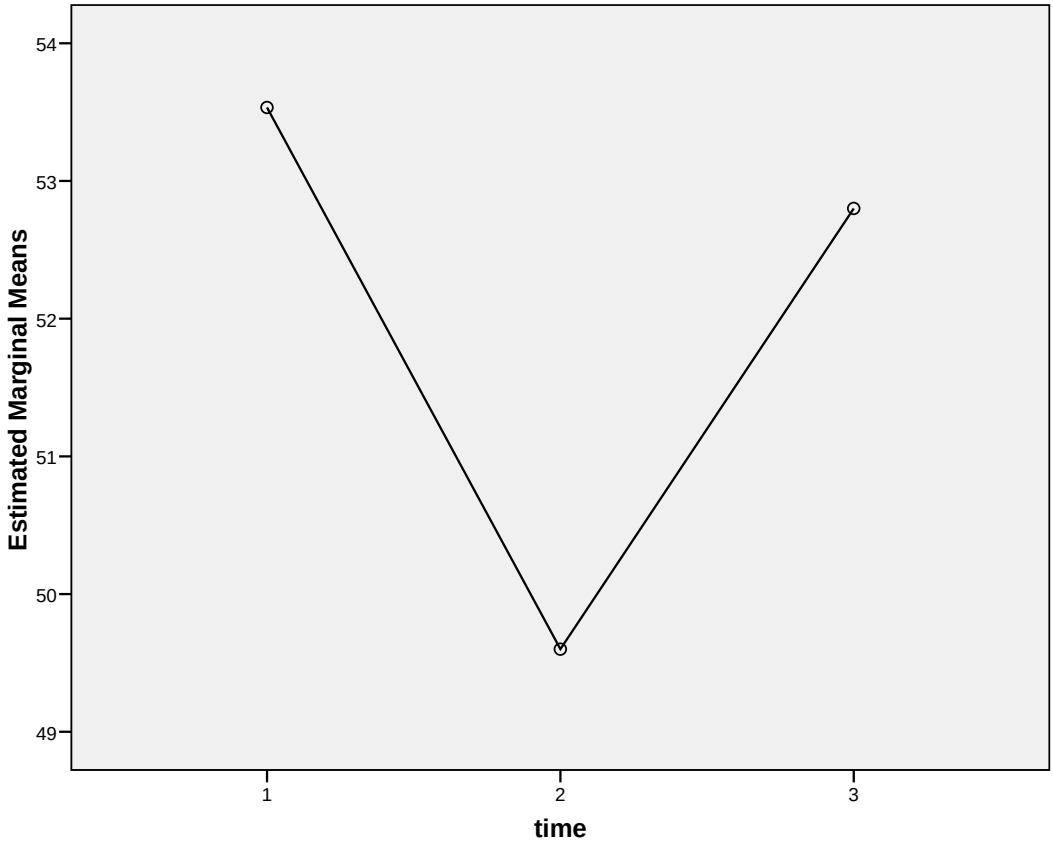


Figure 11: Fighting Spirit scores of early stage breast cancer patients at 4-weeks (baseline), 12-weeks (T1), and 20-weeks (T2) post-treatment

RCIs were calculated to determine positive, negative, and no change without measurement error (Table 29). These results indicate that there was little reliable change observed for FS across 4-12 and 4-20 weeks post-treatment. When reliable improvement in FS scores was observed this occurred between 4-12 weeks post-treatment for two participants, and for one participant 12-20 weeks post-treatment. Worsening FS scores were only observed between 12-20 weeks for two participants in this sample.

Table 29: Reliable Change Indices (RCIs) For Fighting Spirit (FS: MAC) Scores From 4-12 Weeks, 12-20-Weeks, and 4-20 Weeks Post-Treatment

	4-12 weeks	12-20 weeks	4-20 weeks
Positive reliable	2 (13.33%)	1 (6.67%)	1 (6.67%)
Negative reliable	0 (0.00%)	2 (13.33%)	0 (0.00%)
No reliable change	13 (86.67%)	12 (80.00%)	14 (93.33%)

Note. Positive reliable change refers to FS scores improving during the time period; negative reliable change refers to FS scores worsening

5.2.8.4.2 Decreased Helpless/Hopeless, Anxious Preoccupation, Fatalistic, and Avoidant coping responses.

It was hypothesised that negative coping styles would decrease (improve) over the course of time post-treatment. Change across time for the psychological response of Helpless/Hopeless coping, a depressive coping style, were explored. No significant changes were reported across the three assessment points in the post-treatment time frame (Table 21 & 22). However a moderate- to- large effect size was observed. Graphical depiction (Figure 12) suggests a decrease in this style of coping between 4-12 weeks (baseline to T1), with a slight worsening between 12-20 weeks (T1 to T2). No covariates were identified for this variable.

RCIs were calculated to determine positive, negative, and no change without measurement error (Table 30). These results indicate that there was little reliable change observed across 4-12, 12-20, and 4 -20 weeks post-treatment. When improvement in HH scores was observed this occurred between 4-12 weeks post-treatment for 20% of the sample, and between 12-20 weeks for 13.33% of the sample. In contrast worsening in HH

scores was only observed between 12-20 weeks post-treatment, and only occurred for two participants in this sample.

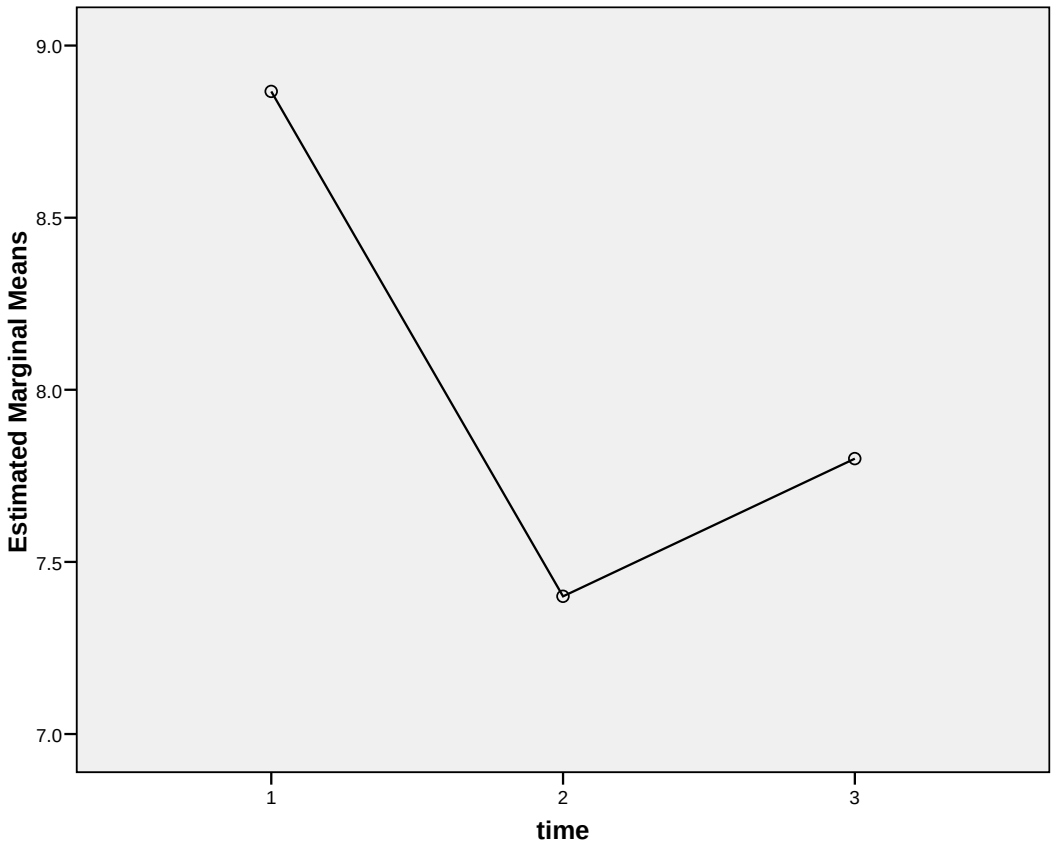


Figure 12: Helpless/Hopeless scores of early stage breast cancer patients at 4-weeks (baseline), 12-weeks (T1), and 20-weeks (T2) post-treatment

Table 30: Reliable Change Indices (RCIs) For Helpless Hopeless (HH: MAC) Scores From 4-12 Weeks, 12 -20 Weeks and 4-20 Weeks Post-Treatment

	4-12 weeks	12-20 weeks	4-20 weeks
Positive reliable	3 (20.00%)	2 (13.33%)	2 (13.33%)
Negative reliable	0 (0.00%)	2 (13.33%)	0 (0.00%)
No reliable change	12 (80.00%)	11 (73.33%)	13 (86.67%)

Note. Positive reliable change refers to HH scores improving during the time period; negative reliable change refers to HH scores worsening

Like HH coping responses across this five month post-treatment timeframe there was no evidence of statistically significant change (Table 21 & 22) in AP scores across the three assessments. Accordingly the reported effect size was small, with graphical depiction (Figure 13) also reflecting the lack of change. Only a slight decrease in scores was observed between 4-12 weeks (baseline to T1), followed by a slight increase between 12-20 weeks (T1 to T2). Overall change was minimal. No covariates were implicated in this analysis.

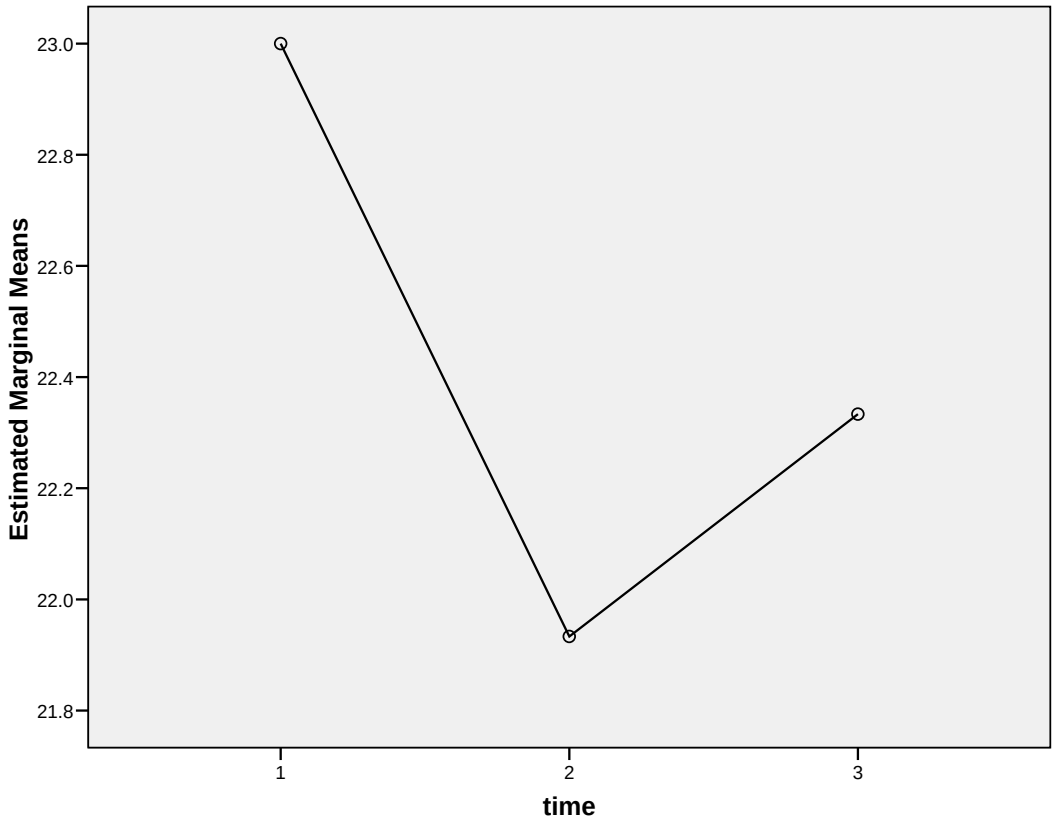


Figure 13: Anxious Preoccupation scores of early stage breast cancer patients at 4-weeks (baseline), 12-weeks (T1), and 20-weeks (T2) post-treatment

Calculation of RCIs (Table 31) also indicated that there was little reliable change observed across 4-12, 12-20, and 4 -20 weeks post-treatment. When reliable improvement in AP scores was observed this only occurred between 4-12 weeks post-treatment for one participant in the sample. In contrast worsening in AP scores was observed between 4 -20 weeks post-treatment for one participant.

Table 31: Reliable Change Indices (RCIs) For Anxious Preoccupation (AP: MAC) Scores From 4-12 Weeks, 12-20 Weeks and 4-20 Weeks Post-Treatment

	4-12 weeks	12-20 weeks	4-20 weeks
Positive reliable	1 (6.67%)	0 (0.00%)	0 (0.00%)
Negative reliable	0 (0.00%)	0 (0.00%)	1 (6.67%)
No reliable change	14 (93.33%)	15 (100.00%)	14 (93.33%)

Note. Positive reliable change refers to AP scores improving during the time period; negative reliable change refers to AP scores worsening

Similarly the F psychological response to cancer evidenced no statistical significant change across the observed post-treatment period (Figure 14). Caution is required on interpretation of this lack of change as a moderate- to- large effect size was evidenced.

RCIs were calculated to determine positive, negative, and no change without measurement error (Table 32). These results indicate that there was little reliable change observed across all three periods, 4-12, 12-20, and 4 -20 weeks post-treatment. Improvement in F scores was observed for only one participant between 12-20 weeks. No worsening F scores were observed.

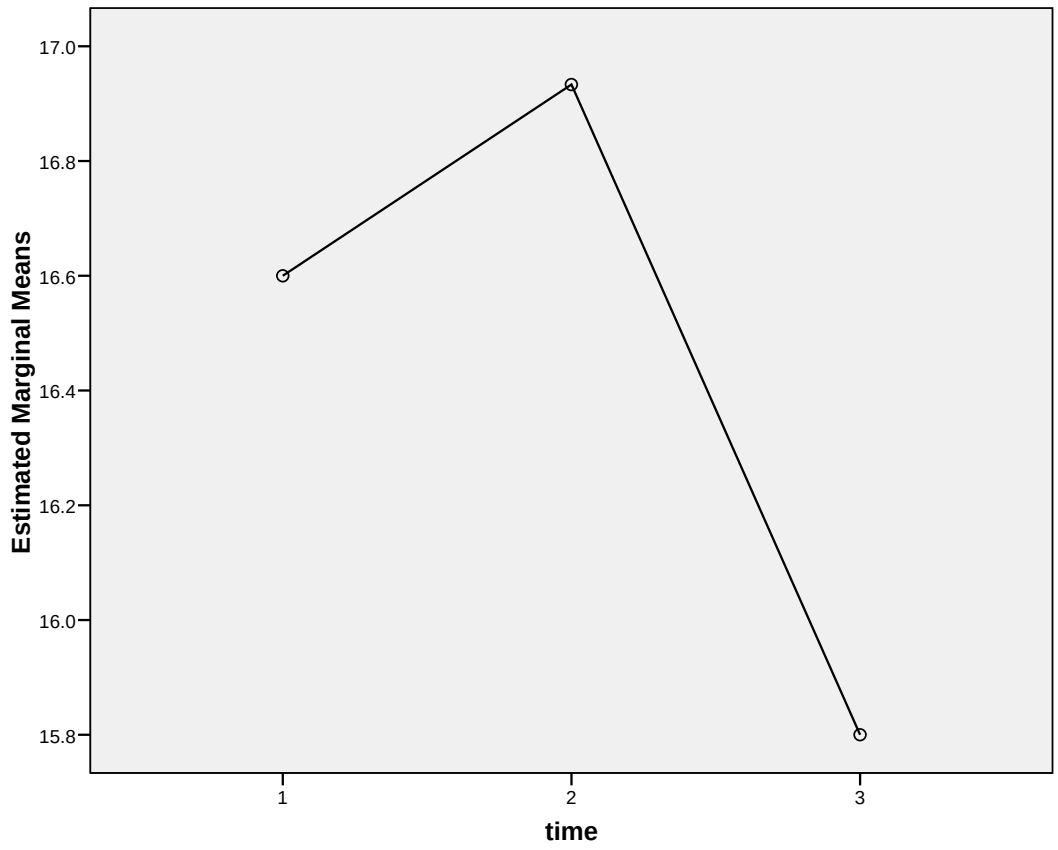


Figure 14: Fatalistic scores of early stage breast cancer patients at 4-weeks (baseline), 12-weeks (T1), and 20-weeks (T2) post-treatment

Table 32: Reliable Change Indices (RCIs) For Fatalistic Coping (F: MAC) Scores From 4-12 Weeks, 12-20-Weeks, and 4-20-Weeks Post-Treatment

	4-12 weeks	12-20 weeks	4-20 weeks
Positive reliable	0 (0.00%)	1 (6.67%)	0 (0.00%)
Negative reliable	0 (0.00%)	0 (0.00%)	0 (0.00%)
No reliable change	15 (100.00%)	14 (93.33%)	15 (100.00%)

Note. Positive reliable change refers to F scores improving during the time period; negative reliable change refers to F scores worsening

For A psychological responses to cancer (Table 21 & 22) there was no statistically significant change reported across the 16-week timeframe. However a moderate effect

size was apparent. Notably alcohol intake was identified as a covariate; it did not contribute any unique significant adjustment to the dependent variable [F (1.62, 11) = 2.37, p = .13, partial η^2 = .15], although a large effect size was evidenced. Health behaviours like hazardous alcohol consumption could be an indicator of more avoidant psychological responses in this population. This scale is comprised of only one item which makes RCI calculations impossible, but graphical depiction is of mean scores attained across the three assessment points suggests little change (Figure 15).

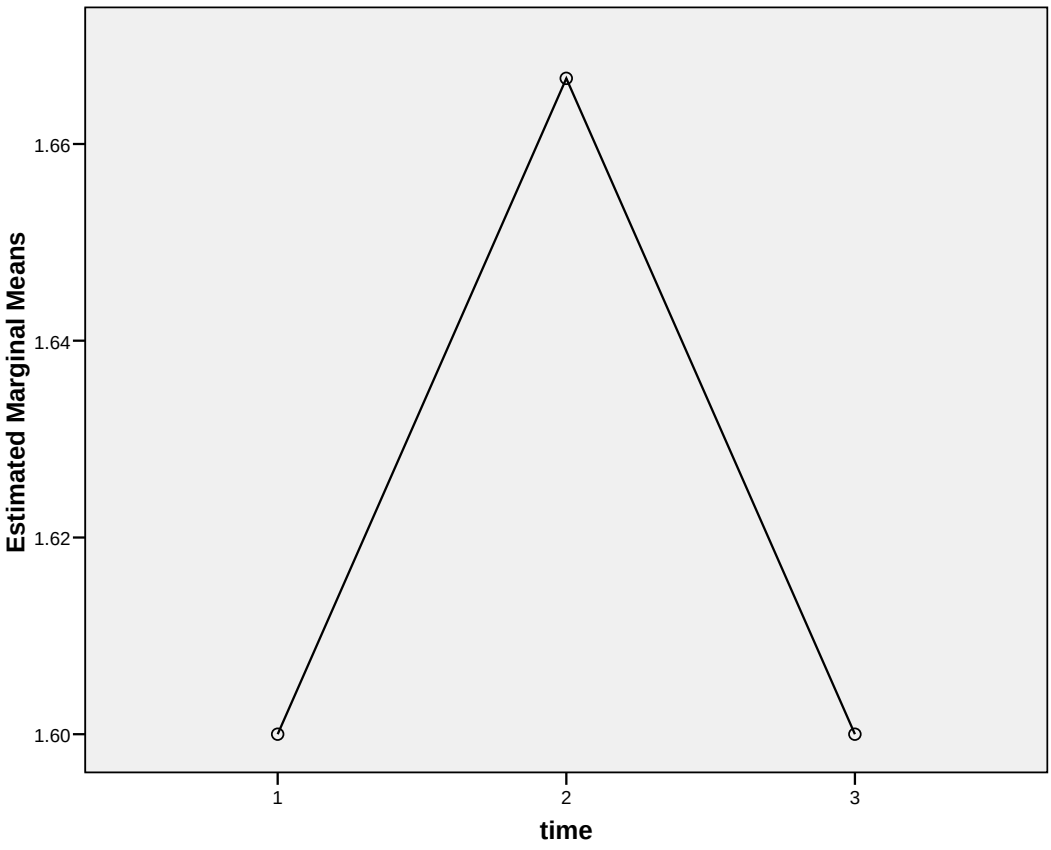


Figure 15: Avoidant coping scores of early stage breast cancer patients at 4-weeks (baseline), 12-weeks (T1), and 20-weeks (T2) post-treatment

5.2.8.5 Decreased cancer-specific trauma over the post-treatment period (v).

The literature exploring the experience of symptoms of posttraumatic stress in the post-treatment period has yielded disparate findings. In the current study using the IES-R measure changes across 4- (baseline), 12- (T1), and 20-weeks (T2) were not statistically significant (Table 21 & 22). Accordingly only a small effect size was evidenced. No covariates were identified. For this sample the experience of trauma symptoms was complex across the period of this study (Figure 16). RCIs were calculated to determine positive, negative, and no change without measurement error (Table 33).These results indicate that there was little reliable change observed across 4 -12 weeks post-treatment. When improvement in trauma scores was observed this occurred early in the post-treatment period between 4-12 weeks post-treatment for two participants. Similarly between 12-20 and 4-20 weeks improvement was observed for 20% of participants. In contrast no reliable negative (worsening) trauma scores were not experience 4 -12 weeks post treatment. Worsening in trauma scores was seen for 2 participants 12-20 and 4 -20 weeks post-treatment.

These findings provide partial support for the hypothesis, but also suggest improvement across time for trauma scores are not simply linear.

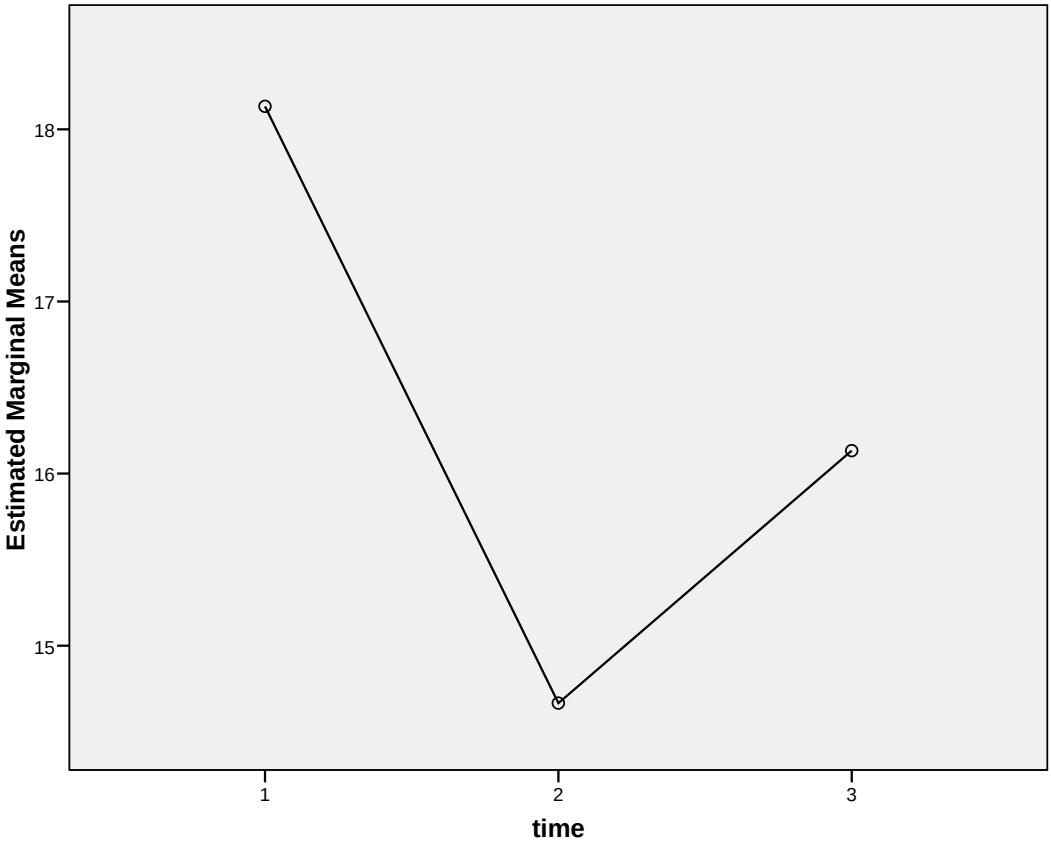


Figure 16: Trauma (IES-R) scores of early stage breast cancer patients at 4-weeks (baseline), 12-weeks (T1), and 20-weeks (T2) post-treatment

Table 33: Reliable Change Indices (RCIs) For Post Traumatic Stress Symptoms (IES-R) Scores From 4-12 Weeks, 12-20-Weeks, and 4-20 Weeks Post-Treatment

	4-12 weeks	12-20 weeks	4-20 weeks
Positive reliable	2 (13.33%)	3 (20.00%)	3 (20.00%)
Negative reliable	0 (100.00%)	2 (13.33%)	2 (13.33%)
No reliable change	13 (86.67%)	10 (66.67%)	10 (66.67%)

Note. Positive reliable change refers to Traumatic Stress symptom scores improving during the time period; negative reliable change refers to Traumatic Stress symptom scores worsening

5.2.9 Hypothesis 2b - pro-oxidant measures will improve over a 20-week post-treatment period.

As with the psychological variables presented above, a series of repeated measure ANOVAs were employed to determine whether biomarkers implicated in The Oxidative Model changed across time. Table 34 refers to pro-oxidant markers and includes means and standard deviations across the three time assessments. The second table (Table 35) includes F tests from repeated measures ANOVAs. Findings associated with change over time and covariates will be discussed for each individual variable.

Table 34: Pro-oxidant Measures: Means and Standard Deviations Across Time

Variable	Norms	Baseline M (SD)	Time 1 M (SD)	Time 2 M (SD)
NT	0.4-1.4 nmol/h/µgDNA	0.40(0.15)	0.39(0.18)	0.47(0.16)
VIT C	50-150 pg/ugDNA	30.25(13.51)	34.25(23.35)	43.00(32.91)
HCY	3-13 umol/L	8.91(2.56)	9.05(2.79)	8.52(2.93)
VIT B12	140-700pmol/L	371.50(101.1	400.79(146.49)	393.50(104.97)
FOLATE	5-45 nmol/L	24.74(9.41)	27.36(8.22)	27.40(9.64)
CHOL	<5.5 mmol/L	5.07(0.77)	5.24(0.89)	5.23(1.11)

Note. Biomarker Abbreviations: 5' –ectonucleotidase (NT), tissue ascorbate (VIT C), homocysteine (HCY), Vitamin B12 (VIT B12), Folate (FOLATE), Cholesterol (CHOL)

Table 35: Analyses of Variance (ANOVA) Change in Pro-oxidant Biomarker Levels over a 20-Week Post-Treatment Period

Variable	n	F	p	Partial η^2
NT*	12	0.73	.48	.07
VIT C	12	1.49	.25	.12
HCY*	13	0.75	.49	.06
VIT B12	14	0.64	.48	.05
FOLATE*	14	0.63	.54	.05
CHOL	14	0.48	.61	.04

Notes. Omnibus F tests reported

Partial η^2 = magnitude of change, .01 = small, .06 = moderate, and .14 = large;

* indicates that Analysis of Covariance performed

Biomarker Abbreviations: 5' –ectonucleotidase (NT), tissue ascorbate (VIT C), homocysteine (HCY), Vitamin B12 (VIT B12), Folate (FOLATE), Cholesterol (CHOL)

5.2.9.1 Increased 5'-ectonucleotidase (i).

NT levels were explored across 4- (baseline), 12- (T1), and 20-weeks (T2) post-treatment. As discussed in the baseline results, NT levels were around the low end of the reference range. A significant change across time was not observed. However a moderate-to- large effect size was reported. Figure 17 shows mean NT levels showing a slight increase across the three assessments, however individual levels suggest that there is a great deal of interindividual variation.

The use of respiratory medication was identified as a covariate. Although this covariate did not contribute a unique significant adjustment [$F(1.78, 10) = 0.65$, $p = .52$, partial $\eta^2 = .06$], a moderate effect size was evidenced.

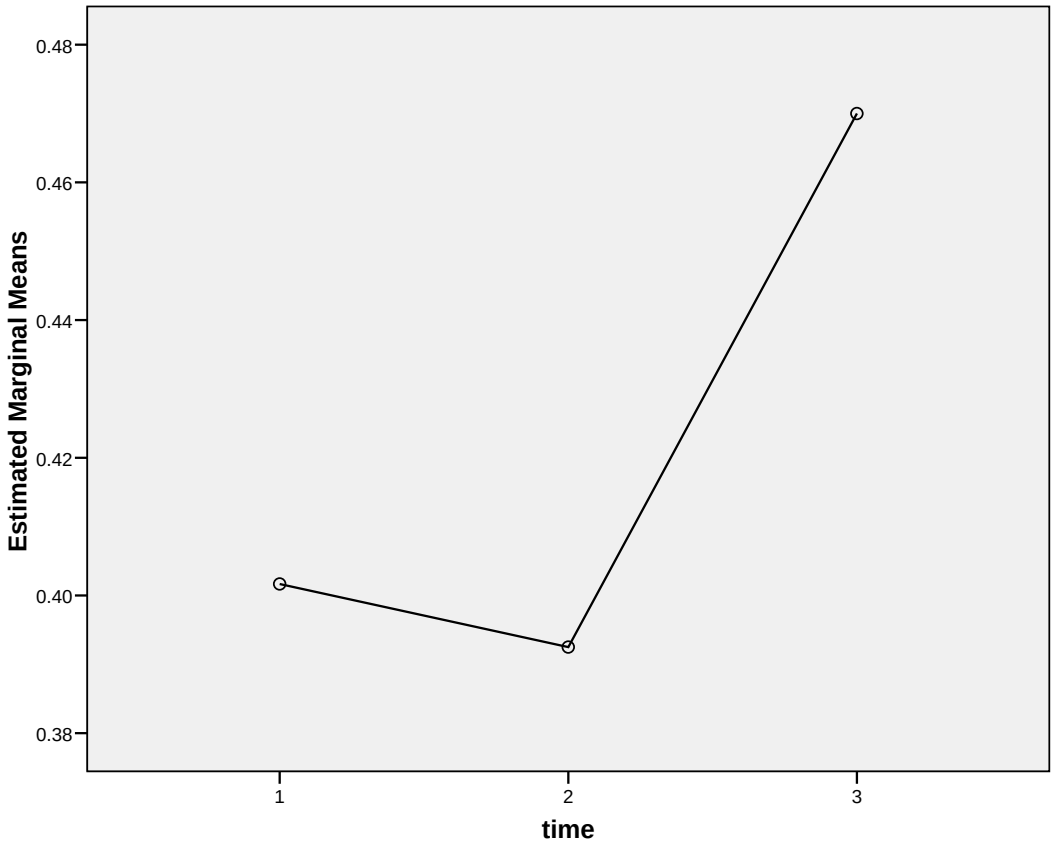


Figure 17: 5' –ectonucleotidase (NT: nmol/h/µgDNA) levels for early stage breast cancer patients at 4-weeks (baseline), 12-weeks (T1), and 20-weeks (T2) post-treatment

5.2.9.2 Increased tissue ascorbate (ii).

Like NT, VIT C levels across the post-treatment period were also around the lower end of the reference range. Change across the post-treatment period for VIT C levels were not significant (Table 34 & 35), however a moderate- to- large effect size was evidenced, so caution is suggested with this interpretation. Trends suggest that there was a sustained increase across the 4-, 12-, and 20-week period (Figure 18). No covariates were detected for inclusion in this analysis.

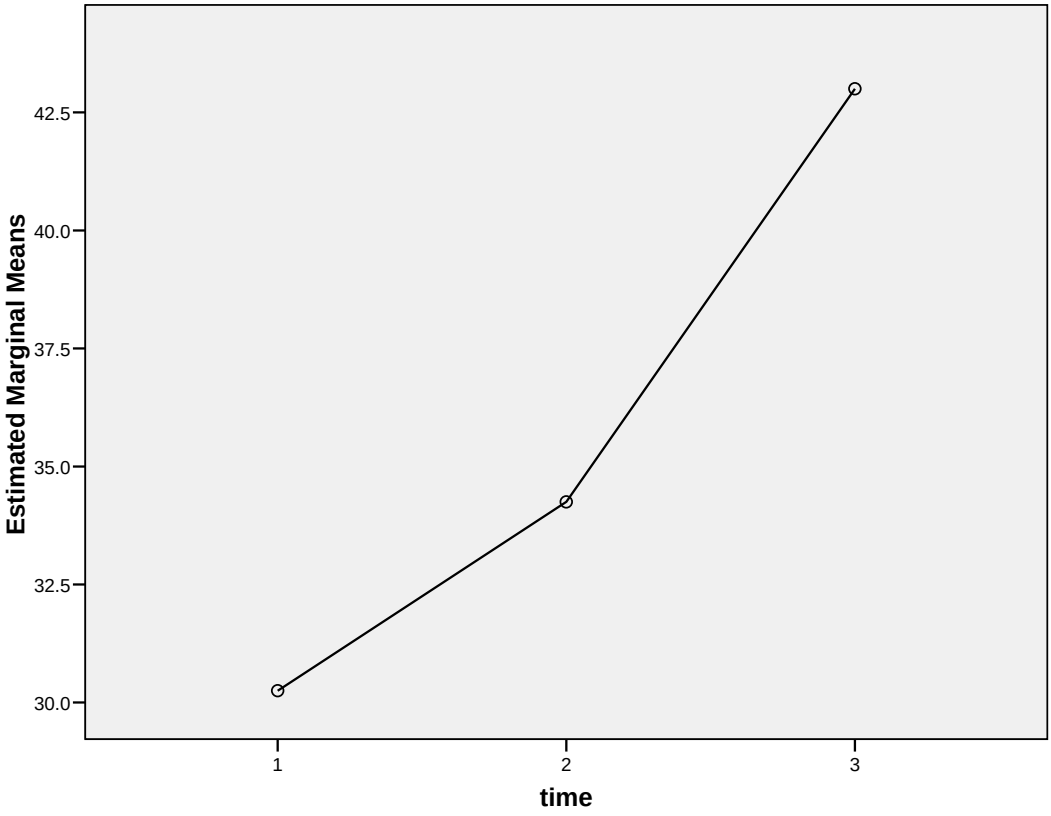


Figure 18: Tissue ascorbate (VIT C: pg/ugDNA) levels for early stage breast cancer patients at 4-weeks (baseline), 12-weeks (T1), and 20-weeks (T2) post-treatment

5.2.9.3 Decreased homocysteine (iii).

HCY levels across the three assesssments in the post-treatment period evidenced no significant change. Higher levels of HCY indicate a pro-oxidant internal state. Graphical representation (Figure 19) suggests HCY levels were stable across assessment points, however observed levels at baseline were already at the high end of the normative reference range. Given the observed lack of statistically significant change over

assesments this suggests the pro-oxidant state remained over the 16-week period. A moderate effect size was observed.

The consumption of vitamins high in antioxidants was identified as a covariate and included in the aforementioned analysis. However it did not contribute unique significant adjustment to the dependent variable [$F(1.98, 10) = 0.26$, $p = .78$, partial $\eta^2 = .02$], and only a small effect size was evidenced.

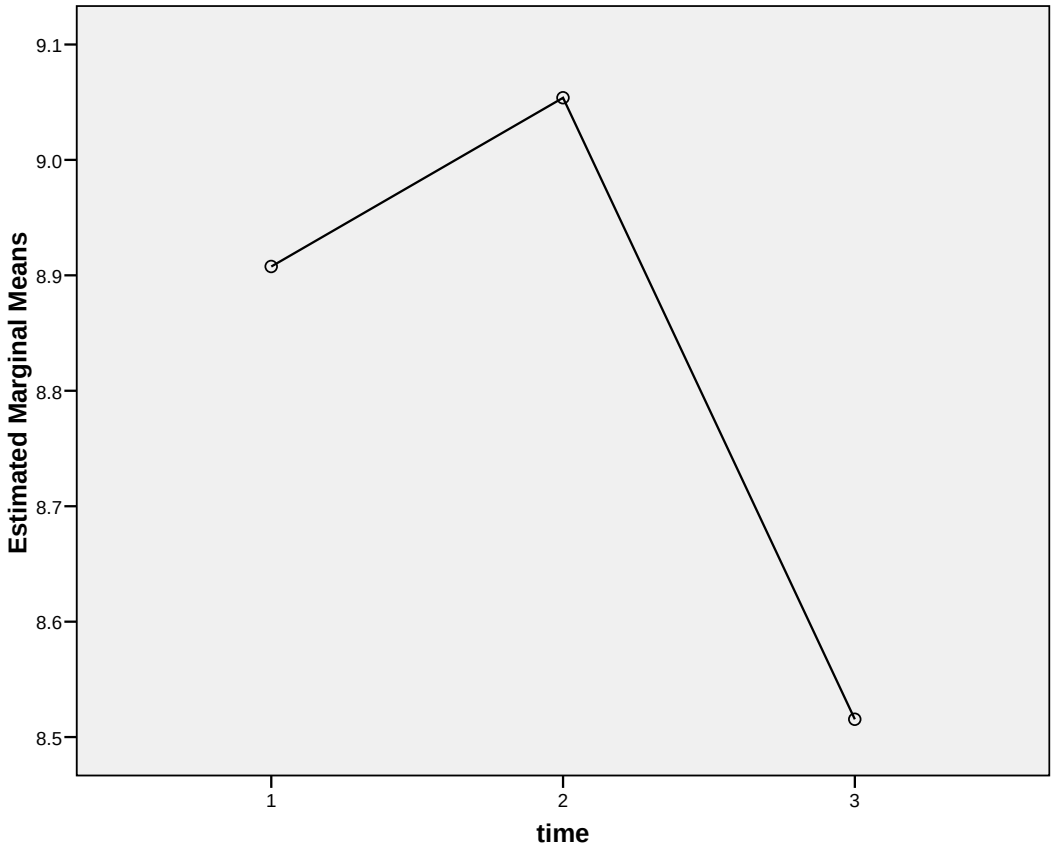


Figure 19: Homocysteine (HCY: umol/L) levels for early stage breast cancer patients at 4-weeks (baseline), 12-weeks (T1), and 20-weeks (T2) post-treatment

5.2.9.4 Increased vitamin B12 & Folate (iv).

Levels of VIT B12 were explored across the three assessments. VIT B12 is considered important in the synthesis of HCY. No significant changes were observed across the 16-week post-treatment period under investigation (Figure 20). A small- to-moderate effect size was reported. No covariates were included in this analysis.

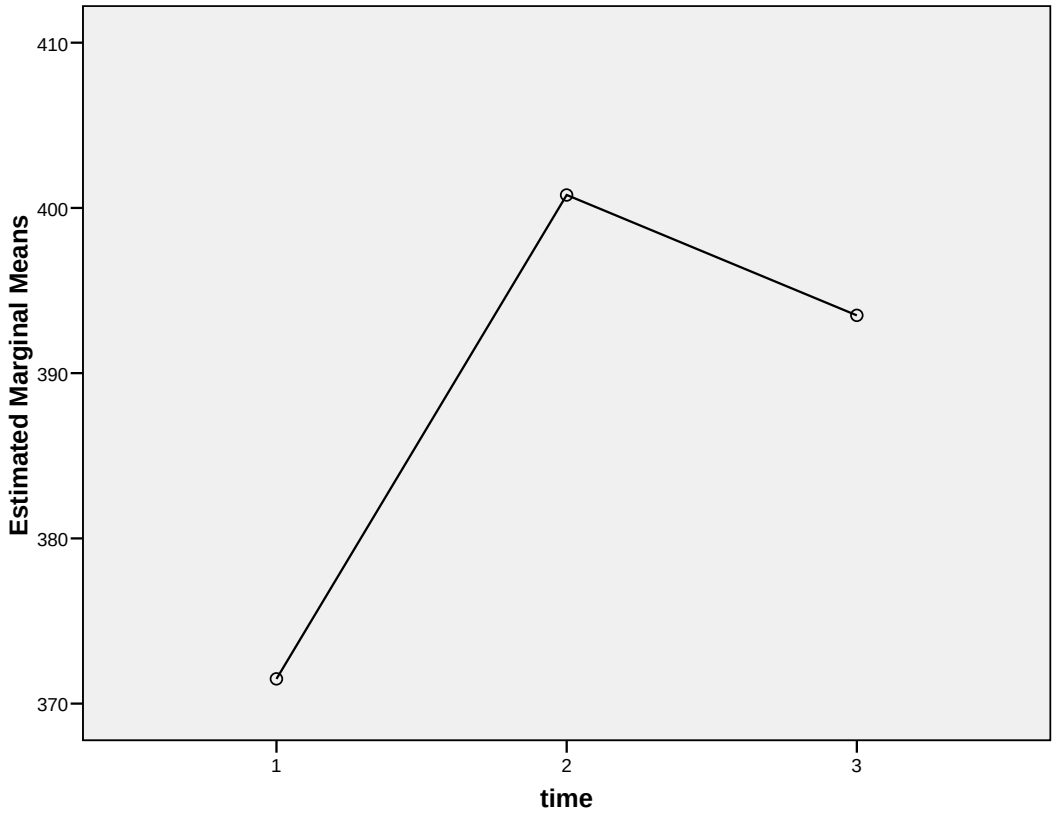


Figure 20: Vitamin B12 (VIT B12: mol/L) levels for early stage breast cancer patients at 4-weeks (baseline), 12-weeks (T1), and 20-weeks (T2) post-treatment

FOLATE levels were also explored (Figure 21). Like VIT B12 , FOLATE is implicated in the synthesis of HCY. No significant changes were observed across the three assessments

over the 16-week period for FOLATE levels (Table 34 & 35). A small- to-moderate effect size was reported.

The use of cardivascular medication was identified as a potential confounding variable. However it did not appear to contribute unique significant adjustment to FOLATE levels [F(1.92, 11) = 0.79, p = .46, partial η^2= .06] , although a moderate effect size was evidenced.

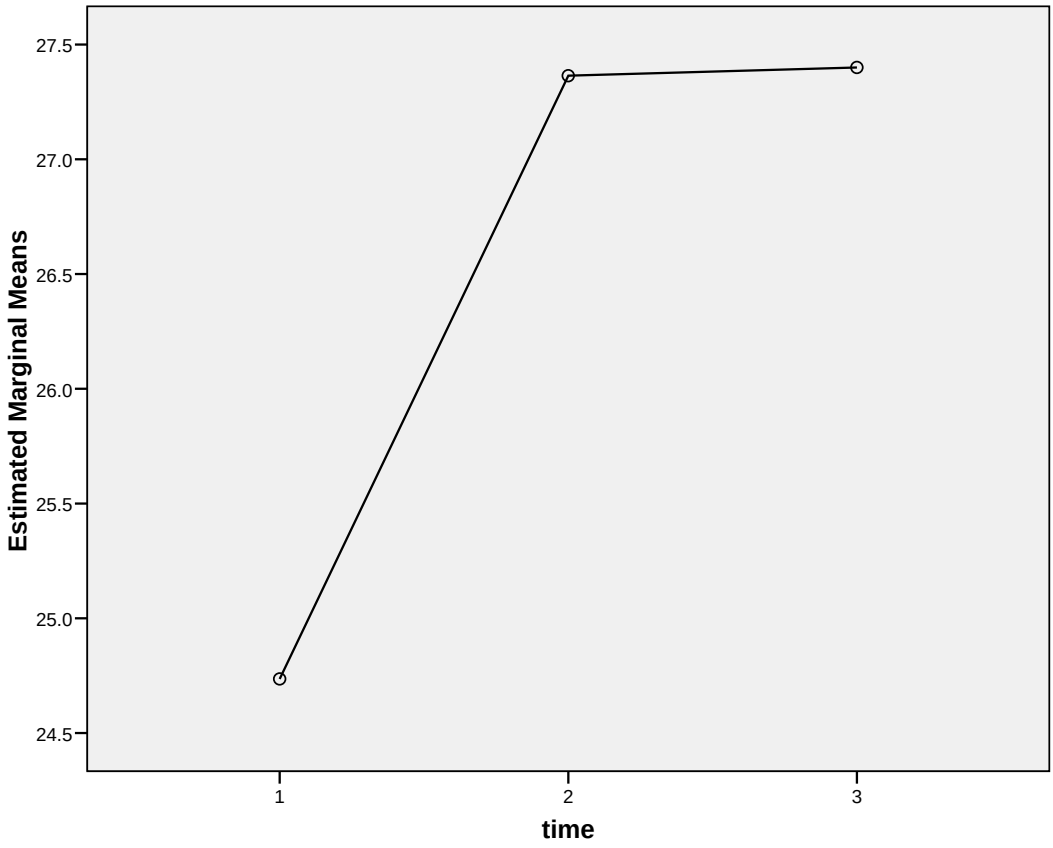


Figure 21: FOLATE (nmol/L) levels for early stage breast cancer patients at 4-weeks (baseline), 12-weeks (T1), and 20-weeks (T2) post-treatment

5.2.9.5 Decreased cholesterol (v).

Cholesterol levels for this sample were around the high end of the reference range. Higher levels indicate a greater risk of cardiovascular incidence. No significant change across time was observed for cholesterol levels in this sample across the 16-week post-treatment period. A small-to-moderate effect size was evidenced. No covariates were included in this analyses based on prior univariate exploration. Figure 22 illustrates a slight worsening in levels between 4-12 weeks, this level remained constant between 12-20 weeks post-treatment.

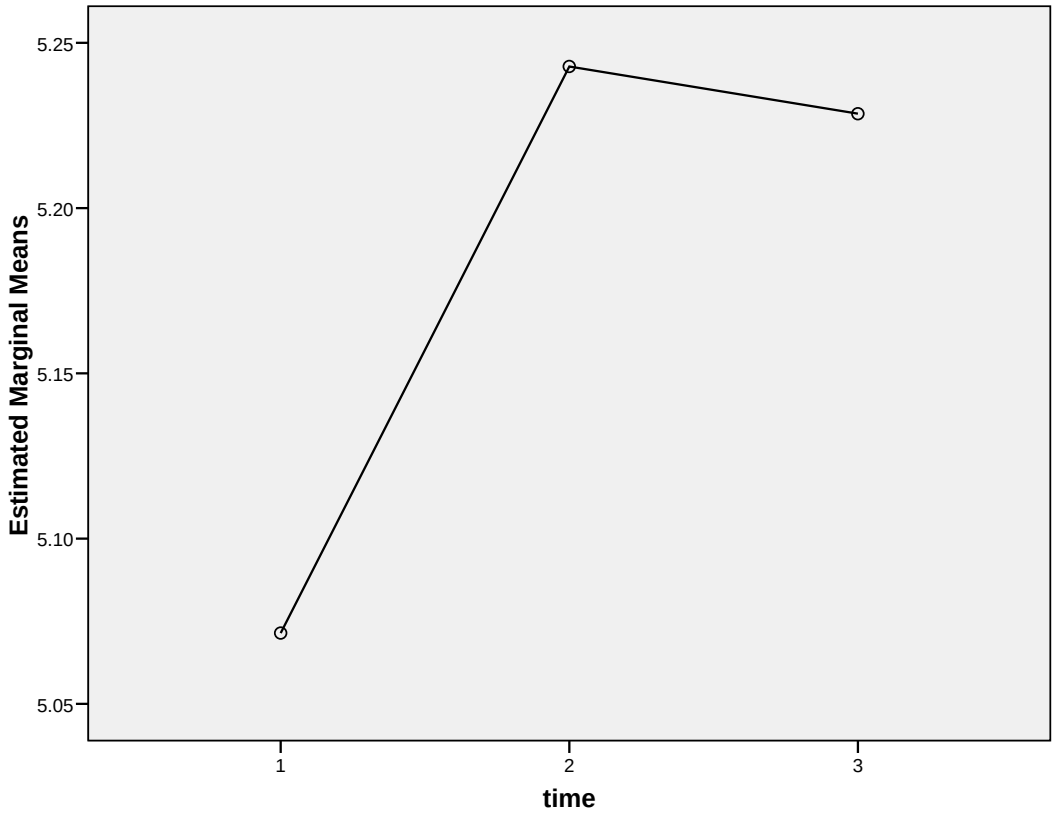


Figure 22: Cholesterol (CHOL: mmol/L) levels for early stage breast cancer patients at 4-weeks (baseline), 12-weeks (T1), and 20-weeks (T2) post-treatment

5.2.10 Hypothesis 2c - pro-inflammatory measures will improve over a 20-week post-treatment period.

Like the previous section pro-inflammatory levels were also explored for this sample across the 16-week post-treatment period. A series of repeated measure ANOVAs were employed to determine whether there were significant changes in cytokine levels for this period. Results will refer to two tables, one of means and standard deviations (Table 36), and the other F tests, p-values, and effect sizes (Table 37). Based on univariate analyses, the only covariates identified were age and endocrine medication use for IL-5 and TNF-β. These will be discussed in the respective cytokine sections.

Table 36: Pro-Inflammatory Measures: Means & Standard Deviations Across Time

Variable	Norms	Baseline M (SD)	Time 1 M (SD)	Time 2 M (SD)
CRP	<6 mg/L	1.77 (2.05)	1.46 (1.66)	1.85 (1.91)
IFN-γ	<365pg/ml	1.44 (2.38)	3.35 (4.29)	1.88 (2.64)
TNF-α	<479pg/ml	2.06 (2.47)	3.74 (3.17)	1.81 (2.60)
IL-1B	<426pg/ml	2.95 (4.30)	9.71 (11.61)	3.83 (5.92)
IL-5	<44pg/ml	2.03 (3.72)	4.80 (5.82)	2.39 (4.46)
TNF-β	<439pg/ml	0.70 (1.77)	2.89 (4.50)	0.41 (1.53)
IL-10	<44pg/ml	3.41 (3.17)	6.04 (4.60)	4.07 (3.41)

Note. Biomarker Abbreviations: C-reactive protein (CRP), Interferon (IFN), Tumour necrosis factor-α (TNF-α), Interleukin-1 (IL-1β), Interleukin-5 (IL-5), Tumour necrosis factor- β (TNF-β), Interleukin-10 (IL-10)

Table 37: Analyses of Variance (ANOVA) Change in Pro-inflammatory Measures Over a 20-week Post-Treatment Period

Variable	n	F	p	Partial η^2
CRP	13	0.58	0.46	.05
IFN-γ	14	1.42	0.26	0.10
TNF-α	14	1.96	0.17	0.13
IL-1B β	14	2.98	0.09	0.19
IL-5*	14	1.09	0.34	0.09
TNF-β*	14	1.09	0.33	0.09
IL-10	14	1.55	0.24	0.11

Note. Biomarker Abbreviations: C-reactive protein (CRP), Cytokine Abbreviations: Interferon (IFN), Tumour necrosis factor-α (TNF-α), Interleukin-1 (IL-1β), Interleukin-5 (IL-5), Tumour necrosis factor- β (TNF-β), Interleukin-10 (IL-10)

Note. Omnibus F tests reported

Partial η^2 = magnitude of change, .01 = small, .06 = moderate, and .14 = large;

* indicates that Analysis of Covariance performed

5.2.10.1 Decreased c-reactive protein (i).

On exploration of CRP across the post-treatment period results indicated no significant change across time. CRP is a marker of inflammation; as a general rule lower level are an indication of good health. Levels of CRP suggest a sustained low level of inflammation. However Figure 23, illustrates just how diverse scores were across participants; however mean CRP levels suggest stability across assessments. Based on univariate investigations no covariates were included in the analysis.

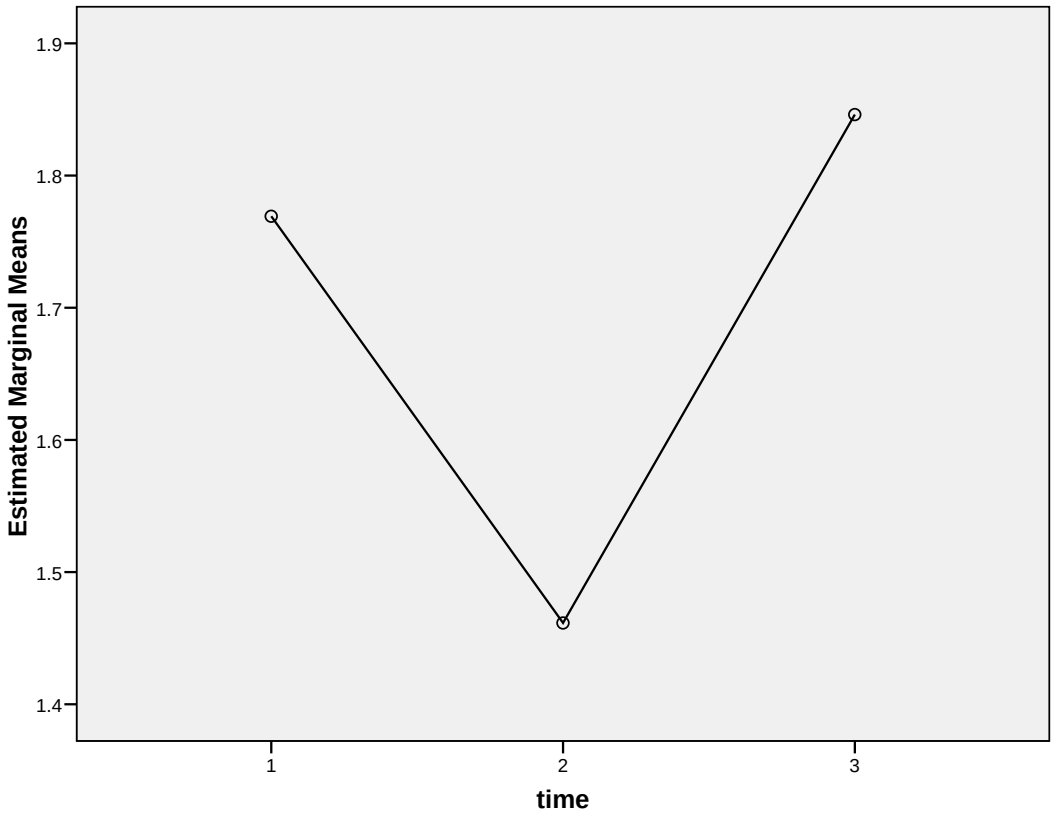


Figure 23: C-reactive protein (CRP: mg/L) levels for early stage breast cancer patients at 4-weeks (baseline), 12-weeks (T1), and 20-weeks (T2) post-treatment

5.2.10.2 Decreased inflammatory cytokines (iii).

5.2.10.2.1 Interferon- γ

IFN-γ levels across the five 16-week post-treatment period were explored. Increased IFN-γ suggests a pro-inflammatory response and a subsequent immune activation. Results from the repeated measures ANOVA (Table 37) indicated that there was no significant change across time for IFN-γ levels; the magnitude of change was .10, indicating a moderate- to- large effect size. Figure 24 illustrates a mean increase in IFN-γ

levels between 4-12 weeks, followed by a decrease between 12-20 weeks (T1 to T2), however individual levels exhibited a great deal of variability. No covariates were included in the analysis, based on univariate investigations. Levels for this sample were within normative ranges.

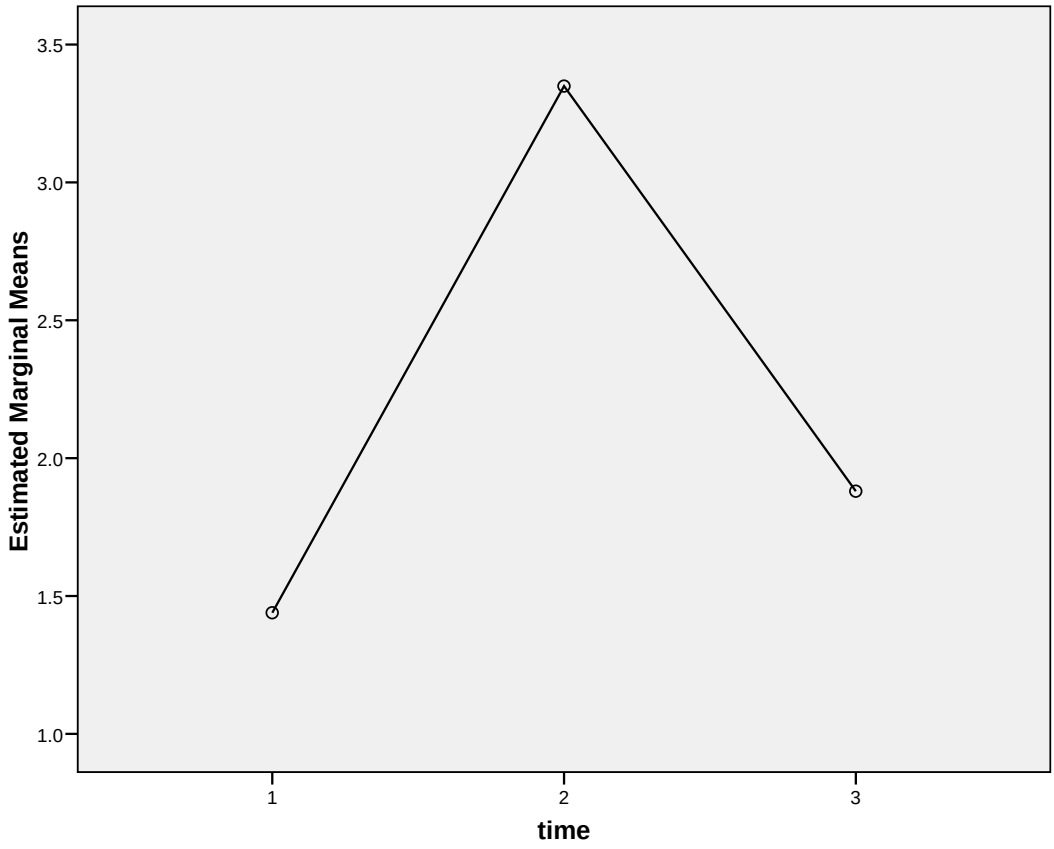


Figure 24: Interferon-γ (IFN-γ: pg/ml) levels for early stage breast cancer patients at 4-weeks (baseline), 12-weeks (T1), and 20-weeks (T2) post-treatment

5.2.10.2.2 Tumor necrosis factor- α

Like IFN-γ levels, graphical exploration of TNF-α levels (Table 36) across this post-treatment period indicated a mean increase (4-12 weeks) followed by a decrease (12-20

weeks) (Figure 25). However repeated measures ANCOVA findings for TNF-α were not significant across time (Table 37) but a moderate- to- large effect size was reported. Based on prior univariate analyses, no covariates were included in this model. TNF-α is a pro-inflammatory cytokine, which activates immune cells (macrophages, etc). Levels of TNF-α for this sample were within normative ranges.

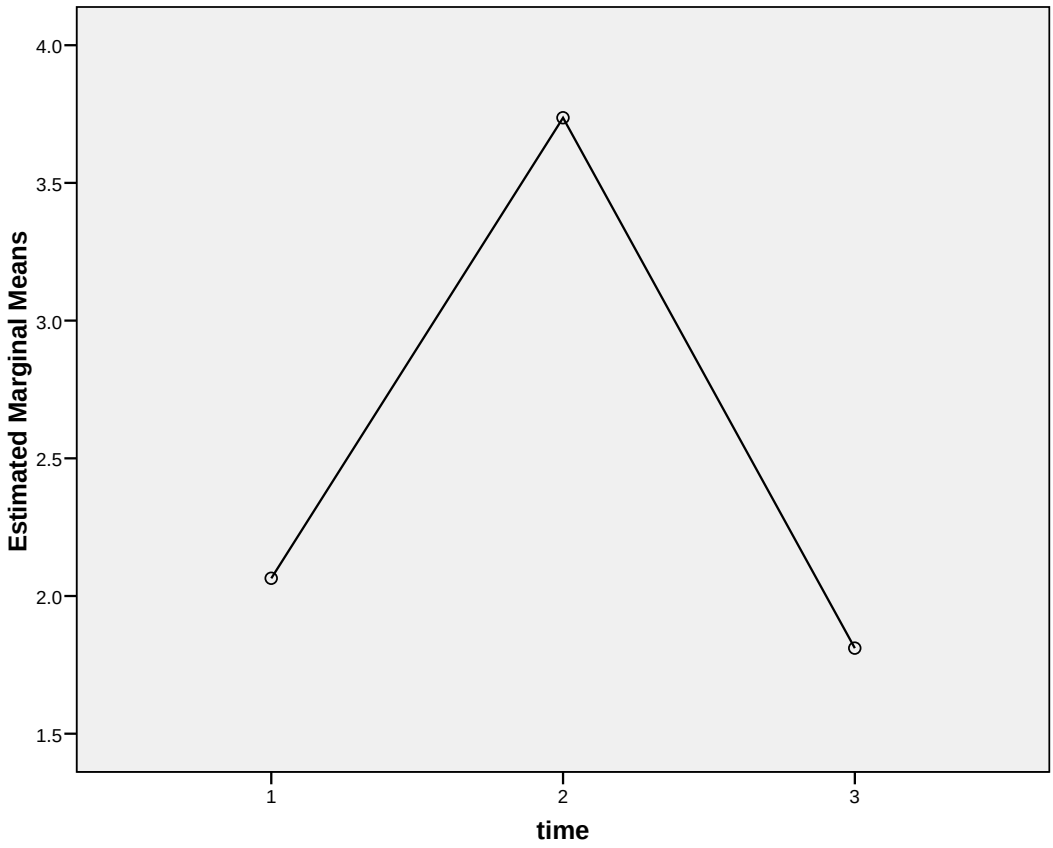


Figure 25: Tumor necrosis factor- α (TNF-α: pg/ml) levels for early stage breast cancer patients at 4-weeks (baseline), 12-weeks (T1), and 20-weeks (T2) post-treatment

5.2.10.2.3 Interleukin- 1β

IL-1β, another pro-inflammatory cytokine, was explored across the 16-week post-treatment period. Like aforementioned cytokines the trend (Figure 26) was for an increase between baseline and T1, followed by a decrease from T1 to T2. Although change in IL-1β levels failed to be significant across time (Table 37), large effect sizes were evidenced (.19). No covariates were included in this analysis.

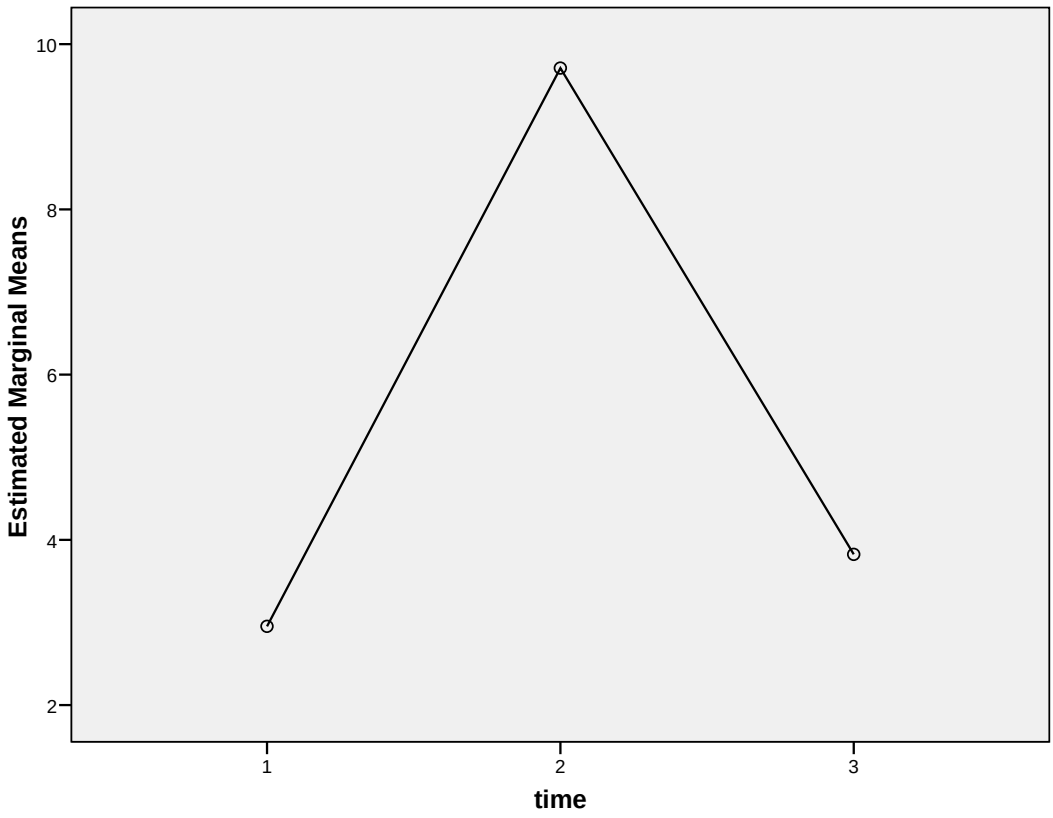


Figure 26: Interleukin-1β (IL-1β : pg/ml) levels for early stage breast cancer patients at 4-weeks (baseline), 12-weeks (T1), and 20-weeks (T2) post-treatment

5.2.10.2.4 Interleukin-5

Post-treatment, IL-5 levels were explored. A repeated measures ANCOVA was performed including age and use of endocrine medication as covariates, based on prior univariate analysis. Results indicate that there was no statistically significant change across time, although a moderate- to- large effect was observed. Figure 27 illustrates an increase between 4-12 weeks, followed by a decrease between 12-20 weeks.

Age [$F(1.38, 11) = 0.57$, $p = .51$, $\eta^2 = .05$] and use of endocrine medication [$F(1.38, 11) = 1.16$, $p = .32$, $\eta^2 = .10$] were included as a covariates, but did not contribute unique significant adjustment to the dependent variable (IL-5). However this result should be cautioned as the use of endocrine medication covariate did evidence a large effect.

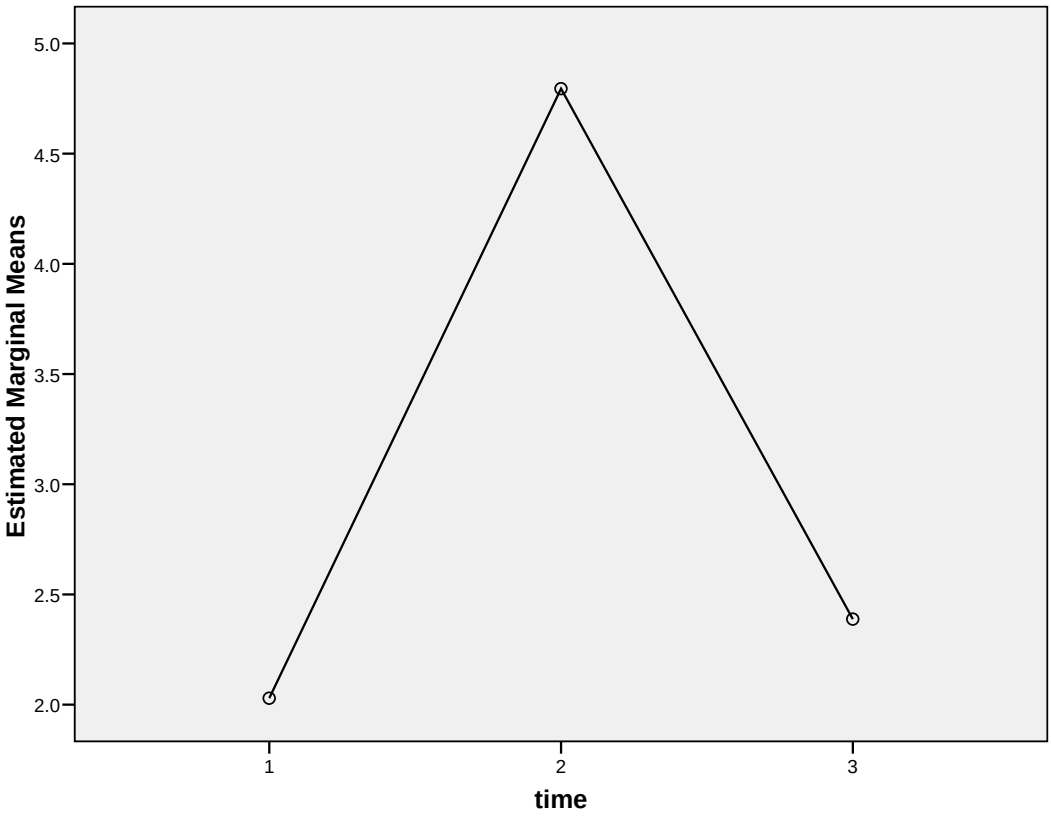


Figure 27: Interleukin-5 (IL-5: pg/ml) levels for early stage breast cancer patients at 4-weeks (baseline), 12-weeks (T1), and 20-weeks (T2) post-treatment

5.2.10.2.5 Tumor necrosis factor- β

TNF-β levels were explored using a repeated measure ANCOVA. Covariates age and endocrine medication use were identified by prior univariate analysis, and included into the model. Results indicated that there was no significant change in TNF-β levels across the 16-week time frame. However a moderate- to- large effect size was evidenced. Graphically (Figure 28), like other cytokines previously discussed, increasing mean levels

were observed at between 4-12 weeks, followed by a decline between 12-20 weeks (T1 to T2)

Age [$F(1.30, 11)=0.38$, $p=.61$, $\eta^2=.03$] as a covariate did not appear to contribute to any unique significant adjustment to TNF-β levels, with a small- to-moderate effect size evidenced. Similarly the covariate for endocrine medication use [$F(1.30, 11)=3.72$, $p=0.7$, $\eta^2=0.25$] did not contribute unique significant adjustment to the dependent variable. However endocrine medication use had a large effect size so caution should be used interpreting this result.

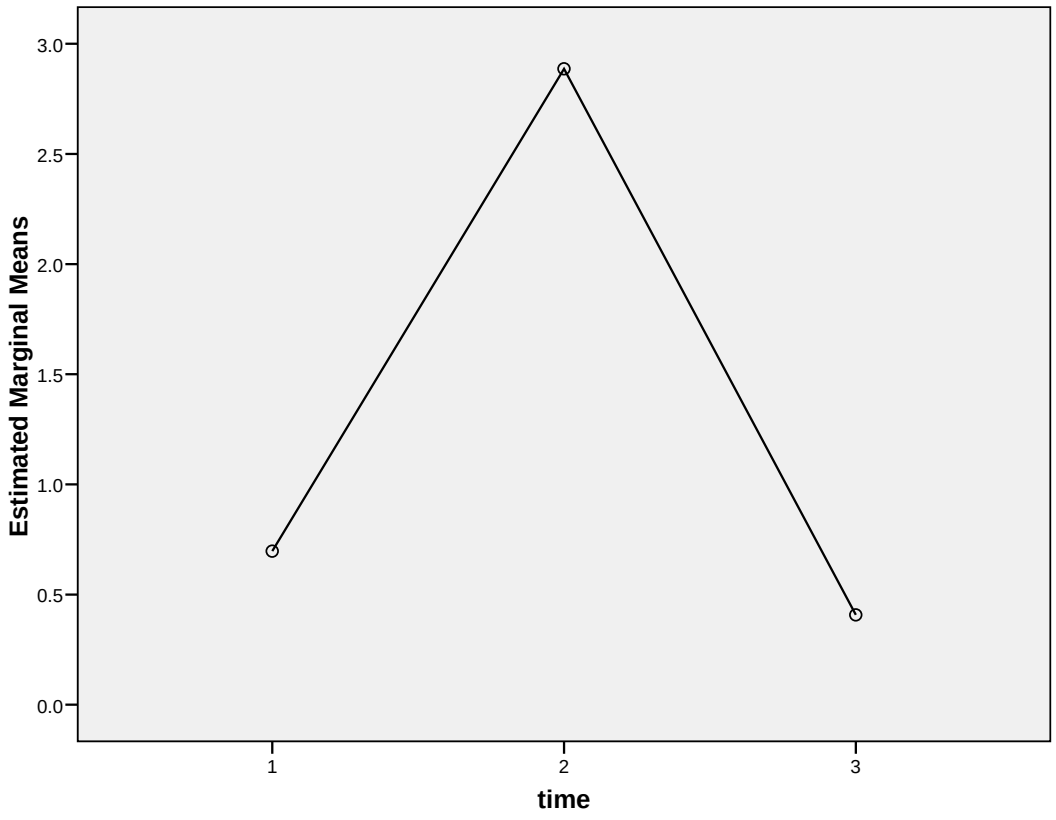


Figure 28: Tumor necrosis factor β (TNF-β: pg/ml) levels for early stage breast cancer patients at 4-weeks (baseline), 12-weeks (T1), and 20-weeks (T2) post-treatment

5.2.10.3 Increased anti-inflammatory cytokine (iv).

IL-10 is an anti-inflammatory cytokine. Changes across the post-treatment period were explored for IL-10. Based on prior univariate analysis no covariates were included in this model. Results of repeated measures ANOVA indicated that there were no significant changes across this time frame. However a moderate- to- large effect size (0.11) was evidenced for time, suggesting caution basing interpretation on the p value alone. Graphical depiction (Figure 29) shows a similar pattern to previous cytokines, associated with an increase between 4-12 weeks.

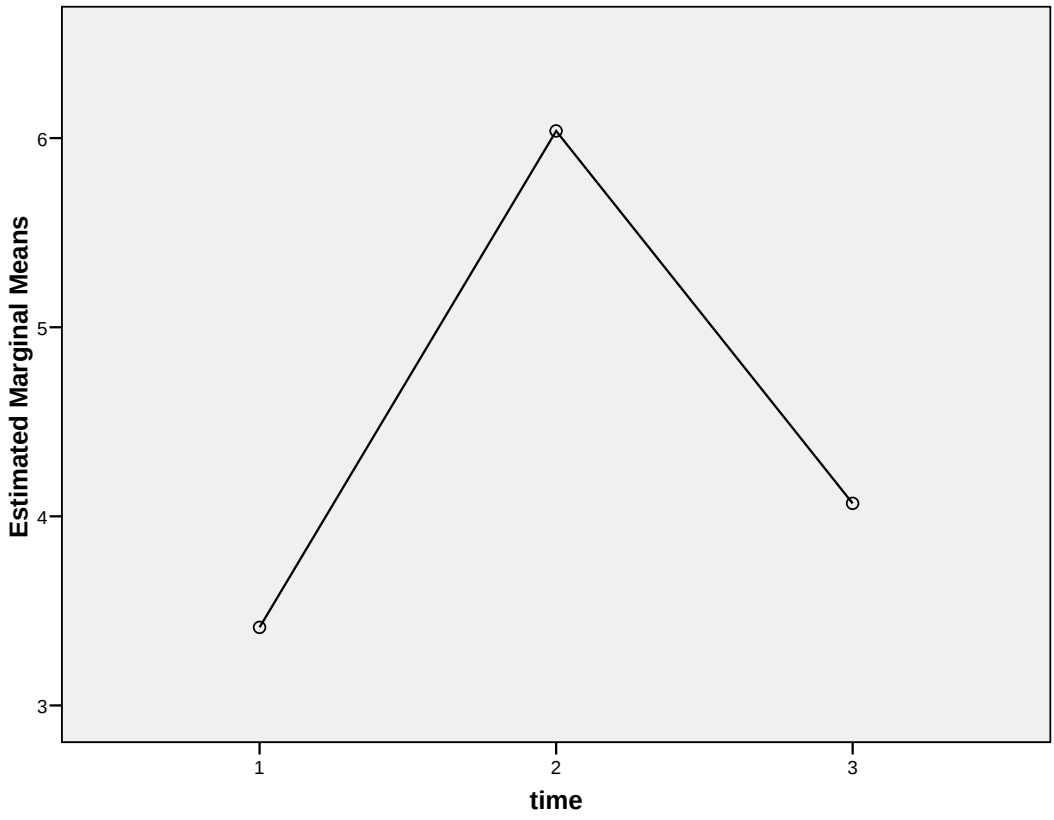


Figure 29: Interleukin-10 (IL-10: pg/ml) levels for early stage breast cancer patients at 4-weeks (baseline), 12-weeks (T1), and 20-weeks (T2) post-treatment

5.3 Discussion

5.3.1 Overview.

The purpose of this study was to explore biopsychosocial factors after adjuvant treatment for early stage breast cancer, in order to learn about beneficial health interventions which can or should be employed during this time. Whether women experienced ongoing psychological distress and emotional dysfunction in this period was of interest. Guided by The Oxidative Model's propositions, about the pro-oxidant and pro-inflammatory impact of chronic stress, the current study was designed to observe PNI

mechanism for breast cancer patients across the 6-months following adjuvant treatment. In addition recruitment of patients and data collection was informative. Following this observational study a full-size intervention was envisioned for patients in the post-treatment setting, through the participating hospital (i.e., yoga, meditation, antioxidant supplements, etc).

5.3.2 Psychological well-being 4-weeks post-treatment.

Mixed findings for psychological distress in the post-treatment period have previously been observed as outlined in Chapter 3. It was anticipated that women in the immediate post-treatment period (4-weeks) would experience poorer psychological adjustment, distress, anxiety, depression, anger, loneliness, trauma, and decreased curiosity. Findings, from the current study, suggest that on the whole women were experiencing minimal levels of psychological distress, as measured by the GHQ-12. Only three of the 17 women recruited reported experiencing severe distress at baseline. This concurs with recent findings suggesting that only a subset of women treated for early stage breast cancer experience distress during the post-treatment period (Costanzo et al., 2007). Post-treatment distress can result from residual treatment side-effects (Mast, 1998) and fear of disease recurrence. Fear of disease recurrence is a considerable concern for women in this timeframe, with Armes and colleagues (2009) identifying this fear to remain a significant stressor for up to 6-months post-treatment. In this sample fear of recurrence was not explored, however for the subset of women experiencing severe psychological distress it is plausible that this was a contributing factor.

Post-treatment psychological distress has been predicted by T-anxiety levels (Bleiker, et al. 2000). For the current study, it was apparent that the sample was psychologically very similar to healthy normative samples on several Trait characteristics, specifically anxiety, depression, curiosity, anger, and anger expression (Spielberger, 1996, 2003). Similarly, measures of State psychological well-being, anxiety, depression, curiosity, anger, and loneliness were also well within normative reference ranges for healthy, general population samples. These descriptive observations at 4-week post-treatment imply that most women in this sample were comparable to women in the broader population with regard to psychological well-being. This could be explained by self-selection bias.

It is likely that this study is influenced by a self-selection bias as these women were doing well quite well psychologically. This is a common problem across all research which employs volunteers. However it is possible that this was a contributing factor to the lack of psychological distress and emotional dysfunction in this group. For women, having completed adjuvant treatment, to voluntarily come back to the treatment setting three additional times over a 6-month period required a certain level of well-being and/or type of personality characteristics. This may account for the psychological picture of this sample being comparable to healthy norms. It is unclear whether women who were not doing well during treatment were less likely to participate. Future studies might consider home visits in this type of population in order to attain a more representative sample.

In addition to normative State and Trait characteristics, this group evidenced minimal cancer-specific trauma in the post-treatment period. Only four women indicated high scores on the revised Impact of Events Scale (IES-R). Past research employed the preceding version of the IES. The IES-R does not provide clinical cut-offs (Weiss & Marmar, 1997) which made it difficult to quantify severity. This sample was comparable to healthy norms. In addition two scales of psychological adjustment also reflected normative sample means; including a measure of psychological defense mechanisms.

The Lifestyle Defense Mechanisms Inventory (LDMI; Spielberger & Reheiser, 2002) assesses the presence of Traits which have been linked to Type C personality. These Traits have been linked to the etiology and progression of cancer (Greer & Watson, 1985) (Kneier & Temoshock, 1993). The scale comprises a Need for Harmony and a Emotional Defensiveness subscale. Extensive research (Eysenck, 1994) has described these Traits to include:

> being over-cooperative, appeasing, unassertive, over-patient, avoiding conflict, suppressing emotions like anger and anxiety, using repression and denial as coping mechanisms, self-sacrificing, predisposed to experience hopelessness and depression. (p. 168)

These types of psychological responses have been shown to differentiate between patients with breast cancer, those with benign tumours, and healthy women. Women with breast cancer have been identified to employ these responses more frequently. Application of the LDMI to the current sample was informative. It clarified that the current

sample were comparable to healthy norms with regard to these Trait psychological responses. Unlike previous research this sample of women could not be distinguishable from healthy women without breast cancer, based solely on these psychological responses.

In line with this finding, psychological adjustment to cancer in the current sample was similar to other mixed cancer samples observed for a mixed sample of men and women (Whitford et al., 2008). Based on the Mental Adjustment to Cancer (MAC) scale, Watson et al. (1989) propose the use of cut-off scores to distinguish clinical 'cases' from 'non-cases'. In this instance 'cases' are defined as scoring 47 or less on the Fighting Spirit subscale in combination with a score of 12 or more on the Helpless/Hopeless subscale. Only one participant in the current sample met this criterion 4-weeks post-treatment.

Scores observed for subscales -Fighting Spirit and Helpless/Hopeless coping responses fell in-between norms for breast cancer patients and norms for healthy women. (Watson, et al. 1989) This suggests that the current sample of women were doing better than other studies of psychological adjustment in breast cancer patients, but not quite as well as healthy women. Fighting Spirit is characterized by a determination to fight the illness and adopt an optimistic attitude. On the other hand, patients scoring high on Helpless/Hopeless coping responses may feel engulfed by knowledge of the diagnosis and have a pessimistic attitude (Waston, Haviland, Greer, Davidson, & Bliss, 1999). Helpless/Hopeless responses to a cancer diagnosis have been shown to exert a significant effect on disease-free survival evidenced at 5-years (Watson, Homewood, Haviland, &

Bliss, 2005; Greer, Morris, & Pettingale, 1979) and have an impact up to 10-years after diagnoses (Watson, et al., 1999). In simple terms, those reporting more Helpless/Hopeless psychological responses had poorer survival outcomes. Recently Fighting Spirit was identified as the sole psychological predicator of survival in a sample of breast cancer patients at 10-years post-diagnosis (Osborne et al., 2004b). However other studies suggest there is little consistent evidence that psychological adjustment styles play an influential role in survival or disease recurrence (Petticrew, Bell, & Hunter, 2002).

5.3.3 Pro-oxidant and pro-inflammatory markers 4-weeks post-treatment.

It was considered that women diagnosed and treated for early stage breast cancer could experience deterioration at both psychological and physiological levels, based on The Oxidative Model. This section will expand on pro-oxidant and pro-inflammatory findings observed 4-weeks post-treatment. In addition the biopsychosocial relationships between Oxidative Model measures will be discussed.

5.3.3.1 Pro-oxidant markers at baseline.

The initial assessment of oxidative biomarkers 4-weeks post-treatment revealed a pro-oxidant state. Three key markers of the model evidenced pro-oxidant levels at baseline - low 5-‘ectonucleotidase (NT), low tissue ascorbate (VIT C), and high homocysteine (HCY) levels.

NT is a central biomarker to The Oxidative Model. It is a key ecto-enzyme responsible for lymphocyte maturation, therefore directly impacting on acquired

immunity and subsequent susceptibility to infections. The low levels observed in the current study have been seen in previous Oxidative Model literature (Blake-Mortimer et al., 1996, 1998b; Hapuarachchi et al., 2003). However unlike the current sample who have relatively low stress levels, similar NT levels were observed in severely stressed samples including occupational stress (Hapuarachchi et al., 2003), major depressive disorders on a low antioxidant diet (Blake-Mortimer et al., 1998b), and academic examination stress (Blake-Mortimer et al., 1996).

Low NT levels observed in these earlier studies were measured following bouts of sustained stress in the 1-3 month period prior to assessment (Blake-Mortimer et al., 1996). In the current study, given the repeated nature of cancer treatment regimes, it is plausible these women they have experienced a similar stressful event sequence as defined by Elliot (1982), despite psychological stress scores discussed previously suggesting otherwise. For instance each of these women had undergone regular treatments that are often painful (i.e., radiation therapy) and/or have negative side-effects like nausea (i.e., chemotherapy). In addition reaching the end of the treatment period also has elements of a chronic stressor given these stressors are defined as ones that pervade one's life forcing one to restructure and/or reinvent themselves. It is possible that stress associated with treatment regimens for this sample in the preceding 3-months plays a part in the observed low NT levels at 4-weeks post-treatment.

A pro-oxidant state was also reflected by very low VIT C in the current sample. According to The Oxidative Model, VIT C is considered to be protective of NT; during

periods of sustained distress VIT C is depleted before NT levels experience depletion. This explains the low levels observed for both these oxidative markers. Similarly, HCY levels were at the high end of their respective reference ranges, further evidence of a pro-oxidant state. Observed levels of NT, VIT C, and HCY suggest that adjuvant treatments, which are often pro-oxidant (Mantovani et al., 2003), in addition to the stressful event sequence/chronic stressor, may have a lasting impact on pro-oxidant states 4-weeks post-treatment.

Cholesterol (CHOL) levels observed for this sample at baseline were on the high end of the reference range. Mean levels bordered on higher than recommended levels (< 5.5mmol/L L). It must be noted that these were not fasted samples, thus food intake in the last 12 hours can influence overall scores. However, higher cholesterol levels suggest co-morbid health conditions, and paired with pro-inflammatory processes, increases risk of atherosclerosis.

5.3.3.1.1 Pro-oxidant markers associated with psychological well-being.

Several large, significant associations were observed between lowered NT and negative psychological states. These provide support for the Oxidative Model. Higher S-depression and T-depression scores were associated with lowered NT levels. This finding concurs with previous Oxidative Model patterns observed in a sample of patients with clinical depression. Conversely higher S-curiosity and T-curiosity scores were associated with improved NT levels reflecting previous unpublished findings observed in an academic sample (Oliver, 2004). Spielberger et al. (1989) proposed that curiosity may be associated

with longer survival. Hapuarachchi and colleagues (2003) identified that higher psychological distress as measured by the GHQ-12 was associated with depleted NT levels. The same pattern of association between distress and NT was found in this study however it evidenced only a moderate association.

For anger measures, higher overall anger (AX-I) and internalized anger control (AC-in) were associated with higher HCY levels, indicating a pro-oxidant state. This finding replicates other findings whereby HCY has been associated with measures of anger and anger expression in healthy female samples (Stoney, 1999; Stoney & Engebretson, 2000). FOLATE and VIT B12 are vital nutrients involved in the synthesis of HCY (Wilcken & Wilcken, 1998). In the current study lowered VIT B12 levels were associated with increased S-anger. This gives further support for the detrimental impact of anger on the internal oxidative balance.

The exploration of CHOL, a novel marker to The Oxidative Model, yielded three large associations. Higher CHOL is a known risk factor for CVD. In the current study higher levels were associated with higher scores on psychological distress, Fatalism, Helpless Hopeless coping responses. The latter two subscales (MAC) explore the presence of depressive and passive psychological responses to cancer. Items include 'I feel that nothing I can do will make a difference' and 'I've left it all to my doctors' indicating patients' loss of control over their current situation. These negative psychological states paired with a marker of CVD provide support for this marker in The Oxidative Model

framework. It must be reiterated that these blood samples were not fasted samples which can influence CHOL levels observed.

These preliminary findings suggest psychological well-being had an impact on pro-oxidant biomarkers in the immediate post-treatment period. Other researchers have also explored this. For women with early stage breast cancer the rate of improvement or resolution of stress during the treatment and post-treatment period has a subsequent impact on enumerative improvements in immune cells (Thornton et al., 2007). Findings from the current study highlight the role of biopsychosocial relationships, and the importance of assessment of psychological well-being in this population.

5.3.3.2 Pro-inflammatory measures at baseline.

Pro-inflammatory measures 4-weeks post-treatment revealed a low level of pro-inflammatory processes. Low- to- moderate levels of C-reactive protein (CRP) were observed at baseline. Circulating CRP levels under 10 mg/L have largely been regarded as clinically insignificant. In recent years, a number of researchers have demonstrated an association between minor elevated CRP (3 and 10 mg/L) and the risk of developing cardiovascular diseases, metabolic syndrome, and cancers (Kushner, Rzewnicki, Damols, 2006). Chronic low-grade inflammatory conditions might be associated with these diseases. Several women in this sample were taking cardiovascular medication at the outset indicating pre-existing conditions. This could account for CRP levels observed at baseline. In addition chemotherapy regimes can have a damaging impact on the cardiovascular system (Shakir & Rasul, 2009).

In Oxidative Model literature elevated CRP, along with NT, has been identified as the strongest correlate of chronic stress in a natural setting (occupational stress) by Happuarachchi and colleagues (2003). In the current study the ongoing treatment regimes experienced by patients are a likely chronic stress scenario. It is possible that this contributed to the levels of inflammation observed, as is proposed by the Model. Elevated CRP has been identified as a determinant predictor of lower survival rates post surgery (Wang & Sun, 2009) for gastric cancer patients. CRP may serve as an additional prognostic predictor for post-treatment monitoring in cancer patients.

Levels of inflammatory cytokines observed 4-weeks post-treatment also suggest a low level of inflammation. However there was evidence of much inter-individual variability for cytokine levels at baseline assessment. This is not surprising given the process of inflammation occurs rapidly, and cytokine responses cascade, with one triggering another and so on. Therefore the discrepancy observed between individuals in the current study is to be expected.

To summarise, at 4-weeks post-treatment, this group of women evidenced levels of pro-inflammatory measures suggesting moderate inflammation. Like observed pro-oxidant measures, the pro-inflammatory processes observed 4-weeks post-treatment could be due to the lasting impact of adjuvant treatment. However biopsychosocial relationships were also evident

5.3.3.2.1 Pro-inflammatory measures associated psychological well-being.

Relationships between pro-inflammatory markers and psychological measures were explored 4-weeks post-treatment. Increased inflammation, as evidenced by higher CRP levels, was associated with higher scores on the Anger expression-out (AX-out) scale, a Trait measure of how frequently angry feelings are expressed in verbally or physically aggressive behaviour. This finding is similar to previous Oxidative Model research whereby occupational stress in a non-clinical sample was associated with increased CRP (Hapuarachchi et al., 2003).

Pro-inflammatory processes were also explored by the measurement of cytokines. The inclusion of cytokines in the current study was to add to the growing knowledge base around their relationship to The Oxidative Model. To date preliminary exploration of inflammatory cytokines has been undertaken across several populations including victims of crime (Pfitzer, 2008), general population (Le, 2004), and student samples (Oliver, 2004).

Observed associations betweens between cytokines in the current study provides evidence for poorer psychological adjustment and an associated pro-inflammatory state. For example, higher reporting of a Helpless/Hopeless response to cancer was associated with increased IL-1 β levels. The main role of IL-1β is the stimulation of acute–phase protein synthesis, increases in plasma proteins that rise in the blood with inflammation (i.e., CRP) and the proliferation of T_H2 cells. The T_H2 response pathway is essential for humoral immunity. The humoral response is extracellular involving activation of factors in the fluids surrounding cells. To understand this mechanism, keep in mind the T_H1 and T_H2

cells downregulate one another (see Chapter 1, cytokine section). It is also associated with the fever response.

Tumor necrosis factor β (TNF-β) is central to all inflammatory responses. Higher TNF-β levels have been reported with psychological distress and symptom distress in breast cancer patients both pre- and post-diagnosis (DeKeyser, Wainstock, Rose, Converse, & Dooley, 1998). However for the current study lower levels of TNF-β were associated with higher T-curiosity. Already in this study, section 5.3.3.1.1, curiosity (both Trait and State) has been associated with higher levels of NT. Paired with decreased inflammation these findings highlight this curiosity as a characteristic associated with an efficient working immune response. Thus T-curiosity may have a protective effect on an individual's immune system and hence survival.

Anger expression scales revealed many associations with inflammatory cytokine levels observed at baseline. Anger expression-in (AX-in) was associated with lower IFN-γ levels (released by T_H1 cells), IL-1β (released by monocytes), and Il-5 (produced by T_H2) cells. AX-in is defined as how often angry feelings were experienced but not expressed. This finding seems counterintuitive given that the T_H1 and T_H2 responses down regulate one another. Spielberger, Sydeman, Owen, and Marsh (1999) suggest that persons with high scores on Anger expression-in, who also have high Anger expression- out scores, may in fact express their anger in some situations while suppressing it in others. This could explain the observed patterns. Further support of is provided with Anger expression-out associated with higher levels of IL-1 β. The complex cytokine associations found around

anger expression styles in the current study highlight the potential for the experience of anger to have both immuno-enhancing and immunosuppressant effects.

5.3.4 Psychological well-being across the post-treatment period.

It has been established that the current sample 4-weeks post-treatment were comparable to healthy norms, with only a couple reporting severe distress, problems with psychological adjustment, and cancer-related trauma. Whether psychological well-being changes over the post-treatment period was a principal research question of this study. The initial research question specifically asked do women suffer from ongoing psychological distress over the post-treatment period. It was anticipated that measures of distress would decrease with the passing of time in the post-treatment period. The influence of demographic, treatment, and health behavior variables during this period was also investigated.

5.3.4.1 Psychological distress, S-anxiety, S-anger, and S-curiosity.

S-anger, S-anxiety, and S-curiosity scores evidenced moderate- to- large effect sizes indicating change over the three post-treatment assessments. Only one variable, psychological distress (GHQ-12; Goldberg, et al, 1978), evidenced statistically significant change across the period. Patterns suggest a rebound with worsening psychological states and distress at 12-week assessment. There are a number of possible explanations for this observed fluctuating pattern. Firstly, the experience of increased distress, S-anxiety, S-

anger and declining S-curiosity at 12-weeks may be linked to lasting side effects of adjuvant treatment.

Providing support for this explanation, having undergone chemotherapy was implicated as a covariate for psychological distress. Treatment side-effects specific to Taxane-based adjuvant chemotherapy have been shown to persist, and contribute to patterns in distress. Over half women in this sample received Taxane-based adjuvant chemotherapy. This type of treatment can remain influential up to two years post-treatment, however most chemotherapy regimes have side-effects which last from 6 to 12- months post-treatment (Thornton et al., 2008). Research supports the psychometric properties of the measure of distress (GHQ-12) employed as it avoids focusing on symptoms of physical illness, however authors suggest that higher cut-offs may be necessary for respondents with somatic symptoms which can inflate scores (Goldberg & Williams, 1988). Somatic symptoms could exacerbate distress in this sample but were not assessed. For this study the GHQ-12 appeared to be a suitable measure of psychological distress.

Secondly, the fluctuating distress patterns observed in the current study could be due to this post-treatment period including a number of potential stressors. It is well documented that resuming 'normal' life after cancer treatment and fear of recurrence (Armes, et al., 2009) are potential sources of distress. However these survivorship issues are often more pertinent in the longer term which may in part explain why levels of distress were not very high across assessments, in the current study. The population from

which this sample was derived had just finished 'active' treatment. This signifies a time when patients began to have less contact with the medical setting where they had received intensive treatment and support for a sustained period. They have often been part of this network for at least 6-months (average 30 weeks) since diagnosis, some for periods of up to a year. This network comprises doctors, nurses, breast care nurses, chaplains, counselors, other patients, volunteers, etc. It must be acknowledged that there is an element of social support which remains underexplored in this setting. It has been suggested that being involved in an active form of treatment creates a social affiliation, active coping (Deshields, et al, 2005) and reassurance from engaging in ongoing treatment (Gurevich et al., 2002; Tjemsland, Soreide, & Malt, 1998). In addition concepts including illness uncertainty and emotional distress have been associated with the cessation of frequent contact with the treatment setting (Mast, 1998).

A third explanation for fluctuating distress patterns is the potential for the treatment setting to be a trigger for conditioned responses. Previous research has observed this effect in patients having undergone chemotherapy, specifically with regard to the experience of anticipatory nausea as a conditioned response to chemotherapy. The psychological characteristic T-anxiety has been associated with the increased experience of nausea (Fredrikson, Furst, Lekander, Rotstein, & Blomgren, 1993). It is reasonable to assume that the conditioned experience of nausea also comes with a level of distress. Although none of the patients in the current sample experienced nausea during assessments, it is plausible that blood-taking could be a reminder of chemotherapy.

Given the longitudinal design of the breast cancer study, women were required to revisit the hospital/treatment setting on a number of occasions. During this time they were asked to discuss their cancer experience, complete questionnaires, as well as having blood taken by a nurse. It is possible they could experience a conditioned response to the treatment setting. For example studies have reported that cancer patients who experience nausea with chemotherapy can experience conditioning so that simply being in the vicinity of treatment sites can trigger nausea (Andrykowski & Gregg, 1992) even after chemotherapy has ceased. Similarly for the participating women it is possible that biomarkers are influenced by conditioned responses. One study has reported an association between high blood pressure and elevated HCY (Rodrigo et al 2003). High HCY was observed in this sample, but as blood pressure was not taken as part of the study it is not possible to account for the effects of hyperextension on HCY levels. It has been suggested that immune cells are influenced by conditioned responses (Fredrikson et al., 1993).

In contrast, revisiting the hospital provided an opportunity for women to meet with the breast care nurse (during blood sample collection). This may have inadvertently extended the perceived supportive network for this sample. This is potentially another reason why these women were not experiencing substantial psychological distress, anxiety, depression anger, or adjustment issues. An additional concern is that those women who do not respond to post-treatment research, like this project, may not be doing as well psychologically. To remedy both the potential influence of conditioned responses, benefits from seeing breast care nurse in the treatment setting, as well as

potentially attain a wider pool of participants, visits to patients at their homes for assessments would be recommended for future studies of this population.

5.3.4.2 Depression, Avoidance and confounding health behaviours.

There was little evidence of change for S-depression scores across the post-treatment period with a magnitude of change less than .01, the lowest possible reference point for a small effect size. For S-depression, two confounding variables were identified. The first covariate (endocrine medication use) did not uniquely significantly adjust S-depression scores. Specifically endocrine medication use had a large negative effect with S-depression. This suggests a benefit of endocrine medication use with lowered depression scores. Endocrine medication use was frequent in this sample with over half taking Tamoxifen or similar. This finding is contradictory to research in the quality of life literature which suggests endocrine medication use to cause affective disorders in breast cancer patients (Coster & Fallowfield, 2002). It is likely that hormonal changes in women cause emotional effects. Fallowfield and colleagues (2002) research recognised that medication and treatment side-effects may be more of a contributing factor but failed to identify a conclusive link between receiving endocrine medication and changes in depressive symptoms. In the current study, endocrine medication use was not identified as a covariate for other affective measures such as S-anxiety, S-anger, S-curiosity, or psychological distress.

The second covariate- hazardous alcohol use- uniquely and significantly adjusted S-depression scores. This warrants some consideration about the relationships between

psychological well-being and health behaviours given the large association observed. Hazardous alcohol use was assessed using the Alcohol Use Disorders Identification Test (AUDIT) outlined in section 5.1.8.1.2. For the current study only the hazardous drinking domain was applied. This pattern of alcohol consumption is defined by increasing the risk of harmful consequences for the user or others. The domains covered for this pattern of alcohol use include 'frequency of drinking', 'typical quantity', and 'frequency of heavy drinking'. Hazardous alcohol use scores in the current sample were low. The role of health behaviours (like alcohol use) for individuals experiencing psychological stress from cancer has previously been explored (Anderson et al., 1994). It has been suggested that psychological or behavioural effects of cancer treatments can be so disruptive that patients become discouraged and fail to complete or comply with treatment regimes.

This pattern of alcohol use was also identified as a covariate for Avoidant coping in response to cancer diagnosis, as measured by the MAC scale. This subscale comprises only one item 'I don't really believe I had cancer' thus reliability is somewhat questionable. Research from social cognitive theory suggest that individuals learn to utilize drinking alcohol as a coping response when they believe other ways of coping are unavailable to them (Abrams & Niaura, 1987). S-depression and Avoidant coping could be indicators of denial about the situation. Engaging in Avoidant behaviour post-treatment presents an issue with adherence to follow-up appointments and additional screening.

5.3.4.3 Loneliness.

A directional hypothesis for patterns in social needs in the post-treatment period was avoided due to the mixed findings from past literature. Firstly, it was thought that resuming 'normal' life, including resuming work, no further time spent at the hospital, less negative treatment side-effects would lead to improved loneliness scores. On the other hand leaving the treatment setting and support network may actually lead to increased loneliness. Findings showed a small effect size over post-treatment assessments. The UCLA Loneliness Scale assesses the uni-dimensional discrepancy between desired and achieved levels of social contact. This remained constant across the post-treatment period. Given that the UCLA does not specify a time frame for respondents, it remains unclear as to whether it is a State or Trait measure. However authors suggest a substantial trait component in UCLA scale scores (Russel et al., 1980). This limited its utility as a measure of change.

5.3.4.4 Mental adjustment to cancer.

The Avoidance subscale derived from the MAC scale evidenced some change across time. A moderate effect size was evidenced. Observed scores suggested greater avoidance between 4-12 weeks and then improvement between 12-20 weeks. Caution should be taken with interpreting this finding, as this scale is comprised of only one item. Subsequently this finding has limited reliability as suggested by the non-significant finding.

Only a small effect size was observed for Fighting Spirit over the three assessments. The exploration of confounding variables for Fighting Spirit yielded interesting findings. Two covariates were identified: the use of cardiovascular medicine and Trastuzumab treatment. Trastuzumab is categorized as an immunomodulator and antineoplastic, also known as Herceptin . In order to be eligible for this study it was required that women have completed adjuvant treatment 4-weeks prior to taking part. Adjuvant treatment was considered standard chemotherapy regimes and/or radiation therapy. However due to the exploratory nature of this study and slow accrual, women undergoing Trastuzumab treatment were included.

Specifically, three of the 17 participants in this study received Trastuzumab. These women were 'post-treatment' for chemotherapy and/or radiation therapy but continued to come to the hospital every three weeks for Trastuzumab for a 12-month period. In contrast to those women who were receiving Trastuzumab, for the other participants' the post-treatment experience is quite different. In the post-treatment period, unless adverse events occur, follow-up is minimal until a screening mammogram occurs. This is usually in the 12-months following the cessation of adjuvant treatment. For this group the average time to mammography varied from 4- to 12-months post-treatment.

Trastuzumab has been associated with cardiotoxicity (Slamon, Leyland-Jones, & Shak, 2002). Correlational findings support this, with those receiving Trastuzumab also more likely to be taking cardiovascular medication. However both medications contributed unique variance to the measure. For higher levels of Fighting Spirit to be

associated by medication regimen suggests that perhaps receiving active treatment and frequent contact with the treatment setting could be psychologically reassuring. Alternately a possible explanation is that women willing to undergo more intensive and extensive course of treatment have higher levels of Fighting Spirit.

Helpless/Hopeless coping responses evidenced a moderate- to- large effect size for change across time. Improvement was observed between 4-12 weeks, and worsening 12-20 weeks. It is feasible this latter period coincided with a follow-up mammogram. This could explain some of the variability observed across responses. At the 20-week assessment point, six participants had already undergone mammography screening, eight had not. If changes in coping responses were due to anxiety due to follow-up medical procedures it would be expected that Fatalistic coping responses, also evidencing a moderate effect size across time, would have a similar pattern. This was not observed; in contrast reliable change indices indicate improvement between 12- to 20- week assessments, with no worsening. Similarly, it could be expected that Anxious Preoccupation would change in a similar manner to Helpless/Hopeless responses. This was not the case with a small effect size observed paired with little reliable change observed to indicate little change for the Anxious Preoccupation coping style over the three assessments.

5.3.4.5 Trauma.

The experience of the breast cancer event as traumatic was explored across the post-treatment period using the Impact of Events Scale, revised version (IES-R). It was

hypothesized that further from the event, IES-R scores would decrease. There was no general trend for IES-R scores over time, reflected in the observed small effect size. Past research, detailed in section 3.5 is conflicting. It suggests that in the immediate post-treatment (12-month) period, the experience of trauma can be long-lasting (Bleiker et al., 2000) (Andrykowski et al., 2000) or decrease over time (Epping-Jordon, Compas, & Osowiecki, 1999). IES-R scores evidenced a great deal of inter-individual variability. Heightened emotional reactivity and high levels of intrusive thoughts in the immediate post-surgery period have been identified as risk factors for PTSD in breast cancer patients up to 12-months later (Tjemsland et al., 1998). The mixed trends observed in the current study suggest that for a sub-group of women cancer-specific trauma is experienced in the first 6-months post-treatment. This could be related to coping styles employed. Alternately whether heightened IES-R scores coincided with medical follow-ups (i.e., mammogram) varied for participants. It is possible this could account for some variability observed across women in the current sample.

To summarise the 6-month post-treatment period is psychologically complex. Trajectories of psychological well-being do not simply improve with distance from the event as was hypothesized.

5.3.5 Pro-oxidant measures across the post-treatment period.

Based on The Oxidative Model's assumptions it was anticipated that measures indicating a pro-oxidant state would be present 4-weeks post-treatment assessment.

Subsequent to this it was hypothesised that this pro-oxidant state would decrease over time.

There was some evidence for change over the post treatment period; HCY, NT, and VIT C all evidenced moderate- to- large effect sizes. These are central biomarkers to The Oxidative Model. For this sample, the predominant observed trends were for increased levels of NT and VIT C. These both suggest an improvement in pro-oxidant state. HCY evidenced only a slight decline, remaining relatively stable across assessments. The reliability of these findings is limited given the lack of statistical significance. Despite this, levels of NT were low and HCY were high in comparison to their normative reference range at 12- and 20-week assessments. This implies a sustained level of internal oxidative stress, with only slight improvement over time.

Past research has linked vitamin consumption, especially supplements high in antioxidants, to improved levels of these biomarkers (i.e., increased NT, VIT C, and decreased HCY) in stressed samples (Blake-Mortimer et al., 1998b) (Oliver, 2004) (Hapuarachchi et al., 2003). In the current study support for this link was attained by one particular influential covariate; lower HCY levels were associated with increased vitamin intake. Participant's regularly consuming supplements with antioxidant properties had lower HCY levels. This provides support for the Model. In contrast, for NT and VIT C no covariates were identified.

Serum levels of VIT B12 and FOLATE did not evidence much change longitudinally over the post-treatment period with only small effect sizes observed. Serum levels

remained within normal reference ranges. Cardiovascular medication use was identified as a confounding variable for FOLATE levels across the longitudinal assessment. VIT B12 and FOLATE are essential for the synthesis of HCY (a risk factor for CVD). One explanation for this finding could be that women requiring cardiovascular medications had higher levels of HCY, subsequently metabolized their FOLATE levels more rapidly resulting in lower levels. Significant negative correlations between HCY and FOLATE in this sample support this explanation. Furthermore given the previous discussion of vitamin supplementation and improved HCY levels, it is apparent that the supplements participants were taking were often rich in antioxidant, and B vitamins including FOLATE.

5.3.5 Pro-inflammatory measures across the post-treatment period.

Low levels of pro-inflammatory processes were observed in the immediate post-treatment period. It was hypothesised that pro-inflammatory processes would decrease over the post-treatment period.

Individual trends for CRP levels over the post-treatment period were mixed and only a small effect was observed across assessments. A low to moderate level of CRP was sustained over the 16-week period. This suggests a low level of inflammation across the post-treatment period. Like CRP, CHOL levels did not evidence change over the post-treatment period and only a small effect sizes was observed. CHOL levels at baseline were on the high end of recommended normative levels. Longitudinally these levels remained stable across the three assessments. Both these biomarkers are risk factors for CVD. A sustained level across assessments suggests cardiovascular conditions were not improving

in this current sample. This is a concern as returning patients to good health post-treatment is vital to log term health. However as assessments were not undertaken pre-diagnosis levels of CVD risk like CRP and CHOL it is impossible to compare their current state to pre-disease states.

Although no significant findings were observed for inflammatory cytokines, moderate- to- large effect sizes were observed for all measures. Patterns of change suggest inflammatory (IL-1, IL- 5, IFN- γ, TNF-α, TNF-β) and anti- inflammatory cytokine (IL-10) increase between 4-12 weeks and then evidenced a decrease between 12-20 weeks. This non-linear pattern was reflected across all cytokines. Given the nature of cytokine release this trend across cytokines measured represents a cascading response whereby one cytokine triggers the release of another and so on.

This pattern of increase at 4-week assessment could be an artifact of immunological rebound as suggested by Osbourne and colleagues (2004). This is an acute increase in immune parameters, in this case cytokines, in response to recent treatment (i.e., chemotherapeutic agents, radiation therapy, or Tamoxifen). In the current study baseline and subsequent assessments were carried out at least 4-weeks post-treatment to remove the likelihood of immunological rebound, but over half the sample was currently taking Tamoxifen. It is possible, although unlikely, at the 12-week assessment when the increase in cytokines was observed, that this represented the effects of acute rebound. Exploration of influential covariates for cytokines supports this rebound notion with endocrine medication use a covariate for both Interleukin-5 and Tumor necrosis factor β

(TNF-β). The influence of medication regimes across the post-treatment period cannot be ruled out as influencing patterns of inflammatory responses.

5.3.6 Limitations.

Conclusions drawn from this study are limited due to the small sample size. An initial calculation based on cancer registry data from the participating hospital, identified 12-months as a feasible period to collect the 30 participants for an adequately powered study. In practice, recruitment was stretched to an 18-month period, only managing to reach just over half of our intended sample size. Inadequate power increases the likelihood of Type II error (Stevens, 2002). However reporting descriptive details, effect sizes, and reliable change indices has made the best use of the data collected.

Recruitment for this current study was informative. Several design issues were identified. Firstly, the resource intensive nature of PNI research paired with opting to recruit from a single hospital site limited the sample size attained. In addition specifying the age of patients (18 – 65 years) decreased the patient recruitment pool. This exclusion criteria plus additional criteria, (1) having sufficient English fluency and literacy, (2) not participating in other research trials, and (3) specific disease stage I –III, (4) not currently experiencing illness, not to mention the non participators were critical in influencing the sample attained. This study also required women to return to the treatment setting to take part creating the potential for the experience of conditioned responses as a negative side-effect. This would potentially contribute to slow accrual. These are all important issues to consider for future studies in this population, during the post-treatment period.

This observational study highlighted a number of additional sampling difficulties in this population difficult. The attempt at the outset was to gather a group comparable on age, diagnosis, and treatment variables. With such stringent eligibility criteria the sample attained were surprisingly heterogeneous on a number of demographic and treatment variables. It is one of the main challenges of both psychoneuroimmunology (Segerstrom & Miller, 2004) and of psychooncology (Anderson et al., 1994; Fox, 1976). Had a larger sample been attained the heterogeneity may not have been so noticeable.

The sample was disparate on age, employment status, as well as health behaviours like vitamin use. In addition treatment type, frequency, and duration varied considerably. Vitamin use falls under the broad category of complimentary and alternative medicine (CAM). This was pertinent to the current theoretical Model as vitamins with antioxidant properties are considered to improve VIT C and NT levels and subsequently improve immune function by way of enabling lymphocyte maturation. Predominantly supplements consumed by this current sample were very high in antioxidant properties plus vitamin B derivatives, although the actual supplements and the number taken (1 – 7) varied considerably between individuals. This raised concern of whether a RCT of multivitamins in this population could be performed.

The effective application of a Placebo group in this population would be difficult for several reasons. Firstly, breast cancer patients were already evidencing a high level of vitamin use. CAM use, which includes vitamin consumption, has been identified as a psychological 'crutch' for cancer patients (Tasaki, Maskarinec, Shumay, & Tatsumara,

2002). Limited evidence, using the Oxidative Model, exists regarding definitive benefits of vitamins during stress. Specifically no confirmatory analysis has been performed to assess the role of vitamin intake on oxidative or inflammatory biomarkers. The current study has identified breast cancer patients post-treatment to be particularly pro-oxidant. However there is not enough evidence to ethically ask patients to cease taking vitamins for the purpose of a trial.

5.3.7 Future directions.

At the outset it was anticipated that this sample would be experiencing significant psychological distress and emotional dysfunction as a result of a cancer diagnosis, surgery, intensive physiological challenge (adjuvant therapy), and leaving the supportive hospital-network. This was not the case. This sample was not experiencing high psychological distress levels like those evidenced in previous Oxidative Model literature, yet they evidenced heightened pro-oxidant and pro-inflammatory states. The Oxidative Model is still in its infancy with inconclusive results across studies. For this Model to reach the next step, a randomised controlled trial of the influence of vitamin consumption during sustained or chronic psychological distress is proposed. The current study has been informative and provided several reasons (i.e. recruitment challenges, additional treatment covariates, sample heterogeneity, ethical application of placebo group) to consider an RCT in an alternative population prior to testing it in a breast cancer sample.

Chapter 6

Stressful Life Events and Multivitamin Use: A Randomised Controlled Trial

6.1 Overview

The Oxidative Model has been proposed (Blake-Mortimer et al., 1996, 1998a, 1998b; Hapuarachchi et al., 2003) linking stress with increased oxidative and inflammatory processes and subsequent immune dysfunction. One of the mechanisms underlying this Model is that the body's antioxidant levels can mediate detrimental effects of stress on pro-oxidant and pro-inflammatory measures. This current study proposes a randomised controlled trial (RCT) of multivitamin supplementation in a sample experiencing chronic stress.

6.2 Aims of the Study

In light of the findings from the first study: *The Psychoneuroimmunology of Breast Cancer Patients Post-Treatment,* the aim of this second study was to provide a further test of The Oxidative Model. Ideally an intervention study in distressed breast cancer patients post-treatment was proposed. Several challenges and limitations arose during the observational study described in Chapter 6. Particular challenges included the slow accrual of patients. Over a 2-year period only 17 were consented to the study. This severely impacted on the power and limited the vigor of findings drawn from this data. In addition, as a group, the sample of breast cancer patients were not experiencing evidenced of sustained distress. Therfore in order to screen and recruit a sufficiently stressed breast cancer sample for a RCT would require a much larger multi-site pool of patients. PNI research is resource intense by its very nature. The

availability of trained staff specific to Oxidative Model who were specialised in biochemical assay techniques required a sufficient population to draw a sample from for this second study.

The breast cancer observational study confirmed previous research whereby patients frequently used vitamin and nutrient supplements. Given The Oxidative Model proposes antioxidant to play a protective role for the immune measure NT; this was identified as a confounding variable for a key biomarker (HCY). In addition the variety and number of vitamin supplements being taken by patients in the previous study was broad. To undertake a RCT of multivitamin use in this sample would involve excluding those already taking supplements, limiting sample size. Alternately researchers could request patients to cease taking supplements. This raises an ethical quandary as taking supplements has been observed to be psychologically beneficial especially for breast cancer patients (Lis, Cambron, Grutsch, Granick, & Giupta, 2006). Physiological benefits have also been proposed although there is disagreement and concern about nutrients and complementary therapies interfering with cancer treatments (Prasad, 2004; Seifried, McDonald, Anderson, Greenwald, & Milner, 2003).

Thus to deal with the shortcomings identified from the previous study, a single-sex group experiencing chronic stress and not currently taking vitamins/supplements was targeted for the RCT. This will inform whether multivitamin supplements have an impact on psychological well-being, pro-oxidant, and pro-inflammatory processes (biomarkers) during periods of chronic stress, as proposed by The Oxidative Model.

Three research questions are proposed. Firstly is increased psychological distress associated with pro-oxidant and pro-inflammatory markers as proposed by

The Oxidative Model? The previous study of found support for distress associated with several biomarkers proposed in the Model and replicating these findings will strengthen the overall Model.

Secondly can multivitamin intake counteract psychological stress, pro-oxidant, and pro-inflammatory processes in a chronic stress sample. The Oxidative Model proposes that antioxidants like those found in multivitamin supplements can deter the negative impact of chronic stress on oxidative and inflammatory measures.

Thirdly, are there influential covariates? The evaluation of covariates in the previous study highlighted just how sensitive biomarkers were to demographic, health behaviours, and treatments. This was one of the first of the first studies to explore and control for these confounding variables. It is important that these are tested in a general population study to further strengthen the evidence base.

6.2.1 Primary hypotheses

1. There will be improvement in *psychological outcomes* for women undergoing stressful life events who were allocated to an active multivitamin group compared to those allocated to a Placebo group.
    a. Improved *psychological outcomes* will be assessed by-
        i. Decreased distress (GHQ-12)
        ii. Decreased state anxiety, depression, anger, and a contemporaneous increase on state curiosity scores (STPI)
        iii. Decreased loneliness scores (UCLA)

2. There will be improvement in *pro-oxidant and pro-inflammatory measures* for women undergoing stressful life events who were allocated to an active multivitamin group compared to those allocated to a Placebo group.
    a. Improved pro-oxidant state will be measured by-
        i. Increased 5'-ectonucleotidase (NT) levels
        ii. Increased Tissue Ascorbate (VIT C) levels
        iii. Increased Total Antioxidant Status (TAS) were analysed by Dr Chalmers using the randox kit which measures the total antioxidant status of serum relative to a vitamin E standard.
        iv. Increased Folate (FOLATE) and Vitamin B12 (VIT B12) levels
        v. Decreased Homocysteine (HCY) levels
    b. Improved pro-inflammatory state will be measured by
        i. Decreased C-Reactive Protein (CRP) levels
        ii. Decreased Cholesterol (CHOL) levels
        iii. Decreased inflammatory cytokines (IL-1β , TNF-α, TNF-β, IL-5, IL-6, and IFN-γ)
        iv. Increased anti-inflammatory cytokine, IL-10.

6.2.2 Secondary hypotheses

3. Pre-intervention, pro-oxidant measures will be associated with higher levels of psychological distress and dysfunctional emotion states-
    a. Decreased NT and VIT C will be associated with increased distress
    b. Increased HCY and decreased FOLATE and VIT B12 will be associated with increased distress and anger

4. Pre-intervention, pro-inflammatory measures will be associated with higher levels of psychological distress and dysfunctional emotion states-
    a. Increased CRP will be associated with increased distress
    b. Increased inflammatory cytokines (IL-1β , TNF-α, TNF-β, IL-5, IL-6, and IFN-γ)will be associated with increased distress

6.3 Method

6.3.1 Site.

This study was conducted at The University of Adelaide, South Australia from 2005 through to 2006. This study was approved by The University of Adelaide Human Research Ethics Committee. In addition this study was registered with the Therapeutic Goods Administration, clinical trial number: BR040502. Consort 2010 guidelines have been adhered to (Appendix O).

Volunteers were recruited from the general population over a 6-month period, using a variety of resources including press-releases, local and regional radio interviews, local newspaper advertisements, and posters displayed around the Adelaide CBD. Women responded to advertisements (Appendix E) and contacted the researcher via email or telephone. On initial contact, participants were provided with a brief description of the study. This was followed up with posting or emailing of a comprehensive participant information sheet outlining the study (Appendix F).

6.3.2 Inclusion criteria.

Women aged between 25 and 45 years were invited to participate in this study. This age range was selected based on previous findings suggesting that oxidative biomarkers, NT in particular, decreases with age. This age range was chosen in order to remove some of the variability which has been associated with ageing processes (Boss et al., 1980) (Segerstrom & Miller, 2004).

6.3.2.1 Stress screen.

In order to obtain a 'stressed' sample, the General Health Questionnaire short version (GHQ-12; Goldberg, 1992) was used as a screening tool for recruitment of participants to this trial (Appendix I). This scale was used based on its success in previous work exploring relationships between stress, the immune system, and multivitamin supplementation (Hapuarachchi et al., 2003).

The GHQ was developed in the 1970s and is one of the principle self-report questionnaires used to measure non-psychotic mental illness in a community or general practice setting (Donath, 2001). Respondents rate themselves according to the degree to which they have experienced symptoms over the past few weeks; the standard scoring method is a binary method. GHQ-12 scores of 0-1 are considered normal, 2-3 as mildly stressed, and 4+ as severely stressed. In this instance 'GHQ score' is an indication of cases at risk of psychological breakdown and is determined by using the binary code (0, 0, 1, 1) to assess whether a symptom is present '1' or not '0'.

Two criterions were set in order to attain a chronic stress sample. Firstly it was necessary to set criteria for eligibility based on stress levels meeting the criterion of ≥3. This criterion incorporates moderate to severe stress, and was chosen to allow

sufficient variability in scores, plus an adequate sample size to be attained. Secondly participants were required to have been experiencing 'stress' for at least one month duration. This was considered necessary in order to concur with the chronic stress scenario highlighted in The Oxidative Model literature (Blake-Mortimer et al., 1996, 1998b; Hapuarachchi et al., 2003). In line with this the wording of the GHQ-12 for screening purposes was altered slightly, instead of:

> *We should like to know if you have had any medical complaints and how your health has been in general, over the last few weeks.*

it was changed to

> *We should like to know if you have had any medical complaints and how your health has been in general, <u>OVER THE LAST FOUR WEEKS</u>.*

Reliability coefficients provided in this chapter are based on this set of instructions described here. Aforementioned it is possible to score the GHQ-12 in two ways; the first scoring method represents a binary coding system described above. The second scoring method uses a Likert-scale rating, ranging from 0-3, i.e., (0) 'not at all/better than usual', (1) 'no more than usual', (2) 'rather more than usual', to (3) 'much more than usual'. In the current study binary scoring was used for screening, and the Likert-scale rating was employed for subsequent pre- and post-intervention assessment. This change in scoring from screening to Likert scoring within the RCT makes scores comparable for the analysis of choice (i.e., ANCOVA).

6.3.3 Exclusion criteria.

It was required that patients could only enter the study if they were free from current infections. Further to this, people suffering from chronic conditions (i.e., severe heart disease or diabetes), autoimmune and inflammatory diseases (e.g., rheumatoid arthritis, Addison's disease, Cushing's disease, Lupus Erythematosus), or taking immunosuppressive medication (e.g., steroids like cortisone) were excluded from participation. These medications are known to influence biomarkers central to the Model.

In addition people taking blood thinning agents such as Warfarin were excluded to avoid adverse consequences of blood taking. For similar reasons women with known allergies, women who were pregnant, recently had a baby, or were breastfeeding were also excluded from this study. To further ensure that women taking part in this study did not suffer from other unknown health conditions, a full blood examination (FBE) was conducted at each assessment point. It was planned that any participants with abnormal blood results would be excluded and referred to their medical clinician; this was not necessary for any women taking part in this study. Self-reported regular multivitamin-takers (> 2 times per week) were excluded from this sample to remove any pre-existing influence.

6.3.4 Withdrawal criteria.

Participants had the right to refuse to continue with the study at any time. Participants experiencing symptoms of infection (i.e., common cold) during pre- and/or post-intervention data collection were excluded from analyses. In addition participants becoming pregnant during the course of the trial were also withdrawn for precautionary reasons. Furthermore any participants experiencing adverse

psychological or physiological side-effects throughout the trial were withdrawn and referred to their GP.

6.3.5 Design.

This study incorporated a parallel group, randomised control trial (RCT) design. Participants were recruited consecutively into the study using only the above inclusion and exclusion criteria in order to ensure, as far as possible, patients reflected the population from which they were drawn. Recruitment was targeted on areas surrounding the Adelaide CBD, in order to ensure participants could access facilities for data collection easily. Participants were randomly allocated to an Active, multivitamin supplement group (Active) or a Placebo group (Placebo). This design is particularly robust, and controls for all threats to internal validity (Campbell & Stanley, 1963). In total, there were two repeated assessments- pre- and post-intervention. The second assessment occurred 8-weeks, 63.27 days (SD = 5.77) after the pre-intervention assessment. This enabled exploration of pre-intervention associations, as well as change across the duration of the study.

6.3.6 Flow of participants.

Flow of participants through the study is presented in Figure 30. Eighty-one women responded and were assessed for eligibility by the researcher. Recruitment to attain a sample of 60 women was ongoing over a 6-month period. Over a quarter of initial respondents (26%) were excluded as they did not meet the eligibility criteria. As discussed previously in the Method, all respondents were screened using the GHQ-12

short version in order to achieve a sample with moderate to severe distress levels. The most common reason for exclusion was inadequate stress level.

Pre-intervention psychological data were collected for 60 participants. Technical difficulties with blood-taking resulted in some missing biochemical data (Table 38). For two participants' blood samples were inadequate, with only enough collected for one set of assays. As a result pro-oxidant markers were missing for one participant at baseline, allocated to the Placebo group. Similarly pro-inflammatory measures for a participant allocated to the Active group were missing. For cytokine assays two sets of data were affected for IFN-γ. This was due to inconclusive assay results, one participant at baseline, and the other post intervention. Both had been randomised to the Placebo group.

Post-intervention assessment occurred 8-weeks following baseline data. Ten women were unable to complete assessment. This was predominantly due to loss of interest. One woman fell pregnant during the trial and was subsequently withdrawn and another withdrew due to the experience of nausea on taking the capsules. Psychological data was collected for the remaining 50 women who completed the trial. Technical difficulties with blood-taking at post-intervention assessment resulted in some missing data (Table 38). Two sets of pro-oxidant markers were missing; one from the Placebo group and the other for an Active group member. Similarly pro-inflammatory measures were missing for another two participants due to inconclusive assays. Full data were obtained for 47 participants for this trial.

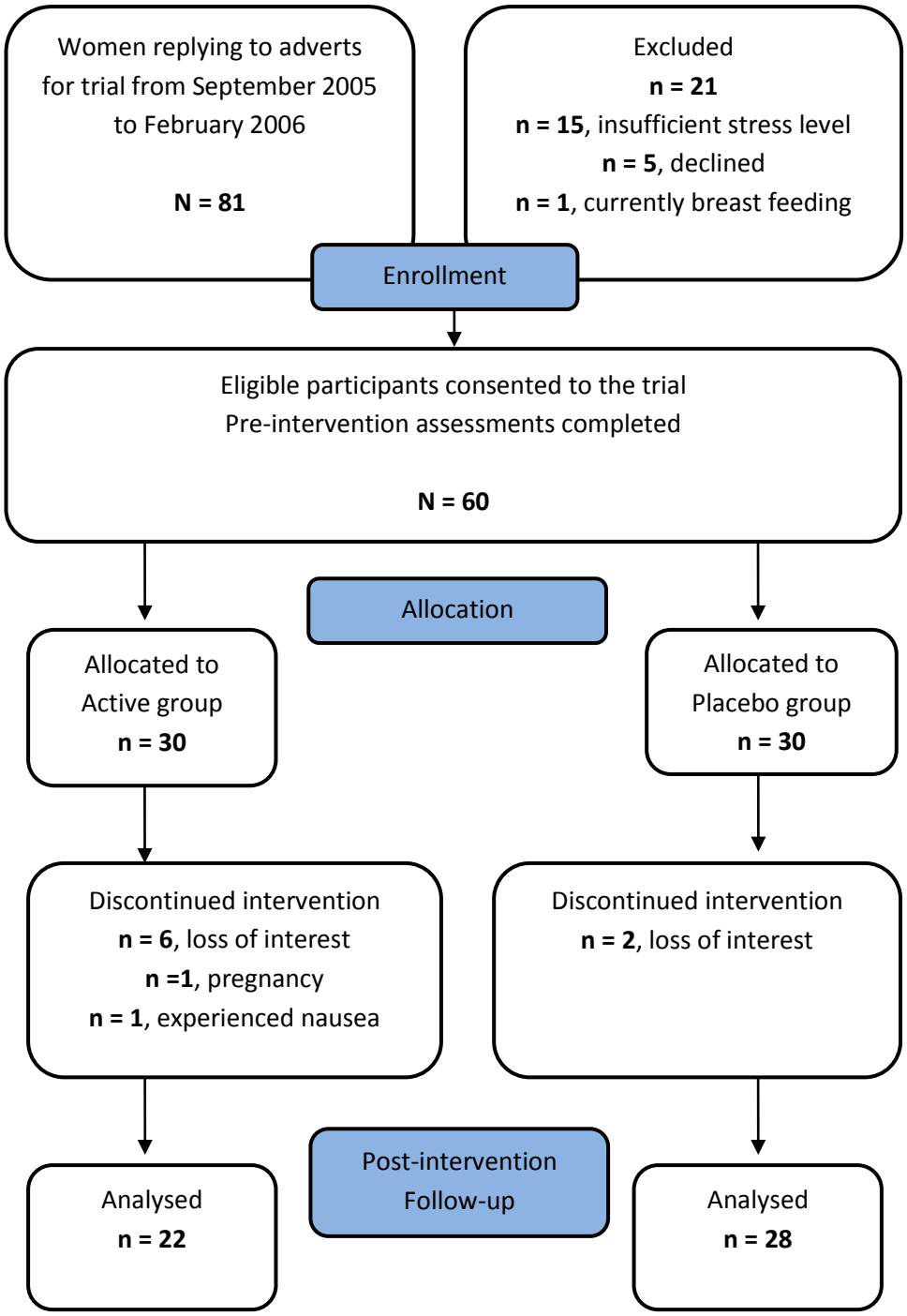


Figure 30: Flow of recruitment, allocation and participation throughout the trial

Table 38: Pre- and Post-Treatment Assessments: The Number of Biochemical Marker Results Available at Each Time Point Due to Blood Collection and Assay Technical Difficulties

Measures	Number of cases obtained			
	Pre-intervention		Post-intervention	
	Active	Placebo	Active	Placebo
	n = 30	n = 30	n = 22	n = 28
HCY	30	30	22	28
CRP	30	30	22	28
VIT B12	29	30	22	28
FOLATE	28	30	21	28
NT	29	30	22	27
VIT C	29	30	22	27
TAS	29	30	21	27
IL-1β	29	29	21	27
IL-5	28	30	21	27
IL-6	29	30	21	27
IFN-γ	28	28	21	26
TNF-α	29	30	21	27
TNF-β	29	30	21	27
IL-10	29	30	21	27

Note. Biomarker Abbreviations: 5' –ectonucleotidase (NT), tissue ascorbate (VIT C), homocysteine (HCY), Vitamin B12 (VIT B12), Folate (FOLATE), Cholesterol (CHOL), C-reactive protein (CRP), Interferon (IFN), Tumor necrosis factor-α (TNF-α), Interleukin-1 (IL-1β), Interleukin-5 (IL-5), Interleukin-6 (IL-6), Interleukin-10 (IL-10), Tumor necrosis factor- β (TNF-β), FBE = full blood examination

6.3.7 Intervention.

Eligible participants were randomised to either intervention or control groups. The intervention group were the multivitamin supplemented (Active) group, the control group the non-active supplement group (Placebo). Pre- and post-intervention data were collected for both groups. Regardless of group allocation participants were

instructed to take one capsule twice a day (morning and night), preferably to be consumed with a food.

6.3.7.1 Active group.

Participants allocated to the Active group were provided with an 8-week supply of multivitamin supplements. The ingredients of these capsules, listed below (Table 39), claim to help 'balance the ups and downs of everyday life'. The manufacturers claim that the combination of ingredients aim to balance the body's systems that respond to stress, replace nutrients depleted during periods of stress, as well as supporting the body's energy production and detoxification of chemicals such as alcohol.

Table 39: Composition of Multivitamin Supplement for the Active Group

Therapeutic Agent	Dosage
Vitamin B1 (Thiamine nitrate)	12.5mg
Vitamin B2 (Riboflavin)	12.5mg
Nicotinamide	25mg
Vitamin B5 (Pantothenic from Calcium pantothenate)	37.5mg
Vitamin B6 (Pyridoxine hydrochloride)	25mg
Vitamin B12 (Cyanocobalamin)	25mcg
Biotin	37.5mcg
Folic acid	150mcg
Vitamin C (Ascorbic acid)	75mg
Magnesium oxide- heavy (Magnesium 62.5 mg)	109mg
Zinc amino acid chelate (Zinc 6mg)	30mg
Withania somnifera (Winter cherry) extract equiv. to dry root	1.5g (1500mg)

Note. This product is registered for use as a natural health product in Australia, under the Therapeutic Goods Administration.

6.3.7.2 Placebo group.

Participants randomised to the control group were provided with an 8-week supply of placebo capsules. The participating multivitamin manufacturer provided placebo capsules comprising un-reactive ingredients, specifically a formulation comprised of calcium dihydrogen phosphate. Placebo capsules were identical to the active supplement capsules in order to ensure that participants were unable to detect any visual difference between them.

6.3.8 Randomisation.

Randomisation of participants was achieved by employing a computer generated, block randomisation technique in order to ensure equivalent numbers were allocated to both Active and Placebo groups. The researcher was provided with identical containers containing the capsules; containers were numbered sequentially to implement the random allocation sequence.

6.3.8.1 Implementation.

The multivitamin manufacturer generated the random allocation sequence. The principal researcher was in charge of recruitment, screening, consenting, and data collection. Pre-intervention, on completion of the preliminary questionnaire and blood collection the researcher consecutively provided each participant with a numbered container of capsules. At the conclusion of the 8-week trial, data collection procedures were repeated. Participants were requested at this follow-up to return all containers regardless of whether empty or with remaining capsules. Thirty-three containers were returned with any remaining capsules (M = 10.04, SD = 8.05, capsules remaining).

6.3.8.2 Blinding.

The manufacturers were blinded to the allocation of participants. Nurses involved in blood-taking, laboratory staff, and biochemists undertaking assays pre and post-intervention were blinded to demographic and psychological outcomes, and group allocation for the entire study.

The principal researcher was blinded to group allocation, until primary and secondary results were analysed. At this time the manufacturer provided information to determine only the distinct groups (i.e., group A and group B). On informing the manufacturer of preliminary findings, the full randomisation schedule was revealed to the researcher.

6.3.9 Data collection procedure.

On initial contact, participants were informed of the objectives of the study and of possible adverse effects that might occur because of participation in the study (i.e., a slight risk of bruising associated with blood taking) and provided a patient information sheet. Participants were asked to complete a consent form once they had decided to participate. The researcher obtaining consent made a conscientious effort to be fully satisfied that the participant had truly understood the nature of participation in the study to which consent was given. Preservation of the confidentiality of patients taking part in this study was maintained at all times.

Participants, once confirmed eligible and consented, were scheduled to take part in this study. All data collection took place at The University of South Australia's Nursing School. Pre-intervention data was collected within 2-4 weeks of the initial

screening. This was dependent on participant scheduling as well as the availability of blood collection resources.

6.3.9.1 Pre- and post-intervention assessment.

Self-report questionnaires and biochemical measures were collected twice. Pre-intervention or baseline data was collected at the outset of the study prior to allocation to the Active or Placebo group. Post-intervention was attained 8-weeks after pre-intervention data after participants had been randomised to Active or Placebo groups, and received the intervention.

Both pre- and post-intervention assessments were taken during the same scheduled hours (between 11.30am and 2.00pm) in order to alleviate the potential influence of circadian fluctuations on biochemical markers.

6.3.9.1.1 Demographic information and health behaviours.

At pre-intervention assessment, participants completed a self-report questionnaire (Appendix G) consisting of a series of standardized psychological scales as well as questions on demographic and health related behaviours previously described in detail in the previous methodology section 5.1.8.1. To summarise, for collection of data on alcohol use, researchers adapted questions from the WHO Alcohol Use Disorders Identification Test (AUDIT (Babor et al., 2001).) For physical activity measurement the International Physical Activities Questionnaire (IPAQ short form: International Physical Activities Questionnaire Committee, 2004) was employed. Both the IPAQ and AUDIT are outlined in detail below. Smoking behavior was assessed from an adapted version of another measure (West, 2004). In summary, health

behaviours assessed included rates of exercise, alcohol, tobacco, and medication use. Post-intervention questionnaires (Appendix H) comprised only psychological questionnaires as it was considered there would be little change in demographic and health behavior data over the 8-week period.

6.3.9.1.2 Psychological measures.

Selection of psychological scales (Table 40) for this trial was based on previous research on The Oxidative Model (Blake-Mortimer et al., 1996, 1998b; Hapuarachchi et al., 2003). The measures employed were the same as those used in the observational study (Chapter 5). Cancer-specific measures were omitted. Table 40 shows each scale and its corresponding reliability relative to this sample for full description please refer to Chapter 5 section 5.18 and 5.19.

Pre-intervention psychological assessment included several standardised psychological measures of trait characteristics including Spielberger's Trait Personality Inventory (STPI; Spielberger, 1996), the Lifestyle Defense Mechanism Scale (LDMS; Spielberger & Reheiser, 2002), and the revised T-anger Expression Inventory (STAXI-2; Spielberger, 2003). These measures assess dispositional characteristics which are considered to be stable across time.

Table 40: Psychological Measures Assessed and their Reliability Coefficients for the Current Study

Instrument	Measures	Scales	Reliability
General Health Questionnaire(GHQ-12)	Psychological distress	GHQ	.88
State Trait Personality Inventory (STPI)	Personality style	S- Anxiety*	.87
		S- Curiosity*	.81
		S- Anger*	.92
		S- Depression*	.86
		T-Anxiety	.87
		T-Curiosity	.90
		T-Anger	.82
		T-Depression	.86
		Anger Expression Out	.75
		Anger Control In	.86
		Anger Expression In	.73
		Anger Index	.61
Lifestyle Defense Mechanisms Scale (LDMS)	Control and suppression of emotions	Rationality & Emotional Defensiveness	.86
		Need for Harmony	.85
UCLA Loneliness	Experience of social isolation	Loneliness*	.92

Note. Reliability reported is based on Cronbach's alpha for the current sample (N = 60)

6.3.9.1.3 Biochemical measures.

Biochemical parameters were assessed from blood samples attained pre- and post-intervention. Approximately 40 ml of blood was collected from each participant. Table 41 provides a summary of biochemical measures collected and materials required for this trial. Blood collection procedures and protocol were followed as outlined in the previous study in Chapter 5. For additional information on assay techniques refer to section 5.1.9.2.

A novel biomarker was also added to this study. Assay techniques for this biomarker were unavailable when the observational study was undertaken. The TAS biomarker indicates capacity of the body's total antioxidant defence system in circulation. A study of lifestyle effects on antioxidant capacity in a healthy adult sample found that for the 8% of participants who consume multivitamins or trace element supplements, their whole-blood resistance to free-radical aggression (in-vitro) was significantly higher than in non-consumers ($p < .00$, $N = 184$; Lesgards et al., 2002). Thus it is proposed that plasma antioxidant status improves blood resistance against free radicals. This study also assessed psychological stress as measured by a 5-item questionnaire concerning workplace demands and intrusion of work concerns into home life. Severe stress was identified as the lifestyle factor that most markedly associated with decreased antioxidant capacity ($p < .00$, $N = 177$) in comparison to weak or moderate distress levels. Mechanisms underlying this finding were not thoroughly investigated.

Table 41: Materials Required for Blood Collection for each Participant at each Time Point

Vacuette for blood collection	Measures
1 x 4ml K3E EDTA vacuette	HCY
1 x 4ml K3E EDTA vacuette	FBE
1 x 9ml Lithium Heparin vacuette	CRP
1 x 8ml serum Sep. Clot Activator vacuette	VIT B12
	FOLATE
1 x 9ml Lithium Heparin vacuette	NT
	VIT C
	TAS
1 x 8ml serum Sep. Clot Activator vacuette	IL-1β
	IL-5
	IL-6
	IFN-γ
	TNF-α
	TNF-β
	IL-10

Note. Biomarker Abbreviations: 5' –ectonucleotidase (NT), tissue ascorbate (VIT C), total antioxidant status (TAS), homocysteine (HCY), Vitamin B12 (VIT B12), Folate (FOLATE), Cholesterol (CHOL), FBE = full blood examination, C-reactive protein (CRP), Interferon (IFN), Tumor necrosis factor-α (TNF-α), Interleukin-1 (IL-1β), Interleukin-5 (IL-5), Interleukin-6 (IL-6), Interleukin-10 (IL-10), Tumor necrosis factor- β (TNF-β).

6.3.10 Statistical methods.

All available data from participants were analysed in the groups they were allocated to regardless of whether they followed the intervention (i.e., consumed the multivitamins/ placebo capsules). Participant data were not excluded from analyses due to non-compliance. This design excluded those participants who withdrew at either pre or post-intervention assessment.

Data were analysed using SPSS version 17. Data were initially screened for missing data, outliers, normality, heterogeneity, and skewness before descriptive statistics were presented for demographic, psychological, and immunological baseline assessments. Frequencies and descriptive statistics were used to report demographic, health behaviour, psychological characteristics, and biomarker levels of the sample. Subsequent Chi-Square tests and Independent Samples t-tests were used to assess differences between groups to see whether randomisation techniques were successful in attaining comparable groups. Mann-Whitney U tests were performed for variables not normally distributed.

Additional screening was completed in order to meet assumptions for between within-subject analyses of covariance (ANCOVA) techniques, i.e., linearity, homogeneity of variances, and reliable measurement of covariates. Prior to inferential statistical analyses, preliminary correlation matrices of psychological, demographic, and health behaviour variables at baseline were performed in order to elucidate any covariates.

Hypotheses 1a (i-iii), 2a (i-v), and 2b (i-iii) were explored using between-within ANCOVAs to assess changes across time and between groups, including interaction effects, whilst controlling for covariates. ANOVAS with repeated measures assessments are particularly susceptible to the violation of the assumption of sphericity with violation causing the test to become too liberal (i.e. an increase in the Type I error rate). To remedy this Mauchly's Test of Sphericity were assessed. If sphericity was violated ($\varepsilon < 0.75$) the more conservative correction Greenhouse-Geisser will be applied. Reporting Greenhouse-Geisser values produces a more valid critical F-value to reduce the increase in Type I error rate.

For non-parametric variables change across time (pre-to post-intervention) were assessed using the Wilcoxon Signed Rank Tests. Group differences post-intervention were explored using Mann Whitney U techniques.

Hypotheses 3 (a, b, and c) and 4 (a, b, and c) were tested using Pearson product-moment correlations and Spearman's rank-order correlations to explore pre-intervention relationships between psychological and biochemical measures.

6.3.10.1 Sample size.

In an attempt to detect medium sized differences between the Active group and the Placebo group, it was anticipated that approximately 64 participants (J. Cohen, 1988) allocated to each group would be needed to achieve 80% power ($\alpha < .05$). This level of power to detect between group differences was considered ample to detect within-group differences across two time points (Stevens, 2002).

Due to the extremely labour intensive assays typical of PNI studies, plus specialist assay techniques confined to one biochemist, it was only possible to collect and successfully assay a small sample size for this study (N = 60). It is worth noting this is the largest sample recruited for single study across all Oxidative Model literature to date. However due to the sample size attained, a power calculation suggests only large effects would be detectable (Rosenthal & Rosnow, 1991). Therefore results should be viewed with caution as Type II errors could be likely. Consequently instead of simply relying on null hypothesis significance testing, effect sizes (partial eta squared [η^2] and phi coefficients [ϕ]) were also calculated to determine the magnitude of change over time (and the differences between groups). Type 1 errors are also possible although given the sample size the likelihood for a Type II error is more critical. For future reference, according to Cohen (1988), for partial eta squared, .01 represents a small effect, .06 a moderate effect, and .14 a large effect. For phi coefficients, .10 represents a small effect, .30 a moderate effect, and .50 a large effect

6.3.10.2 Reliable change indices calculation.

For the main psychological parametric variables (distress, S-anxiety, S-anger, S-depression, S-curiosity, and loneliness) for those evidencing small or larger effects over time, reliable change indices (RCIs) were calculated across the two time points. RCIs cannot be calculated for non-parametric variables as means and standard deviations are required. Furthermore, they could not be calculated for biochemical variables as reliability coefficients from continuous scales need to be specified, thus mean change scores will be calculated instead. Specifically, positive, negative, and no reliable change was calculated for individual participants. This time period included change from pre-

intervention to post-intervention. Analyses were based on those described by Evans, Margison, and Barkham (1998) using the following formula:

$$RC = \frac{X_2 - X_1}{SE_{diff}}$$

In the above formula, X2 is the post-test or Time 2 score and X1 is the pre-test or baseline score. The SEdiff refers to the standard error of the difference between the two test scores and is calculated using the formula below where SD1 refers to the pre-test or baseline standard deviation and the r refers to the internal consistency (Cronbach's alpha) of the measurement tool as assessed by the current research.

$$SE_{diff} = SD_1 \sqrt{2} \sqrt{1-r}$$

RCIs are often used to determine meaningful change in clinical situations, such as following a therapeutic intervention. They are still considered useful in research as they show the extent of change evidenced by an individual outside of that which could be attributed to measurement error or variability of the assessment tool or intervention being used. Reliable change suggests that actual change was likely to occur 95% of the time.

6.4 Results

6.4.1 Data screening.

6.4.1.1 Normality.

Normality was assessed via histograms, Q-Q plots, and measures of skewness (Pallant, 2007). Although there were some minor variations, most variables were normally distributed and were assessed as such. S-anger scores were positively skewed. In a general population sample this is expected in as few individuals will exhibit high. Variables which did not meet normality criteria were the inflammatory cytokines (IL-1β, TNF-α, TNF-β, IL-5, IL-6, and IFN-γ). For the current study inflammatory cytokines evidenced a wide range of scores, unlike the previous study of breast cancer patients. This wide range of scores, paired with variability between individuals exacerbated the skewness and non normal distribution of these measures. The observed skewness for these variables was positive, which reflects the pattern of inflammation, specifically these responses are either not activated (i.e. very low or zero cytokine levels) or alternately a rapid cascade is triggered occurring over a matter of hours; this is when very high levels of cytokines are observed.

Transformations were considered but not undertaken for a number of reasons. Firstly, any transformation would have had to be across all time points to achieve repeated score comparisons, possibly forcing transformations of 'normally' distributed variables. In the case of cytokine patterns observed skewness varied between pre- and post-assessments within individual measures between assessments. It was deemed transformations would interfere unnecessarily with these 'real life' patterns in the

data, for example a high score pre-intervention followed by a low score post-intervention.

Secondly, in order to keep results interpretable data transformations are not universally recommended, as interpretation of analyses using transformed variables can be more difficult (Tabachnick & Fidell, 2007). This is especially important when comparing scores to normative means or ranges of a variable. In study 1, the observed inflammatory cytokine levels fit a normal probability distribution. In the current sample, of healthy stressed women, the observed levels across individuals did not fit. Therefore, it was decided that these variables be analysed using nonparametric tests due to the reliance of on fewer assumptions. Non-parametric methods are more robust, but this increased robustness comes at a cost. In cases where a parametric test would be appropriate, non-parametric tests have less power. In other words, a larger sample size can be required to draw conclusions with the same degree of confidence. Effect sizes for non-parametric results will be reported.

6.4.1.2 Outliers.

Variables were further checked by conversion to standardized scores (z-scores) with any scores above 3.29 considered outliers. For CRP and cytokines, several scores bordering on 3.29 were found. Due to the nature of these physiological markers, extremely high scores for short periods of time are not indicators of abnormality, rather an indicator of an inflammatory response normal in a healthy functioning immune system. For this reason these 'borderline' outliers were included in further analyses, unless participants informed the researcher of recent symptoms of illness in

the days preceding assessment (i.e., cold/flu). Borderline outliers were not considered to sufficiently impact on the distribution of variables and as such none were ruled out.

6.4.1.3 Attrition analysis.

Refer to Figure 29 of section 6.3.6, for flow of participants through the study. Forty-eight women completed the entire 8-week intervention, including both pre- and post-treatment assessments. The drop out rate (16.7%) was higher than anticipated. An attrition analysis was undertaken to assess demographic, health behaviours, psychological and biomarker characteristics of 'non-completers'.

Results indicated minimal differences for demographic and health behaviours between those completing the 8-week trial compared to non-completers (Table 42 and 43). Moderate effect sizes were observed for education and occupation. Completers tended to be better educated, and all participants who were students completed the trial. For health behaviours, participants who dropped out of the study had lower levels of antidepressant and cardiovascular medication use, evidencing large effect sizes. In contrast a small effect suggested non-completers were more likely to not currently be taking any medications.

Psychologically groups did not differ significantly, and effect sizes reflected this also (Table 44 and 45). Similarly there was little physiological disparity (Table 46 and 47) observed. The only biomarker evidencing significant change between completers and non-completers was TAS (t (57) = -2.38, p = .02 η^2 = .10). Those dropping out of the trial had higher serum antioxidant levels pre-intervention than those remaining in the study. This effect size was moderate- to- large. Attrition analysis identified non-

completers comprised more Active group participants compared to Placebo group as reflected by the small-to-moderate effect size observed for group allocation (Table 42).

Table 42: Demographic Information of Completers Compared to Non-completers (N=60)

Variable	Completers n = 50(%)	Non-Completers n = 10 (%)	*t* or χ^2	df	p	ϕ
Age in years, M (SD)	37.18 (6.41)	40.10 (6.26)	*t* (1.03)		.31	.02*
Marital Status						.
Married	24 %)	7(%)				
Defacto	8 (%)	0 (%)				
Single	9 (%)	1 (%)				
Divorced	9 (%)	2 (%)	χ^2 (2.72)	3	.44	.21
Education Level						
Secondary	7 (%)	5 (%)				
Vocational	7 (%)	1 (%)				
Tertiary	36 (%)	4 (%)	X^2 (6.78)	2	.03	.34
Occupation						
Professionals	17 (%)	2 (%)				
Management	14 (36.36%)	3 (%)				
Clerical & Service	7 (%)	4 (%)				
Home Duties	2 (%)	1 (%)				
Student	10 (%)	0 (%)	X^2 (5.91)	4	.21	.31
Workload						
Full Time	31 (%)	5 (%)				
Part Time	15 (%)	5 (%)				
Casual Employment	3 (%)	0 (0.00%)				
Other	1 (%)	0 (0.00%)	X^2 (2.00)	3	.57	.18
Children	32 (%)	9 (%)	X^2 (1.54)	1	.22	.21
Group Allocation						
Active	22 (%)	8 (%)				
Placebo	28 (%)	2 (%)	X^2 (3.00)	1	.08	.27

Note. Φ = phi coefficient is a measure of effect size and can range from 0 to 1, with .10 for small, .30 for medium and .5 for large applies (Cohen, 1988). *η^2 reported guidelines are .01 = small effect, .06 = moderate effect, and .14 = large effect.

Table 43: Health Behaviour Variables of Completers Compared to Non-Completers (N = 60)

Variable	Completers n = 50 (%)	Non-Completers n = 10(%)	t or χ^2	df	p	ϕ
Alcohol use M (SD)	4.32 (1.93)	4.22 (2.39)	(t) 0.14	58	.89	*.00
Smoker	6 (%)	1 (%)	X^2 (0.00)	1	.99	.02
Physical Activity						
Inactive	15 (%)	3 (%)				
Minimally Active	22(%)	7(%)				
Health Enhancing	13 (%)	0(0.00%)	X^2 (3.77)	2	.15	.25
Medication						
None	24 (%)	7 (%)	X^2 (0.85)	1	.20	.16
Antidepressants	4(18.18%)	0 (0.00%)	X^2 (3.34)	1	.07	.33
Respiratory	4 (%)	0 (0.00%)	X^2 (0.05)	1	.82	.12
Cardiovascular	2 (%)	0 (0.00%)	X^2 (0.00)	1	.34	.23
Contraceptive	16 (%)	2 (%)	X^2 (0.57)	1	.71	.10

Note. Φ = phi coefficient is a measure of effect size and can range from 0 to 1, with .10 for small, .30 for medium and .5 for large applies. * η^2 reporting guidelines are .01 = small effect, .06 = moderate effect, and .14 = large effect (Cohen, 1988)

Alcohol usage measured with AUDIT employing hazardous drinking items (scores range 0-12); **Contraceptive** medications include all forms of hormonal contraception oral, intra-uterine and implants.

Table 44: Trait Psychological Characteristics of Completers Compared to Non-Completers (N = 60)

Psychological Measure	Normative Sample		Current Sample		df	t	p	η^2
	N	M (SD)	Completers n = 50 M (SD)	Non-completers n = 10 M (SD)				
T-anxiety	133	17.98 (5.45)	20.96 (5.36)	21.30 (5.64)	58	-0.18	.86	.00
T-curiosity	133	28.86 (5.73)	27.34 (5.42)	25.00 (5.27)	58	1.25	.22	.03
T-anger	133	18.13 (4.82)	21.50 (6.14)	22.50 (6.31)	58	-0.47	.64	.00
T-depression	171	14.79 (5.05)	19.38 (5.67)	19.80 (6.96)	58	-0.21	.84	.00
AX-out	952	14.79 (3.78)	14.88 (3.60)	15.50 (3.72)	58	-0.50	.62	.00
AX-in	952	15.69 (4.38)	16.48 (3.96)	15.90 (4.23)	58	0.42	.68	.00
AC-out	952	21.52 (4.91)	22.08 (5.30)	22.44 (3.61)	58	-0.20	.84	.00
AC-in	952	23.28 (5.82)	20.70 (5.13)	20.80 (3.97)	58	-0.06	.95	.00
AX-index	952	28.59 (13.02)	36.58 (14.24)	36.44 (11.95)	58	0.03	.98	.00
R/ED	585	34.13 (5.52)	32.96 (6.14)	33.20 (3.80)	58	-0.12	.91	.00
N/H	577	35.60 (5.74)	35.04 (6.11)	36.10 (6.81)	58	-0.49	.63	.00

Note. Abbreviations: anger expression out (AX-out), anger expression in (AX-in), anger control out (AC-out), anger control in (AC-in), anger expression index (AX-index), R/ED = Rationality & Emotional defensiveness, N/H = Need for Harmony ;

Normative trait data (STPI) derived from normal female college sample, age unspecified (Spielberger, 1996); Normative Anger expression data (STAXI-2) derived from a female sample, age unspecified (Spielberger, 2003); Normative Lifestyle Defense Mechanisms data (LDMS) derived from a female sample, age unspecified (Spielberger, 2002)

η^2 reporting guidelines are .01 = small effect, .06 = moderate effect, and .14 = large effect (Cohen, 1988)

Table 45: State Psychological Variables of Completers Compared to Non-Completers (N = 60).

Psychological Measure	Normative Sample		Current Sample					
	N	M (SD)	Completers n = 50 M (SD)	Non-completers n = 10 M (SD)	df	t	p	η^2
Psychological distress		N/A	16.12 (5.91)	13.30 (3.89)	58	1.44	.15	.03
S-anxiety	133	19.06 (5.75)	21.14 (5.40)	20.70 (5.83)	58	0.23	.82	.00
S-curiosity	133	26.17 (5.45)	24.58 (4.53)	24.40 (6.28)	58	0.11	.92	.00
S-depression	133	14.79 (5.05)	19.22 (5.33)	17.90 (4.77)	58	0.73	.47	.01
S-anger[1]	133	14.24 (5.75)	12.76 (4.92)	11.70 (2.67)	58	0.66	.51	.01
Loneliness	240	36.30 (2.80)	43.74 (10.29)	41.00 (11.51)	58	0.75	.45	.01

Note. Normative data based on normal female college sample (N = 133), age unspecified (Spielberger, 1996), UCLA Loneliness normative data derived combined sample of men and women(Steptoe, et al. 2004)

η^2 reporting guidelines are .01 = small effect, .06 = moderate effect, and .14 = large effect (Cohen, 1988)

Table 46: Pro-oxidant Biomarkers of Completers Compared to Non-Completers (N = 60)

Measure	Normal Range	Current Sample		df	t	p	η^2
		Completers	Non-completers				
		n = 50	n = 10				
		M (SD)	M (SD)				
NT	0.4-1.4 nmol/h/µgDNA	0.69 (0.21)	0.78 (0.26)	57	-1.21	.23	.03
VIT C	50-150 pg/ugDNA	68.10 (17.56)	59.89 (13.70)	57	1.33	.20	.03
TAS	1.30-1.77 umol/L	1.55 (0.17)	1.70 (0.20)	57	-2.38	.02	.10
HCY	3-13 umol/L	7.33 (1.53)	8.02 (1.74)	58	-1.27	.21	.03
VIT B12	140-700pmol/L	401.32 (183.82)	316.33 (105.52)	57	1.34	.19	.03
FOLATE	5-45 nmol/L	28.76 (9.44)	23.63 (6.93)	56	1.55	.13	.04
CHOL	<5.5 mmol/L	4.98 (0.91)	4.96 (0.90)	58	0.08	.94	.00

Note. Biomarker Abbreviations: 5' –ectonucleotidase (NT), tissue ascorbate (VIT C), total antioxidant status (TAS), homocysteine (HCY), Vitamin B12 (VIT B12), Folate (FOLATE), Cholesterol (CHOL)

η^2 reporting guidelines are .01 = small effect, .06 = moderate effect, and .14 = large effect (Cohen, 1988)

.

Table 47: Inflammatory measures of Completers Compared to Non-Completers (N = 60)

Measure	Normal Range	Current Sample		t/U	p	η^2
		Completers	Non-completers			
		n = 50	n = 10			
		Median (Range)	Median (Range			
CRP	<6.0 mg/L	2.27 (2.90)*	3.10 (4.80)*	(t) -0.73	.47	*
TNF-β	<439pg/ml	9.38 (0.00 – 445.08)	8.72 (0.00 – 15.20)	(U) 420.00	.50	.02
IFN-γ	<365pg/ml	46.68 (13.26 – 203.31)	61.64 (23.07 – 131.42)	(U) 378.00	.23	.03
IL-5	<44pg/ml	8.00 (4.96 – 19.15)	11.27 (6.24 – 14.27)	(U) 392.50	.70	.06
IL-10	<44pg/ml	14.87 (0.00 – 34.88)	16.22 (0.00 – 27.46)	(U) 381.55	.99	.11
IL-6	<149pg/ml	17.96 (0.00 – 43.08)	12.82 (5.88 – 19.21)	(U) 379.00	.23	.11
IL-1β	<426pg/ml	38.60 (6.65 – 1051.27)	23.40 (8.32 – 37.04)	(U) 375.50	.10	.09
TNF-α	<479pg/ml	16.90 (4.36 – 556.68)	15.16 (6.88 – 32.64)	(U) 390.00	.50	.09

Note. Biomarker Abbreviations: C-reactive protein (CRP), Interferon (IFN), Tumor necrosis factor-α (TNF-α), Interleukin-1 (IL-1β), Interleukin-5 (IL-5), Interleukin-6 (IL-6), Interleukin-10 (IL-10), Tumor necrosis factor- β (TNF-β).

*Mean And Standard Deviations reported for CRP.

η^2 reporting guidelines are .01 = small effect, .06 = moderate effect, and .14 = large effect (Cohen, 1988)

6.4.2 Descriptive statistics for trial participants.

Table 48 describes demographic details for the 50 participants who completed the 8-week trial. Information is presented based on their group allocation. The mean age of all participants was 37.18 years (SD = 6.41; median = 43; range = 24 to 45). The majority of participants enrolled in this trial were tertiary-educated professionals, working full-time, married or living in defacto relationships, with children. On exploration using Independent samples t-tests and chi-square analyses no significant differences were observed between active and Placebo groups on these demographic variables. According to effect sizes, some disparity was observed between groups for Occupational categories. This is likely due to participants reporting their occupation as Home Duties all allocated to the Active group. All participants (80%) identifying their occupation as student were allocated to the Placebo group.

Table 48: Participant Demographic Information by Group Allocation (n=50)

Variable	Active n = 22(%)	Placebo n = 28 (%)	t or χ^2	df	p	ϕ
Age in years, M (SD)	38.23 (6.62)	36.36 (6.24)	t (1.03)		.31	.02*
Marital Status						.
Married	11 (50.00%)	13 (46.42%)				
Defacto	3 (13.63%)	5 (17.86%)				
Single	2 (9.10%)	7 (25.00%)				
Divorced	6 (27.33%)	3 (10.71%)	χ^2 (3.78)	3	.29	.26
Education Level						
Secondary	3 (13.63%)	4 (14.29%)				
Vocational	4 (18.18%)	3 (10.71%)				
Tertiary	15 (68.18%)	21 (75.00%)	X^2 (0.57)	2	.75	.11
Occupation						
Professionals	7 (31.82%)	10 (35.71%)				
Management	8 (36.36%)	6 (21.42%)				
Clerical & Service	3 (13.63%)	4 (14.29%)				
Home Duties	2 (9.09%)	0 (0.00%)				
Student	2 (9.09%)	8 (28.57%)	X^2 (5.95)	4	.21	.34
Workload						
Full Time	15 (68.18%)	16 (57.14%)				
Part Time	6 (27.27%)	9 (32.14%)				
Casual Employment	1 (4.54%)	2 (7.14%)				
Other	0 (0.00%)	1(3.57%)	X^2 (1.26)	3	.74	.16
Children	15 (68.18%)	17 (60.71%)	X^2 (0.06)	1	.59	.08

Note. Φ = phi coefficient is a measure of effect size and can range from 0 to 1, with .10 for small, .30 for medium and .5 for large applies (Cohen, 1988). *η^2 reported guidelines are .01 = small effect, .06 = moderate effect, and .14 = large effect.

Table 49 presents the health behaviour characteristics of the two groups at baseline. Exploration of demographic and health behaviour variables between the Active group compared to the Placebo group revealed few differences, and small effect sizes. There were no significant differences between the groups on Age, Exercise, Alcohol Use, or Smoking, as assessed by Independent Samples T-Test and Chi Square analyses. As indicated, Smoking and Alcohol Use in this sample were low. A difference was noted between groups for Antidepressant medication usage. All four women taking Antidepressant medication were allocated to the Active group; subsequently a Chi-square test for independence revealed a moderate effect size but a non significant association between randomisation and antidepressant medication use.

Table 49: Participant Health Behaviour Variables by Group Allocation (n = 50)

Variable	Active n = 22 (%)	Placebo n = 28(%)	t or χ^2	df	p	ϕ
Alcohol use M (SD)	4.45 (2.28)	4.21 (1.64)	t (0.43)		.67	.00*
Smoker	2 (9.09%)	4 (14.29%)	X^2 (0.02)	1	.90	.08
Physical Activity						
Inactive	8 (36.36%)	7 (25.00%)				
Minimally Active	8(36.36%)	14(50.00%)				
Health Enhancing Activity	6 (27.27%)	7 (25.00%)	X^2 (1.08)	2	.58	.15
Medication						
None	9 (40.90%)	15 (53.57%)	X^2 (0.37)	1	.55	.13
Antidepressants	4(18.18%)	0(0.00%)	X^2 (3.34)	1	.07	.33
Respiratory	2 (9.09%)	2 (7.14%)	X^2 (0.00)	1	.80	.04
Cardiovascular	2 (9.09%)	0 (0.00%)	X^2 (0.81)	1	.34	.23
Contraceptive	7 (31.82%)	9 (32.14%)	X^2 (0.00)	1	.99	.00

Note. Φ = phi coefficient is a measure of effect size and can range from 0 to 1, with .10 for small, .30 for medium and .5 for large applies; *η^2 reported guidelines are .01 = small effect, .06 = moderate effect, and .14 = large effect (Cohen, 1988).

Alcohol usage measured with AUDIT employing hazardous drinking items (scores range 0-12); **Contraceptive** medications include all forms of hormonal contraception oral, intra-uterine and implants.

6.4.3 Pre-existing differences between active and placebo groups

Variables measured for psychological trait, state, pro-oxidant, and pro-inflammatory variables were explored comparing pre-intervention scores between Active and Placebo groups. In addition for psychological variables normative data has been included, and for biomarkers normal reference ranges. Overall randomisation appears to have been successful in attaining comparable groups.

6.4.3.1 Psychological variables.

No statistically significant differences were observed at baseline between randomised groups on psychological (Table 50 and 51), pro-oxidant (Table 52), or pro-inflammatory measures (Table 53) as assessed by Independent Samples T-tests and Chi square analyses. Small effect sizes were observed for the variables -T-anxiety, T-anger, and AX-out. On further investigation the Active group had slightly higher scores for these variables. Similarly S-anger scores showed small effect size, indicating the Placebo group had slightly higher scores pre-intervention.

In comparison to normative data, the current sample was considered similar. Slightly higher mean scores were observed for T-anxiety, T-anger, T-depression, and AX-index (Table 50). Similarly the observed mean scores for State characteristics (Table 51), Loneliness and S-depression were higher than normative data. This is not surprising given eligibility for this study screened for a required level of psychological distress in the month preceding the trial.

Table 50: Pre-intervention Comparisons between Trait Psychological Characteristics for Active and Placebo Groups (n = 50)

Psychological Measure	Normative Sample		Current Sample		df	t	p	ϕ
	N	M (SD)	Active n = 22 M (SD)	Placebo n = 28 M (SD)				
T-anxiety	133	17.98 (5.45)	21.73 (5.55)	20.30 (5.16)	48	1.04	0.31	0.14
T-curiosity	133	28.86 (5.73)	26.33 (5.55)	27.57 (5.32)	48	-0.88	0.38	0.12
T-anger	133	18.13 (4.82)	22.63 (6.37)	20.70 (5.81)	48	1.23	0.23	0.16
T-depression	171	14.79 (5.05)	19.83 (6.63)	19.07 (5.02)	48	0.51	0.62	0.07
AX-out	952	14.79 (3.78)	15.50 (3.99)	14.47 (3.14)	48	1.12	0.27	0.15
AX-in	952	15.69 (4.38)	16.50 (4.27)	16.27 (3.72)	48	0.23	0.82	0.03
AC-out	952	21.52 (4.91)	21.57 (4.83)	22.72 (5.29)	48	-0.88	0.38	0.12
AC-in	952	23.28 (5.82)	20.67 (4.52)	20.77 (5.38)	48	0.47	0.94	0.01
AX-index	952	28.59 (13.02)	37.77 (13.02)	35.31 (14.72)	48	0.68	0.50	0.09
R/ED	585	34.13 (5.52)	33.67 (5.35)	32.33 (6.22)	48	0.89	0.38	0.12
N/H	577	35.60 (5.74)	35.27 (6.02)	35.17 (6.45)	48	0.06	0.95	0.01

Note. Abbreviations: anger expression out (AX-out), anger expression in (AX-in), anger control out (AC-out), anger control in (AC-in), anger expression index (AX-index), R/ED = Rationality & Emotional defensiveness, N/H = Need for Harmony ; Normative trait data (STPI) derived from normal female college sample, age unspecified (Spielberger, 1996); Normative Anger expression data (STAXI-2) derived from a female sample, age unspecified (Spielberger, 2003); Normative Lifestyle Defense Mechanisms data (LDMS) derived from a female sample, age unspecified (Spielberger, 2002)

Φ = phi coefficient is a measure of effect size and can range from 0 to 1, with .10 for small, .30 for medium and .5 for large applies

Table 51: Pre-intervention Comparisons between State Psychological Variables for Active and Placebo Groups (n = 50).

Psychological Measure	Normative Sample		Current Sample		df	t	p	ϕ
	N	M (SD)	Active n = 22 M (SD)	Placebo n = 28 M (SD)				
Psychological distress		N/A	15.50 (5.77)	15.80 (5.71)	48	-0.20	.84	.03
Loneliness	240	36.30 (2.80)	44.33 (9.38)	42.23 (11.48)	48	0.78	.44	.10
S-anxiety	133	19.06 (5.75)	21.07 (5.60)	21.07 (5.34)	48	0.00	.00	.00
S-curiosity	133	26.17 (5.45)	24.07 (5.27)	25.03 (4.31)	48	-0.78	.44	.10
S-depression	133	14.79 (5.05)	18.73 (5.28)	19.27 (5.25)	48	-0.39	.70	.05
S-anger[1]	133	14.24 (5.75)	12.30 (4.79)	12.87 (4.50)	48	388.50	.33	.13

Note. Normative data based on normal female college sample (N = 133), age unspecified (Spielberger, 1996), UCLA Loneliness normative data derived combined sample of men and women(Steptoe, et al. 2004)

Φ = phi coefficient is a measure of effect size and can range from 0 to 1, with .10 for small, .30 for medium and .5 for large applies

6.4.3.2 Biomarkers.

The pro-oxidant biomarker TAS was the only pro-oxidant biomarker investigated which suggested a disparity between the groups, with a small- to-moderate effect size observed. Specifically the Active group tended to have higher levels than the Placebo group on this biomarker pre-intervention.

For inflammatory measures non-parametric analyses were performed for variables that were not normally distributed to assess the presence of pre-existing group differences. For these variables Mann-Whitney U tests were performed. Results (Table 53) suggest there were no obvious discrepancies between the active and Placebo groups' inflammatory measures at the outset. For this current sample pro-oxidant and pro-inflammatory measures were within normative reference ranges.

Table 52: -intervention Comparisons between Pro-oxidant Biomarkers for Active and Placebo Groups (n = 50)

Measure	Normal Range	Current Sample		df	t	p	ϕ
		Active	Placebo				
		n = 22	n = 28				
		M (SD)	M(SD)				
NT	0.4-1.4 nmol/h/µgDNA	0.71 (0.22)	0.70 (0.22)	48	1.03	.31	.04
VIT C	50-150 pg/ugDNA	65.90 (15.08)	67.77 (19.22)	48	-0.42	.68	.06
TAS	1.30-1.77 umol/L	1.61 (0.19)	1.53 (0.16)	48	1.73	.10	.22
HCY	3-13 umol/L	7.39 (1.45)	7.50 (1.71)	48	-0.28	.78	.04
VITB	140-700pmol/L	387.00 (197.73)	389.67 (155.91)	48	-0.06	.95	.01
FOLATE	5-45 nmol/L	28.26 (8.70)	27.69 (9.85)	48	0.23	.82	.03
CHOL	<5.5 mmol/L	5.03 (0.94)	4.93 (0.87)	48	0.43	.67	.06

Note. Biomarker Abbreviations: 5' –ectonucleotidase (NT), tissue ascorbate (VIT C), total antioxidant status (TAS), homocysteine (HCY), Vitamin B12 (VIT B12), Folate (FOLATE), Cholesterol (CHOL)

Φ = phi coefficient is a measure of effect size and can range from 0 to 1, with .10 for small, .30 for medium and .5 for large applies.

Table 53: Pre-intervention Comparisons between Inflammatory measures for Active and Placebo Groups (n = 50)

Measure	Normal Range	Current Sample		t/U	p	ϕ
		Active	Placebo			
		n = 22	n =28			
		Median (Range)	Median (Range)			
CRP	<6.0 mg/L	1.85 (2.32)	2.60 (3.28)	(t) -0.91	.37	.02
TNF-β	<439pg/ml	9.93 (0.00 – 341.36)	9.02 (0.00 – 445.08)	(U) 420.00	.82	.02
IFN-γ	<365pg/ml	47.92 (23.07 – 203.31)	43.67 (13.26 – 150.59)	(U) 378.00	.82	.03
IL-5	<44pg/ml	7.60 (4.96 – 17.27)	8.04 (5.08 – 19.15)	(U) 392.50	.67	.06
IL-10	<44pg/ml	14.20 (0.00 – 27.46)	15.11 (0.00 – 34.88)	(U) 381.55	.42	.11
IL-6	<149pg/ml	12.32 (0.00 – 43.08)	18.30 (0.00 – 40.16)	(U) 379.00	.40	.11
IL-1β	<426pg/ml	25.51 (8.32 – 1051.27)	37.04 (6.65 – 963.72)	(U) 375.50	.49	.09
TNF-α	<479pg/ml	15.60 (5.40 – 534.48)	17.16 (4.36 – 556.68)	(U) 390.00	.48	.09

Note. Biomarker Abbreviations: Cholesterol (CHOL), C-reactive protein (CRP), Interferon (IFN), Tumor necrosis factor-α (TNF-α), Interleukin-1 (IL-1β), Interleukin-5 (IL-5), Interleukin-6 (IL-6), Interleukin-10 (IL-10), Tumor necrosis factor- β (TNF-β).

*Mean And Standard Deviations reported for CRP.

For variables not normally distributed variables Mann-Whitney U tests were performed.

Φ = phi coefficient is a measure of effect size and can range from 0 to 1, with .10 for small, .30 for medium and .5 for large applies)

6.4.4 Covariates.

Before conducting the principal analyses data were checked for the contribution of covariates that could potentially be related to psychological stress, pro-oxidant, or pro-inflammatory outcomes, or both. The variables examined were measures of demographic information (i.e., age), medication use, health behaviours (i.e., exercise), and other behavioural measures (i.e., smoking, alcohol intake, vitamin intake, etc) that have been shown to be associated with psychological wellbeing, biomarkers, and immune measures in similar studies (Boss et al., 1980; Hapuarachchi et al., 2003; Lesgards et al., 2002).

The relationships between these variables and each of the psychological, pro-oxidant, and pro-inflammatory outcome variables were examined. As in the observational study in Chapter 5, several issues required consideration.

Firstly significant associations among variables were discovered. Covariates were only included if it was considered they contributed uniquely to the reduction in error variance. Therefore for the covariates with high correlations (i.e., age, Contraceptive and Cardiovascular medication use) further exploration was required to determine whether they were contributing uniquely to the dependent variable.

Secondly, due to the sample size, and being cognizant to maintain as much power as possible, covariates were added to an ANCOVA only if they were indicated to influence the dependent variable with a correlation of $r \geq .20$. This indicates at least a small- to – moderate effect according to Cohen (1988). This is an arbitrary cut-off

score, but it was considered that adding variables with too small an effect would compromise power.

Furthermore for each outcome variable an independent ANCOVA was performed separately to allow for the inclusion of influential covariates for that specific variable. This was done to minimize any further power loss by adding all covariates to all ANCOVAS. As a result, each of the longitudinal analyses was discussed separately to allow for discussion of covariates specific to that analysis.

6.4.4.1 Covariates influencing psychological well-being.

It was anticipated that demographic and health behaviours could influence psychological state measures. Subsequently a correlation matrix was performed to explore these relationships (Appendix J) to show which variables were to be added as covariates in later analyses. Subsequent exploration of influential health behaviours revealed that each of the psychological dependent variable of interested was affected by at least one demographic or health behaviour variable.

Psychological distress evidenced a small, non significant effect of age ($r = .24$, $p = .09$, $n = 50$), suggesting older women in this sample were experiencing more distress. Similarly age evidenced a moderate, significant relationship with loneliness scores ($r = .33$, $p = .02$, $n = 50$) suggesting that older participants were more dissatisfied with their social interactions. Similarly Respiratory medication use was associated with higher Loneliness scores as indicated by a small, non significant association ($r = .27$, $p = .06$, $n = 50$). S-anxiety evidenced a small positive, albeit non significant association with

smoking (r = .21, p = .15, n = 50). Despite smoking levels being low in the overall sample, this pattern proposes smokers experienced more anxiety.

Medication use impacted on several psychological state measures. Women taking Cardiovascular medications had higher S-depression scores with a small, non-significant association observed (r = .26, p = .07, n = 50). Similarly a moderate, significant association was observed between Cardiovascular medication use and S-anger (r = .30, p = .03, n = 50). For S-curiosity, Respiratory medication use and Exercise had a moderate, significant positive association with S-curiosity scores (r = .31, p = .04, n = 60; r = .22, p = .13, n = 50) respectively. In contrast Contraceptive medication use was identified as a small negative, albeit non significant association with S-curiosity scores(r = -.24, p = .09, n = 50).

6.4.4.2 Covariates influencing pro-oxidant and pro-inflammatory measures.

Similarly the influence of demographic and health behaviour variables and pro-oxidant measures was explored (Appendix K). It was apparent that there were several variables that were influential to biomarker levels.

NT levels evidenced small associations, albeit non significant, with smoking (r = .27, p = .06, n = 50) and age (r = -.26, p = .07, n = 50). NT levels are documented to decline with increasing age (Boss, et al. 1980), but smoking finding seems counterintuitive as smoking promotes oxidative processes within the body (Lesgards, et al. 2002).

VIT C scores were associated with a small positive association with exercise, bordering on significance (r = .27, p = .06, n = 50). Improved nutrition paired with

exercise suggests that this is a pattern of a healthy lifestyle. In contrast TAS levels, indicative of the body's antioxidant capacity, were observed to be associated with increasing age. A moderate, significant association was observed (r = .33, p = .02, n = 50).

TAS also evidenced small to moderate, non significant association with Contraceptive use (r = -.20, p = .16, n = 50) and Cardiovascular medication use (r = .27, p = .06, n = 50). Hormonal contraceptive use also evidenced a moderate, significant association with NT (r = .40, p = .01, n = 50).

HCY levels had small positive, non significant associations with alcohol use (r = .20, p = .16, n = 50), exercise (r = .20, p = .15, n = 50). Suggesting both these health behaviours were associated with increased HCY. Furthermore moderate positive, non significant associations were observed with age (r = .25, p = .09, n = 50) and smoking (r = .27, p = .06, n = 50). These are expected as both ageing and smoking are considered to impair oxidative mechanisms. Similarly, FOLATE (r = -.25, p = .08, n = 50) and VIT B12 (r = -.23, p = .11, n = 50) evidenced a small to moderate negative, non significant association with smoking. Higher inflammatory measure CRP was associated with Smoking (r = .27, p = .06, n = 50), Alcohol use (r = .26, p = .07, n = 50), and Contraceptive use (r = .23, p = .10, n = 50). Cholesterol had two small positive, associations with Respiratory medication use (r = 0.29, p = .04, n = 50) and Antidepressant use (r = 0.27, p = .05, n = 50). These both reached significance.

Once identified, covariates were controlled for in the subsequent analyses of dependent variables.

6.4.5 Hypothesis 1a - Psychological outcomes for women undergoing stressful life events who were allocated to the active supplement group compared to those allocated to a placebo.

Between within-subject ANOVA techniques were employed to explore changes across time and group, including any interaction effects, for psychological measures. Influential covariates identified in the preliminary correlation matrices were included in analyses. Pre- and post-intervention means and standard deviations are presented (Table 54). F-tests are also presented (Table 55). Following these tables, each variable will be presented and discussed separately with regard to observed change based on group allocation, time, or interaction effects.

Table 54: Psychological Measures Pre- and Post-Intervention for Active and Placebo Groups

Psychological measure	Time	n	Active group M(SD)	n	Placebo group M(SD)
Psychological Distress	Pre	30	16.45 (6.03)	30	15.89 (6.02)
	Post	22	8.59 (5.98)	28	10.33 (5.24)
S-anxiety	Pre	30	21.00 (5.52)	30	21.25 (5.40)
	Post	22	17.27 (5.99)	28	18.04 (3.88)
S-curiosity	Pre	30	23.91 (4.88)	30	25.11 (4.25)
	Post	22	27.18 (5.13)	28	26.11 (4.60)
S-depression	Pre	30	19.23 (5.46)	30	19.21 (5.32)
	Post	22	16.00 (5.02)	28	16.32 (4.42)
S-anger	Pre	30	12.57 (5.46)	30	13.11 (4.68)
	Post	22	10.52 (2.40)	28	10.78 (1.28)
Loneliness	Pre	30	45.45 (9.70)	30	42.30 (10.89)
	Post	22	41.73 (9.78)	28	41.37 (8.91)

Table 55: Psychological Variables: Between-Within Analyses of Covariance (ANCOVA) Results

Psychological variable	Within Subjects effects								Between Subjects effects			
	Multivariate Tests											
	Time				Interaction				Group			
	F	df	p	Partial η^2	F	df	p	Partial η^2	F	df	p	Partial η^2
Psychological Distress	0.44	46	.51	.01	0.48	46	.49	.01	0.40	46	.53	.01
S-anxiety	8.73	47	**.01**	.16	0.15	47	.70	.00	0.15	47	.70	.00
S-curiosity	0.00	45	.98	.00	1.90	45	.18	.04	0.01	45	.95	.00
S-depression	7.93	47	**.01**	.14	0.07	45	.79	.00	0.14	47	.71	.00
S-anger	5.37	47	**.03**	.11	6.61	47	.47	.01	1.02	47	.31	.02
Loneliness	3.65	45	.06	.08	4.47	45	**.04**	.09	0.08	45	.78	.00

Note: Bold values show significance. Partial η^2 are effect size statistics and indicate the proportion of variance of the dependent variable that is explained by the independent variable. Values can range from 0-1. Strength of the effect sizes is based on the following guidelines -Small = .01, Medium = .06, Large = .14 (Cohen 1988)

6.4.5.1 Psychological distress.

A repeated measures ANCOVA was used to test the hypothesis that Psychological Distress scores would improve over time more so for the Active group compared to the Placebo group. A covariate included in this analysis based on univariate investigations was age.

Results indicated that there was a small, non significant change over time (Table 55) suggesting there was little change between pre- and post-intervention scores. Similarly the main effect for group evidenced a small, non-significant change suggesting that only a small degree of the change in scores over time was likely due to group allocation. The interaction between time and group showed a small, non-significant effect suggesting that the trajectory of change over time was slightly different for each group. According to Figure 31 and Table 54, both the Active and Placebo groups commenced pre-intervention with similar psychological distress scores and these initial differences were not significant (showing a less than small effect size, $\phi = .03$).

According to Figure 31 the trajectory of change in scores from pre- to post-intervention for the Active group's decrease (improve) more sharply for psychological distress scores more than the Placebo group's. Based on these results, the administration of multivitamins slightly favored improved psychological distress for the Active group compared to the Placebo group supporting the proposed hypothesis. However, results should be viewed with caution as the effect was small and non-significant there somewhat unreliable given the compromised power. The covariate in this analysis indicated that age only contributed a small, non significant impact on

Psychological Distress scores, F (1, 46) = 0.66, p = .42, partial η2 = .01 It did not uniquely, significantly adjust Psychological Distress scores.

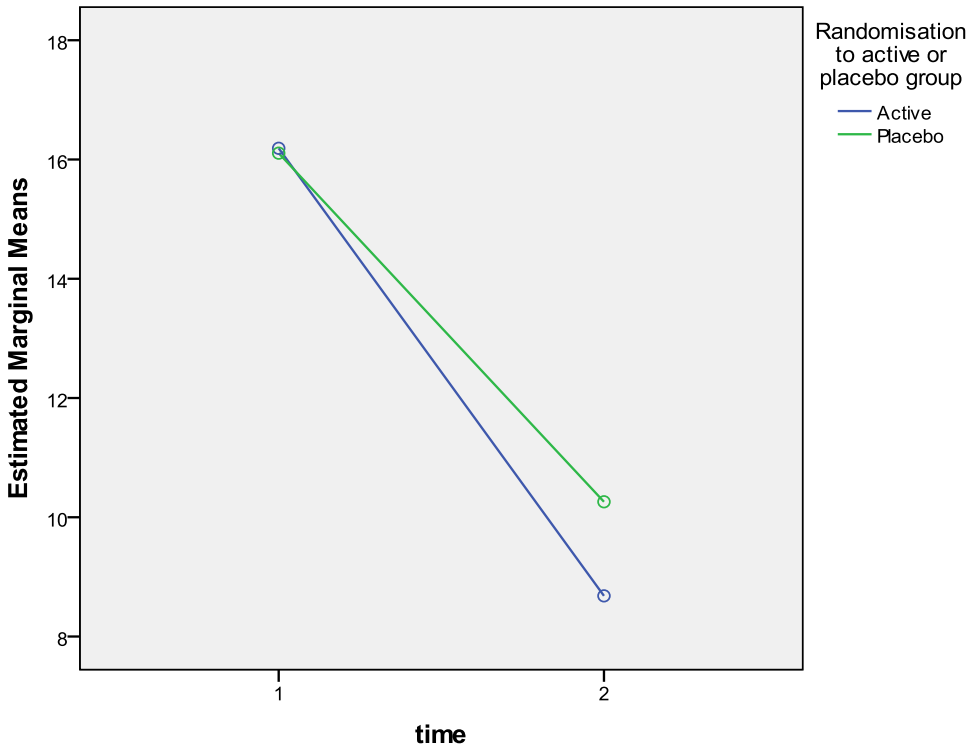


Figure 31: Psychological Distress Levels Across Time for the Active and Placebo Groups

Due to the small effect for group, RCIs were calculated to determine positive, negative, and no change without measurement error (Table 56). These results indicate that improvement was observed for both groups, post-intervention. However over half of the Placebo group no change or worsening (3%) distress scores post-intervention. In comparison the over 60% of the Active group evidenced a positive change between pre to post intervention distress scores. This supports the hypothesis that distress will improve for women allocated to the Active group compared to the Placebo group.

Table 56: Reliable Change Indices (RCIs) for Psychological Distress Pre- to Post-Intervention

	Active group (n = 22) n (%)	Placebo group (n = 28) n (%)
Positive reliable change	15 (68.18%)	11 (40.74%)
Negative reliable change	3 (13.64%)	1 (3.70%)
No reliable change	4 (18.18%)	15 (55.56%)

Note. Positive reliable change refers to psychological distress scores improving during the time period; negative reliable change refers to psychological distress scores worsening

6.4.5.2 S-Anxiety.

A repeated measures ANCOVA was used to test the hypothesis that S-Anxiety scores would improve (decrease) over time more so for the Active group compared to the Placebo group. A covariate included in this analysis, based on univariate investigation was smoking.

Results indicated that there was a large, significant change over time (see Table 55). However the main effect for group evidenced a less than small, non-significant change suggesting that only a minor amount of the change in scores over time was likely due to group allocation. The interaction term evidenced little impact, with a less than small, non-significant change suggesting that the trajectory of change over time was similar for each group. Specifically, both groups commenced pre-intervention with very similar S-Anxiety scores, reflected in the pre-intervention analyses (Table 51) these initial differences were not significant (showing a less than small effect size, ϕ = .00). According to Figure 32 and Table 54 (of means and standard deviations), change in scores from pre- to post-intervention declined (improved) at a similar trajectory for both the Active (19.3 %) and Placebo (16.6 %) Group S-Anxiety scores. Based on these

results, the administration of multivitamins to the Active group only slightly improved S-Anxiety compared to the Placebo group supporting the proposed hypothesis. Results should be viewed with caution as the effect was small and non-significant there somewhat unreliable given the compromised power. The covariate in this analysis indicated that smoking did not uniquely, significantly adjust S-anxiety scores. It evidenced a small impact according to the effect size, F (1, 47) = 0.53, p = .47, partial η2 =.01.

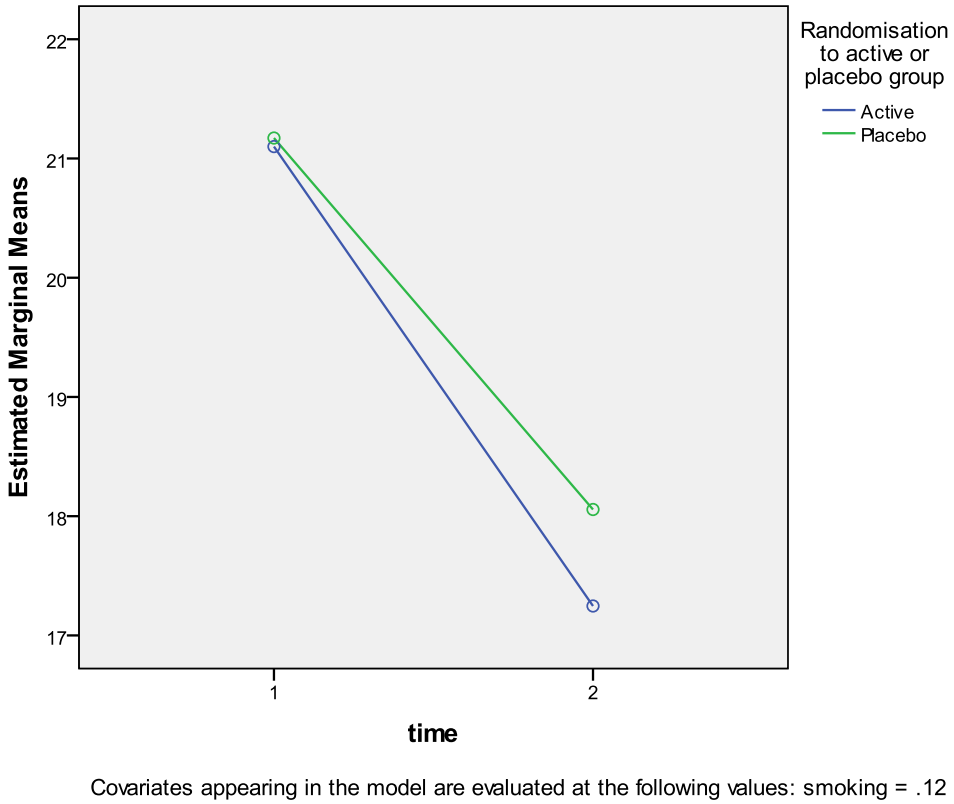


Figure 32: S-anxiety Levels Across Time for the Active and Placebo Groups

Due to the small effect for group, RCIs were calculated to determine positive, negative, and no change without measurement error (Table 57). These results indicate for a majority of the participants there was no reliable change in S-anxiety scores over

time. There were a small number of participants who had a positive reliable change with only slightly more improvement observed in the Placebo group as compared with the Active group. No worsening was observed in the Active group supporting the current hypothesis.

Table 57: Reliable Change Indices (RCIs) for S-anxiety Pre- to Post-Intervention

	Active group (n = 22) n (%)	Placebo group (n = 28) n (%)
Positive reliable change	6 (27.27%)	8 (28.57%)
Negative reliable change	0 (0.00%)	3 (10.71%)
No reliable change	16 (72.73%)	17 (60.71%)

Note. Positive reliable change refers to S-anxiety scores improving during the time period; negative reliable change refers to S-anxiety scores worsening

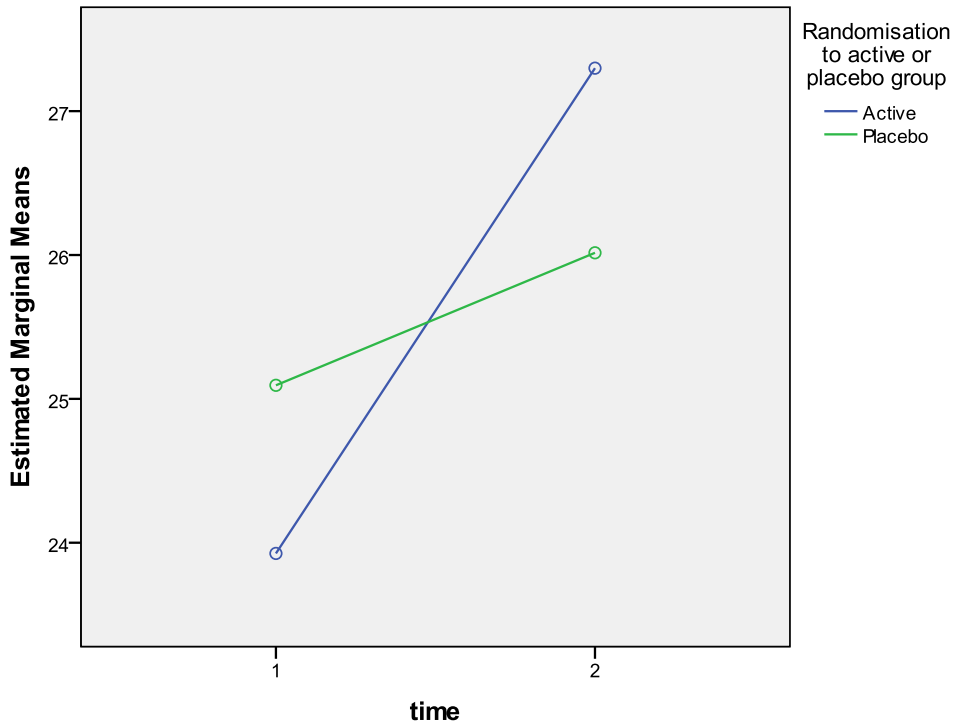
6.4.5.3 S-Curiosity.

A repeated measures ANCOVA was used to test the hypothesis that S-curiosity scores would increase over time for the Active group more so than the Placebo group. Three covariates were included in this analysis, based on univariate investigations- use of respiratory medications, hormonal contraceptive medications and exercise.

Results indicated that there was a less than small, non significant change over time for S-curiosity scores (Table 55). The main effect for group evidenced a less than small, non significant outcome suggesting that change in scores over time was not due to group allocation. These main effects are complicated by the interaction term. Specifically, the interaction between time and group showed a small to moderate, non-significant effect suggesting that the trajectory of change over time for each group was different. Specifically, the Active group commenced pre-intervention with slightly

lower (worse) S-curiosity scores than the Placebo group. According to pre-intervention analyses (Table 51) these initial differences were small, but not significant (showing only a small effect size, $\phi = .10$). According to Figure 33 and Table 54 (of means and standard deviations), the change in scores from pre- to post-intervention then cross over with the Active group's S-curiosity scores increasing (12.61 %) more than the Placebo group's (5.66 %). Based on these results, the administration of multivitamins to the Active group could be attributed to improved S-curiosity scores observed compared to the Placebo group supporting the proposed hypothesis.

The covariate in this analysis indicated that the use of respiratory (i.e. Ventolin inhalers) and hormonal contraceptive medications did not uniquely, significantly adjust S-curiosity scores, although the effect for both covariates verged on being moderate, $F (1, 47) = 2.78$, $p = .10$ partial $\eta^2 = 06$ and $F (1, 45) = 2.43$, $p = .13$, partial $\eta^2 = .05$ respectively. The amount of exercise contributed a large, significant contribution to S-curiosity scores, $F (1, 45) = 10.64$, $p = .00$, partial $\eta^2 = .19$. However, results should be viewed with caution, as the effect was small and non-significant. Due to a less than small effect for time, RCIs were not calculated to determine positive, negative, and no change without measurement error.



Covariates appearing in the model are evaluated at the following values: IPAQ = 1.96, Resp = .08, OC = .32

Figure 33: S-curiosity Levels Across Time for the Active and Placebo Groups

6.4.5.4 S-depression.

A repeated measures ANCOVA was used to test the hypothesis that S-depression scores would decrease (improve) over time for the Active group more so than the Placebo group. A covariate in this analysis, based on univariate investigations, was use of cardiovascular medication.

Results indicated that there was a large, significant change over time (Table 55). The main effect for group evidenced a less than small, non-significant change suggesting that only a minor amount of the change in S-depression scores over time was likely due to group allocation. The interaction term evidenced little impact, with a less than small, non-significant change suggesting that the trajectory of change over

time was similar for both groups. Specifically, the Placebo group commenced pre-intervention with slightly higher (worse) S-depression scores, although as reflected in the pre-intervention analyses (Table 51) these initial differences were not significant (showing a less than small effect size, $\phi = .05$).

According to Figure 34 and Table 54 (of means and standard deviations), change in scores from pre- to post-intervention declined (improved) at a similar trajectory for both the Active (17.9 %) and Placebo (16.3 %) Group S-depression scores. Based on these results, the administration of multivitamins to the Active group did not convincingly improve S-depression compared to the Placebo group. Results should be viewed with caution as the effect was small and non-significant therefore somewhat unreliable given the compromised power. The covariate in this analysis indicated that cardiovascular medication use did not uniquely, significantly adjust S-depression scores, although it evidenced a small impact according to the effect size, $F (1, 47) = 1.05$, $p = .31$, partial $\eta^2 = .02$.

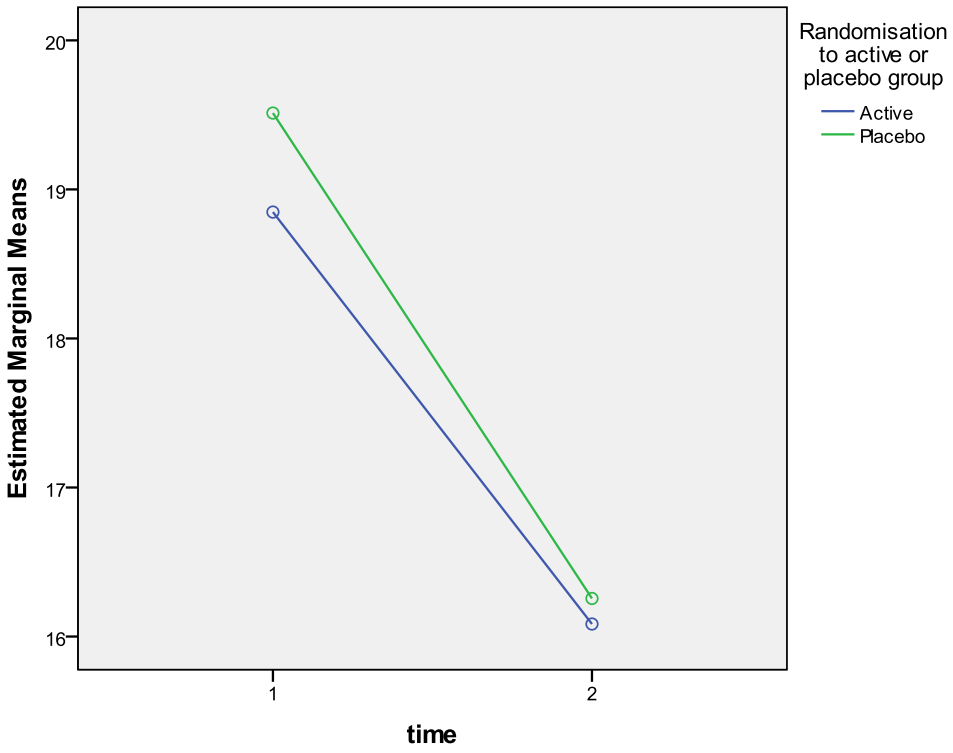


Figure 34: S-depression Levels Across Time for the Active and Placebo Groups

Due to the small effect for group, RCIs were calculated to determine positive, negative, and no change without measurement error (Table 58). These results indicate that for the majority of participants there was no reliable change in S-depression scores over time. There were only a small number of participants who had a positive reliable change (8%) and there appeared no benefit to allocation to the Active group over the Placebo group for improving S-depression scores.

Table 58: Reliable Change Indices (RCIs) for S-depression Pre- to Post-Intervention

	Active group (n = 22) n (%)	Placebo group (n = 28) n (%)
Positive reliable change	2 (9.09%)	2 (7.14)
Negative reliable change	0 (0.00%)	0 (0.00%)
No reliable change	20 (90.91%)	20 (71.43%)

Note. Positive reliable change refers to S-depression scores improving during the time period; negative reliable change refers to S-depression scores worsening

6.4.5.5 S-anger.

A repeated measures ANCOVA was used to test the hypothesis that S-depression scores would decrease (improve) over time for the Active group more so than the Placebo group. A covariate in this analysis, based on univariate investigations, was use of cardiovascular medication.

Results indicated that there was a moderate- to -large, significant change over time (Table 55). The main effect for group evidenced a less than small, non-significant change suggesting that only a minor amount of the change in S-anger scores over time was likely due to group allocation. The interaction term evidenced little impact, with a less than small, non-significant change suggesting that the trajectory of change over time was similar for both groups. The Placebo group commenced pre-intervention with slightly higher (worse) S-anger scores, although as reflected in the pre-intervention analyses (Table 51) these initial differences were not significant (showing a less than small effect size, $\phi = .05$). According to Figure 35 and Table 54 (of means and standard deviations), change in scores from pre- to post-intervention declined (improved) at a similar trajectory for both the Active and Placebo group S-anger scores.

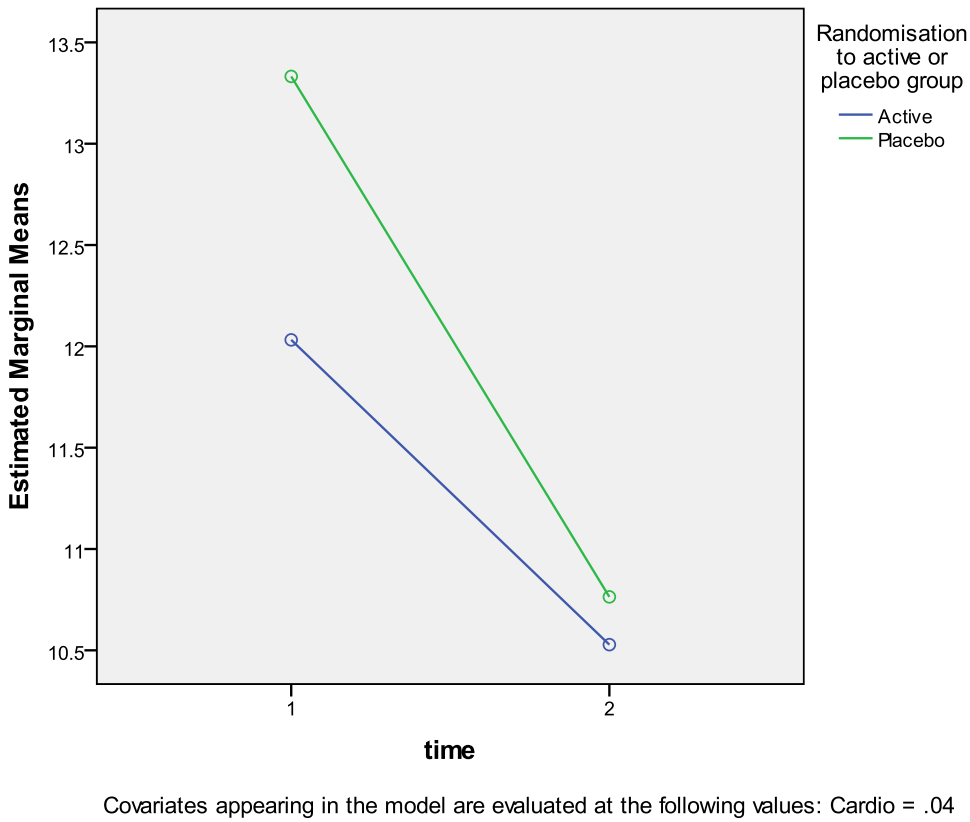


Figure 35: S-anger Levels Across Time for the Active and Placebo Groups

Based on these results, the administration of multivitamins to the Active group did not convincingly improve S-anger compared to the Placebo group. Results should be viewed with caution as the effect was small and non-significant therefore somewhat unreliable given the compromised power. The covariate in this analysis indicated that cardiovascular medication use did uniquely and significantly adjust S-depression scores. It evidenced a moderate impact according to the effect size, F (1, 47) = 4.03, p = .05, partial η^2 =.08. This suggested that Cardiovascular medication use explained up to 8% of variance in S-anger scores.

Due to the small effect for group, RCIs were calculated to determine positive, negative, and no change without measurement error (Table 59). These results indicate that for the majority of participants there was no reliable change in S-anger scores over time. There were an equal number of participants who had a positive and negative reliable change in both the Active and Placebo groups, but there appeared no benefit to allocation to the Active group over the Placebo group for improving S-anger scores.

Table 59: Reliable Change Indices (RCIs) for S-anger Pre- to Post-Intervention

	Active group (n = 22) n (%)	Placebo group (n = 28) n (%)
Positive reliable change	9 (41.00%)	9 (32.14%)
Negative reliable change	2 (9.09%)	2 (7.14%)
No reliable change	11 (50.00%)	17 (60.71%)

Note. Positive reliable change refers to S-anger scores improving during the time period; negative reliable change refers to S-anger scores worsening

6.4.5.6 Loneliness.

A repeated measures ANCOVA was used to test the hypothesis that Loneliness scores would improve (decrease) over time for the Active group more so than the Placebo group. Two covariates were included in this analysis, based on univariate investigations, respiratory medication and age.

Results indicated that there was a less than moderate to large, non significant change over time for Loneliness scores (Table 55). The main effect for group evidenced a less than small, non significant outcome suggesting that change in scores over time was not due to group allocation. These main effects are complicated by the interaction

term. Specifically, the interaction between time and group showed a moderate to large, significant effect suggesting that the trajectory of change over time for each group was different. Specifically, the Active group commenced pre-intervention with slightly higher (worse) Loneliness scores than the Placebo group. According to pre-intervention analyses (Table 50) these initial differences were small, but not significant (showing only a small effect size, $\phi = .10$).

Figure 36 and Table 54 (of means and standard deviations), indicate the change in scores from pre- to post-intervention then cross over with the Active group's Loneliness scores decreasing (7.5 %- improving) more than the Placebo group's (3.9 %). Based on these results, the administration of multivitamins to the Active group could be attributed to improved Loneliness scores observed compared to the Placebo group supporting the proposed hypothesis. Results should be viewed with caution as the effect was small and non-significant there somewhat unreliable given the compromised power.

The covariates in this analysis indicated that the use of respiratory medications (i.e. Ventolin inhalers) did not uniquely, significantly adjust Loneliness scores, although the effect was small to moderate in influence $F (1, 45) = 1.32$, $p = .27$ partial $\eta^2 = .03$. However age contributed a large, significant contribution to Loneliness scores, $F (1, 45) = 7.08$, $p = .01$, partial $\eta^2 = .14$. This suggested that age explained up to 14% of variance in Loneliness scores.

Due to the small effect for group, RCIs were calculated to determine positive, negative, and no change without measurement error (Table 60). These results indicate that there was 100% no reliable change in Loneliness scores over time.

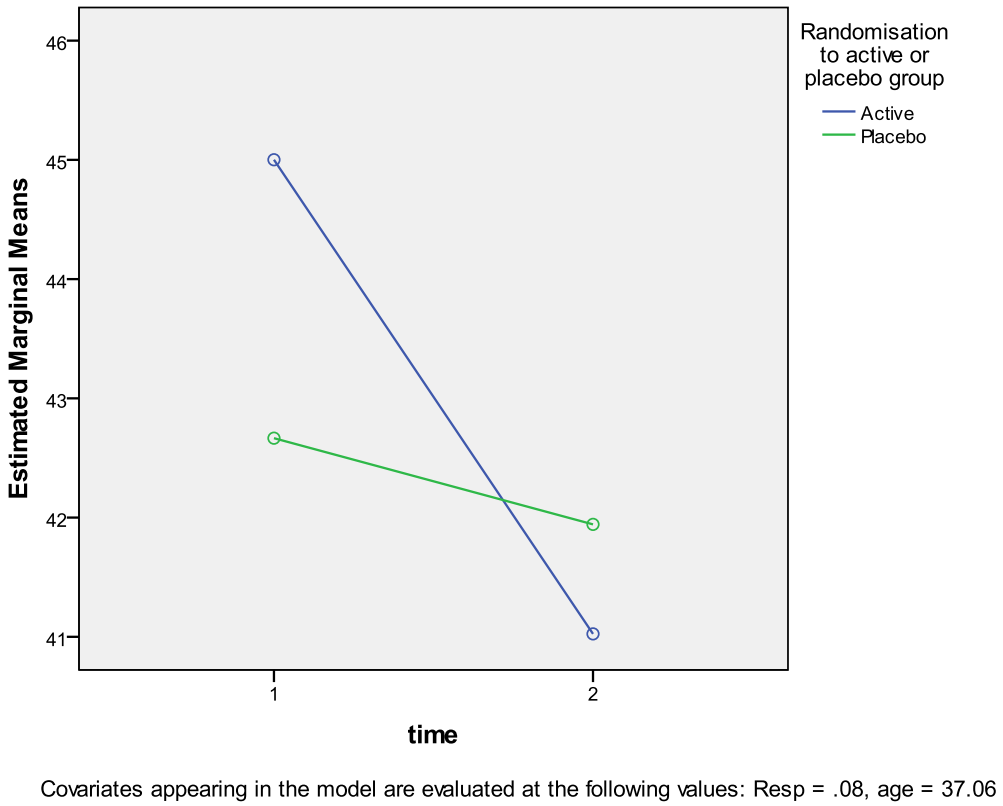


Figure 36: Loneliness Levels Across Time for the Active and Placebo Groups

Table 60: Reliable Change Indices (RCIs) for Loneliness Pre- to Post-Intervention

	Active group (n = 22) n (%)	Placebo group (n = 28) n (%)
Positive reliable change	0 (0.00%)	0 (0.00%)
Negative reliable change	0 (0.00%)	0 (0.00%)
No reliable change	22 (100.00%)	27 (100.00%)

Note. Positive reliable change refers to loneliness scores improving during the time period; negative reliable change refers to loneliness scores worsening

6.4.6 Hypothesis 2a - Pro-oxidant biomarkers for those allocated to the active multivitamin group compared to those allocated to the Placebo group.

Between within-subject ANOVA techniques were employed to explore changes across time and between groups for pro-oxidant measures. Influential covariates identified in the preliminary correlation matrices were included in analyses. Pre- and post-intervention means and standard deviations are presented (Table 61) followed by F values (Table 62). Each variable will be discussed separately.

Table 61: Pro-oxidant Measures Pre- and Post-Intervention for Active and Placebo Groups

Biomarker	Time	n	Active group M (SD)	n	Placebo group M (SD)
NT	Pre	29	0.67 (0.20)	30	0.72 (0.22)
	Post	22	0.79 (0.17)	27	0.82 (0.31)
VIT C	Pre	29	67.09 (15.70)	30	69.85 (18.81)
	Post	22	70.68 (16.18)	27	68.30 (16.78)
TAS	Pre	29	1.59 (0.19)	30	1.50 (0.14)
	Post	22	1.62 (0.13)	27	1.65 (0.12)
HCY	Pre	30	7.10 (1.22)	30	7.52 (1.73)
	Post	22	6.45 (1.18)	28	7.35 (1.59)
VIT B12	Pre	29	418.00 (211.78)	30	388.21 (161.34)
	Post	22	507.14 (204.60)	28	372.46 (142.93)
FOLATE	Pre	28	28.71 (8.43)	30	28.25 (9.97)
	Post	21	39.72 (11.25)	28	28.51 (9.67)
CHOL	Pre	30	5.05 (0.94)	30	4.93 (0.90)
	Post	22	4.86 (0.97)	28	4.81 (0.82)

Note. Biomarker Abbreviations: 5' –ectonucleotidase (NT), tissue ascorbate (VIT C), total antioxidant status (TAS), homocysteine (HCY), Vitamin B12 (VIT B12), Folate (FOLATE), Cholesterol (CHOL)

Table 62: Biomarker Variables: Between-Within Analyses of Covariance (ANCOVA) Results

	Within Subjects effects								Between Subjects effects			
	Multivariate Tests											
Biomarker	Time				Interaction				Group			
	F	df	p	Partial η2	F	df	p	Partial η2	F	df	p	Partial η2
NT	6.78	44	**.01**	.13	0.39	44	.54	.01	0.04	44	.84	.00
VIT C	0.22	46	.64	.01	0.72	44	.40	.02	0.01	46	.91	.00
TAS	3.27	43	.08	.07	1.40	43	.24	.03	.42	43	.52	.01
HCY	0.00	44	.99	.00	3.21	44	.08	.07	3.85	44	.06	.08
CHOL	0.59	46	.45	.01	0.02	46	.90	.00	0.16	46	.70	.00
FOLATE	14.12	45	**.00**	.24	15.17	45	**.00**	.25	4.95	45	**.03**	.10
VIT B12	9.76	47	**.00**	.17	19.57	47	**.00**	.29	2.39	47	.13	.05
CHOL	0.59	46	.45	.01	0.02	46	.90	.00	0.16	46	.70	.00

Note: Biomarker Abbreviations: 5' –ectonucleotidase (NT), tissue ascorbate (VIT C), total antioxidant status (TAS), homocysteine (HCY), Vitamin B12 (VIT B12), Folate (FOLATE), Cholesterol (CHOL)

Bold values show significance. Partial η^2 are effect size statistics and indicate the proportion of variance of the dependent variable that is explained by the independent variable. Values can range from 0-1. Strength of the effect sizes is based on the following guidelines -Small = .01, Medium = .06, Large = .14 (Cohen 1988)

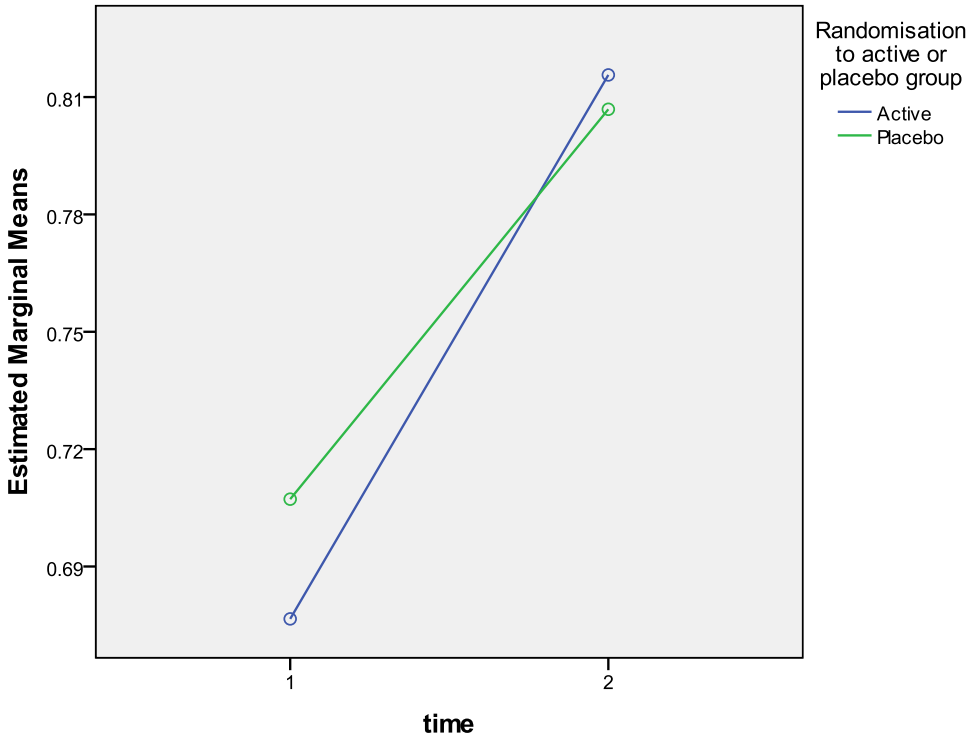
6.4.6.1 5'- ectonucleotidase (NT).

A repeated measures ANCOVA was used to test the hypothesis that NT levels would increase over time for the Active group more so than the Placebo group. Based on univariate analysis, three covariates were included in this analysis: hormonal contraceptive use, smoking, and age.

Results indicated that there was a large, significant change over time for NT levels (see Table 62). The Active group evidenced NT levels to be increased (improved) by 15% between pre- and post-intervention assessments, compared with a 10% increase for the Placebo group. The main effect for group evidenced a less than small, non significant outcome suggesting that change in scores over time was unlikely to be due to group allocation. As can be seen in Figure 37 and following the means and standard deviations presented in Table 61, the Active group commenced pre-intervention with slightly lower NT levels than the Placebo group, although according to pre-intervention analysis conducted earlier in this chapter , these pre-intervention differences were not significantly different (showing a less than small effect size, ϕ = .04). The interaction effect between time and group showed no effect implying that the NT level changes were in the same direction for both groups.

The covariates in this analysis indicated that smoking did not uniquely, significantly adjust NT scores, although the effect was small to moderate in influence $F(1, 44) = 1.71$, $p = .20$ partial $\eta^2 = .04$. However age and hormonal contraceptive use both contributed a large, significant contribution to NT levels, $F(1, 44) = 5.75$, $p = .02$, partial $\eta^2 = .12$ and, $F(1, 44) = 6.17$, $p = .02$, partial $\eta^2 = .12$ respectively. This suggests

that both contraceptive use and age each explained up to 12%, of unique variance in NT levels.



Covariates appearing in the model are evaluated at the following values: smoking = .12, age = 37.06, OC = .33

Figure 37: NT Levels Across Time for the Active and Placebo Groups

To further clarify individual change across time and group, Figure 38 plots NT scores pre-test versus post-test for individuals in the Active (crosses) and Placebo (dashes) groups. The solid 45 degree line represents a line of no change between pre- to post-intervention. Individuals above the 45 degree line showed improved levels of NT pre-to post-intervention while individuals located under the 45 degree line report lower or worsening NT levels pre- to post-intervention.

Consistent with the results at the main effect for time level, Figure 38 shows that the majority of participants in both Active and Placebo groups evidenced

improved NT levels between pre- to post intervention. Improvement slightly favoured the Active group. Based on these results the administration of multivitamins to the Active group increased NT levels only slightly more than the Placebo group, providing partial support for the proposed hypothesis.

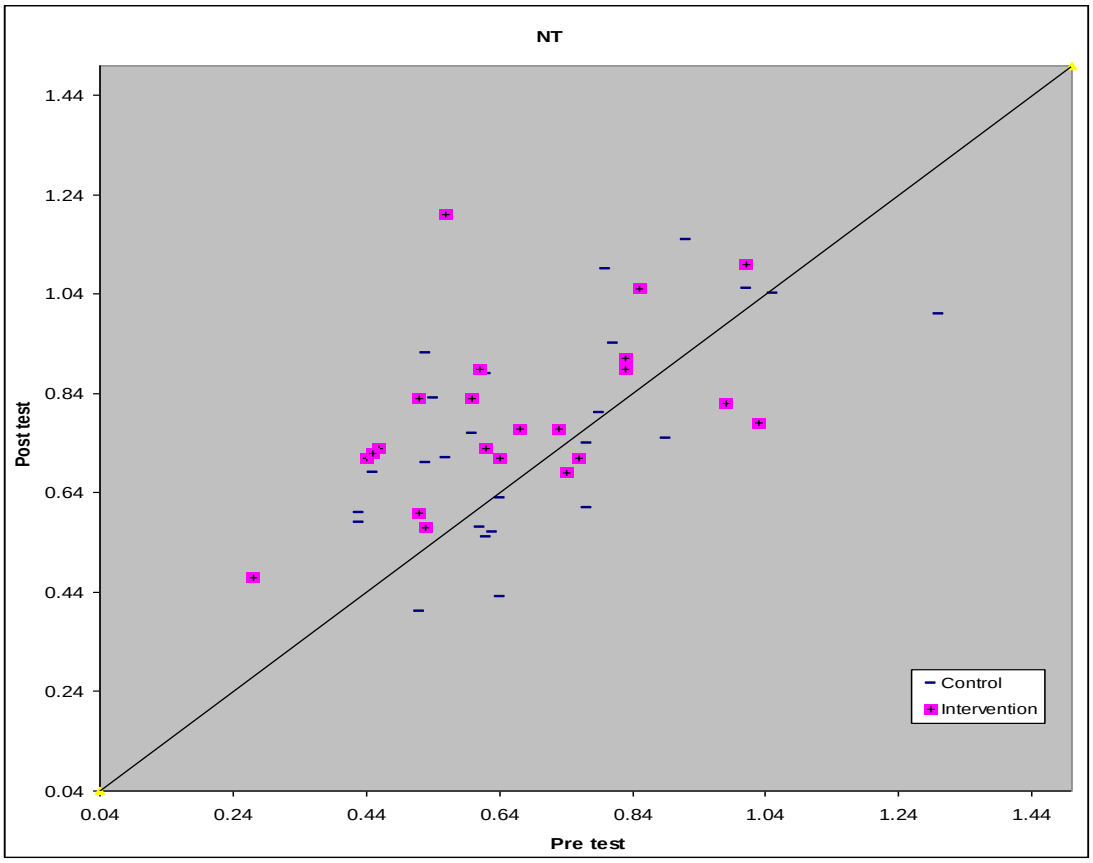


Figure 38: 5' –ectonucleotidase (NT) pre and post intervention scores

6.4.6.2 Tissue ascorbate (VIT C).

A repeated measures ANCOVA was used to test the hypothesis that VIT C levels would improve over time more so for the Active group compared to the Placebo group. A covariate included in this analysis, based on univariate investigations was exercise.

Results indicated that there was small, non significant effect in VIT C scores over time (Table 62), suggesting scores changed little over the 8-week intervention. On viewing Figure 39, it is apparent that this is not the case, with mean scores for the Active group increasing and Placebo group scores decreasing (Table 61). The changes observed were small and subsequently could not be attributed to group allocation based on the small, non significant main effect of group on VIT C observed. The interaction between time and group showed no effect suggesting that VIT C level changes had a similar trajectory, although in opposite directions, for both groups.

The covariate in this analysis indicated that exercise levels did not uniquely, significantly adjust VIT C scores, although it evidenced a moderate to large impact according to the effect size, $F(1, 46) = 3.41$, $p = .07$, partial $\eta 2 = .07$.

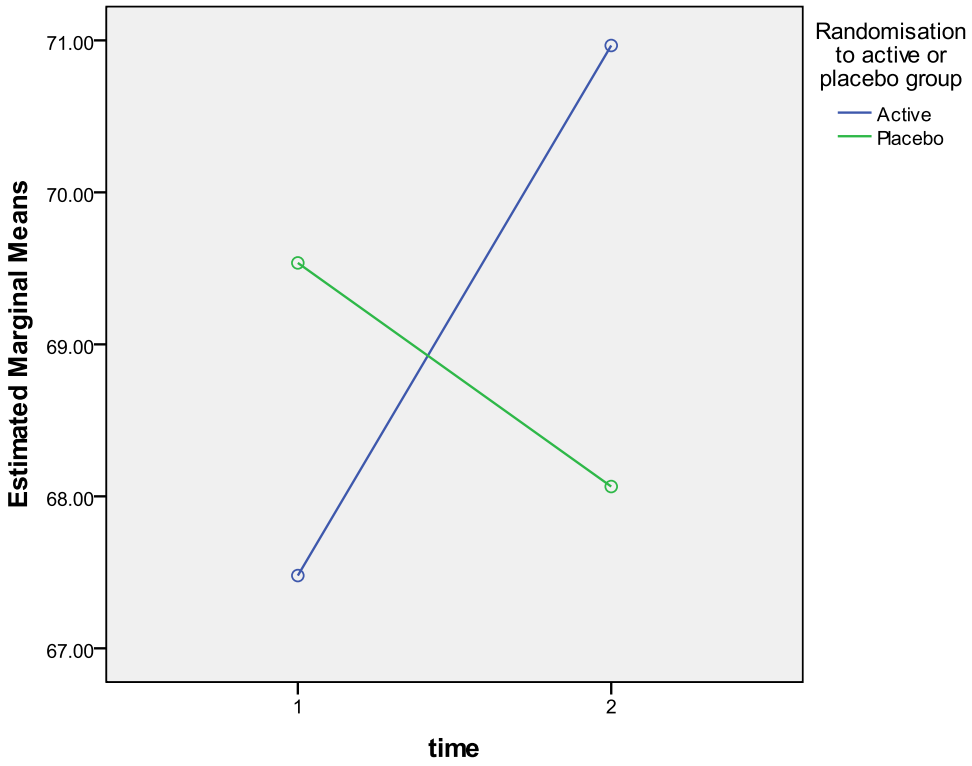


Figure 39: VIT C Levels Across Time for the Active and Placebo Groups

To further clarify individual change across time and group, Figure 40 plots VIT C scores pre-test versus post-test for individuals in the Active (crosses) and Placebo (dashes) groups. The solid 45 degree line represents a line of no change between pre- to post-intervention. Individuals above the 45 degree line showed improved levels of VIT C pre-to post-intervention while individuals located under the 45 degree line report lower or worsening VIT C levels pre- to post-intervention.

Consistent with the results at the main effect for time and group level on mean VIT C scores, Figure 40 shows that there was no pattern of improvement solely for the Active group compared to the Placebo group. Individual change on VIT C levels across time and group was mixed. Based on these findings, the administration of

multivitamins to the Active group did not conclusively improve VIT C levels as compared to the Placebo group. This finding does not support the proposed hypothesis.

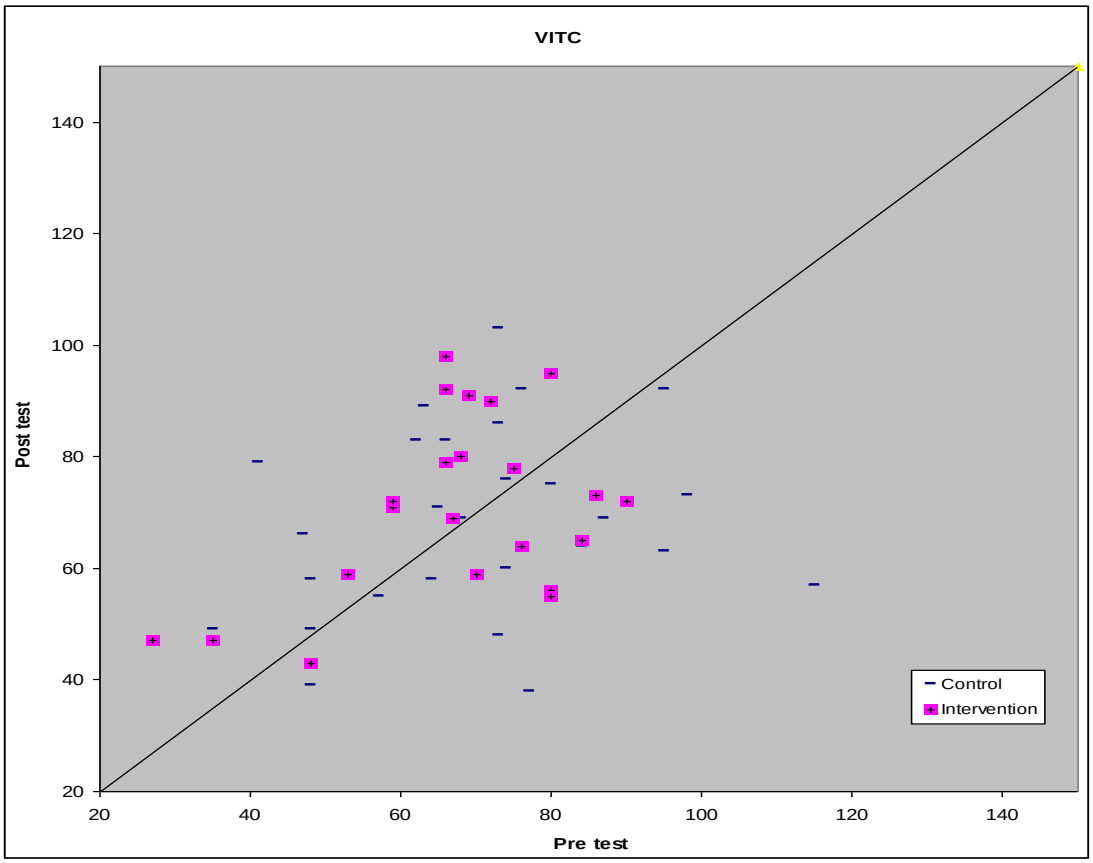


Figure 40: Tissue ascorbate (VIT C) pre and post intervention scores

6.4.6.3 Total antioxidant status (TAS).

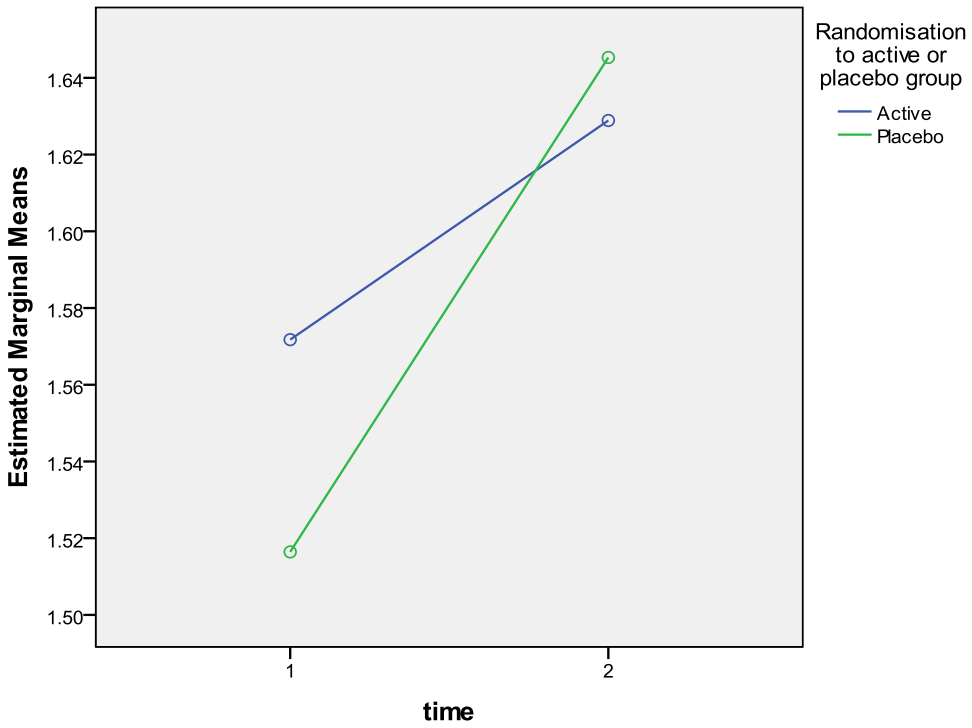
A repeated measures ANCOVA was performed to test the hypothesis that TAS levels would improve over time more so for the Active group compared to the Placebo group. Based on univariate investigations three covariates were added to this analsysis, hormonal contraceptive use, cardiovascular medication use and age.

Results indicated a moderate to large increase on TAS scores over time, verging on significance (Table 62). This increase in scores was unlikely due to group allocation,

as the main effect for group evidenced a small, non significant impact on TAS level. However there was a small to medium interaction effect between time and group allocation, suggesting the change in scores for each group were different.

Specifically, as can be seen in Figure 41 and following the means and standard deviations presented in Table 61, the Placebo group commenced pre-intervention with lower TAS scores than the Active group. According to pre-intervention analysis differences were not significant, however it is worth noting that a small to moderate effect size was observed (ϕ= .22, see Table 52). This suggests that pre-existing group differences potentially contributed to the changes observed. Subsequently the trajectory of increase for TAS scores for the Placebo group is much steeper than the Active group trajectory contributing to the interaction effect.

The covariates in this analysis indicated that the use of cardiovascular medication did not uniquely, significantly adjust TAS scores, $F (1, 43) = 0.0$, $p = .99$, partial $\eta 2 = .00$. Age although not reaching significance evidenced a small to moderate impact, $F (1, 43) = 1.52$, $p = .22$, partial $\eta 2 = .03$, and hormonal contraceptive use a moderate to large effect bordering on significance, $F (1, 43) = 3.69$, $p = .06$, partial $\eta 2 = .08$.



Covariates appearing in the model are evaluated at the following values: age = 37.10, Cardio = .04, OC = .31

Figure 41: TAS Levels Across Time for the Active and Placebo Groups

To further clarify individual change across time and group, Figure 42 plots TAS scores pre-test versus post-test for individuals in the Active (crosses) and Placebo (dashes) groups. The solid 45 degree line represents a line of no change between pre- to post-intervention. Individuals above the 45 degree line showed improved levels of TAS pre-to post-intervention while individuals located under the 45 degree line report lower or worsening TAS levels pre- to post-intervention.

Consistent with the results at the main effect for time on mean TAS scores, Figure 42 shows that there was improvement for both groups. Based on these findings, the administration of multivitamins to the Active group did not conclusively improve TAS levels as compared to the Placebo group. This finding does not support the proposed hypothesis.

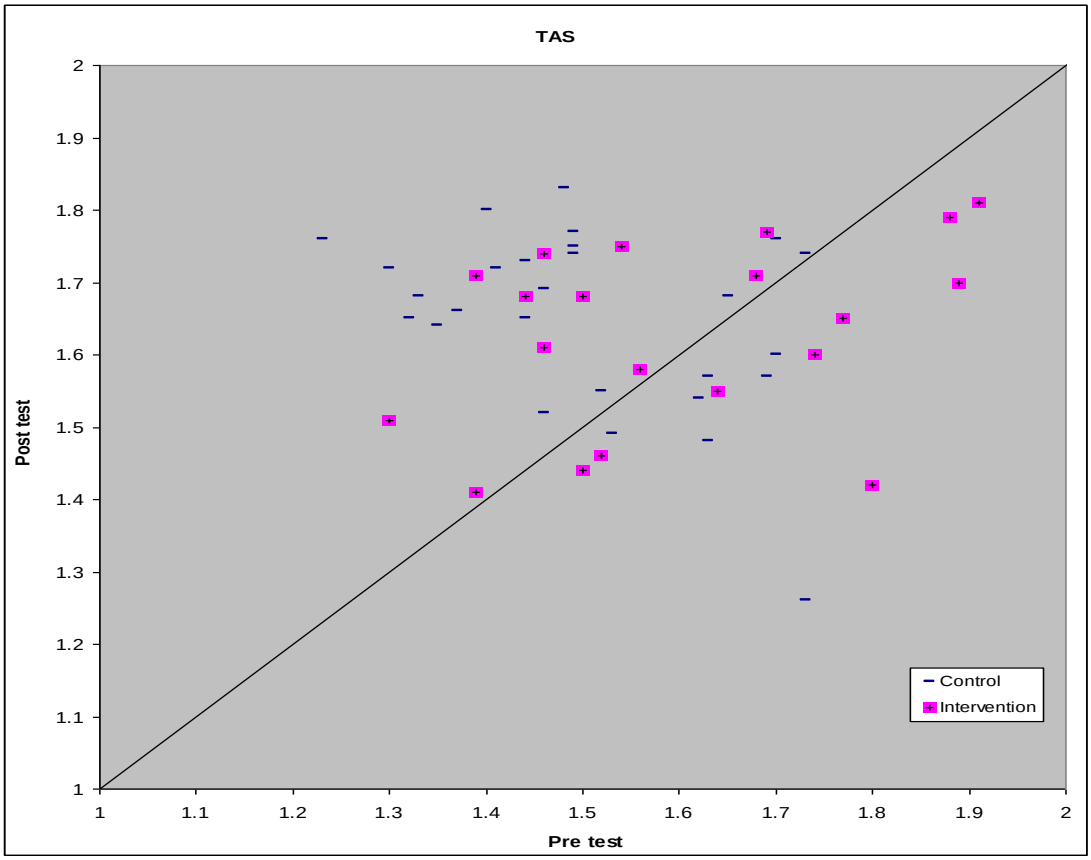


Figure 42: Total antioxidant status (TAS) pre and post intervention scores

6.4.6.4 Homocysteine (HCY).

A repeated measures ANCOVA was used to test the hypothesis that HCY levels would decrease over time for the Active group more so than for the Placebo group. Four covariates were included in this analysis based on univariate investigation: alcohol use, exercise, age and smoking.

Results show that there was a less than small, non significant main effect for time (Table 62). The small effects observed across time appear to be influenced by group allocation as the main effect for group evidenced a moderate to large effect, bordering on significance. These main effects are complicated by the interaction term. Specifically, the interaction between time and group showed a moderate- to-large, non-significant effect suggesting that the trajectory of change over time for each group

was different impacting main effects. Specifically, the Active group commenced pre-intervention with slightly lower (improved) HCY scores than the Placebo group. According to pre-intervention analyses (Table 52) these initial differences were not significant (showing only a small effect size, ϕ = .04).

According to Figure 43 and Table 61 (of means and standard deviations), the change in scores from pre- to post-intervention have quite different trajectories, with the Active group's HCY levels improving (8.7%) more than the Placebo group's (1%). Based on these results, the administration of multivitamins to the Active group could be attributed to improved HCY scores, thus providing support for the proposed hypothesis.

Covariates included in this analysis indicated that age contributed a large, significant impact on HCY levels, $F(1, 44) = 6.48$, $p = .02$, partial $\eta^2 = .13$. Alcohol use did not contribute unique significant influence on HCY levels, however it evidenced a small impact according to the effect size, $F(1, 44) = 0.27$, $p = .60$, partial $\eta^2 = .01$. For the covariates smoking, $F(1,44) = 1.25$, $p = .27$, partial $\eta^2 = .03$) and exercise, $F(1, 44) = 2.75$, $p = .10$, partial $\eta^2 = .06$ neither significantly adjusted HCY levels. The small to moderate effect sizes observed suggest there to be a slight level of impact.

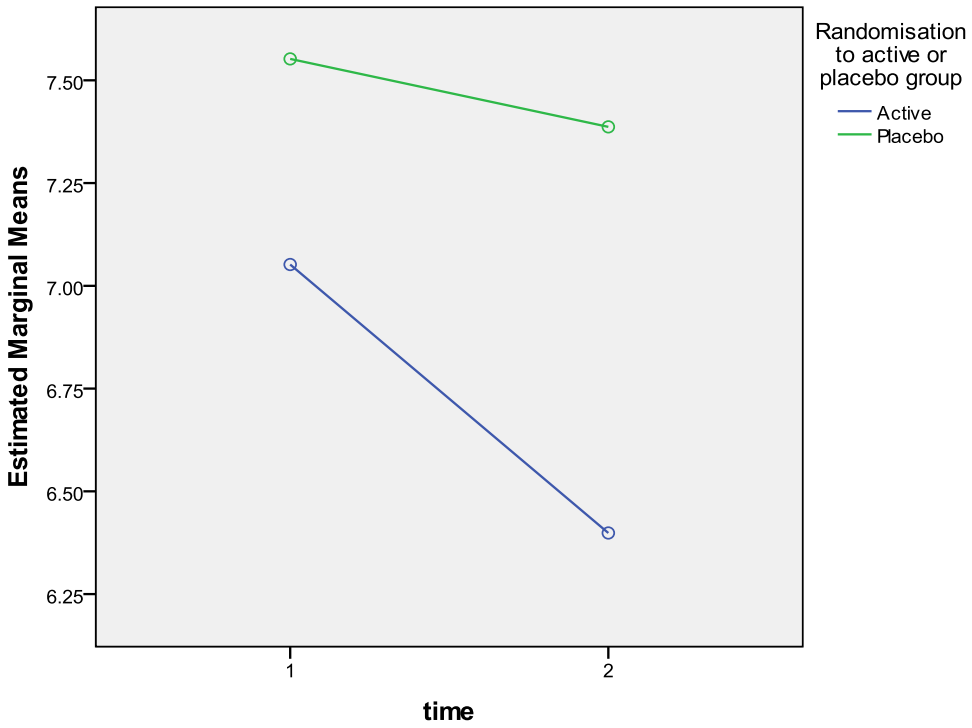


Figure 43: HCY Levels Across Time for the Active and Placebo Groups

To further clarify individual change across time and group, Figure 44 plots HCY scores pre-test versus post-test for individuals in the Active (crosses) and Placebo (dashes) groups. The solid 45 degree line represents a line of no change between pre- to post-intervention. Individuals above the 45 degree line showed worsened levels of HCY pre-to post-intervention while individuals located under the 45 degree line report lower or improved HCY levels pre- to post-intervention.

Consistent with the results at the main effect for group mean HCY scores, Figure 43 shows that there was largely improvement (decreases) observed for the Active group. These overall findings suggest that the administration of multivitamins to

the Active group improved HCY levels as compared to the Placebo group. This finding supports the proposed hypothesis.

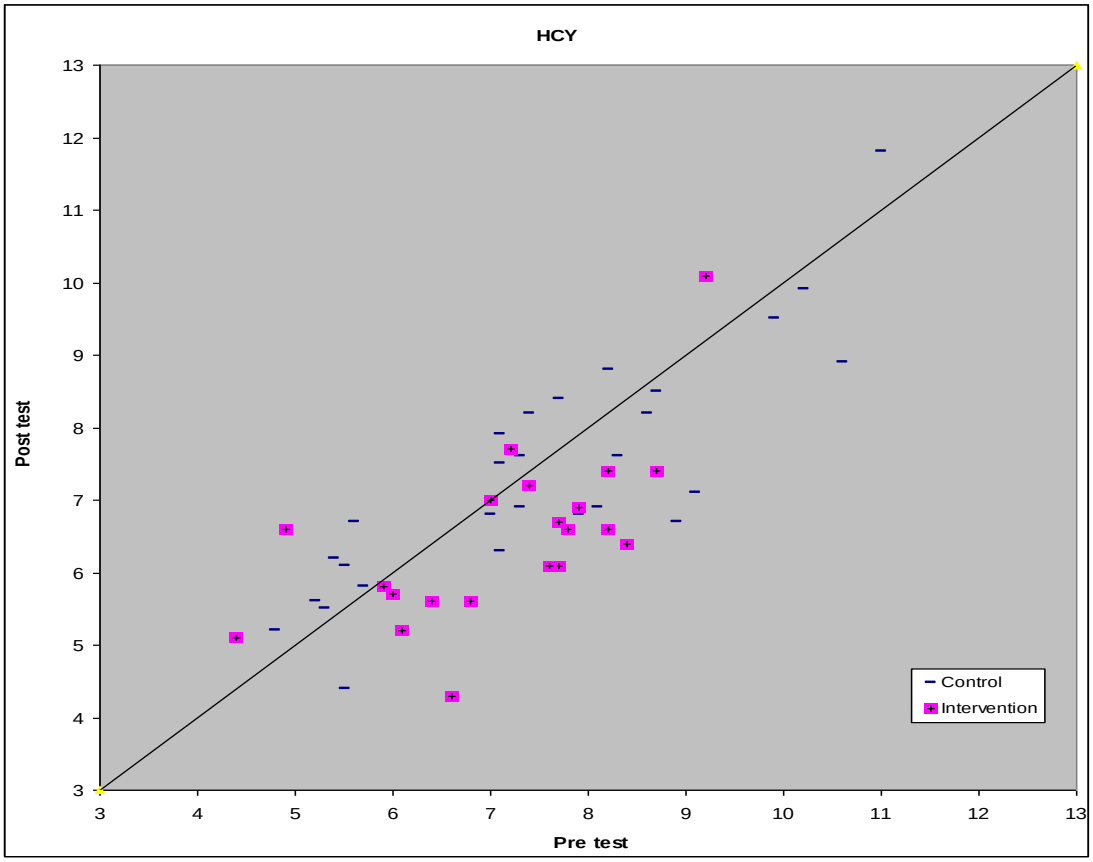


Figure 44: Homocysteine(HCY) pre and post intervention scores

6.4.6.5 Folate.

A repeated measures ANCOVA was used to test the hypothesis that FOLATE levels would improve over time for those in the Active group compared with the Placebo group. A covariate included in this analysis, base on univariate analysis, was smoking.

Results indicated that there was a large, significant change over time for FOLATE levels (Table 62). Furthermore this change over time was likely due to group

allocation given the moderate to large difference between intervention arms. There was also a large, significant interaction effect between time and group allocation, suggesting that the change in scores for each group were highly different, impacting the strength of change over time.

Specifically as can be seen in Figure 45 and following the means and standard deviations in Table 61, the Active group and the Placebo group commenced intervention with similar FOLATE scores (showing a less than small effect size ϕ= .03) The change in scores from pre- to post-intervention for the Active group increase substantially (28.4%), while the Placebo group's scores appear to increase only minimally.

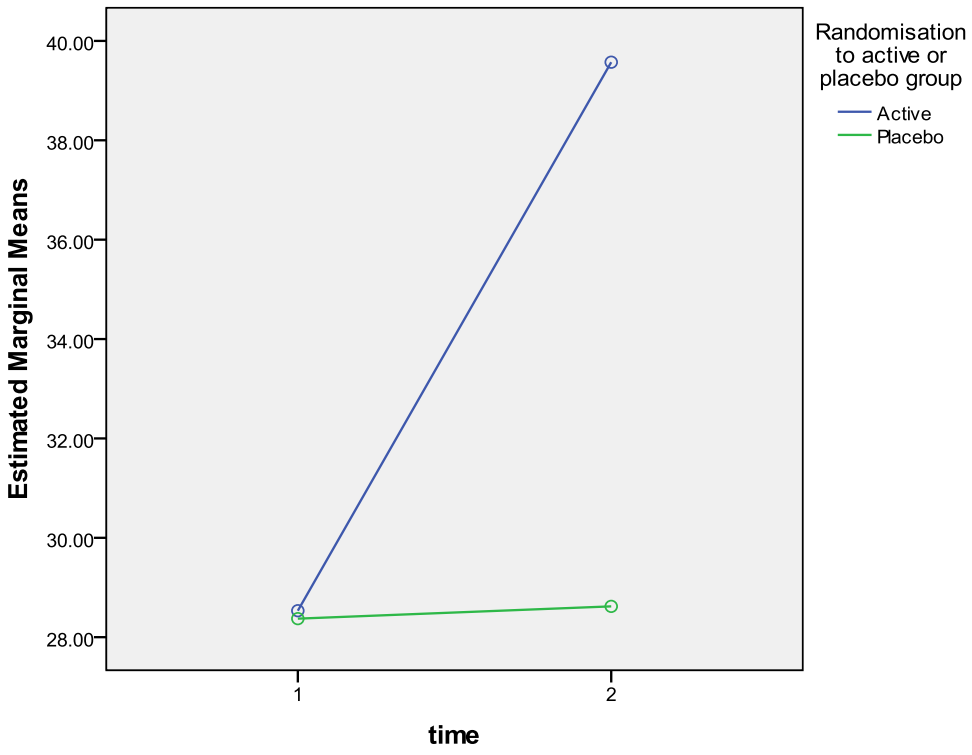


Figure 45: FOLATE Levels Across Time for the Active and Placebo Groups

The covariate included in this analysis, smoking, did not uniquely significantly adjust FOLATE scores, although it evidenced a moderate impact according to the effect size, $F(1, 45) = 2.96$, $p = .09$, partial $\eta 2 = .06$.

To further clarify individual change across time and group, Figure 46 plots FOLATE scores pre-test versus post-test for individuals in the Active (crosses) and Placebo (dashes) groups. The solid 45 degree line represents a line of no change between pre- to post-intervention. Individuals above the 45 degree line showed improved FOLATE levels pre-to post-intervention while individuals located under the 45 degree line report worsened FOLATE levels pre- to post-intervention. Consistent with the results at the main effects for time and group mean FOLATE scores, Figure 46 shows that there was largely improvement for the Active group.

These findings suggest that the administration of multivitamins to the Active group improved FOLATE levels as compared to the Placebo group. This also gives a good indication of participant compliance. This finding supports the proposed hypothesis.

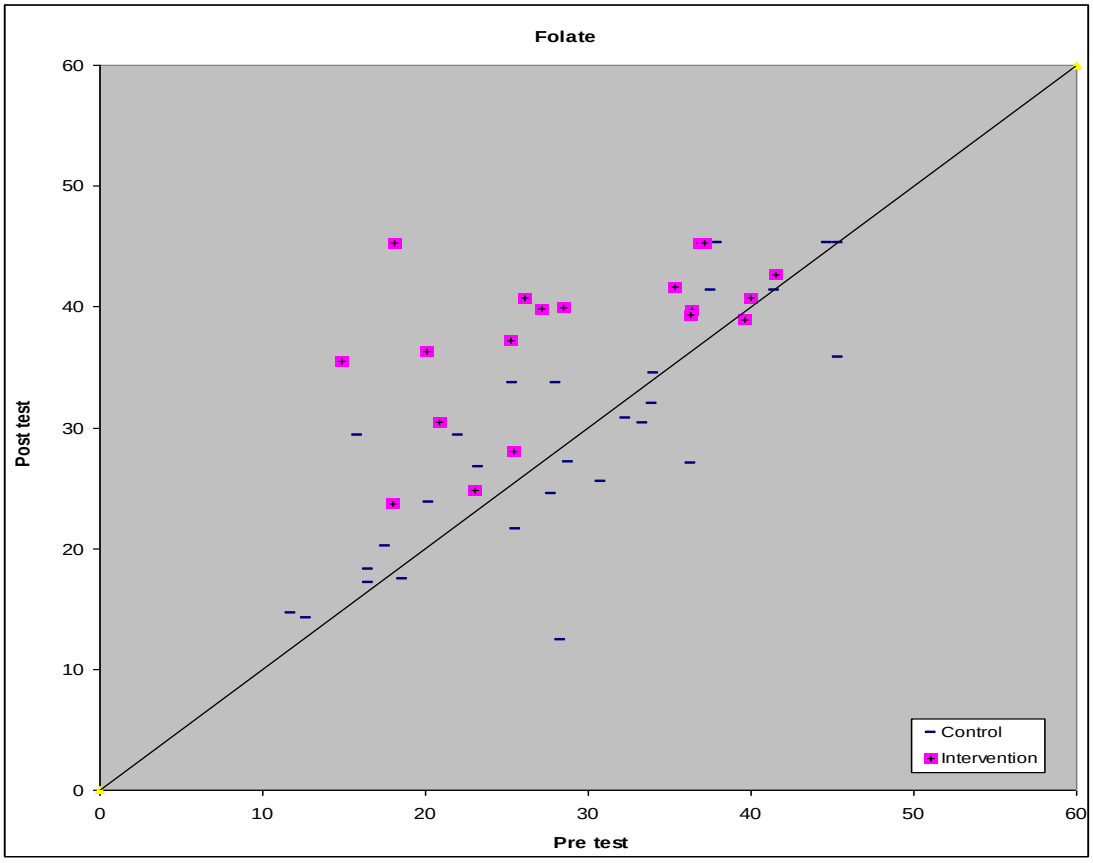


Figure 46: Folate pre and post intervention scores

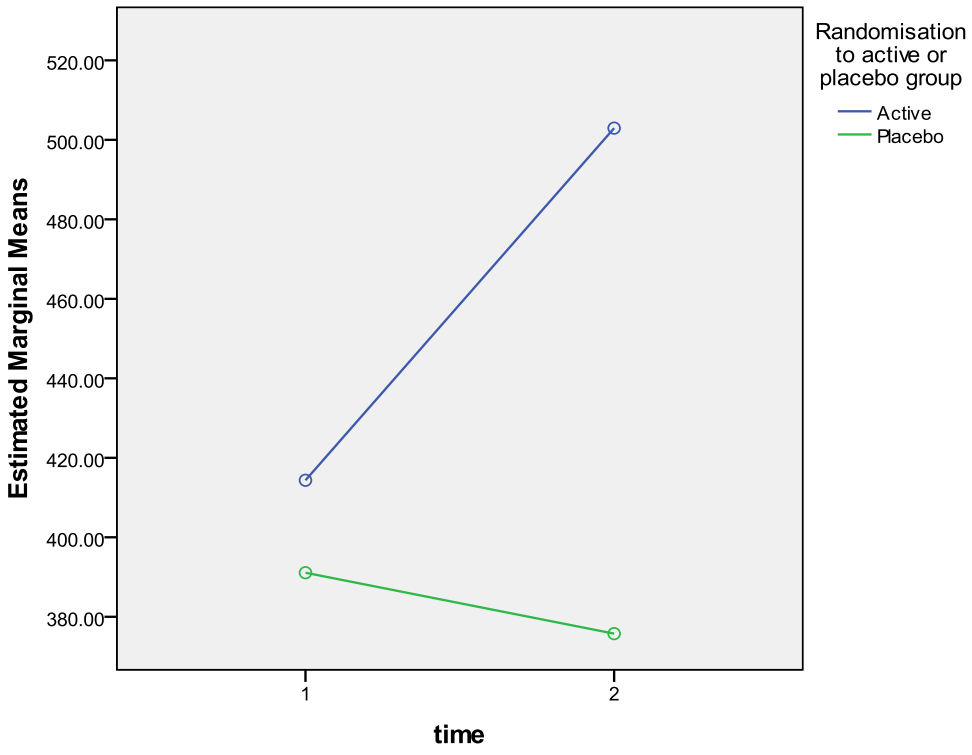
6.4.6.6 VIT B12 levels.

A repeated measures ANCOVA was conducted to test the hypothesis that VIT B12 levels would improve over time more so for the Active group compared to the Placebo group. A covariate included in this analysis, base on prior univariate investigations, was smoking.

Results indicated a large, significant increase in VIT B12 over time (Table 62). The main effect for group evidenced a small-to-moderate, non significant outcome suggesting that the change in scores over time was not due to group allocation. These main effects are complicated by the interaction term. Specifically, the interaction

between time and group showed a large, significant effect suggesting that the trajectory of change over time for each group was different impacting main effects.

Specifically, the Active group commenced pre-intervention with slightly higher VIT B12scores than the Placebo group. According to pre-intervention analyses (see Table 52) these initial differences were not significant (showing only a small effect size, ϕ = .01). Subsequently according to Figure 47 and Table 61 (of means and standard deviations), the change in scores from pre- to post-intervention then take quite different trajectories, with the Active group's VIT B12 scores improving (increased 17%) whilst the Placebo group's VIT B12 worsened (declined 3.4%). Based on these results, the administration of multivitamins to the Active group could be attributed to improved VIT B12 scores, providing support for the proposed hypothesis. This is also a good indicator of compliance.



Covariates appearing in the model are evaluated at the following values: smoking = .12

Figure 47: VIT B12 Levels Across Time for the Active and Placebo Groups

The covariate included in this analysis, smoking, did not uniquely significantly adjust VIT B12 scores, although it evidenced a moderate impact according to the effect size, $F (1, 47) = 3.29$, $p = .08$, partial $\eta 2 = .07$.

To further clarify individual change across time and group, Figure 48 plots VIT B12 scores pre-test versus post-test for individuals in the Active (crosses) and Placebo (dashes) groups. The solid 45 degree line represents a line of no change between pre- to post-intervention. Individuals above the 45 degree line showed improved VIT B12 levels pre-to post-intervention while individuals located under the 45 degree line report worsened VIT B12 levels pre- to post-intervention. Consistent with the results at the main effects for time and group mean VIT B12 scores, Figure 48 shows that there was largely improvement for the Active group.

These findings suggest that the administration of multivitamins to the Active group improved VIT B12 levels as compared to the Placebo group. This finding supports the proposed hypothesis.

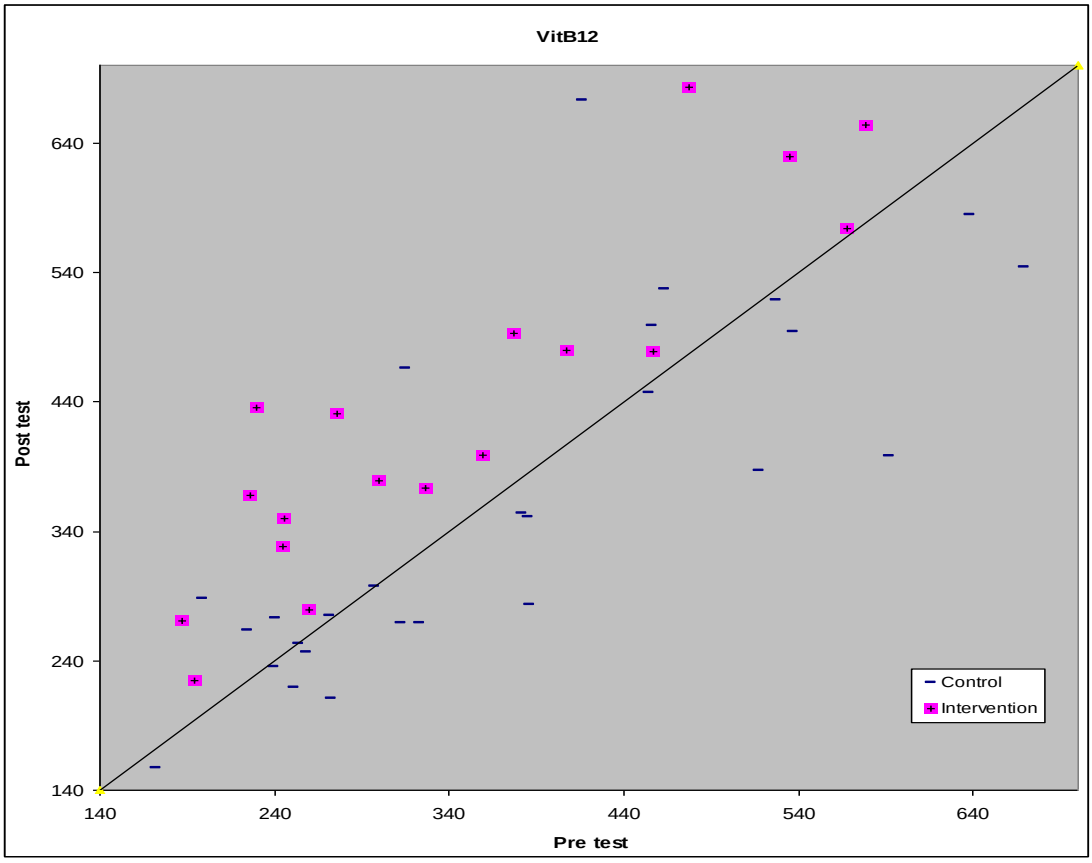
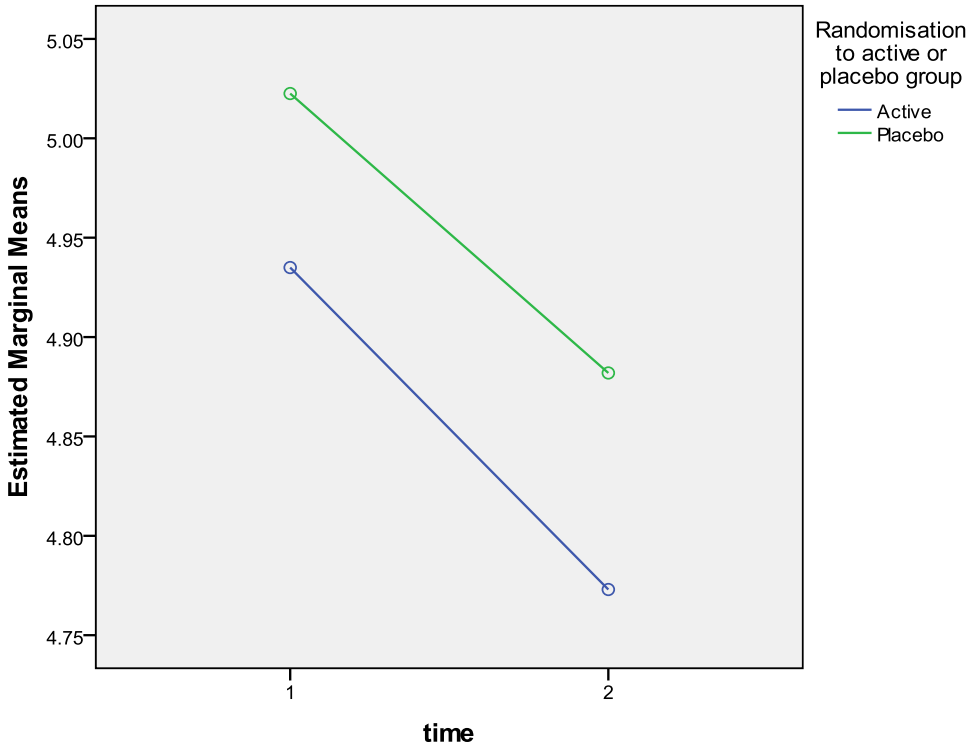


Figure 48: Vitamin B12 Levels Across Time for the Active and Placebo Groups

6.4.6.7 Cholesterol (CHOL).

A repeated measures ANCOVA was used to test the hypothesis that CHOL levels would decrease over time for the Active group more so than for the Placebo group. Covariates included in this analysis, based on univariate analysis, were antidepressant and respiratory medication use.

Results indicated that there was only a small, non significant change in CHOL levels over time (Table 62). The main effect for group showed no effect suggesting group allocation did not influence CHOL levels. Concurrently the interaction effect between time and group showed no effect suggesting score changes were in the same direction (Figure 49).



Covariates appearing in the model are evaluated at the following values: Resp = .08, AnitDep = .08

Figure 49: CHOL Levels Across Time for the Active and Placebo Groups

The covariates in this analysis indicated that antidepressant medication use uniquely, significantly adjusted CHOL scores, $F (1, 46) = 4.23$, $p = .05$, partial $\eta 2 = .08$. Respiratory medication use evidenced a small- to- moderate effect it did not uniquely, significantly adjust CHOL scores, $F (1, 46) = 2.47$, $p = .12$, partial $\eta 2 = .05$.

To further clarify individual change across time and group, Figure 50 plots CHOL scores pre-test versus post-test for individuals in the Active (crosses) and Placebo (dashes) groups. The solid 45 degree line represents a line of no change between pre- to post-intervention. Individuals above the 45 degree line showed worsened CHOL levels pre-to post-intervention while individuals located under the 45 degree line report improved CHOL levels pre- to post-intervention. Figure 50 and Table 61, show that consistent with the results at the main effects for time and group CHOL scores are clustered around the line of no change, there was largely no improvement for either the Active or Placebo Group.

These findings suggest that the administration of multivitamins to the Active group did not improve CHOL levels as compared to the Placebo group. This finding does not provide support for the proposed hypothesis.

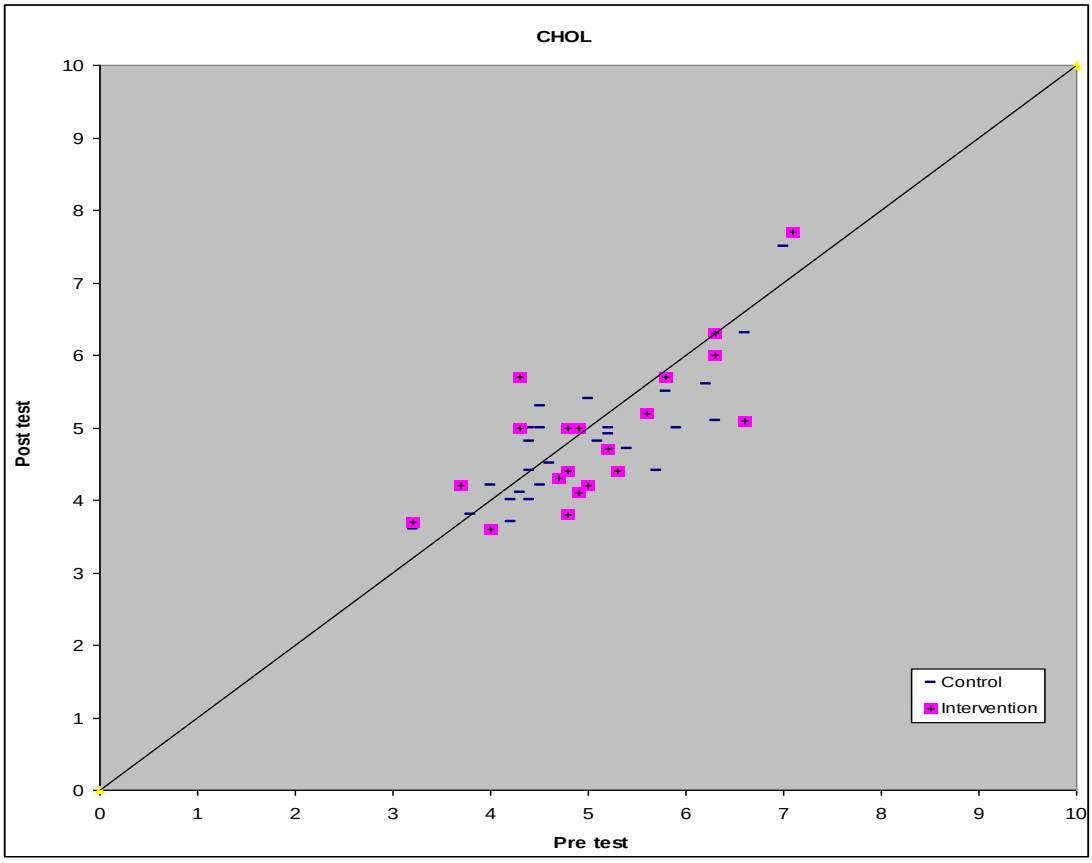


Figure 50: Cholesterol (CHOL) pre and post intervention scores

6.4.7 Hypothesis 2b - Pro-inflammatory measures for those allocated to the active multivitamin group will be lower compared to those allocated to the placebo group.

Inflammatory cytokines in the current sample were not normally distributed. This is not unexpected as pro-inflammatory responses by their very nature being acute and occurring over a matter of hours rather than days or weeks. This is expected in a well-functioning immune system. Hence exploration of these markers requires non-parametric techniques.

6.4.7.1 Covariates influencing inflammatory measures.

Potential confounding variables (like age, exercise, alcohol intake, etc) which have been shown to influence other normally distributed variables were explored also (Appendix L). Although nonparametric statistics are not sophisticated as to be able to control for possible confounders, by identifying them at the outset subsequent findings can be interpreted in the context of any potential influences.

Higher levels of exercise were moderately, significantly associated with lower levels of IL-5 ($r = -.22$, $p = .104$, $n = 58$). In contrast increased scores of hazardous alcohol use ($r = .27$, $p = .04$, $n = 57$) were significantly associated with higher IL-5 levels, verging on a moderate effect size.

Higher levels of IFN-γ levels was moderately associated with Antidepressant medication use ($r = .30$, $p = .03$, $n = 58$) and smoking, suggesting this to be pro-inflammatory. In contrast the use of hormonal contraceptive medications evidenced a moderate inverse association ($r = -.22$, $p = .10$, $n = 56$) with IFN- γ levels.

IL-10 levels were negatively associated with exercise (r = -.30, p = .01, n= 59). IL-10 is an anti-inflammatory cytokine. This suggests increased exercise was associated with lower anti-inflammatory cytokines in the current sample. Further interpretation of these variables in the non-parametric analyses should be treated with caution due to these confounders.

6.4.7.2 Pre- to post-intervention change for pro-inflammatory measures.

Based on preliminary exploration of inflammatory variables at the outset of this result section (Table 53) no significant differences were observed for cytokine levels at baseline between the groups. Assessment of change over time was undertaken and median scores are reported for each cytokine for Active and Placebo groups pre- and post-intervention (Table 63). Pre- to post-intervention changes for cytokine levels over the 8-week trial was explored using Wilcoxon Signed Rank Tests, followed by exploration of post-intervention levels, using Mann Whitney U techniques (Table 64). The Wilcoxon Signed Rank Test converts individual scores to ranks and then compares them (Pallant, 2001). Effect sizes are reported alongside p-values.

Table 63: Assessment of Change in Inflammatory Cytokine Levels for Active and Placebo Groups Pre- To Post-Intervention

Biomarker	Reference Range	Time	Active group					Placebo group				
			n	Median	z	p	r	n	Median	z	p	r
TNF-β	<439pg/ml	Pre	29	10.04				30	8.92			
		Post	21	7.06	-2.97	**.00**	**.40**	27	7.81	-0.12	.90	.02
IFN-γ	<365pg/ml	Pre	28	47.84				28	43.16			
		Post	21	59.00	-0.16	.88	.02	26	63.57	**-2.31**	**.02**	**.31**
IL-5	<44pg/ml	Pre	28	7.57				30	10.05			
		Post	21	6.98	-0.15	.88	.02	27	9.35	-0.76	.46	.10
IL-10	<44pg/ml	Pre	29	14.02				30	15.20			
		Post	21	14.10	-0.11	.91	.01	27	13.20	-1.45	.15	.19
IL-6	<149pg/ml	Pre	29	12.32				30	18.04			
		Post	21	8.24	-1.48	.14	.20	27	12.80	-1.87	.06	.26
IL-1β	<426pg/ml	Pre	29	38.60				29	52.28			
		Post	21	13.32	-2.80	**.05**	**.38**	27	23.40	-1.73	.08	.23
TNF-α	<479pg/ml	Pre	29	15.60				30	18.68			
		Post	21	10.84	-2.38	**.02**	**.31**	27	15.67	-0.91	.36	.12

Note. Biomarker Abbreviations: C-reactive protein (CRP), Interferon (IFN), Tumor necrosis factor-α (TNF-α), Interleukin-1 (IL-1β), Interleukin-5 (IL-5), Interleukin-6 (IL-6), Interleukin-10 (IL-10), Tumor necrosis factor- β (TNF-β).

Bold values show significance. p-values based on Wilcoxon Signed Rank Test; Effect sizes reported r using Cohen (1988) criteria of .1 = small effect, .3 = medium effect, and .5 = large effect

Table 63 presents the pre-to post-intervention changes for Active and Placebo group cytokine levels. For the Active group medium- to- large effect sizes which reached significance were observed between pre- and post-intervention for IL-1β ($z = -2.80$, $p = .05$, $r = .38$), TNF-α ($z = -2.38$, $p = .02$, $r = .31$), and TNF-β ($z = -2.97$, $p = .00$, $r = .40$). The trend was for decreases across these cytokines. In addition a small- to-medium effect was observed for IL-6 but did not reach significance.

In contrast the Placebo group evidenced a medium effect size for increased IFN-γ between pre- (Md = 43.16) and post-intervention (Md = 63.57) which reached significance ($z = -2.31$, $p = .02$, $r = .31$). In addition small to medium effect sizes were observed for decreased IL-6 and IL-1β, although not reaching significance.

These findings suggest decreased inflammation was observed for both the Active and Placebo groups, but favored those receiving the Active supplement. In contrast increased IFN- γ levels were observed to increase for both groups, reaching significance for the Placebo group. These mixed findings provide partial support for the current hypothesis.

Table 64: Post-Intervention Comparisons of Inflammatory Cytokine Levels between Active and Placebo Groups

Biomarker	Reference Range	Time	Active group		Placebo group		Mann Whitney U	z	p	r
			n	Median	n	Median				
TNF-β	<439pg/ml	Pre	29	10.04	30	8.92				
		Post	21	7.06	27	7.81	207.50	-1.09	.27	.16
IFN-γ	<365pg/ml	Pre	28	47.84	28	43.16				
		Post	21	59.00	26	63.57	211.00	-0.83	.41	.12
IL-5	<44pg/ml	Pre	28	7.57	30	10.05				
		Post	21	6.98	27	9.35	225.00	-0.70	.48	.10
IL-10	<44pg/ml	Pre	29	14.02	30	15.20				
		Post	21	14.10	27	13.20	254.50	.96	.96	.06
IL-6	<149pg/ml	Pre	29	12.32	30	18.04				
		Post	21	8.24	27	12.80	197.00	-1.33	.18	.19
IL-1β	<426pg/ml	Pre	29	38.60	29	52.28				
		Post	21	13.32	27	23.40	210.00	-1.04	.30	.15
TNF-α	<479pg/ml	Pre	29	15.60	30	18.68				
		Post	21	10.84	27	15.67	170.50	-1.92	**.05**	.30

Note. Biomarker Abbreviations: C-reactive protein (CRP), Interferon (IFN), Tumor necrosis factor-α (TNF-α), Interleukin-1 (IL-1β), Interleukin-5 (IL-5), Interleukin-6 (IL-6), Interleukin-10 (IL-10), Tumor necrosis factor- β (TNF-β).

Bold values show significance. Effect sizes reported r using Cohen (1988) criteria of .1 = small effect, .3 = medium effect, and .5 = large effect

6.4.7.3 Post-intervention cytokine comparisons between the active and placebo group.

Post-intervention comparisons between the Active and Placebo group cytokine levels are presented in Table 64. It was hypothesised that the Active group members would have lower cytokine levels post-intervention compared to the Placebo group. Group comparisons revealed several cytokines (TNF-β, IL-6, IL-1β, and IFN-γ) evidenced small to medium effect sizes. In addition a medium effect reaching statistical significance was observed for TNF-α. Significantly lower levels of TNF-α were observed for the Active group (Md = 15.60, n = 21) compared to those allocated to the Placebo group (Md = 18.24, n = 27), z = -1.92, p = .05 with a medium effect size (r = .30) observed. Graphical depiction (Figure 51) of these post-intervention cytokine levels by group indicates lower inflammatory cytokines levels for those allocated to the Active group. These findings provide support for the hypothesis.

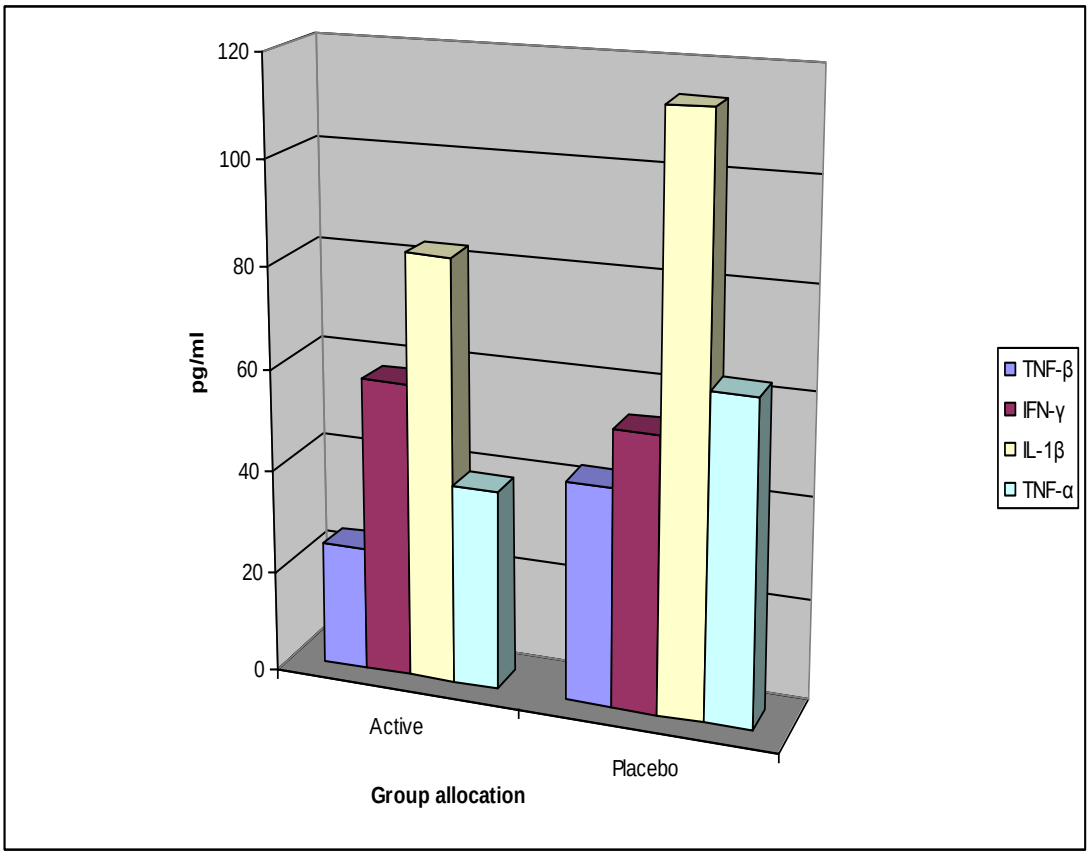


Figure 51: Post-Intervention Mean TNF-β, IFN- γ, IL-1β, TNF-α Levels For Active and Placebo Groups

6.4.8 Hypothesis 3a and 3b- Pre-intervention pro-oxidant and pro-inflammatory measures will be associated with higher levels of psychological distress and dysfunctional emotion states.

The hypotheses at the outset of this chapter explicitly focused on exploring associations of psychological distress and anger measures with measures of pro-oxidant and pro-inflammatory measures. Relationships were investigated using Pearson product-moment correlations. Preliminary analyses were performed to ensure no violation of the assumptions of normality, linearity and homoscedasticity. If violations to normality were

observed Spearman's rank order correlations were employed. Partial correlations were performed controlling for variables previously identified as potential confounders.

At pre-intervention higher distress scores were associated with higher VITB levels, there was a small positive correlation between the two variables [r = .25, N = 59, p = .03]. HCY was found to be associated with higher internalized anger expression (AC—in) scores (r = .27, n = 60, p = .04) (Appendix M). Higher distress scores were not associated with increased inflammatory cytokines (Appendix N).

6.5 Discussion

6.5.1 Overview.

The current study aimed to explore the role of multivitamin supplementation for women experiencing stressful life events, using The Oxidative Model as a guide. The Model proposes that with increased stress and emotional dysfunction there is an associated pro-oxidant and pro-inflammatory imbalance. It was anticipated multivitamin supplement employed in this trial would balance the body systems, specifically those that respond to stress, by replacing nutrients that are in high demand during times of stress.

It was hypothesized that allocation to the Active group (the multivitamin supplement) would result in improvement in psychological, pro-oxidant, and pro-inflammatory outcomes across an 8-week trial. There was a significant proportional decrease in inflammatory cytokines across the trial for those allocated to the Active group. Yet this study was unable to definitively confirm multivitamin supplementation during

stressful life events was beneficial in the context of decreased oxidative stress or improved psychological wellbeing. Furthermore observed associations between biopsychosocial outcomes were low and not statistically significant and subsequently did not support the proposed hypotheses or previous findings. There were significant associations between psychological outcomes at baseline as hypothesized.

6.5.2 Psychological well-being.

As predicted there was a significant decrease in GHQ, S-anxiety, S-depression, scores across pre- and post-intervention assessments. This improved psychological state was not unique to those allocated to the Active group as hypothesized. Psychological well-being improved for all participants. There are two possibilities that may account for these observations. Firstly, these findings could suggest that over the course of time, stressors experienced by participants may have been resolved independent of allocation to either Active/Placebo group. Improvement may be solely through the passage of time and the natural resolution of stressful life events. Concurrently, volunteering to take part in a study about stress might have influenced or facilitated how participants viewed and addressed their current experience.

Secondly, there is also the placebo-effect whereby the act of an intervention, in this case taking capsules, alters perceptions with regard to improved health and well-being in turn influencing participants' mood scores. Another mechanism, known as the Hawthorne Effect refers to a form of reactivity whereby subjects improve or modify an aspect of their behavior simply in response to the fact that they are being studied, not in

response to any particular experimental manipulation (McCarney et al. 2007). It is often difficult in clinical trials to quantify the influence of the extra attention by researchers or higher levels of clinical surveillance.

The Hawthorne Effect applies equally to treatment and control arms. In the current study the improvements for psychological distress favored the active treatment arm, but other improved mood states were observed across the entire sample. Although the Hawthorne Effect should not affect assessment of the difference between intervention and control groups, it may result in an inflated estimate of effect size in routine clinical settings by over-estimating response in both groups.

Potentially enrollment in an RCT focused on psychological stress and physical health, subsequent interactions with researchers, and self-report questions around their personal experience of psychological stress was enough to have an impact on lifestyle measures like diet, exercise, alcohol consumption, smoking, etc. Changes to lifestyle (i.e., diet, exercise, and alcohol intake) might have been implemented by participants over the course of the intervention which was not measured as part of this study. As a result, these changes potentially contributed to the overall improvement in psychological well-being.

S-curiosity did not markedly increase across the trial as proposed at the outset. Psychological components surrounding curiosity include concepts like exploratory behaviour, sensation-seeking, reaction to novel stimuli, and feelings of interest or uncertainty. This construct is less well understood in PNI research. Curiosity is identified as a positive personality trait for this study. Participants had curiosity scores (State and

Trait) comparable to a normative sample at the outset so it is possible that their stress experience was not sufficient to dampen their curiosity. S-anger scores were clustered at the low values (positively skewed) for participants in the current study. This 'flooring effect' limited the potential amount of improvement possible. Slight decreases were observed but again these were not confined to the Active group.

Loneliness was the one psychological measure which suggested a possible influence of group allocation with a small to moderate effect size observed. Although this was not statistically significant, participants in the Active group evidenced a larger decrease in loneliness levels than the Placebo group. Scores on the UCLA loneliness scale were slightly elevated but remained within one standard deviation of normative samples, and were comparable to women of a similar age. Loneliness is a psychological experience related to social isolation and a perceived lack of companionship. Research has suggested that loneliness is a psychological factor that relates to biological responses that are potentially relevant to health (neuroendocrine, cardiovascular, inflammatory stress), (Steptoe, Owen, Kunz-Ebrecht, & Brydon, 2004) although causal conclusions have not been drawn.

Loneliness was associated with increased psychological distress replicating previous findings (Nolen-Hoeksema & Ahrens, 2002). This indicated a perceived social support element to the stress experience of women in this sample. Loneliness has been found to be independent of social and demographic determinants, and is independently associated with emotion states (Steptoe et al., 2004). Thus its role is potentially prejudicial

to psychological well-being. This is an area which requires further exploration with regard to the relevance of this construct to the Oxidative Model. Loneliness was confounded by age suggesting that as participants' age increased they required more social support and/or their desired social contact level was not being met. Past research suggests that middle-aged adults report more loneliness than younger or older adults (Steptoe et al., 2004). Older adults tend to decrease the size of their social networks and rather focus their efforts on maintaining these connections.

6.5.3 Pro-oxidant markers.

Primary hypotheses were partially supported, given that NT, HCY, FOLATE, and VIT B12 levels all showed improvement across the eight-week trial. An increase in NT indicates the body's increased acquired immune status, specifically with regard to lymphocyte maturation and proliferation. NT levels significantly increased for all participants regardless of group allocation. Those allocated to the Active group had proportionally higher NT levels post-intervention it could not be attributed to group allocation or pre-existing differences.

Based on previous research by Blake-Mortimer and Colleagues (1996) who observed that a group of patients diagnosed with major depressive disorders placed on antioxidant rich multivitamin supplements, by their clinician, had NT values that were twofold higher than non supplemented patients. Similar findings were observed in a healthy occupational stress sample (Hapuarachchi et al., 2003). It was anticipated that NT levels would increase, due to the increased antioxidant supply resulting from multivitamin

supplementation. Exploration of a causal relationship in the current study did not provide support for this. Although it is worth mentioning that due a small sample, higher than expected attrition, and missing data statistically the possibility of Type II errors becomes more likely. Despite this both the Active and the Placebo group oxidative marker levels improved rendering it impossible to attribute this improvement specifically to multivitamin supplementation.

Baseline NT values observed in this trial were more similar to those attained previously (Hapuarachchi et al., 2003) from a 'normal' (non-stressed control) sub-group rather than a mild/severely stressed groups. This potentially indicates inconsistencies in specificity resulting from the screening tool used (GHQ) in both of these studies. Similarly observed NT values in the Honours student group (Blake-Mortimer, et al. 1998), during a high stress period, were comparable to NT levels observed in 'normal' samples in other populations (Happuarachchi et al., 2003). Although this biomarker appears to be associated with stress and negative mood states, inconsistencies are apparent across stress scenarios. Identifying a sufficient screening measure for stress remains a challenge. This limits the usefulness of the NT biomarker. Additionally it is difficult to recognize clinical significance for NT values for this study compared to previous literature due to the disparity between observed levels across the studies. It is likely that covariates, like age, were influential in past research but went uncontrolled for in analyses.

Unlike past research on The Oxidative Model(Blake-Mortimer et al., 1996, 1998b; Hapuarachchi et al., 2003), age and other influential health and demographic measures

were statistically controlled for in this trial. Age was especially relevant for NT values in this sample of 25 to 45 year olds, accounting for 11% of variance. Although normal immune functions decline with age, there is no specific biomarker for the ageing immune system (Boss et al., 1980). Similarly exercise rate accounted for nearly 10% variance in VIT C levels. These confounding variables identified in this study indicate that demographics and lifestyle choices with regard to health behaviours are salient factors for PNI theoretical models. Health behaviours were found to influence all aspects of wellbeing both psychological and physiological. Happuarachchi (2003) raised this notion in commenting on the multivitamin-takers in his study having either a sense of control in their lives or a perception that the taking of multivitamins enhanced their sense of wellbeing. Finding out reasons why people take multivitamins might implicitly be linked to their perception of wellbeing and thus physical health.

Similarly HCY levels improved as observed by a significant decrease for all women in this study. As a marker of oxidation and cardiovascular disease (CVD) a decrease indicates an improved pro-oxidant state. This decrease was across all participants but the trajectory of decline suggests the Active group experienced greater improvement. An association between measures of internalised anger expression and HCY was replicated (Hapuarachchi et al., 2003; Stoney, 1999; Stoney & Engebretson, 2000). Both HCY and Anger measures for this current sample were well within the normative range for a normal, healthy sample of women.

FOLATE and VIT B12 levels significantly increased for the Active group, supporting the proposed hypotheses. FOLATE and VIT B12 levels increased across time and could be attributed to group allocation. Specifically, the Active group showed significantly improved FOLATE and VIT B12 levels over time, an effect not observed in the Placebo group. The supplement employed for this study was rich in both these nutrients. This finding suggests that there was a sufficient rate of compliance. However interpretation of this finding is taken with caution as there was a significant difference at the outset of the study for FOLATE levels between groups. This potentially contributed to the size of the improvement at follow-up. Both VIT B12 and FOLATE may have further influenced other biomarkers in this study although the timeframe for eliciting clinically relevant change remains to be defined for many of these markers.

It was anticipated that VIT C and TAS levels would increase in the Active group as a result of additional multivitamins to these participants' diets. This was not observed. VIT C was the sole antioxidant contribution in the multivitamin supplement used for this trial although Withania somnifera (winter cherry), an ayervedic herb, has reported antioxidant properties (Mishra, Singh, & Dagenais, 2000). The lack of a significant finding for VIT C levels could be explained in three ways.

Firstly, the level of VIT C within the current supplement when compared with previous studies of the Oxidative Model (Blake-Mortimer et al., 1996, 1998b) was substantially lower. The amount of VIT C ingested per day in this trial was 150mg. Levels ingested in other studies ranged from 1-4g per day (Blake-Mortimer, 1998, 1996), 10

times the amount. One study included VIT C at these levels with other antioxidants like Vitamin A (3mg), Vitamin E (>300mg) and Co-enzyme Q10 (Blake-Mortimer et al., 1998b). This might have impacted on the efficacy of the multivitamin used to increase antioxidant status. However this is potentially why there was not a significant change in total antioxidant status (TAS) observed across the study. Yet similar types of supplements were observed to have an impact in one Oxidative Model study (Hapuarachchi et al., 2003). Specific levels of antioxidant contributions were not outlined in this research making further comparisons difficult.

Secondly, it is possible that as NT levels were observed to increase across the study and as VIT C is considered protective of NT that the amounts of VIT C ingested were oxidized rapidly. This does not account for lack of finding a difference between groups. Thirdly, at baseline VIT C levels were within the normative range and thus participants may have already had a sufficient dietary intake of VIT C therefore further addition of VIT C did not contribute to significant change across this trial.

6.5.4 Pro-inflammatory measures

As hypothesized pro-inflammatory cytokine levels decreased significantly in the Active group compared to the Placebo group but this was not observed across all cytokines. Pre to post-intervention decreased TNF-β levels were observed for the Active group. In contrast the Placebo group evidenced an increase for IFN-γ. On further exploration observing the two groups separately, the Active group had a significantly greater number of participants whose levels of TNF-α decreased post-intervention.

Similarly moderate- to- large decreases for IL-6, IL-1β, and a concurrent increase in IFN-γ levels favoured the Active group. These findings support previous research (Maes et al., 1998; Paik et al., 2000). Measurement of acute phase proteins is a useful marker of inflammation and is commonly used across medical contexts. These findings suggest a decrease in inflammation, specific to the Active group. It is important to consider the acute phase response (and inflammation) as a dynamic homeostatic process that involves all of the major systems of the body, in addition to the immune, cardiovascular, and central nervous system. These findings could be attributed to the influence of the multivitamin supplement, although the mechanism for decreasing inflammation is less well understood in this Model.

One possible mechanism is the addition of the ayervedic herb, Withania Somnifera to the multivitamin. As previously discussed this component of the multivitamin supplement has reported antioxidant-like effects, but importantly its composition has been described as steroidal in nature; this proposed effect is supported by the decreased inflammatory cytokines observed in the Active group. In the Placebo group a significant proportion of participants with increased IFN-γ levels were observed post treatment. This is an anti-inflammatory cytokine. This suggests whilst the Placebo groups' inflammatory cytokine levels were not significantly reduced, their levels of this anti-inflammatory cytokine actually increased. This pattern was also observed in the Active group.

This does not aid in confirming the anti-inflammatory nature of the multivitamin supplement for this study although the Cytokine Shift Model puts forward that chronic

stress has a simultaneous enhancement and suppression of the immune response. It does so by altering patterns of cytokine secretion (Marshall et al., 1998). Cytokines can be defined by the types of cells they are secreted by. For example, Th-1 (Helper T cells) cells secrete IFN-γ, TNF-α and TNF-β. These are the cytokines which evidenced decreases for the Active group in the current sample. These cytokines are responsible for cell mediated immune responses, which includes the activation and recruitment of macrophages, natural killer cells and other T cells. *Cell-mediated* responses are the most effective against intracellular pathogens such as viruses and cancer cells. They are considered part of the innate immune response, the first line of defense. The Cytokine Shift Model of PNI suggests that cytokines are key components in connecting immune changes to psychological state (Segerstrom & Miller, 2004)

Cytokines serve as chemical messengers within the immune system and across other systems including the nervous system. Notably the acute phase response usually lasts only a few days; in cases of chronic or recurring inflammation, an aberrant continuation of some aspects of the acute phase response may contribute to the underlying tissue damage, and may also lead to further complications i.e. cardiovascular diseases. Findings from the current study are suggestive of multivitamin supplementation linked to decreased inflammation and decreased innate immune responses which are by their nature pro-oxidant.

It was expected that CHOL and CRP, both markers of CVD, would decrease indicating an additional benefit of the increased multivitamin intake of the Active group.

This was not observed. This could be explained by a number of reasons. The time frame is quite different for both these markers. For cholesterol the eight week trial may not have been sufficient for lowering this marker. At the same time diet largely affects cholesterol levels and this was something that was not recorded. In addition cholesterol measures attained were not from fasted samples (at least 12 hours without food) and as a result are not as accurate; a high fat meal prior to participating might have influenced subsequent levels. Cholesterol levels recorded were only total cholesterol rather than LDL (bad) and HDL (good) cholesterol. It may be more important to know levels of good and bad cholesterol as opposed to an overall reading.

Alternately CRP levels increase quickly and dramatically and fluctuate rapidly; changes observed only give a window of the last 24 hours and thus might not give a true representation of the biomarker during periods of stress, although we were more interested in these measures to determine relationships amongst the biochemical measures as opposed to relying on a significant change result.

6.5.5 Relationships between psychological biochemical variables.

Unlike previous studies little or no association was found between psychological measures and biomarkers of a pro-oxidant state. Specifically psychological distress was not associated with any significant change in NT, HCY, TAS, VIT C, FOLATE, or VIT B12. It is possible that the lack of association could be attributed to lack of variability in psychological measures and biochemical measures. Means for both were comparable to those reported by Happuarachhci and colleagues (2003). A non-stressed comparison

group was used as a control which would have added to the variability of measures in that study. In addition in the current study statistically controlling for confounding variables removed some of the variance.

In addition few significant associations were observed for pro-inflammatory measures. Specifically psychological stress, as measured by GHQ, was not associated with changes in cytokine levels. Again this may be due to lack of variability from this sample. On comparison with Happuarachchi's (2003) correlational findings, we were unable to replicate findings of decreased NT and an associated increase in psychological stress and GHQ scores. Similarly we were unable to reproduce the association between increased anger expression measures and HCY expressed in this study, as found by others (Stoney, 1999; Stoney & Engebretson, 2000).

6.5.6 Limitations

6.5.6.1 Stress definition.

This study provides suggestive evidence but remains unable to confirm the benefits of multivitamin supplementation during stressful life events in the context of improved psychological, oxidative, and inflammatory states. There are several possible explanations for this.

Firstly, whether the sample was adequately stressed to be defined as a chronically stressed group is debatable. Screening and eligibility criteria used were based on previous

Oxidative Model literature (Hapuarachchi et al., 2003), specifically using the GHQ-12 measure. However the GHQ-12 wording was changed to inquire into well-being in the past month as opposed to the last 2-weeks. The impact of this change was not assessed but it is possible that the validity of these responses could be questionable. In light of the stressor taxonomy initially outlined (Segerstrom & Miller, 2004), it is likely that this sample of women were experiencing either 1) a brief-naturalistic stressor, when a person confronts a real-life short term challenge, i.e. academic examinations, 2) stressful event sequences, those based on a focal event and a related series of challenges, i.e. bereavement, or 3) a chronic stress scenario where stressors pervade a person's life forcing one to restructure their role or identity i.e. caring for a spouse with dementia. In a comprehensive meta-analysis (Segerstrom & Miller, 2004) these three stress experiences were found to elicit different immune changes.

For example a brief naturalistic stressor types was shown to reliably change the profile of cytokine production via a shift in T_H1-type and T_H2-type cytokines ratios. On the other hand, stressful event sequence type stressors did not elicit robust patterns of immune changes, and furthermore chronic stressors furthermore did not have any systematic relationship with studies of enumerative measures (simple counts of cells of different subtypes in the correct proportions) of the immune system but rather had systematic changes associated with functional measures (the ability of cells to perform activities). Potentially having differing stress experiences within one group could make distinguishing specific immune changes very complex. Only enumerative counts were used in the current study.

Secondly, participants taking part in this study may not have been 'chronically stressed' despite attempts to screen for this. Given that the sample for the current study were well-educated and had the time and inclination to volunteer to take part in a research project over a two-month period further supports this possibility. In adfition volunteers may have had adequate resources – emotional and social support to sustain them through a period of stress. The Oxidative Model relies on a chronic stress scenario. Yet the lack of clear definitions in previous Oxidative Model research makes it difficult to compare the stress experience across the various groups employed for these studies.

Furthermore the GHQ-12, the screening tool used to establish the stress sample for the current study, is a phenomenological measure. This means that it does not define what 'stress' is but relies on the participant filling in the questionnaire to interpret the meaning of stress from their own vantage point (their own experience and/or intuitive ideas). This would normally be sufficient however for Oxidative Model research this psychological measure has yielded disparate biomarker levels with moderate to severe stress levels in the current sample evidencing higher NT levels than low stress controls in previous research (Hapuarachchi et al., 2003).

Like the GHQ the extensive battery of self-report symptom-based scales are transparent and it is easy for a respondent to either disguise their symptoms (faking good or social desirability) or exaggerate their symptoms (faking bad or malingering). As the participants were aware of the nature of the research there may have been a level of exaggeration. Furthermore observed baseline NT levels (≈0.70) were comparable to both

a ‘normal’ stress scenario in past literature (Hapuarachchi et al., 2003) using the GHQ, and a high stress student sample (Blake-Mortimer et al., 1996). In addition the conventional dichotomous (0,0,1,1) scoring system, as was used in this study and by Happuarachchi and colleagues (2003), the response of ‘no more than usual’ to negatively-worded questions such as ‘been feeling unhappy or depressed’ is scored zero. This potentially rules out the chronicity of an individual’s experience of stress. Revised scoring of the GHQ to score negative items as 0,1,1,1 has been suggested to account for the presence of chronic problems rather than good health. This is the chronic –GHQ scoring method (C-GHQ). Studies have not always supported the superiority of the C-GHQ over the conventional scoring methods in reducing the number of false negatives (Piccinelli, Bisoffi, Bon, Cunico, & Tansella, 1993). Best results were observed by combining GHQ and C-GHQ case criteria and considering a respondent to be a ‘case’ according to either a GHQ ≥ 3 or a C-GHQ ≥ 5.

Defining the experience of stress is a difficulty in all PNI research as discussed in Chapter 1. In the current study this limits the ability to evaluate the impact of nutrients on biomarker levels. Careful consideration is required for future Oxidative Model research to find adequate psychometric tools for assessing ‘stress’ and to identify ‘casedness’. Currently The Oxidative Model attempts to remedy this by exploring a variety of psychological States and Traits that arbitrate the relationship between a stressor and an immune parameter. A consistent measure of stress across studies needs to be further investigated. Future research should ideally include a measure of perceived stress and additionally how one perceives their experience in relation to their peers.

6.5.6.2 Antioxidant contribution.

As discussed in section 6.5.3, the antioxidant contribution in this multivitamin supplement was not considered high in comparison to previous Oxidative Model studies. Recent research has suggested that VIT C may act as both a pro-oxidant and as an antioxidant in vivo (Bjelakovic, Nikolova, Gluud, Simonetti, & Gluud, 2007). Only biologically relevant doses of antioxidants in a healthy population should be considered (Chandra, 1992, 1999) and exceeding those levels commonly found in a balanced diet or above the recommended daily intake should be carefully monitored for adverse side effects. The difficulty lies in finding a balance.

6.5.6.3 Immune system adaptability.

It is feasible that in a healthy population The Oxidative Model may not be as relevant given that the homeostasis mechanisms of the body remain flexible and adaptive. This limits the generalisability of these findings to other populations.

In a healthy system chronic stress has a simultaneous enhancement and suppression of the immune response. As suggested by the Cytokine Shift Model (reviewed in section 1.3.5) it does this by altering patterns of cytokine secretion. Cytokines serve as chemical messengers within the immune system and across other systems including the endocrine and nervous systems. They are able to function to maintain homeostasis but also drive the inflammatory responses and hence have the ability to promote acute or chronic distress in specific tissue and organ systems (Coico et al., 2003). Thus in a normally functioning body the systems are capable of sufficient resource redistribution to cope

with perceived stressors. For example the organized pattern of suppression of cytokines implicated in a T_H1 but not T_H2 immune responses in healthy individuals undergoing brief stressors may reflect regulation in a healthy immune system (Segerstrom & Miller, 2004). The array of stressors experienced by this sample, according to the stressor taxonomy, made it difficult to define clear systematic changes even though a screening tool like the GHQ was employed specifically to nullify this.

6.5.6.4 Timeframe of biomarkers.

Shifts in biomarker levels, especially inflammatory meaaures, observed in this study should be viewed cautiously as enumerative measures only give a snapshot of broader patterns of change; measuring enumerative changes (increases and decreases) in all of these markers does not represent the whole picture. Whether these changes indicate compromised immune function is theoretically unclear, specifically as observed changes were relatively small for most biomarkers in the current study. This is a common criticism of enumerative PNI studies (S. Cohen & Herbert, 1996). Potentially enumerative and functional aspects of biomarkers deserve concurrent assessment, although time and resources often dictate this. It is challenging to form an accurate picture using biomarkers with different timeframes, like oxidative and inflammatory measures. make it difficult to form an accurate picture in a single blood sample. Enumerative measures do not give any insight into the functional ability of these markers within the body's systems. Furthermore biochemical mechanisms may not be linear. Future research should include a third data

collection point, like used in Study 1 of this dissertation, to assist further elaborate on the biomarker trajectories.

6.5.6.5 Changes in health behaviours.

Although health behaviors were assessed at pre-intervention, they were not re-assessed post-treatment. There is a possibility that over a two month period participants (un)consciously altered their patterns of behaviour with regard to medication use, exercise, smoking, and alcohol intake. These were identified as influential to several oxidative and inflammatory biomarkers. Lifestyle changes over the 8-week trial may have gone unaccounted for.

Similarly dietary changes, which contribute to antioxidant intake, were not sufficiently measured in the current study. Allergies or specialized dietary concerns were noted but in future Oxidative Model studies, food intake would need to be measured to account for VIT C, FOLATE, VIT B12 contributions. Food frequency questionnaires have been found to be useful for large scale epidemiological studies. They have proven to be a reliable means of assessing dietary antioxidant intake in large populations (McCarty, De Paola, Livingston, & Taylor, 1997). On a small scale over a short time frame (8-weeks) they appear to be relatively robust in a large sample (N>150)(Xinying, Noakes, & Keogh, 2004). Future research using the Oxidative Model should assess antioxidant intake via diet.

6.5.7 Future directions

6.5.7.1 Normative levels established.

This study has established normative levels of Oxidative Model biomarkers for a healthy sample of stressed women. This provides an excellent comparison for future research on this model. The current study is the largest single sample that The Oxidative Model has been applied to.

6.5.7.2 Covariate exploration.

Covariates were explored in this study in order to get a clearer picture of the mechanisms influencing biomarkers in The Oxidative Model. This is novel to Oxidative Model literature which has largely ignored these confounding variables. For example given the variability of NT levels across stress studies this further supports the need to account for influential health and demographic variables in order to attain a clearer picture of clinical implications.

Several variables integral to The Oxidative Model were observed to be influenced by demographics and health behaviors. Most notable, age was identified as a confounder for pro-oxidant measure, NT. This was anticipated as age dependency of NT had been established in much earlier research (Boss et al., 1980). Past research has suggested that NT activity begins to fall significantly after the age of 40. Although decreases were observed, given the relatively young sample and narrow age range of this sample (25-45 years) it was not anticipated that would be an influence. It was also observed that age was also a confounding variable across psychological measures, specifically.

Similarly the variable termed ‘medication use’ was identified as an influential confounder. Further exploration allowed identification of specific medication relevant to specific variables. Interestingly hormonal contraceptive use was associated with higher NT levels. This might partially be explained by younger women (<39) being more likely to be controlling their fertility. Antidepressant use was associated with higher cholesterol levels. Interestingly this supports the association of cardiovascular medication use with state depression scores. This may have been indicative of the influence of the condition linking poor health and depression therefore the need for antidepressants. Causality cannot be assumed.

For over two decades biochemical research on the fundamental markers in the Oxidative Model has acknowledged confounding variables, like age (Boss et al., 1980). However only one other unpublished dissertation attempted to control for this in statistical techniques. Although statistical power becomes an issue when we control for influencing health behaviors, as a result of doing so findings for the current study is robust and provides a template for future research design in larger samples.

6.5.7.3 Robust design.

This study was also novel in its field as it was a RCT as opposed to an observational study design. Furthermore a repeated measures design was incorporated. measures were assessed pre- and post-intervention providing additional robustness given that each participant acted as their own control across the duration of the study. Given the inter-individual variation observed across previous studies, this is a design which should be

employed for future Oxidative Model research. The small sample size meant Type II errors could be likely. Instead of simply relying on null hypothesis significance testing, effect sizes (partial eta squared [η^2] and phi coefficients [ϕ]) were also calculated to determine the magnitude of change over time.

The lack of associations of biomarkers and psychological variables in this study is likely due to a lack of variability, given this were a healthy sample of women. Past literature on the Oxidative Model (Blake-Mortimer et al., 1996) (Blake-Mortimer et al., 1998b; Hapuarachchi et al., 2003) has often relied on control groups. It is possible that this adds the variability needed to observe such associations.

6.5.7.4 Biologically relevant antioxidant levels.

Findings from the current study provide suggestive evidence for improvement in biomarkers, although this could not be fully accounted for by allocation to the Active group. Importantly the current study assessed the role of multivitamins at recommended daily intake levels as opposed to previous Oxidative Model literature which observed much higher levels(Blake-Mortimer et al., 1998b), or unspecified levels(Hapuarachchi et al., 2003), as discussed in section 6.5.6.2.

6.5.8 Conclusion

Based on the overall findings from the current study partial support has been observed for supplementation with multivitamin supplements for women experiencing moderate to severe stress. Psychological well-being improved for women in this study

was not constrained to those allocated to the Active group. Similarly physiological improvement was identified for pro-oxidant markers (NT, HCY, FOLATE, and VIT B12) although only FOLATE and VIT B12 could be attributed to membership to the Active group. Decreased pro-inflammatory levels could be attributed to allocation to the Active group as evidenced by lowered IL-1β, TNF-α, and TNF-β. This provides partial support for benefits of supplements during stress to avoid deleterious pro-oxidant and pro-inflammatory states. This study has added to The Oxidative Model, it provides useful design and statistical strategies for future investigations.

Chapter 7

General Discussion

7.1 Overview

This dissertation began with a review and critique of a psychoneuroimmunology (PNI: see section 1.1) to highlight some of the issues facing researchers in this area as they attempt to understand the complex processes between psychological well-being and physical health. These include inter-individual variability as well as difficulties categorizing and defining stress. A biopsychosocial model- The Oxidative Model- was introduced (see section 2.2). This theoretical model outlines an approach to stress and health, whereby the experience of sustained or chronic stress is linked to oxidative stress and inflammation within the body.

It is via these oxidative and inflammatory mechanisms after a sustained period of psychological stress (at least 6-8 weeks), that individuals become vulnerable to infection. If stress conditions are sustained for a longer period, the combination of oxidative stress and inflammation contributes to an individual's risk of certain types of disease such as arthritis, obesity, type 2 diabetes, and allergies {Osborne, 2003 #84}. The Oxidative Model proposes supplementation with multivitamins during periods of ongoing psychological strain could ameliorate oxidative and inflammatory damage. This proposition prior to this dissertation had yet to be formerly tested.

A novel chronic stress sample in which to test The Oxidative Model was proposed (see section 3.1). Breast cancer patients have been identified as a population experiencing psychological distress. This dissertation focused on the cessation of active treatment. For a subset of women this has been identified as a period of psychological strain predominantly manifesting as distress, poorer psychological adjustment, depression, and anxiety. This has been connected to the cessation of regular contact with their treatment team and fear of relapse. The period after cessation of treatment also has the added advantage that adjuvant treatments like chemotherapy and/or radiation have finished and disruption due to treatment on inflammatory and immune markers which we wished to measure have had time to stabilise.

Any kind of 'immunology' or physiological marker research, like The Oxidative Model, becomes increasingly difficult to do with patients undergoing these types of treatments. In addition the inclusion of interventions, like vitamin supplementation, during adjuvant treatments has the potential to negatively interact with adjuvant treatments. Breast cancer patients in the post-treatment period were investigated using The Oxidative Model with the idea that the more quickly breast cancer patients can resume good levels of oxidative, inflammatory, and psychological well-being post-treatment, the less risk of further illness such as infection occurring.

This was a longitudinal, observational study. Primarily of interest was whether women in the post-treatment period were experiencing psychological distress, oxidative stress and heightened pro-inflammatory states and, importantly, did this change across

time. Following this study an intervention study of vitamin supplementation during periods of chronic stress was completed to test assumptions of The Oxidative Model (Chapter 6).

7.2 Breast Cancer Patients Post-treatment

For the majority of women treated for breast cancer, the period following the cessation of active treatment (chemotherapy and/or radiation) was not one of sustained psychological strain. This was observed across the spectrum of measures, from general measures (i.e., GHQ-12, Spielberger's STPI, UCLA loneliness) to cancer-specific measures (IES-R, MAC). However for a subset of women, high levels of psychological distress, as measured by the GHQ-12 and cancer-specific stress (IES-R), were observed. This sustained distress experienced by a subset of women is reflected in previous findings (Bleiker et al., 2000; Cordova et al., 1995; Costanzo et al., 2007; B.L. Green et al., 1996) . In this study this finding was purely observational, based on comparisons with normative data. This would have been strengthened had an age-matched control group been assessed.

7.2.1 Evidence of oxidative stress and inflammation.

Despite this apparent lack of distress across women 4-weeks post-treatment, evidence of oxidative stress and inflammation were apparent. Specifically levels of NT and VIT C were observed similar to mean levels observed in a chronically depressed sample (Blake-Mortimer et al., 1996)and HCY levels comparable to workers experiencing occupational strain (Hapuarachchi et al., 2003). This is in spite of this sample having

normal serum nutrient levels of VIT B12 and FOLATE. Levels of these nutrients reflect those of normal 'healthy' populations, well within the normal reference ranges. In contrast minimal inflammation was observed as suggested by low cytokine levels; however CRP levels - a marker of inflammation- were moderate to high.

Individual trajectories for psychological distress might in part explain the lack of statistical findings for mean change in measures of oxidative stress and inflammation for this sample. Thornton and colleagues (2007) tracked early stage breast cancer patients' experience of stress (subjective and emotional) along the treatment/post-treatment continuum. They observed perceived stress at baseline to lead to different trajectories for the proliferative responses of immune cells up to 18-months later. Trajectories in the current sample also appeared to be varied. Reasons for this variability could not be discerned by the self-report questionnaire responses alone. Sources associated with distress- like the experience of more health complaints, having an illnesses other than cancer, and symptom distress during the post-treatment period were not explored in this study, and may have proved useful as has been suggested (Bleiker et al., 2000; Mast, 1998). Future research would ideally use a mixed methods approach incorporating interviews, in a larger sample order to clarify sources of increased/decreased distress.

7.2.2 Curiosity, depression, oxidative stress, and inflammation.

Subscales of Spielberger's personality inventory (Spielberger, 1996) yielded several relevant correlations supporting Oxidative Model patterns. For breast cancer patients, post-treatment depression was associated with poorer (lower) NT levels. In contrast,

increased curiosity was observed with improved (higher) NT levels. This dichotomy suggests positive psychological mood states and cognitions to have just as much influence on oxidative stress as negative mood. This has been reflected in one other unpublished dissertation (Oliver, 2004). The experience of curiosity has been suggested as a potential emotional vital sign (Spielberger & Reheiser, 2009). Specifically as a motivator of exploratory behavior, curiosity often contributes to effective personal adjustment and successful adaptation to environmental stimuli.

This is one of the first studies to explore relationships between psychological measures of distress and inflammatory cytokines in the post-treatment period. At the time of this dissertation, no literature existed specifically of curiosity levels in breast cancer patients in the post-treatment period. However other positive states and traits have been explored. For instance one study suggests enhancing optimism in post-operative breast cancer patients has been linked to optimal immune function during treatment (Ah, Kang, & Carpenter, 2007). In the current study lower levels of the inflammatory cytokine, TNF-β, were also associated with higher curiosity, suggesting a decreased pro-inflammatory response with this personality characteristic. On the other hand, the psychological response to cancer of Helpless/Hopeless coping was linked to heightened inflammatory responses as expressed through increased IL-1β levels. Research on inflammatory cytokines during the breast cancer diagnostic phase has observed heightened levels of TNF-α to be associated with more psychological distress as well as symptom distress (DeKeyser et al., 1998).

The association of depression with lowered NT indicates greater oxidative stress and is in line with previous finding by Blake-Mortimer and colleagues of a clinically depressed sample (1996). In breast cancer patients post-treatment, Deshields and colleagues (2005) observed improved rates of depression to occur over time. Despite this improvement, approximately 25% continued to score above the clinical cut-off over a 6-month period reflecting depressive symptoms had not been resolved. Bleiker and colleagues (2000) suggest that personality characteristics, like anxiety are the best predictors of psychological distress in the post-treatment period; large associations were evidenced supporting this with anxiety associated with oxidative (lower NT) measures in this sample.

The correlations discussed in this dissertation reflect previous Oxidative Model findings but being correlations must be interpreted cautiously and causality not assumed. In addition the number of variables in the matrices and subsequent number of correlations is subject to the threat of Type I error. Little or no association was found between psychological measures and biomarkers of a pro-oxidant or pro-inflammatory state for women experiencing chronic stress recruited for the RCT. It is possible that the lack of association could be attributed to lack of variability in psychological measures and biochemical measures. However it is feasible that The Oxidative Model is more relevant in disease- challenged samples like the post-treatment cancer patients in the current study. This is reflected in the founding Oxidative Model studies of newly diagnosed HIV patients (Chalmers & Hare, 1990) or in clinical samples of patients with diagnosed major

depressive disorder (Blake-Mortimer et al., 1996) or post traumatic stress disorder (Jolly, 2004; Pfitzer, 2008).

7.3 Testing the Oxidative Model

This dissertation focused on testing The Oxidative Model. This was in two stages, firstly to assess its applicability to a sample of post-treatment, breast cancer patients. The second phase was to test the role of vitamin supplementation during periods of distress in preventing exacerbation of oxidative and inflammatory processes. An overarching issue which covered both investigations was the influence of confounding variables on measures used in The Oxidative Model.

7.3.1 Covariate exploration.

Past Oxidative Model research has largely ignored the potential influence of confounding variables despite there being evidence in the literature of the influence of age(Boss et al., 1980), vitamin use (Hapuarachchi et al., 2003), and smoking (Lesgards et al., 2002) . Studies in this dissertation were mindful of this weakness and set out to explore these potential covariates (Table 65) prior to analyses. Additionally the study of early-stage breast cancer patients post treatment took into account additional treatment variables (surgery, chemotherapy, radiation and ongoing hormone therapies and medication use).

Table 65: Covariate Exploration for Studies of Post-Treatment Breast Cancer Patients, and Healthy Women Experiencing Stress

	Covariate Type	Variable	Psychological Variables	Pro-oxidant Biomarkers	Pro-inflammatory Biomarkers
Study 1	Behavioural	Alcohol use	↑ S-depression, ↑ Avoidant Coping		
Breast Cancer Patients		Vitamin Use		↓ HCY	
	Demographic	Age			↓ Il-5, ↓TNF-β
	Medication	Endocrine	↑ S-depression		↑ Il-5, ↓TNF-β
		Cardiovascular	↑ Fighting Spirit	↓ FOLATE	
		Respiratory		↑ NT	
	Treatment	Herceptin	↑ Fighting Spirit		
		Chemotherapy	↑ Distress, ↓ S-anger		
Study 2	Behavioural	Exercise			↓IL-5, IL-6
Healthy women		Alcohol Use			↑ IL-5
	Demographic	Age	↑ loneliness	↓ NT, ↑ TAS,	
	Medication	Cardiovascular	↑ S-anger		
		Respiratory	↓ S-curiosity	↑ CHOL	
		Antidepressant		↑ CHOL	↑IFN-γ

Note. Biomarker Abbreviations: 5' –ectonucleotidase (NT), tissue ascorbate (VIT C), total antioxidant status (TAS), homocysteine (HCY), Folate (FOLATE), Cholesterol (CHOL), Interferon-γ (IFN-γ), Interleukin-5 (IL-5), Interleukin-6 (IL-6), Tumor necrosis factor- β (TNF-β).

It was anticipated that behavioural and health variables would largely influence biomarkers, for example the influence of increased age depleting NT levels was predicted (Boss et al., 1980). However psychological variables showed evidence of confounding by health behaviours including alcohol, medication and vitamin use, as well as exercise levels. Inflammatory cytokines also evidenced influence from confounders. These variables considered a 'nuisance' to analysis of the dependent variables reveal just how sensitive many of the biomarkers employed in the Model are. Future research in this area should continue to explore the influence of these confounders. The physical and psychological benefits of health behaviours in post-treatment cancer samples provide some ideas for future interventions to improve patient health and well-being.

7.3.2 Recruitment for a randomised controlled trial.

A number of recruitment considerations were revealed from the breast cancer study. Firstly limited stress was observed in this sample. Secondly vitamin usage in this sample was already high and created an ethical complexity in proposing a blinded RCT where participants would be asked to take a placebo. Thirdly slow patient accrual, paired with the resource intensive data collection and biochemical assays was not ideal for collecting a sufficient sample in a reasonable timeframe. A relevant and readily available population was sought to complete a RCT to test The Oxidative Model's key proposition- that vitamin supplementation would improve the oxidative and inflammatory impact of chronic stress.

The findings from the breast cancer study lead to the question of why there was such a disparity between oxidative stress markers and nutrients supposedly meant to alleviate this physiological state. The Oxidative Model suggests that the body utilizes available nutrients to remedy or ameliorate pro-oxidant states like low NT, high HCY and high CRP. Observed nutrient levels in this sample (VIT B12 and FOLATE) should have been sufficient to reinstate the internal oxidative balance in this sample. Despite this, oxidative stress and inflammation were evident. It is a plausible hypothesis that this was due to persistent treatment side-effects.

This finding did not provide support for a vitamin supplementation intervention based on The Oxidative Model's propositions. Additional multivitamins for this particular sample would be superfluous as the observed serum levels should have been sufficient, given they were well within normal reference ranges, even approaching the high end. Furthermore and excess nutrients can be detrimental (Chandra, 1992, 1999). In addition 60% women in this sample were already taking a dietary supplement at the time of the study. This would account for the serum nutrient levels observed. However despite the observation of sufficient nutrient levels, oxidative and inflammatory processes dominated. A controlled evaluation of vitamin supplementation during chronic stress was undertaken in a general population sample in order to test the Model further.

7.3.3 Vitamin supplementation during periods of psychological distress.

Another principal aim of this dissertation was to test the role of vitamin supplementation in a chronically stressed sample. In particular, whether providing

sufficient nutrient and antioxidants during periods of stress counteract the pro-oxidant pro-inflammatory processes as The Oxidative Model suggests was explored. For the general population sample it was difficult to assess the benefits of vitamin supplementation during stress as the sample were best described as a healthy, well-nourished group with no nutrient deficiencies. Subsequently the findings from the RCT in a sample of 'stressed' women were not conclusive although provided some support for vitamin supplementation in the context of The Oxidative Model.

Findings suggest some benefit from supplementation as observed across oxidative biomarkers, with large effect sizes suggesting improved HCY levels for those participants in the vitamin group. However the primary Oxidative Model biomarker NT improved for all participants regardless of group allocation, either with the course of time or some other mechanism (i.e., Placebo or Hawthorne effects).

This dissertation highlighted NT to remain the most reliable marker of psychological distress in The Oxidative Model, similar to previous research by Happuarachchi and colleagues (2003). In both the breast cancer and general population samples it yielded several associations in the expected directions. Ideally biomarkers should be highly reproducible and measureable (i.e. display change). High test-retest reliability is desirable because it indicates that the biomarker measurement is likely to be measuring an actual phenomenon (i.e., the impact of chronic stress), rather than other confounding processes. NT meets these criteria as it is a marker which takes 6-8 weeks to decline/increase.

In contrast, inflammatory cytokines are incredibly volatile and fluctuate over a matter of hours. Lower inflammatory cytokine levels were observed for those participants in the Active group. Confounding processes, like an acute stress response, influence inflammatory variables more so than oxidative measures, like NT. Ideally biomarkers should also serve as a marker of illness/infection. The findings from this study wrongly assume oxidative and inflammatory mechanisms to be detrimental. In an Allostatic Load Model the body shifts resources from one mechanism to another. For example in the face of depleted acquired immune function innate responses increase. These are often more inflammatory states, but whether this shift translates to increased illness/infection or stress-related disease was not explored sufficiently in the current study. Suggestive findings around positive mood states like curiosity is a definite future direction for the Model given the observed patterns with NT. PNI research often looks at the negative implications of stress. Of interest who is doing well under 'stress' and what it is that makes them more resilient could be the next step in Oxidative Model research.

7.3.4 Allostatic load.

The general population sample was considered to have high levels of distress but biomarker levels were not suggestive of significant oxidative stress or inflammation. The breast cancer sample, in contrast, was considered to have a low levels of psychological distress but clearly had evidence of oxidative stress (low NT, VIT C, and high HCY) and inflammation (moderate to high CRP) which did not evidence improvement over the 6-

month post-treatment period. The disparity between these samples is counterintuitive yet compelling in the context of The Oxidative Model.

Women comprising the breast cancer sample were not experiencing high levels of psychological distress. However there was evidence of increased oxidative and inflammatory challenge. This is counterintuitive to The Oxidative Model propositions where higher levels of stress are associated with pro-oxidant and pro-inflammatory states. Biomarkers indicating oxidative stress (low NT, VIT C, and high HCY) and inflammation (moderate CRP) remained over the 6-month period. These two findings together suggest residual side-effects of treatment rather than chronic distress was influential over the 6-months post-treatment, as previously mentioned. It is also feasible that this is a result of Allostatic Load. As discussed at the outset of this dissertation (section 1.3.6), Allostasis is the process whereby an organism maintains physiological stability by changing parameters of its internal state to match environmental demands (Juster et al., 2010; McEwen, 1998b), where stability is maintained through change. Allostatic Load was proposed by McEwen (1998) and refers to the state where normal processes for maintaining the body's internal balance fail to disengage/shut off. Importantly if components of any of these systems (i.e. immune, oxidative, inflammatory) are out of balance (i.e. due to chronic stress or residual treatment side-effects) an Allostatic state results.

Despite the availability of sufficient serum nutrient levels (VIT B12, and FOLATE) plus the passage of time (6-months) this was not substantial in the breast cancer sample

to rectify the oxidative balance. This suggests the Allostatic Load for this population is significant. This approach proposes that when physiological systems are under repeated stress over time (environmental, physical, and psychosocial) and unable to adapt (Seeman et al., 2001), the patterns of physiological response remain at heightened level (e.g. inflammatory processes). It is thought that without sufficient recovery, often compensatory mechanisms are activated (innate vs. acquired immunity).

This model suggests that with frequent chronic challenge, dysregulation is evidenced across several major physiological systems including the HPA axis, sympathetic nervous system & immune function (Schulkin, 2004). Repeated cumulative activation over time is what leads to Allostatic Load and has been associated with neural, endocrine & immune stress mediators which are key in various organ diseases (McEwen, 1998a, 1998b). This is what is likely to be occurring in the breast cancer sample post-treatment. Some examples of allostatic states include chronic hypertension, flattened cortisol rhythms in major depression, and sustained elevation of inflammatory cytokines accompanied by low cortisol in chronic fatigue syndrome (McEwen, 2005).

This Allostatic Load framework is further supported given the observed ease by which the healthy general population 'stressed' samples oxidative and inflammatory measures showed improvement over a short period (8-weeks) despite already being within normal reference ranges pre-intervention. In addition improvement was often seen across both groups only slightly favouring the Active group. The Oxidative Model has largely been tested in healthy samples (students and academic staff). The mechanisms

might be quite different in a diseased population or in this case, people recuperating from a significant illness like breast cancer. The immune system in healthy samples is robust to fluctuations in stress and is protected by efficient homeostatic systems that evidence plasticity and flexibility. Allostatic states have the capacity to cause wear and tear on regulatory systems throughout the brain and body. Therefore during recovering from breast cancer or other significant illnesses, The Oxidative Model is highly relevant and a measure of how well the body is restoring to some level of internal oxidative and inflammatory balance. How long this process takes remains unclear but findings from this dissertation suggest longer than 6-months following the cessation of adjuvant treatment.

7.4 Strengths

Based on the review and critique of Oxidative Model literature (section2.3) this dissertation identified and made several steps to rectify limitations observed in previous research. Firstly the sample employed for the RCT is the largest sample based on The Oxidative Model framework to date. Like a lot of PNI literature, Oxidative model research is commonly limited to small samples. Every effort has been made in this dissertation to account for this by the reporting of effect sizes as well as p -values. The use of statistical significance testing to evaluate both immune and psychological change provides no information on the variability of changes within the sample. The existence of statistical change is often unrelated to the clinical importance of changes in these measures (Jacobson & Traux, 1991). For example, knowing the clinical relevance of increasing

biomarkers, like NT or HCY, by a certain % and this changing infection rates would help to plan future sample sizes to effectively test the model.

For this reason this dissertation endeavoured to report Reliable Change Indices (RCI). The RCI proposes that pre-test scores from scales be subtracted from their post-test scores, then the difference divided by standard error. Although this approach works for standardized psychological measures, there are a number of challenges in applying this technique to more novel biomarkers. Firstly, it relies on a scale with standardized items. Although this would be sufficient for well-known psychological measures of emotion, stress and well-being, it is not appropriate for single item measures like biomarkers. The Oxidative Model does, however, propose an assortment of biomarkers implicated in oxidative stress. It is not out of line to envision these as part of a future scale of oxidative stress, i.e. Oxidative Stress= [HCY + C-RP + NT - (VITC + VIT B12)]. In this way, pre-test and post-test scores could be displayed in a scatter plot giving further interpretation of improvement (above the line) or deterioration (below the line), as well as reliability (with outliers easy to pick)(Jacobsen & Traux, 1991).

Secondly, both studies in this dissertation are the first to employ samples of the same sex, specifically women only in order to avoid differences in stress-responses between men and women. For instance it has been argued that the physiology of the stress-response can be quite different in females, who are typically less aggressive, and having dependent young often precludes the option of fight-or flight (Taylor et al., 2000). Hormonal differences between men and women (i.e., oestrogen and testosterone levels)

and the influence these may have on biomarkers implicated in the Model is another reason for studying same-sex samples.

Both studies in this dissertation have used repeated measures assessment. Previous research suggested that multiple assessments of the same individuals yield the most informative results (Arthurson, 2003; Blake-Mortimer et al., 1996) and control for inter-individual variability as opposed to cross-sectional cohorts (Hapuarachchi et al., 2003; Jolly, 2004; Pfitzer, 2008). Studies in this dissertation employed repeated-measure methods. These consisted of 3 assessments at 8-week intervals for the breast cancer observational study, and 2 assessments, pre- and post-intervention (8-weeks apart) for the general population RCT.

PNI research which has revealed significant links between psychological and immune changes have largely relied on comparisons of stressed samples to control groups (Segerstrom & Miller, 2004). Similarly, significant results, based on markers implicated in the Oxidative Model have also relied on this technique. This dissertation opted not to employ healthy controls for either study as comparison groups. As there is great 'inter-individual' differences, psychological and immune/biochemical change should be considered at the level of the individual (Segerstrom, 2003). This has by far a greater chance of elucidating underlying mechanisms and in the case of The Oxidative Model might better untangle some of the cause of the disparity between previous studies.

The use of covariates was considered a strength of this dissertation and has added much to understanding the role of demographic and health behavior variables in the

context of The Oxidative Model. Covariates are required to be measures a priori, i.e., before treatment or intervention. This protocol was followed for both studies. Demographics, health behaviours, and treatment variables were collected prior to participants commencing each study. It is highly likely that across the course of each of the projects, participants changed on a number of these variables (exercise, complementary and alternative medicine use, medication use, smoking, alcohol, etc). For example in the case of the breast cancer sample, as women recovered from the treatment side-effects exercise levels might increase. Similarly the RCT general population sample may have altered health behaviours in response to taking part in this research project. Future research methods using The Oxidative Model should take changes across time in health and behavior variables into consideration.

7.5 Limitations

The main limitation is that this dissertation is based on two relatively underpowered studies. Like many PNI studies, the resource intensive requirements are a constant challenge. Despite this, this dissertation has endeavoured to collect, assay and collate biochemical and psychological measures under a broader theoretical framework- The Oxidative Model. A key challenge at the outset was to identify a 'chronic stress' sample. Two attempts were made. The first, by identifying a potential chronic stressor or stressful event sequence using the taxonomy outlined (Elliot & Eisdorfer, 1982), specifically breast cancer patients in the post-treatment period. The breast cancer sample was not screened for distress at the outset, and subsequent assessments indicated the

majority were not experiencing high levels of distress. The second attempt to identify a 'chronic stress' sample followed criteria outlined in by Happuarachchi, and colleagues (2003). Prior to enrolment in the study all participants were screened for baseline distress as part of the eligibility criteria. This attained a sample experiencing moderate to severe psychological distress in the past month, as measured by the GHQ-12.

Despite the measure of psychological distress (GHQ-12) previously proving useful at determining stress-related oxidative stress (Hapuarachchi et al., 2003; Oliver, 2004; Pfitzer, 2008), this was not the case in this dissertation. Self-report measures of psychological distress for future Oxidative Model research require careful consideration. The breast cancer patients post-treatment were much worse off with regard to oxidative and inflammatory measures than the healthy sample who reported sustained stress for a month preceding baseline assessment. A combination of identifying a chronic stressor or stressful event sequence, plus screening for distress as an eligibility criteria could be the key in establishing a sufficient 'stress' sample. An objective evaluation of the stressor plus the subjective evaluation by self-report and/or interview is recommended for future Oxidative Model research.

It is clear that the breast cancer sample showed some supportive correlations in expected directions proposed by The Oxidative Model. However the general population 'stressed' sample did not yield any correlations in support of Oxidative Model mechanisms. Screening criteria at the outset for participants experiencing moderate to severe levels of stress at baseline is thought to have removed the variability necessary for

finding correlations with biomarkers. The high-functioning, healthy, age restricted range of the general population sample meant that psychological measures had to be associated with very small amounts of variance in biomarker levels; a challenging task even for the most reliable of measures.

7.6 Implications of this research

Although the current breast cancer sample was not ideally a chronic stress sample, observing this group using the Oxidative model has been informative. The observational study of breast cancer patients adds to the growing body of PNI research employing The Oxidative Model framework. It provides a thorough and comprehensive picture of the psychological, pro-oxidant and pro-inflammatory journey across the 6-month post-treatment period. It explored descriptively as well as statistically, the influence of treatment, demographic, and health behaviours on psychological and immunological measures. Health care professionals working with early stage breast cancer patients would be well advised to identify patients experiencing distress during treatment, as it remains influential for a subset of patients post-treatment.

With regard to The Oxidative Model, manifestations of distress, like depression, are implicated and have been shown to be associated with lower NT levels in the post-treatment period. Lowered NT is considered an indicator of increased susceptibility to infection due to its role as a maturational enzyme for lymphocytes (Bastian et al., 1984). Given poor health and illness other than cancer in the post-treatment period is linked with increased emotional distress (Bleiker et al., 2000; Mast, 1998), NT offers a plausible

mechanism underlying this observation, which remains to be explored. It is important that individuals experiencing psychological and physiological strain are identified as soon as possible as they are in a physiologically vulnerable period.

The findings from this research should also prompt health care professionals to have a dialogue with patients around vitamin consumption, and other alternative therapies. A majority (66%) of this small sample was taking some sort of dietary supplement. There is suggestion that more engagement with CAM therapies is associated with more psychological distress. Burstein and colleagues (1999) observed women who begin to use dietary supplements after receiving a diagnosis of breast cancer were experiencing greater distress 3 -months after diagnosis, and greater fears of recurrence 12-months post-diagnosis. This was in comparison to regular dietary supplement takers irrespective of a cancer diagnosis. It was unclear in this study whether the breast cancer sample employed for this dissertation had started using supplements in response to their positive diagnosis with breast cancer.

This dissertation is the first attempt at a confirmatory analysis using The Oxidative Model Framework to evaluate the role of vitamin supplementation during periods of chronic stress. Speculation around the potential benefits of multivitamin supplements high in antioxidants influencing oxidative measures have been proposed over the past 15 years (Blake-Mortimer et al., 1996, 1998b; Hapuarachchi et al., 2003). In the general population study, those allocated to an Active group had suggestive improvements in HCY and NT, paired with significantly lower inflammatory cytokine levels

than those in the Placebo group after an 8-week intervention. This shows partial support for the Model. Similarly regular vitamin-taking was identified as a covariate for HCY levels in the sample of breast cancer patients post-treatment. Both these findings suggest mediating effects of taking multivitamins on Oxidative Model biomarkers. Yet a definitive causal relationship between multivitamin supplementation and improved oxidative states during psychological distress remains unproven. This thesis brings out the case for careful exploration of complementary interventions, like multivitamin use, in the post-treatment period for breast cancer patients experiencing ongoing distress.

References

Abrams, D. B., & Niaura, R. S. (1987). Social Learning Theory. In H. T. Blane & K. E. Leonard (Eds.), *Psychological theories of drinking and alcoholism* (pp. 131-178). New York: Guilford Press.

Ah, D. V., Kang, D.-H., & Carpenter, J. S. (2007). Stress, optimism, and social support: Impact on immune responses in breast cancer. *Research in Nursing & Health, 30*(1), 72-83.

AIHW. (2006). *Breast cancer in Australia: an overview, 2006*. Canberra: National Breast Cancer Centre.

AIHW. (2009). *Breast cancer in Australia: an overview, 2009*. Canberra, Australia.

Anderson, B. L., Farrar, W. B., Golden-Kruetz, D., Kutz, L. A., McCullum, R., Courtney, M. E., et al. (1998). Stress and immune responses after surgical treatment for regional breast cancer. *Journal of the National Cancer Institute, 90*(1), 30-62.

Anderson, B. L., Farrar, W. B., Golden-Kruetz, D. M., Glaser, R., Emery, C. F., Crespin, T. R., et al. (2004). Psychological, behavioural, and immune changes after a psychological intervention: a clinical trial. *Journal of Clinical Oncology, 22*(17), 3570-3580.

Anderson, B. L., Kiegolt-Glaser, J. K., & Glaser, R. (1994). A biobehavioral model of cancer stress and disease course. *Am Psychol, 49*, 389-404.

Andrykowski, M. A., & Cordova, M. J. (1998). Factors Associated with PTSD Symptoms Following Treatment for Breast Cancer: Test of the Anderson Model. *Journal of Traumatic Stress, 11*(2), 189-203.

Andrykowski, M. A., Cordova, M. J., McGrath, P. C., Sloan, D. A., & Kenady, D. E. (2000). Stability and change in posttraumatic stress disorder symptoms following breast cancer treatment: a 1-year follow-up. *Psycho oncology, 9*, 69-78.

Arora, N. K., Gustafson, D. H., Hawkins, R. P., McTavish, F., Cella, D. F., Pingree, S., et al. (2001). Impact of surgery and chemotherapy on the quality of life of younger women with breast carcinoma: a prospective study. *Cancer, 92*(5), 1288-1298.

Arthurson, C. (2003). *The relationship between psychological distress and biochemical markers in students undergoing exams.* Unpublished Honours, The University of Adelaide, Adelaide.

Babor, T. F., Higgins-Biddle, J. C., Saunders, J. B., & Monteiro, M. G. (2001). *The Alcohol Use Disorders Identification Test: guidelines for use in primary care* (Second Edition ed.): (Second edition): WHO.

Balkwill, F., & Mantovani, A. (2001). Inflammation and Cancer; back to Virchow? *Lancet, 357*, 539-545.

Barnes, P. F., Abrams, J. S., Lu, S., Sieling, P. A., Rea, T. H., & Modlin, R. L. (1993). Patterns of cytokine production by mycobacterium-reactive human T-cell clones. *Infect Immun, 61*(1), 197- 203.

Bastian, J. F., Ruedi, J. M., MacPherson, G. A., Golembesky, H. E., O'Connor, R. D., & Thompson, L. F. (1984). Lymphocyte ecto-5'-nucleotidase activity in infancy: increasing activity in peripheral blood B cells precedes their ability to synthesize IgG in vitro. *Journal of Immunology, 132*(4), 1767- 1772.

Bjelakovic, G., Nikolova, D., Gluud, L. L., Simonetti, R. G., & Gluud, C. (2007). Mortality in Randomized Trials of Antioxidant Supplements for Primary and Secondary Prevention. *Journal of The American Medical Association, 297*(8), 842-857.

Blake-Mortimer, J. S., Winefield, A. H., & Chalmers, A. H. (1996). The Relationship between Psychological stress and Lymphocyte 5'-ectonucleotidase. *International Journal of Stress Management, 3*(4).

Blake-Mortimer, J. S., Winefield, A. H., & Chalmers, A. H. (1998a). The effect of depression in an animal model on 5'-ectonucleotidase, antibody production and tissue ascorbate. *Journal of General Psychology, 125*(2), 129-146.

Blake-Mortimer, J. S., Winefield, A. H., & Chalmers, A. H. (1998b). Evidence for Free-radical mediated reduction of lymphocytic 5'-ectonucleotidase during stress. *Human Sciences press, 1998*.

Bleiker, E. M., Pouwer, F., van der Ploeg, H. M., Leer, J. W., & Ader, H. J. (2000). Psychological distress two years after diagnosis of breast cancer: Frequency and Prediction. *Patient Educ Couns, 40*, 209-217.

Boss, G. R., Thompson, L. F., Spielberg, H. L., Pichler, W. J., & Seegmiller, J. E. (1980). Age dependency of lymphocyte ecto-5'-nucleotidase activity. *Journal of Immunology, 125* (679-682.).

Bovbjerg, D. H., & Valdimarsdottir, H. B. (1998). Psychoneuroimmunology: Implications for Psycho-oncology. In J. C. Holland (Ed.), *Psycho-Oncology*. New York: Oxford University Press.

Brady, M. J., Cella, D. F., & Mo, F. (1997). Reliability and Validity of the Functional Assessment of Cancer Therapy- Breast Quality of life Instrument. *Journal of Clinical Oncology, 15*, 974-986.

Brenner, H., & Hakulinen, T. (2004). Are patients with breast cancer before age 50 years ever cured? *J Clin Oncol, 22*(3), 432-438.

Broadhead, W. E., Gehlbach, S. H., de Gruy, F. V., & Kaplan, B. H. (1988). The Duke-UNC Functional Social Support Questionnaire. Measurement of social support in family medicine patients. *Medical Care, 26*(7), 709-723.

Burstein, H. J., Gelber, S., Guadagnoli, E., & Weeks, J. C. (1999). Use of alternative medicine by women with early-stage breast cancer. *N Engl J Med, 340*(22), 1733-1739.

Campbell, D., & Stanley, J. (1963). *Experimental and quasi-experimental designs for research*. Chicago, IL: Rand-McNally.

Carlson, E. D., & Chamberlain, R. M. (2005). Allostatic load and health disparities: a theoretical orientation. *Research in Nursing & Health, 28*, 306-315.

Cella, D. F., Fallowfield, L. J., Barker, P., Cuzick, J., Locker, G., & Howell, A. (2006). Quality of life of postmenopausal women in the ATAC (armiadex, tamoxifen, alone or in combination) trial after completion of 5 years adjuvant treatment for early stage breast cancer. *Breast Cancer Research and Treatment, 100*, 273-284.

Chalmers, A. H., & Hare, C. (1990). A semi-automated method for 5'-ectonucleotidase measurement in lymphocytes. *Immunological cell biology*(68), 75-79.

Chalmers, A. H., Hare, C., Woolley, G., & Frazer, I. H. (1990). Lymphocyte ectoenzyme activity compared in healthy persons and patients seropositive to or at high risk of HIV infection. *Immunol Cell Biol, 68*, 81-85.

Chalmers, A. H., Winefield, A. H., & Blake-Mortimer, J. S. (2003). The pro-oxidant state and psychological stress. *Environmental Health Perspectives, 11*(1).

Chandra, R. K. (1992). Effect of vitamin and trace-element supplementation on immune responses and infection in elderly subjects. *Lancet, 340*(8828), 1124-1127.

Chandra, R. K. (1999). *Nutrition and immunology: from the clinic to cellular biology and back again.* Paper presented at the Nutrition Society and the Nutritional Immunology Affinity Group of the British Society for Immunology, Harrogate.

Chiappelli, F., Manfrini, E., Franceschi, C., Cossarizza, A., & Black, K. L. (1994). Steroid regulation of cytokines: Relevance for Th1 and Th2 shift? In E. R. de Kloet, E. C. Azmitia & P. W. Landfields (Eds.), *Annals of the New York Academy of Sciences: Vol. 746. Brain corticosteroid receptors: Studies on the mechanism, function, and neurotoxicity of corticosteroid action* (Vol. 746, pp. 204-215). New York: New York Academy of Sciences.

Cohen, J. (1988). *Statistical power analysis for the behavioral sciences* (2nd Ed.). Hillsdale, NJ: Lawrence Erlbaum Associates.

Cohen, S., & Herbert, T. B. (1996). Health Psychology: Psychological Factors and Physical Disease from the Perspective of Human Psychoneuroimmunology. *Annual Review of Psychology, 47*, 113-142.

Cohen, S., & Williamson, G. M. (1983). A global measure of perceived stress. *Journal of Health and Social Behavior, 24*, 385-396.

Coico, R., Sunshine, G., & Benjamini, E. (2003). *Immunology: A Short Course. Fifth Edition*: John Wiley & Sons, Inc.

Cordova, M. J., & Andrykowski, A. M. (2003). Response to cancer diagnosis and treatment: posttraumatic stress and posttraumatic growth. *Semin Clin Neuropsychiatry, 8*(4), 286-296.

Cordova, M. J., Andrykowski, A. M., Kenady, D. E., McGrath, P. C., Sloan, D. A., & Redd, W. H. (1995). Frequency and Correlates of Post Traumatic Stress Disorder like Symptoms after treatment for Breast Cancer. *Journal of Consulting and Clinical Psychology, 63*, 981-986.

Costanzo, E. S., Lutgendorf, S. K., Mattes, M. L., Trehan, S., Robinson, C. B., Tewfik, F., et al. (2007). Adjusting to life after treatment: distress and quality of life following treatment for breast cancer. *British Journal of Cancer, 97*, 1625-1631.

Coster, S., & Fallowfield, L. J. (2002). The impact of endocrine therapy on patients with breast cancer: a review of the literature. *The Breast, 11*(Special Article), 1-12.

Coussens, L. M., & Werb, Z. (2002). Inflammation and Cancer. *Nature, 420*, 860-867.

Danner, M., Kasl, S. V., Abramson, J., & Vaccarino, V. (2003). Association Between Depression and Elevated C-Reactive Protein. *Psychosomatic Medicine, 65*, 347-356.

De Haes, J., Van Knippenberg, F., & Nejit, J. (1991). Measuring Psychological and Physical Distress in Cancer Patients: Structure and Application of the Rotterdam Checklist. *British Journal of Cancer, 64*, 353-356.

Dean, C., & Surtees, P. G. (1989). Do psychological factors predict survival in breast cancer? *Psychosom Res, 33*, 561-569.

DeKeyser, F. G., Wainstock, J. M., Rose, L., Converse, P. J., & Dooley, W. (1998). Distress, symptom distress, and immune function in women with suspected breast cancer. *Oncological Nursing Forum, 25*(8), 1415-1422.

Derogatis, L. R., & Spencer, P. M. (1982). The Brief Symptom Inventory (BSI). In *Clinical Psychometric Research*. Baltimore, MD.

Deshields, T., Tibbs, T., Fan, M., Bayer, L., Taylor, M., & Fisher, E. (2005). Ending treatment: the course of emotional adjustment and quality of life among breast cancer survivors immediately following radiation therapy. *Support Care Cancer, 13*, 1018-1026.

Dhabhar, F. S., & McEwen, B. S. (1997). Acute stress enhances while chronic stress suppresses cell-mediated immunity in vivo: A potential role for leukocyte trafficking. *Brain, Behavior, and Immunity, 11*, 286-306.

Donath, S. (2001). The validity of the 12-item General Health Questionnaire in Australia: a comparison between three scoring methods. *Aust N Z J Psychiatry, 35*(2), 231-235.

Dopp, J. M., Miller, G. E., Myers, H. F., & Fahey, J. L. (2000). Increased natural killer-cell mobilization and cytotoxicity during marital conflict. *Brain, Behavior, and Immunity, 14*, 10-26.

Elliot, G. R., & Eisdorfer, C. (1982). *Stress and human health: An analysis and implications of research. A study by the Institute of Medicine, National Academy of Sciences*. New York: Springer Publishing.

Epping-Jordon, J. E., Compas, B. E., & Osowiecki, D. M. (1999). Psychological adjustment in breast cancer: process of emotional distress. *Health Psychology, 18*, 315-326.

Eysenck, H. J. (1994). Cancer, personality and stress: prediction and prevention. *Advances in Behavior Research and Therapy, 16*, 167-215.

Fallowfield, L. J., Hall, A., Maguire, P., & Baum, M. (1990). Psychological outcomes of different treatment policies in women with early breast cancer outside a clinical trial. *British Medical Journal, 301*(22), 575-580.

Fallowfield, L. J., Hall, A., Maguire, P., Baum, M., & A'Hern, R. P. (1994). Psychological effects of being offered choice of surgery for breast cancer. *British Medical Journal, 309*, 448.

Fernandez-Ballesteros, R., Ruiz, M., & Grade, S. (1998). Emotional expression in healthy women and those with breast cancer. *British Journal of Health Psychology, 3*, 41-50.

Feuerstein, M. (2007). Optimizing cancer survivorship. *J Cancer Surviv, 1*, 1-4.

Figley, C. R. (1978). *Stress Disorders among Vietnam Veterans*. New York: Brunner/Mazel.

First, M. B., Spitzer, R. L., Gibbon, M., & Williams, J. B. (1995). Structured Clinical Interview for DSM-IV Axis I Disorders- Patient Edition (SCID-I/P) (Version 2.0). New York: Biometrics Research Department.

Fox, B. H. (1976). Psychosocial epidemiology of cancer. In J. W. Cullen, B. H. Fox & R. N. Isom (Eds.), *Cancer: The Behavioural Dimension* (pp. 11-22). New York: Raven Press.

Fredrikson, M., Furst, C. J., Lekander, M., Rotstein, S., & Blomgren, H. (1993). Trait anxiety and anticipatory immune reactions in women receiving adjuvant chemotherapy for breast cancer. *Brain Behavior and Immunity, 7*(1), 79-90.

Friso, S., Jacques, P. F., Wilson, P. W., Rosenberg, I. H., & Selhub, J. (2001). Low circulating vitamin B(6) is associated with elevation of the inflammation marker C-reactive protein independently of plasma homocysteine levels. *Circulation, 103*(23), 2788-2791.

Gil, L., Seems, W., Mazurek, B., gross, J., Schroeder, P., & Voss, P. (2006). Age-associated analysis of oxidative stress parameters in human plasma and erythrocytes. *Free Radical Research, 40*(5), 495-505.

Ginzburg, K., Wrensch, M., Rice, T., Farren, G., & Spiegel, D. (2008). Breast Cancer and Psychosocial Factors: early stressful life events, social support, and well-being. *Psychosomatics, 49*, 407-412.

Glaser, R. (2007). Stress and Immunity. In R. Ader (Ed.), *Psychoneuroimmunology* (Vol. 2, pp. 705). New York: Elsevier Inc.

Goldberg, D. (1978a). *General health Questionnaire (GHQ-12)*. Winsor: NFER-NELSON.

Goldberg, D. (1978b). *General Health Questionnaire (GHQ-60)*. Winsor: NFER-NELSON.

Goldberg, D. (1992). *General health Questionnaire (GHQ-12)*. Windsor: NFER-NELSON.

Golden-Kruetz, D. M., Browne, M. W., Frierson, G. M., & Anderson, B. L. (2004). Assessing stress in cancer patients; a second order factor analysis for the perceived stress scale. *Assessment, 11*, 216-223.

Goldsby, R. A., Kindt, T. J., & Osbourne, B. A. (2000). *Kuby Immunology*. New York: Freeman, W. H.

Green, B. L. (1996). Trauma History Questionnaire. In B. H. Stamm & M. D. Lutherville (Eds.), *Measurement of Stress, Trauma, and Adaptation* (pp. 366-369).

Green, B. L., Rowland, J. H., Krupnick, J. L., Epstein, S. A., Stocketon, P., Stern, N. M., et al. (1996). Prevalence of Posttraumatic Stress Disorder in Women with Breast Cancer. *Psychosomatics, 39*, 102-111.

Greer, S., & Watson, M. (1985). Towards a psychobiological model of cancer: psychological considerations. *Journal of Psychosomatic Research, 20*, 773-777.

Grossarth-Maticek, R. (1980). Psychosocial predictors of cancer and internal disease: An overview. *Psychotherapy and Psychosomatics, 33*, 122-128.

Gurevich, M., Devins, G. M., & Rodin, G. M. (2002). Stress response syndromes and cancer: conceptual and assessment issues. *Psychosomatics, 43*(4), 259-281.

Hall, A., A'Hern, R. P., & Fallowfield, L. J. (1999). Are we using appropriate self-report questionnaires for detecting anxiety and depression in women with early breast cancer? *Eur J Cancer, 35*(1), 79-85.

Hapuarachchi, J. R., Chalmers, A. H., Winefield, A. H., & Blake-Mortimer, J. S. (2003). Biochemical Changes with Psychological Stress. *Behavioural Medicine, 29*(2), 52-59.

Henderson, S., Duncan-Jones, P., & Byrne, D. (1980). Measuring Social Relationships. The Interview Schedule for Social Interaction. *Psychological Medicine, 10*, 723-734.

Herbert, T. B., & Cohen, S. (1993). Stress and Immunity in humans: a meta-analytic review. *Psychosomatic Medicine* (55), 364-379.

Holland, J. C. (2002). History of Psycho-Oncology: Overcoming Attitudinal and Conceptual Barriers. *Psychosomatic Medicine, 64*, 206-221.

Holmes, S. (1989). Use of a modified symptom distress scale in assessment of the cancer patient. *International Journal of Nursing Studies, 26*(1), 69-79.

Horowitz, M. J., Wilner, N., & Alvarez, W. (1979). Impact of Events Scale: A measure of subjective stress. *Psychosomatic Medicine, 41*, 209-218.

Jacobsen, N. S., & Traux, P. (1991). Clinical Significance: A Statistical Approach to Defining Meaningful Change in Psychotherapy Research. *Journal of Consulting and Clinical Psychology, 59*(1), 12-19.

Jacobson, N. S., & Traux, P. (1991). Clinical Significance: A Statistical Approach to Defining Meaningful Change in Psychotherapy Research. *Journal of Consulting and Clinical Psychology, 59*(1), 12-19.

Johnston, M., Wright, S. J., & Weinmann, J. (1995). *Measures in health psychology: a user's portfolio* Winsor: NFER-Nelson.

Jolly, A. (2004). *Acute and Traumatic Stress and the Oxidative Stress Model.* The University of Adelaide, Adelaide.

Juster, R. P., McEwen, B. S., & Lupien, S. J. (2010). Allostatic load biomarkers of chronic stress and impact on health and cognition. *Neuroscience and Biobehavioural Reviews, 35*(1), 2-16.

Karnofsky, D. A., & Burchenal, J. H. (1949). The clinical evaluation of chemotherapeutic agents in cancer. In C. M. Macleod (Ed.), *Evaluation of Chemotherapeutic Agents* (pp. 199-205). New York: Columbia University Press.

Katz, M. M., & Lyerly, S. B. (1963). Methods for measuring adjustment and social behaviour in the community: I. Rationale, description, discriminate validity and scale development. *Psychol Rep, 13*, 503-535.

Katzung, B. G., Masters, S. B., & Trevor, A. J. (1992). *Basic and Clinical Pharmacology. 11th Edition*: McGraw-Hill / Lange.

Keller, S. E., Schleifer, S. J., Bartlett, J. A., Shiflett, S. C., & Rameshwar, P. (2000). Stress, Depression, Immunity and Health. In K. Goodkin & A. P. Visser (Eds.), *Psychoneuroimmunology: Stress, Mental Disorders, and Health* (pp. 1-26). Washington DC: American Psychiatric Press Inc.

Kiegolt-Glaser, J. K., & Glaser, R. (1999). Psychoneuroimmunology and Cancer: Fact or Fiction *Eur J Cancer, 35*(11).

Kiegolt-Glaser, J. K., McGuire, L., Robles, T. F., & Glaser, R. (2002). Psychoneuroimmunology and Psychosomatic Medicine: Back to the Future. *Psychosomatic Medicine, 64*, 15-28.

Kneier, A. W., & Temoshock, L. (1993). Repressive coping reaction in patients with malignant melanoma compared to cardiovascular disease patients. *Journal of Psychosomatic Research, 28*, 145-155.

Kornblith, A. B., Herndon, J. E., Weiss, R., Chunfeng Zhang, M. S., Zuckerman, E. L., Rosenberg, S., et al. (2003). Long-term adjustment of survivors of early stage breast carcinoma, 20 years after adjuvant chemotherapy. *Cancer, 98*(4), 679-689.

Ladas, E. J., Jacobsen, J. S., Kennedy, D. D., Teel, K., Fleischauer, A. T., & Kelly, K. M. (2004). Antioxidants and cancer therapy: a systematic review. *J Clin Oncol, 22*(3), 517-528.

Laufer, E. M., Hartman, T. J., Baer, D. J., Gunter, E. W., Dorgan, J. F., Campbell, W. S., et al. (2004). Effects of moderate alcohol consumption on folate and vitamin B12 status in postmenopausal women. *European Journal of Clinical Nutrition, 58*, 1518-1524.

Lazarus, R. S., & Folkman, S. (1984). *Stress, appraisals, and coping*. New York: Springer.

Le, P. (2004). *An evaluation of the effects of yoga on psychological stress and biochemical measures.* The University of Adelaide, Adelaide.

Lebel, S., Rosberger, Z., Edgar, L., & Devins, G. M. (2007). Comparison of four common stressors across the breast cancer trajectory. *Journal of Psychosomatic Research, 63*, 225-232.

Lecrubier, Y., Sheehan, D., Weiller, E., Amorim, P., Bonara, I., Sheehan, K., et al. (1997). The MINI International Neuropsychiatric Interview (M.I.N.I.) a short Diagnostic Structured Interview: Reliability and Validity According to the CIDI. *European Psychiatry, 12*(224-231).

Lekander, M., Furst, C. J., Rotstein, S., Blomgren, H., & Fredrikson, M. (1996). Social Support and Immune Status During and After Chemotherapy for Breast Cancer. *Acta Oncologica, 35*(1), 31-37.

Lengacher, C. A., Bennett, M. P., Kip, K. E., Keller, R., Lavance, M. S., Smith, L. S., et al. (2002). Frequency of use of complementary and alternative medicine in women with breast cancer. *Oncological Nursing Forum, 29*(10), 1445-1452.

Lesgards, J., Durand, P., Lassarre, M., Lesgards, G., Lanteaume, A., Prost, M., et al. (2002). Assessment of Lifestyle Effects on the Overall Antioxidant Capacity of Healthy Subjects. *Environmental Health Perspectives, 110*(5), 479-486.

Lis, C. G., Cambron, J. A., Grutsch, J. F., Granick, J., & Giupta, D. (2006). Self-reported quality of life in users and nonusers of dietary supplements in cancer. *Support Care Cancer, 14*, 193-199.

Liu, R. T., & Alloy, L. B. (2010). Stress generation in depression: A systematic review of the empirical literature and recommendations for future study. *Clinical Psychology Review, 30*, 582-593.

Logan, J. G., & Barksdale, D. J. (2008). Allostasis and allostatic load: expanding the discourse on stress and cardiovascular disease. *J Clin Nur, 17*(7b), 201-208.

Luecken, L. J., & Compas, B. E. (2002). Stress, Coping and Immune Function in Breast Cancer. *Annals of Behavioral Medicine, 24*(4), 336-344.

Maes, M., Song, C., Lin, A., de Jong, R., van Gastel, A., Kenis, G., et al. (1998). The effects of psychological stress on humans: increased production of pro-inflammatory cytokines and Th-1 response in stress-induced anxiety. *Cytokine, 10*(4), 313-318.

Maier, S. F., Watkins, L. R., & Fleshner, M. (1994). Psychoneuroimmunology: The Interface between Behavior, Brain, and Immunity. *American Psychologist, 49*(12), 1004-1017.

Mantovani, G., Maccio, A., Madeddu, C., Mura, L., Massa, E., Camboni, P., et al. (2003). Reactive Oxygen Species, antioxidant mechanisms, and serum cytokine levels in cancer patients: impact of an Antioxidant treatment. *Journal of Environmental Pathology, Toxicology and Oncology, 22*(1), 17-28.

Marshall, G. D., Agarwall, S. K., Lloyd, C., Cohen, L., Henniger, E. M., & Morris, G. J. (1998). Cytokine dysregulation associated with exam stress in healthy medical students. *Brain Behavior and Immunity, 12*, 297-307.

Martin, D. P. (1997). *The Healing Mind*. New York: St Martins Press.

Marucha, P. T., Crespin, T. R., Shelby, R. A., & Anderson, B. L. (2005). TNF-α levels in cancer patients relate to social variables. *Brain, Behavior, and Immunity, 19*(6), 521-525.

Mast, M. E. (1998). Survivors of breast cancer: illness uncertainty, positive reappraisal, and emotional distress. *Oncological Nursing Forum, 25*(3), 555-562.

McCarty, C. A., De Paola, C., Livingston, P. M., & Taylor, H. R. (1997). Reliability of a food frequency questionnaire to assess dietary antioxidant intake. *Opthalmic Epidemiology, 4*(1), 33-39.

McEwen, B. S. (1998a). Protective and damaging effects of stress mediators. *The New England Journal of Medicine, 338*, 171-179.

McEwen, B. S. (1998b). Stress, adaptation, and disease: allostasis and allostatic load. *Annals New York Academy of Sciences, 840*, 33.

McEwen, B. S. (2005). Stressed or stressed out: What is the difference? *J Psychiatry Neurosci, 30*(5), 315-318.

McKinley, E. D. (2000). Under Toad Days: Surviving the Uncertainty of cancer Reoccurrence. *Annals International Medicine, 133*, 479-480.

McNair, D. M., Lorr, M., & Droppleman, L. F. (1971). *Profile of Mood States (POMS)*: Multi-Health Systems Inc.

Melamed, S., Shirom, A., Toker, S., Berliner, S., & Shapira, I. (2004). Associations of fear of terror with low grade inflammation among apparently healthy employed adults. *Psychosomatic Medicine, 66*, 484-491.

Miller, A. H., Ancoli-Israel, S., Bower, J. E., Capuron, I., & Irwin, M. (2008). Neuroendocrine-Immune Mechanisms of Behavioural Comorbidities in Patients with Cancer. *Journal of Clinical Oncology, 26*(6), 971-982.

Miller, G. E., Cohen, S., & Herbert, T. B. (1999). Pathways linking major depression and immunity in ambulatory female patients. *Psychosom Med, 61*, 850-860.

Miller, R. G., Sutherland, A. G., Hitchinson, J. D., & Alexander, D. A. (2001). C-reactive protein and interleukin 6 receptor in post-traumatic stress disorder: a pilot study. *Cytokine, 13*(4), 253-255.

Mishel, M. H. (1988). Uncertainty in illness. *Image: Journal of Nursing Scholarship, 20*, 225-232.

Mishra, L. C., Singh, B. B., & Dagenais, S. (2000). Scientific Basis for the Therapeutic Use of Withania somnifera (Ashwagandha): A Review. *Alternative Medicine Review, 5*(4), 334-346.

Moinpour, C. M., Feigl, P., Metch, B., Hayden, K. A., Meyskens, F. L., & Crowley, J. (1989). Quality of Life end points in cancer clinical trials: review and recommendations. *J Natl Cancer Inst, 81*, 485-495.

Mullan, F. (1985). Seasons of Survival: Reflections of a Physician with Cancer. *New England Journal of Medicine, 313*(4), 270-273.

Mundy, E. A., Blanchard, E. B., Cirenza, E., Gargiulo, J., Maloy, B., & Blanchard, C. G. (2000). Posttraumatic stress disorder in breast cancer patients following autologous bone marrow transplantation or conventional cancer treatments. *Behaviour Research and Therapy, 38*, 1015-1027.

Murtaugh, M. P., Baarsch, M. J., Scamurra, R. W., & Lin, G. (1996). Inflammatory Cytokines in animal health and disease. *Veterinary Immunology and Immunopathology, 54*, 45-55.

Myint, A., Leonard, B. E., Steinbusch, H., & Kim, Y. (2005). Th1, Th2 and Th3 cytokine alterations in major depression. *Journal of Affective Disorders, 88*(2005), 167-173.

Nolen-Hoeksema, S., & Ahrens, C. (2002). Age differences and similarities in the correlates of depressive symptoms. *Psychol Aging, 17*(1), 116-124.

Oliver, J. M. (2004). *An Evaluation of the Effects of Nutrient Supplementation during Academic Examinations.* Unpublished Dissertation, The University of Adelaide, South Australia.

Ollonen, P., Lehtonen, J., & Eskelinen, M. (2005). Stressful and adverse life experiences in patients with breast symptoms; a prospective case-control study in Kuopio, Finland. *Anticancer Res, 25*(1B), 531-536.

Olver, I. (1998). *Conquering Cancer: Your Guide to Treatment and Research*. St Leonards: Allen & Unwin.

Osborne, R. H., Sali, A., Aaronson, N. K., Elsworth, G. R., Mdzewski, B., & Sinclair, A. (2004a). Immune Function and Adjustment Style: Do they predict survival in breast cancer? *Psycho-Oncology, (in press)*.

Osborne, R. H., Sali, A., Aaronson, N. K., Elsworth, G. R., Mdzewski, B., & Sinclair, A. (2004b). Immune Function and Adjustment Style: Do they predict survival in breast cancer? *Psycho-Oncology*.

Paik, I., Toh, K., Lee, C., Kim, J., & Lee, S. (2000). Psychological stress may induce increased humoral and decreased cellular immunity. *Behavioural medicine, 26*(3), 1-4.

Pallant, J. (2007). *SPSS Survival Guide: A step-by-step guide to data analysis using SPSS for Windows (Version 15)*. Sydney: Allen & Unwin.

Peakman, M., & Vergani, D. (1997). *Basic and Clinical Immunology*. New York: Churchill Livingstone.

Perneger, T. V. (1998). What's wrong with Bonferroni adjustments? *British Medical Journal, 316*, 7139.

Petticrew, M., Bell, R., & Hunter, D. (2002). Influence of psychological coping on survival and recurrence in people with cancer: a systematic review. *British Medical Journal, 325*(7372), 1066-1069.

Pfitzer, B. (2008). *A Step Towards a Broader Understanding of Complex Traumatization in Victims of Crime: Psychological and Physical Health Impacts and Implications for Psychological Interventions and Treatment Evaluation.* The University of Adelaide, Adelaide.

Piccinelli, M., Bisoffi, G., Bon, M. G., Cunico, L., & Tansella, M. (1993). Validity and test-retest reliability of the Italian version of the 12-item General Health Questionnaire in general practice: a comparison between three scoring methods. *Compr Psychiatry, 34*(3), 198-205.

Potter, P. J. (2007). Breast biopsy and distress: feasibility of testing a Reiki intervention. *Journal of Holistic Nursing, 25*(4), 238-248.

Prasad, K. N. (2004). Multiple Dietary Antioxidants Enhance the Efficacy of Standard and Experimental Cancer Therapies and decrease their Toxicity. *Integrative Cancer Therapies, 3*(4), 310-322.

Rabin, B. S. (1999). *Stress, immune function and health: the connection*. New York: Wiley-Liss, Inc.

Radloff, L. S. (1977). The CES-D Scale: a Self-Report Depression Scale for Research in the General Population. *Applied Psychological Measures, 1*, 385-401.

Ridker, P. M., Buring, J. E., Shih, J., Matias, M., & Hennekens, C. H. (1998). Prospective Study of C - reactive protein and the Risk of Future Cardiovascular Events among Apparently Healthy Women. *Circulation, 98*, 731-733.

Ross, R. (1999). Atherosclerosis--an inflammatory disease. *N Engl J Med, 340*(2), 115-126.

Russel, D., Peplau, L. A., & Cutrona, C. E. (1980). The revised UCLA Loneliness Scale: concurrent and discriminant validity evidence. *Journal of Personality and Social Psychology, 39*, 472-480.

Russel, D., Peplau, L. A., & Ferguson, M. L. (1978). Developing a measure of loneliness. *Journal of Personality Assessment, 42*(3), 290-294.

Sali, A. (1997). Psychoneuroimmunology. Fact or Fiction. *Aust Fam Physician, 26*(11), 1291-1294, 1296-1299.

Sammarco, A. (2001). Psychosocial stages and quality of life of women with breast cancer. *Cancer Nurs, 24*(4), 272-277.

Sapolsky, R. (2004). *Why Zebras Don't Get Ulcers: The Acclaimed Guide To Stress, Stress-Related Disease And Coping* (Vol. 3). USA: Henry Holt & Co.

Sarafino, E. P. (1998). *Health Psychology: Biopsychosocial Interactions* (3 ed.): Wiley.

Schleifer, S. J. (2007). PNI and cancer: Are we there yet? *Brain, Behavior, and Immunity, 21*, 393-394.

Schnipper, H. H. (2001). Life after breast cancer. *Journal of Clinical Oncology, 19*(15), 3581-3584.

Schulkin, J. (2004). *Allostasis, Homeostasis, and the Costs of Physiological Adaptation*. New York: Cambridge University Press.

Seeman, T. E., McEwen, B. S., Rowe, J. W., & Singer, B. H. (2001). *Allostatic load as a marker of cumulative biological risk: MacArthur studies of successful ageing*. USA, 1998: Proc. Natl. Acad. Sci.

Seematter, G., Binnert, C., Martin, J. L., & Tappy, L. (2004). Relationship between stress, inflammation and metabolism. *Current Opinions in Clinical Nutrition and Metabolic Care, 7*(2), 169-173.

Segerstrom, S. C. (2003). Individual differences, immunity, and cancer: lessons from personality psychology. *Brain Behavior and Immunity, 17*, S92-S97.

Segerstrom, S. C., & Miller, G. E. (2004). Psychological stress and the human immune system: a meta-analytic study of 30 years of inquiry. *Psychol Bull, 130*(4), 601-630.

Seifried, H. E., McDonald, S. S., Anderson, D. E., Greenwald, P., & Milner, J. A. (2003). The Antioxidant Conundrum in Cancer. *Cancer Research*(63), 4259-4298.

Selye, H. (1956). *The Stress of Life*. U.S.A: Longmans, Green and Co Ltd.

Selye, H. (1975). Confusion and controversy in the stress field. *J Human Stress, 1*(2), 37-44.

Shakir, D. K., & Rasul, K. I. (2009). Chemotherapy Induced Cardiomyopathy: Pathogenesis, Monitoring and Management. *Journal of Clinical Medicine Research, 1*(1), 8-12.

Shankar, A., Wang, J. J., Rochtchina, E., Yu, M. C., Kefford, R., & Mitchell, P. (2006). Association Between Circulating White Blood Cell Count and Cancer Mortality: A Population-Based Cohort Study (Vol. 166, pp. 188-194).

Shaver, P. R., & Brennan, K. A. (1991). Measures of Depression and Loneliness. In J. P. Robinson, P. R. Shaver & L. S. Wrightsman (Eds.), *Measures of social psychological attitudes, Vol. 1: Measures of personality and social psychological attitudes*. San Diego, CA: Academic Press.

Sheehan, D., Lecrubier, Y., Hamett-Sheehan, K., Janavas, J., Weiller, E., Bonara, L. I., et al. (1997). Reliability and Validity of the MINI International Neuropsychiatric Interview (M.I.N.I.): According to the SCID-P. *European Psychiatry, 12*, 232-241.

Sheehan, D. V., Lecrubier, Y., Hamett-Sheehan, K., Amorim, P., Janavas, J., Weiller, E., et al. (1998). The MINI International Neuropsychiatric Interview (M.I.N.I.): The Development and Validation of a Structured Diagnostic Psychiatric Interview. *J Clin Psychiatry, 59*((suppl 20)), 22-23.

Slamon, D. J., Leyland-Jones, B., & Shak, S. (2002). Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer over expression. *N Engl J Med, 344*(11), 783-792.

Song, C., Kenis, G., van Gastel, A., Bosmans, E., Lin, A., de Jong, R., et al. (2004). Influence of psychological stress on immune-inflammatory variables in normal humans. Part 2. Altered serum concentrations of natural anti-inflammatory agents and soluble membrane antigens of monocytes and T lymphocytes. *Psychiatry Research, 85*(3), 293-303.

Spanier, G. B. (1976). Measuring dyadic adjustment: new scales for assessing the quality of married and similar dyads. *J Marriage Fam, 38*, 15-28.

Spielberger, C. D. (1996). *Preliminary Manual for the State-Trait Personality Inventory (STPI).* Tampa, FL: Human Resources Institute, University of Florida.

Spielberger, C. D. (2003). The revised and expanded STAXI-2. In J. Blake-Mortimer (Ed.), *Psychological Assessment resources, Inc (PAR)*. Lutz, Florida: Psychological Assessment resources, Inc (PAR).

Spielberger, C. D., & Reheiser, E. C. (2002). Preliminary Test Manual for the Lifestyle Defense Mechanisms Inventory.

Spielberger, C. D., & Reheiser, E. C. (2009). Assessment of Emotions: Anxiety, Anger, Depression, and Curiosity. *Applied Psychology: Health and Well-being, 1*(3), 271-302.

Spitzer, R. L., Kroenke, K., & Williams, J. B. (1999). The Patient Health Questionnaire Study Group (1999): Validation and Utility of a Self-report Version of Prime-MD: the PHQ Primary Care Study. Primary Care Evaluation of Mental Disorders. Patient Health Questionnaire. *Journal of the American Medical Association, 282*, 1737-1744.

Spitzer, R. L., Williams, J. B., & Gibbon, M. (1990). *Structured Clinical Interview for DSM-III-R-Non-Patient Edition (SCID-NP, Version 1.0)*. Washington, DC.

Steptoe, A., Owen, N., Kunz-Ebrecht, S. R., & Brydon, L. (2004). Loneliness and neuroendocrine, cardiovascular, and inflammatory stress responses in middle-aged men and women. *Psychoneuroendocrinology, 29*, 593-611.

Sternberg, E. M., Chrousos, G. P., Wilder, R. L., & Gold, M. D. (1992). *The Stress Response and the Regulation of Inflammatory Disease*. Paper presented at the Combined Clinical Staff Conference.

Stevens, J. P. (2002). *Applied Multivariate statistics for the Social Sciences* (4th ed.). New Jersey: Lawrence Erlbaum Associates.

Stoney, C. M. (1999). Plasma homocysteine levels increase in women during psychological stress. *Life Science, 64*(25), 2359-2365.

Stoney, C. M., & Engebretson, T. O. (2000). Plasma homocysteine concentrations are positively associated with hostility and anger. *Life Science*(66), 2267-2275.

Tabachnick, B. G., & Fidell, L. S. (2007). *Using Multivariate Statistics (5th Edition)*. Boston: Pearson Education.

Tasaki, K., Maskarinec, G., Shumay, D., & Tatsumara, Y. (2002). Communication between physicians and cancer patients about complementary and alternative medicine: Exploring patients' perspectives. *Psycho-Oncology, 11*, 212-220.

Taylor, S., Klein, L., Lewis, B., Gruenwald, T., Gurung, R., & Updegraff, J. (2000). Biobehavioral responses to stress in females: tend and befriend, not fight- or- flight. *Psychological Review, 107*, 411.

Thornton, L. M., Anderson, B. L., Crespin, T. R., & Carson, W. E. (2007). Individual Trajectories in stress covary with immunity during recovery from cancer diagnosis. *Brain Behavior and Immunity, 21*(2), 185-194.

Thornton, L. M., Anderson, B. L., Schuler, T. A., & Carson, W. E. (2009). A psychological intervention reduces inflammatory markers by alleviating depressive symptoms: secondary analysis of a randomised controlled trial. *Psychosomatic Medicine, 71*, 715-724.

Thornton, L. M., Carson, W. E., Shapiro, C. L., Farrar, W. B., & Anderson, B. L. (2008). Delayed Emotional Recovery After Taxane-based Chemotherapy. *Cancer, 113*(3), 638-647.

Tierney, W. M., & McKinley, E. D. (2002). When the Physician-Researcher gets Cancer: Understanding cancer, Its Treatment, and Quality of Life from the Patients Perspective. *Medical Care, 40*(6), III20 - III27.

Tjemsland, L., Soreide, J. A., & Malt, U. F. (1998). Post traumatic distress symptoms in operable breast cancer III: status one year after surgery. *Breast Cancer Research and Treatment, 47*, 141-151.

Van der Ploeg, H. M., Defares, P. B., & Spielberger, C. D. (1980). *Manual for the Dutch Adaption of the STAI-Y*. Unpublished manuscript.

van Oostrom, M. A., Tijhuis, M. A. R., De Haes, J. C. J. M., Tempelaar, R., & Kromhout, D. A. (1995). A measurement of social support in epidemiological research: the social experiences checklist tested in a general population in the Netherlands *J Epidemiol Community Health, 49*, 518-524.

Vardy, J. (2009). *The Challenge- Current Trials in Exercise and Cancer*. Paper presented at the Clinical Oncology Society of Australia's 36th Annual Scientific Meeting.

Vickerberg, S. M. (2003). The Concerns About Recurrence Scale (CARS): a systematic measure of women's fears about the possibility of breast cancer recurrence. *Ann Behav Med, 25*, 16-24.

Wang, C. S., & Sun, C. F. (2009). C-reactive protein and malignancy: clinical-pathological association and therapeutic implication. *Chang Gung Med J, 32*(5), 471-482.

Ware, J. E., Snow, K. K., & Kosinski, M. (2000). *SF-36 Health Survey: Manual Interpretation Guide*. Lincoln RI: Quality Metric.

Watson, M., Greer, S., Young, J., Inayat, G., Burgess, C., & Robertson, B. (1989). Mental Adjustment to Cancer (MAC) Scale. Users Manual. In *Measures in Health Psychology: A Users Portfolio* Royal Marsden Hospital, Sutton Surrey, UK: CRC Psychological Medicine Research Group.

Weathers, F. W., Huska, J. A., & Keane, T. M. (1991). The PTSD Checklist- Civilian version (PCL-C). Boston, MA: National Centre for PTSD, Boston Veterans Affairs Medical Center.

Weiss, D. S., & Marmar, C. (1997). The Impact of Events Scale- Revised. In J. P. Wilson & T. M. Keane (Eds.), *Assessing psychological trauma and PTSD*. New York: Guilford Press.

Wenzel, L. B., Fairclough, D. L., & Brady, M. J. (1999). Age-related differences in the quality of life of breast carcinoma patients after treatment. *Cancer, 86*, 1768-1774.

West, R. (2004). ABC of smoking cessation: assessment of dependence and motivation to stop smoking. *British Medical Journal, 328*, 338-339.

Whitford, H. S., Olver, I. N., & Peterson, M. J. (2008). Spirituality as a core domain in the assessment of quality of life in oncology. *Psycho oncology, 17*(11), 1121-1128.

Wing, J., Cooper, J., & Satorius, N. (1974). *Measurement and Classification of Psychiatric Symptoms*. Cambridge: Cambridge University Press.

Xinying, P. X., Noakes, M., & Keogh, J. (2004). Can food frequency questionnaires be used to capture dietary intake data in a 4 week clinical intervention trial? *Asia Pac J Clin Nutr, 13*(4), 318-323.

Zigmond, A. S., & Snaith, R. P. (1983). The Hospital Anxiety and Depression Scale. *Acta Psychiatrica Scandinavia 67*(6), 361-370.

Appendices

THE UNIVERSITY OF ADELAIDE AUSTRALIA

SUB CRUCE LUMEN

Appendix A

Health and Wellbeing after Breast Cancer

Psychological Stress and Immunity

An exploratory study of physical, emotional and social factors

Participant Information Sheet

Professor Ian Olver MD, Clinical Director, RAH Chief Executive officer, The Cancer Council of Australia

Jodie Oliver, PhD Candidate, RAH protocol 051006, Version 4, 15 September 2006

You have been invited to participate in a study exploring elements of psychological wellbeing and immunity during cancer. This is a study conducted by Professor Ian Olver, Clinical Director RAH Cancer Centre and Jodie Oliver, a PhD candidate at the Psychology Department at the University of Adelaide. Before agreeing to participate in the study, it is important that you read and understand the following explanation of the study and procedures. Prior to agreeing to participate, you will be asked to sign a form indicating that you consent to take part in this study. However, if you chose to participate, you have the right to withdraw from the study at any time.

What is this study about?

This study is funded by the RAH. The purpose of this study is to explore immunological and psychological factors that are experienced after treatment for breast cancer, in order to discover what beneficial health interventions can or should be employed during this time. This study will examine whether factors like emotional stress are associated with blood markers of immunity. The study will involve a questionnaire and you will also be interviewed by the researcher about your current levels of stress.

Who can take part in this study?

Women aged between 18 and 60 years will be invited to participate in this study. For the purpose of this research, we are looking for:

Women who have undergone treatment at the RAH for stage 1-2 breast cancer

We will not be able to include you in this study if you are:

- Physically unwell at the time of testing. Please inform us if you are suffering from a cold or flu at the time of testing.
- Suffer from any of the following medical illnesses- severe heart disease, diabetes or rheumatoid arthritis; Addison's disease, Cushing's disease and lupus
- Taking medication that suppresses the immune system.
- Suffering from a psychotic disorder such as schizophrenia
- Currently taking warfarin
- Please indicate any medication or vitamins you are currently taking

What does this study involve?

Each person will be asked to complete three assessments. The first assessment will be taken 4 weeks after your last treatment of either adjuvant chemotherapy or radiation therapy.

The second assessment will be taken 8 weeks following this, and the third session a further 8 weeks later.

- A Registered Nurse will take a small blood sample (40ml) at each session.
- You will be asked to take part in an interview which assesses whether you are suffering from clinical depression or anxiety.
- You will be asked to fill out a questionnaire consisting of various psychological factors which may contribute to life stress

Each assessment will take approximately 1 hour of your time

Precautionary advice and possible adverse effects

Whenever a blood sample is taken there is a slight risk of bruising. Relevant biochemical blood measures will be analysed following the procedure. If any blood abnormalities are detected, you will be advised to consult your oncologist as a precautionary measure. If you have emotional concerns or concerns about your mental health I would encourage you to consult with the Cancer Centre psychologist, Tony DiBlasio. Alternately you can locate a psychologist by contacting the Australian Psychological Society referral service on 1800 333 497 or referral@psychology.org.au

Human Research Ethics Committee Contact

If you have any ethical concerns regarding this study please refer to the contact the Human Research Ethics Committee's Secretary on phone (08) 8303 6028.

Benefits & Feedback

You will be informed of the results of your blood tests. However, the results of your test will have no benefit to you. Upon completion of the study, a summary sheet of the overall results will be available.

Voluntary Participation and Confidentiality

Participation in the study is voluntary. You are free to withdraw from the project at any time and this will not affect your medical treatment now or in the future. The information that you provide is strictly confidential. The results of this study are part of research that may be published in an aggregated form, but will not personally identify you. The data will be stored securely in locked filing cabinets (as required for seven years).

If you agree to participate in the study you will be asked to sign a Consent form. If you have any questions or concerns, at any time before, during or after the study, please do not hesitate to contact:

Jodie Oliver on 8303 5884 or Email: jodie.oliver@adelaide.edu.au

Professor Ian Olver on Telephone: + 61 8 9036 3110 or Email: ian.olver@cancer.org.au

Appendix B

Dear Participant,

Thank you for taking the time to take part in this research project.

Please read the following instructions before completing the questionnaire.

1. Please complete all questions.
2. Please respond as honestly as you can.
3. Place a tick in the box or underline the answer that you feel is most appropriate.
4. Your immediate response is often the best.
5. If you make a mistake simply put a cross through it and mark your correct response.

If you have any other queries with regard to this questionnaire, please let me know.

THE UNIVERSITY OF ADELAIDE HUMAN RESEARCH ETHICS COMMITTEE

STANDARD CONSENT FORM FOR PEOPLE WHO ARE SUBJECTS IN A RESEARCH PROJECT

1. I, ……*(please print name)*

   consent to take part in the research project entitled:

   Health & Wellbeing after Breast Cancer

2. I acknowledge that I have read the attached Information Sheet entitled:

   Health & Wellbeing after Breast Cancer

3. I have had the project, so far as it affects me, fully explained to my satisfaction by the research worker. My consent is given freely.

4. Although I understand that the purpose of this research project is to improve our knowledge of psychological stress and risk of illness, it has also been explained that my involvement may not be of any benefit to me.

5. I understand the purpose of the study and my involvement in it.

6. I have been informed that, while information gained during the study may be published, I will not be identified and my personal results will not be divulged.

7. I understand that I am free to withdraw from the project at any time and that this will not affect my medical treatment, now or in the future.

8. I am aware that I should retain a copy of this Consent Form, when completed, and the attached Information Sheet (a copy can be sent to you at any time during the study, at your request).

……

(signature) *(date)*

WITNESS

I have described to …….. *(name of subject)*

the nature of the procedures to be carried out. In my opinion she/he understood the explanation.

Status in Project**:** …**Researcher**…….

Name: ……**Jodie Oliver**……

…….

(signature) *(date)*

a) DEMOGRAPHIC QUESTIONNAIRE

Office Use Only ID _____

Name _____

Date of Birth ___/____/__

1. Do **you currently smoke cigarettes, cigars, pipes or any other tobacco products? Please specify**

Daily ☐

☐

At least weekly (not daily) ☐

☐

Less Often than weekly

Or not at all

2. On average how many cigarettes do you smoke per day (daily) or each week (weekly)?

Enter number of cigarettes per day _____

OR

Enter number of cigarettes each week _____

3. During the last 7 days, on how many days did you do vigorous physical activities like heavy lifting, digging, aerobics or fast bicycling?

Days per week _____

No vigorous physical activities ☐

4. **How much time did you usually spend doing <u>vigorous</u> physical activities on one of those days?**

Hours per day _____

Minutes per day _____

Don't know/not sure ☐

5. During the last 7 days, on how many days did you do <u>moderate</u> physical activities like carrying light loads, bicycling at a regular pace, or doubles tennis? Do not include walking.

Days per week _____

No moderate physical activities ☐

6. How much time did you usually spend doing <u>moderate</u> physical activities on one of those days?

Hours per day _____

Minutes per day _____

Don't know/not sure ☐

7. During the last 7 days, on how many days did you <u>walk</u> for at least 10 minutes at a time?

Days per week _____

No walking ☐

8. **How much time did you usually spend <u>walking</u> on one of those days?**

Hours per day _____

Minutes per day _____

	Don't know/not sure	☐
9. How often do you have a drink containing alcohol?	(0) Never	☐
	(1) Monthly or less	☐
	(2) 2 to 4 times a month	☐
	(3) 2 to 3 times a week	☐
	(4) 4 or more times a week	☐
10. **How many drinks containing alcohol do you have on a typical day when you are drinking?**	(0) 1 or 2	☐
	(1) 3 or 4	☐
	(2) 5 or 6	☐
	(3) 7, 8 or 9	☐
	(4) 10 or more	☐
11. How often do you have six or more drinks on one occasion?	1) Never	☐
	2) Less than monthly	☐
	3) Monthly	☐
	4) Weekly	☐
	5) Daily or almost daily	☐
12. Do you take any other substances?	Yes	☐
(i.e. Marijuana/Cannabis **,** Cocaine, Speed, Ecstasy, Amphetamines, Opioids, Hallucinogens)	No	☐

If yes, please specify how much and how often?

_____

13. Diet

Do you have any special dietary requirements?

No ☐ Yes ☐

Please specify_____

(e.g. vegetarian, vegan, gluten free, lactose intolerant etc)

14. PLEASE LIST ANY MEDICATION WHICH YOU ARE CURRENTLY TAKING

Name of medication and mg	Dose (How many per day?)	Reason for medication
1.		
2.		
3.		
4.		
5.		
6.		
7.		
8.		
9.		

15. PLEASE LIST ANY VITAMIN, ANTIOXIDANT OR NUTRIENT WHICH YOU ARE CURRENTLY TAKING

Name	Dose (How many per day?)	Reason for taking
1.		
2.		
3.		
4.		
5.		
6.		
7.		
8.		
9.		

a) We should like to know if you have had any medical complaints and how your health has been in general, OVER THE LAST FEW WEEKS. Please answer ALL the questions simply by <u>circling</u> the answer which you think most nearly applies to you. Remember that we want to know about PRESENT AND RECENT complaints, not those that you had in the past. It is important that you try to answer ALL the questions.

Have you recently……

	been able to concentrate on whatever you're doing?	**Better than usual**	**Same as usual**	**Less than usual**	**Much less than usual**
	lost much sleep over worry?	**Not at all**	**No more than usual**	**Rather more than usual**	**Much more than usual**
	felt that you are playing a useful part in things?	**More so than usual**	**Same as usual**	**Less useful than usual**	**Much less useful**
	felt capable of making decisions about things?	**More so than usual**	**Same as usual**	**Less so than usual**	**Much less than usual**
	felt constantly under strain?	**Not at all**	**No more than usual**	**Rather more than usual**	**Much more than usual**
	felt you couldn't overcome your difficulties?	**Not at all**	**No more than usual**	**Rather more than usual**	**Much more than usual**
	been able to enjoy your normal day-to-day activities?	**More so than usual**	**Same as usual**	**Less so than usual**	**Much less than usual**
	been able to face up to your problems?	**More so than usual**	**Same as usual**	**Less so than usual**	**Much less able**
	been feeling unhappy and depressed?	**Not at all**	**No more than usual**	**Rather more than usual**	**Much more than usual**
	been losing confidence in yourself?	**Not at all**	**No more than usual**	**Rather more than usual**	**Much more than usual**
	been thinking of yourself as a worthless person?	**Not at all**	**No more than usual**	**Rather more than usual**	**Much more than usual**
	been feeling reasonably happy, all things considered?	**More so than usual**	**About same as usual**	**Less so than usual**	**Much less than usual**

b) A number of statements that people have used to describe themselves are given below. Read each statement and then circle the appropriate value to indicate how you GENERALLY feel. There are no right or wrong answers. Do not spend too much time on any one statement but give the answer which seems to describe how you GENERALLY feel.

			Not at all	*Somewhat*	*Moderately so*	*Very much so*
Ax-	1	I am a steady person	1	2	3	4
C+	2	I feel like exploring my environment	1	2	3	4
Ag+	3	I am quick-tempered	1	2	3	4
D+	4	I feel gloomy	1	2	3	4
Ax-	5	I feel satisfied with myself	1	2	3	4
C+	6	I am curious	1	2	3	4
Ag+	7	I have a fiery temper	1	2	3	4
D-	8	I feel happy	1	2	3	4
Ax+	9	I get in a state of tension or turmoil as I think over my recent concerns and interests	1	2	3	4
C+	10	I feel interested	1	2	3	4
Ag+	11	I am a hot-headed person	1	2	3	4
D+	12	I feel depressed	1	2	3	4
Ax+	13	I wish I could be as happy as others seem to be	1	2	3	4
C+	14	I feel inquisitive	1	2	3	4
Ag+	15	I get angry when I'm slowed down by others mistakes	1	2	3	4
D+	16	I feel sad	1	2	3	4
Ax+	17	I feel like a failure	1	2	3	4
C+	18	I feel eager	1	2	3	4
Ag+	19	I feel annoyed when I am not given recognition for doing good work	1	2	3	4
D-	20	I feel hopeless	1	2	3	4
Ax+21	21	I feel nervous and restless	1	2	3	4
C+	22	I am in a questioning mood	1	2	3	4

Ag+	23	I fly off the handle	1	2	3	4
D+	24	I feel low	1	2	3	4
Ax+	25	I feel secure	1	2	3	4
C+	26	I feel stimulated	1	2	3	4
Ag+	27	When I get mad, I say nasty things	1	2	3	4
			Not at all	Somewhat	Moderately so	Very much so
D-	28	I feel whole	1	2	3	4
Ax+	29	I lack self-confidence	1	2	3	4
C-	30	I feel disinterested	1	2	3	4
Ag+	31	It makes me furious when I am criticized in front of others	1	2	3	4
D+	32	I feel safe	1	2	3	4
Ax+	33	I feel inadequate	1	2	3	4
C+	34	I feel mentally active	1	2	3	4
Ag+	35	When I get frustrated, I feel like hitting someone	1	2	3	4
D-	36	I feel peaceful	1	2	3	4
Ax+	37	I worry too much over something that really does not matter	1	2	3	4
C-	38	I feel bored	1	2	3	4
Ag+	39	I feel infuriated when I do a good job and get a poor evaluation	1	2	3	4
D-	40	I enjoy life	1	2	3	4

c) Everyone feels angry or furious from time to time, but people differ in the ways that they react when they are angry. A number of statements are listed below which people use to describe their reactions when they feel *angry* or *furious.* Read each statement and then write the appropriate number that indicates how often you GENERALLY react or behave in the manner described when you are feeling angry or furious. There are no right or wrong answers. Do not spend too much time on any one statement.

(1) = Almost never (2) = Sometimes (3) = Often (4) = Almost always

1.	**I control my temper**	**()**
2.	**I express my anger**	**()**
3.	**I take a deep breath and relax**	**()**
4.	**I keep things in**	**()**
5.	**I am patient with others**	**()**
6.	**If someone annoys me, I'm apt to tell him or her how I feel**	**()**
7.	**I try to calm myself as soon as possible**	**()**
8.	**I pout or sulk**	**()**
9.	**I control my urge to express my angry feelings**	**()**
10.	**I lose my temper**	**()**
11.	**I try to simmer down**	**()**
12.	**I withdraw from people**	**()**
13.	**I keep my cool**	**()**
14.	**I make sarcastic remarks to others**	**()**
15.	**I try to soothe my angry feelings**	**()**
16.	**I boil inside, but I don't show it**	**()**
17.	**I control my behaviour**	**()**
18.	**I do things like slam doors**	**()**
19.	**I endeavor to become calm again**	**()**
20.	**I tend to harbor grudges that I don't tell anyone about**	**()**
21.	**I can stop myself from losing my temper**	**()**
22.	**I argue with others**	**()**
23.	**I reduce my anger as soon as possible**	**()**
24.	**I am secretly quite critical of others**	**()**
25.	**I try to be tolerant and understanding**	**()**
26.	**I strike out at whatever infuriates me**	**()**
27.	**I do something relaxing to calm down**	**()**
28.	**I am angrier than I am willing to admit**	**()**
29.	**I control my angry feelings**	**()**
30.	**I say nasty things**	**()**
31.	**I try to relax**	**()**
32.	**I'm irritated a great deal more than people are aware of**	**()**

d) A number of statements are listed below which people have used to describe their relations with others. Read each statement and then circle the appropriate number to the right of the statement to indicate how <u>often</u> you <u>generally</u> feel or react in the manner described. There are no right or wrong answers. Do not spend too much time on any one statement but give the answer that seems best to describe how you <u>generally feel or react</u>.

	<u>Almost Never</u>	<u>Some times</u>	<u>Often</u>	<u>Almost Always</u>
1. I try to do what is sensible and logical.	**1**	**2**	**3**	**4**
2. When I am in a situation in which I strongly disagree with other people, I try not to show my emotions.	**1**	**2**	**3**	**4**
3. I am available to help someone I care about with even the smallest problem.	**1**	**2**	**3**	**4**
4. My aim in life is to live for my dearest friends and family members, without demanding anything for myself.	**1**	**2**	**3**	**4**
5. I try to understand people and their behaviour.	**1**	**2**	**3**	**4**
6. If anyone deeply hurts my feelings, I still try to treat them reasonably and to understand their behaviour.	**1**	**2**	**3**	**4**
7. When I care about someone I go out of my way to make that person happy.	**1**	**2**	**3**	**4**
8. I will accept difficulties and ignore my own needs in order to have harmonious relationships with others.	**1**	**2**	**3**	**4**
9. I try to act rationally in my relations with others.	**1**	**2**	**3**	**4**
10. I try to understand other people even if I do not like them.	**1**	**2**	**3**	**4**
11. It is important for me to do everything possible to have harmonious relationships with people I care about.	**1**	**2**	**3**	**4**
12. I am willing to make personal sacrifices to maintain smooth relationships with people I care about.	**1**	**2**	**3**	**4**

	Almost Never	Some times	Often	Almost Always
13. I use intelligence and reason to overcome conflicts or disagreements with other people.	**1**	**2**	**3**	**4**
14. If someone acts against my needs and desires, I still try to understand him/her.	**1**	**2**	**3**	**4**
15. When I can't be with my close friends, I enjoy talking with them on the phone.	**1**	**2**	**3**	**4**
16. I feel responsible for making my relationships with others go as smoothly as possible.	**1**	**2**	**3**	**4**
17. My behaviour in most life situations is logical and reasonable, and not influenced by my emotions.	**1**	**2**	**3**	**4**
18. My use of reason and logic prevents me from attacking others, even if there are good reasons for doing so.	**1**	**2**	**3**	**4**
19. It is very important to me to make my dear ones happy.	**1**	**2**	**3**	**4**
20. When there is a conflict between my own needs and taking care of someone important to me, I will sacrifice my own needs to help the other person.	**1**	**2**	**3**	**4**
21. I succeed in avoiding arguments with others by using reason and logic (often contrary to my feelings).	**1**	**2**	**3**	**4**
22. If someone deeply hurts my feelings, I may attack them or respond purely emotionally.	**1**	**2**	**3**	**4**
23. I want to have only harmonious relations with my best friend.	**1**	**2**	**3**	**4**
24. It is very important to me to get along with people who are dear to me.	**1**	**2**	**3**	**4**

Everyone's experience of events is different. This section provides an opportunity to write in your own words about *your experience of breast cancer*.

Appendix C

Dear Participant,

Thank you for taking the time to take part in this research project.

Please read the following instructions before completing the questionnaire.

- Please complete all questions.
- Please respond as honestly as you can.
- Place a tick in the box or underline the answer that you feel is most appropriate.
- Your immediate response is often the best.
- If you make a mistake simply put a cross through it and mark your correct response.

Checklist	**Office Use Only**
Questionnaires Completed	☐
Blood Sample	☐
Saliva Sample	☐
Interview	☐
Schedule Next Appointment	☐
	_____

a. Impact of Event Scale (EIS-R) (Weiss & Marmar, 1997)

b. GHQ 12 (Goldman, 1982).

c. A number of statements that people have used to describe themselves are given below. Read each statement and then circle the appropriate value to indicate how you feel RIGHT NOW, that is, AT THIS MOMENT. There are no right or wrong answers. Do not spend too much time on any one statement but give the answer which seems to BEST describe your PRESENT FEELINGS.

			Not at all	*Somewhat*	*Moderately so*	*Very much so*
Ax-	1	1 I feel calm	1	2	3	4
C+	2	I am in a questioning mood	1	2	3	4
Ag+	3	I am furious	1	2	3	4
D-	4	I feel strong	1	2	3	4
Ax+	5	I am tense	1	2	3	4
C+	6	I feel curious	1	2	3	4
Ag+	7	I feel like banging on the table	1	2	3	4
D+	8	I feel blue	1	2	3	4
Ax1	9	I feel at ease	1	2	3	4
C+	10	I feel interested	1	2	3	4
Ag+	11	I feel angry	1	2	3	4
D+	12	I feel miserable	1	2	3	4
Ax+	13	I am presently worrying over possible misfortunes	1	2	3	4
C+	14	I feel inquisitive	1	2	3	4
Ag+	15	I feel like kicking somebody	1	2	3	4
D+	16	I feel downhearted	1	2	3	4
Ax+	17	I feel nervous	1	2	3	4
C+	18	I feel like exploring my environment	1	2	3	4
Ag+	19	I feel like breaking things	1	2	3	4
D-	20	I feel alive	1	2	3	4
Ax+	21	I am jittery	1	2	3	4
			Not at all	**Somewhat**	**Moderately so**	**Very much**

						SO
C+	22	I feel stimulated	1	2	3	4
Ag+	23	I am mad	1	2	3	4
D+	24	I feel sad	1	2	3	4
Ax+	25	I am relaxed	1	2	3	4
C+	26	I feel mentally active	1	2	3	4
Ag+	27	I feel irritated	1	2	3	4
D-	28	I feel safe	1	2	3	4
Ax+	29	I am worried	1	2	3	4
C-	30	I feel bored	1	2	3	4
Ag+	31	I feel like hitting someone	1	2	3	4
D+	32	I feel gloomy	1	2	3	4
Ax+	33	I feel steady	1	2	3	4
C+	34	I feel eager	1	2	3	4
Ag+	35	I feel annoyed	1	2	3	4
D-	36	I feel healthy	1	2	3	4
Ax+	37	I feel frightened	1	2	3	4
C-	38	I feel disinterested	1	2	3	4
Ag+	39	I feel like swearing	1	2	3	4
D-	40	I feel hopeful about the future	1	2	3	4

d. A number of statements are given below which describe people's reactions to having cancer. Please circle the appropriate number to the right of each statement, indicating how far it applies to you at present. For example, if the statement definitely does not apply to you then you should circle 1 in the first column.

	Definitely does not apply to me	Does not apply to me	Applies to me	Definitely applies to me
1. I have been doing things that I believe will improve my health e.g. changed my diet.	1	2	3	4
2. I feel I can't do anything to cheer myself up.	1	2	3	4
3. I feel that problems with my health prevent me from planning ahead.	1	2	3	4
4. I believe that my positive attitude will benefit my health	1	2	3	4
5. I don't dwell on my illness	1	2	3	4
6. I firmly believe that I will get better	1	2	3	4
7. I feel that nothing I can do will make a difference	1	2	3	4
8. I've left it all to my doctors	1	2	3	4
9. I feel that life is hopeless	1	2	3	4
10. I have been doing things that I believe will improve my health e.g. exercising	1	2	3	4
11. Since my cancer diagnosis, I now realize how precious life is and am making the most of it	1	2	3	4
12. I've put myself in the hands of God	1	2	3	4
13. I have plans for the future e.g. holidays, jobs, housing	1	2	3	4
14. I worry about the cancer returning or getting worse	1	2	3	4
15. I've had a good life; what's left is a bonus	1	2	3	4
16. I think my state of mind can make a	1	2	3	4

	Definitely does not apply to me	Does not apply to me	Applies to me	Definitely applies to me
lot of difference to my health				
17. I feel that there is nothing I can do to help myself	1	2	3	4
18. I try to carry on my life as I've always done	1	2	3	4
19. I would like to make contact with others in the same boat	1	2	3	4
20. I am determined to put it all behind me	1	2	3	4
21. I have difficulty in believing that this has happened to me				
22. I suffer great anxiety about it	1	2	3	4
23. I am not very hopeful about the future	1	2	3	4
24. At the moment I take one day at a time	1	2	3	4
25. I feel like giving up	1	2	3	4
26. I try to keep a sense of humour about it	1	2	3	4
27. Other people worry about me more than I do	1	2	3	4
28. I think of other people who are worse off	1	2	3	4
29. I am trying to get as much information as I can about cancer	1	2	3	4
30. I feel that I can't control what is happening	1	2	3	4
31. I try to keep a very positive attitude	1	2	3	4
32. I keep quite busy, so I don't have time to think about it	1	2	3	4
33. I avoid finding out more about it	1	2	3	4
34. I see my illness as a challenge	1	2	3	4

	Definitely does not apply to me	Does not apply to me	Applies to me	Definitely applies to me
35. I feel fatalistic about it	**1**	**2**	**3**	**4**
36. I feel completely at a loss about what to do	**1**	**2**	**3**	**4**
37. I feel very angry about what has happened to me	**1**	**2**	**3**	**4**
38. I don't really believe that I had cancer	**1**	**2**	**3**	**4**
39. I count my blessings	**1**	**2**	**3**	**4**
40. I try to fight the illness	**1**	**2**	**3**	**4**

e. The following statements describe how people sometimes feel. For each statement, please indicate how often you feel the way described by writing a number in the space provided.

(1) = never (2) = rarely (3) = sometimes (4) = always

1.	**How often do you feel you are 'in tune' with the people around you?**	**( )**
2.	**How often do you feel you lack companionship?**	**( )**
3.	**How often do you feel there is no one you can turn to?**	**( )**
4.	**How often do you feel alone?**	**( )**
5.	**How often do you feel part of a group of friends?**	**( )**
6.	**How often do you feel you have a lot in common with the people around you?**	**( )**
7.	**How often do you feel you are no longer close to anyone?**	**( )**
8.	**How often do you feel your interests and ideas are not shared by those around you?**	**( )**
9.	**How often do you feel outgoing and friendly?**	**( )**
10.	**How often do you feel close to people?**	**( )**
11.	**How often do you feel left out?**	**( )**
12.	**How often do you feel your relationships with others are not meaningful?( )**	
13.	**How often do you feel no one really knows you well?**	**( )**
14.	**How often do you feel isolated from others?**	**( )**
15.	**How often do you feel you can find companionship when you want it?**	**( )**
16.	**How often do you feel there are people who really understand you?**	**( )**
17.	**How often do you feel shy?**	**( )**
18.	**How often do you feel people are around you but not with you?**	**( )**
19.	**How often do you feel there are people you can talk to?**	**( )**
20.	**How often do you feel there are people you can turn to?**	**( )**

f. Is there anything regarding the questionnaire you have completed that you would like to comment on?

Thank You for taking the time to fill in this questionnaire.

Correlation- Covariate exploration- Psychological variables

	age	smoking	IPAQ	alcohol	Immunomodulator	cardiovascular	psychotropic	Analgesics	osteoarthrits	endocrine	respiratory	AO_use_
GHQ_1	.034	.040	-.236	.293	-.080	-.320	.042	-.039	-.039	-.393	.009	-.185
IES_1	.039	-.014	-.250	.415	-.006	-.329	-.003	-.050	-.050	-.191	-.073	-.256
Sanx_1	-.040	-.214	-.389	.359	-.061	-.194	.140	.229	.229	-.222	-.028	-.020
Scur_1	-.182	-.036	.309	-.460	.312	.222	-.134	-.417	-.417	.255	.370	-.236
Sang_1	-.001	-.196	-.321	-.115	-.259	-.256	-.051	-.035	-.035	-.093	-.128	-.463
Sdep_1	.009	.206	-.198	.656(**)	.009	-.156	.137	.243	.243	-.520(*)	-.230	.070
fighting spirit 1	.386	-.374	-.149	-.162	.567(*)	.586(*)	-.055	.102	.102	.123	-.106	.025
helpless-hopelessness 1	.063	-.086	-.314	.396	.030	-.313	-.235	-.074	-.074	-.242	-.361	.015
anxious preoccupation 1	.057	-.355	-.474	.164	.137	-.029	-.266	-.052	-.052	-.137	-.114	-.274
fatalistic 1	.509	.201	-.146	.145	.164	.056	.356	.149	.149	-.493	.082	-.164
avoidant 1	.158	.250	-.102	.590(*)	.177	-.075	-.294	-.200	-.200	-.177	-.294	.000
ucla_1	-.114	.132	-.294	.283	-.186	-.259	.341	-.004	-.004	-.245	.137	-.314

* Correlation is significant at the 0.05 level (2-tailed).** Correlation is significant at the 0.01 level (2-tailed).

Correlation – Covariate exploration pro-oxidant and pro-inflammatory markers

	age	smoking	IPAQ	alcohol	Immunomodulator	cardiovascular	psychotropic	Analgesics	osteoarthrits	endocrine	respiratory	AO_use
nt1	.046	-.004	.291	-.462	.181	.136	.005	-.371	-.371	.206	.565(*)	-.095
vitc1	.102	.502	.372	-.015	-.500	-.485	.336	-.142	-.142	.255	.422	-.276
LO1	.158	-.103	-.203	-.082	-.085	.058	-.117	-.058	-.058	.290	-.207	-.324
vitb1	-.182	.155	.415	.162	.194	-.100	-.125	-.423	-.423	.197	.248	.204
folate1	-.333	-.298	.030	.124	-.157	-.549(*)	-.508	-.406	-.406	.360	.092	.453
chol1	.293	.308	.066	.054	.248	.010	.208	.073	.073	-.441	-.144	-.048
IFNg_1	-.401	-.077	.296	-.069	-.183	-.420	-.273	-.186	-.186	.462	.225	.199
TNFa_1	-.324	.128	.056	.186	-.129	-.289	-.030	-.249	-.249	.026	.432	-.107
IL1b_1	-.453	-.162	.168	.183	-.075	-.463	-.301	-.205	-.205	.314	.238	.179
IL5_1	-.589(*)	-.321	-.023	.124	-.044	-.387	-.252	-.171	-.171	.531(*)	.127	.240
IL10_1	-.490	-.244	.006	.069	.069	-.193	-.125	.145	.145	.134	-.007	.364
LT_1	-.646(**)	-.250	.270	-.060	.002	-.301	-.196	-.134	-.134	.707(**)	.288	.354

* Correlation is significant at the 0.05 level (2-tailed).** Correlation is significant at the 0.01 level (2-tailed).

Stressed Women?

THE UNIVERSITY OF ADELAIDE AUSTRALIA

Are you Juggling Work? Family? Social Life?

Volunteers Needed to take part in a clinical trial on reducing the physical impact of stress on health, through supplementation with vitamins.

Are you a woman aged between 25- 45 years?

Are you interested in taking part in the trial?

The trial involves two assessments over an 8 week period.

There is no cost for vitamins provided

For more information please phone: 8303 5884

jodie.oliver@adelaide.edu.au

Appendix F

Participant Information Sheet

An evaluation of the possible benefits of taking vitamins during stress

You have been invited to participate in a study evaluating the effects of taking nutrient supplementation during everyday stress. This is a study conducted by Dr Jane Blake-Mortimer, Senior Lecturer, Psychology Department, University of Adelaide. Before agreeing to participate in the study, it is important that you read and understand the following explanation of the study and procedures. If you choose to participate, you have the right to withdraw from the study at any time.

What is this study about?

This study is funded by the manufacturers of the vitamins being tested. The ultimate purpose of this study is to examine the potential health benefits of a Women's D-Stress Formula during stress. This study will examine whether psychological stress decreases immunity, increases the incidence of infection and increases your risk of heart disease as indicated by examining factors such as cholesterol. Nutrient supplementation with vitamins may protect the body against infections. This study will examine relationships between stress, immunity, infection and risk of heart disease (eg. cholesterol) and the use of vitamins. Prior to agreeing to participate, you will be asked to sign a form indicating that you consent to take part in this study.

Who can take part in the trial?

Premenopausal women aged between 25 and 45 years in the community will be invited to participate in this study. For the purposes of this research trial we will not be able to include you in this study if you are:

1. Physically unwell at the time of testing. Please inform us if you are suffering from a cold or flu at the time of testing.
2. Suffer from any of the following medical illnesses- heart disease, diabetes or cancer, rheumatoid arthritis, Addison's disease, Cushing's disease and lupus
3. Taking medication that suppresses the immune system.
4. Pregnant or breast feeding. Please inform us if there is any possibility that you may be pregnant
5. Suffering from a psychotic disorder such as schizophrenia
6. Currently taking warfarin
7. Regularly taking vitamin supplements (2+ times per week)

What does the study involve?

Each person will be asked to complete two assessments. The first assessment will be taken at the beginning of the trial and the second assessment will be taken 8 weeks after the intervention. A registered nurse will take a small blood sample (30ml) at the beginning of the trial and at the conclusion of the trial (8 weeks later)

- You will be asked to swill 15ml of distilled water in your mouth for 30 seconds and expel liquid into a container.

- You will be asked to fill out a questionnaire consisting of various psychological factors which may contribute to life stress
- If you choose to take part in this assessment, after the initial assessment you will be randomly allocated to one of two groups. One group will receive nutrient supplement containing a combination of vitamin B, C and E and medicinal herbs. The other group will receive a placebo.

The placebo will consist of a similar looking tablet to the vitamin, but does not contain any vitamins. You will not be aware of whether you are taking the vitamin supplement or the placebo tablet. In this way we can examine whether taking vitamins is helpful during times of life stress. You will be asked to take a dose of two tablets a day after a meal. The vitamin supplements will be provided free of charge.

- Each assessment will take approximately an hour of your time.

Precautionary advice and possible adverse effects

Whenever a blood sample is taken there is a slight risk of bruising. As a precaution blood thinning agent such as aspirin, NSAID and gingko should not be taken 3 days prior to giving a blood sample. Ingestion of the vitamins used in this study at this dose is not known to have any adverse side-effects. Relevant biochemical blood measures will be analysed following the procedure. If any blood abnormalities are detected, you will be advised to consult your GP as a precautionary measure.

Feedback

Upon completion of the study, a summary sheet of the results will be available. Individual results will be available on request.

Voluntary Participation and Confidentiality

Participation in the study is voluntary. You are free to withdraw from the project at any time and this will not affect your medical treatment now or in the future.

The information that you provide is strictly confidential. The results of this study are part of research that may be published in an aggregated form, but will not personally identify you. The data will be stored securely in locked filing cabinets (as required for seven years)

If you agree to participate in the study you will be asked to sign a Consent form. If you have any questions or concerns, at any time before, during or after the study, please do not hesitate to contact:

Jodie Oliver on 8303 5884

jodie.oliver@adelaide.edu.au

Human Research Ethics Committee Contact

If you have any ethical concerns regarding this study please contact the Human Research Ethics Committee on: Tel: 8303 6028

APPENDIX G

Dear Participant,

Thank you for taking the time to take part in this research project.

Please read the following instructions before completing the questionnaire.

- Please complete all questions.
- Please respond as honestly as you can.
- Place a tick in the box or underline the answer that you feel is most appropriate.
- Your immediate response is often the best.
- If you make a mistake simply put a cross through it and mark your correct response.

Checklist	**Office Use Only**
Questionnaires Completed	☐
Blood Sample	☐
Saliva Sample	☐
Vitamins	☐
Retesting date confirmed	☐
	_____

THE UNIVERSITY OF ADELAIDE HUMAN RESEARCH ETHICS COMMITTEE

STANDARD CONSENT FORM FOR PEOPLE WHO ARE SUBJECTS IN A RESEARCH PROJECT

1. I, …… *(please print name)*

   consent to take part in the research project entitled:

   An evaluation of the possible benefits of taking vitamins during stress

2. I acknowledge that I have read the attached Information Sheet entitled:

   An evaluation of the possible benefits of taking vitamins during stress

3. I have had the project, so far as it affects me, fully explained to my satisfaction by the research worker. My consent is given freely.

4. Although I understand that the purpose of this research project is to improve our knowledge of psychological stress and risk of illness, it has also been explained that my involvement may not be of any benefit to me.

5. I understand the purpose of the study and my involvement in it.

6. I have been informed that, while information gained during the study may be published, I will not be identified and my personal results will not be divulged.

7. I understand that I am free to withdraw from the project at any time and that this will not affect my university studies, now or in the future.

8. I am aware that I should retain a copy of this Consent Form, when completed, and the attached Information Sheet.

   ……..

   (signature) *(date)*

WITNESS

I have described to …….. *(name of subject)*

the nature of the procedures to be carried out. In my opinion she/he understood the explanation.

Status in Project**:** …….

Name: …….

(signature) *(date)*

DEMOGRAPHIC QUESTIONNAIRE

Office Use Only	ID	_____

Name _____

Date of Birth ___/____/____

Do you live? On your own ☐ Family ☐ Partner ☐ Shared Accommodation ☐

Do you have children? Yes ☐ No ☐

How many? _____

Country of Birth (please specify) _____

Education Primary ☐ Secondary ☐ Tafe ☐ Tertiary ☐ Other ☐ _____

1. Do you currently smoke cigarettes, cigars, pipes or any other tobacco products? Please specify Daily ☐ At least weekly (not daily) ☐ Less Often than weekly ☐ Or not at all ☐	**3. During the last 7 days, on how many days did you do <u>vigorous</u> physical activities like heavy lifting, digging, aerobics or fast bicycling?** Days per week _____ No vigorous physical activities ☐
2. On average how many cigarettes do you smoke per day (daily) or each week (weekly)? *Enter number of cigarettes per day* _____ *or* *Enter number of cigarettes each week* _____	**4. How much time did you usually spend doing <u>vigorous</u> physical activities on one of those days?** Hours per day _____ Minutes per day _____ Don't know/not sure ☐

5. During the last 7 days, on how many days did you do moderate physical activities like carrying light loads, bicycling at a regular pace, or doubles tennis? Do not	**9. How often do you have a drink containing alcohol?** (0) Never ☐

include walking. Days per week _____ No moderate physical activities ☐	(1) Monthly or less ☐ (2) 2 to 4 times a month ☐ (3) 2 to 3 times a week ☐ (4) 4 or more times a week ☐
6. How much time did you usually spend doing <u>moderate</u> physical activities on one of those days? Hours per day _____ Minutes per day _____ Don't know/not sure ☐	**10. How many drinks containing alcohol do you have on a typical day when you are drinking?** (0) 1 or 2 ☐ (1) 3 or 4 ☐ (2) 5 or 6 ☐ (3) 7, 8 or 9 ☐ (4) 10 or more ☐
7. During the last 7 days, on how many days did you <u>walk</u> for at least 10 minutes at a time? Days per week _____	**11. How often do you have six or more drinks on one occasion?** (0) Never ☐ (1) Less than monthly ☐ (2) Monthly ☐ (3) Weekly ☐

No walking ☐	(4) Daily or almost daily ☐
8. How much time did you usually spend walking on one of those days? Hours per day _____ Minutes per day _____ Don't know/not sure ☐	**12. Do you take any other substances?** If yes, please specify how much and how often Marijuana/Cannabis…… Cocaine…… Speed/Ecstasy/Amphetamines…… Opioids/Hallucinogens…….. . Other (please specify)……..

PLEASE LIST ANY MEDICATION WHICH YOU ARE CURRENTLY TAKING

Name of medication and mg e.g. Lasix 20mg tablet	**Dose (How many per day?)**	**Reason for medica**

12. <u>Menstrual Cycle</u>

How long is your cycle? (21 to 40 days or more, 28 is average)_____ Don't know/not sure

Currently what day of your cycle are you at? _____ Don't know/not sure (count the first day of menstruation as day one)
Not Applicable

13. <u>This section provides an opportunity to write in your own words about your personal experience of stress.</u>

Everyone's experience of events is different.

Describe how stressed you have been lately and what sort of things have influenced this.

14. We should like to know if you have had any medical complaints and how your health has been in general, OVER THE LAST FEW WEEKS. Please answer ALL the questions simply by <u>circling</u> the answer which you think most nearly applies to you. Remember that we want to know about PRESENT AND RECENT complaints, not those that you had in the past. It is important that you try to answer ALL the questions.
Have you recently……

	been able to concentrate on whatever you're doing?	**Better than usual**	**Same as usual**	**Less than usual**	**Much less than usual**
	lost much sleep over worry?	**Not at all**	**No more than usual**	**Rather more than usual**	**Much more than usual**
	felt that you are playing a useful part in things?	**More so than usual**	**Same as usual**	**Less useful than usual**	**Much less useful**
	felt capable of making decisions about things?	**More so than usual**	**Same as usual**	**Less so than usual**	**Much less than usual**
	felt constantly under strain?	**Not at all**	**No more than usual**	**Rather more than usual**	**Much more than usual**
	felt you couldn't overcome your difficulties?	**Not at all**	**No more than usual**	**Rather more than usual**	**Much more than usual**
	been able to enjoy your normal day-to-day activities?	**More so than usual**	**Same as usual**	**Less so than usual**	**Much less than usual**
	been able to face up to your problems?	**More so than usual**	**Same as usual**	**Less so than usual**	**Much less able**
	been feeling unhappy and depressed?	**Not at all**	**No more than usual**	**Rather more than usual**	**Much more than usual**
	been losing confidence in yourself?	**Not at all**	**No more than usual**	**Rather more than usual**	**Much more than usual**
	been thinking of yourself as a worthless person?	**Not at ll**	**No more than usual**	**Rather more than usual**	**Much more than usual**
	been feeling reasonably happy, all things considered?	**More so than usual**	**About same as usual**	**Less so than usual**	**Much less than usual**

15. A number of statements that people have used to describe themselves are given blow. Read each statement and then circle the appropriate value to indicate how you feel RIGHT NOW, that is, AT THIS MOMENT. There are no right or wrong answers. Do not spend too much time on any one statement but give the answer which seems to BEST describe your PRESENT FEELINGS.

			Not at all	*Somewhat*	*Moderately so*	*Very much so*
Ax-	1	I feel calm	1	2	3	4
C+	2	I am in a questioning mood	1	2	3	4
Ag+	3	I am furious	1	2	3	4
D-	4	I feel strong	1	2	3	4
Ax+	5	I am tense	1	2	3	4
C+	6	I feel curious	1	2	3	4
Ag+	7	I feel like banging on the table	1	2	3	4
D+	8	I feel blue	1	2	3	4
Ax1	9	I feel at ease	1	2	3	4
C+	10	I feel interested	1	2	3	4
Ag+	11	I feel angry	1	2	3	4
D+	12	I feel miserable	1	2	3	4
Ax+	13	I am presently worrying over possible misfortunes	1	2	3	4
C+	14	I feel inquisitive	1	2	3	4
Ag+	15	I feel like kicking somebody	1	2	3	4
D+	16	I feel downhearted	1	2	3	4
Ax+	17	I feel nervous	1	2	3	4
C+	18	I feel like exploring my environment	1	2	3	4
Ag+	19	I feel like breaking things	1	2	3	4
D-	20	I feel alive	1	2	3	4

Ax+21	21	I am jittery	1	2	3	4
C+	22	I feel stimulated	1	2	3	4
Ag+	23	I am mad	1	2	3	4
D+	24	I feel sad	1	2	3	4
Ax+	25	I am relaxed	1	2	3	4
C+	26	I feel mentally active	1	2	3	4
Ag+	27	I feel irritated	1	2	3	4
D-	28	I feel safe	1	2	3	4
Ax+	29	I am worried	1	2	3	4
C-	30	I feel bored	1	2	3	4
Ag+	31	I feel like hitting someone	1	2	3	4
D+	32	I feel gloomy	1	2	3	4
Ax+	33	I feel steady	1	2	3	4
C+	34	I feel eager	1	2	3	4
Ag+	35	I feel annoyed	1	2	3	4
D-	36	I feel healthy	1	2	3	4
Ax+	37	I feel frightened	1	2	3	4
C-	38	I feel disinterested	1	2	3	4
Ag+	39	I feel like swearing	1	2	3	4
D-	40	I feel hopeful about the future	1	2	3	4

16. The following statements describe how people sometimes feel. For each statement, please indicate how often you feel the way described by writing a number in the space provided.

(1) = never (2) = rarely (3) = sometimes (4) = always

1.	**How often do you feel you are 'in tune' with the people around you?**	**( )**
2.	**How often do you feel you lack companionship?**	**( )**
3.	**How often do you feel there is no one you can turn to?**	**( )**
4.	**How often do you feel alone?**	**( )**
5.	**How often do you feel part of a group of friends?**	**( )**
6.	**How often do you feel you have a lot in common with the people around you?**	**( )**
7.	**How often do you feel you are no longer close to anyone?**	**( )**
8.	**How often do you feel your interests and ideas are not shared by those around you?**	**( )**
9.	**How often do you feel outgoing and friendly?**	**( )**
10.	**How often do you feel close to people?**	**( )**
11.	**How often do you feel left out?**	**( )**
12.	**How often do you feel your relationships with others are not meaningful?**	**( )**
13.	**How often do you feel no one really knows you well?**	**( )**
14.	**How often do you feel isolated from others?**	**( )**
15.	**How often do you feel you can find companionship when you want it?**	**( )**
16.	**How often do you feel there are people who really understand you?**	**( )**
17.	**How often do you feel shy?**	**( )**
18.	**How often do you feel people are around you but not with you?**	**( )**
19.	**How often do you feel there are people you can talk to?**	**( )**
20.	**How often do you feel there are people you can turn to?**	**( )**

17. OPTIONAL SECTION

Is there is anything regarding the questionnaires that you have completed that you would like to comment on?

APPENDIX H

Dear Participant,

Thank you for taking the time to take part in this research project.

Please read the following instructions before completing the questionnaire.

- Please complete all questions.
- Please respond as honestly as you can.
- Place a tick in the box or underline the answer that you feel is most appropriate.
- Your immediate response is often the best.
- If you make a mistake simply put a cross through it and mark your correct response.

Checklist	**Office Use Only**
Questionnaires Completed	☐
Blood Sample	☐
Saliva Sample	☐
Vitamin Container Collected	☐
	_____

DEMOGRAPHIC QUESTIONNAIRE

Office Use Only ID _____

Name _____

Date of Birth ___/____/___

1. Have you suffered from any illness or cold/flu like infections since you began taking the capsules for this study?

Yes ☐

No ☐

Please describe _____

_____

_____

_____

2. <u>Menstrual Cycle</u>

Currently what day of your cycle are you at?

_____

(count the first day of menstruation as day one)

Don't know/not sure ☐

Not Applicable ☐

3. <u>This section provides an opportunity to write in your own words about your personal experience of stress.</u>

Describe in relation to your experience of stress how you have been over the last 8 week period ……

We should like to know if you have had any medical complaints and how your health has been in general, OVER THE LAST FEW WEEKS. Please answer ALL the questions simply by circling the answer which you think most nearly applies to you. Remember that we want to know about PRESENT AND RECENT complaints, not those that you had in the past. It is important that you try to answer ALL the questions.

Have you recently……

	been able to concentrate on whatever you're doing?	**Better than usual**	**Same as usual**	**Less than usual**	**Much less than usual**
	lost much sleep over worry?	**Not at all**	**No more than usual**	**Rather more than usual**	**Much more than usual**
	felt that you are playing a useful part in things?	**More so than usual**	**Same as usual**	**Less useful than usual**	**Much less useful**
	felt capable of making decisions about things?	**More so than usual**	**Same as usual**	**Less so than usual**	**Much less than usual**
	felt constantly under strain?	**Not at all**	**No more than usual**	**Rather more than usual**	**Much more than usual**
	felt you couldn't overcome your difficulties?	**Not at all**	**No more than usual**	**Rather more than usual**	**Much more than usual**
	been able to enjoy your normal day-to-day activities?	**More so than usual**	**Same as usual**	**Less so than usual**	**Much less than usual**
	been able to face up to your problems?	**More so than usual**	**Same as usual**	**Less so than usual**	**Much less able**
	been feeling unhappy and depressed?	**Not at all**	**No more than usual**	**Rather more than usual**	**Much more than usual**
	been losing confidence in yourself?	**Not at all**	**No more than usual**	**Rather more than usual**	**Much more than usual**
	been thinking of yourself as a worthless person?	**Not at ll**	**No more than usual**	**Rather more than usual**	**Much more than usual**
	been feeling reasonably happy, all things considered?	**More so than usual**	**About same as usual**	**Less so than usual**	**Much less than usual**

4. A number of statements that people have used to describe themselves are given blow. Read each statement and then circle the appropriate value to indicate how you feel RIGHT NOW, that is, AT THIS MOMENT. There are no right or wrong answers. Do not spend too much time on any one statement but give the answer which seems to BEST describe your PRESENT FEELINGS.

			Not at all	*Somewhat*	*Moderately so*	*Very much so*
Ax-	1	I feel calm	1	2	3	4
C+	2	I am in a questioning mood	1	2	3	4
Ag+	3	I am furious	1	2	3	4
D-	4	I feel strong	1	2	3	4
Ax+	5	I am tense	1	2	3	4
C+	6	I feel curious	1	2	3	4
Ag+	7	I feel like banging on the table	1	2	3	4
D+	8	I feel blue	1	2	3	4
Ax-	9	I feel at ease	1	2	3	4
C+	10	I feel interested	1	2	3	4
Ag+	11	I feel angry	1	2	3	4
D+	12	I feel miserable	1	2	3	4
Ax+	13	I am presently worrying over possible misfortunes	1	2	3	4
C+	14	I feel inquisitive	1	2	3	4
Ag+	15	I feel like kicking somebody	1	2	3	4
D+	16	I feel downhearted	1	2	3	4
Ax+	17	I feel nervous	1	2	3	4
C+	18	I feel like exploring my environment	1	2	3	4
Ag+	19	I feel like breaking things	1	2	3	4
D-	20	I feel alive	1	2	3	4

Ax+	21	I am jittery	1	2	3	4
C+	22	I feel stimulated	1	2	3	4
Ag+	23	I am mad	1	2	3	4
D+	24	I feel sad	1	2	3	4
Ax-	25	I am relaxed	1	2	3	4
C+	26	I feel mentally active	1	2	3	4
Ag+	27	I feel irritated	1	2	3	4
D-	28	I feel safe	1	2	3	4
Ax+	29	I am worried	1	2	3	4
C-	30	I feel bored	1	2	3	4
Ag+	31	I feel like hitting someone	1	2	3	4
D+	32	I feel gloomy	1	2	3	4
Ax-	33	I feel steady	1	2	3	4
C+	34	I feel eager	1	2	3	4
Ag+	35	I feel annoyed	1	2	3	4
D-	36	I feel healthy	1	2	3	4
Ax+	37	I feel frightened	1	2	3	4
C-	38	I feel disinterested	1	2	3	4
Ag+	39	I feel like swearing	1	2	3	4
D-	40	I feel hopeful about the future	1	2	3	4

5. The following statements describe how people sometimes feel. For each statement, please indicate how often you feel the way described by writing a number in the space provided.

(1) = never (2) = rarely (3) = sometimes (4) = always

1.	**How often do you feel you are 'in tune' with the people around you?**	**( )**
2.	**How often do you feel you lack companionship?**	**( )**
3.	**How often do you feel there is no one you can turn to?**	**( )**
4.	**How often do you feel alone?**	**( )**
5.	**How often do you feel part of a group of friends?**	**( )**
6.	**How often do you feel you have a lot in common with the people around you?**	**( )**
7.	**How often do you feel you are no longer close to anyone?**	**( )**
8.	**How often do you feel your interests and ideas are not shared by those around you?**	**( )**
9.	**How often do you feel outgoing and friendly?**	**( )**
10.	**How often do you feel close to people?**	**( )**
11.	**How often do you feel left out?**	**( )**
12.	**How often do you feel your relationships with others are not meaningful?**	**( )**
13.	**How often do you feel no one really knows you well?**	**( )**
14.	**How often do you feel isolated from others?**	**( )**
15.	**How often do you feel you can find companionship when you want it?**	**( )**
16.	**How often do you feel there are people who really understand You?**	**( )**
17.	**How often do you feel shy?**	**( )**

18. How often do you feel people are around you but not with you? ( )

19. How often do you feel there are people you can talk to? ( )

20. How often do you feel there are people you can turn to? ( )

6. OPTIONAL SECTION

Is there is anything regarding the questionnaires that you have completed that you would like to comment on?

APPENDIX I

We would like to know how your health has been in general, OVER THE LAST 4 WEEKS. Please answer ALL the questions simply by underlining the answer which you think most nearly applies to you. Remember that we want to know about PRESENT AND RECENT complaints, not those that you had in the past. It is important that you try to answer ALL the questions.

Have you in the last 4 weeks……

1.	**been able to concentrate on whatever you're doing?**	Better than usual	Same as usual	Less than usual	Much less than usual
2.	**lost much sleep over worry?**	Not at all	No more than usual	Rather more than usual	Much more than usual
3.	**felt that you are playing a useful part in things?**	More so than usual	Same as usual	Less useful than usual	Much less useful
4.	**felt capable of making decisions about things?**	More so than usual	Same as usual	Less so than usual	Much less than usual
5.	**felt constantly under strain?**	Not at all	No more than usual	Rather more than usual	Much more than usual
6.	**felt you couldn't overcome your difficulties?**	Not at all	No more than usual	Rather more than usual	Much more than usual
7.	**been able to enjoy your normal day-to-day activities?**	More so than usual	Same as usual	Less so than usual	Much less than usual
8.	**been able to face up to your problems?**	More so than usual	Same as usual	Less so than usual	Much less able
9.	**been feeling unhappy and depressed?**	Not at all	No more than usual	Rather more than usual	Much more than usual
10.	**been losing confidence in yourself?**	Not at all	No more than usual	Rather more than usual	Much more than usual
11.	**been thinking of yourself as a worthless person?**	Not at all	No more than usual	Rather more than usual	Much more than usual
12.	**been feeling reasonably happy, all things considered?**	More so than usual	About same as usual	Less so than usual	Much less than usual

Overall is this better than usual OR is this worse than usual? If you have any concerns please call me on 8303 5884 or 0405 385 814

APPENDIX J

Correlations

Variables=ghq_pre

ghq_pre	Pearson Correlation	1
	Sig. (2-tailed)	
	N	50
age	Pearson Correlation	.241
	Sig. (2-tailed)	.091
	N	50
smoking	Pearson Correlation	.045
	Sig. (2-tailed)	.756
	N	50
IPAQ	Pearson Correlation	-.058
	Sig. (2-tailed)	.687
	N	50
hazardous alcohol use (0-12)	Pearson Correlation	.120
	Sig. (2-tailed)	.407
	N	50
Cardio	Pearson Correlation	.048
	Sig. (2-tailed)	.740
	N	50
Resp	Pearson Correlation	-.019
	Sig. (2-tailed)	.898
	N	50
OC	Pearson Correlation	-.087
	Sig. (2-tailed)	.546
	N	50
AnitDep	Pearson Correlation	.057
	Sig. (2-tailed)	.694
	N	50

Correlations

Variables=state anxiety pre intervention

state anxiety pre intervention	Pearson Correlation	1
	Sig. (2-tailed)	
	N	50
age	Pearson Correlation	.018
	Sig. (2-tailed)	.901
	N	50
smoking	Pearson Correlation	.209
	Sig. (2-tailed)	.145
	N	50
IPAQ	Pearson Correlation	.036
	Sig. (2-tailed)	.802
	N	50
hazardous alcohol use (0-12)	Pearson Correlation	-.047
	Sig. (2-tailed)	.744
	N	50
Cardio	Pearson Correlation	.014
	Sig. (2-tailed)	.924
	N	50
Resp	Pearson Correlation	-.035
	Sig. (2-tailed)	.808
	N	50
OC	Pearson Correlation	.078
	Sig. (2-tailed)	.589
	N	50
AnitDep	Pearson Correlation	-.104
	Sig. (2-tailed)	.471
	N	50

Correlations

Variables=state curiosity pre intervention

state curiosity pre intervention	Pearson Correlation	1
	Sig. (2-tailed)	
	N	50
age	Pearson Correlation	-.083
	Sig. (2-tailed)	.566
	N	50
smoking	Pearson Correlation	-.116
	Sig. (2-tailed)	.421
	N	50
IPAQ	Pearson Correlation	.216
	Sig. (2-tailed)	.132
	N	50
hazardous alcohol use (0-12)	Pearson Correlation	.004
	Sig. (2-tailed)	.978
	N	50
Cardio	Pearson Correlation	-.026
	Sig. (2-tailed)	.855
	N	50
Resp	Pearson Correlation	.307*
	Sig. (2-tailed)	.030
	N	50
OC	Pearson Correlation	-.242
	Sig. (2-tailed)	.091
	N	50
AnitDep	Pearson Correlation	-.120
	Sig. (2-tailed)	.405
	N	50

*. Correlation is significant at the 0.05 level (2-tailed).

Correlations

Variables=state depression pre intervention

state depression pre intervention	Pearson Correlation	1
	Sig. (2-tailed)	
	N	50
age	Pearson Correlation	.163
	Sig. (2-tailed)	.258
	N	50
smoking	Pearson Correlation	.160
	Sig. (2-tailed)	.268
	N	50
IPAQ	Pearson Correlation	-.099
	Sig. (2-tailed)	.493
	N	50
hazardous alcohol use (0-12)	Pearson Correlation	-.076
	Sig. (2-tailed)	.598
	N	50
Cardio	Pearson Correlation	.262
	Sig. (2-tailed)	.066
	N	50
Resp	Pearson Correlation	-.110
	Sig. (2-tailed)	.446
	N	50
OC	Pearson Correlation	-.086
	Sig. (2-tailed)	.555
	N	50
AnitDep	Pearson Correlation	.127
	Sig. (2-tailed)	.378
	N	50

Correlations

Variables=state anger pre intervention

state anger pre intervention	Pearson Correlation	1
	Sig. (2-tailed)	
	N	50
age	Pearson Correlation	-.093
	Sig. (2-tailed)	.520
	N	50
smoking	Pearson Correlation	-.020
	Sig. (2-tailed)	.892
	N	50
IPAQ	Pearson Correlation	.129
	Sig. (2-tailed)	.371
	N	50
hazardous alcohol use (0-12)	Pearson Correlation	-.095
	Sig. (2-tailed)	.512
	N	50
Cardio	Pearson Correlation	.304*
	Sig. (2-tailed)	.032
	N	50
Resp	Pearson Correlation	-.016
	Sig. (2-tailed)	.914
	N	50
OC	Pearson Correlation	.016
	Sig. (2-tailed)	.911
	N	50
AnitDep	Pearson Correlation	-.137
	Sig. (2-tailed)	.343
	N	50

*. Correlation is significant at the 0.05 level (2-tailed).

Correlations

Variables=loneliness pre intervention

loneliness pre intervention	Pearson Correlation	1
	Sig. (2-tailed)	
	N	50
age	Pearson Correlation	.332*
	Sig. (2-tailed)	.018
	N	50
smoking	Pearson Correlation	-.063
	Sig. (2-tailed)	.663
	N	50
IPAQ	Pearson Correlation	-.172
	Sig. (2-tailed)	.232
	N	50
hazardous alcohol use (0-12)	Pearson Correlation	.062
	Sig. (2-tailed)	.670
	N	50
Cardio	Pearson Correlation	.156
	Sig. (2-tailed)	.281
	N	50
Resp	Pearson Correlation	-.268
	Sig. (2-tailed)	.060
	N	50
OC	Pearson Correlation	-.159
	Sig. (2-tailed)	.269
	N	50
AnitDep	Pearson Correlation	.015
	Sig. (2-tailed)	.919
	N	50

*. Correlation is significant at the 0.05 level (2-tailed).

APPENDIX K

Correlations

Variables=5'-ectonucleotidase pre intervention

5'-ectonucleotidase pre intervention	Pearson Correlation	1
	Sig. (2-tailed)	
	N	50
age	Pearson Correlation	-.261
	Sig. (2-tailed)	.067
	N	50
smoking	Pearson Correlation	.266
	Sig. (2-tailed)	.062
	N	50
IPAQ	Pearson Correlation	.110
	Sig. (2-tailed)	.445
	N	50
hazardous alcohol use (0-12)	Pearson Correlation	-.021
	Sig. (2-tailed)	.883
	N	50
Cardio	Pearson Correlation	-.149
	Sig. (2-tailed)	.303
	N	50
Resp	Pearson Correlation	-.072
	Sig. (2-tailed)	.622
	N	50
OC	Pearson Correlation	.397**
	Sig. (2-tailed)	.004
	N	50
AnitDep	Pearson Correlation	.086
	Sig. (2-tailed)	.553
	N	50

**. Correlation is significant at the 0.01 level (2-tailed).

Correlations

Variables=Tissue Ascorbate pre intervention

Tissue Ascorbate pre intervention	Pearson Correlation	1
	Sig. (2-tailed)	
	N	50
age	Pearson Correlation	-.140
	Sig. (2-tailed)	.332
	N	50
smoking	Pearson Correlation	.101
	Sig. (2-tailed)	.487
	N	50
IPAQ	Pearson Correlation	.271
	Sig. (2-tailed)	.057
	N	50
hazardous alcohol use (0-12)	Pearson Correlation	-.109
	Sig. (2-tailed)	.453
	N	50
Cardio	Pearson Correlation	.093
	Sig. (2-tailed)	.522
	N	50
Resp	Pearson Correlation	.032
	Sig. (2-tailed)	.824
	N	50
OC	Pearson Correlation	.028
	Sig. (2-tailed)	.846
	N	50
AnitDep	Pearson Correlation	.104
	Sig. (2-tailed)	.471
	N	50

Correlations

Variables=Total antioxidant status pre intervention

Total antioxidant status pre intervention	Pearson Correlation	1
	Sig. (2-tailed)	
	N	50
age	Pearson Correlation	.330*
	Sig. (2-tailed)	.019
	N	50
smoking	Pearson Correlation	-.144
	Sig. (2-tailed)	.319
	N	50
IPAQ	Pearson Correlation	-.157
	Sig. (2-tailed)	.275
	N	50
hazardous alcohol use (0-12)	Pearson Correlation	-.004
	Sig. (2-tailed)	.978
	N	50
Cardio	Pearson Correlation	.265
	Sig. (2-tailed)	.063
	N	50
Resp	Pearson Correlation	-.051
	Sig. (2-tailed)	.723
	N	50
OC	Pearson Correlation	-.200
	Sig. (2-tailed)	.164
	N	50
AnitDep	Pearson Correlation	.074
	Sig. (2-tailed)	.608
	N	50

*. Correlation is significant at the 0.05 level (2-tailed).

Correlations

Variables=Homocysteine pre intervention

Homocysteine pre intervention	Pearson Correlation	1
	Sig. (2-tailed)	
	N	50
age	Pearson Correlation	.245
	Sig. (2-tailed)	.086
	N	50
smoking	Pearson Correlation	.269
	Sig. (2-tailed)	.059
	N	50
IPAQ	Pearson Correlation	.204
	Sig. (2-tailed)	.154
	N	50
hazardous alcohol use (0-12)	Pearson Correlation	.202
	Sig. (2-tailed)	.159
	N	50
Cardio	Pearson Correlation	.063
	Sig. (2-tailed)	.663
	N	50
Resp	Pearson Correlation	-.070
	Sig. (2-tailed)	.631
	N	50
OC	Pearson Correlation	-.082
	Sig. (2-tailed)	.569
	N	50
AnitDep	Pearson Correlation	-.109
	Sig. (2-tailed)	.453
	N	50

Correlations

Variables=Folate pre intervention

Folate pre intervention	Pearson Correlation	1
	Sig. (2-tailed)	
	N	49
age	Pearson Correlation	-.166
	Sig. (2-tailed)	.256
	N	49
smoking	Pearson Correlation	-.254
	Sig. (2-tailed)	.078
	N	49
IPAQ	Pearson Correlation	.032
	Sig. (2-tailed)	.829
	N	49
hazardous alcohol use (0-12)	Pearson Correlation	-.119
	Sig. (2-tailed)	.416
	N	49
Cardio	Pearson Correlation	.106
	Sig. (2-tailed)	.470
	N	49
Resp	Pearson Correlation	.168
	Sig. (2-tailed)	.249
	N	49
OC	Pearson Correlation	.040
	Sig. (2-tailed)	.787
	N	49
AnitDep	Pearson Correlation	.043
	Sig. (2-tailed)	.771
	N	49

Correlations

Variables=Folate pre intervention

Folate pre intervention	Pearson Correlation	1
	Sig. (2-tailed)	
	N	49
age	Pearson Correlation	-.166
	Sig. (2-tailed)	.256
	N	49
smoking	Pearson Correlation	-.254
	Sig. (2-tailed)	.078
	N	49
IPAQ	Pearson Correlation	.032
	Sig. (2-tailed)	.829
	N	49
hazardous alcohol use (0-12)	Pearson Correlation	-.119
	Sig. (2-tailed)	.416
	N	49
Cardio	Pearson Correlation	.106
	Sig. (2-tailed)	.470
	N	49
Resp	Pearson Correlation	.168
	Sig. (2-tailed)	.249
	N	49
OC	Pearson Correlation	.040
	Sig. (2-tailed)	.787
	N	49
AnitDep	Pearson Correlation	.043
	Sig. (2-tailed)	.771
	N	49

Correlations

Variables=Cholesterol pre intervention

Cholesterol pre intervention	Pearson Correlation	1
	Sig. (2-tailed)	
	N	50
age	Pearson Correlation	-.010
	Sig. (2-tailed)	.943
	N	50
smoking	Pearson Correlation	.136
	Sig. (2-tailed)	.347
	N	50
IPAQ	Pearson Correlation	-.108
	Sig. (2-tailed)	.457
	N	50
hazardous alcohol use (0-12)	Pearson Correlation	.126
	Sig. (2-tailed)	.385
	N	50
Cardio	Pearson Correlation	.071
	Sig. (2-tailed)	.622
	N	50
Resp	Pearson Correlation	.291*
	Sig. (2-tailed)	.041
	N	50
OC	Pearson Correlation	-.135
	Sig. (2-tailed)	.350
	N	50
AnitDep	Pearson Correlation	.274
	Sig. (2-tailed)	.054
	N	50

*. Correlation is significant at the 0.05 level (2-tailed).

Correlations

Variables=Vitamin B12 pre intervention

Vitamin B12 pre intervention	Pearson Correlation	1
	Sig. (2-tailed)	
	N	50
age	Pearson Correlation	-.065
	Sig. (2-tailed)	.655
	N	50
smoking	Pearson Correlation	-.229
	Sig. (2-tailed)	.109
	N	50
IPAQ	Pearson Correlation	-.181
	Sig. (2-tailed)	.207
	N	50
hazardous alcohol use (0-12)	Pearson Correlation	-.059
	Sig. (2-tailed)	.682
	N	50
Cardio	Pearson Correlation	.051
	Sig. (2-tailed)	.724
	N	50
Resp	Pearson Correlation	.170
	Sig. (2-tailed)	.237
	N	50
OC	Pearson Correlation	-.154
	Sig. (2-tailed)	.287
	N	50
AnitDep	Pearson Correlation	-.087
	Sig. (2-tailed)	.547
	N	50

Correlations

Variables=C-reactive protein pre intervention

C-reactive protein pre intervention	Pearson Correlation	1
	Sig. (2-tailed)	
	N	50
age	Pearson Correlation	-.120
	Sig. (2-tailed)	.408
	N	50
smoking	Pearson Correlation	.265
	Sig. (2-tailed)	.063
	N	50
IPAQ	Pearson Correlation	.034
	Sig. (2-tailed)	.814
	N	50
hazardous alcohol use (0-12)	Pearson Correlation	.261
	Sig. (2-tailed)	.067
	N	50
Cardio	Pearson Correlation	-.091
	Sig. (2-tailed)	.531
	N	50
Resp	Pearson Correlation	-.105
	Sig. (2-tailed)	.467
	N	50
OC	Pearson Correlation	.234
	Sig. (2-tailed)	.103
	N	50
AnitDep	Pearson Correlation	.049
	Sig. (2-tailed)	.736
	N	50

APPENDIX L

Correlations

Variables2=Interferon gamma pre intervention

Spearman's rho	Interferon gamma pre intervention	Correlation Coefficient	1.000
		Sig. (2-tailed)	.
		N	48
	age	Correlation Coefficient	.057
		Sig. (2-tailed)	.701
		N	48
	smoking	Correlation Coefficient	.204
		Sig. (2-tailed)	.164
		N	48
	IPAQ	Correlation Coefficient	-.191
		Sig. (2-tailed)	.194
		N	48
	hazardous alcohol use (0-12)	Correlation Coefficient	.072
		Sig. (2-tailed)	.628
		N	48
	Cardio	Correlation Coefficient	-.090
		Sig. (2-tailed)	.541
		N	48
	Resp	Correlation Coefficient	-.120
		Sig. (2-tailed)	.418
		N	48
	OC	Correlation Coefficient	-.163
		Sig. (2-tailed)	.269
		N	48
	AnitDep	Correlation Coefficient	.321*
		Sig. (2-tailed)	.026
		N	48

*. Correlation is significant at the 0.05 level (2-tailed).

Correlations

Variables2=Interleukin-1 beta pre intervention

Spearman's rho	Interleukin-1 beta pre intervention	Correlation Coefficient	1.000
		Sig. (2-tailed)	.
		N	50
	age	Correlation Coefficient	-.031
		Sig. (2-tailed)	.831
		N	50
	smoking	Correlation Coefficient	-.038
		Sig. (2-tailed)	.791
		N	50
	IPAQ	Correlation Coefficient	-.033
		Sig. (2-tailed)	.818
		N	50
	hazardous alcohol use (0-12)	Correlation Coefficient	.226
		Sig. (2-tailed)	.115
		N	50
	Cardio	Correlation Coefficient	.071
		Sig. (2-tailed)	.625
		N	50
	Resp	Correlation Coefficient	.082
		Sig. (2-tailed)	.573
		N	50
	OC	Correlation Coefficient	-.088
		Sig. (2-tailed)	.545
		N	50
	AnitDep	Correlation Coefficient	-.003
		Sig. (2-tailed)	.986
		N	50

Correlations

Variables2=Tumor necrosis factor alpha pre intervention

Spearman's rho	Tumor necrosis factor alpha pre intervention	Correlation Coefficient	1.000
		Sig. (2-tailed)	.
		N	50
	age	Correlation Coefficient	.140
		Sig. (2-tailed)	.332
		N	50
	smoking	Correlation Coefficient	.023
		Sig. (2-tailed)	.872
		N	50
	IPAQ	Correlation Coefficient	-.105
		Sig. (2-tailed)	.470
		N	50
	hazardous alcohol use (0-12)	Correlation Coefficient	.137
		Sig. (2-tailed)	.342
		N	50
	Cardio	Correlation Coefficient	-.046
		Sig. (2-tailed)	.751
		N	50
	Resp	Correlation Coefficient	-.169
		Sig. (2-tailed)	.242
		N	50
	OC	Correlation Coefficient	-.116
		Sig. (2-tailed)	.423
		N	50
	AnitDep	Correlation Coefficient	.128
		Sig. (2-tailed)	.377
		N	50

Correlations

Variables2=Tumor necrosis factor beta pre intervention

Spearman's rho	Tumor necrosis factor beta pre intervention	Correlation Coefficient	1.000
		Sig. (2-tailed)	.
		N	50
	age	Correlation Coefficient	-.006
		Sig. (2-tailed)	.965
		N	50
	smoking	Correlation Coefficient	-.045
		Sig. (2-tailed)	.757
		N	50
	IPAQ	Correlation Coefficient	.031
		Sig. (2-tailed)	.832
		N	50
	hazardous alcohol use (0-12)	Correlation Coefficient	.059
		Sig. (2-tailed)	.684
		N	50
	Cardio	Correlation Coefficient	.113
		Sig. (2-tailed)	.434
		N	50
	Resp	Correlation Coefficient	.013
		Sig. (2-tailed)	.930
		N	50
	OC	Correlation Coefficient	.048
		Sig. (2-tailed)	.743
		N	50
	AnitDep	Correlation Coefficient	.015
		Sig. (2-tailed)	.916
		N	50

Correlations

Variables2=Interleukin-5 pre intervention

Spearman's rho	Interleukin-5 pre intervention	Correlation Coefficient	1.000
		Sig. (2-tailed)	.
		N	49
	age	Correlation Coefficient	-.151
		Sig. (2-tailed)	.299
		N	49
	smoking	Correlation Coefficient	.043
		Sig. (2-tailed)	.770
		N	49
	IPAQ	Correlation Coefficient	-.360*
		Sig. (2-tailed)	.011
		N	49
	hazardous alcohol use (0-12)	Correlation Coefficient	.179
		Sig. (2-tailed)	.219
		N	49
	Cardio	Correlation Coefficient	-.139
		Sig. (2-tailed)	.342
		N	49
	Resp	Correlation Coefficient	-.063
		Sig. (2-tailed)	.666
		N	49
	OC	Correlation Coefficient	.143
		Sig. (2-tailed)	.326
		N	49
	AnitDep	Correlation Coefficient	-.123
		Sig. (2-tailed)	.398
		N	49

*. Correlation is significant at the 0.05 level (2-tailed).

Correlations

Variables2=Interleukin-10 pre intervention

Spearman's rho	Interleukin-10 pre intervention	Correlation Coefficient	1.000
		Sig. (2-tailed)	.
		N	50
	age	Correlation Coefficient	.049
		Sig. (2-tailed)	.733
		N	50
	smoking	Correlation Coefficient	-.023
		Sig. (2-tailed)	.872
		N	50
	IPAQ	Correlation Coefficient	-.395**
		Sig. (2-tailed)	.005
		N	50
	hazardous alcohol use (0-12)	Correlation Coefficient	-.042
		Sig. (2-tailed)	.774
		N	50
	Cardio	Correlation Coefficient	-.004
		Sig. (2-tailed)	.981
		N	50
	Resp	Correlation Coefficient	-.092
		Sig. (2-tailed)	.525
		N	50
	OC	Correlation Coefficient	.106
		Sig. (2-tailed)	.466
		N	50
	AnitDep	Correlation Coefficient	-.026
		Sig. (2-tailed)	.860
		N	50

**. Correlation is significant at the 0.01 level (2-tailed).

Correlations

Variables2=Interleukin-6 pre intervention

Spearman's rho	Interleukin-6 pre intervention	Correlation Coefficient	1.000
		Sig. (2-tailed)	.
		N	50
	age	Correlation Coefficient	-.004
		Sig. (2-tailed)	.978
		N	50
	smoking	Correlation Coefficient	-.049
		Sig. (2-tailed)	.735
		N	50
	IPAQ	Correlation Coefficient	-.283*
		Sig. (2-tailed)	.046
		N	50
	hazardous alcohol use (0-12)	Correlation Coefficient	.063
		Sig. (2-tailed)	.664
		N	50
	Cardio	Correlation Coefficient	.134
		Sig. (2-tailed)	.352
		N	50
	Resp	Correlation Coefficient	-.036
		Sig. (2-tailed)	.805
		N	50
	OC	Correlation Coefficient	.089
		Sig. (2-tailed)	.538
		N	50
	AnitDep	Correlation Coefficient	.064
		Sig. (2-tailed)	.659
		N	50

*. Correlation is significant at the 0.05 level (2-tailed).

APPENDIX M

Correlations

Control Variables			GHQ	NT
age	GHQ	Correlation	1.000	-.022
		Significance (1-tailed)	.	.434
		df	0	56
	NT	Correlation	-.022	1.000
		Significance (1-tailed)	.434	.
		df	56	0

		GHQ	HCY
GHQ	Pearson Correlation	1	-.014
	Sig. (1-tailed)		.459
	N	60	60
HCY	Pearson Correlation	-.014	1
	Sig. (1-tailed)	.459	
	N	60	60

		GHQ	CRP
GHQ	Pearson Correlation	1	-.052
	Sig. (1-tailed)		.347
	N	60	60
CRP	Pearson Correlation	-.052	1
	Sig. (1-tailed)	.347	
	N	60	60

		GHQ	VITC
GHQ	Pearson Correlation	1	-.166
	Sig. (1-tailed)		.104
	N	60	59
VITC	Pearson Correlation	-.166	1
	Sig. (1-tailed)	.104	
	N	59	59

		GHQ	FOLATE	VITB
GHQ	Pearson Correlation	1	-.053	.253(*)
	Sig. (1-tailed)		.348	.027
	N	60	58	59
FOLATE	Pearson Correlation	-.053	1	.290(*)
	Sig. (1-tailed)	.348		.014
	N	58	58	58
VITB	Pearson Correlation	.253(*)	.290(*)	1
	Sig. (1-tailed)	.027	.014	
	N	59	58	59

* Correlation is significant at the 0.05 level (1-tailed).

HCY

	Pearson Correlation	Sig. (1-tailed)	N
AXO	.004	.487	60
AXI	.001	.496	60
ACO	-.016	.451	59
ACI	-.047	.360	60
ANG_EXP_INDEX	.027	.420	59
state anger	.056	.334	60
tAng	-.061	.321	60
HCY	1		60

APPENDIX N

Correlations

GHQ

Spearman's rho

	Correlation Coefficient	Sig. (2-tailed)	N
GHQ	1.000	.	60
IL1b	.060	.655	58
TNFa	.152	.251	59
L_T	.146	.269	59
IL5	.085	.525	58
IL6	.099	.456	59
IL10	-.078	.557	59
IFNg	.085	.531	56

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Section No
Title and abstract			
	1a	Identification as a randomised trial in the title	6.6
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	6.3.5
Background and objectives	2a	Scientific background and explanation of rationale	6.1
	2b	Specific objectives or hypotheses	6.2
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	6.3.5
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	n/a
Participants	4a	Eligibility criteria for participants	6.3.2 & 6.3.3
	4b	Settings and locations where the data were collected	6.3.1
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	6.3.7
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	6.2.1 & 6.2.2
	6b	Any changes to trial outcomes after the trial commenced, with reasons	n/a
Sample size	7a	How sample size was determined	6.3.10.1
	7b	When applicable, explanation of any interim analyses and stopping guidelines	n/a

Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	6.3.8
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	6.3.8
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	6.3.8
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	6.3.8
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	6.3.8.1
	11b	If relevant, description of the similarity of interventions	6.3.8
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	6.3.10
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	6.3.10
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	6.3.6
	13b	For each group, losses and exclusions after randomisation, together with reasons	6.3.6
Recruitment	14a	Dates defining the periods of recruitment and follow-up	6.3.1
	14b	Why the trial ended or was stopped	n/a
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	6.4.2
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	6.3.6

Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	6.4.5 to 6.4.7
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	6.4.5 to 6.4.7
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	6.4.8
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	6.3.6
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	6.5.6
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	6.5.6.3
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	6.5
Other information			
Registration	23	Registration number and name of trial registry	6.3.1
Protocol	24	Where the full trial protocol can be accessed, if available	n/a
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	n/a