The Effect of a Hatha Yoga Practice on Factors Related to Chronic Stress

A thesis submitted for the Degree of

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by

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ABSTRACT

This thesis investigated the efficacy of an eight-week randomised waitlist controlled yoga intervention for middle-aged Australian women reporting chronic stress and psychological distress (N = 116). The research included two primary components. The first was a process evaluation of the implementation and quality of a standardised yoga protocol in a chronically stressed female population. The second component involved three outcome evaluation studies conducted to explore the longitudinal effects of yoga practice on psychological mental health variables and physiological variables, including a pilot study that explored biochemical markers of stress (i.e., inflammation proteins and deoxyribonucleic acid [DNA] methylation).

Paper 1 reports on a process evaluation that includes discussion regarding the development of an eight week secular yoga intervention and the underpinning theory, evaluation of fidelity and quality of its implementation, and examination of causal mechanisms and contextual factors associated with clinically significant improvement in distress (reported by 43% of women in the yoga intervention). These reported improvements in distress were comparable to psychotherapy, and participation in yoga classes was associated with improved positive and negative affect. It was concluded that yoga intervention was feasible for treatment of distress and was positively received by participants.

Paper 2 presents an evaluation of psychophysiological indicators of health following completion of the yoga intervention. Compared to a control group, practicing yoga was found to be associated with increased positive affect, and decreased levels of distress and stress. Additionally, decreased waist circumference and increased flexibility were demonstrated. These findings indicate that an eight-

week yoga intervention is associated with psychological and physiological benefits that exceed those attributable to the effects of time.

Paper 3 presents results of a longitudinal study that explored mental health variables at baseline, post-test and follow-up (one month) time-points. A strong effect of time was indicated as distress was found to decrease in both the yoga and control group, although positive affect was only benefited in the yoga group. Improvements reported at post-test were not robustly seen at follow-up indicating the benefits did not persist without continued regular yoga practice.

Paper 4 reports on a small pilot study (N = 28) that investigated the effect of yoga on biochemical variables associated with stress. Compared with the control group, women in the yoga group exhibited moderately higher levels of serum interleukin-6 (IL-6) and expressed less methylation in the tumor necrosis factor (*TNF*) region. Mental health variables were found to be moderately associated with C-reactive protein (CRP) and the methylation of *IL-6* (region 1), *CRP* and *LINE-1* (global methylation). Although the findings indicated some early methylation changes, the methodological constraints of the study only allow for preliminary insights and need to be further explored using larger samples.

In conclusion, this thesis demonstrated that an eight-week secular yoga intervention was associated with some short-term mental health benefits in distressed women and is a feasible treatment option. However, it did not appear that the benefits were robustly maintained beyond engagement with the yoga classes. Some evidence for molecular effects were indicated by tests involving specific biochemical markers of immunity. This thesis provides support for the potential value of larger scale trials examining efficacy of yoga practice in treating stress-related illness.

DECLARATION

I, Kaitlin Harkess, certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution 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. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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Kaitlin Harkess

Signed: _____ Date: _____

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This thesis is an embodiment of the work to which I have dedicated the last four years of my life. This period of time has provided me ample opportunity to develop my skills as an academic and clinician, as well as growing my character as a person. I will forever be grateful to have had the opportunity to take my education to this level and recognise that this fortune is due to the strong support network I have around me – I would like to express my sincere thanks to all who have helped me along this journey.

I thank my academic supervisors Paul Delfabbro and Sarah Cohen-Woods who have provided me invaluable guidance through this research project and provided their counsel as I navigated the clinical portion of my degree. Thank you both so much for assisting me in developing my skills as an academic researcher and writer. I have particularly enjoyed the humour always contained within the late-night email threads! Paul, thank you for your contribution to this project throughout – your dedication to supporting this project navigate hurdles and your availability and prompt communication has ensured that I was able to accomplish what I had hoped. I am also particularly grateful for your assistance in developing my skills as a statistician, of which I feel quite proud. Sarah, thank you for opening up new opportunities to this project, which I could not have dreamed when the project was conceived. Your excitement in the topic and energetic approach is inspiring, and I have no doubt that this modelling has aided my academic development, as it has contributed to this thesis.

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DEDICATION

For my mum, who herself wished for the opportunity to undertake Doctoral studies and whose influence has no doubt inspired my own path. I love you forever.

For all my family, friends, and fellow yogis who seek their breath on the yoga mat.

OVERVIEW

Introduction

The prevalence of mental health disorders is increasing and 'The Global Burden of Disease' report by the World Health Organisation estimates that by 2020 mental diseases, including stress-related disorders, will be the second leading cause of disease (Kalia, 2002; Kessler et al., 2009). These observations have been borne out in prevalence statistics which show that the general population is reporting increased levels of stress and distress, and decreased levels of well-being (Cassey & Ling, 2014; Australian Bureau of Statistics, 2015). Chronic stress is also known to have physiological impacts as it can negatively affect multiple systems of the body, including the gastrointestinal, cardio-respiratory and immune systems (Chrousos, 2009; Cohen, Janicki-Deverts, & Miller, 2007).

Research indicates that physical activity buffers the negative effects of stress on mental and physical health (Zschucke, Renneberg, Dimeo, Wüstenberg, & Ströhle, 2015), and is associated with increased well-being (Hassmen, Koivula, & Uutela, 2000). Mindfulness/meditation is also associated with enhanced psychological well-being (Brown & Ryan, 2003). Although a number of individual activities are capable of reducing stress and promoting mental and physical health, increasing attention has been directed toward examining the practice of yoga due to its integration of physical activity and mental focus to help individuals achieve overall feelings of well-being. More broadly, activities such as yoga are consistent with current theoretical interest in biopsychosocial models of public health and a move away from traditional mind-body dichotomy emphasised in Western societies for many decades. A focus on physical and mental health is also reflected in the growing influence of multidisciplinary fields, such as psychoneuroimmunology, which have emerged in recognition of empirical

evidence for bidirectional communication between the brain and immune system, as well as epigenetics which proposes that environmental experiences may influence activation of underlying genetic structures.

Despite recent interest in physical and mental health, relatively few studies have examined the efficacy of yoga as a clinical intervention and most have been plagued with methodological limitations, such as lack of standardised protocols, control groups, and biological measures, which are considered to be the objective 'gold-standard' of Western science (Field, 2011). These problems, in particular a lack of standardised protocols, make it difficult to replicate interventions and compare different interventions. Thus, an important motivation for this research was to develop a standardised approach for examining the psychotherapeutic potential of yoga.

Outline of Thesis and Aims of the Project

This research evaluated the effectiveness of yoga as a psychotherapeutic intervention for mental health in a chronically stressed population. As stress and distress are known to have both psychological and biophysiological effects, a multidisciplinary approach was taken in designing the series of papers used in this research, specifically a psychoneuroimmunological perspective was considered. The project had several aims:

- First, to evaluate the efficacy of yoga as a mind-body psychotherapeutic intervention, focusing on a chronically stressed female population. At present, many yoga interventions have not benefitted from the use of a standardised protocol, so the first aim of this investigation was to design a standardised protocol for a yoga intervention and to evaluate its implementation.

- Second, as the practice of yoga combines physical exercise and meditation, both of which are linked to enhanced psychological well-being, this study examined the psychotherapeutic benefit of a yoga practice itself. The focus was on its potential to decrease psychological distress in a chronically stressed population, along with an exploration of other changes related to health and broader indices of quality of life.
- Third, this study evaluated whether regular yoga practice is associated with changes in physiological and/or biochemical parameters (related to immunity), which have been linked to maladaptive psychological states, such as stress, anxiety and depression.

This research is one of the first attempts to address current limitations in the literature and evaluate yoga from a mental health perspective in various formats (process, psychological, biophysiological). This research presents the first process evaluation conducted of a yoga intervention conducted in a community population, and results of a registered clinical trial (ACTRN12616000612415), which include the first exploration of yoga and epigenetic modifications. Findings from one process evaluation, and one clinical trial (which utilised psychological, physiological, and biochemical outcome measures) were reported in four papers, presented here as separate chapters.

Chapter 1 provides an introduction to the psychological and biophysiological impacts of stress, the field of mind-body communication, and literature on yoga and its relationship with mental health. The aims of the thesis are then detailed. Chapters 2 to 4 present the four papers of this thesis which include opening and closing statements connecting each study and interpret them in the context of the broader research aims of this thesis. Chapter 5 summarises the findings of each study and

presents a discussion of broader research implications. The limitations of the research are presented along with potential future directions.

Each chapter includes tables and figures numbered consecutively; however the references for all chapters are located at the end of this thesis. A copy of the demographics questionnaire used is included as Appendix A, and a copy of the standardised yoga intervention is included as Appendix B.

Conventions

The nature of this project is multi-disciplinary, but was designed from a psychotherapeutic perspective. The dissertation was written presuming the reader is familiar with the field of clinical psychology, and less familiar with biologically based fields. Accordingly, where it is reasoned appropriate by the writer, a superficial overview is provided to assist reader comprehension.

Outline of Candidate

This research project was undertaken to fulfil requirements of Doctor of Philosophy/Master of Psychology (Clinical). The candidature program (*4 years full-time study*) combined the Master of Psychology (Clinical) course-load (*2 years full-time study*) and Doctor of Philosophy research program (*3 years full-time study*). The four papers that form this body of work, the PhD 'Structured Program', six compulsory Master of Psychology (Clinical) courses, and three Master of Psychology (Clinical) placements (representing a total of 2011 hours of clinical work), were successfully completed within four years of equivalent full-time study. This thesis fulfils the requirement of the Doctor of Philosophy degree.

CHAPTER 1. INTRODUCTION AND LITERATURE REVIEW

1.1 Preamble

This chapter provides an overview of the literature on psychological stress, its underlying biophysiological effects, mind-body communication and the biopsychosocial model of healthcare. This is followed by a review of literature specifically exploring yoga and mental health outcomes, including evaluation of the methodological quality of studies previously conducted. The underlying mechanisms of the effects of yoga practice are considered and the relationship with chronic stress. The chapter concludes with a detailing of thesis aims introduced in the overview.

1.2 The Stress Epidemic

High levels of psychological stress and distress (anxiety and/or mood disorders) are increasingly reported in Australia and worldwide (Cassey & Ling, 2014; Hammen, 2005; Australian Bureau of Statistics, 2015). It has been argued many industrialised countries are facing an 'epidemic' of stress (Kalia, 2002; Epel, 2009). This is concerning as a considerable body of research has established causal relationships between high chronic stress levels and development of clinical anxiety and depression (Angst & Vollrath, 1991; Breslau, Schultz, & Peterson, 1995; Brown, Bifulco, Harris, & Bridge, 1986). It is recognised that a modest amount of stress can be considered a normal part of an individual's life and can motivate adaptive coping strategies and resilience (a state of 'eustress'). However, an accumulation of stress over time may transition to a maladaptive state that may lead to poor mental and physical health (McEwen, 2002; Faravelli & Pallanti, 1989; Finlay-Jones & Brown, 1981; Hammen, 2005; MacLeod & Mathews, 1988; Rapee, 1991). The relationship between adaptive capacity and stress level, depicted in Figure 1, indicates that

adaption and potential personal growth is most likely when people face moderate levels of stress. On the other hand, high levels of stress can lead to a state of 'distress', which is further defined below.

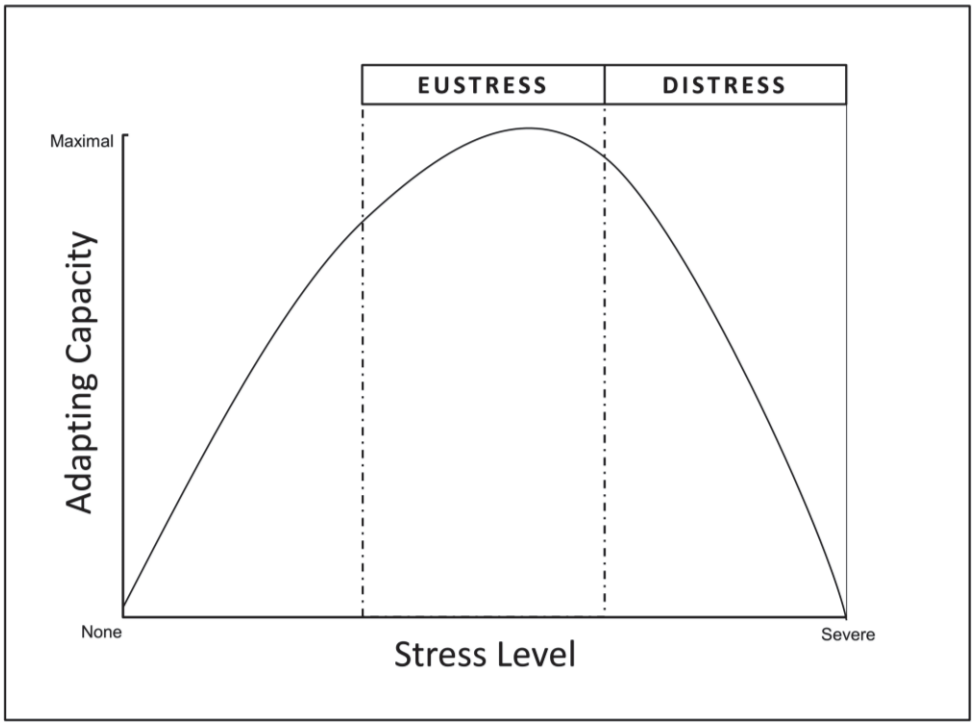

Figure 1. Hypothetical depiction of the relationship between stress, eustress, distress, and adaptive capacity.

1.3 Defining Stress

In the field of psychology stress has been defined as the impact on an individual's emotions that stems from the pressure life exerts (McEwen, 2002). A broader definition presented by Martin (1998) is "the state arising when an individual perceives that the demands placed upon them exceed (or threaten to exceed) their capacity to cope, and therefore threaten their well-being" (p. 118). This definition captures subjective interpretation (one's perception) of experience, and behavioural and biological alterations (interruption of homeostasis) that arise as a consequence of

a stressor. Individual psychological, behavioural, and physiological differences affect how a stressor is perceived.

A range of factors influence stress reactions, ranging from genetics and demographics, such as gender, developmental stage, and physiological and psychological history (McEwen & Stellar, 1993). Stress can be triggered by a multitude of stimuli including physical stressors, such as trauma, infection, and inflammation, and psychological stressors, such as fear, anxiety, and disappointment. Psychological stressors can themselves elicit physiological responses and have an impact on individual homeostasis.

1.3.1 Homeostasis and Allostasis

Stress has been defined as the interruption of homeostasis (Sterling, 2012). *Homeostasis* refers to the adjustment of physiological processes by which organisms tend to maintain internal equilibrium. While homeostasis describes the body at a physical equilibrium, individuals' bodies are far from static. Since the environment is continuously changing, a steady state is cultivated though continuous physiological responses referred to as allostasis. *Allostasis* is the process of continuous fluctuation of vital functions in response to environment (Sterling, 2012).

Allostasis is inherently unstable, continuously involving the organs and tissues that generate physiological responses (Sterling, 2012). The impact of this 'wear and tear' on the individual is called *allostatic load* and is hypothesised to be linked to pathology, one of the mechanisms by which stress leads to disease (McEwen & Stellar, 1993). Physiological systems suffer pressure and changes in operation as a result of the continuous strain chronic stress places on maintaining homeostasis (McEwen & Stellar, 1993).

An example of allostatic load is the experience of increased stress levels when students undertake examinations. At the conclusion of the exam period, the load becomes more likely to yield negative outcomes as the body takes time to return to homeostasis (McEwen & Stellar, 1993). Thus, it is not uncommon for an individual to experience illness at the conclusion of an extended period of great stress, during 'the recovery period' (McEwen & Stellar, 1993). This is in contrast with situations where there may be no conclusion of the stressor (chronic stress) and where bodily systems do not obtain short-term respite.

1.3.2 Taxonomy of Stress

In an attempt to capture the diversity of stress types, Elliot and Eisdorfer (1982) devised a popular taxonomy of the stress experience. This taxonomy is helpful in classifying an experience of stress and investigating associated physiological responses. It comprises of five categories defined by duration and course of the stressor (e.g., discrete vs. continuous):

1. Acute time-limited stressors are considered brief challenges an individual faces, such as that of mental arithmetic;
2. Brief naturalistic stressors are short-term challenges, like undergoing an examination;
3. A stressful event sequence is a life-changing event followed by additional challenges which the individual knows will subside in the future; e.g., the loss of a spouse;
4. Chronic stressors are those that give no sense the challenge will end and consequently permeate one's existence, leading to the restructuring of identity or social roles. An example is if one were to become disabled.

5. Distant stressors are traumatic events that occurred in the past, yet still affect the body, e.g., post-traumatic stress disorder.

Chronic stressors are considered particularly detrimental to the psychological and physical health of an individual. The lack of reprieve from these stressors results in sustained endocrine imbalance (McEwen & Stellar, 1993), and is linked to long-term psychological and physiological changes, such as maladaptive emotional states (e.g. depression), increased inflammation, and decreased immune functioning (Cohen et al., 2007). These effects are thought to develop in response to the allostatic load placed on the body.

Most chronic stress is reported to have a psychological component (Whitesman, 2008). Unlike physical stressors where effects are more likely to be acute and time-limited, psychological stress can often continue for longer resulting in a longer recovery period. This effect has been observed in the immune dysregulation of long-term carers of family members with Alzheimer's disease; in response to influenza vaccinations, this group has suppressed lymphocyte proliferation, natural killer cells and antibody responses, which continues for a duration of years after the conclusion of their caregiving relative to age, sex and socioeconomic matched controls (Kiecolt-Glaser, Glaser, Gravenstein, Malarkey, & Sheridan, 1996).

1.3.3 Psychological Distress

The psychological consequence of experiencing high levels of stress is often a state of distress (as depicted in Figure 1). While stress and distress are not always distinguished systematically, there have been attempts to differentially operationalise them, both in terms of biological and psychological concepts, as shown in Figure 2 (Ridner, 2004).

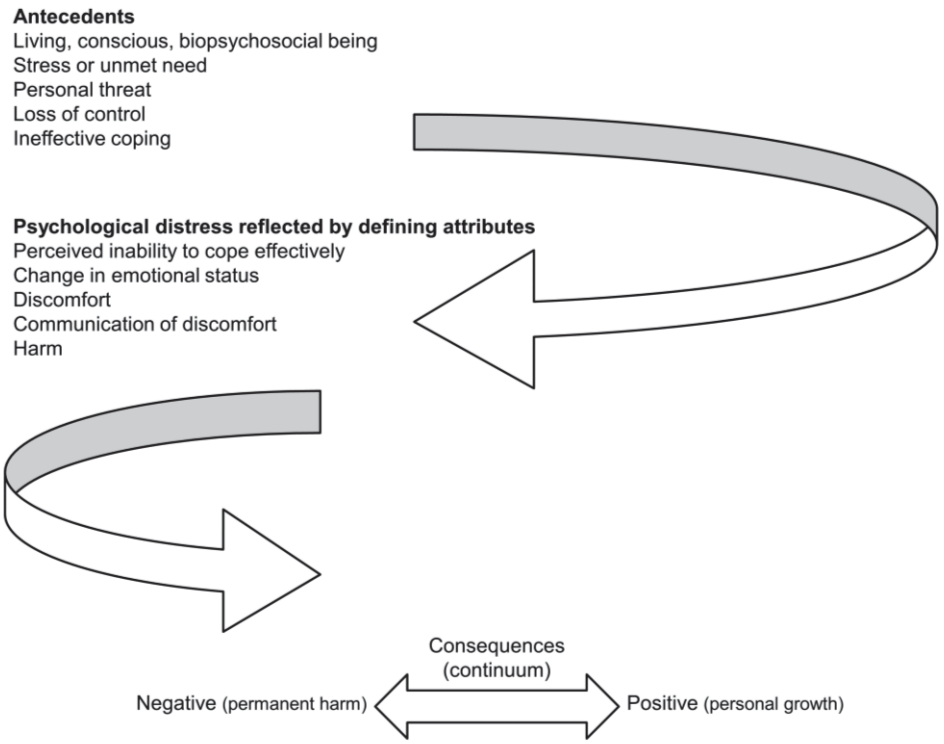

Figure 2. Antecedents, attributes and consequences of the concept of psychological distress. Reproduced with permission from "Psychological Distress: Concept Analysis," by S. H. Ridner, 2004, Journal of Advanced Nursing, 45(5), p. 543. Copyright 2004 by Blackwell Publishing Ltd.

Ridner (2004) conceptualises stress as "a non-specific biological response to a demand or stressor that is not necessarily harmful to the individual" and distress as "a non-specific, biological or emotional response to a demand or stressor that is harmful to the individual" (p. 539). Thus, psychological distress is the experience of discomforting emotional states in response to demands or stressors (Ridner, 2004), often characterised by the experience of high levels of anxiety and depressive symptoms (Andrews & Slade, 2001; Kessler & Mroczek, 1994). This definition captures the progression of an adaptive state into a maladaptive state often associated with chronic stress that is likely to overwhelm an individual's capacity to cope psychologically and physiologically. Additionally, psychological distress can evoke a cascade of physiological consequences.

1.3.4 Biophysiological Response to Stress

In response to a physical or psychological stressor, a cascade of neural, chemical and hormonal changes occur within the body in preparation to cope with the stressor (McEwen & Stellar, 1993). These changes affect various parts of the body, such as the autonomic, cardiovascular, gastrointestinal, and immune systems. Energy is generated by an increase in oxygen delivered to the lungs, which coincides with an increase in glucose deployed to the heart and large skeletal muscles (McEwen & Stellar, 1993). Simultaneously, energy-consuming systems, such as the gastrointestinal tract and components of the immune system, are down-regulated (Whitesman, 2008). Communication between, and systematic changes within, these systems enable lifesaving behaviours, such as the 'fight or flight' response (McEwen & Stellar, 1993). This response stems from the sympathetic nervous system and is closely related to emotions (Jevning, Wallace, & Beidebach, 1992).

Whitesman (2008) proposes two communication channels through which perception of a stressor elicits a physiological response. The first is a key communication channel between the brain and immune system via the hypothalamic-pituitary-adrenal (HPA) axis. The communication channel is bidirectional so the mind/brain can affect immune system functioning. Conversely, the mind/brain is influenced by immune system activation, such as during illness. The effect of a stressor on immune function can be measured biochemically in peripheral blood (Whitesman, 2008). The second pathway is through the connection of functional neuroendocrine-immune systems. In this pathway, bidirectional communication is enabled through common molecular languages, such as immune cells expressing receptors for hormones and neurotransmitters, which is then regulated by inflammatory processes or chronic stress (Heijnene, 2007). This molecular language

is also utilised by the parasympathetic nervous system, which has been implicated in immune system regulation through the vagus nerve.

The vagus nerve is the main nerve of the parasympathetic nervous system and has a role in a number of metabolic functions including regulating heart rate, gastrointestinal functions, and the immune system (Pavlov & Tracey, 2012). Specifically, decreased vagus nerve activity is implicated in the inflammatory response of the immune system (Pavlov & Tracey, 2012).

1.3.5 The Immune System

As the immune system is particularly complex, a brief overview of the immune system, with a focus on responses relevant to this thesis, is presented here. The word immune is derived from the Latin term *immunis*, which means 'exempt' (Goldsby, Kindt, Osborne, & Kuby, 2003). The immune system is an organization of cells, tissues, and organs that have evolved to defend the body from invading pathogenic microorganisms, such as bacteria, parasites, and fungi, which can cause infections and cancer (Cota & Midwinter, 2009; Goldsby, et al., 2003 Mackay, Rosen, Delves, & Roitt, 2000). Most cells in the immune system are white blood cells that develop in the bone marrow. Through their response to different cytokines (proteins that affect interactions between cells) they grow into specific immune cells.

Innate (natural) immunity is *non-specific*, which means that immune defences lack immunological memory and remain unchanged in spite of previous encounters with an antigen (Delves & Roitt, 2000). Two lines of defence are associated with innate immunity: first-line, or external, defences, such as the skin; and airway defences, such as the sneeze reflex (Cota & Midwinter, 2009). Second-line, or

internal, defences include inflammation and soluble proteins (e.g., acute-phase proteins).

An inflammatory response is triggered when a pathogen passes through the first line of defence. Next, neutrophils and macrophages move to the area of infection. Put simply, these are ‘defender cells’ that ‘eat’ the pathogen. Macrophages secrete a number of proteins including cytokines and interleukins, such as tumour necrosis factor alpha (TNF) and interleukin-6 (IL-6), which are explored in this thesis. TNF and IL-6 are central to developing an immune response. They not only contribute to the inflammatory response, but also activate lymphocytes, and in the case of TNF, secrete factors that kill specific cells and stimulate the liver to produce acute phase proteins (e.g., C-reactive protein [CRP], which is also explored in this thesis). While inflammation protects the body from pathogen, if the production of these pro-inflammatory molecules is unregulated surrounding tissues can be damaged. For example, in the case of rheumatoid arthritis, macrophages and neutrophils overproduce pro-inflammatory molecules that invade the joints and cause inflammation. While immune responses are initiated by antigens and regulated by cytokines, the central nervous system (CNS) is physically connected to the immune system and may exert a physiological effect (Maier, Watkins, & Fleshner, 1994). This means a psychological stressor can elicit an inflammatory response in the body (Zachariae, 2009).

1.4 Mind-Body Communication

It is postulated that bidirectional communication occurs between the brain and immune system, such that behavioural-psychological processes affect immune function, and vice-versa (Maier et al., 1994). Hence, the mind-body interaction via the

immune system affects one's health, as physical health depends strongly on immune function (Martin, 1998). In turn, the brain makes contact with the immune system through the peripheral nervous system, which connects the CNS to limbs and organs of the body. In the Maier et al. (1994) review, innervations of the parasympathetic and sympathetic nervous systems (SNS; i.e., autonomic nervous system) with visceral organs, such as the stomach and the heart, are noted as a potential channel of communication, along with connection of the SNS with organs of the immune system. Immune organs and cells have *catecholamine* receptors. Catecholamine and norepinephrine are released by sympathetic nerve terminals, which make contact with lymphocytes (Maier et al., 1994).

A second path to communicate with peripheral organs is through the release of hormones by the brain (i.e., the hypothalamus and pituitary; Wrona, 2005). This release is tigered by internal and external stimuli including psychological stress, and leads the endocrine glands to secrete hormones into blood circulation (Wrona, 2005). Hormones travel to different organs and bind to specific hormone receptors (Maier et al., 1994). This pathway is particularly pertinent to determining stress levels, as stress is often identified through increased levels of hormones in the blood (Ehlert Gaab & Heinrichs, 2001; George, Everyly, & Lating, 2012). For example, the adrenal glands produce *corticosteroids* called glucocorticoids, for which some innate immune cells have receptors. This path is termed the hypothalamic-pituitary-adrenal (HPA) axis. Corticotrophin releasing hormone (CRH) is released by the hypothalamus after a stress response. CRH travels to the pituitary gland where the release of adrenocorticotrophic hormone (ACTH) is stimulated. ACTH then stimulates the adrenal gland which produces glucocorticoids (e.g., cortisol), as mentioned earlier (Maier et al., 1994). Innate immune responses are activated by glucocorticoids, which

mobilise and stimulate neutrophils. In a healthy response, glucocorticoids communicate with the brain to decrease CRH, ACTH, and further glucocorticoid release; however, under chronic stress the decrease does not occur and the allostatic load on the organism is increases (Zachariae, 2009). Cortisol interacts with glucocorticoid receptors to supress inflammatory protein production (i.e., cytokines), this immunosuppressive activity weakens the immune system and increases the likelihood of illness (Herbert & Cohen, 1993).

The multifaceted relationship between psychological stress, the SNS, HPA axis and immune system is depicted in see Figure 3. In effect, psychological events give rise to neural activity and the experience of stress, or psychological distress, activates the SNS and the HPA axis (the 'stress system'). The paths between the 'stress system' and the immune system demonstrate it is possible for the brain to influence the immune system (Maier et al., 1994;). Just as psychological events can trigger SNS and HPA axis responses, which then influences the immune system, sustained activation of the immune system results in sickness behaviour and depressive symptoms (Dantzer, O'Connor, Freund, Johnson, & Kelley, 2008). These bidirectional paths between the SNS, HPA axis and immune system demonstrate a connection between the experience of stress, psychological distress, and physical disease (Wrona, 2006).

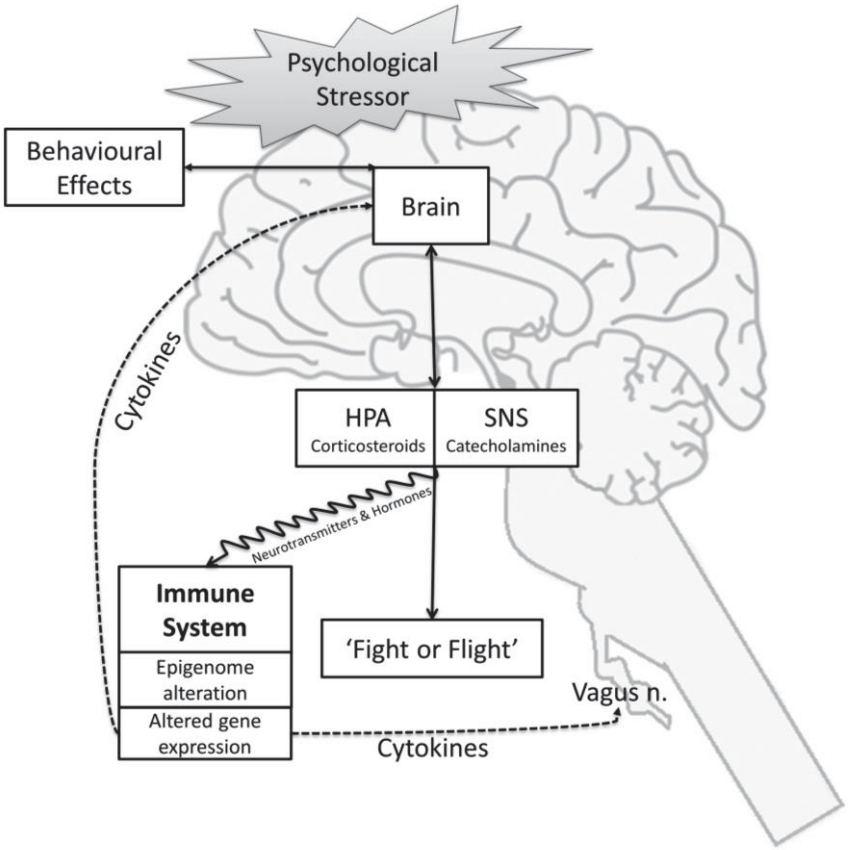

Figure 3. Simplified depiction of nervous system and immune system interaction.

Just as there is a link between stress and distress, there is a link between relaxation and wellness. The relaxation response is the body's natural antagonist to the fight or flight response. Elicitation of the relaxation response requires four elements: a quiet environment; an object to dwell upon; a passive attitude; and, a comfortable position (Benson, Greenwood, & Klemchuk, 1975). Successful attainment of the response induces parasympathetic nervous system activation as measured by decreased heart rate, breathing, and metabolism (Benson et al., 1975). Accordingly, it has been proposed that a reduction in SNS activity follows regular elicitation of the relaxation response (Benson et al., 1975). It is also possible that the relaxation response may be due to stimulation of the vagus nerve. The vagus nerve is

one of the main channels of parasympathetic nervous system communication (e.g., stimulates digestion, airway constriction, decrease heart rate, and modifies attention; Friedman & Thayer, 1998). Evoking the relaxation response though mind-body practices may then influence multiple systems, including behavioural, neural, endocrine, and immune processes.

1.4.1 Psychoneuroimmunology

The mind-body connection is a relatively new concept in Western psychotherapy and clinical practices. Historically, the West has followed the dichotomy poised in Descartes' philosophy of mind-body dualism. In the field of psychotherapy, the focus has traditionally been on the cognitive factors of behaviour, rather than on the physical aspects; hence, the 'talking cure' was propagated by Freud (Salmon, Lush, Jablonski, & Sephton, 2009). The consequence of a focus on mind-body dualism was a lack of acceptance of movement therapies in the West. In contrast, Eastern traditions have long considered that the mind and body are connected as part of a continuum of communication, resulting in establishment of a number of somatically-based practices, such as yoga and tai-chi, in the East.

As Western science progresses, the dichotomy between mind and body is disintegrating as multilevel multidisciplinary areas of investigation are emerging. At the intersection of psychology, immunology, and neurosciences, the field of psychoneuroimmunology (PNI) posits that "bidirectional pathways connect the brain and the immune system and provide the foundation for neural, endocrine, and behavioural effects on immunity" (Ader, 2001, p. 94). As discussed earlier, investigation into the relationships between various behavioural, neural, endocrine and immune processes has produced numerous examples of bidirectional

communication between the mind and body. Stress can lead to changes in immune function resulting in increased susceptibility to immunologically-mediated diseases. It is now accepted that coronary heart disease, cancer, lung ailments, accidental injuries, cirrhosis of the liver, and suicide, are among the burdens of which stress is a major contributor (Selye, 2013). Such findings show that the immune system interacts dependently with other systems in the body and with psychological factors (Ader & Cohen, 2001).

1.4.2 The Field of Epigenetics

Towards the end of last century increasing attention was given to understanding the role of an individual's genetic/epigenetic signature (Holliday, 2006). Research investigating genetic risk factors in mood disorders, such as depression, has furthered understanding of the mind-body communication system (Caspi, et al., 2003). The focus was primarily on exploring the link between deoxyribonucleic acid (DNA) and predisposition to psychopathology. DNA is contained within the nucleus of cell bodies and provides the information needed for development and function of living organisms. DNA can be broken down into specific codes that guide production of specific proteins; these codes are identified as genes (Parent, Zhang, & Meaney, 2012).

While DNA contains codes for expression of proteins, such as hormones that mediate physiological activity, not all genes are expressed at all times. Specifically, 'silent' genes require a signal to 'turn on' and produce proteins (Parent et al., 2012). This form of gene expression is controlled by epigenomic factors, which includes a number of chemical compounds and proteins that attach to DNA and direct genome activity. Epigenetics refers to changes in gene-expression that do not alter the

underlying DNA sequence, as the prefix ‘epi’ means over, or in addition to (Rivera & Bennett, 2010). Epigenetic regulation is critical to healthy development in utero and tissue differentiation, and can be stimulated by environmental exposure, such as chemicals, disease, ageing, and psychological stress (Parent et al., 2012). One type of epigenetic modification is histone modification where DNA wraps around proteins (histones) for compaction in cells. Modification takes place when proteins bind an epigenetic factor to the histone ‘tail’, which tells other proteins if the region of DNA can be accessed, thus transcribed and translated (Holliday, 2006). Another modification is DNA methylation, which refers to methyl tagging of DNA at the 5’ cytosine residue in cytosine rings located at specific sites called CpG dinucleotide. DNA methylation typically repress gene expression by preventing access to transcription factors (Holliday, 2006; Mathews & Janusek, 2011; Razin, 1998). It is this ‘flagged’ DNA that provides protein coding information about what genes will (or will not) be transcribed into messenger ribonucleic acid (mRNA) through gene expression (Mathews & Janusek, 2011), which is subsequently translated into proteins, as shown in Figure 4. Consequently, as DNA within each cell is practically identical, it is the epigenome, rather than the genome, that determines differentiation of various cells and cellular physiological responses.

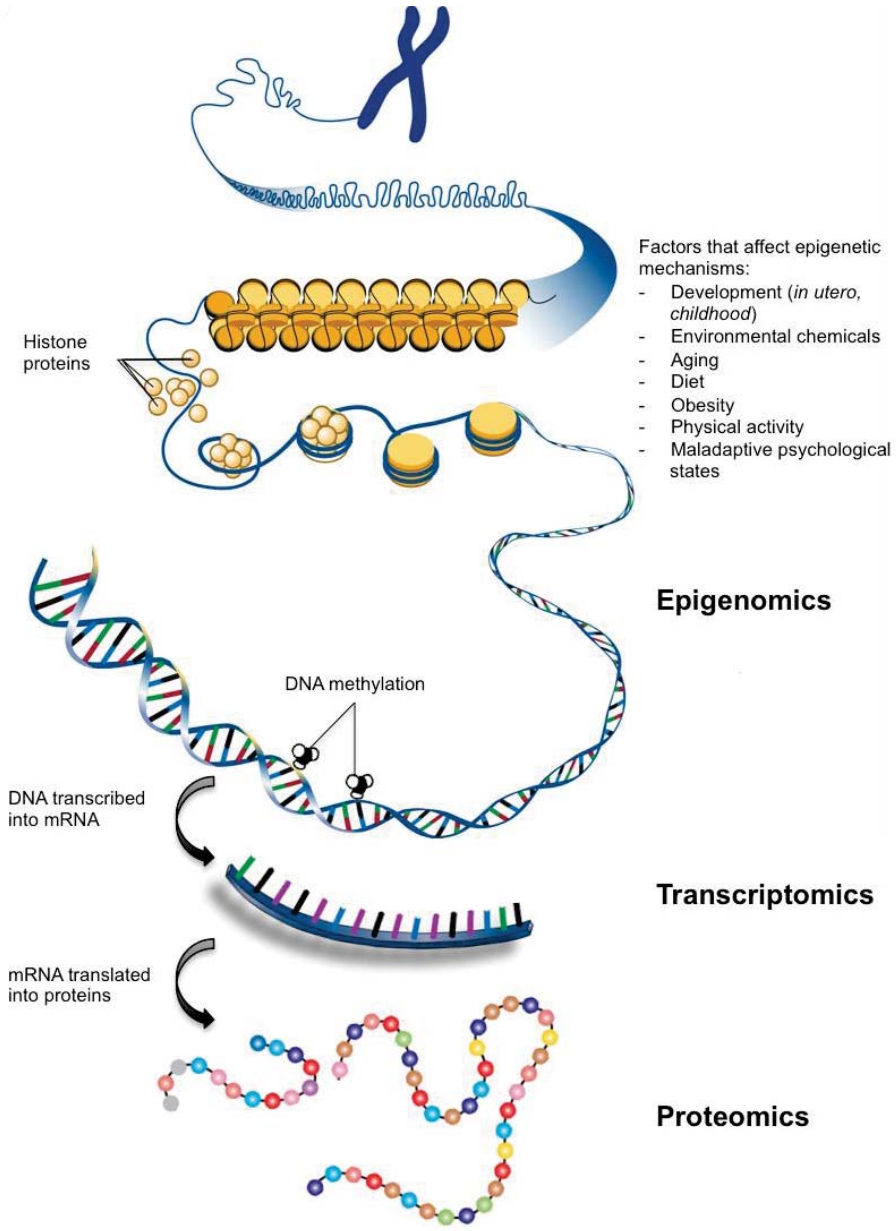

Figure 4. Scientific illustration of cellular processes affected by epigenetic mechanisms. Compilation of images sourced from Darryl Leja of the National Human Genome Research Institute, www.genome.gov. Accessed May 7, 2016.

Epigenetic alterations can affect an individual's health, which may result in physical disease (e.g., cancer, diabetes, or autoimmune) and/or psychiatric illness. DNA methylation change is the most studied epigenetic biomarker of psychological distress (Docherty & Mill, 2008; Sananbenesi & Fischer, 2009; Toyokawa, Uddin, Koenen, & Galea, 2012; Unternaehrer et al., 2012), and has been the focus of a recent

review reporting a link between exercise and altered DNA methylation (Horsburg, 2015). Evidence in support of genomic effects of mind-body therapy remains largely undeveloped, although at least one cross-sectional study demonstrated changes in DNA methylation patterns associated with long-term practice of tai chi (Ren et al., 2012). Another study showed a reduction in expression of histone deacetylase genes and decreased expression of proinflammatory genes following an eight-hour meditation session, however DNA methylation was not investigated in this study (Kaliman et al., 2014). For these reasons epigenetic changes may be useful as biomarkers to evaluate responses to mind-body therapies and the relationship of such therapies with the immune system.

1.4.3 How Stress Connects Psychoneuroimmunology and Epigenetics

Stress evokes physiological responses, including altering nervous, endocrinological, and immune system functions (Eskandari & Sternberg, 2002) which are explored in PNI. It has also been demonstrated that the environment, including stressful psychosocial experiences, can have epigenetic consequences that impact on psychological outcomes and disease vulnerability (Feinberg, 2008; Foley et al., 2009; Gluckman, Hanson, Cooper, & Thornburg, 2008; Gluckman, Hanson, & Pinal, 2005; Handel, Ebers, & Ramagopalan, 2010). This relationship between environment, mind and body captured by epigenetics demonstrates that analysis of epigenetic molecular processes contributes to understanding the complex PNI networks and indicates a bidirectional relationship between these two fields (Mathews & Janusek, 2011). It appears that molecular changes to the epigenome is communicated through neurotransmission, with the post-synaptic gene evoking a molecular cascade of biochemical activity that can last for days, weeks, or a lifetime (Stahl, 2013).

Alterations in the ‘stress-response’ cellular communication process may be mediated in part by cytokines, so it is possible that stress induced changes in cytokines may be associated with epigenetic variation which can impact gene expression, and in turn, protein (e.g., cytokine) expression. All of the cellular processes involved in the ‘stress-response’ cascade are considered candidates for modification by drugs; however, non-chemical interventions, such as psychotherapy, have also demonstrated promise (Stahl, 2013). This indicates mind-body interventions may provide therapeutic agency.

Another mind-body link between PNI and emerging epigenetic research is the concept that individual differences in the body’s ‘stress-response’ influence how people react to stress. For example, physiological stress reactivity of the HPA axis, and vagal tone, is suggested to be epigenetically mediated by prenatal and childhood environments (Propper et al., 2008; Weaver, Meaney, & Szyf, 2006) and may be considered conceptually in terms of adaptive change, which then contributes to the body’s allostatic load (Mathews & Janusek, 2011). However, some unfavourable epigenetic developments may be reversible and can have an important role in an individual’s stress response. Thus, an individual’s reaction to stress may be conceptualised in two ways: self-regulation as dictated epigenetically through epigenetic modification, and through immunological changes instigated by the physiological systems PNI captures. Equally, an intervention that attenuates a stress response may be conceptualised in both ways, thus the call for investigations linking epigenetics and PNI in conditions of psychosocial stress and behavioural factors (Mathews & Janusek, 2011). Essentially, there is an increased need for mind-body interventions and models of healthcare conducive to this need.

1.4.4 Biopsychosocial Model of Healthcare

The biopsychosocial model of healthcare presently endorsed by the American Psychological Association focusses on the whole person (Kersting & Association, 2005) and was previously argued for adoption within clinical psychology (Gilbert, 1995). This model is designed to integrate physical and mental health, rather than dichotomise the two, as was the tendency in the past. With this paradigm shift, Eastern somatically-based practices, such as yoga, are becoming more prevalent in the West. Specifically, Western medical sciences and yogic practices now share common goals in achievement of an individual's best physical and mental health and a model of mind-body connection.

1.5 Yoga

Yoga continues to be practiced and accepted in India's healthcare system; however, acceptance into mainstream Western healthcare settings faces challenges due to cultural, spiritual, and social origins in India (Salmon et al., 2009). As with other mindfulness-based interventions, the incorporation of yoga into empirical clinical psychology requires scientific conceptualisation, which entails separation from religious and spiritual traditions from which yoga was derived (Hayes, 2002).

While formal acceptance into healthcare systems is slow, the practice of yoga is nonetheless becoming increasingly popular in the Western world. Nearly a third of mid-life and older adults in America engage in complementary health approaches (Johnson, Jou, Rhee, Rockwood, & Upchurch, 2016), of which practicing yoga is one of the most popular. Yoga practitioners in the West are reportedly predominantly well-educated, middle-aged females (Birdee et al., 2008; Ding & Stamatakis, 2014; Penman, Cohen, Stevens, & Jackson, 2012). It has recently been reported that yoga is

one of the most popular organised physical activities for Australian females, with over 273,000 women participating in 2009-10 (Australian Bureau of Statistics, 2015). Interestingly, 76% of Australian general practitioners (GPs) consider yoga to be beneficial, though only 55% of GPs report referring or suggesting it to patients in the previous 12-months (Cohen et al., 2005).

Yoga was developed in south Asia sometime between 150 and 500 AD (Alter, 2004). Yoga is a holistic approach to an individual's well-being, encompassing physical, spiritual, psychological, and social dimensions. The name yoga is derived from the Sanskrit root *yuj*, which means to attach and yoke, to concentrate on, or to create union (Iyengar, 1965). Khalsa, Shorter, Cope, Wyshak, & Sklar (2009) define yoga as "a holistic system of mind-body practices for mental and physical health [that] incorporates multiple techniques including meditation, breathing exercises, sustained concentration, and physical postures that develop strength and flexibility" (p. 279). Yoga is defined in the *Yoga Sutras* as "the inhibition of the modifications of the mind" (Taimni, 1961, p. 6). To reach stillness, or peace, of the mind the *Yoga Sutras* prescribe the eight limbs of yoga to be practiced by practitioners/yogis (Brown & Gerbarg, 2009). These limbs are as follows:

1. Attitudes towards others/restraints (yamas),
2. Rituals/self-observances (niyamas),
3. Physical practice of postures (asana),
4. Breathing practice (pranayama),
5. Withdrawal of the senses (pratyahara),
6. Concentration (dharana),
7. Meditation (dhyana), and
8. State of enlightenment (samadhi).

This eight limbs category of yoga is broadly encompassing and often considered a lifestyle. It is not necessarily reflective of what has become known as 'yoga' in the West. Western yoga tends to be the body-oriented practice of '*hatha'* yoga which includes the following limbs: the postures (asana), the breathing (pranayama), and meditation (dhyana). This is considered to be a more physical practice than other forms of yoga, although the key to effective yoga practice still lies in one's focused attention (Iyengar, 1965; Taimni, 1961). A more palatable entry point for the Western individual, *hatha* yoga is a practice comprised of three empirically-based interventions for stress reduction: exercise, breathing, and meditation, which are explored below. For ease of readability 'hatha yoga' as a broad category is referred to as 'yoga' in this thesis. This is additionally pertinent due to confusion that often develops due to a specific style of *hatha* yoga having the same name. Refer to Figure 5, below, for clarification of styles of yoga discussed in this thesis.

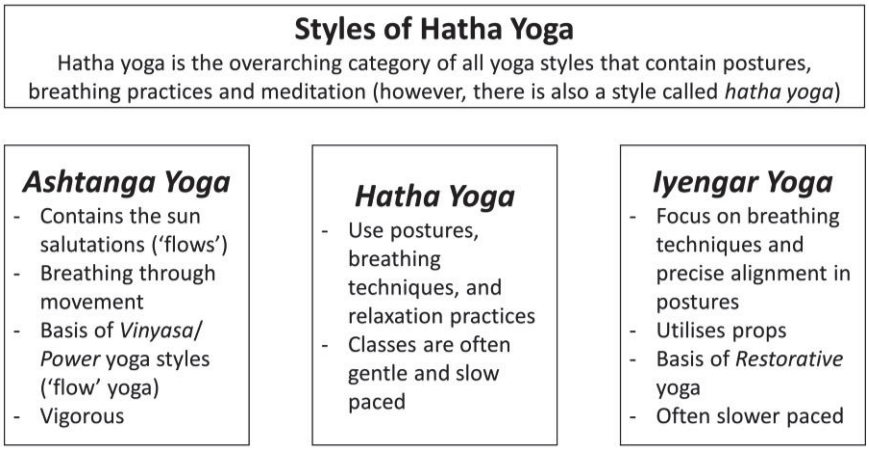

Figure 5. Brief overview of hatha yoga styles commonly evaluated. Developed based on "Prescribing Yoga," by M. Hayes and S. Chase, 2010, Primary Care, 37(1), p. 31-47. Copyright by Elsevier. And "Physical and perceptual benefits of yoga asana practice: results of a pilot study," by V. S. Cowen and T. B. Adams, 2005, Journal of Bodywork and Movement Therapies, 9, p. 211-219.

1.5.1 Physical activity

Extensive research supports the antidepressant and anxiolytic effects of exercise (e.g., Cooney et al., 2013; Kvam, Kleppe, Nordhus, & Hovland, 2016; Landers & Petruzzello, 1994; Petruzzello, Landers, Hatfield, Kubitz, & Salazar, 1991; Rebar et al., 2015), as well as improving well-being, cognitive functioning and physical health (Carek, Laibstain, & Carek, 2011). Stress resilience ensuing from exercise is also characterised by psychobiological effects (Salmon, 2001). Preliminary evidence indicates that exercise modifies DNA methylation associated with inflammation (Horsburgh, Robson-Ansley, Adams, & Smith, 2015), although this has not been studied in relation to yoga specifically. Yoga has been found to demonstrate at least equal, and sometimes superior, efficacy at improving health-related outcome measures that range from flexibility and balance, to improved kidney function, decreasing anxiety and stress, and improvement in mood and well-being (Ross & Thomas, 2010).

1.5.2 Yogic breathing

Breath regulation helps yogis balance sympathetic and parasympathetic nervous systems, anchor attention, and guide the flow of their asanas (Salmon et al., 2009). As the yogis' breathing becomes deeper and slower, they are reportedly able to 'breath into' their asanas, finding deeper levels, as opposed to when they are tense and breathing is more shallow (Salmon et al., 2009). Thus, regulated deep breathing generates a feeling of relaxation and general well-being. Interventions focused on yogic breathing have demonstrated improvements in well-being (Jyotsna et al., 2012) and decreased measures of stress, anxiety and depression (Brown & Gerbarg, 2009; Kjellgren, Bood, Axelsson, Norlander, & Saatcioglu, 2007).

1.5.3 Meditation

Meditation has been reported to improve practitioners' well-being and decrease psychological stress and distress (Goyal et al., 2014; Marchand, 2012). One particularly well researched style of meditation is mindfulness-mediation. Mindfulness-based interventions (MBIs) are flourishing in the field of clinical psychology, supported by exponential growth in scientific literature that demonstrates clinical efficacy in enhancing positive health outcomes (Cullen, 2011; Demarzo, Cebolla, & Garcia-Campayo, 2015; Salmon et al., 2009), particularly for reducing anxiety, depression, and stress (Khoury et al., 2013). Mindfulness is defined as "the state of being attentive to and aware of what is taking place in the present" (Brown & Ryan, 2003, p. 822). Yoga is similarly defined as an attempt "to create a state in which we are always present – really present – in every action, in every moment," (Desikachar, 1999, p. 6). For this reasons, yoga is considered a type of 'mindful exercise' (La Forge, 2005).

1.5.4 Mindful Exercise

Classification as a form of mindful exercise requires expression of five factors thought to enhance the practitioners' mind-body connection: 1) meditative/contemplative, 2) proprioceptive awareness, 3) breath-centring, 4) anatomic alignment, such as spine, trunk and pelvis or proper physical form, 5) energy-centric. Practicing mindfulness increases an individual's 'mindfulness skills', and is often measured by questionnaires, as are many constructs in clinical psychology. An individual's predisposition or willingness to sustain a state of mindfulness may differ along with the individual's capacity to achieve this form of awareness which may be enhanced or dulled by various inter- and intra-personal

factors (Brown & Ryan, 2003). Accordingly, it follows that engagement in yoga practice may affect an individual's mindfulness skill through the five factors of mindful exercise mentioned above (La Forge, 2005).

A number of studies support that yoga practice is related to mindfulness enhancement (Field, 2011). Positive effects on mindfulness were demonstrated in an eight week randomised waitlist controlled yoga intervention (Shelov, Suchday, & Friedberg, 2009). This is corroborated by a cross-sectional study which examined the effect of duration of yoga practice on participant levels of mindful attention (Brisbon & Lowery, 2011). Brisbon and Lowery (2011) found that longer duration of practice correlated with higher levels of mindfulness and that higher levels of stress were correlated with lower levels of mindfulness. However, causation cannot be inferred in cross-sectional studies and the potential mediation effect of mindfulness on perceived stress has since been explored in a pilot study examining a non-randomised, four month yoga intervention with young adults (Gard et al., 2012). Gard et al. (2012) revealed that the effect of group was not mediated by mindfulness. Thus, mindfulness may be conceptualised as a clinical outcome measure in and of itself. As mindfulness-based interventions have demonstrated enhanced clinical and non-clinical outcomes (Gotink et al., 2015), it is suggested that engagement in yoga practice may have comparable outcomes However, further studies are needed to scientifically validate the practice of yoga from the standpoint of the Western healthcare system (Salmon et al., 2009).

1.5.5 Yoga as an Evidence-Based Intervention

A number of small randomised controlled trials (RCTs) indicate yoga may be a successful therapy for a wide range of conditions, including psychological ailments

(e.g., stress, anxiety, and depression) and physiological health problems (e.g., pain syndromes, cardiovascular conditions, and immune conditions). Additionally, there has been investigation into the potential of yoga to improve well-being and quality of life (Field, 2011; Büssing, Michalsen, Khalsa, Telles, & Sherman, 2012). Considering 'stress epidemic' concerns mentioned earlier, it is promising that research exploring this mind-body intervention is growing exponentially. However, to date, the literature is inadequate from a methodological perspective (Field, 2011; Elwy, Groessl, Eisen et al., 2014)[1].

The methological limitations often reported in yoga-based mind-body intervention research include small sample size insufficient to obtain statistical power; a lack of standardised protocols in conducting interventions making studies difficult to replicate; heterogeneity of interventions conducted ranging from individual or group settings, dynamic to gentle, varying sessions (daily or weekly), and differing duration; and a general lack of the use of biological markers considered to be more objective than psychological measures alone. Notwithstanding these limitations, the mind-body effects demonstrated by varying yoga interventions are promising indicators of potential utility to address decreased well-being, and increased levels of stress and distress being reported (Cassey & Ling, 2014). Thus, closer evaluation of the literature which has explored mental health outcomes is warranted.

[1] It is important to note that this thesis is not exploring practices of transcendental meditation and MBIs, like mindfulness based stress reduction (MBSR). While such practices do have a yoga component included and share a philosophically common thread, they are more directly focused on meditative elements than *hatha* yoga practice.

1.6 Yoga and Mental Health

One of the most common reasons for an individual to engage in a complementary therapy such as yoga is reportedly due to experiencing a mental health problem, such as depression, anxiety, or stress (Pilkington, Kirkwood, Rampes, & Richardson, 2005). As with the literature on yoga as a whole, research examining the utility of yoga as a psychotherapeutic intervention, or adjunct treatment for mental health, has methodological limitations. Varying populations (i.e., clinical and community), interventions (e.g. dynamic vs gentle, daily vs weekly), and outcomes measured is highlighted as contributing to the complexity of interpreting findings in this area. To this end, this section is divided according to mental health outcomes investigated (i.e., well-being, psychological distress, and perceived stress)[2], although it is recognised that it is common for yoga trials to explore more than one outcome measure. The focus here is primarily directed towards methodologically strong studies; however, in order to develop a clear depiction of the breadth of research relevant to this thesis, other studies are included when necessary.

1.6.1 Well-being

Well-being is a longstanding concept that integrates mental health (mind) and physical health (body; Dunn, 1973), often used to explore how an individual perceives his or her own life (Diener & Seligman, 2004; Frey & Stutzer, 2010). Well-being is associated with many health, social, and economic outcomes (Ostir, Markides, Black, & Goodwin, 2000; Tov & Diener, 2008). Participation in yoga

[2] Psychiatric disorders beyond those commonly conceptualised as psychological distress (i.e., anxiety and depression) are not explored here. If the reader is interested he or she is referred to Cabral, Meyer, and Ames (2011) review of yoga's use as an adjunct therapy for major psychiatric disorders.

practice has been found to promote well-being in community populations, such as British university staff (Hartfiel, Havenhand, Khalsa, Clarke, & Krayer, 2011), German women reporting distress (Michalsen et al 2005, 2012), and older persons (Bonura, 2011). However, other studies involving medical populations, such as cancer patients (Lin, Hu, Chang, Lin, & Tsauo, 2011), have yielded mixed results. A meta-analysis comprised of 10 RCTs exploring the effects of yoga on psychological health, quality of life, and physical health of patients with cancer demonstrated yoga was associated with improvements in anxiety, depression, and stress levels, but was not related to a significant change in quality of life, although a small positive effect was seen (Lin et al., 2011). These results need further verification, as small sample sizes, poor methodology, and lack of consistency in outcome measures used (e.g. positive and negative affect were often explored as surrogates for well-being) were reported as limitations of the studies.

Improvement in reported well-being has also been demonstrated in 'medically stable' (i.e., not having limitations from symptoms) heart failure patients who took part in a *hatha* yoga intervention (Pullen et al., 2010). In this randomised control trial the yoga group also demonstrated significantly lower inflammation following a two month period of yoga practice. Interestingly, in heart failure patients, whose symptoms resulted in activity impairments ranging from 'marked limitations' to 'no limitations', yoga was found to improve inflammatory markers (IL-6, and high-sensitivity [hs]CRP), but did not significantly improve well-being, though significance was within the 'trend range' (Pullen et al., 2008). This may indicate that changes in inflammation precede changes in perceived well-being, or it could be reflective of the impact stress (symptoms of illness) has on perceptions of well-being.

Well-being has been theorised to have both a cognitive component (i.e., satisfaction with life as a whole and various domains) and an affective component (Diener, Suh, Lucas, & Smith, 1999). Affect is the expression of emotions, such as being excited or proud (positive), or being irritable or scared (negative). Positive affect has been called the 'hallmark of well-being' and has been found to mediate a relationship between happiness and success (Lyubomirsky, King, & Diener, 2005). Negative affect co-occurs with chronic stress and has adaptive significance in prompting motivation and action to address a stressor (Folkman & Moskowitz, 2000). Both positive and negative affect have been explored in the context of short-term effects of yoga practice. For instance, positive affect increases pre-to post-restorative yoga practice in beginner and advanced practitioners (Kiecolt-Glaser et al., 2010). Interestingly, a decrease in negative affect was also noted, but this decrease was more substantial for beginners (Kiecolt-Glaser et al., 2010) indicating differential affective states between the two groups. A similar pattern was reported in a large trial evaluating a week long yoga intervention for youth which involved a two hour *hatha* yoga class in the morning coupled with a two hour lecture in the evening (Narasimhan, Nagarathna, & Nagendra, 2011). In light of testing being conducted on the final day of the trial and lack of a control group, these results may point to the immediate effects of yoga practice. Decreased negative affect following *hatha* yoga practice was also demonstrated in healthy undergraduate students by West Otte, Geher, Johnson, and Mohr (2004). Interestingly, no difference in positive affect was demonstrated, yet an increase in positive affect was negatively correlated with salivary cortisol (West et al., 2004), indicating a relationship between positive mood state and the body's homeostasis, which was not affected by 90-minute *hatha* yoga

practice. It appears that an immediate benefit to mood follows yoga practice, though both positive and negative affect are not necessarily equally impacted.

1.6.2 Psychological Distress

As described in detail previously, psychological distress is an emotional state characterised by the experience of high levels of anxiety and depressive symptoms (Andrews & Slade, 2001; Kessler & Mroczek, 1994). A number of studies have explored the relationship between yoga and various elements of psychological distress in differing populations, ranging from breast cancer patients (Rao et al., 2015; Vadiraja et al., 2009), to community women reporting distress (Michalsen et al 2005, 2012) and mildly depressed university students (Woolery, Myers, Sternlieb, & Zeltzer, 2004), to those with treatment resistant depression (Uebelacker, Tremont, et al., 2010).

Pilkington et al. (2005) reviewed RCT trials that examined yoga as a treatment for varying severities of depression (e.g. symptoms to clinical diagnosis). Despite some cautions about sample size which ranged from 10-25 per group, it was concluded that yoga is potentially beneficial and can lead to reductions in symptoms of anxiety and depression. These findings were similarly supported by Uebelacker et al. (2010) who included eight trials in their review. Their main concerns regarding interpretation were that: (a) trials explored various styles of yoga (e.g., passive vs. dynamic) and little is known about the individual efficacy of different yoga styles, which has been noted to hamper the use of meta-analysis (Büssing et al., 2012; Cramer, Lauche, Langhorst, & Dobos, 2013); and (b) trials have not clearly differentiated between clinical diagnosis of major depressive disorder and depressive symptoms, thus non-clinical populations might expect diminished return when

compared to outpatient populations. Cramer et al. (2013) has since concluded, based on 12 RCTs, that there is moderate evidence for utilising yoga as an auxiliary treatment for those with depressive disorders and elevated depression levels. However, high-quality methodological trials exploring clearly defined depressive populations are warranted to generate greater understanding of the relationship between yoga and improvements in depressive symptoms (Cramer et al., 2013; Büssing et al., 2012).

While yoga has proved efficacious for those with elevated symptoms of depression, results are not as clear in those reporting less-severe symptoms (Cramer et al., 2013). For instance, a cohort of mildly depressed university students (N = 28) randomly assigned to an *Iyengar* yoga intervention demonstrated a reduction in depressive and anxiety symptoms over a five week period (once-weekly class), while the control group did not show this improvement (Woolery et al., 2004). Similar results were reported in a three month, three-armed RCT (yoga group with 12 sessions, yoga group with 24 sessions, and a waitlist control), which explored the effects of an *Iyengar* yoga class in a larger sample (N = 72) of women reporting high levels of perceived stress (Michalsen et al., 2012). The yoga groups showed improvements in measures of depression, anxiety, and perceived stress, although no difference was reported between those assigned to weekly, or twice-weekly, yoga class intervention. Michalsen et al. (2013) suggested the lack of dosage effect was due to limited compliance in the twice-weekly group. However, a mid-length *Iyengar* yoga RCT that randomly assigned 65 women with mild depression and anxiety to a two month intervention (twice-weekly classes) or a waitlist control did not demonstrate improvement in depression scores for either group, although an improvement in anxiety was demonstrated in the yoga group alone (Javnbakht &

Hejazi Kenari, & Ghasemi, 2009). While these findings highlight the difficulty of interpreting the effect of yoga on mood, they support yoga as an anxiolytic.

The anxiolytic effect of yoga has been highlighted in systematic reviews exploring the effectiveness of yoga for anxiety and anxiety disorders (Kirkwood, Rampes, Tuffrey, Richardson, & Pilkington, 2005; Li & Goldsmith, 2012). However, methodological caveats exist; i.e., sample sizes, lack of randomisation and utilisation of a control group. Additionally, anxiolytic effects of yoga are not always demonstrated in adquently powered RCTs. A ten week *hatha* yoga intervention for a community population of women reporting mild to moderate stress levels did not demonstrate improved anxiety status when compared with a relaxation intervention as an active control (Smith, Hancock, Blake-Mortimer, & Eckert, 2007). Though the anxiety levels of both groups decreased following intervention, there was no control group against which to explore the effect of time; nor was adherence to weekly practice controlled for in the Smith et al. (2007) trial. These results indicate difficulty interpreting findings of non-actively controlled studies, though active controls allow for 'noninferiority trials' of yoga (i.e., test if yoga intervention is as beneficial as established treatment).

Active control groups (counselling) have been used in a number of randomised trials to evaluate the effect of six week gentle yoga interventions on symptoms of psychological distress in post-operative breast cancer (Banerjee et al., 2007; Rao et al., 2015; Vadiraja et al., 2009), with all demonstrating a positive effect on mood in the yoga group. Vadiraja et al. (2009) also reported improvements in anxiety, perceived stress, positive and negative affect, and quality of life. However, while Banerjee et al. (2007) also reported improvements in anxiety symptoms, they did not find an effect for perceived stress. The lack of consistent evidence of yoga

intervention modulation on outcomes of psychological stress and distress is further highlighted in a large scale RCT exploring 200 breast cancer survivors assigned to a 12 week, twice-weekly, 90-minute *hatha* yoga class or a waitlist control (Kiecolt-Glaser et al., 2014). The Kiecolt-Glaser et al. (2014) trial found no between group difference in depressive symptoms post-treatment, or at three month follow-up, but this study did not include measures of anxiety or stress. Interestingly, while few researchers have examined the underlying mechanisms through which yoga may influence mind-body (Uebelacker, Epstein-Lubow & Gaudiano et al., 2010), the trials by Banerjee et al. (2007), Vadiraja et al. (2009), and Kiecolt-Glasser et al (2014) demonstrate plausible biological mechanisms in breast cancer patients.

Varied biological mechanisms have been explored in yoga intervention trials with breast cancer patients. Kiecolt-Glasser et al. (2014) found less lipopolysaccharide-stimulated cytokines (IL-6, IL-1 Beta, and TNF) in the yoga group at the three month follow-up for those practicing at least 29 minutes per day showing the greatest effect, which was not immediately evident following conclusion of yoga intervention, suggesting that for breast cancer survivors a longer duration of regular practice is needed to see immune changes that are not necessarily reflected by mood. In contrast, at conclusion of the yoga intervention benefits were demonstrated by Banerjee et al. (2007), with less radiation-induced DNA damage in those practicing yoga, indicating yoga attenuated some of the allostatic load breast cancer treatment may place on the body. Additionally, decreased salivary cortisol levels were reported with yoga (Vadiraja et al., 2009) which supports the theory that yoga induces decreased stress reactivity through the HPA axis (Uebelacker et al., 2010), consistent with the hypothesis of relaxation response evocation (Benson et al., 1975). Another study involving a clinical population observed no decrease in cortisol for women

suffering rheumatoid arthritis who partook in a non-randomised ten week yoga intervention although decreased symptoms of depression and perceived pain were noted (Bosch, Traustadottir, Howard, & Matt, 2009). This inconsistency of biochemical and physiological markers has been addressed more specifically by Li and Goldsmith (2012) in a review exploring the effects of yoga on anxiety and stress in clinical and non-clinical populations. Their review reports on ten trials that measured cortisol levels, and found that most showed no yoga practice effect. However, these authors highlight the difficulty in measuring cortisol, due to levels fluctuating through the day, and report a number of caveats similar to trials exploring depression; namely, limited sample sizes, lack of randomisation and utilisation of a control group.

1.6.3 Perceived stress

As defined earlier, psychological stress is the subjective evaluation of threat, and perceived stress is conceptualised as the degree to which an individual views their life as uncontrollable, unpredictable and overwhelming (Cohen, Kamarck, & Mermelstein, 1983). A recent review by Sherma (2014) reported that yoga has potential as an effective stress management intervention, which is supported by the review of Li and Goldsmith (2012). Strong support for the efficacy of yoga as a stress management intervention is indicated by reports of improvement in stress symptoms in actively controlled trials. Specifically, *Ashtanga*-based yoga proved more effective than physical exercise for decreasing stress in a large (N = 112) RCT of women undergoing menopause (Chattha, Raghuram, Venkatram, & Hongasandra, 2008), a gentle yoga was found to be more effective than standard prenatal exercises in a large (N = 90) RCT of pregnant women (Satyapriya et al., 2009), *hatha* yoga was found to

be as effective as relaxation in a large (N = 119) RCT of community women (Smith et al., 2007), and *Kundalini*[3] yoga was found to be as effective as cognitive behaviour therapy (CBT) in a smaller (N = 33) RCT (Granath, Ingvarsson, von Thiele, & Lundberg, 2006). These trials indicate yoga is at least as effective as established stress-reduction interventions.

Yoga's use for stress management has also been explored physiologically and in waitlist controlled trials. In addition to positive changes in psychological perception of stress, women in the trial conducted by Satyapriya et al. (2009) showed lower heart rate variability, a measure of autonomic nervous system function, relative to a relaxation group. Decreased perceptions of stress following participation in yoga intervention have been reported in other female populations including post-operation breast cancer patients (Banerjee et al., 2007), and community populations reporting distress (Michalsen et al., 2005; Michalsen et al., 2012). On the other hand, a 10 week intervention in a population of metabolic syndrome patients observed no difference in stress levels of those who participated in restorative yoga intervention relative to a waitlist control (Cohen, Chang, Grady, & Kanaya, 2008), which highlights inconsistency in effects. This inconsistency may be associated with the various populations explored or the interventions conducted.

As previously noted various styles of yoga have been explored, yet little is known about individual efficacy of yoga styles (Uebelacker, Epstein-Lubow, et al., 2010). A small (N = 26) pilot study of healthy adults explored the longitudinal effect of yoga practice in general, as well as specific practice of *Ashtanga* (a dynamic style) and *hatha* yoga (a gentle style; Cowen & Adams, 2005). Measures were taken before

[3] The exploration of yoga and psychotherapy by Granath et al. (2006) used a style of yoga heavily grounded in spiritual concepts concerning movement of energy through chakras in the spine, which is outside the scope of this review.

commencement of a six week, twice-weekly yoga class (for each of the two styles), and at conclusion. In general, yoga practice showed an association with decreased perceived stress levels (Cowen & Adams, 2005). However, on analysis of each group individually, only the *Ashtanga* group demonstrated significant decrease in stress, though the effect for both groups was large. But a small number of observations (n = 9 *Ashtanga*, and n = 8 *hatha* yoga participants) may indicate this study was limited by power. Nonetheless, the significance noted in the *Ashtanga* group may reflect that this yoga style includes sun salutations (a dynamic sequence of postures that 'flow' together) which may improve cardio-respiratory fitness, while more static postures performed in *hatha* yoga may not result in the same level of cardio-respiratory fitness (Hagins, Moore, & Rundle, 2007). Thus, *Ashtanga* yoga may affect perceived stress by evoking an aerobic component involving increased heart rate and metabolic expenditure which is linked with an anxiolytic effect (Petruzzello et al., 1991). However, even when yoga does not increase heart rate and metabolic expenditure there are still links to positive health benefits, indicating other mechanisms, such as 'relaxation response', are involved in these benefits (Hagins et al., 2007).

In addition to studies reporting improvements in perceived stress following participation in multiple yoga classes, immediate effects of yoga on stress levels have been explored. West et al. (2004) examined perceived stress by comparing participation in a 90-minute *hatha* yoga class to a dance class and biology lecture as controls. These authors found that yoga and dance class participation were largely associated with decreased perceived stress. Salivary cortisol levels measured before and after intervention decreased after yoga class participation, increased after dance class, and did not change after the biology lecture. This indicates that yoga may differentially impact the HPA axis, and that perceived stress is not necessarily

reflective of cortisol levels. As participants were undergraduate students enrolled in African dance, *hatha* yoga, and introductory biology classes there was no allocation to conditions, so it is possible that personality may be a confounding variable on activity choice. Further, this study did not explore effects of continuous practice. However, the effects of a long-term yoga practice have been explored cross-sectionally.

Differences in stress levels and mindfulness were explored in beginner (less than five years' experience, $n = 24$) and advanced (greater than five years' experience, $n = 28$) yoga practitioners (Brisbon & Lowery, 2011). Advanced practitioners demonstrated significantly lower stress levels and higher levels of mindfulness (Brisbon & Lowery, 2011) suggesting that how one perceives stress and utilises mindfulness skills may be associated with long-term yoga practice. Potential changes in perception of stress, or 'stress management', reported with yoga practice have also been investigated in studies designed to explore underlying biological mechanisms.

Difference in biochemical markers between beginner ($n = 25$) and advanced ($n = 25$) yoga practitioners was examined before, during, and after an *Iyengar* yoga class (Kiecolt-Glaser et al., 2010). It was showed that positive affect increased following participation in yoga class, although no differences in inflammatory and endocrine response measures were found. However, advanced yoga practitioners experience less stress-related change to acute stressors than their less experienced counterparts as lipopolysaccharide-stimulated (LPS) serum IL-6 levels were 41% higher in beginners, and beginners were 4.75 times more likely to have detectable levels of hsCRP. Kiecolt-Glaser et al. (2010) suggest that a possible mechanism for potential stress reduction benefits of *Iyengar* yoga practice is minimisation of inflammatory response elicited by stressful stimuli. It is possible that stressors are perceived as less

challenging by advanced yoga practitioners, thus yoga expertise may be a mediating factor of psychological and biochemical changes. While, Kiecolt-Glaser et al. (2010) is limited by cross-sectional study design, it should be noted that serum IL-6 is also discussed in the context of a three month *hatha* yoga intervention in breast cancer patients (Kiecolt-Glaser et al., 2014). In this trial no effect was observed after three months of intervention, but an effect was evident at three month follow-up (six months from baseline). While it is difficult to disentangle the positive effects of yoga as breast cancer patients and community population differ significantly in health-status, it appears probable there is an effect of yoga on markers of inflammation. It is also worth noting that the Kiecolt-Glasser et al. (2010, 2014) studies investigated LPS stimulated IL-6, and not 'native' levels. LPS stimulation may more accurately reflect how the body responds to a pathogen or infection, whereas 'native' non-stimulated levels may more closely represent the natural 'non-invaded' body-state.

1.6.4 Theorised Biological Mechanisms

In general, very little research has examined the underlying mechanisms of various effects of yoga practice (Field, 2011). Evidence indicates that yoga psychologically and physiologically affects stress levels (Cowen & Adams, 2005). The psychophysiological response, or 'relaxation response' (McCall, 2007) of yoga practice has been linked to reduction in sympathetic nervous system tone (Bower, 2005). This effect of yoga was systematically addressed by Innes, Bourguignon, and Taylor (2005) who proposed a two part physiological model of how yoga may elicit a hypometabolic state.

The first part of the two part physiological model proposed by Innes et al. (2005) proposes that slow movement patterns of yoga postures stimulate pressure

receptors which elicit a ‘relaxation response’ via the parasympathetic nervous system. This response is mainly communicated through the vagus nerve which connects cardiac control centres of the brain stem with the sinoatrial node, the heart’s intrinsic pacemaker (Powers & Howley, 2004), thus decreasing heart rate and blood pressure. It has also been suggested that increased vagal activity is associated with reduced cortisol levels (Field, 2011), suggesting a link with HPA activity, as well as turning off production of proteins associated with inflammation, such as TNF. This leads to the second component of the Innes et al. (2005) model which hypothesises that participation in yoga intervention results in less perceived stress and increased feelings of well-being. In turn, a reduction in perceived stress and improved feeling of well-being may decrease reactivity of the HPA axis and SNS (Innes et al., 2005), thereby mitigating the impact of stress on the immune system. The result is a restorative, energy-conservation effect which is normally balanced through parasympathetic activation, in contrast to allostatic load elicited by stress-related responses which may damage body systems. If it is the case that yoga curtails effects of chronically activating the HPA and SNS (McEwen & Stellar, 1993), biomarkers may be used to may be measure potential effects on the autonomic nervous system, HPA axis, and immune system.

This link between mind-body interventions and immune system health has been explored in a small number of gene expression studies. A review by Saatcioglu et al. (2013) reports that gene expression changes observed in response to mind-body interventions mirror those seen with improved responses to environmental stress, particularly in immune cells. However, only three studies were included in the review conducted by Saatcioglu et al. (2013), and none explored body oriented styles of yoga (i.e., *hatha* yoga). However, more recently Bower et al. (2014) demonstrated that a 12

week RCT *Iyengar* yoga intervention for breast cancer survivors (N = 31) resulted in reduced transcription of pro-inflammatory markers and an increase in anti-inflammatory markers. Interestingly, no changes in serum proteins (i.e., CRP or IL-6) were demonstrated, indicating that different 'omics' (proteomics, transcriptomics, epigenomics) may be capturing different mechanisms of yoga intervention impact. While gene expression changes reported in response to yoga practice must be governed by the epigenome, to date, there is an absence of studies examining the epigenetic modification effect of yoga. As discussed earlier, two mind-body interventions, meditation and tai chi, have been evaluated and both demonstrate promising epigenetic alterations (Ren et al., 2012; Kaliman et al., 2014). Thus, it appears that mind-body interventions have a measurable effect at the epigenetic level which warrants further investigation.

1.7 The Proposed Role of Yoga in Chronic Stress

This literature review presents evidence that yoga may contribute to mental and physical health by evoking a bidirectional ('top-down' and 'bottom-up') interaction between the brain, nervous system, and immune system (Taylor, Goehler, Galper, Innes, & Bourguignon, 2010). While a complex network is likely at play, it is proposed that engagement in yoga practice immediately improves mood, perception of stress, and develops mindfulness skills. The cumulative effect of engagement in regular yoga practice may result in improved mindfulness capacities, increased sense of well-being, and decreased perceptions of stress and distress. This may be mediated by decreased sympathetic nervous system reactivity, and/or increased parasympathetic activation, which mediates release of inflammatory cytokines through layered interactions with the HPA axis, immune system, and epigenome,

thereby affecting allostatic load. The literature reviewed above has begun to explore plausible psychological and biological mechanisms through which yoga practice may improve mental health and several studies provide support for the suggestion that yoga evokes a mind-body effect on indices of stress. On the other hand, findings are not always clear as inconsistent results are reported in a range of studies of varying methodological limitations. Accordingly, in advancing understanding of mind-body therapies, such as yoga, researchers should be directed by an empirical framework that provides a template to evaluate underlying mechanisms (Taylor et al., 2010). The research undertaken for this thesis proposes that the framework in Figure 3 provides a basic template to evaluate psychological markers of chronic stress and mental health, and underlying mechanisms of yoga intervention.

In conclusion, high levels of psychological stress and distress and decreased levels of well-being are being increasingly reported in the global community (Cassey & Ling, 2014). In addition to negative impacts on mental health, high levels of stress and distress negatively affect immune system health and contribute to disease susceptibility (Segerstrom & Miller, 2004) through allostatic load placed on the body (McEwen & Stellar, 1993). Consequently, there is a need to find alternative, or adjunct, treatments that adequately address the layered effect of stress. Mind-body interventions, such as yoga practice, are increasingly utilised to treat stress and depressive symptoms due to proposed mechanisms which may curtail negative effects of chronic stress. Considering the cost-effectiveness of administering yoga intervention compared to psychotherapy or biomedical interventions this mind-body intervention warrants further exploration. However, current literature has been criticised methodologically and high-quality trials are needed, particularly

standardised interventions conducive to replication needed for research purposes (Yang, 2007).

1.8 Aims of Thesis

The aim of this thesis is to contribute to knowledge of feasible and effective mental health interventions for community populations from a mind-body perspective. In particular, this research was designed to build upon the literature relating to the utility of yoga as an intervention for Australian women reporting chronic stress and psychological distress. This thesis draws on guidelines for community health interventions (Moore et al., 2013) and theories that explore the mind-body connection, such as mindfulness-based interventions, mindfulness-based exercise, and psychoneuroimmunology. The studies in this thesis address specific under-developed areas of research, which are identified in the following section.

1.8.1 Gaps in the Literature and Specific Research Aims

An initial literature search revealed that, although a number of trials evaluating efficacy of yoga as an intervention to improve mental health outcomes have been conducted, the majority are limited methodologically. The specific aims of this thesis were to address three gaps identified in the literature review, namely: (a) the limited use of empirically-based standardised protocols, detailing of yoga intervention conduct, and evaluation of yoga intervention implementation quality; (b) the limited conduct of sufficiently powered clinical trials evaluating mental health and well-being outcomes in community populations; and, (c) the limited use of biological markers to examine mechanisms of yoga intervention effect.

The first gap identified is the lack of standardised yoga intervention protocols (Sherman, 2012). This is important given the complexity and variability of yoga

intervention in regards to possible dose, style, and setting, which indicates there are multiple interacting components possible (Moore et al., 2015; Sherman, 2012). Indeed, recent Medical Research Council (MRC) guidelines for complex interventions suggest that process evaluations are conducted to assess "fidelity and quality of implementation, clarify causal mechanisms and identify contextual factors associated with variation in outcomes" (Craig et al., 2008, p. 3). Until now, formal process evaluation has only been conducted in three yoga intervention trials. One was in the context of a multifaceted wellness intervention at a workplace (Strijk, Proper, van der Beek, & van Mechelen, 2011) and gave limited details about the yoga practice itself. The other two explored very different populations, incarcerated adolescent girls (Harris & Malone, 2014) and adolescent sex offenders (Derezotes, 2000), which limits generalisability to a community population. Thus the first aim of this research was to conduct a process evaluation to facilitate understanding of how yoga interventions are developed and analysed and to provide a template which can be replicated.

The second gap identified is the limited use of appropriately powered randomised-controlled yoga intervention trials evaluating mental health outcomes (Cramer, Lauche, Langhorst, & Dobos, 2013; Field, 2011; Li & Goldsmith, 2012; Patel, Newstead, & Ferrer, 2012). While large trials have been conducted, as detailed in the literature review, the majority have been underpowered. Additionally, a limited number of RCTs have been conducted and the majority of these have used clinical populations (Li & Goldsmith, 2012). As yoga is generally practiced within the community setting, it is important to understand therapeutic benefits it provides to these populations (Sherman, 2012). This is particularly important as it has been observed that Australians are reporting decreased levels of well-being along with

increased levels of stress and distress (Cassey, 2013). Women report higher levels of stress and health issues (e.g., maintaining a healthy lifestyle and mental health; Cassey, 2013). Furthermore, it is noted that middle-aged women are the most common yoga practitioners in Australian communities (Penman et al., 2012). The gender differences in stress and yoga are relevant in considering the aims of this research project as a whole (i.e., biological markers) as sex differences in biological markers are established (El-Maarri, 2007; Rohleder, Schommer, Hellhammer, Engel, & Kirschbaum, 2001). These potential confounds of gender are avoided by the use of a single-sex population. Thus the second aim of this research is evaluation of the effect of yoga intervention on mental health outcomes, in a community population of women reporting high levels of stress and distress, through the conduct of a clinical trial sufficiently powered to detect meaningful effects.

The third gap identified was a lack of biological measures (biomarkers) used to explore mechanisms of effect. This is particularly relevant as Western science considers biomarkers to be more objective than psychological measures alone (Field, 2011). Of the limited studies evaluating yoga using biomarkers to capture 'stress' there is great variability in the measures used (i.e., capturing SNS, HPA axis or immune system activity) and mixed results are reported (Li & Goldsmith, 2012; Ross & Thomas, 2010; Sharma, 2014). For example, cortisol has been widely used to capture HPA axis activity yet demonstrates unclear results; although this is, at least in part, due to fluctuating levels through the day and resultant measurement difficulty (Li & Goldsmith, 2012). While there is no clear biomarker that can directly allow inferences regarding 'stress' or 'distress', this literature review has presented evidence that individuals suffering chronic stress are immunocompromised (Glaser & Kiecolt-Glaser, 2005; Kiecolt-Glaser et al., 1996; Maes et al., 1998). Thus the final aim of this

research is to develop understanding of the biophysiological mechanisms of yoga through an exploratory study of ‘stress system’ biomarkers, principally exploring markers of immune system function.

CHAPTER 2. PAPER 1

2.1 Preamble

This first paper documents the development of a standardised yoga intervention for a community population of middle-aged women. It describes the implementation of a RCT which provides insight into the effectiveness of an intervention. Development of standardised interventions of this nature are importance, not only because of the methodological rigour, but also because it assists with replication (Yang, 2007). Process evaluations of this nature, which document and describe the development of the intervention are important because of the complexity of yoga interventions; in particular, variability in the style of yoga administered; the dose; and, the duration of yoga practice (Field, 2011; Li & Goldsmith, 2012). In support of this approach, evidence suggests that psychological and biophysiological parameters reportedly differ according to the style of yoga practiced (Cowen & Adams, 2005; Hagins et al., 2007). Moreover, as mechanisms for therapeutic change are complex, comprising behavioural, psychological, and biological effects (Uebelacker, Epstein-Lubow, et al., 2010), results of this study help inform interpretation of outcome evaluations discussed in the following chapters. It is worth noting that the decision to include only women in this study was based on consideration of this research project as a whole in terms of providing a more homogenous sample, and in particular, the planned use of biochemical measures. Given research has demonstrated an association between sex and immune function and pro-inflammatory cytokine production (Eikelenboom, Killestein, Uitdehaag, & Polman, 2005; Rohleder, Schommer, Hellhammer, Engel, & Kirschbaum, 2001), it was deemed that greater clarity and statistical power could be achieved by examining the effects of yoga practice on one sex.

Process Evaluation of a Secular Yoga Intervention with Clinical Reductions of Participant Reported Distress

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Submitted manuscript:

Evaluation and Program Planning

Ms Kaitlin N. Harkess (Candidate)

I was responsible for the conception of this study, literature review, conducting the intervention, data collection and analysis, manuscript drafting, preparation. I was also responsible for submission, was corresponding author and was primarily responsible for revisions to the paper. My overall percentage (%) of contribution to the paper is 85%.

Signed: Kaitlin Harkess

Professor Paul Delfabbro, Dr Sarah Cohen-Woods (Co-authors)

We were the supervisors of the research program to which this manuscript belongs. We collaborated with Ms. Harkess in the development of the content and structure of the manuscript and assisted with editing and proof-reading. Ms. Harkess was responsible for the development and administration of the intervention; the

collection of and analysis of data; and, writing this manuscript. Our role was to comment on drafts, make suggestions on the presentation of material in the paper, and to provide editorial input. We also provided advice on responding to comments by the journal reviews and editor. We hereby give our permission for this paper to be incorporate in Ms. Harkess's submission for the degree of Doctor of Philosophy from the University of Adelaide.

Signed: Paul Delfabbro

Signed: Sarah Cohen-Woods

Ms Elli Curtis (Co-author)

I was the research assistant who assisted in coding of data for this study, as well as providing critical feedback on the manuscript drafts Ms. Harkess produced.

Signed: Elli Curtis

Abstract

Objectives

The purpose of this paper was to report the theory underpinning a secular yoga intervention, evaluate the fidelity and quality implementation, and explore the causal mechanisms and contextual factors associated with clinically significant outcomes.

Method

The study consisted of 116 women (35-65 years) reporting psychological distress, of which 60 were randomly allocated to the eight-week yoga intervention (16 classes). Formative and summative process-evaluation data were collected. These included measurement of alliance, intervention satisfaction, attendance, and quantitative feedback.

Results

All 16 yoga sessions were available to the participants, demonstrating acceptable reach and dosage, with an average attendance of 11 classes. Significant improvement was demonstrated for both positive and negative affect, following each class. At eight weeks clinically significant improvement in psychological distress was reported by 43% of participants (33% reported reliable change).

Conclusions

Implementation of a secular yoga intervention in distressed women is feasible and positively received. Immediate effects of yoga on affect were reported, and change in negative affect was associated with treatment outcomes. The clinically significant improvements reported are comparable to psychotherapy, indicating that further outcome evaluation is warranted.

High levels of psychological stress and distress are increasingly reported worldwide (Cassey & Ling, 2014; Australian Bureau of Statistics, 2015), and a causal relationship between high levels of stress and the development of clinical anxiety and depression is established (e.g., Hammen, 2005; MacLeod & Mathews, 1988; Rapee, 1991). Chronic stress has a physiological effect impacting the function of multiple systems, including the gastrointestinal, cardiorespiratory and immune systems (Chrousos, 2009). It further affects an individual's perception of well-being and mental health, which highlights multiple pathways between psychological stress and poor ill-health (Cohen et al., 2007).

Exercise protects against the negative effects of stress on mental and physical health (Zschucke, Renneberg, Dimeo, Wüstenberg, & Ströhle, 2015), including having anti-inflammatory effects (Petersen & Pedersen, 2005) and reducing risk of cardiovascular disease (CVD; Penedo & Dahn, 2005). Exercise is also associated with increased well-being (Hassmen et al., 2000) and has been suggested as a plausible psychiatric intervention for cases where conventional clinical interventions are not appropriate (Salmon, 2001).

In recent years, there has been increased interest in the effect of mindfulness meditation on physical and psychological well-being (e.g., Beauchamp-Turner & Levinson, 1992; Brown & Ryan, 2003; Carmody & Baer, 2008; Keng, Smoski, & Robins, 2011; Marchand, 2012; van den Hurk, Janssen, Giommi, Barendregt, & Gielen, 2010). This has led to the development of a broad range of mindfulness-based interventions (MBIs), including standardised psychological interventions (Chiesa & Malinowski, 2011). In activities such as yoga, the practice of mindfulness is coupled with exercise (La Forge, 2005), which has been theorised to produce additional benefits in some populations (Uebelacker, Epstein-Lubow, et al., 2010).

Yoga has been investigated as a therapeutic intervention for maladaptive psychological states, such as stress, anxiety and depression, with encouraging results (Balasubramaniam, Telles, & Doraiswamy, 2012; Cabral et al., 2011; Chong, Tsunaka, & Chan, 2011; Kirkwood et al., 2005; Louie, 2014; Pilkington et al., 2005). Biophysiological parameters have also been investigated, and positive effects have been observed such as reduced inflammation (Kiecolt-Glaser et al., 2014; Kiecolt-Glaser et al., 2010; Kiecolt-Glaser et al., 2012), cortisol levels (West et al., 2004), blood pressure (BP), heart rate (HR), and body mass index (BMI; Cowen & Adams, 2005; Sujatha & Judie, 2014). However, the benefits achieved are reported to differ according to the style of yoga practiced (Cowen & Adams, 2005).

However, interpretation of the literature in this field is difficult, as the style of yoga administered can vary greatly, from the dynamic physical styles commonly practiced in the West to slower restorative styles (Hayes & Chase, 2010). Furthermore, the 'dose' provided (i.e., frequency and duration of yoga practice) may also impact likelihood of therapeutic change.

In a community population of yoga practitioners, the most common dose reported is a weekly or twice weekly practice in class durations between 60-70 minutes or 90-100 minutes (Penman et al., 2012). Most, or all, yoga practices are reportedly done in classes, with a lack of time being the most common reason reported for ceasing practice (78.1%; Penman et al., 2012). The majority of Western yoga interventions conducted are reflective of community practice, involving weekly or twice weekly classes of 60-90 minutes, with a median duration of eight weeks (Sherman, 2012). Investigation into the 'minimal' or 'optimal dose' for therapeutic change needs to be established, allowing for promotion of maximal effect (Sherman, 2012).

The specific mechanisms for therapeutic change are complex and have been attributed to biological, psychological, and behavioural effects (Uebelacker, Epstein-Lubow, et al., 2010), indicating that multidisciplinary investigation is prudent. The wide breadth of yoga styles, doses, and potential mechanisms of change make it challenging to interpret study findings, specifically in understanding the relationship between the elements of the yoga intervention conducted and the outcomes reported. This highlights the importance of monitoring and documenting intervention implementation; namely, conducting a process evaluation (Saunders, Evans, & Joshi, 2005).

Process Evaluation

Process evaluations provide insight about an intervention's success or failure through monitoring and documentation of its implementation (Saunders et al., 2005). While randomised controlled trials (RCT) are highly regarded methods to measure the effectiveness of an intervention, they do not provide information to enable replication of the intervention, information on the fidelity and quality of implementation, or the mechanisms of impact that would inform whether the outcomes can be replicated (Moore et al., 2015). It is argued that a clear understanding of why an intervention was, or was not effective, is necessary to truly test its impact, as well as to furthering the development of effective interventions that translate to real world settings and thus improving the health and well-being of our communities (Durlak & DuPre, 2008). Unfortunately, evaluation of interventions is not thought to reach stakeholders adequately, and it has consequently been recognised as a priority in behavioural medicine by the Medical Research Council (MRC; Craig et al., 2008), with guidelines for conducting evaluations of interventions effectively being subsequently discussed (Moore et al., 2013). At current, there are few published mental health interventions

that have documented and evaluated implementation, and despite recommendations (Sherman, 2012), a limited number of yoga studies plan systematic documentation, or evaluation of the intervention delivered and received (Chen, Tseng, Ting, & Huang, 2007; Skoro-Kondza, Tai, Gadelrab, Drincevic, & Greenhalgh, 2009).

Study Aims

This paper details the design of a yoga intervention and reports on the process evaluation of a randomised waitlist controlled trial in a community population of distressed women who reported to be chronically stressed, known as the Yoga for Stress intervention. The processes for acquiring qualitative and quantitative data are delineated along with procedures for summarising and presenting the data. Interpretation of the relationship between the program components and outcomes is discussed, as are lessons we have learned through conducting this process evaluation.

Method

Research Design and Study Population

This process evaluation has been conducted to examine the Yoga for Stress intervention, a RCT evaluating a standardised yoga intervention aimed at decreasing levels of stress and distress and increasing well-being in a community population of distressed middle-aged women (N = 116). Stratification was determined by Psychological Distress Categories (Moderate, High, and Very High), as measured by the Kessler Psychological Distress Scale (K10; Andrews & Slade, 2001). Inclusion criteria were: 1) female aged 35-65 years, 2) body mass index (BMI) < 30, 3) reporting chronic stress and experiencing moderate to very high levels of psychological distress for at least one month (as measured by the K10), 4) able to commit to attendance at 2 yoga classes a week for the duration of the intervention

(eight weeks), and 5) written informed consent. A subset of this population (n = 35) were randomly allocated to have blood samples taken for biological outcome measures. Inclusion criteria for this arm of the study were; 1) between 35-50 years, 2) not having undergone menopause or having symptoms of menopause, 3) no reported illness over the two weeks prior to testing, and 4) not pregnant or breastfeeding. Participants were randomised to an intervention group (n = 60; blood sample n = 16) or a control group (n = 56; blood sample n = 19) by Research Randomizer (Urbaniak, 2013) and, once the deadline of acceptance of new participants into the study had passed, all participants were allocated a random identification number which was then used for the allocation process. The intervention lasted eight weeks and had a one month follow-up period. Baseline, post-test, and follow-up measures all took the same format, which was an online questionnaire and an in-person assessment of physiological measures, which was conducted at The University of Adelaide. The study's protocol was approved by the Human Research Ethics Committee of The University of Adelaide.

Process Evaluation Method

This process evaluation has a systematic approach that follows previously reported guidelines (Linnan & Steckler, 2002; Moore et al., 2013; Saunders et al., 2005). The following measures are included: (a) Description of the intervention and its causal assumptions, (b) information on the implementation process (How delivery was achieved, and what was delivered: fidelity, dose delivered), (c) exposure of participants to the intervention (reach, dose received), (d) mechanisms of impact (participant's response to the intervention) and participant's emotional and cognitive experience (affect, alliance, evaluation), (e) context of the intervention, and (f) recruitment.

Development

After reviewing the literature of yoga intervention programs and recommendations for their development, the first author, a qualified yoga instructor with 7 years teaching experience, was responsible for the development of the Yoga for Stress standardised intervention protocol (See Figure 1 for a brief outline of the class components), as well as for teaching all yoga classes. The research team, as a whole, was responsible for development of the study, including outcomes and process evaluation.

Introduction	
Complete PANAS	
Guided meditation (breath awareness)	~5 minutes
Warm-up exercises	~6 minutes
Asanas (postures)	
Sūrya-Namaskāra A (Sun-Salutation A)	~12 minutes
Sūrya-Namaskāra B (Sun-Salutation B)	~12 minutes
Standing postures (changed according to class theme)	~12 minutes
Floor postures (changed according to class theme)	~12 minutes
Śavāsana (relaxation posture)	~5 minutes
Poem Reading	
Conclusion	
Complete PANAS	
Total Duration:	~60 minutes

Figure 1. Outline of yoga class structure.

Theoretical framework. As with yoga, the technologies of acceptance and mindfulness were originally drawn from a religious and spiritual discipline (Hayes, 2002; Sherman, 2012). In order for these practices to incorporate into empirical clinical psychology, it has been argued that they must be separated from their religious and spiritual traditions and evaluated and conceptualised scientifically (Hayes, 2002). A scientific approach has fostered the secular development of numerous mindfulness-based interventions (MBIs), and evidence of their efficacy in clinical practice has been growing exponentially (Cullen, 2011; Goyal et al., 2014; Khoury et al., 2013). It has been recognised that MBIs are complex interventions as their implementation has a high degree of variability that needs to be considered when determining the most effective models for delivery models in healthcare (Demarzo et al., 2015). Process evaluations are useful for obtaining such information.

The technology of yoga. Yoga is considered to be a mindful exercise program (La Forge, 2005) and, like other MBIs, scientific evidence of efficacy is emergent (e.g., Balasubramaniam et al., 2012; Cramer et al., 2013; Froeliger, Garland, Modlin, & McClernon, 2012; Woodyard, 2011). The "active ingredients" of yoga as treatment for mood disorders are suggested to be mindfulness promotion and exercise (Uebelacker, Epstein-Lubow, et al., 2010). The focus of 'yoga' has shifted, as it historically contained practices focusing on attaining enlightenment through the performance of hymns and rituals and adherence to prescribed ethical behaviours (Hayes & Chase, 2010). Through many centuries, it has evolved into the behavioural practice that utilises physical movement and concentrative elements, which is now recognised in the West as yoga (Hayes & Chase, 2010), which can be effectively practiced without spiritual components. It is this physical practice of yoga (often referred to as *hatha yoga*) that is currently being examined as a plausible therapy for a

range of conditions (Hayes & Chase, 2010). Yoga is increasingly being practiced in a secular form, as it becomes integrated into schools and healthcare settings (e.g., Khalsa, Hickey-Schultz, Cohen, Steiner, & Cope, 2012; Khanna & Greeson, 2013).

Secular philosophy. The inclusiveness of the secular approach of MBIs was used to guide the development of this yoga protocol. The typical modern entry points to yoga are thought to be postures and breath regulation (Gard, Noggle, Park, Vago, & Wilson, 2014), which were included in the protocol, along with meditative exercise components. This protocol did not include discussion of specific yogic ethical attitudes and behaviours, or advanced religious or spiritual components sometimes entailed in a yoga discipline (Gard et al., 2014).

Physical movement component. A dynamic yoga style called *Ashtanga* has demonstrated enhanced physical benefit (increased heart rate) and decreased perceived stress when compared with gentle yoga and relaxation-based yoga (Cowen & Adams, 2005). The *Ashtanga* practice features a common series of postures called the sun salutations, which are performed in time with one's breath cycle. The sun salutations' physicality has been specifically investigated and demonstrated to have an energy cost of 6.7 metabolic equivalents (MET; Carroll, Ring, Hunt, Ford, & Macintyre, 2003), whilst other postures, such as those considered more restorative, have been found to give rise to METs less than 2.19 (Ray, Pathak, & Tomer, 2011). Inclusion of the sun salutations was deemed suitable for this intervention due to the stress reduction and exercise they have previously demonstrated. Looking specifically at exercise, it has historically been argued that to maximise psychological benefits, there are a number of requirements (Berger, 1996), detailed in Table 1, which are present in this intervention design.

Table 1

Berger's Taxonomy to Maximise Psychological Benefits of Exercise

Major Requirements	
Pleasing and Enjoyable	
Mode Characteristics	- Aerobic or rhythmical abdominal breathing - Absence of interpersonal competition - Closed, predictable, or temporally and spatially certain activity
Practice Requirements	- Intensity: moderate - Duration: at least 20 to 30 minutes - Frequency: regularly included in weekly schedule

Note: Adapted from "Psychological Benefits of an Active Lifestyle: What We Know and What We Need to Know," by B. B. Berger, 1996, *Quest, 48*(3), p. 335. Copyright 1996 by the American Academy of Kinesiology and Physical Education.

Dosage. The yoga intervention consisted of 16 hour-long sessions, which took place over eight weeks (2 classes a week), reflective of current yoga interventions (Sherman, 2012) and community practice (Penman et al., 2012). Competing time commitments have been reported to be the most common reason for ceasing a yoga practice (Cohen, Penman, Pirotta, & Da Costa, 2005), and a lack of adherence to twice-weekly practice has been hypothesised to contribute to the lack of difference seen between weekly and twice-weekly practice (Michalsen et al., 2012). The positive impact of weekly classes on stress and distress has been supported by a number of additional studies (e.g., Cowen & Adams, 2005; Moadel et al., 2007; Sujatha & Judie, 2014) and is considered reflective of what the general population is able to fit into their schedule (Amin & Goodman, 2014). Consequently, the protocol defined completion of this yoga intervention as an average of weekly practice (eight classes), whilst the offering of the twice-weekly classes was suggested to improve the reach of the intervention, particularly considering very few yoga practitioners report engaging in practice outside of class participation (Penman et al., 2012).

It was theorised that dosage would be further mediated by motivation. Self-determination theory (SDT) suggests that motivation to engage in a behaviour is based on the needs of competence, autonomy, and relatedness, and these needs form a continuum of internal to external motive (Deci & Ryan, 1985). In both community and psychiatric populations, intrinsic motivation has been found to be positively related to engagement in physical activity and decreased symptomatology during the activity, while extrinsic motivation has been negatively correlated with engagement (Biddle & Mutrie, 2007; Sorensen, 2006). This suggests motivational mechanisms will be associated with both adherence and outcomes in the current study.

Description of Protocol

The classes commenced with an active mindfulness meditation (i.e., 'Mindfulness of breath') and then moved into a brief series of warm-up postures on the floor. This was followed by the Sun salutations. Next, a series of standing postures were performed; these postures were different each class and were often performed more slowly, as more instruction was entailed (See Figure 1 for an outline of the class structure). The series of classes was designed to accommodate novice practitioners, such that the first class was led as a complete introduction (with a strong focus on teaching the components of the sun salutations), and further classes slowly progressed in their degree of difficulty and variety, to keep participants interested. However, the classes were also designed to progress at such a rate that participants were still able to participate if they had missed previous classes. Where a posture was not accessible to a participant, modifications were provided. All classes were piloted in a small sample (N = 2) prior to instruction, ensuring timing and cohesion.

Each yoga session was coupled with a handout detailing the classes theme and new postures (For example, the theme of one session was "Warrior Pose," and a

handout was provided that explained how to perform each of the Warrior Postures taught in the class). In the event a participant missed a session, the handout was provided to them at their next attendance.

Data Collection

Demographic measures. Only demographic and process evaluation measures will be discussed in this paper; program outcomes will not be presented. At the baseline assessment, participants responded to a demographic instrument that assessed their age, nationality, education, annual income, neighbourhood and relationship status. Additionally, motivation for exercise was measured at baseline by the Motivation for Physical Activity Questionnaire (MPAQ), which has an internal consistency of 0.73-0.88 (Frederick, Morrison, & Manning, 1996).

Process evaluation measures. Process evaluation measures included the following: 1) attendance sign-in forms coupled with head-counts performed by the instructor, 2) a Positive and Negative Affect Schedule (PANAS; Watson, Clark, & Tellegen, 1988), which consists of two mood scales that measure an individual's positive and negative affect (administered before and after all the yoga classes), 3) a yoga session completion checklist, completed by the instructor, 4) a modified Revised Helping Alliance Questionnaire (HAq-II; Luborsky et al., 1996) was used to assesses the extent to which the participant experienced the yoga instructor (rather than therapist) as helpful (administered at a mid-intervention evaluation and at a final evaluation), 5) a Course Satisfaction Questionnaire that evaluated participant's satisfaction with the yoga classes based on qualitative and quantitative assessment of the following domains: if expectations were met, assessment of the content, assessment of the instructor's performance, if questions were answered adequately, duration and rhythm, general organisation, and overall assessment. A physical activity

log was provided when the study commenced to gain information on physical activity outside of the face-to-face yoga classes (dose received); however, participants had difficulty completing this weekly log, resulting in a small sample size, so it is not considered in this paper. A formative evaluation was conducted at the mid-point of the intervention (Class 8) and the summative evaluation was scheduled after the intervention's completion. The process-evaluation plan is detailed in Table 2. The plan details the following: the process-evaluation questions asked; the data sources; the measures and procedures used; the timing of data collection; data analysis and synthesis; and reporting used to assess fidelity, dose delivered, dose received, reach, recruitment and context are detailed in Table 2.

Table 2

Process Evaluation Plan for the Yoga for Stress Intervention Implementation

	Process-Evaluation Question	Data Sources	Tools/Procedures	Timing of Data Collection	Data Analysis or Synthesis	Reporting
Fidelity	To what extent was each standardised class implemented as planned? (session plan, scheduled day/time)	Instructor	Self-reported check-list for each class, ethnographic observations	Instructor reports after each class	Narrative description of check-list and observations	Formative – weekly feedback to instructor on class organisation; Summative – summarized overall
	To what extent did the participants perceive a helping alliance was formed with the instructor?	Participants	HAq-II (modified)	Taken at the mid-intervention review and final-class	Calculated levels of HA at mid-intervention review and at final-class	Formative- formal feedback on instructor at mid-intervention; Summative – reported total
Dose delivered	To what extent were all components of the class (including handouts) implemented?	Instructor	Self-reported check-list for each class and written observation	Instructor reports after each class	Narrative description of check-list and observations	Formative – feedback to self on class organisation; Summative – summarised overall

Table continues

Table 2 Continued

	Process-Evaluation Question	Data Sources	Tools/Procedures	Timing of Data Collection	Data Analysis or Synthesis	Reporting
Dose received cont.	How many classes did participants attend? How many participants attended 1 class per week (per-protocol), how many attended all classes?	Instructor, and participants	Attendance check-list and headcount	Taken for each class	Look at number of participants in each class/total number of classes attended	Formative – report bi-weekly for each class; summative – report overall
	Did participants report an affective change from taking place in the class?		PANAS		Calculate levels of PA and NA pre-class and post-class	Summative – calculated for each class individually, and overall
	Were participants satisfied with the various components of the classes and overall?		Course Satisfaction Questionnaire and open-ended questions	Taken at the mid-intervention review and final-class	Calculated levels of satisfaction and identify feedback themes through quantitative analysis at mid-intervention review and at final-class	Formative- formal feedback to instructor at mid-point; summative – reported by component and total

Table continues

Table 2 Continues

	Process-Evaluation Question	Data Sources	Tools/Procedures	Timing of Data Collection	Data Analysis or Synthesis	Reporting
Reach	How many participants had contact with some portion of the intervention?	Instructor	Attendance check-list and headcount	Taken for each class	Look at number of participants participating in at least one session	Summative – report overall
Recruitment	What procedures were followed to recruit participants to the intervention?	Researchers	Researchers document all recruitment activities	After each activity	Narrative description of procedures	Formative – examined weekly to inform recruitment progress; summative- described for intervention overall
Context	What were barriers and facilitators to implementing the yoga classes' standardised protocol?	Instructor Participants	Written observations Reported perceptions	Written at the end of the course Reported at midpoint and end of course	Narrative description of perceived barrios and facilitators	Summative- reported after intervention
	Were specific motivations for participating related to intervention implementation or outcome?	Researchers	MPAQ	MPAQ taken at baseline, measures of PA, participation and satisfaction compiled at post-test.	Calculate correlations	Summative – calculated after intervention

Table continues

Table 2 Continued

	Process-Evaluation Question	Data Sources	Tools/Procedures	Timing of Data Collection	Data Analysis or Synthesis	Reporting
Context cont.	Can any mechanisms of change be identified?	Researchers	All qualitative and quantitative data	MPAQ taken at baseline, measures of PA, participation and satisfaction compiled at post-test, quantitative data collected at mid-point and post-test.	Calculate correlations, and Narrative description of themes	Summative – calculated and compiled after intervention

Note: Adapted from "Developing a process-evaluation plan for assessing health promotion program implementation: a how-to guide," by R. P. Saunders, M. H. Evans, & P. Joshi, 2005, *Health Promotion Practice, 6*(2), 134-147.
PANAS = Positive and Negative Affect Schedule; HAq-II = Revised Helping Alliance Questionnaire; MPAQ = Motivation for Physical Activity Questionnaire.

Statistical Analysis

First, to examine relationships, correlation analysis was used. Where appropriate, paired-sample t-tests were used for continuous variables. For all analysis, SPSS version 21.0 was used. Statistical significance was defined as $p < .05$.

Second, to obtain an indicator of clinical outcome in this sample, reliable change and clinically significant change scores were calculated for K10. The reliable change index (RC) is a standardised measure which indicates if improvement or decline for an individual is greater than might be due to measurement error, while clinically significant change index (CSC) is the cut-off point for which the person's score is within the normal range, rather than the clinical (Evans, Margison, & Barkham, 1998).

To obtain the K10 outcome scores, the RC was estimated as a 6.16 point change (rounded to 6 points) using reliable coefficients reported for a female Australian normative group (16-85 years) in the 2007 National Survey of Mental Health and Wellbeing (Slade, Grove, & Burgess, 2011). Using the same sample norms, the CSC cut-off was estimated to be 17.86 (rounded to 18 points).

Results

Demographic characteristics of participants who took part in the Yoga for Stress intervention are shown in Table 3. There are no statistical differences between the group as a whole (intent-to-treat; ITT) and participants who completed the intervention per-protocol (PP; attendance at a minimum of eight classes) for any demographic charactistics.

Table 3

Baseline Demographic Characteristics of Intervention Participants

	Yoga (ITT)[a]	Yoga (PP)[b]
	M (*SD*)	*M* (*SD*)
Age (years)	48.38 (8.47)	49.20 (7.93)
	n (%)	n (%)
Education		
High School (no degree)	6 (10.0)	6 (13.0)
High School Degree	4 (6.7)	3 (6.5)
Vocational School	14 (23.3)	11 (23.9)
Bachelor's Degree	29 (48.3)	21 (45.7)
Master's Degree	6 (10.0)	4 (8.7)
Doctorate Degree	1 (1.7)	1 (2.2)
Relationship Status		
Single (never married)	9 (15.0)	6 (13.0)
Married/common-law	27 (45.0)	21 (47.8)
Separated or Divorced	16 (26.7)	17 (37.0)
Declined to answer	1 (1.7)	1 (1.7)
Have Children	42 (70.0)	35 (76.1)

Note: [a] ITT = Intent-to-treat (full sample, N =60); [b] PP = Adherence to Protocol (per-protocol, N = 46).

Dose Delivered

A full dose of 16 sessions was delivered (100%). In the event that the instructor was ill or an unavoidable circumstance came up, it was expected that make-up classes would be offered at the end; however this was not needed. All classes were delivered on time, and handouts were always provided. There were no issues with yoga mat avaliability. All intervention components were delivered as planned.

Fidelity

The intervention protocol was followed entirely, and classes ran to the allocated time schedules. Handouts were provided for all classes, and when a participant missed a class, she was provided the handouts at the next class she attended. It was observed that participants were generally at the same fitness level and, as beginners to yoga, they reported enjoying the depth of instruction for new postures and then more advanced cues as their skills improved.

Therapeutic relationship is known to be an important predictor of treatment outcomes (Lambert & Barley, 2001; Martin, Garske, & Davis, 2000). The HAq-II has good internal consistency, with a Cronbach alpha coefficient reported of .90 (Luborsky et al., 1996). As we modified the questionnaire, we checked to ensure the reliability was acceptable and found a Cronbach's alpha coefficient of.74 at the mid-point evaluation and.71 at the final evaluation. While less internal consistency than the original, these values are considered acceptable. At the mid-point evaluation, participants reported a mean of 5.4 on the modified HAq-II 6-point scale (1 = strongly disagree to 6 = strongly agree), which suggests that they generally agree with positive statements about their relationship with the instructor. The mean at the final evaluation was 5.5, which suggests agreement to strong agreement. The positive

endorsement of a helping alliance suggests that the relationship students perceived with their instructor did not hinder their progress in the yoga intervention.

Adverse Events

Two adverse events were reported by one participant; one at the first and one at the second class. The participant reported what she described as a 'shock reaction', where she reported a headache and aches throughout her body. In both cases, she recovered within the day but chose not to participate in further classes. No other adverse events are known to have resulted from this study.

Dose Received

Of the 60 allocated to the yoga intervention 46 (76.7%) completed the program per-protocol (once-weekly class or 50%). Three participants (5.0%) attended all classes (twice-weekly) and 36 (60%) attended an average of 1.5 classes/week (12 classes). The average number of classes attended was 10.7 (65.9%). One participant did not attend any yoga classes and discontinued the intervention because she felt she could not make time for it, and 2 participants only attended 2 classes; both of these participants dropped out, one due to an adverse reaction (described above) and one due to time constraints. Between the two classes offered each evening (described below) the total attendance fluctuated from 49 to 31 participants, with an average attendance of 38.4 (64.0%).

Affective change was also used as a tool to measure the dose received of the yoga intervention. Paired-sample t-tests were conducted to evaluate the impact of the intervention on participants' scores of PA and NA. First, t-tests were conducted to determine if there was a significant mean difference in compiled pre-yoga and post-yoga scores each class. There was a statistically significant increase in PA from pre-yoga (M = 27.41, SD = 5.61) to post-yoga (M = 31.48, SD = 7.03), t (58) = 6.15, p

<.001 (two-tailed). The mean increase in the PA scores was 4.06 with a 95% confidence interval ranging from 5.39 to 2.74. The eta squared statistic (.39) indicating a large effect.

There was a statistically significant decrease in NA from pre-yoga (*M* = 13.54, *SD* = 3.37) to post-yoga (*M* = 11.05, *SD* = 1.72), *t* (58) = 8.18, *p* <.001 (two-tailed). The mean decrease in the NA scores was 2.49 with a 95% confidence interval ranging from 1.88 to 3.09. The eta squared statistic (.54) indicating a large effect.

To ensure this effect was consistent across the 16 classes, paired-sample t-tests were conducted for each class individually. For each of the 16 classes there was a statistically significant increase in PA from pre-yoga to post yoga, along with a statistically significant decrease in NA (see Figure 2), supporting an immediate effect of yoga.

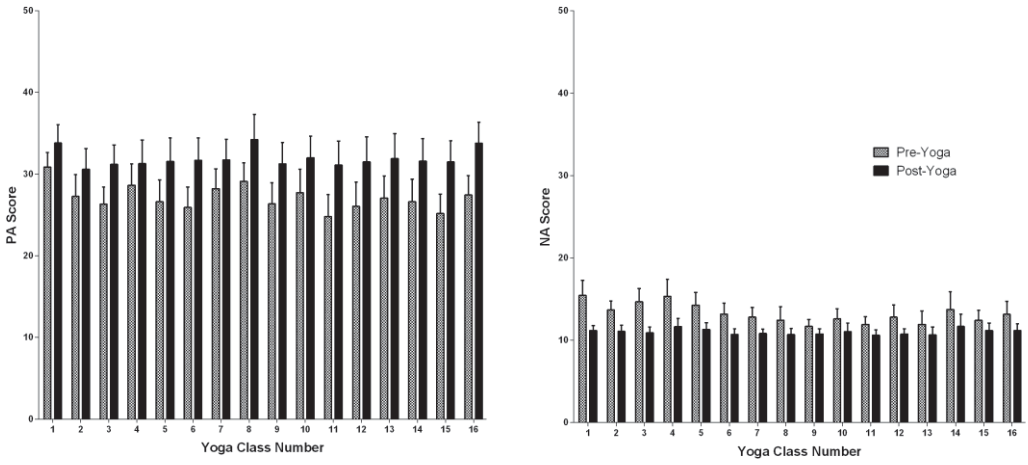

Figure 2. Mean positive affect (PA) and negative affect (NA) scores and 95% confidence intervals for Pre-Yoga (N = 28 - 49) and Post-Yoga (N = 26 - 49).

Finally, therapeutic alliance and course satisfaction were also used to measure the dose received of the yoga intervention. The mid-point perceived therapeutic alliance (*M* = 65.19, *SD* = 3.72) was comparable to the endpoint course satisfaction

(*M* = 66.59, *SD* = 0.93). With participants reporting a mean in the upper range of perceived alliance, suggesting they generally "Agreed" or "Strongly Agreed" with the statements on the modified HAq-II (total possible satisfaction = 72). Responses for the individual questions can be seen in Figure 3.

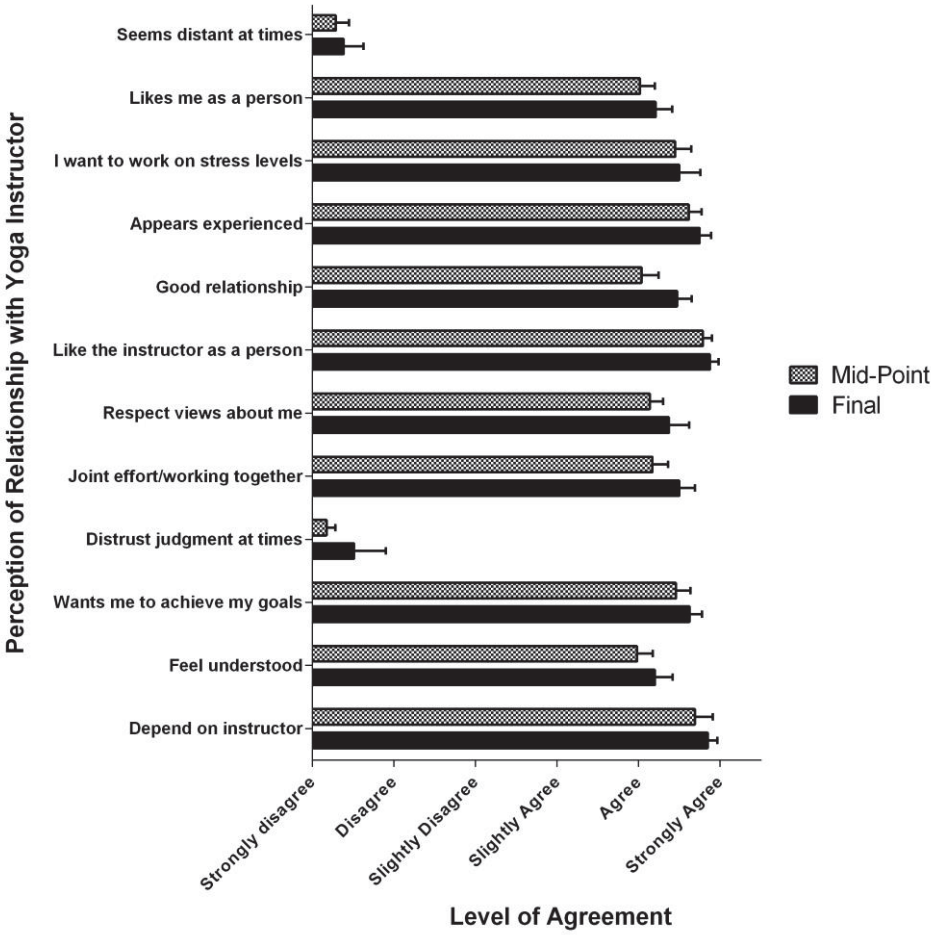

Figure 3. Mean modified HAq-II scores and 95% confidence intervals for the mid-Point (N = 48) and final (N=37) process evaluations.

The mid-point course satisfaction (*M* = 26.36, *SD* = 2.31) was comparable to the endpoint course satisfaction (*M* = 27.54, *SD* = 0.76). With participants reporting a mean in the upper range of satisfaction, suggesting they generally found the course

components “Very Good” (total possible satisfaction = 28). Responses for the individual questions can be seen in Figure 4.

Figure 4. Mean Course Satisfaction scores and 95% confidence intervals for the mid-point (N = 39) and final (N = 53) process evaluations.

Reach

Of the 60 allocated to the yoga intervention 59 (98.3%) had contact with the intervention.

Recruitment

Recruitment for this study was not difficult, given the community population used and the media the study received. The University of Adelaide put a segment in their alumni newsletter and put out a press release providing information about the study, which resulted in 3 radio interviews of the first author and a segment in a local news program. Potential participants were directed to a website, where they could get more information about the study and make contact with the researchers. As cost is considered a major barrier to participation in yoga in Australia (Penman et al., 2012), offering 16 yoga classes free of charge likely enhanced our recruitment. Just over 300

potential participants registered their interest in participating; however, only 116 met the inclusion criteria and were invited to participate in this study.

Context

The Yoga for Stress intervention was implemented from March through July 2011, with recruitment starting in March. The intervention took place in April through June, with a one-month follow-up in July. Classes took place on Monday and Friday evenings, and each night had two time blocks to which participants were randomly assigned (5:30-6:30 and 7:00- 8:00 timeslot; n = 25.) However, if a participant had a timetable conflict with her timeslot, it was accommodated. There were no statistically different demographics or outcomes found between classes. Participants reported that they generally found the evening class times generally suitable to their schedules, although they voiced a preference for classes not to have been conducted on a Friday. Classes were held within the city of Adelaide, Australia, at a local community centre. Of note is that though this was a central location, there was ample free parking around this location. Participants did not voice any concerns with the location, and a number reported the parking to be helpful. Yoga mats were provided for all participants, which was reportedly appreciated, as it allowed them to come straight from work and did not require they allocate any funds to purchasing one.

In the first week of class, numbers were higher (20 – 25) and some participants reported to the instructor that they had found it intimidating. They also reported that as the weeks progressed and the average class size became smaller (15 – 19) they found it more comfortable. One participant reported that another's personal hygiene was concerning to her. It was addressed by the concerned participant relocating herself to a different area of the room.

Mechanisms of Impact

There are a number of possible causal pathways that may explain how the effects of the intervention occurred. Both qualitative and quantitative data is presented. Directional analysis (i.e., paths analysis) is not feasible due to sample size, so correlational data is presented.

Participants' reported response to the intervention. Participants' reported enjoying the yoga class and finding it effective for stress management. They reported the class design to be achievable and that it progressed at a pace commensurate with their level of experience. It was reported that there was enjoyment of learning new postures every week, as well as benefiting from the repetition of previous postures and sequences. Participants reported that they found the class size too large at the commencement of the intervention; however they also reported that the class sizes at the conclusion of the intervention were perceived to be acceptable. Some of this situation perception may be due to person factors (i.e., knowledge of the environment, level of comfort), in addition to the situation effects (Rauthmann, 2012).

Yoga teachers' observations of the intervention. Participants were observed to engage in more discussion with others post-class and often reported feeling more relaxed. No interpersonal competition was observed in the class, and it was noted that participants became very warm towards each other as the classes progressed. The design of the class appeared to be appropriate to the participants' level, in that it was observed that the postures and speed at which they were introduced was achievable for most participants. Generally, their postural progress was observed to improve greatly from the beginning to the end of the class and when this feedback was conveyed to them, they reported their agreement. It was observed that through the sun salutations and standing postures, a light sweat was developed by a number of

participants, suggesting that the class was evoking an aerobic effect for at least 30 minutes. It was observed that there was very little personal space due to the numbers and room size at the beginning of the intervention; however, through the intervention some participants stopped attending or attended less regularly, which offered participants more personal space. This was perceived to improve the ease for instruction, because it became easier for the instructor to clearly see all participants and offer feedback.

Affective response. The PA and NA pre- and post-yoga class measures support a single yoga class as leading to the immediate enhancement of positive mood and alleviation of negative mood, as previously reported in exercise literature in both clinical and community samples (Yeung, 1996). This suggests yoga may be an effective short-term mood-regulation strategy in this community population.

The qualitatively reported and observed theme of increased self-efficacy (i.e., posture improvement) highlights a plausible mechanism for the increased PA and decreased NA, which has previously been reported following an exercise session of as little as 10 minutes of aerobic exercise (Rudolph & Butki, 1998) and at a moderate intensity (Treasure & Newbery, 1998). Future research may wish to quantitatively measure self-efficacy and determine if the benefit is maximised immediately post class, or if there is a benefit seen over a longer duration (i.e., through the intervention).

Motivation, affect, alliance, satisfaction, and adherence. Two-tailed correlation analysis was conducted to examine associations of hypothesised predictors of outcome (See Table 4), and significant correlations are discussed.

Table 4

Pearson Product-moment Correlations Between Measures of Motivation, Affect, Evaluation and Outcome

Measure		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	Fitness	-	.70**	.42**	.62**	.31*	.42**	-.25	.03	.00	.04	-.08	.08	.30	-.01	.33*	-.11
2	Appearance		-	.33**	.43**	.17	.32*	-.25	.07	.02	.09	-.11	-.08	.07	.04	.40*	.12
3	Enjoyment			-	.78**	.12	.20	-.14	-.03	-.08	.01	.09	.04	.14	-.05	.27	-.21
4	Challenge				-	.15	.19	-.10	-.03	-.02	-.02	.12	-.01	.19	-.02	.20	-.13
5	PA Pre					-	.70**	.14	-.04	.01	-.07	-.05	.03	.38*	.02	.27	.14
6	PA Post						-	-.61**	-.07	-.07	-.05	.16	.07	.34*	.16	.44**	.13
7	PA Improve							-	.05	.12	-.01	-.27*	-.06	-.03	-.20	-.31	-.02
8	NA Pre								-	.76**	.88**	-.22	-.10	.00	.04	.06	.53**
9	NA Post									-	.36**	-.10	-.05	.16	.12	.13	.39**
10	NA Improve										-	-.25	-.11	-.17	-.04	-.05	.48**
11	Attendance											-	.33*	.09	.14	-.08	-.15
12	Mid Sat												-	.30	.54**	.06	.00
13	Post Sat													-	.11	.05	-.03
14	Mid HAq-II														-	.40*	.23
15	Post HAq-II															-	.38*
16	Post K10																-

Note: Interpretation of the correlation strength according to Cohen (1988) is: Small r =.10 to .29; medium r =.30 to .49; large r =.50 to 1.0.

While intrinsic motivation to engage in exercise has been found to predict affect and satisfaction (Frederick et al., 1996) and adherence (Richard, Frederick, Lepes, Rubio, & Kennon, 1997), the associations between motivation and adherence is weak in this sample (Table 4). However, a significant association of attendance and improvement in PA from pre-yoga class to post may reflect the previously noted proposition that affective responses influence adherence (Williams, 2008). The only measure that was associated with adherence was mid-point (formative) satisfaction, which may have been related to the retention benefits reported when administering feedback informed therapy (Duncan, Miller, & Sparks, 2011; Duncan et al., 2003; Miller, Duncan, & Hubble, 2005) and supports the inclusion of formative feedback in evaluation.

Interestingly, the extrinsic motivation factor of Fitness was most robustly associated with PA (pre- and post-class) and with the final-point therapeutic alliance. The extrinsic motivation of Appearance was also associated with PA post-class and final-point therapeutic alliance. Level of PA (pre- and post-class) was associated with final-point course satisfaction, and PA post-class was associated with final-point therapeutic alliance. Mid-point therapeutic alliance was also associated with mid-point satisfaction. Though there was a positive association between final-point therapeutic alliance and PA post-yoga, final-point therapeutic alliance was associated with increased K10. Additionally, K10 was associated with NA pre- and post-class, as well as improvement from pre- to post-class. The correlations demonstrated indicated that there was no association between negative affect measures with measures of yoga enjoyment (i.e., satisfaction and therapeutic relationship), as might have been expected.

Outcome Indication

The 50-point (minimum possible score is 10-points) K10 scale (psychological distress scale) was administered before and after the yoga intervention (See Figure 5 for distribution of scores). The Cronbach's alpha of the scale at Time 1 was .82. A K10 score of 10-15 = Low, 16-21 = Moderate, 22-29 = High, 30-50 = Very high.

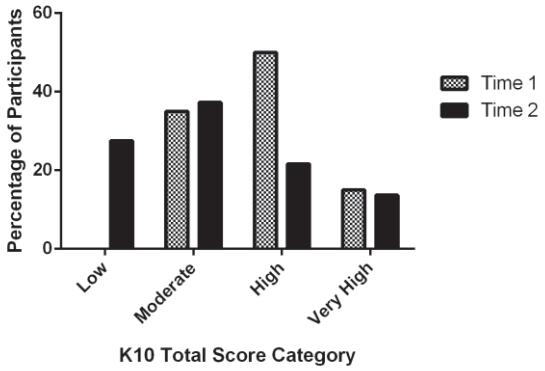

Figure 5. Change in the distribution of the K10 score at baseline and post-intervention.

The percentages of women reporting reliable and clinically significant change (RCSC) from baseline to post-test are presented in Table 5. Psychological distress was reliably improved in 33% of women and clinically improved (crossing the cut-off value distinguishing clinical and non-clinical populations) in 43% of women. K10 scores reliably worsened in 4% of women. At one month follow-up, clinical change was reported by 35.4% of participants (when considered against baseline status).

Table 5

Proportion of Women Showing Reliable and Clinical Change in Psychological Distress between Baseline and Post-test Assessment

		Change Category		
Method	N	Improvement	No change	Deterioration
Reliable change (RC)	51[a]	33.3% (17)	64.7% (33)	3.9% (2)
Clinically sig. change (CSC)	47[b]	42.6% (20)	55.3% (26)	2.2% (1)
RC & CS (RCSC)	47[bc]	25.5% (12)	74.5% (35)	NA

Note: [a]Nine participants did not complete the K10 measure at post-test and are excluded from analysis;. [b]It is not possible to assess the CSC of women who were not in the clinical population at the first time point and remained there (n = 4), so they were excluded from analysis; [c]It is not possible to assess women who did not demonstrate the RC (a change in magnitude greater than 6-points), so they were excluded from analysis.

Discussion

The Yoga for Stress intervention presented in this paper evaluated the process of a yoga intervention for stress reduction in a community population of middle-aged women. Process evaluation guidelines were followed and evaluation of fidelity, dose delivered, dose received, reach, recruitment, and context was conducted (Moore et al., 2015; Saunders et al., 2005).

As planned, 2 yoga classes a week were delivered for eight weeks at a local community centre, where 5% of participants completed all of the classes offered (twice-weekly practice) and 77% of participants completed the program per-protocol (an average of a weekly practice). These adherence rates are acceptable and comparable to other yoga interventions that have examined weekly and twice-weekly practice (Michalsen et al., 2012), as well as to that of community practitioners (Penman et al., 2012). Factors that may have contributed to the high rate of per-

protocol completion include the convenient location of the classes, the post-work hour session times, and twice-weekly offerings of classes. This study indicates a greater reach of yoga classes (98.4%) than reported in a workplace intervention of aging (≥ 45 years old) hospital workers (70.6%; Strijk et al., 2011). However, it is possible that the lower percentage of engagement with yoga in the Strijk et al. (2011) study may be reflective of their sample self-selection being motivated by engagement with another arm in their intervention, an assessment with a Personal Vitality Coach, which had a higher reach (89.6%). Our study also demonstrates a higher attendance rate (64.0% compared with 51.7%); however, the Strijk et al. (2011) study was conducted in two locations, and the one which was reportedly more proximal to the participants' workplace had a comparable attendance rate (63.2%). This further highlights the effect of location, which is reported as a barrier in the less-proximal arm of the Strijk et al. (2011) study.

In similar population studies (Penman et al., 2012), the most reported barrier to attending classes was a lack of time, which was attributed to vocational and household demands. Similarly, in our study, lack of time was a barrier and may be related to the study population: middle aged women, who were generally working and raising children. Another less frequently reported barrier to the Friday night timeslot was social obligations, and it is suggested that it is a less agreeable evening to schedule classes than other weeknights. Based on the literature of exercise and self-determination theory, it was expected that internal motivation would be associated with intervention adherence (Deci & Ryan, 1985). However, in this sample, neither internal nor external motivations are associated with adherence. Only external motivation factors are associated with affect, suggesting that in this sample the attainment of contingent outcomes (Deci, 1971) has a stronger association to mood

than pleasure and satisfaction. In particular, medium strength correlations were observed between fitness and both pre- and post-PA, whereas appearance was correlated with post-PA.

In other clinical studies, participants' ratings of therapeutic alliance have been argued to be the single largest predictor of therapeutic outcome (Bachelor & Horvath, 1999; Horvath, 2006). In contrast, the association demonstrated in this study indicates a stronger alliance is correlated with higher psychological distress at the conclusion of the intervention. It is possible that this may reflect an adverse reaction to the cessation of the intervention, due to the perceived loss of attachment to the yoga class setting and the instructor, which suggests a possible adverse effect of a strong therapeutic alliance. Interestingly, the only outcome that was associated with adherence was mid-point satisfaction. It may be the case that satisfaction with the yoga classes predicts attendance, or it may be that engaging in the mid-point evaluation was perceived to be beneficial by participants and improved their experience of the yoga classes. Such an effect would support reports that retention and outcomes in therapy are improved when feedback on progress and outcomes is measured (Duncan et al., 2011; Duncan et al., 2003; Miller et al., 2005).

As this study offered participants regular yoga classes and did not charge for participation, it overcame commonly reported reasons for discontinuing a yoga practice (Penman et al., 2012). However, effective strategies to increase participation in spite of the time commitment are still lacking. Future yoga interventions may consider addressing this issue, possibly by coordinating with workplaces to offer yoga classes at work, as suggested by Strijk et al. (2011) or including children in the class or an adjacent class. None-the-less, the acceptability of the yoga intervention was high. Participants rated the instructor and the various aspects of the intervention

positively. The acceptability of the implementation could have been related to the intervention's sequential design, which focused on making the practice accessible to beginners through clear instruction and then by building on skills to improve participants' sense of self-efficacy. Furthermore, the delivery of the intervention was reported to have been conducted such that participants felt adequately supported; that the content was relevant; and, because the instructions were clear.

In addition to the demonstrated feasibility and acceptability of the yoga intervention, the outcome indication showed that psychological distress was reliably reduced in 33.3% of cases and coupled with clinical improvement in 28.2% of participants. A statistically different adherence rate was not found in these participants, which suggests that improvement is seen with attendance at an average of 11 yoga classes. Comparative data are scarce, in that yoga studies conducted in community populations and service evaluations have not historically measured clinical improvement, as has been recommended (Evans et al., 1998). For this reason, it would be beneficial for future studies to consider its inclusion, with an aim to evaluate yoga as an evidence-based clinical intervention**.**

When clinically significant change has been examined in studies of brief psychotherapy, it has been estimated that 8 therapy sessions provide a 30% improvement rate, whereas 16 sessions increases the recovery rate to approximately 50% (Shapiro et al., 2003). Survival analysis conducted in an outpatient population has reported that an exposure of 11 to 16 sessions led to 50% of their clients achieving CSC (a similar rate to our study) and that 35% obtained CSC at a median of 11 sessions (Anderson & Lambert, 2001). Similar rates have also been reported in an Australian population comprising predominantly women seeking psychological services (primarily for a mood or anxiety disorder; Harnett, O'Donovan, & Lambert,

2010). In the sample, it was estimated that RC would be achieved for 50% of the population after 10 sessions; however, CSC was achieved by 34% due to dropouts (Harnett et al., 2010). While survival analysis was not conducted, we have demonstrated similar rates of improvement in psychological distress with attendance at an average of 10-11 yoga classes. Considering the reported studies have been conducted in an outpatient population, a reduced response might have been expected in this current study because of the inclusion of "functional" (non-clinical) range as opposed to the "dysfunctional" (clinical; Harnett et al., 2010). However, the majority of participants who took part in this intervention reported psychological distress scores that placed them in the dysfunctional range. They may have enrolled in this study during a crisis and thus a stronger effect may have been seen than if multiple pre-intervention measures had been taken (Evans et al., 1998). It is also that case that uncontrolled studies are often reported, so the effect of time's impact on the outcome is not captured.

There are some limitations of this intervention that should be discussed: The self-selection of the sample indicates all participants are likely highly motivated for change; in particular, to engage in interventions to lower their stress levels. Further, given that the intervention took place promptly after recruitment, a steeper regression to the mean is more likely to have occurred than if the study design had a longer wait time (Evans et al., 1998). Regular measures of psychological distress were not included in the study design, which inhibited the performance of a class-by-class survival analysis that would complement CSC analysis and provide more information about the relative impact of each yoga class. No clear comparison of weekly and twice-weekly practice was possible due to the small number of participants that completed yoga classes twice-weekly, although this also highlights adherence issues

beyond weekly practice. Despite these limitations, the yoga intervention evaluation provides insights into the mechanisms that influence the outcomes observed.

Lessons Learned

The following recommendations for yoga interventions are suggested based on the results reported:

To

- use a secular approach based on an MBI and physical activity energy expenditure theoretical framework
- use a session-by-session protocol that has strong continuity
- use a qualified yoga instructor to facilitate participant perception of a strong helping alliance
- take regular measures of satisfaction to ensure feedback informed treatment (which may foster adherence)
- utilise a central location with easily accessible transport and parking
- offer classes on weeknight evenings (Monday through Thursday; avoid Friday)
- start with smaller class sizes to ensure the participants' comfort
- identify the time-constraint barriers in the population of the study and consider ways to overcome them (offering multiple session times or considering workplace or family-centred classes)
- include a longer wait from recruitment and/or a second pre-intervention measure to control for the regression to the mean effect (larger effect of time)
- take regular outcome measures to facilitate survival analysis

Conclusions

The yoga intervention was implemented as planned with respect to the protocol adherence, dose delivery, and dose received. Indicators of reach suggested that most participants had contact with the intervention, and the adherence rates were acceptable with the majority attending a weekly yoga class, whereas limited time availability was a commonly reported barrier for not attending class's twice-weekly. The participants reported satisfaction with the intervention and a strong helping alliance with the teacher. They also reported sadness at the conclusion of the classes, as well as a desire to continue practicing yoga. Participation in yoga classes was correlated with significant changes in levels of affect. NA levels are clearly associated with the level of psychological distress reported at post-test, whilst levels of PA show smaller correlations with measures of adherence, satisfaction, and alliance.

This study contributes to the growing body of yoga literature; in particular, it enhances understanding of the feasibility and acceptability of a secular mindfulness-based exercise approach. While yoga has been evaluated in the context of multi-faceted vitality interventions (Strijk et al., 2011), to our knowledge, this is the first yoga intervention in a community population to conduct a process evaluation. The conduct of a process evaluation is crucial in this field, given the multitude of styles of yoga commonly practiced (Hayes & Chase, 2010) and the likelihood that there are differing mechanisms of impact and populations that are most impacted. The presented evaluation enhances the body of literature by providing information on the utilisation of a secular yoga approach in a reportedly chronically-stressed community population of middle-aged women. As a result, implementation and evaluation of similar yoga programs in other populations is warranted.

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2.2 Summary

This was the first process evaluation conducted on a yoga intervention administered in a community population of middle-aged women. Results of this evaluation demonstrated that an intervention corresponding with median duration of Western yoga interventions (i.e., eight weeks) and at the lower bound of doses (i.e., weekly or twice weekly, 60 minute classes) was acceptable and feasible in this population (Sherman, 2012). The paper included details on development of this intervention using a secular framework and information sufficient to enable replication. On the whole, participants were positively disposed towards the intervention and complied with participation protocol. Therefore, the protocol described could be applied successfully to real-world settings. In sum, this type of brief, low-cost, group yoga intervention warrants further investigation in community settings (Durlak & DuPre, 2008). Emphasis in the present research is upon psychological outcomes, but it is likely that other outcome measures could also be considered.

CHAPTER 3. PAPER 2

3.1 Preamble

The evaluation reported in Study 1 (Chapter 2) indicates that the yoga intervention was implemented successfully and documented how this was achieved. This chapter (Paper 2) explores the association between participation in yoga intervention and improvement in measures of mental health and physiological markers of allostatic load (body composition, blood pressure, and heart rate). The study method involved application of a randomised waitlist controlled trial and recording of measures at pre- and post-intervention.

Brief Report on the Psychophysiological Effects of a Yoga Intervention for Chronic Stress: Preliminary Findings

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I was responsible for the conception of this study, literature review, conducting the intervention, data collection and analysis, manuscript drafting, preparation. I was also responsible for submission, was corresponding author and was primarily responsible for revisions to the paper. My overall percentage (%) of contribution to the paper is 85%.

Signed: Kaitlin Harkess

Professor Paul Delfabbro, Dr Jane Mortimer, Dr Sarah Cohen-Woods (Co-authors)

We were the supervisors of the research program to which this manuscript belongs. We collaborated with Ms. Harkess in the development of the content and structure of the manuscript and assisted with editing and proof-reading. Ms. Harkess was responsible for the development

and administration of the intervention; the collection of and analysis of data; and, writing this manuscript. Our role was to comment on drafts, make suggestions on the presentation of material in the paper, and to provide editorial input. We also provided advice on responding to comments by the journal reviews and editor. We hereby give our permission for this paper to be incorporate in Ms. Harkess's submission for the degree of Doctor of Philosophy from the University of Adelaide.

Signed: Paul Delfabbro

Signed: Jane Mortimer

Signed: Sarah Cohen-Woods

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I was the research assistant who assisted in coding of data for this study, as well as providing critical feedback on the manuscript drafts Ms. Harkess produced.

Signed: Zara Hannaford

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Journal of Psychophysiology, Published online July 27, 2016.

CHAPTER 4. PAPER 3

4.1 Preamble

The results reported in Study 2 (Chapter 3) indicated that practicing yoga regularly over eight weeks was associated with improved psychological distress, perceived stress and positive affect, compared against the control group. Interestingly, a between group difference in measures of physical activity was not found. The principal aim of this third paper was to monitor mental health over time, including after yoga intervention cessation. Utilising a one month follow-up period allowed exploration of the association between lack of, or option of, regular contact with the yoga intervention and mental health outcomes. A one month follow-up period was deemed sufficient to detect sustained differences in scores, particularly given that K10 is a measure designed to capture an individual's level of psychological distress over the previous four weeks (Kessler & Mroczek, 1994). Additionally, the one month time period was practical given timing of school terms in South Australia as many participants had school-aged children.

Conducting a one month follow-up was considered an important contribution to the literature due to the limited number of yoga and mental health trials that had been conducted at the time of study conceptualisation. This approach is consistent with previous evaluations of established interventions, such as cognitive behaviour therapy (CBT) and mindfulness-based stress reduction (MBSR), both of which have been investigated in terms of short-term effectiveness in the treatment of psychological distress, such as anxiety, as well as for periods extending beyond the intervention itself (DiMauro, Domingues, Fernandez, & Tolin, 2013; Miller, Fletcher, & Kabat-Zinn, 1995).

The Longitudinal Mental Health Benefits of a Yoga Intervention in Women Experiencing Chronic Stress: A Clinical Trial

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I was responsible for the conception of this study, literature review, conducting the intervention, data collection and analysis, manuscript drafting, preparation. I was also responsible for submission, was corresponding author and was primarily responsible for revisions to the paper. My overall percentage (%) of contribution to the paper is 85%.

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Professor Paul Delfabbro, Dr Sarah Cohen-Woods (Co-authors)

We were the supervisors of the research program to which this manuscript belongs. We collaborated with Ms. Harkess in the development of the content and structure of the manuscript and assisted with editing and proof-reading. Ms. Harkess was responsible for the development and administration of the intervention; the collection of and analysis of data; and, writing this manuscript. Our role was to comment on drafts, make suggestions on the presentation of material in the paper, and to provide editorial input. We also provided advice

on responding to comments by the journal reviews and editor. We hereby give our permission for this paper to be incorporate in Ms. Harkess's submission for the degree of Doctor of Philosophy from the University of Adelaide.

Signed: Paul Delfabbro

Signed: Sarah Cohen-Woods

Abstract

Background and Objectives

Chronic stress contributes to psychopathology and the practice of yoga is suggested to decrease stress and improve well-being. However, the literature often reports methodological problems (cross-sectional designs, sample sizes ≤ 20, and limited exploration of community populations). The aim of this study was to address these limitations and evaluate the potential psychological benefits of yoga to a non-clinical population.

Methods

Women (N = 116) reporting chronic stress participated in this longitudinal study. Participants were allocated to a twice-weekly, hour-long yoga class for a period of two months, or a waitlist-control. Indicators of psychological well-being were measured at baseline, post-test and 1-month follow-up.

Results

Psychological distress decreased over time in both groups, however the control group experienced decreases in positive effect compared with the yoga group. Curvilinear trends were observed, indicating that trajectories of improvement seen at post-test were not robustly seen at follow-up.

Conclusion

The study indicates that short-term yoga practice may yield some benefits to stressed individuals, but that evaluation over a longer-term of practice may be required to determine the optimal dose for improvements and maintenance. Differential treatment effects may be difficult to detect in studies with populations that may already be motivated to improve their health.

In recent years an increased prevalence in mental disorders has been reported globally. The World Health Organization (WHO) has estimated the projected lifetime risk of experiencing a mental disorder to be between 17-49% (Kessler et al., 2007). Mental disorders are found to commonly occur in the general population and have significant societal costs (Kessler et al., 2009) and, by 2020, it is predicted that mental illnesses, including stress-related disorders, will constitute the leading burden of disease worldwide (Kalia, 2002; Kessler et al., 2009, Mathers, Fat, & Boerma, 2008). These observations follow reports that the level of stress and distress experienced by the general population is increasing, whilst levels of well-being are decreasing (Cassey & Ling, 2014). Although stress may be a motivating and useful experience in the short-term and can lead to adaptive responses and resilience, stress that lasts over extended periods is concerning as it is physiologically detrimental and can contribute to maladaptive psychological states, including clinical anxiety and depression.

In addition to affecting mental health, chronic stress can lead to widespread dysfunctions in the body, affecting the digestive system (Mathers, 2008), endocrine system (Cohen et al., 2007), and immune system (Glaser & Kiecolt-Glaser, 2005; Segerstrom & Miller, 2004). Encouragingly, it has been demonstrated that regular exercise engagement buffers the negative impact of stress on mental and physical health (Zschuck, Renneberg, Dimeo, Wüustenberg, & Ströhle, 2015). Exercise enhances psychological well-being (Hassmen et al., 2000), and has demonstrated positive effects on symptoms of depression and other mood states, such as improving self-perceptions, self-efficacy, and general well-being (Fox, 2000; Penedo & Dahn, 2005).

There is also evidence that stress reduction can be effected through psychological methods, including meditation (Goyal et al., 2014). Meditation is not currently a formal therapy, although its practice has been linked to enhanced psychological well-being (Brown

& Ryan, 2003). Recent meta-analysis has demonstrated that in diverse populations, it provides small to moderate reductions in the negative affect dimensions of psychological stress, such as 10-20% reductions in depression and 5-10% decreases in anxiety (Goyal et al., 2014). These findings suggest that meditation-based activities may have a role in addressing psychological distress. While a number of individual activities may reduce stress and promote mental and physical health, yoga has been proposed as a potentially useful approach to reducing psychological distress due to its integration of physical exercise and meditation. Physical, spiritual, psychological, and social elements are introduced through the utilisation of postures (asanas) that focus on strength, flexibility, and balance, co-ordinated with breathing (pranayama) and meditation (Amin & Goodman, 2014).

Over the past decade, there has been a proliferation of interest in the efficacy of yoga interventions to address both the physical and psychological consequences of stress (Cohen et al., 2005; Penman et al., 2012). Recently, a self-regulation framework has been proposed to help model a myriad of psychological and physical health benefits that have been linked to the practice of yoga (Gard et al., 2014). The use of yoga is suggested to couple the top-down processing (Beauregard, 2007) used in psychotherapies like cognitive behaviour therapy (CBT; Beck, 1990) with the bottom-up transformation of advanced meditation (van den Hurk et al., 2010). Specifically, yoga is thought to target cognitive process (e.g., thoughts, feelings, beliefs) by enhancing meta-awareness, and developing self-regulation tools such as reframing and reappraising negative cognitions. These tools are coupled with mindfulness-related skills in ‘third-wave’ CBT (Baer, 2005). Mindfulness-related skills involve more formal concentrative practices including meditation (Cahn & Polich, 2006), which is thought to target physiological pathways such as the autonomic nervous system and cardiorespiratory system, as well as emotion-generative processes (Gard et al., 2014; Vestergaard-Poulsen et al., 2009).

Practising yoga has indeed been associated with overall increases in well-being, quality of life, and positive affect, alongside decreases in negative affect, levels of stress, and, psychological distress (i.e., symptoms of anxiety and depression; Pilkington et al., 2005; Woodyard, 2011). Further studies have provided support for yoga as a potential treatment, or adjunct treatment, for psychiatric disorders, such as depression, anxiety, post-traumatic stress and schizophrenia (Balasubramaniam et al., 2012; Cabral et al., 2011; Cramer et al., 2013), Kirkwood et al., 2005; Li & Goldsmith, 2012). For example, a small, randomised study examining yoga and CBT for stress management found that both treatments proved equally efficacious (Granath et al., 2006). Another study used a CBT intervention enriched with yoga (Y-CBT) in a population of treatment resistant sufferers of generalised anxiety, yielding improvements in anxiety, depression and quality of life (Khalsa, Greiner-Ferris, Hofmann, & Khalsa, 2014). A limitation of these psychotherapeutically focused studies is small sample sizes (N = 33 and N = 22, respectively), and the latter's lack of a control group. This is broadly reflective of the literature, with most possessing small sample sizes, no randomised or control groups, and using a non-standardised intervention of varying durations, meaning it is difficult to compare one study to another (Sharma, 2014).

Most studies of the efficacy of yoga have been conducted using clinical populations, such as those who have been diagnosed with breast cancer or psychiatric disorders (e.g., Balasubramaniam et al., 2012; Cabral et al., 2011; Harder, Parlour, & Jenkins, 2012; Pilkington, Kirkwood, Rampes, & Richardson, 2005; Sadja & Mills, 2013), which is not reflective of the community populations reporting increased levels of stress and psychological distress. A further limitation of yoga literature thus far is that, while established interventions such as cognitive behaviour therapy (CBT) and mindfulness-based stress reduction (MBSR) have demonstrated effectiveness in the treatment of psychological distress for periods beyond the intervention itself (DiMauro, Domingues, Fernandez, & Tolin,

2013; Miller, Fletcher, & Kabat-Zinn, 1995), the psychological impact of yoga intervention beyond cessation of yoga practice has not been evaluated (Li & Goldsmith, 2012). Accordingly, there is a need for investigations involving larger studies involving randomised controlled designs that enable assessment of the sustained psychological benefits of yoga in the general population (Gard et al., 2014; Li & Goldsmith, 2012).

A further consideration in evaluating yoga as an intervention is the frequency and duration of the intervention provided. Several studies have provided support for a minimum of once-weekly yoga practice being sufficient for psychological benefits, such as a reduction in stress or distress (Banerjee et al., 2007; Cowen & Adams, 2005; Michalsen et al., 2005 & 2012; Moadel et al., 2007; Satyapriya et al., 2009; Sujatha & Judie, 2014; West et al., 2004). Additionally, established group interventions, such as MBSR, have traditionally used an 8-week intervention period, which is reflective of the median duration of Western yoga interventions (Sherman, 2012).

The Present Study

This paper presents the longitudinal psychotherapeutic outcome of yoga practice in a sample of middle-aged women, who work in largely professional occupations. This population is reported to commonly experience high levels of chronic stress and to be characteristic of community yoga users (Birdee et al., 2008; Diener et al., 1999; Nolen-Hoeksema et al., 1999; Penman et al., 2012) and using a single-sex population was deemed prudent to avoid the potential confound of gender. The intervention was an 8-week, moderate intensity yoga class (practicing twice-a-week) with baseline (pre-intervention) and post-intervention and follow-up (1 month after) measures. In line with post-test outcomes in the population (Harkess et al., 2016), it was hypothesised that after accounting for the duration of the intervention (time) and other potentially influential variables, yoga would influence both cognitive and emotional facets of mental health. These effects would be reflected in

decreased levels of perceived stress and psychological distress, increase mindfulness, and improve well-being (measured by an increase in subjective well-being and positive affect and a decrease in negative affect). Further, we planned to explore the trajectory of any effects observed in the yoga group across the multiple assessment periods (pre-, post-intervention, and follow-up).

Method

Study Design

This study used a longitudinal, stratified, randomised, waitlist-control trial design. Participants were encouraged to attend two yoga classes a week; however, completion of the yoga intervention per protocol (PP) was defined as attendance at an average of 1 class each week (8 classes), which was met by 46 women (14 did not receive the intervention PP, only 3 completed all classes). To account for not all participants receiving the allocated intervention both PP and intention-to-treat analysis (ITT), were conducted and are described below. Practicing 1 class a week is more reflective of what the population can fit into their training schedule (Amin & Goodman, 2014) and has been demonstrated to be sufficient to have a positive influence on stress and psychological distress (e.g. Cowen & Adams, 2005; Moadel et al., 2007; Sujatha & Judie, 2014). The control group did receive any treament and were requested to withold yoga practice until after completion of the study (they were encoraged to continue with their normal activities). Measures were collected at baseline (April 2013), post-treatment (July 2013), and a 1-month follow-up (August 2013) in a testing lab at The University of Adelaide.

This trial was approved by the Human Research Ethics Committee of The University of Adelaide, and all participants gave informed consent. In addition, this trial has been registered at the Australian and New Zealand Clinical Trials Registry (ANZCTR), under the

registration number ACTRN12616000612415. The study was initiated as a portion of a PhD Dissertation and registration as a Clinical Trial was not compulsory. However, with increased recognition of the importance of transparency and dissemination in a timely manner we decided to make the study accessible to the public and register the trial at ANZCTR. The authors confirm that all ongoing and related trials for this intervention are registered.

Randomisation and Stratification

Participants were randomly allocated to the intervention group or to the control group using Research Randomizer (Urbaniak, 2013). Stratification was based on the level of psychological distress reportedly experienced using Psychological Distress Categories (Moderate, High, and Very High), as measured by the K10 (Andrews & Slade, 2001). Scores in the 'Low' category indicate that the individual was not be experiencing significant feelings of distress and was therefore not included in this study.

Participants

The CONSORT flow diagram (Figure 1) illustrates recruitment and retention for this study. Eligible participants were females between the ages of 35 and 65 years old, non-obese (as measured by BMI), and experiencing symptoms of depression and/or anxiety, or some form of depression and/or anxiety for at least one month (as indicated by moderate to very high levels of psychological distress Andrews & Slade, 2001; Australian Bureau of Statistics, 2003; Kessler & Mroczek, 1994). Potential participants who had undertaken regular yoga practice over the previous year were excluded. The first author was in charge of screening potential participants.

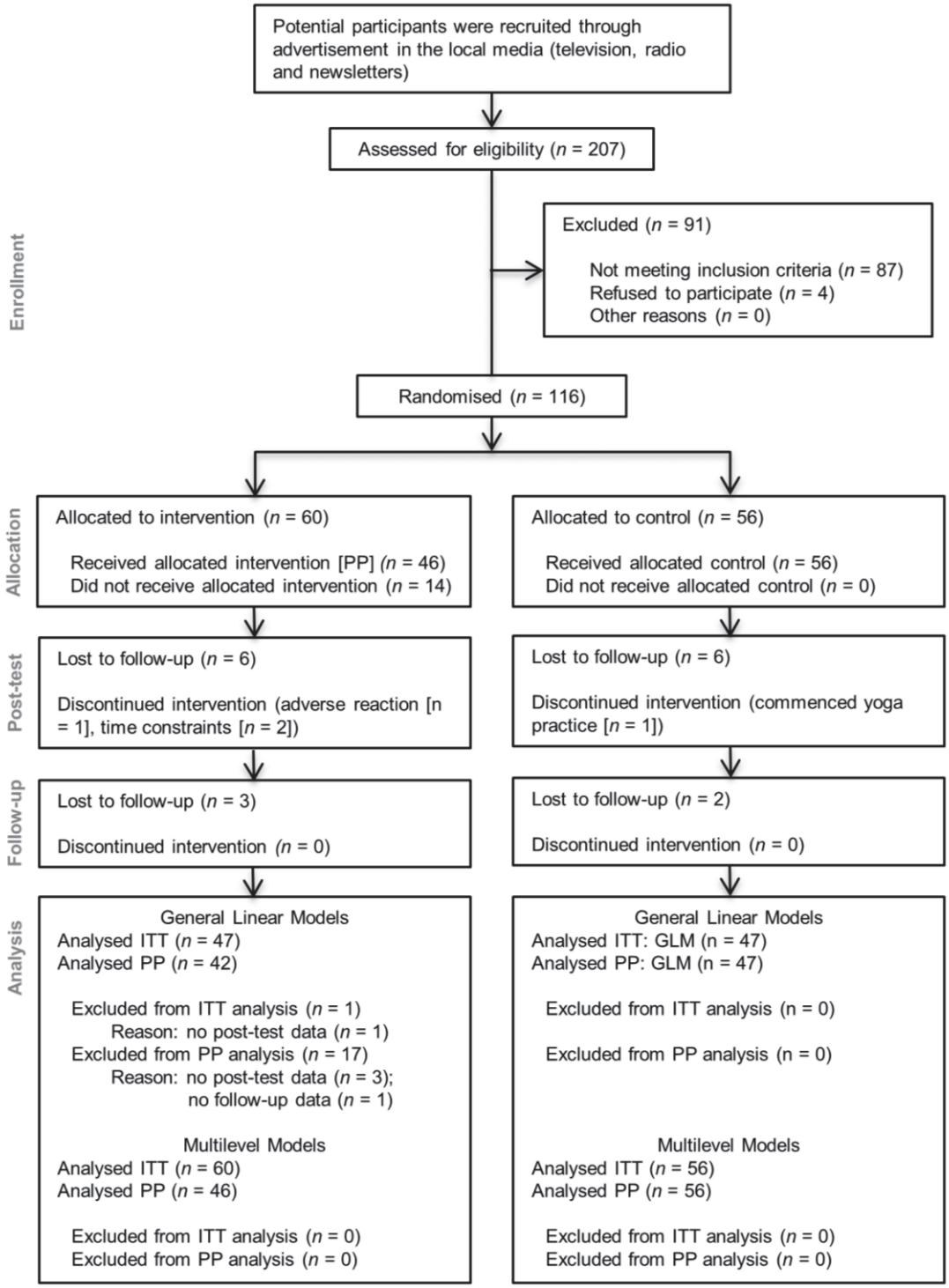

Figure 1. CONSORT flow diagram.

Power analysis (.80) indicated a total of 84 participants was needed to detect a minimum effect (f = 0.35), which is considered to be a meaningful, so a minimum of 96 participants was sought due to the common drop-outs in exercise interventions. See Table 1 below for the characteristics of the study participants, including detailing of participants included in per-protocol (PP) analysis and intention-to-treat (ITT) analysis, which is described in detail below.

Table 1

Baseline Characteristics of Study Participants

	Total Sample (*N* = 116) *n* (%)	Control (*n* = 56) *n* (%)	Yoga (PP) (*n* = 46) *n* (%)	Yoga (ITT) (*n* = 60) *n* (%)
Level of Education				
High School (no degree)	13 (11.2)	7 (12.5)	6 (13.0)	6 (10.0)
High School Degree	8 (6.9)	4 (7.1)	3 (6.5)	4 (6.7)
Vocational School	21 (18.1)	7 (12.5)	11 (23.9)	14 (23.3)
Bachelor's Degree	56 (48.3)	27 (48.2)	21 (45.7)	29 (48.3)
Master's Degree	14 (12.1)	8 (14.2)	4 (8.7)	6 (10.0)
Doctorate Degree	4 (3.4)	3 (5.4)	1 (2.2)	1 (1.7)
	M (*SD*)	*M* (*SD*)	*M* (*SD*)	*M* (*SD*)
Age (years)	47.86 (8.22)	47.30 (7.98)	49.20 (7.93)	48.38 (8.47)
WHtR	.526 (.077)	.525 (.078)	.521 (.070)	.526 (.076)
MET	1442 (1253)	1500 (1245)	1654 (1973)	1387 (1269)

Note. PP = Per-protocol analysis (received allocated intervention); ITT = Intention-to-treat analysis; WHtR = waist-to-height ratio; MET = Metabolic Equivalence of Task.

Measures

Psychological measures were collected via an online survey, and physiological measures were collected in person at The University of Adelaide. The measures used were (a) Kessler Psychological Distress Scale (K10; Kessler & Mroczek, 1994), which gives a global measure of psychological distress based on questions about anxiety and depression symptoms over the previous four weeks; (b) Perceived Stress Scale (PSS;. Cohen et al., 1983), which measures the degree to which situations in one's life are appraised as stressful; (c) The Mindfulness Attention Awareness Scale (MAAS; Brown & Ryan, 2003), which measures people's tendency to be mindful of moment to moment experience; (d) The Psychological Wellbeing Index – Adult (PWI-A; International Wellbeing Group, 2006); which is a measure of subjective well-being focusing on cognitive evaluations in different areas of life (standard of living, health, achieving in life, relationships, safety, community-connectedness, future security and spirituality/religion); (e) Positive and Negative Affect Schedule (PANAS; Watson et al., 1988), which consists of two high activation mood scales, which measure people's positive and negative affect; (f) the International Physical Activity Questionnaire (IPAQ; Craig et al., 2003; IPAQ Web site, 2005), which is a measure of the physical activity taken over the past week in a number of domains. From the IPAQ the energy cost of participants' weekly physical activities is calculated as the Metabolic Equivalence of Task (MET) from the IPAQ (IPAQ Web site, 2005); and (h) the waist-to-height ratio (WHtR; Cox & Whichelow, 1996; Janssen et al., 2002; Savva et al., 2013), which is a measure to detect central obesity, in particular visceral fat, and the health risks associated with it.

Procedure

The yoga condition was comprised of 16 one-hour yoga classes that took place twice a week over a period of 8 weeks. Yoga classes were conducted at a local community centre by the first author, a certified yoga instructor with 7 years' teaching experience (Yoga

Australia - Level 2 Member). The classes followed a standardised structure and were Ashtanga-based, commencing with a guided meditation; followed by Sun-Salutations (a series of postures that flow together), standing postures, and floor postures, and concluding with a relaxation posture. Ashtanga yoga has demonstrated cardiovascular benefit, and is considered a dynamic style, relative to other gentle and relaxation based yoga styles (Carroll et al., 2003; Cowen & Adams, 2005). Further, it has been demonstrated that the dynamic practice of Ashtanga yoga is associated with benefits beyond a gentler Hatha yoga practice (Cowen & Adams, 2005). Two adverse events were reported by one participant. During attendance at the first and second class, this participant reported developing a headache and aches throughout her body, which she described as a 'shock reaction'. In both cases, she recovered within that day and chose not to participate in further classes. No other adverse events were reported.

Baseline measures were taken in the two-weeks prior to the yoga intervention's first class. After the conclusion of the yoga classes, post-test measures were taken (8 weeks post-baseline), see Harkess et al., 2016. A follow-up was conducted 4 weeks after the post-test (12 weeks post-baseline).

Statistical Analysis

SPSS-v.22 statistical software package was used to conduct all statistical analyses, with an alpha level of .05. A number of analytic strategies can be used to examine this type of longitudinal data; each addresses a specific research problem and is situationally preferable. In psychological literature, there are two preferred methods for examining change and both were utilised to thoroughly examine longitudinal differences in outcome variables between the yoga-intervention and control groups. Mean change in the outcome variable from baseline to follow-up was examined to see if it differs between the two groups. To do this a 2 x 3 mixed factorial design with a between-subjects factor of Group (yoga or control) and a within

subjects-factor of Time (baseline, post intervention, or follow-up) was conducted. A benefit of mixed-model ANOVA is that it provides a reliable measure of effect size as well as contrasts of significant effects that indicate the trajectory of time (i.e. linear or quadratic trajectories). However, generalised linear models have been criticised for violating the assumption of independence of observations. To address this concern, mixed-level models (mixed effect models and marginal models) with maximum likelihood (ML) estimation were also used to analyse the intervention data (Bryk & Raudenbush, 1992). Mixed-level models are appropriate for analysis of this data as cases are nested within the individual, so there is a lack of independence between observations obtained at each time point (two-level hierarchy). In addition, these models are more robust to missing data and unbalanced designs (Krueger & Tian, 2004).

To account for the attrition bias in estimating treatment effect, an intention-to-treat (ITT) analysis was run on all outcome variables, in addition to per-protocol (PP) analysis, which was conducted to estimate maximum treatment efficacy (Armijo-Olivo et al., 2009; Gupta, 2011; Lesaffre & Verbeke, 2005). Separate models were conducted for each outcome variable. While a number of participants did discontinue attendance at the yoga class they still attended the post-test and follow-up (see Figure 1), so it was possible to ascertain the practical value of being able to offer yoga in this population (Lesaffre & Verbeke, 2005).

We did not adjust for multiple testing. Exact *p* values are presented along with the effect size for mixed-model ANOVA and confidence intervals for mixed models.

Results

Results of the mixed-model ANOVA will be presented first, followed by results of multi-level models (mixed effect and marginal).

Mixed-model ANOVA

Mixed between-within subjects analysis of variances (ANOVA) were conducted to assess the impact of the yoga intervention on various outcome measures, across three time points (pre-intervention, post-intervention and one-month follow-up). The outcome variables used were changes in psychological distress, stress, well-being, and positive and negative affect measures. Each outcome variable was evaluated in an intent-to-treat and per-protocol analysis.

Intent-to-treat analysis. All participants were included in the construction of ITT models based on the original randomisation, regardless of protocol adherence. There was a significant interaction of Group (yoga vs control) and Time in relative to positive affect (η_ρ^2 = .07). No other significant interactions were observed, but subjective well-being changes indicated a medium effect size (η_ρ^2 = .06). There was a substantial main effect of Time for psychological distress (η_ρ^2 = .23), perceived stress (η_ρ^2 = .27), and subjective well-being (η_ρ^2 = .12). The main effect of Group was not found significant, which suggested no difference in effectiveness between participation in the yoga intervention and the control group. No effect was seen for mindfulness or negative effect. Descriptive statistics and ANOVA outcomes are detailed in Table 2.

Table 2

Raw Mean, SD and ANCOVA of Psychological Outcome Variables of Total Sample and Groups

	K10			PSS			MAAS		
	Pre	Post	Foll-up	Pre	Post	Foll-up	Pre	Post	Foll-up
	M (*SD*)	*M* (*SD*)	*M* (*SD*)	*M* (*SD*)	*M* (*SD*)	*M* (*SD*)	*M* (*SD*)	*M* (*SD*)	*M* (*SD*)
Control[a]	24.27 (5.43)	22.53 (5.9)	21.11 (5.15)	26.0 (5.4)	24.23 (5.63)	22.87 (7.15)	3.55 (0.76)	3.65 (0.82)	3.60 (0.82)
Yoga (PP)[b]	23.31 (4.91)	19.40 (6.24)	19.52 (6.27)	26.40 (5.05)	21.93 (6.87)	22.87 (7.37)	3.46 (0.83)	3.72 (0.79)	3.73 (0.78)
Group*Time (PP)	F(2, 86) = 2.42, p = .095 ηρ2 = .05 (small)			F(2,86) = 2.37, p = .099 ηρ2 = .05 (small)			F(2, 86) = .41, p = .665 ηρ2 < .01 (small)		
Time (PP)	F(2,86) = 15.53, p < .001 ηρ2 = .27 (large)			F(2,86) = 20.99, p < .001 ηρ2 = .33 (large)			F(2, 865) = 1.94, p = .150 ηρ2 < .04 (small)		
Group (PP)	F (1,87) = 229.41, p = .071 ηρ2 = .04 (small)			F (1, 87) = .81, p = .371 ηρ2 = .01 (small)			F (1, 87) = .19, p = .665 ηρ2 < .00 (small)		
Yoga (ITT)[c]	23.55 (5.22)	20.23 (6.88)	20.40 (7.08)	26.42 (4.86)	22.21 (6.85)	22.40 (7.71)	3.53 (0.80)	3.72 (0.82)	3.71 (0.79)
Group*Time (ITT)	F(2, 91) = 2.16, p = .121 ηρ2 = .05 (small)			F(2, 91) = 2.17, p = .121 ηρ2 = .05 (small)			F(2, 91) = .492, p = .613 ηρ2 = .01 (small)		
Time (ITT)	F(2, 91) = 13.29, p < .000 ηρ2 = .23 (large)			F(2, 91) = 16.88, p < .000 ηρ2 = .27 (large)			F(2, 91) = 2.46, p < .091 ηρ2 = .05 (small)		
Group (ITT)	F (1, 92) = 1.28, p = .260 ηρ2 = .01 (small)			F (1, 92) = .93, p = .534 ηρ2 < .00 (small)			F(1, 92) = 12, p = .736 ηρ2 < .00 (small)		

Table Continues

Table 2 Continued

	PWI-A			POS Affect			NEG Affect		
	Pre	Post	Foll-up	Pre	Post	Foll-up	Pre	Post	Foll-up
	M (*SD*)	*M* (*SD*)	*M* (*SD*)	*M* (*SD*)	*M* (*SD*)	*M* (*SD*)	*M* (*SD*)	*M* (*SD*)	*M* (*SD*)
Control[a]	50.89 (11.48)	53.76 (9.93)	55.00 (0.40)	32.49 (7.19)	29.78 (8.20)	29.64 (8.20)	13.91 (4.54)	13.33 (4.3)	12.76 (3.77)
Yoga (PP)[b]	48.68 (11.94)	54.65 (12.45)	52.65 (9.86)	32.45 (7.00)	32.5 (7.4)	33.01 (7.73)	13.39 (4.64)	11.79 (2.92)	12.24 (3.30)
Group*Time (PP)	F(2,65) = 1.50, p = .230 ηρ2 = .04 (small)			F(2,80) = 2.61, p = .080 ηρ2 = .06 (medium)			F(2, 80) = 1.0, p = .374 ηρ2 = .02 (small)		
Time (PP)	F(2,65) = 4.67, p = .013 ηρ2 = .13 (medium)			F(2,80) = 1.53, p = .223 ηρ2 = .04 (small)			F(2, 80) = 2.33, p = .104 ηρ2 = .06 (medium)		
Group (PP)	F (1,66) = .32, p = .573 ηρ2 = .01 (small)			F (1,81) = 2.05, p = .156 ηρ2 = .03 (small)			F(1, 81) = 1.17, p = .194 ηρ2 = .02 (small)		
Yoga (ITT)[c]	47.86 (11.56)	53.72 (12.6)	51.36 (10.99)	32.48 (6.73)	32.30 (6.84)	33.36 (7.30)	13.30 (4.34)	12.5 (4.93)	12.53 (3.57)
Group*Time (ITT)	F(2, 70) = 2.08, p = .133 ηρ2 = .06 (medium)			F(2, 86) = 3.12, p = .049 ηρ2 = .07 (medium)			F(2, 86) = .29, p = .751 ηρ2 = .01 (small)		
Time (ITT)	F(2, 70) = 4.84, p = .011 ηρ2 = .12 (medium)			F(2, 86) = 1.68, p = .193 ηρ2 = .04 (small)			F(2, 86) = 1.82, p = .169 ηρ2 = .04 (small)		
Group (ITT)	F(1, 71) = 1.07, p = .304 ηρ2 = .02 (small)			F(1, 87) = 2.44, p = .122 ηρ2 = .03 (small)			F(2, 87) = .71, p = .401 ηρ2 = .01 (small)		

Note: PWI-A = The Psychological Wellbeing Index – Adult, POS Affect = Positive, NEG Affect = Negative Affect, PP = Per-Protocol Analysis, ITT = Intent-to-treat Analysis, Foll-up = Follow-up, η_ρ^2 = partial eta squared.
[a]n = between 37 and 47, [b]n = between 31 and 42, [c]n = between 36 and 47.
Field (2013) suggests that small, medium, and large effect sizes correspond to: small = 0.01; medium = 0.06; and, large = 0.14.

Per-protocol analysis. Only participants who adhered to protocol were included in the construction of PP models; specifically, those in the yoga group were required to have attended a minimum of one yoga class per week. There were no significant Group x Time interactions, although positive affect indicated a trend and demonstrated a medium effect size ($\eta_\rho^2 = .06$). The Time main effect was significant for psychological distress ($\eta_\rho^2 = .27$), perceived stress ($\eta_\rho^2 = .33$), and subjective well-being ($\eta_\rho^2 = .13$), but no such effect for mindfulness, positive affect or negative effect. The Group main effect was not significant. Descriptive statistics and mixed ANOVA outcomes are detailed in Table 2.

Multi-level Models (Mixed and Marginal)

Following the proposed method suggested by Singer and Willett (2003) models were constructed sequentially to determine if the increasing polynomial complexity enhanced model fit, as assessed by the Akaike Information Criterion (AIC), Bayesian Information Criterion (BIC), and Log-Linear Likelihood Ratio (-2LLR), where non-significant values indicate that the number of variables contained in the model does not improve the fit from the previous model with fewer variables. In the first stage of analysis, we constructed mixed effects that modelled time as a random effect. These models were tested first to determine if they provided enough control to deal with the non-independence of subject's residuals (*between* subject). The models we constructed were: (1) a base intercept only model (Model 1) to examine mean differences in the outcome across individuals (i.e. the starting value of the outcome variable), (2) an unconditional growth model (Model 2) that serves as a baseline linear model for growth curves (i.e. the slope of the growth curve over time), in the case where the mixed ANOVA result indicated a significant quadratic function of time, (3) a quadratic growth factor was used to construct a second-order polynomial model (Model 3) to estimate the rate of change, and (4) a conditional model (Model 4) to examine if the predictor (group was examined as a time-invariant covariate) was related to the growth parameters (i.e.,

initial status, linear growth, and quadratic growth). The second stage involved examining *within* subject variance by altering the covariance structure of the residuals in 3 different marginal models (AKA, the population averaged models) with time as an independent variable (Models 6, 7, and 8) which examined the following residual covariance structures: Unstructured (UN), Compound Symmetry (CS), and First-Order Autoregressive (AR1). In this second stage time was modelled as a repeated variable, which yields enhanced model fit if there is extra non-independence or non-consistent variance among the residuals that is not accounted for in growth curve models. Successful model convergence was achieved for all models reported.

The covariates of age, energy expenditure, blood pressure and heart-rate were examined, but these factors are not included in the final models because they were not found to approach significance, nor were they statistically relevant when included. This supports the previous observation of these factors not being found to differ between groups in this population (Harkess, 2016). The measurement of time was adjusted to account for the unequal measurement intervals. Resultant model parameters (fixed effects) indicated the unit differences in scores on the outcome measure associated with a unit increase in the value of a predictor variable.

Analysis of multi-level models. Please see Table 3 for an overview of relevant ITT and PP analysis for each measure. Please see Supplementary Material (Tables 4 through 9) for a detailed depiction of relevant ITT and PP analysis for each measure (for ease of readability, these tables are included at the end of this Chapter).

Table 3

Overview of Multi-level Models Representing Change in Psychological Outcome Variables

	K10[a]		PSS[a]		PWI-A[a]	
	ITT	PP	ITT	PP	ITT	PP
	Coef. (SE)	Coef. (SE)	Coef. (SE)	Coef. (SE)	Coef. (SE)	Coef. (SE)
Fixed effects						
Intercept	24.39 (1.59)***	23.64 (1.76)***	27.95 (1.46)***	27.95 (1.66)***	42.90 (3.80)***	43.85 (4.22)***
Time (Lin)	-5.74 (1.94)**	-6.34 (2.08)**	-6.88 (2.22)**	-6.59 (2.44)**	11.26 (4.42)*	9.88 (4.91)*
Time (Quad)	1.48 (0.59)*	1.57 (0.64)*	1.71 (.75)*	-0.82 (0.51)	-3.10 (1.30)*	-2.60 (1.42)*
Group	-0.12 (1.01)	.25 (1.08)	-.95 (.94)	-0.95 (1.02)	3.26 (2.43)	2.79 (2.60)
Group*Time (Lin)	2.61 (1.23)*	2.91 (1.29)*	3.18 (1.41)*	3.04 (1.51)*	-4.74 (2.81)*	-4.05 (3.04)
Group*Time (Quad)	-0.83 (0.38)*	-0.87 (0.39)*	-.94 (.48)*	-0.82 (0.51)	1.47 (0.82)*	1.22 (0.88)
Log Likelihood	-14.55	-16.63	-5.91	-6.32	-48.11	-20.76
LLR-2 Log Likelihood[c]	1856.73	1684.48	1905.63	1738.31	2080.81	1900.34
Δ -2 Log Likelihood[d]	$p < .05$	$p < .05$			$p < .05$	$p < .05$

Table Continues

Table 3 Continued

	POS Affect[b]		NEG Affect[b]		MAAS[b]	
	ITT	PP	ITT	PP	ITT	PP
	Coef. (SE)	Coef. (SE)	Coef. (SE)	Coef. (SE)	Coef. (SE)	Coef. (SE)
Fixed effects						
Intercept	31.85 (1.98)***	32.31 (2.23)***	13.00 (1.23)***	12.65 (1.39)***	3.34 (.22)***	3.48 (0.25)***
Time (Lin)	1.69 (0.74)*	1.46 (.79)*	-0.27 (0.49)	-0.41 (0.54)	0.14 (.07)*	0.11 (0.07)
Group	-0.09 (1.27)	-0.32 (1.37)	0.49 (0.79)	0.68 (0.86)	0.12 (.14)	0.05 (0.15)
Group*Time (Lin)	-1.34 (.47)**	-1.23 (0.49)*	-0.04 (0.31)	0.02 (0.33)	-0.06 (.04)	-0.05 (0.04)
Log Likelihood	-9	-7.52	-21.77	-18.22	-2.23	11.65
LLR-2 Log Likelihood[c]	1983.52	1804.85	1694.21	1509.41	563.81	506.35
Δ -2 Log Likelihood[d]	$p < .05$	$p < .05$	$p < .05$	$p < .05$		

Note: Coef = Coeficient; PWI-A = The Psychological Wellbeing Index – Adult, POS Affect = Positive, NEG Affect = Negative Affect, PP = Per-Protocol Analysis, ITT = Intent-to-treat Analysis, [a] Model 5, (Unstructured (UN) Covariance Structure Marginal Model), evaluated against Model 3 (Quadratic Growth Curve Model), [b] Model 5, (Unstructured (UN) Covariance Structure Marginal Model), evaluated against Model 2 (Unconditional Growth Curve Model), [c]i.e., deviance, [d]*p-value.*
*p<.05, **p < .01, ***p<.001.

Time and Time^2. Intent-to-treat (ITT) and per-protocol (PP) analysis both indicated equivalent outcomes for the dependant variables, with the exception of PWI-A, so for simplicity ITT and PP models will not be defined unless the significance of the results differ. The addition of linear time improved the 'change from baseline' model fit for all psychological variables tested. Following results of mixed-model ANOVAs, quadratic time (Time^2) was included for all K10, PSS, PQI-A, though none of the models improved significantly with its addition. However, we retained quadratic time as it was theoretically predicted that there would be an interaction for the yoga Group and Time^2 (due to the post-follow-up period vs the pre-to-post). The statistically significant variance components for K10, and PWI-A suggest that participants differ substantially from the average linear change over time.

Addition of Group and Group-by-Time interactions. The addition of Group and interaction between Group and Time variables significantly improved K10, PWI-A, PA and NA, but did not improve PSS or MAAS.

Mixed or marginal models. It was found that only PA had a significantly better fit when controlling for between subject variability, rather than within. This suggests less variability in participants' PA over time than the other psychological variables tested. In the marginal model, Group was a significant predictor of linear changes in PA score but was not associated with the initial status. The control group showed a faster rate of change as compared with the yoga group. K10, PQI-A and NA models were best fit by the UN covariance structure marginal model, where within subject variability was controlled.

Main effects demonstrated by significant models. K10 and the ITT PQI-A model demonstrated main effects of Time and Time^2, as well as interactions for Group x Time, and Group x Time^2, specifically, the yoga group had a faster rate of linear change (decrease) and a slower rate of quadratic change (upturn), relative to the control group, while the PWI-A ITT

indicated the yoga group had a slower rate of linear change (increase) but a quicker quadratic rate (downturn). PP PQI-A demonstrated main effects for Time and Time^2, and NA did not demonstrate any main effects.

Discussion

The aim of this study was to investigate the longitudinal impact of an 8-week, moderate-intensity yoga intervention on chronically stressed women's psychological well-being (at baseline, post-test, and a one-month follow-up). A mixed-ANOVA approach revealed a main effect of time, with women reporting decreased psychological distress and perceived stress, alongside increased subjective well-being, regardless of group. As expected, both quadratic and linear trajectories of change were indicated for distress, stress, and well-being, though only linear was indicated for PA. No main effects of group were found. The only significant Group x Time interaction was observed for PA. The clearly observed effect for time observed across both groups may reflect the fact that women who chose to participate were actively seeking stress reduction and had a "readiness" to change attitude that would support engagement in health-promoting behaviours (Mann, de Ridder, & Fujita, 2013), such as physical activity. This is supported by the observation that including energy expenditure as a covariate in multilevel models did not significantly improve any models, and by previous analysis in this cohort that showed that the two groups were generally matched in terms of the estimated energy expenditure reported over the period of the study (Harkess et al., 2016).

It is possible that a longer intervention duration was necessary to maintain effects seen on distress, stress and PA at post-test (Harkess et al., 2016) through the follow-up period. To the authors' knowledge, this was the first study to examine the effects of a brief yoga intervention on psychotherapeutic measures in a follow-up assessment, as called for in a

review of yoga on anxiety and stress (Li & Goldsmith, 2012). One previous RCT has conducted a follow-up assessment in a community population at 6-weeks after a 10-week yoga intervention in a community population (Smith et al., 2007). However, they did not report on how the effect of yoga was maintained as their purpose was to compare yoga and relaxation participants (Smith et al., 2007). It was reported that relaxation participants had a more significant follow-up effect, plausibly due to the challenge of incorporating yoga into daily life beyond formal classes (Smith et al., 2007). Further, while CBT and MBSR demonstrate that the psychotherapeutic benefit is maintained beyond intervention cessation, it likely that the case self-regulation tools instructed in CBT are implemented beyond therapy and this continues to affect top-down processing. By contrast, MBSR requires continued home practice and this gives rise to bottom-up transformation. This highlights the importance of future research exploring the association between home yoga practice, or continued yoga practice, and outcomes, as well as the longevity of effects reported from varying intervention durations.

A further consideration in evaluating intervention durations is that not all dispositions demonstrated the same trajectory. For example, it has been reported that levels of mindfulness did not change over the 8-weeks in a clinical population (early-stage breast and prostate cancer patients; Brown & Ryan, 2003). This was attributed to longer durations of time being necessary to detect change in this disposition, which is supported by their finding that mindfulness levels of Zen meditation students were not related to current practice, but to the years they had practised (Brown & Ryan, 2003). It is likely that intervention effects on different domains have varied minimum intervention necessary to produce change (MINC) and to maintain effects beyond the conclusion of formal intervention. At current, the optimal durational frequency and duration of yoga has yet to be determined.

The MINC frequency of practice is suggested to be weekly, offering twice-weekly yoga classes is not reported to be more beneficial than once-weekly, due to limited compliance (Michalsen et al., 2012). Previous analysis of this cohort (Harkess et al., 2016) supported a minimum of once-weekly yoga practice for maximal benefit. However, robust difference between PP and ITT analysis is not indicated in the current study, which may be attributed to the inclusion of the follow-up period (1 month following cessation of the formal yoga classes) in analysis and the large variability in the follow-up group (relative to the previous time points). This larger variability in the follow-up group may also be attributable to some continuing with yoga practice independently, and others not. Previously, the group was homogenous in yoga practice (none at the start, and either experimental or wait-list group during the active part of the study). While previous studies have not conducted follow-ups (Li & Goldsmith, 2012), which would allow us to compare how differing intervention lengths effect outcomes over time, it seems probable that this study did not administer the MINC to impact on various systems of self-regulation beyond weekly participation in the intervention itself.

The moderate Group x Time effect for positive affect did not reach significance in the mixed-model ANOVA (PP p = .080), but was supported by multilevel models. Previous studies have demonstrated that PA increases with yoga (Danhauer et al., 2009; Narasimhan et al., 2011; Tolbanos Roche & Mas Hesse, 2014; Vadiraja et al., 2009); contrarily the trajectory here suggests PA decreased in the control group. Considering the decreased distress and stress and increased well-being seen in both groups, it is interesting to postulate why this may be the case. One possibility is that it may be a seasonal effect; namely, the study commenced in autumn, and the follow-up took place in winter. Cold weather is known to assist in the survival of bacteria (Handley & Webster, 1995), as well as having adverse effects on the immune system. Winter is a time when the normal population experiences

increased levels of anxiety and depression, a subset being vulnerable to seasonal affective disorder (SAD; Lansdowne & Provost, 1998; Partonen & Lönnqvist, 1998). As PA is protective of illness (Cohen et al., 2003) and is found to be diminished in depressed individuals (Folkman & Moskowitz, 2000), it is plausible that yoga buffered the seasonal effect on positive affect, supporting a bottom-up effect. Alternatively, it could be due to anticipation of yoga being sufficient in the wait-list group to have some effect.

Multi-level models demonstrated group was a significant predictor of linear and quadratic changes in distress and well-being (ITT). The interactions indicate that the yoga group had a faster decrease in distress to post-test, but their rate of change slowed, while the control group's continued until follow-up. Similar trends have been characterised by previous research findings that when people are engaged in physical activity, they report less symptoms of depression; but when they cease exercising, they report more symptoms of depression than those who maintain an exercise program (Babyak et al., 2000). However, the current study design does not allow for formal testing of the effects of ceasing yoga versus continuation. Another contribution to this effect may be the control group would be anticipating the start of their round of yoga classes.

Contrastingly, the yoga group and control groups showed similar growth in subjective well-being (ITT analysis) from baseline to post-test, but unexpectedly there was a decrease in the yoga practitioners' well-being upon the completion of the yoga intervention, whilst the control group's well-being continued to increase. It may be that the yoga classes are perceived to increase in value to the person's well-being once they are unavailable (Brock, 1968), and may be reflective of reported barriers to continuing a yoga practice, namely cost and availability (Penman et al., 2012).

Limitations

There are some limitations to this study which merit consideration when interpreting the results. First, due to the nature of the intervention, it was not possible for participants to be blind to their group allocation; due to timeframe constraints the follow-up period was of a brief duration (1 month), further, in our efforts to follow trends in the psychotherapy (e.g MBSR**),** our intervention was only 8 weeks, which may not have provided the MINC. A second limitation was that the research focused on self-selected middle-aged women in an educated population. Although this may be reflective of those who do generally practice yoga (Birdee et al., 2008), further resarch examining other populations will highlight if these results are generalisable to a broader community. Additionally, the women self-selected so they were likely ready to make life changes to reduce stress experienced, which may explain clear time effect. Furthermore, the wait-list control design may have impacted on outcomes measured as at the conclusion of the first round of yoga classes which was the time that the intervention group ceased to be offered further yoga classes, a resource known to be limited by availability and cost, while the control group was approaching the ofference of yoga classes. This may be avoided in future by allowing the intervention group to continue practising with the control group, which would also be interesting as it would allow the evaluation of outcomes at multiple time points. A further option is to commence the second round of classes after a longer duration, so their effect and cognition is less affected at post-test and follow-up assessments. A final consideration is the use of a no-treatment control, which does not allow for controlling for variables such as attention to reported levels of stress, or contact with a caring yoga teacher. While this study indicates benefit for participating in the yoga intervention, future research may utilise an active-control, which would allow for more refined exploration of the effects of a yoga intervention compared with participation in another intervention aimed at stress-reduction.

Conclusions

In conclusion, the findings of this study indicate that at a 1-month follow-up assessment, participation in a weekly 60-minute yoga class taking place over two months only yielded small and largely non-significant changes in psychological measures for women with chronic stress. There was some evidence of psychotherapeutic outcome improvements in the intervention group compared to the control at post-test, however these effects were not robustly captured when the follow-up assessment was modelled. In light of the cost-effectiveness and physical benefit of engaging in aerobic and resistance training exercise (Wanderley et al., 2013), further validation of this finding over a longer duration and with other samples may have important implications. Given that yoga very likely provides physical benefits, or is comparable with other stress reduction interventions (i.e. psychotherapy), it could provide a multifaceted intervention (targeting both cognitive, emotional, and behavioural output as well as autonomic output; (Gard et al., 2014). Further research into yoga's MINC, mechanisms, and potential to enrich psychotherapy (e.g. Khalsa et al., 2014 with CBT) may be beneficial in global efforts to address the negative effects of chronic stress.

Supplementary Material

Table 4

Comparison of fitting alternate multilevel models representing change in psychological distress over time

	Intent-to-Treat Analysis			
	Model A[a]		Model B[b]	
	Coefficient (SE)	95% CI	Coefficient (SE)	95% CI
Fixed effects				
Intercept	24.21 (0.51)***	[23.20, 25.21]	24.39 (1.59)***	[21.25, 27.53]
Time (Lin)	-1.85 (0.58)**	[-3.00, -0.69]	-5.74 (1.94)**	[-9.58, -1.89]
Time (Quad)	0.25 (0.19)	[-0.14, 0.63]	1.48 (0.59)*	[0.30, 2.66]
Group			-0.12 (1.01)	[-2.13, 1.89]
Group*Time(Lin)			2.61 (1.23)*	[0.16, 5.05]
Group*Time(Quad)			-0.83 (0.38)*	[-1.58, -0.08]
Log Likelihood	-58.34		-14.55	
LLR-2 Log Likelihood[c]	1871.280		1856.730	
Δ -2 Log Likelihood[d]	$p < .05$		$p < .05$	
AIC	1885.280		1880.730	
BIC	1911.460		1925.600	
DF	7		12	

Table continues

Table 4 Continues

	Per-Protocol Analysis			
	Model A[a]		Model A[a]	
	Coefficient (SE)	95% CI	Coefficient (SE)	95% CI
Fixed effects				
Intercept	24.03 (0.54)***	[22.96, 25.10]	23.64 (1.76)***	[20.15, 27.13]
Time (Lin)	-1.89 (0.61)**	[-3.10, -0.68]	-6.34 (2.08)**	[-10.48, -2.21]
Time (Quad)	0.30 (0.24)	[-0.19, 0.78]	1.57 (0.64)*	[0.31, 2.83]
Group			0.25 (1.08)	[-1.89, 2.40]
Group*Time (Lin)			2.91 (1.29)*	[0.34, 5.48]
Group*Time (Quad)			-0.87 (0.39)*	[-1.66, -0.09]
Log Likelihood	-55.11		-16.63	
LLR-2 Log Likelihood[c]	1701.110		1684.480	
Δ -2 Log Likelihood[d]	$p < .05$		$p < .05$	
AIC	1715.110		1708.480	
BIC	1740.650		1752.270	
DF	9		12	

Note. [a] Model 3 (Quadratic Growth Curve Model), evaluated against Model 1 (Intercept Only Model) [b] Model 5, (Unstructured (UN) Covariance Structure Marginal Model), evaluated against Model 3 (Quadratic Growth Curve Model), [c]i.e., deviance, [d]*p-value.*
*p<.05, **p < .01, ***p<.001.

Table 5

Comparison of fitting alternate multilevel models representing change in perceived stress over time

	Intent-to-Treat Analysis			
	Model A[a]		Model B[b]	
	Coefficient (SE)	95% CI	Coefficient (SE)	95% CI
Fixed effects				
Intercept	26.54 (0.46)***	[25.62, 27.46]	27.95 (1.46)***	[25.05, 30.85]
Time (Lin)	-2.12 (0.72)**	[-3.55, -0.69]	-6.88 (2.22)**	[-11.29, -2.46]
Time (Quad)	0.30 (0.24)	[-0.19, 0.78]	1.71 (0.75)*	[0.21, 3.20]
Group			-0.95 (0.94)	[-2.80, 0.91]
Group*Time (Lin)			3.18 (1.41)*	[0.38, 5.99]
Group*Time (Quad)			-0.94 (0.48)*	[-1.89, 0.01]
Log Likelihood	-68.21		-5.91	
LLR-2 Log Likelihood[c]	1911.540		1905.630	
Δ -2 Log Likelihood[d]	$p < .05$			
AIC	1925.540		1929.630	
BIC	1951.720		1974.510	
DF	7		12	

Table continues

Table 5 Continued

	Per-Protocol Analysis			
	Model A[a]		Model A[b]	
	Coefficient (SE)	95% CI	Coefficient (SE)	95% CI
Fixed effects				
Intercept	26.48 (0.50)***	[25.49, 27.47]	27.95 (1.66)***	[24.65, 31.25]
Time (Lin)	-1.91 (0.74)*	[-3.38, -0.45]	-6.59 (2.44)**	[-11.43, -1.75]
Time (Quad)	0.20 (0.25)	[-0.29, 0.70]	-0.82 (0.51)	[-1.84, 0.19]
Group			-0.95 (1.02)	[-2.97, 1.08]
Group*Time (Lin)			3.04 (1.51)*	[0.03, 6.04]
Group*Time (Quad)			-0.82 (0.51)	[-1.84, 0.19]
Log Likelihood	-62.23		-6.32	
LLR-2 Log Likelihood[c]	1744.630		1738.310	
Δ -2 Log Likelihood[d]	p < .05			
AIC	1758.630		1762.310	
BIC	1784.170		1806.100	
DF	7		12	

Note. [a] Model 3 (Quadratic Growth Curve Model), evaluated against Model 1 (Intercept Only Model) [b] Model 5, (Unstructured (UN) Covariance Structure Marginal Model), evaluated against Model 3 (Quadratic Growth Curve Model), [c]i.e., deviance, [d]*p-value.*
*p<.05, **p < .01, ***p<.001.

Table 6

Comparison of fitting alternate multilevel models representing change in subjective well-being over time

	Intent-to-Treat Analysis			
	Model A[a]		Model B[b]	
	Coefficient (SE)	95% CI	Coefficient (SE)	95% CI
Fixed effects				
Intercept	47.64 (1.18)***	[45.31, 50.00]	42.90 (3.80)***	[35.35, 50.45]
Time (Lin)	4.00 (1.26)**	[1.50, 6.47]	11.26 (4.42)*	[-2.50, 20.03]
Time (Quad)	-0.82 (0.41)*	[-1.63, -0.01]	-3.10 (1.30)*	[-5.66, -0.52]
Group			3.26 (2.43)	[-1.56, 8.08]
Group*Time(Lin)			-4.74 (2.81)*	[-10.32, 0.83]
Group*Time(Quad)			1.47 (0.82)*	[-0.16, 3.10]
Log Likelihood	-27.10		-48.11	
LLR-2 Log Likelihood[c]	2101.820		2080.810	
Δ -2 Log Likelihood[d]	$p < .05$		$p < .05$	
AIC	2115.820		2104.810	
BIC	2141.390		2148.640	
DF	7		12	

Table continues

Table 6 Continued

	Per-Protocol Analysis			
	Model A[a]		Model A[a]	
	Coefficient (SE)	95% CI	Coefficient (SE)	95% CI
Fixed effects				
Intercept	48.06 (1.24)***	[45.60, 50.53]	43.85 (4.22)***	[35.45, 52.25]
Time (Lin)	3.47 (1.34)*	[0.82, 6.13]	9.88 (4.91)*	[0.13, 19.64]
Time (Quad)	-0.64 (0.43)	[-1.50, 0.22]	-2.60 (1.42)*	[-5.42, 0.23]
Group			2.79 (2.60)	[-2.38, 7.95]
Group*Time (Lin)			-4.05 (3.04)	[-10.08, 1.97]
Group*Time (Quad)			1.22 (0.88)	[-0.53, 2.97]
Log Likelihood	-23.10		-20.76	
LLR-2 Log Likelihood[c]	1921.100		1900.340	
Δ -2 Log Likelihood[d]	$p < .05$		$p < .05$	
AIC	1935.100		1924.340	
BIC	1960.050		1967.110	
DF	7		12	

Note. [a] Model 3 (Quadratic Growth Curve Model), evaluated against Model 1 (Intercept Only Model) [b] Model 5, (Unstructured (UN) Covariance Structure Marginal Model), evaluated against Model 3 (Quadratic Growth Curve Model), [c]i.e., deviance, [d]*p-value.*
*p<.05, **p < .01, ***p<.001.

Table 7

Comparison of fitting alternate multilevel models representing change in positive affect over time

	Intent-to-Treat Analysis			
	Model A[a]		Model B[b]	
	Coefficient (SE)	95% CI	Coefficient (SE)	95% CI
Fixed effects				
Intercept	31.73 (0.63)***	[30.47, 32.98]	31.85 (1.98)***	[27.92, 35.78]
Time (Lin)	-0.31 (0.25)	[-0.79, 0.18]	1.69 (0.74)*	[0.21, 3.16]
Group			-0.09 (1.27)	[-2.59, 2.42]
Group*Time(Lin)			-1.34 (0.47)**	[-2.28, -0.40]
Log Likelihood	-8.41		-9.00	
LLR-2 Log Likelihood[c]	1992.520		1983.520	
Δ -2 Log Likelihood[d]	$p < .05$		$p < .05$	
AIC	2004.520		1999.520	
BIC	2026.800		2029.230	
DF	7		8	

Table continues

Table 7 Continued

	Per-Protocol Analysis			
	Model A[a]		Model B[b]	
	Coefficient (SE)	95% CI	Coefficient (SE)	95% CI
Fixed effects				
Intercept	31.82 (0.68)***	[30.47, 33.18]	32.31 (2.23)***	[27.89, 36.74]
Time (Lin)	-0.41 (0.25)	[-0.91, 0.10]	1.46 (0.79)*	[-0.10, 3.03]
Group			-0.32 (1.37)	[-3.04, 2.41]
Group*Time(Lin)			-1.23 (0.49)*	[-2.20, -0.26]
Log Likelihood	-8.90		-7.52	
LLR-2 Log Likelihood[c]	1812.370		1804.850	
Δ -2 Log Likelihood[d]	$p < .05$		$p < .05$	
AIC	1824.370		1820.850	
BIC	1846.090		1849.820	
DF	7		8	

Note. [a] Model 2 (Unconditional Growth Curve Model), evaluated against Model 1 (Intercept Only Model) [b] Model 5, (Unstructured (UN) Covariance Structure Marginal Model), evaluated against Model 2 (Unconditional Growth Curve Model), [c]i.e., deviance, [d]*p-value.*
*p<.05, **p < .01, ***p<.001.

Table 8

Comparison of fitting alternate multilevel models representing change in negative affect over time

	Intent-to-Treat Analysis			
	Model A[a]		Model B[b]	
	Coefficient (SE)	95% CI	Coefficient (SE)	95% CI
Fixed effects				
Intercept	13.75 (0.40)***	[12.95, 14.54]	13.00 (1.23)***	[10.55, 15.44]
Time (Lin)	-0.36 (0.18)*	[-0.71, -0.01]	-0.27 (0.49)	[-1.25, 0.71]
Group			0.49 (0.79)	[-1.07, 2.05]
Group*Time(Lin)			-0.04 (0.31)	[-0.66, 0.58]
Log Likelihood	-6.54		-21.77	
LLR-2 Log Likelihood[c]	1715.980		1694.210	
Δ -2 Log Likelihood[d]	$p < .05$		$p < .05$	
AIC	1727.980		1714.210	
BIC	1750.270		1751.350	
DF	6		10	

Table continues

Table 8 Continued

	Per-Protocol Analysis			
	Model A[a]		Model B[b]	
	Coefficient (SE)	95% CI	Coefficient (SE)	95% CI
Fixed effects				
Intercept	13.85 (0.45)***	[12.95, 14.74]	12.65 (1.39)***	[9.89, 15.42]
Time (Lin)	-0.46 (0.19)*	[-0.83, -0.09]	-0.41 (0.54)	[-1.48, 0.67]
Group			0.68 (0.86)	[-1.03, 2.38]
Group*Time(Lin)			0.02 (0.33)	[-0.64, 0.69]
Log Likelihood	-18.71		-18.22	
LLR-2 Log Likelihood[c]	1527.630		1509.410	
Δ -2 Log Likelihood[d]	$p < .05$		$p < .05$	
AIC	1539.630		1529.410	
BIC	1561.360		1565.610	
DF	6		10	

Note. [a] Model 2 (Unconditional Growth Curve Model), evaluated against Model 1 (Intercept Only Model) [b] Model 5, (Unstructured (UN) Covariance Structure Marginal Model), evaluated against Model 2 (Unconditional Growth Curve Model), [c]i.e., deviance, [d]*p-value.*
*p<.05, **p < .01, ***p<.001.

Table 9

Comparison of fitting alternate multilevel models representing change in mindfulness over time

	Intent-to-Treat Analysis			
	Model A[a]		Model B[b]	
	Coefficient (SE)	95% CI	Coefficient (SE)	95% CI
Fixed effects				
Intercept	3.52 (0.07)***	[3.38, 3.66]	3.34 (.22)***	[2.91, 3.78]
Time (Lin)	0.05 (0.02)*	[0.01, 0.09]	0.14 (.07)*	[0.01, 0.27]
Group			0.12 (.14)	[-0.16, 0.40]
Group*Time(Lin)			-0.06 (.04)	[-0.15, 0.02]
Log Likelihood	-13.09		-2.23	
LLR-2 Log Likelihood[c]	566.040		563.810	
Δ -2 Log Likelihood[d]	$p < .05$			
AIC	578.040		579.810	
BIC	600.480		609.720	
DF	6		8	

Table continues

Table 9 Continued

	Per-Protocol Analysis			
	Model A[a]		Model B[b]	
	Coefficient (SE)	95% CI	Coefficient (SE)	95% CI
Fixed effects				
Intercept	3.56 (0.08)***	[3.41, 3.71]	3.48 (0.25)***	[3.00, 3.97]
Time (Lin)	0.04 (0.02)*	[-0.01, 0.08]	0.11 (0.07)	[-0.03, 0.25]
Group			0.05 (0.15)	[-0.25, 0.35]
Group*Time(Lin)			-0.05 (0.04)	[-0.13, 0.04]
Log Likelihood	10.56		11.65	
LLR-2 Log Likelihood[c]	507.440		506.350	
Δ -2 Log Likelihood[d]	$p < .05$			
AIC	519.440		522.350	
BIC	541.330		551.550	
DF	6		8	

Note. [a] Model 2 (Unconditional Growth Curve Model), evaluated against Model 1 (Intercept Only Model) [b] Model 5, (Unstructured (UN) Covariance Structure Marginal Model), evaluated against Model 2 (Unconditional Growth Curve Model), [c]i.e., deviance, [d]*p-value.*
*p<.05, **p < .01, ***p<.001.

4.2 Summary

Results indicated that most between group differences reported in Study 2 (Chapter 3) were not maintained, which indicates that eight weeks of contact with a yoga intervention was not positively associated with cognitive evaluations of psychological distress and perceived stress one month later. These findings suggest that yoga may evoke similar mechanisms to exercise where continuing engagement is necessary to maintain therapeutic benefit (Babyak et al., 2000). The most robust effect demonstrated longitudinally was positive association between positive affect and participation in the yoga group. This result is surprising given the Group x Time effect for well-being indicated that the yoga group reported decreased well-being (of which positive affect is the emotional component) following intervention cessation. A potential explanation may be that participation in the yoga group was associated with a particularly negative cognitive evaluation of life domains upon no longer having access to regular yoga classes, a negative side-effect worth exploration in future studies. However, this interpretation is speculative in that detailed qualitative data were not collected to ascertain reasons for changes in well-being post intervention. In summary, it appears that positive associations of psychological distress and stress reported in Study 2 were not maintained through the follow-up period which strengthens the view that changes that occurred during yoga intervention were due to the intervention.

CHAPTER 5. PAPER 4

5.1 Preamble

The paper presented in this chapter reports on the relationship between markers of immunity and yoga intervention, which was explored longitudinally in Study 3 (Chapter 4). The need to measure biological markers has been highlighted in reviews of this field due to improved objectivity and ability to capture underlying biological mechanisms of yoga (i.e., effect on allostatic load negatively impacted by stress). As discussed in the Literature Review (Chapter 2), a number of biomarkers of the sympathetic nervous system (SNS) and hypothalamus-pituitary-adrenal (HPA) axis have been used to infer 'stress', as have immunological proteins. While difficulties with measuring cortisol have been reported (Li & Goldsmith, 2012), an inflammatory response assessed by serum proteins has been associated with stress and measured in previous yoga trials (Glaser & Kiecolt-Glaser, 2005; Kiecolt-Glaser et al., 1996; Maes et al., 1998; Pullen et al., 2008; Pullen et al., 2010). In light of psychological markers identified in previous studies, it was decided that markers of inflammation (serum interleukin-6 [IL-6], tumor necrosis factor [TNF], and C-reactive protein [CRP]) be collected and explored longitudinally as a pilot trial (n = 28). The reason for not taking blood samples from all participants was two-fold. Firstly, using immunological markers as surrogate markers of stress/distress in a community sample of middle-aged women warranted a smaller 'proof of concept' clinical trial due to the invasive nature of blood sampling and likelihood that power in the trial as a whole may be negatively impacted by participant lack of willingness to provide a blood sample. Secondly, limited fiscal resources necessitated a smaller sample size or exploration of a more limited number of biological markers.

At the time of commencing recruitment for the yoga intervention Saatcioglu (2013) published a review on gene expression in yoga cognitive-behavioural practices. As noted in

the Literature Review, while the trials reviewed were more meditative than physical in nature, the multi-levelled effect mind-body interventions may have on immune cells was highlighted. During administration of the yoga intervention the opportunity to perform analysis of deoxyribonucleic acid (DNA) methylation became available, and following ethics approval, consent for genetic testing was sought from participants providing blood samples at the post-treatment time point. Consequently, a more detailed picture of immune pathways was captured, although baseline data of DNA methylation was not collected. The complexity of explicating and reporting these findings warranted preparation of this final paper.

Preliminary Indications of the Effect of a Brief Yoga Intervention on Markers of Inflammation and DNA Methylation in Chronically Stressed Women

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Translational Psychiatry

Ms Kaitlin N. Harkess (Candidate)

I was responsible for the conception of this study; the literature review; conducting the intervention; data collection and analysis; and, manuscript preparation and drafting. Dr Cohen-Woods was the corresponding author, and I was involved in assisting with submission and revision of this paper. Dr Joanne Ryan developed and conducted DNA methylation assays based on the literature review I conducted. My overall percentage (%) of contribution to the paper is 85%.

Signed: Kaitlin Harkess

Professor Paul Delfabbro and Dr Sarah Cohen-Woods (Co-authors)

We were the supervisors of the research program to which this manuscript belongs. We collaborated with Ms. Harkess in the development of the content and structure of the manuscript and assisted with editing and proof-reading. Ms. Harkess was responsible for the development and administration of the intervention; the collection of and analysis of data; and, writing this manuscript. Our role was to comment on drafts, make suggestions on the presentation of material in the paper, and to provide editorial input. We also provided advice on responding to comments by the journal reviews and editor. We hereby give our permission for this paper to be incorporate in Ms. Harkess's submission for the degree of Doctor of Philosophy from the University of Adelaide.

Signed: Paul Delfabbro

Signed: Sarah Cohen-Woods

Dr Joanne Ryan (Co-author)

I provided laboratory support, performed the methylation experiments (non-statistical), provided guidance in interpretation of analysis, as well as providing critical feedback on the manuscript drafts Ms. Harkess produced.

Signed: Joanne Ryan

Abstract

Yoga is associated with reduced stress and increased well-being, although the molecular basis for these benefits is not clear. Mounting evidence implicates the immune response, with current studies focused on protein immune markers (such as cytokines) in clinical populations. To explore the molecular impact, this pilot study uses a subsample (n = 28) from a randomised waitlist control trial investigating the impact of an eight week yoga intervention in a community population of women reporting psychological distress (N = 116). We measured interleukin-6 (IL-6), tumor necrosis factor (TNF), and C-reactive protein (CRP) protein levels, and DNA methylation of these genes and the global indicator, *LINE-1*. Correlations between these and psychological variables were explored, identifying moderate correlations with CRP protein levels, and methylation of *IL-6*, *CRP* and *LINE-1*. Many cytokine samples were below detection, however a trend of moderate between group effect for elevated IL-6 in the yoga group was reported. Methylation analyses applied cross-sectional and non-controlled longitudinal analyses. Waist-to-height ratio and age were covaried. We demonstrated reduced methylation of the *TNF* region in the yoga group relative to the waitlist control group. No other genes demonstrated a significant difference. Longitudinal analysis further supported these results. This study is one of the first to explore yoga and immunological markers in a non-clinical population, and is the first study to explore DNA methylation. These findings indicate further research into molecular impact of yoga on markers of immune function is warranted, with larger studies required.

Yoga is an increasingly popular technique combining physical activity, meditation, and breathing practices ("moving mindfulness"; La Forge, 2005), and is often practiced as a treatment/adjunct treatment for psychiatric conditions (Birdee et al., 2008). A growing body of psychological literature demonstrates practicing yoga improves subjective well-being and positive feelings, and reduces levels of stress, distress, and negative feelings, including clinical symptoms of depression and anxiety (e.g., Cramer et al., 2013; Li & Goldsmith, 2012; Patel et al., 2012).

Inflammation has been demonstrated to be associated with depression and exposure to stressors, specifically including the action of the inflammatory cytokines interleukin-6 (IL-6) and tumour necrosis factor (TNF; Hickie & Lloyd, 1995; Juster, McEwen, & Lupien, 2010; McEwen, 2006), and the acute-phase protein C-Reactive Protein (CRP; Gimeno et al., 2009; Howren, Lamkin, & Suls, 2009; Penninx et al., 2003). Further these have been postulated to be impacted by both exercise, and psychological therapies. Anti-inflammatory factors are modified by participation in moderate exercise (Horsburgh et al., 2015), and with participation in a Mindfulness-Based Stress Reduction (MBSR) intervention (Carlson, Speca, Patel, & Goodey, 2003). Biochemical evidence indicates practices such as yoga reduce inflammatory responses associated with stressful situations (Kiecolt-Glaser et al., 2014; Kiecolt-Glaser et al., 2010). However our current understanding of the molecular mechanisms involved in the modulatory effect of yoga remains limited.

Inflammation changes reported in the literature may, in part, be determined by epigenetic processes that impact gene expression, and ultimately protein expression. The epigenome regulates gene expression, and can be altered by environmental factors such as stress (Tsankova, Renthal, Kumar, & Nestler, 2007). Epigenetic changes are increasingly recognised as relevant biomarkers for mental illness, with DNA methylation the most widely studied (Docherty & Mill, 2008; Sananbenesi & Fischer, 2009; Toyokawa, Uddin, Koenen, &

Galea, 2012; Unternaehrer et al., 2012). Changes in DNA methylation have been associated with poor physical health, and high levels of inflammation (Bayarsaihan, 2011; Colotta, Allavena, Sica, Garlanda, & Mantovani, 2009; Iliopoulos, Hirsch, & Struhl, 2009; Perwez Hussain & Harris, 2007). As epigenetic changes are potentially reversible, they may be used for evaluation of responses to clinical therapies (Levenson, 2010).

Emerging studies of mind-body therapies (MBTs), including yoga-based interventions, are increasingly exploring mechanisms (Banerjee et al., 2007; Black et al., 2013; Bower et al., 2014; Lavretsky et al., 2013); however most studies focus on gene-expression (Niles, Mehta, Corrigan, Bhasin, & Denninger, 2014). Thus whilst a change in gene-expression, and therefore a biological effect may be reported, the mechanism of this effect remains unknown. Only two epigenetic studies currently exist in the MBT literature, and indicate interventions conceptually similar to yoga may be correlated with epigenetic change. Specifically, an eight-hour meditation session has been reported to rapidly alter global modification of histones, and reduce expression of histone deacetylase and pro-inflammatory genes (Kaliman et al., 2014). DNA methylation changes in six age-related CpG sites have also been reported in a cross-sectional study of Australian female long-term tai chi practitioners (Ren et al., 2012). However, no studies have investigated the relationship between a psychophysiological intervention, such as yoga, on indicators of genome-wide DNA methylation (which can be explored broadly utilising a repetitive element sequence as a surrogate, such as LINE-1; 36), and DNA methylation patterns of immune candidate genes such as *TNF*, *IL-6*, and *CRP*, candidates implicated in psychological distress and to be altered by MBSR and yoga practice. DNA methylation in these genes have been investigated in the context of inflammatory conditions (rheumatoid arthritis) and engagement in physical activity, age, pollution exposure, and weight-related factors (Campión, Milagro, Goyenechea, & Martínez, 2009; Cordaux & Batzer, 2009; Kirchner et al., 2014; Morabia et al., 2012; Nile,

Read, Akil, Duff, & Wilson, 2008; Plant, Wilson, & Barton, 2014; Stefani et al., 2013; Zhang et al., 2012). Whilst findings have been mixed, they have demonstrated that DNA methylation changes are observed in relation to physical factors and across relatively short time periods.

The objective of this pilot study is twofold: 1) to examine the epidemiological effect of a yoga intervention on markers of inflammation (IL-6, TNF, and CRP); and 2) to examine, for the first time, if participation in a yoga intervention (a mind-body therapy) is associated with altered levels of estimated global DNA methylation (represented by methylation of the interspersed repeat *LINE-1*) or changes to methylation patterns of the *IL-6*, *TNF* and *CRP* genes. Specifically, we have conducted a longitudinal analysis on protein markers of inflammation, comparing distressed middle-aged women who have engaged in a 2-month yoga intervention with a waitlist control group. Second, we have conducted a cross-sectional analysis of between-group DNA methylation profiles comparing post-yoga intervention group with the waitlist group. Finally, we have conducted a longitudinal analysis of the waitlist group's DNA methylation profiles to corroborate the cross-sectional analysis.

Method

Participants and Procedure

This study represents a subsample (n = 28) of a larger clinical trial (N = 116), which explores the psychophysiological effects of a yoga intervention in women reporting psychological distress (as measured by a score of 16+ on Kessler Psychological Distress Scale [K10]; Kessler & Mroczek, 1994), and utilises a stratified, randomised waitlist-control trial design. Psychophysiological results are reported elsewhere (Harkess et al., 2016). The parent study explores the psychophysiological effects of participation in an average of a one-

hour yoga class per week for a period of eight weeks. The study utilised a stratified, randomised waitlist-control trial design (described in detail: Harkess, Delfabbro, Curtis, & Cohen-Woods, Submitted). Within the parent study, a subsample of participants were randomly allocated to provide serum samples for analysis of cytokines (IL-6 and TNF) and high-sensitivity CRP (hsCRP; $n = 35$; $n = 7$ lost to follow-up). Women were eligible for this pilot study if they were: healthy, free from acute infection for two weeks prior to biochemical assessment, and if they had refrained from drinking alcohol in the 48 hours prior to biochemical assessment. Additional exclusion criteria were serious physiological illnesses that would interfere with interpretation of biochemical data (e.g., anaemia, diabetes, cardiovascular diseases, blood cancers, inflammatory bowel diseases, autoimmune diseases, asthma being treated with steroids, immunodeficiency); having a BMI >30; meeting the criteria for substance abuse or dependence; undergoing menopause; having a serious psychological illness; or, having engaged in a regular yoga practice within the previous year. Biological samples were only available for this subsample.

Selection for epigenetic analysis in this subgroup is based on participants (1) already consented to provide blood; (2) meeting the inclusion/exclusion criteria described; and (3) giving informed consent to their blood sample being used for genetic analysis prior to the post-treatment evaluation. Participants who fulfilled the first two criteria were identified and randomly allocated into this portion of the study using Research Randomizer (Urbaniak, 2013). The mean age of participants in this subsample ($M = 41.21$, $SD = 4.14$) is younger than the parent study ($M = 48.14$, $SD = 8.22$), but participants are not appreciably different in terms of other demographic or clinical variables. This trial has been approved by the Human Research Ethics Committee of the University of Adelaide; all participants gave informed consent. This trial is registered at the Australian New Zealand Clinical Trial Registry (ANZCTR): ACTRN12616000612415.

Yoga intervention. The yoga intervention comprised eight weeks of twice-weekly, hour-long yoga classes (the total number of classes offered was 16). Per-protocol completion was considered attendance at eight classes, as weekly practice reflects the average community practitioners' engagement (Birdee et al., 2008; Penman et al., 2012). For further details see Harkess, Delfabbro, Curtis and Cohen-Woods (Submitted).

Study design. The study analyses involve two parts. The first utilises a randomised trial design to compare protein markers of inflammation of the participants who completed the yoga intervention to those of the control group (IL-6, TNF, and hsCRP). The second utilises a cross sectional trial design to compare DNA methylation patterns of participants who completed the yoga intervention to those of the control group at the post-treatment assessment. This is due to consent and ethics for genetic analysis being granted after initiation of the study, but prior post-treatment data and sample collection. DNA methylation patterns are also explored longitudinally in a non-controlled trial design, with the waitlist control group examined from post-treatment and follow-up time points, until after the completion of the second round of yoga classes utilising our standardised protocol. To avoid confusion, we will refer to these as 'waves' (see Figure 1).

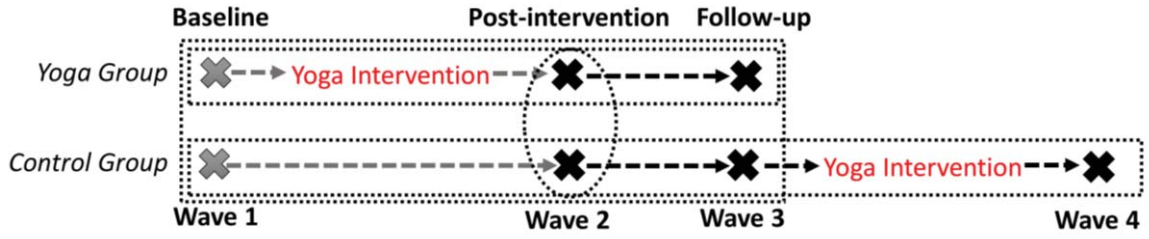

Figure 1. A visual depiction of the parent study and the current sub-study to explicate the analyses conducted. Grey 'X' markings depict where only serum samples were available for analysis (inflammatory markers), and black 'X' markings indicate that both serum and whole bloods were available for analysis (inflammatory markers and DNA methylation). The perforated rectangles indicate the longitudinal analyses conducted (where possible), and the perforated oval indicates the conduct of cross-sectional analysis.

Sample collection. Assessment included completing online surveys including demographic and psychological variables (detailed below), which participants completed prior to an in-person assessment. The in-person assessment involved physiological tests (i.e., waist and height measurements) and collection of blood samples through routine venepuncture at baseline (Wave 1), post-test (Wave 2), one month follow-up (Wave 3), and waitlist control intervention post-test (Wave 4). Participants were requested to abstain from stimulants, such as coffee, on the day of testing. At wave 1 the phlebotomist drew 21 ml intravenous blood sample from each participant. Each sample provided 3 ml for a complete blood picture (CBP) analysis (to screen for abnormalities), 9 ml for cytokine analysis, and 9 ml for hsCRP analysis. At waves 2, 3, and 4 the phlebotomist drew a total of 30 ml, with 9ml extra to allow for genetic analysis. VACUETTE® Plastic K3EDTA tubes (purple top) were used for CBP and genetic analysis of samples, and VACUETTE® Z Serum Sep Clot Activator (white top) were used for cytokines and hsCRP. CBP was analysed on the day of testing. To avoid problems with drift and inter-assay variability, samples for hsCRP and cytokines were centrifuged as per manufacturer's protocol, and serum was frozen at -80°C until the study was completed (post-Wave 4). For DNA analysis, whole blood samples were aliquoted into seven to eight eppendorfs, each containing 1ml volume of whole blood, and stored at -80°C for DNA extraction and analysis as required. The remaining 1ml was stored in RNAlater™ (Lifebiosciences, Thermofisher) and stored at -20°C for future gene expression analysis.

Mental health variables. Using a set of secondary analyses, we also explored correlations between biochemical outcomes (protein and DNA methylation inflammatory candidate markers) and psychological variables that have already demonstrated between-group effects in this population (Harkess et al., 2016). Specifically, the study explores outcome scores at post-test on the (a) Kessler Psychological Distress Scale (K10), which

gives a global measure of psychological distress based on questions about anxiety and depression symptoms (Kessler & Mroczek, 1994); and the (b) Perceived Stress Scale (PSS), which measures the degree to which situations in one's life are appraised as stressful (Cohen et al., 1983); and Positive Affect of the Positive and Negative Affect Schedule (PANAS), which is a mood scale that measure people's positive affect (Watson & Clark, 1997).

Protein Analysis

The study determined hsCRP serum concentration using the Beckman Coulter AU2700 analyser (Olympus, Germany, Beckman Coulter, Krefeld, Germany), and the Beckman Coulter CRP Latex method (immune-turbidimetric test) following the manufacturer's recommended protocol. A highly sensitive application that has a dynamic range of 0.08 to 80 mg/L was used. Samples from all 4 Waves were run by one individual in batches of 20-30 over two days. Calibration was performed as required and Quality Control samples were run in accordance with SA Pathology protocols (internal quality controls were reported to be between 7 – 9% at the time of analysis).

Cytokine (IL-6 and TNF) serum concentrations were measured by cytokine capturing beads, using the BD cytometric Bead Array (CBA) Human Enhanced Sensitivity Master Buffer kit and following the manufacturer's recommended protocols. Sensitivity of this kit is reported between the range of 0.27 to 200 pg. The samples were analysed by flow cytometry on the BD Canto1 flow cytometer. Quality control was performed daily, using Cytometer Setup and Tracking (CTS) beads and an assay utilising the reported kit to determine if proper cytokine readings were taken. A number of samples demonstrated levels below the 0.274 pg threshold for detection (IL-6: Wave 1 = 11; Wave 2 =10; Wave 3 = 10; Wave 4 =6; and TNF: Wave 1 = 16; Wave 2 =16; Wave 3 = 15; Wave 4 = 7).

Methylation Analysis

Methylation assays were designed with Epidesigner software (www.epidesigner.com) and covered key regions found to be differentially methylated in previous studies investigating other exposures or disease outcomes: *TNF* (Campión et al., 2009; Cordero et al., 2010; Gowers et al., 2011; Kirchner et al., 2014; Plant et al., 2014); *IL-6* (Kirchner et al., 2014; Morabia et al., 2012; Nile et al., 2008; Stefani et al., 2013; Zhang et al., 2012); previously reported LINE-1 primers (Flotho et al., 2009); and, the *CRP* assay was designed to target the CpG sites in the promoter region (See supplementary Table 6 for the assay designs). Cleavage patterns were determined using the Bioconductor MassArray package in R (www.bioconductor.org). DNA was extracted using the QIAamp DNA Mini Kit (QIAGEN), and bisulphite converted using the MethylEasy^{TM} Xceed Kit (Genetic Signatures, Darlinghurst, Australia). Samples were PCR amplified and assayed in triplicate. DNA methylation was quantified using the SEQUENOM MassARRAY (San Diego, CA, USA) and methylation ratios calculated using EpiTyper software (v.1.2; SEQUENOM). Further PCR protocol details and conditions are included in supplementary materials (Tables 7–9).

The mean methylation from three technical replicates for each sample was determined; outlying values (deviation of ±10% methylation from median) were discarded. Any individual with only one methylation datapoint following outlier identification was excluded from cross-sectional analyses. In longitudinal analysis, discarding these individuals limited sample size with multiple datapoints (i.e., sample was <6), so we retained single methylation data-point individuals for the purpose of this pilot study (n = 10), with sensitivity analyses excluding these individuals in the supplementary data (Supplementary Tables 1 – 5).

Statistical Analysis.

Statistical analyses were conducted using SPSS for Windows, version 21, software (SPSS, Chicago, IL). The non-normal IL-6 and TNF distributions were dealt with by utilising non-parametric statistical tests.

IL-6 and TNF protein marker analysis. With no non-parametric equivalent to a two-way ANOVA we used two Friedman Tests for longitudinal analyses to investigate change over time within each group (yoga and waitlist control groups, separately) which allowed use of all available data within the study: (Analyses 1) to investigate change over time in Waves 1,2,3 in the yoga group; (Analyses 2) to investigate change over time in Waves 1,2,3, and 4 in the waitlist control group (yoga was engaged in with the waitlist control group between wave 3 and 4). To compare between-group differences post-intervention at Wave 2 on IL-6 and TNF protein levels a cross-sectional analysis was applied: (Analyses 3) using Mann-Whitney U Tests.

hsCRP analysis. (Analyses 4) A mixed between-within subjects analysis of variance (ANOVA) was conducted to assess the impact of the yoga intervention on hsCRP levels. This included data from Waves 1, 2, and 3 for both yoga and waitlist control groups. (Analyses 5) A one-way repeated measures ANOVA was used to investigate if change over time was observed for hsCRP in the waitlist control group following yoga exposure; this included three waves prior to yoga (1, 2, and 3) with the final wave post-yoga (Wave 4).

DNA methylation. For each immune candidate (*IL-6*, *TNF*, *CRP*), a mean percentage of methylation was calculated across all CpG sites in each region assayed. Two sets of analyses were conducted: (Analyses 6) an analysis of covariance (ANCOVA model) was used to evaluate cross-sectional outcome measures for DNA methylation data, with yoga as the predictor (no covariates). A second ANCOVA was run to control for potential confounders (age and waist-to-height ratio at Wave 2). We conducted two analyses due to the

small sample size (N = 28) and the exploratory nature of this study. Utilising the two analyses allows examination of the impact of additional covariates on the F-value, which is sensitive to degrees of freedom. (Analyses 7) To evaluate change across time following yoga intervention we were restricted to utilising the waitlist control group only for longitudinal analysis as we did not have DNA methylation data for Wave 1; a t-test was conducted, with the mean of the two pre-intervention results (Wave 2 and Wave 3) compared with post-intervention methylation (Wave 4).

Secondary exploratory analyses of measures of mental health.

(Analysis 8) We were restricted to correlational analyses due to insufficient numbers to enable regression-based analyses (Cassey & Ling, 2014). The protein biomarkers, IL-6 and TNF, exhibit non-normal distributions, thus non-parametric correlational analyses were applied. A Spearman rank-order correlation was performed to explore post-test associations of inflammatory protein markers, DNA methylation, and mental health outcome variables (Analyses 8).

Effect Size and Significance.

As recommended by Perneger (1998), we discuss the results in regard to both statistical significance and effect size (where possible), specifically Spearman's r (Small = .10 Medium = .30, Large =.50), partial eta squared ($\eta_{\rho}^{2;}$ Small = .01, Medium = .06, Large = .138), and Cohen's d (Small = .02, Medium = .50, and Large = .80). Each hypothesis has been considered individually (Perneger, 1998).

Results

Characteristics of the Participants

As displayed in Table 1, the main characteristic of the participants did not differ significantly between the yoga and control groups, including in energy expenditure (METs), indicating equal engagement in physical activity. All participants who participated in the blood sampling were Caucasian, therefore, we did not control for ethnicity.

Table 1

Baseline Participant Characteristics

	Overall (*N* = 26)		Control (*n* = 15)		Yoga (*n* = 11)	
	Mean	*SD*	Mean	*SD*	Mean	*SD*
Age (years)	41.12	4.28	40.8	4.36	41.55	4.34
Waist-to-Height	0.5	0.07	0.48	0.08	0.52	0.07
BMI	24.78	4.96	24.2	5.38	25.57	4.46
METS	3162	7988	4080.4	10516	1910	1302
K10	23.69	5.22	24.2	5.45	23	5.06
WBCC[a]	6.29	1.59	6.28	1.04	6.32	2.19
hsCRP[a]	1.43	1.23	1.73	1.45	1.02	0.73
	No.	%	No.	%	No.	%
Marital status						
Single	4	15.4	2	13.3	2	18.2
Common-law/Married	17	69.2	10	66.7	8	72.7
Separated/Divorced	3	11.5	2	13.3	1	9.1
Declined to answer	1	3.8	1	6.7	-	-
Parous	16	61.5	7	46.7	8	72.7
Education level						
High school or less	4	15.3	1	6.7	3	27.3
Vocational school	4	15.4	2	13.3	2	18.2
University graduate	14	53.8	9	60.0	5	45.5
Postgraduate	4	15.4	3	20.0	1	9.1

Note: BMI = body-mass index; METS= Metabolic Equivalent; K10 = Psychological Distress; WBCC = White blood cell count; hsCRP = high-sensitivity C-reactive protein.

[a] Levels are reported in untransformed units.

Analysis of Inflammatory Markers

Analyses 1: The Friedman Test indicated there was no evidence of a longitudinal difference in IL-6 or TNF across the three time-points (X^2 (2, n = 11) = 2.34, p = .310; X^2 (2, n = 11) = 0.50, p = .779). Analyses 2: The Friedman Test indicated there was no evidence of a difference in IL-6 or TNF across the four time-points (X^2 (3, n = 9) = 0.57, p = .904; X^2 (3, n = 9) = 2.10, p = .551). Analyses 3: A Mann-Whitney U Test revealed a non-significant, but moderate effect size suggesting that at post-test IL-6 levels were higher in the intervention group (*Md* = 1.33, n = 11) than in the control group (*Md* = 0.00 n = 15), U = 49.0, z = -1.79, p = .073, r = .35). There was no evidence for differences in TNF levels (non-detectable: intervention: *Md* = 0.00, n = 11; control: *Md* = 0.00, n = 15), U = 78.0, z = -0.27, p = .790, r = .05).

Analysis 4: A mixed between-within subjects ANOVA demonstrated non-significant effect, but good effect size for differences in hsCRP over time, Wilks' Lambda = .75, F (2, 19) = 3.17, p = .065, η_ρ^2 = .25; though there was no evidence of a group by time interaction, Wilks' Lambda = .91, F (2, 19) = 0.91, p = .421, η_ρ^2 = .09. The means and standard deviations are presented in Table 2. Analysis 5: A one-way repeated measures ANOVA (analysis 5) indicated there was no effect for time, Wilks' Lambda = .54, F (3, 5) = 1.42, p = .342, η_ρ^2 = .46 . The means and standard deviations are presented in Table 3.

Table 2

Descriptive Statistics for hsCRP for Varying Timepoints

	Yoga Intervention Group		Waitlist Control Group		Total Group	
	n	Mean (*SD*)	*n*	Mean (*SD*)	*N*	Mean (*SD*)
Baseline (Wave 1)	10	1.00 (0.76)	12	1.49 (1.38)	22	1.27 (1.14)
Post-Test (Wave 2)	10	1.05 (0.81)	12	0.99 (0.49)	22	1.02 (0.64)
Follow-up (Wave 3)	10	1.79 (1.59)	12	1.50 (1.24)	22	1.63 (1.38)

Table 3

Descriptive Statistics for hsCRP for Pre-Intervention Time Points and Post-Test

Time Period	*N*	Mean	*SD*
Pre-Test (Wave 1)	8	1.56	1.67
Pre-Test (Wave 2)	8	0.84	0.29
Pre-Test (Wave 3)	8	1.45	1.49
Post-Test (Wave 4)	8	1.00	0.96

Analysis of DNA Methylation

Please see Table 4 for depiction of the cross-sectional analysis (Wave 2) Analysis 6, described below.

Table 4

Results of DNA Methylation Cross-Sectional ANCOVA analyses

Promoter Region	Mean (*SD*) Control	*n*	Mean (*SD*) Yoga	*n*	Main effect Yoga vs Control	Effect with Covariates (Age and WHtR) Yoga vs Control	Covariates
IL-6$_1$							
CpG 1	0.898	13	0.921	9	F(1, 20) = 5.29, p = .032*	F(3, 18) = 4.30, p = .053†	age ηρ2 = .11
	(0.024)		(0.021)		ηρ2 = .21	ηρ2 = .19	WHtR ηρ2 = .02
CpG 2/3	0.931	10	0.924	8	F(1, 16) = .768, p = .394	F(3, 14) = 1.12, p = .307	age ηρ2 = .07
	(0.013)		(0.019)		ηρ2 = .05	ηρ2 = .07	WHtR ηρ2 = .04
CpG 4/5/6	0.928	14	0.933	9	F(1, 21) = 0.59, p = .452	F(3, 19) = 0.27, p = .609	age ηρ2 = .02
	(0.016)		(0.009)		ηρ2 = .03	ηρ2 = .01	WHtR ηρ2 = .11
Mean	0.853	14	0.926	9	F(1, 21) = 0.78, p = .387	F(3, 19) = 0.72, p = .406	age ηρ2 = .10
	(0.246)		(0.011)		ηρ2 = .04	ηρ2 = .04	WHtR ηρ2 = .00
IL-6$_2$							
CpG 1	0.035	15	0.037	11	F(1, 24) = 0.24, p = .626	F(3, 22) = 0.14, p = .717	age ηρ2 = .02
	(0.009)		(0.010)		ηρ2 = .01	ηρ2 = .00	WHtR ηρ2 = .04
CpG 2	0.006	15	0.003	11	F(1, 24) = 0.91, p = .349	F(3, 22) = 1.22, p = .281	age ηρ2 = .05
	(0.009)		(0.005)		ηρ2 = .04	ηρ2 = .05	WHtR ηρ2 = .04
CpG 4/5/6	0.033	15	0.034	11	F(1, 24) = 0.04, p = .852	F(3, 22) = 0.01, p = .931	age ηρ2 = .04
	(0.011)		(0.009)		ηρ2 = .00	ηρ2 = .00	WHtR ηρ2 = .00
CpG 7/8	0.078	15	0.084	11	F(1, 24) = 0.56, p = .463	F(3, 22) = 0.44, p = .515	age ηρ2 = .00
	(0.020)		(0.015)		ηρ2 = .02	ηρ2 = .02	WHtR ηρ2 = .01
Mean	0.038	15	0.040	11	F(1, 24) = 0.13, p = .720	F(1, 22) = 0.05, p = .824	age ηρ2 = .04
	(0.008)		(0.007)		ηρ2 = .01	ηρ2 = .00	WHtR ηρ2 = .03

Table continues

Table 4 Continued

Promoter Region	Mean (*SD*) Control	n	Mean (*SD*) Yoga	n	Main effect Yoga vs Control	Effect with Covariates (Age and WHtR) Yoga vs Control	Covariates
TNF							
CpG 1	0.829	13	0.748	6	F(1, 17) = 3.45, p = .081†	F(3, 15) = 2.32, p = .148	age ηρ2 = .04
	(0.079)		(0.108)		ηρ2 = .17	ηρ2 = .13	WHtR ηρ2 = .00
CpG 2	0.814	13	0.738	8	F(1, 19) = 4.30, p = .052†	F(3, 17) = 4.56, p = .049*	age ηρ2 = .00
	(0.079)		(0.086)		ηρ2 = .19	ηρ2 = .21	WHtR ηρ2 = .04
CpG 4/5/6	0.142	13	0.106	9	F(1, 20) = 1.69, p = .208	F(3, 18) = 2.51, p = .131	age ηρ2 = .00
	(0.074)		(0.043)		ηρ2 = .09	ηρ2 = .12	WHtR ηρ2 = .20*
CpG 8	0.231	14	0.210	10	F(1, 22) = 0.28, p = .599	F(3, 20) = 0.33, p = .573	age ηρ2 = .02
	(0.096)		(0.091)		ηρ2 = .01	ηρ2 = .02	WHtR ηρ2 = .02
CpG 9	0.089	13	0.075	10	F(1, 21) = 0.63, p = .437	F(3, 19) = 0.57, p = .458	age ηρ2 = .01
	(0.050)		(0.030)		ηρ2 = .03	ηρ2 = .03	WHtR ηρ2 = .00
CpG 12	0.087	13	0.073	8	F(1, 19) = 0.39, p = .537	F(3, 17) = 0.49, p = .495	age ηρ2 = .17
	(0.060)		(0.031)		ηρ2 = .02	ηρ2 = .03	WHtR ηρ2 = .05
Mean	0.367	15	0.322	11	F(1, 24) = 5.68, p = .025*	F(3, 22) = 6.16, p = .021*	age ηρ2 = .00
	(0.048)		(0.046)		ηρ2 = .19	ηρ2 = .22	WHtR ηρ2 = .09
CRP							
CpG 1	0.875	12	0.885	10	F(1, 20) = 0.32, p = .579	F(3, 18) = 0.18, p = .675	age ηρ2 = .12
	(0.048)		(0.031)		ηρ2 = .02	ηρ2 = .01	WHtR ηρ2 = .00
CpG 2	0.733	12	0.740	10	F(1, 20) = 0.06, p = .803	F(3, 18) = 0.01, p = .937	age ηρ2 = .33
	(0.064)		(0.058)		ηρ2 = .00	ηρ2 = .00	WHtR ηρ2 = .22
CpG 4	0.726	12	0.715	10	F(1, 20) = 0.15, p = .701	F(3, 18) = 0.39, p = .539	age ηρ2 = .14
	(0.074)		(0.051)		ηρ2 = .01	ηρ2 = .02	WHtR ηρ2 = .07
Mean	0.717	13	0.709	11	F(1, 22) = 0.01, p = .934	F(3, 20) = 0.01, p = .908	age ηρ2 = .01
	(0.220)		(0.237)		ηρ2 = .00	ηρ2 = .00	WHtR ηρ2 = .02

Table continues

Table 4 Continued

Promoter Region	Mean (*SD*) Control	n	Mean (*SD*) Yoga	n	Main effect Yoga vs Control	Effect with Covariates (Age and WHtR) Yoga vs Control	Covariates
LINE-1							
CpG 1	0.693	15	0.692	11	F(1, 24) = 0.00, p = .958	F(3, 22) = 0.00, p = .974	age ηρ2 = .06
	(0.020)		(0.017)		ηρ2 = .00	ηρ2 = .00	WHtR ηρ2 = .07
CpG 2	0.721	15	0.724	11	F(1, 24) = 0.32, p = .577	F(3, 22) = 0.25, p = .626	age ηρ2 = .00
	(0.014)		(0.009)		ηρ2 = .01	ηρ2 = .01	WHtR ηρ2 = .04
CpG 3	0.607	15	0.605	11	F(1, 24) = 0.20, p = .662	F(3, 22) = 0.24, p = .632	age ηρ2 = .00
	(0.012)		(0.015)		ηρ2 = .01	ηρ2 = .01	WHtR ηρ2 = .04
Mean	0.674	15	0.674	11	F(1, 24) = 0.00, p = .997	F(1, 22) = 0.00, p = .982	age ηρ2 = .02
	(0.014)		(0.012)		ηρ2 = .00	ηρ2 = .00	WHtR ηρ2 = .07

Note: WHtR = Waist-to-height ratio; IL-6 = interleukin 6; TNF = tumor necrosis factor; CRP = C-reactive protein; LINE-1 = global methylation indicator. **p < .01; *p < .05; †p <.10 (two-tailed tests).

Regions of methylation and yoga. No between group differences in mean methylation across *CRP* or the *IL-6* regions was observed in either ANCOVA model (no covariates, or with age and WHtR as covariates). A significant main effect of Group was found for the mean methylation of *TNF* which explained 19% of the variance. Women in the yoga group demonstrated a 4.5% lower level of methylation relative to the waitlist control group (see Table 4). The main effect of the group on *TNF* remained (yoga group with lower methylation) when covariates age and waist-to-height ratio were included in the analysis.

Individual CpG units and yoga. No significant differences in methylation at individual CpG units were demonstrated for *CRP* and *IL-6*$_{2;}$, differences in mean methylation for *IL-6*$_1$ CpG site 1 were observed between groups (2.3% higher methylation in the yoga group), but this association was reduced with the inclusion of age and WHtR in the model. There appeared to be some differences between groups at individual *TNF* sites, but this varied depending on the inclusion of covariates. Only one covariate, WHtR, demonstrated a close to significant association with the *TNF* CpG site 4/5/6 (p = .050; 20% of variance explained).

Global DNA marker *LINE-1*. No evidence for differences in methylation at individual *LINE-1* CpG units, nor the over-all mean, was demonstrated. Covariates were also not associated with differences in *LINE-1* methylation.

Longitudinal DNA Methylation Analysis

Please see Figure 2 for depiction of longitudinal analysis of the waitlist control group (Analysis 7). Sample sizes for the longitudinal analyses are small (ranging from 10 to 11). As some longitudinal techniques used to compare groups are unreliable in small sample sizes, we conducted paired sample t-tests to explore pre- to post-intervention (average of Wave 2 and 3 to Wave 4) effects to ascertain if findings

corroborated cross-sectional analyses already presented. Results of all analyses and descriptive statistics are presented in the supplementary material (reference Supplementary Tables 1 - 5), and we present psychological outcomes that demonstrated medium or large effects of change following yoga (Cohen, 1988).

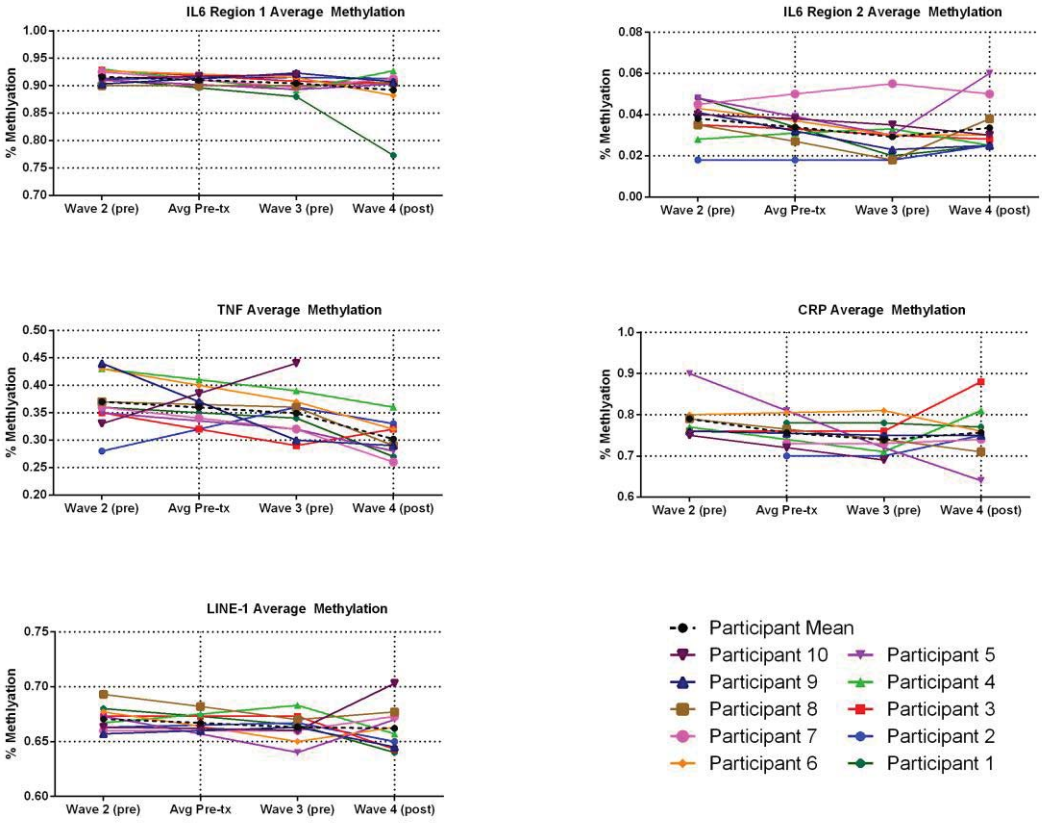

Figure 2. Longitudinal IL-6 (regions 1 and 2), TNF and CRP methylation patterns, and LINE-1 global methylation pattern, for all participants with mean shown as black perforated line.

Regions of methylation. The yoga intervention was associated with a reduction in *TNF* methylation (Cohen's d = 1.68) and decreased *IL-6₁* methylation (Cohen's d = 0.53), although this didn't reach significance.

Individual CpG units. Significant associations indicating decreased methylation at the post-yoga time point was demonstrated for *TNF* CpG site 1 (Cohen's d = 1.11) and 4/5/6 (Cohen's d = 1.00).

Global DNA marker *LINE-1*. No evidence of a difference for time was seen at individual *LINE-1* CpG sites or for the mean.

Exploratory Analyses of Measures of Mental Health

Analyses 8: As shown in Table 5, there is strong correlation between perceived stress and psychological distress. Moderate correlations are demonstrated between subjective well-being and perceived stress and psychological distress. A moderate and significant correlation between global DNA marker *LINE-1* methylation and perceived stress is reported. A number of other moderate size correlations are observed, however significance was not achieved, possibly due to limited power.

Table 5

Results of Spearman's Rank-Order Correlation

	K10	PSS	SWB	PA	hsCRP	IL6	TNF	*IL6*$_1$	*IL6*$_2$	*TNF*	*CRP*	*LINE 1*
Questionnaires												
K10	-											
PSS	.654**	-										
SWB	-.445*	-.128	-									
PA	-.097	-.273	-.025	-								
Proteins												
hsCRP	.089	.337†	.113	.199	-							
IL6	-.061	.040	-.188	.001	-.003	-						
TNF	-.034	.245	-.028	-.149	.059	.608**	-					
DNA methylation												
IL6$_1$	-.031	.096	.400†	-.099	-.119	.069	.312	-				
IL6$_2$	.006	.082	.290	-.093	.121	-.219	-.107	-.020	-			
TNF-α	.242	.154	.046	-.011	.001	-.222	.017	.129	.038	-		
CRP	-.237	-.053	.364†	-.398†	-.031	-.214	.021	.126	.323†	.211	-	
LINE 1	.126	.409*	-.125	-.382†	.202	-.114	-.057	-.008	.074	.053	.384†	-

Note: K10 = Psychological Distress; PSS = Perceived Stress; SWB = Subjective Well-being; PA = Positive Affect; IL-6 = interleukin 6; TNF = tumor necrosis factor; CRP = C-reactive protein; LINE-1 = global methylation indicator. **p < .01; *p < .05; †p <.10 (two-tailed tests)

Discussion

This prospective pilot trial explored the relationship between yoga, psychophysiological health indicators, and inflammatory protein and methylation markers in a stressed female community population. This study was unique in exploring DNA methylation, and correlations between methylation and inflammatory markers with potential to indicate a functional relationship. The DNA methylation component was however included retrospectively, meaning longitudinal analysis was only possible with the waitlist participants pre- and post- yoga intervention. Overall, the study found that an eight-week yoga intervention, requiring at least weekly practice, is associated with some changes in immune protein and DNA methylation biomarkers. The yoga group demonstrated lower DNA methylation of the *TNF* region as a whole, and at specific sites, in cross-sectional analysis relative to the control group. This was further supported by decreased methylation seen post-yoga in the longitudinal analysis of the waitlist control group that later participated in the yoga intervention. Meaningful effect sizes in both protein and methylation analysis were demonstrated, as were associations between psychological variables and biochemical measures; however these were not found to be significant. Lack of significance may be attributed to limited statistical power of the study. Nonetheless, these results indicate that participation in an eight-week yoga intervention may have differential impacts on the methylation responses of the immune-candidate genes investigated, and that further investigation in better powered samples is important.

Of note, we did not find evidence of associations between yoga and serum measures of inflammation. Similarly, a large-scale trial did not demonstrate an association between anxiety and biomarkers of inflammation in females (Vogelzangs, Beekman, De Jonge, & Penninx, 2013), which contrasts the associations reported in

depression (Leonard & Maes, 2012; Loftis, Huckans, Ruimy, Hinrichs, & Hauser, 2008). To this end it should be considered that this is a non-clinical community population in which biomarkers of inflammation were generally low, reflected by the 'bottoming out' of inflammatory cytokines. Nonetheless the moderate effect indicating higher IL-6 levels in the yoga group is interesting insofar as it has a well-known role in the pro-inflammatory processes, but is increasingly recognised in healing and regeneration activities (Scheller, Chalaris, Schmidt-Arras, & Rose-John, 2011). For instance, Eyre et al. reports that increased IL-6 has a role in the neuroprotective effect of exercise on mood (Eyre, Papps, & Baune, 2013). A large effect for time on overall levels of CRP was demonstrated, though we did not find evidence of meaningful between-group difference. The general decrease at post-test may be reflecting a sample bias we have discussed elsewhere (Harkess et al., 2016). Namely at baseline women were reporting chronic stress and moderate-to-high levels of distress (potentially indicated by high levels of acute-phase proteins), however they self-selected for this study which indicates motivation to change. This was supported by the overall decrease in stress and distress at post-test (Harkess et al., 2016), and could account for a change in CRP over time independent of participation in yoga. Additionally, at post-test perceived stress was found to be positively associated with global DNA marker *LINE-1* methylation. While this is not consistent with some literature, indicating elevated methylation correlates with positive health outcomes (White et al., 2013; Wilhelm et al., 2010), it is consistent with literature demonstrating hypermethylation in stressed populations (Rusiecki et al., 2012).

This study reports a robust association between engagement with an eight week yoga intervention and reduction in mean methylation of *TNF* (5.5%), however there is no evidence for sizeable correlations between the *TNF* methylation and

serum, or psychological measures making it difficult to infer causal relationships. We do however report a moderate association with WHtR, parsimonious with previous reports of methylation of *TNF* being associated with leanness/ weight-loss previously (Campión et al., 2009; Kirchner et al., 2014). This could potentially account for our reported hypomethylation of *TNF* in our yoga group, and following yoga intervention. However including WHtR as a covariate did not alter the reported association, in fact it strengthened it suggesting that the reduction observed in *TNF* methylation is not simply attributable to change in body composition in our sample following yoga. Yoga may be associated with a positive alteration on the inflammatory system that is not detectable immediately in serum analysis, and not directly responsible for reported positive psychological effects of yoga.

This sample was relatively homogeneous in variables that have been reported as risk factors for differential global DNA marker of methylation (as measured by *LINE-1*; e.g., Chalitchagorn et al., 2004; El-Maarri et al., 2007; El-Maarri et al., 2011; Hsiung et al., 2007; Kim et al., 2010; White et al., 2013; Wilhelm et al., 2010; Zhang et al., 2011; Zhu et al., 2012). Namely, it was comprised of Caucasian females, aged between 35-50 years old, with BMI's < 30, with nil reported substance abuse problems, and comparable between group physical activity levels (nil between group differences in METs discussed in greater detail elsewhere; Harkess et al., 2016). The yoga and control group were well matched for WHtR, and age. Inclusion of WHtR, which was associated with *TNF* methylation at CpG 4/5/6, improved detection of differences in *TNF* methylation following our yoga intervention. Thus, while the lack of statistical correlation and between group effects could be attributable to the limited statistical power in our sample, the medium to large effect sizes reported are plausibly attributable to the yoga intervention, and not to previously implicated lifestyle factors

due to a lack of variability of these factors in our groups (i.e., ethnicity, WHtR, age). This study presents strengths for investigation of biological biomarkers, in the selection of a homogenous population, longitudinal sampling, and the first study to investigate *DNA* methylation in context of a yoga intervention. However, there are limitations.

Limitations

There are a number of limitations to this study which should be considered. The first is that it was a pilot study with only limited statistical power. As a result, this limited statistical analyses that could be undertaken (e.g., we did not meet the sample size assumption required to conduct a regression, nor exploration of mediation/moderation), in addition to having low power when measuring group differences (e.g., ANOVA cell sizes of 30 are required for 80% power; VanVoorhis & Morgan, 2007). Second, there was no baseline DNA methylation measure, which means we cannot draw causal conclusions about between group differences. DNA methylation investigated was from DNA extracted from peripheral whole blood, which is in keeping with our simultaneous exploration of serum markers of inflammation. However, we cannot draw inferences about the effects on specific tissues, including the brain. We only explored a limited number of immune candidate genes (2 regions of *IL-6* and 1 region of *TNF* and *CRP* each), and, as demonstrated by *IL-6*, different regions may indicate different trends. The lack of association between DNA methylation markers and serum markers of inflammation makes it difficult to interpret functional impact, although this could be due to statistical power and that methylation impacts less immediately in serum protein expression levels. It is notable that a number of cytokine samples were below the detection limit in protein analyses, which is likely due to the non-clinical nature of this sample as well as a technical

limitation of the sensitivity of currently available assays. We did not use an active control group, and while between group METs were equivalent, we cannot rule out the attentional effects of engagement with the yoga teacher and the class environment. To address some of these concerns, we focused on the presentation of effect sizes where available and also analysed the data from two different perspectives to examine the reliability of the findings (cross-sectional and longitudinal).

As levels of stress are reported to be increasing in community populations (Cassey & Ling, 2014), an increased prevalence of stress-related disease is likely to follow (Cohen et al., 2007; McEwen, 2006; McEwen & Stellar, 1993; Segerstrom & Miller, 2004). Therefore, future prospective studies should continue to explore the relationship of stress and biomarkers of inflammation in community populations. We recommend replicating our study in a much larger sample and including analysis of DNA methylation profiles at baseline. A variety of active-controls would also be beneficial to assist in disentangling the potentially different effects of different styles of yoga, exercise, and meditation. Additionally, we recommend exploring other candidate genes that may demonstrate involvement in the inflammatory response that has been associated with maladaptive psychological states and/or epigenomic methods that enable network analyses. Finally, we would recommend an experimental design that could differentiate more clearly between regressions to the mean (i.e., entering the study when distress levels are maximal and a natural decrease with time as opposed to intervention) and an experimental effect would be one that took a number of pre-intervention measures.

Conclusions

Alongside the increased levels of stress and prevalence of stress-related disease reported, there has been increased engagement in Mind-Body Therapies

(MBTs), of which yoga is the most utilised (Clarke, Black, Stussman, Barnes, & Nahin, 2015). While gene-expression studies in the MBT literature suggests a relationship with the immune system (Saatcioglu, 2013), further research into the underlying mechanisms, including possible epigenetic mechanisms, has been called for (Niles et al., 2014). To the best of our knowledge, this is the first study to investigate the role of yoga on epigenetic change, and the first MBT study to investigate DNA methylation in immune candidates' methylation (*IL-6, TNF,* and *CRP*). While this pilot study is small and exploratory, it nevertheless indicates that in a non-clinical chronically stressed community population, practicing a minimum of a once weekly, hour long yoga class, is associated with differential methylation patterns despite the wait-list control group reporting similar energy expenditure to the yoga group. This suggests these changes may not be related to energy expenditure, but some aspect of the yoga engagement. However more definitive conclusions cannot be made without an active control group in future studies. Specifically, we report that engaging in a yoga intervention may affect female participants' serum levels of IL-6 and their epigenetic profile of immune candidates, specifically *TNF*. These findings warrant further large-scale research and contribute to the growing literature seeking to explore underlying epigenetic mechanisms and the relationship between MBT and the immune system (Niles et al., 2014). Additionally, they contribute to the growing body of literature seeking to explore biomarkers of inflammation in clinical and non-clinical conditions of distress.

5.2 Summary

Results of this pilot study indicated that yoga may be associated with some changes at the biochemical level captured by biological markers of immunity. Despite limited statistical power, this was the first study to explore DNA methylation in the context of yoga intervention and between group difference of reduced methylation in the *TNF* region was the most robust effect. This suggests capture of an early response of the 'inflammatory reflex' modulated by vagus nerve stimulation (Wang, Yin, & Yao, 2016), discussed in the Literature Review (Chapter 1) as a hypothesised mechanisms by which yoga may evoke change in the HPA axis (Innes et al., 2005). However, DNA methylation in specific organs more directly involved in such regulation (e.g., brain) needs to be explored. Additionally, between group DNA methylation analysis was cross-sectional and it may also have been the case that yoga was associated with ameliorating effects of distress on DNA methylation. Although longitudinal analyses of the waitlist control group prior to, and following, yoga intervention corroborated these findings, larger longitudinal studies with control groups are warranted to examine potential pathways of this effect. Such understanding may also be useful in explicating the role of serum IL-6 as a pro- or anti-inflammatory biomarker (Bonaz, Sinniger, & Pellissier, 2016a), and in determining if longer intervention duration is required for minimum intervention needed to produce change (MINC) in serum markers of inflammation, or if the non-clinical nature of this population limits applicability. Alternatively, it may be the case that for serum cytokines to be useful markers they may need to be stimulated, as was the case in the studies of Kiecolt-Glasser et al. (2010, 2014). Detecting cytokines in this study was challenging as this was a non-clinical sample, thus lipopolysaccharide stimulation would circumvent this issue. However, it is worth noting that the

phenotype of stimulated cytokines is more ‘immune response’ as opposed to general ‘immunity’. In summary, this study addressed a commonly reported gap in yoga literature by exploring biological mechanisms which may underlie the effects of yoga practice. Specifically, some evidence of an immunological effect was provided, but the pilot nature of this investigation means findings need to be treated with caution.

Supplementary Material

Additional Statistical Analysis.

Supplementary Table 1.

Analysis of Longitudinal IL-6$_1$ Methylation

		All samples included				Only samples assayed in triplicate within 10% of the individual's mean			
		CpG 1	CpG 2	CpG 4/5/6	Mean	CpG 1	CpG 2	CpG 4/5/6	Mean
	N	9	9	9	9	6	3	6	9
Pre-Tx	Mean	0.894	0.914	0.923	0.909	0.889	0.914	0.92	0.909
	SE	0.007	0.011	0.005	0.003	0.006	0.012	0.01	0.003
Post-Tx	Mean	0.879	0.887	0.911	0.892	0.884	0.927	0.93	0.701
	SE	0.009	0.025	0.015	0.015	0.011	0.012	0.01	0.133
Effect	Cohen's d	0.63	0.46	0.38	0.53	0.21	0.60	0.36	0.74
t-test	p	.236	.281	.457	.252				

Note: **$p < .01$; *$p < .05$; †$p < .10$ (two-tailed tests); Pre-Tx = the averaged pre-intervention methylation (Wave 2 and Wave 3); Post-Tx = post-intervention methylation (Wave 4). Significance tests were not applied for reduced sample sizes due to low *N*.

Supplementary Table 2.

Analysis of Longitudinal IL-6₂ Methylation

		All samples included					Only samples assayed in triplicate within 10% of the individual's mean				
		CpG 1	CpG 2	CpG 4/5/6	CpG 7/8	Mean[a]	CpG 1	CpG 2	CpG 4/5/6	CpG 7/8	Mean
	N	10	10	10	10	10	9	9	9	9	10
Pre-Tx	Mean	0.032	0.006	0.028	0.053	0.034	0.031	0.006	0.028	0.068	0.034
	SE	0.002	0.002	0.003	0.004	0.003	0.002	0.002	0.003	0.007	0.003
Post-Tx	Mean	0.034	0.002	0.034	0.064	0.034	0.032	0.002	0.037	0.067	0.031
	SE	0.004	0.002	0.007	0.010	0.004	0.004	0.002	0.007	0.010	0.005
Effect	Cohen's d	0.22	0.63	0.38	0.47	<0.00	0.14	0.68	0.53	0.06	0.20
t-test	p	.520	.138	.283	.260	.988					

Note: $^{**}p < .01$; $^{*}p < .05$; †$p < .10$ (two-tailed tests); Pre-Tx = the averaged pre-intervention methylation (Wave 2 and Wave 3); Post-Tx = post-intervention methylation (Wave 4). Significance tests were not applied for reduced sample sizes due to low *N*.

Supplementary Table 3.

Analysis of Longitudinal TNF Methylation

		All samples included							Only samples assayed in triplicate within 10% of the individual's mean						
		CpG 1	CpG 2	CpG 4/5/6	CpG 8	CpG 9	CpG 12	Mean	CpG 1	CpG 2	CpG 4/5/6	CpG 8	CpG 9	CpG 12	Mean
	N	9	9	9	9	9	9	9	1	2	4	5	5	4	8
Pre-Tx	Mean	0.849	0.788	0.118	0.209	0.075	0.072	0.357	0.910	0.838	0.130	0.240	0.075	0.069	0.358
	SE	0.016	0.035	0.018	0.026	0.011	0.015	0.011	.	0.043	0.038	0.039	0.008	0.023	0.012
Post-Tx	Mean	0.733	0.730	0.077	0.159	0.070	0.048	0.302	0.700	0.810	0.078	0.158	0.090	0.040	0.200
	SE	0.046	0.034	0.008	0.015	0.015	0.012	0.011		0.050	0.017	0.027	0.025	0.011	0.035
Effect	Cohen's d	1.11	0.55	1.00	0.80	0.12	0.59	1.68	N/A	0.42	0.90	1.09	0.37	0.79	2.06
t-test	p	.042*	.241	.019*	.203	.806	.130	.002**							

Note: $^{**}p < .01$; $^{*}p < .05$; †$p < .10$ (two-tailed tests); Pre-Tx = the averaged pre-intervention methylation (Wave 2 and Wave 3); Post-Tx = post-intervention methylation (Wave 4). Significance tests were not applied for reduced sample sizes due to low *N*.

Supplementary Table 4.

Analysis of Longitudinal CRP Methylation

		All samples included				Only samples assayed in triplicate within 10% of the individual's mean			
		CpG 1	CpG 2	CpG 4	Mean	CpG 1	CpG 2	CpG 4	Mean
	N	9	9	9	9	4	4	4	7
Pre-Tx	Mean	0.870	0.720	0.692	0.761	0.893	0.723	0.703	0.606
	SE	0.020	0.023	0.016	0.012	0.028	0.011	0.023	0.079
Post-Tx	Mean	0.899	0.688	0.697	0.757	0.880	0.723	0.645	0.763
	SE	0.021	0.043	0.031	0.022	0.041	0.065	0.056	0.029
Effect	Cohen's d	0.47	0.31	0.06	0.07	0.2	1.2	0.67	1.00
t-test	p	.392	.466	.916	.892				

Note: $^{**}p < .01$; $^{*}p < .05$; †$p < .10$ (two-tailed tests); Pre-Tx = the averaged pre-intervention methylation (Wave 2 and Wave 3); Post-Tx = post-intervention methylation (Wave 4). Significance tests were not applied for reduced sample sizes due to low *N*.

Supplementary Table 5.

Analysis of Longitudinal Global Methylation (LINE-1).

		CpG 1	CpG 2	CpG 3	Mean
	N	10	10	10	10
Pre-Tx	Mean	.684	.716	.602	.667
	SE	.004	.002	.003	.003
Post-Tx	Mean	.681	.711	.596	.662
	SE	.009	.006	.005	.006
Effect	Cohen's d	0.12	0.36	0.49	0.32
t-test	p	.801	.440	.348	.523

Note: $^{**}p < .01$; $^{*}p < .05$; †$p < .10$ (two-tailed tests); Pre-Tx = the averaged pre-intervention methylation (Wave 2 and Wave 3); Post-Tx = post-intervention methylation (Wave 4). Note all samples were successfully assayed in triplicate within 10% of the mean.

Supplementary Table 6.

Genetic regions and primer sequences analysed for DNA methylation analysis.

Genetic locus	Primer type	Primer sequence	Target size (bp)	No. of detectable CpG sites (over no. of units)
IL-6$_1$	Forward	aggaagagagGAGATATTATTTTGAGGGAAGAGGG	309	6 (3)
	Reverse	cagtaatacgactcactatagggagaaggctACCTACATAAACCCCAAATCTCCTA		
IL-6$_2$	Forward	aggaagagagTAGGATTGGAGATGTTTGAGGTTTA	234	7 (4)
	Reverse	cagtaatacgactcactatagggagaaggctAACAACACAACTAAAAACCTACCTCT		
TNF-α	Forward	aggaagagagTTTGGTTTTTAAAAGAAATGGAGGT	410	8 (6)
	Reverse	cagtaatacgactcactatagggagaaggctACTTCTCTCCCTCTTAACTAATCCTC		
CRP	Forward	aggaagagagTTTTAATATTGTTTGTTGGGGTAGG	314	3 (3)
	Reverse	cagtaatacgactcactatagggagaaggctCATCTCCAAAAACTATCAAATTTCC		
LINE-1	Forward	aggaagagagTTTATATTTTGGTATGATTTTGTAG	103	3 (3)
	Reverse	cagtaatacgactcactatagggagaaggctTCACCACCAAACCTACCCTAAA		

Supplementary Table 7.

PCR Reaction Protocol for All Assays

Reagent	Volume for 1 well (µl)
2x FastStart PCR Master Mix	7.5
Forward primer (10µM)	0.6
Reverse primer (10µM)	0.6
Nuclease-free water	5.3
DNA (25ng/µl)	1
Total	15.0

Supplementary Table 8.

PCR Conditions Protocol for IL-6, TNF, and CRP

Stage	Cycles	Temperature	Time
1	1	95°C	10 minutes
2	5	95°C	10 seconds
		54-60°C	30 seconds
		72°C	2 minutes
3	40	95°C	10 seconds
		58-62°C	30 seconds
		72°C	1.5 minutes
4	1	72°C	7 minutes
		4°C	∞

Supplementary Table 9.

PCR Conditions Protocol for LINE-1

Step	Temperature (°C)	Time	Cycle	Concept
1	95	10 mins	1	Denaturation
2 a	95	10 seconds		Denaturation
b	58	30 seconds	5	Annealing
c	72	2 mins		Extension
3 a	95	10 seconds		Denaturation
b	61	30 seconds	30	Annealing
c	72	90 seconds		Extension
4	72	7 minutes	1	Extension
5	4	∝	∝	Finish

CHAPTER 6. DISCUSSION

6.1 Overview

The aim of this research was to explore feasibility and effectiveness of yoga as a mind-body intervention in a community population of Australian women reporting chronic stress and psychological distress. Papers presented in this thesis detail development of an eight week secular yoga intervention, examine feasibility of conducting the intervention in this population, and explore associations between participation and measures of mental health and well-being, along with physiological and biological outcomes.

A process evaluation and three quantitative outcome studies were conducted resulting in the four papers which comprise the main body of this thesis. Outcomes of studies suggest that the intervention developed was feasible, and that participants reported an affective benefit immediately following participation. Exploration of changes in psychological distress reported in the intervention group demonstrated reliable and clinically significant improvements comparable to published evaluations of psychotherapy. The yoga group demonstrated healthier levels of perceived stress, distress and positive affect following an eight week yoga intervention relative to controls. However, these improvements were not robustly maintained following cessation of regular yoga practice which indicates that regular and maintained yoga practice is associated with cognitive and affective mental health benefits. Additionally, there is evidence indicating yoga is associated with a physiological effect, as indicated by increased flexibility and decreased waist circumference. At the molecular level, a biological effect was reported in between-group differences in biological markers of immune function. Psychological and biophysiological effects are of interest given the comparable levels of physical activity reported in both the yoga and control group, indicating positive effects may be specifically attributable to effects of the yoga intervention.

This final thesis chapter reviews the findings of each study, explores the theoretical implications of this work in regard to mind-body interventions, stress, and public health policy, discusses limitations of the studies, and outlines recommendations for future research.

6.2 Review of Thesis Findings

6.2.1 Process Evaluation of a Secular Yoga Intervention with Clinical Reduction of Participant Reported Distress

As the stress levels of Western communities are rising (Cassey & Ling, 2014), yoga has increasingly been trialled as an intervention for improved mental health (e.g., Field, 2011; Sharma, 2014). However, process evaluations have rarely been conducted on yoga intervention administration, despite yoga meeting Medical Research Council (MRC) guidelines as a complex intervention (Craig et al., 2008). In this research, a process evaluation was conducted and reported in Study 1 (Chapter 2) which contains sufficient information to enable replication of the intervention, information on the fidelity and quality of implementation, and some exploration of possible mechanisms of change (Moore et al., 2015). However, the sample size was not sufficient for path analysis. Discussion of this evaluation details the process of designing a standardised secular, body-oriented yoga intervention. Results demonstrate that most women who participated in the intervention were able to participate in an hour long yoga session at least once per week. These features are consistent with engagement levels reported by community practitioners and suggests similarities in the nature of the population (Birdee et al., 2008; Penman et al., 2012). While the intervention was found to be acceptable, participant feedback indicated that smaller class sizes would have been preferable and that the time commitment was a barrier to participation.

As recommended by Evans et al. (1998), clinical improvement was measured in this research. It was demonstrated that 33% of participants reported a reliable reduction in levels

of psychological distress, and 28% reported clinically significant improvement. Women reported attendance at an average of 11 classes, which indicates a rate of clinical improvement similar to that reported for brief psychotherapy interventions (Shapiro et al., 2003). However, it is possible that a 'regression to the mean' may have played a role in that participants enrolled for the intervention when their distress was in the dysfunctional range and the yoga classes commenced within six weeks of the first participant's enrolment.

The data reported in process evaluation suggests that the standardised yoga intervention based on a secular, body-orientated (focusing on a higher energy expenditure) theoretical framework was designed and administered appropriately. The conduct of this evaluation meets MRC standards (Moore et al., 2015) and demonstrates clear within-subject effects. Specifically, an immediate association between yoga and mood was demonstrated, while a pre- to post-treatment effect on psychological distress was demonstrated.

6.2.2 Brief Report on the Psychophysiological Effects of a Yoga Intervention for Chronic stress: Preliminary Findings

In addition to the lack of standardised protocols exploring yoga and mental health in the community reported in the literature, poor study design is a commonly reported limitation. The purpose of this study (Study 2) reported in Chapter 3 was to address some of these limitations and evaluate outcomes of the yoga intervention reported in Chapter 2 (Study 1: Process Evaluation). This study involved a clinical trial with a sample size sufficient to detect meaningful effects on mental health and well-being outcomes, and physiological measures associated with allostatic load. The yoga group demonstrated decreased levels of psychological distress, perceived stress, and an increase in positive affect if they practiced per-protocol (i.e., average of one class per week), whereas only positive affect was significant regardless of practice. This highlights the association between regular yoga practice and

improved cognitive appraisals of distress and stress, and that any amount of contact with the intervention is associated with improved mood state.

The findings of this study are important given that the level of physical activity in the control and yoga intervention groups was similar which suggests that the waitlist control group engaged in activities other than yoga. This was further supported by the lack of between group difference in heart rate and blood pressure, although the yoga group demonstrated a smaller waist-to-height ratio and increased flexibility. Thus, reported mental health benefits may be an intervention specific effect. Conversely, no effect was found for mindfulness, an unexpected result given the conceptualisation of yoga as 'mindful exercise' (La Forge, 2005) and the body of evidence discussed in the Literature Review that supports positive association between yoga and mindfulness (e.g., Field, 2011).

6.2.3 A Randomised Trial of the Psychological Effects of a Yoga Intervention for Chronic Stress

The purpose of the study (Study 3) reported in Chapter 4 was to conduct a follow-up study to explore the association between yoga intervention and psychological outcome variables post-treatment. Previous research examining yoga interventions has rarely conducted follow-up investigations to examine the residual benefits of participating in a yoga intervention.

Results demonstrated that most effects of yoga intervention diminished over time (i.e., between group differences in psychological distress and perceived stress), although the yoga group maintained a higher level of positive affect than the control group. This effect is of interest as it supports the result demonstrated in the intent-to-treat analysis conducted in Study 2 (Chapter 3) in that having the opportunity to engage in yoga classes (not necessarily regular practice) provides affective benefit. The cognitive effect (i.e., psychological distress

and perceived stress) trajectory reported in these studies is reflective of Smith et al.'s (2007) trial, where yoga was controlled with relaxation in community population, and no difference between group difference in level of stress was reported at the six week follow-up, though the relaxation group had better mental health. This is important because at conclusion of the Smith et al. (2007) yoga intervention analysis demonstrates that the yoga group was associated with better mental health, which suggests that participation in regular classes is important for maximal effect.

6.2.4 The Effect of a Brief Yoga Intervention on Markers of Inflammation and DNA Methylation in Chronically Stressed Women: A Pilot Study

The purpose of the pilot study reported in Chapter 5 (Study 4) was to further explore the underlying mind-body mechanisms of yoga intervention, specifically longitudinal biological effects. Limited research has previously explored immunological outcomes of yoga and mental health, and this was the first study to explore immune candidate gene deoxyribonucleic acid (DNA) methylation in yoga intervention participants. This study explored the epidemiological effects on serum markers of inflammation (interleukin-6 [IL-6], tumor necrosis factor [TNF], and C-reactive protein [CRP]), DNA methylation profiles of regions in the *IL-6, TNF,* and *CRP* genes and *LINE-1* (a proxy for global methylation), and associations of these biological outcomes with psychological variables. Baseline measures of DNA methylation were not collected which limited our ability to draw conclusions about baseline status or causality. Meaningful associations were demonstrated between biological and psychological measures; however this study was underpowered. The most robust association was lower mean *TNF* methylation in the yoga group, as opposed to control. As this study demonstrated a likely relationship between biomarkers of inflammation and mental health, further large-scale studies are recommended.

6.3 Implications

6.3.1 The Role of Yoga in Well-being and Mental Illness Prevention

The feasibility of implementing a yoga intervention for mental health concerns was demonstrated alongside a high level of acceptability in Study 1. Interestingly, while the intervention administered was conceptualised as a mindfulness-based intervention (MBI; Hayes, 2002), no changes to participant mindfulness capacity were demonstrated in psychological outcome studies (Study 2 and Study 3). This is in contrast to *hatha* yoga being associated with increased mindfulness in a previous study in a healthy population following a similar dosage (Shelov et al., 2009), and also a cross-sectional study demonstrating that advanced yoga practitioners reported greater mindfulness than beginners (Brisbon & Lowery, 2011). It was hypothesised in Study 3 that as a 'mindful-exercise' yoga would improve an individual's mindfulness capacity through 'bottom-up' attention to the body coupled with 'top-down' modulation of attention/cognition (Gard et al., 2014), a similar process to mindfulness meditation (Kerr, Sacchet, Lazar, Moore, & Jones, 2013). Consequently, results presented in these studies seem to contradict previous studies and do not support the conceptualisation of yoga as a MBI. However, it may also be the case that an eight week *Ashtanga* based yoga intervention is insufficient to evoke change in mindfulness. Further research to determine if dosage effects or the lack of explicit instruction on mindfulness impacted these results is warranted, as is investigation into the psychological mechanisms of yoga intervention on psychological health, as described below.

Increased levels of distress and stress, and decreased levels of well-being, have been reported in recent years (Cassey & Ling, 2014). Yoga is theorised to be a plausible intervention to address this phenomenon (see Literature Review, Chapter 2). While yoga has been broadly found to improve levels of well-being in community populations (Bonura, 2011; Hartfiel et al., 2011; Michalsen et al., 2005; Woodyard, 2011), no association was

previously demonstrated post-treatment (Study 2). However, a clear association between positive affect and yoga was demonstrated, as reported in previous trials which evaluated the immediate effect of yoga participation (Kiecolt-Glaser et al., 2010; Narasimhan et al., 2011), and following a mid-length (ten week) intervention (Danhauer et al., 2009). This is important as positive affect is considered to be the affective component of subjective well-being, indicating an affective benefit to well-being. However, finding no benefit to the cognitive component of well-being may have obscured the affective benefit when measuring well-being as whole construct. In Study 2 it was hypothesised that a lack of positive well-being effect may be in part due to conceptualising attendance at the yoga class as another stressor; conversely, the mixed-models analysis presented in Study 3 demonstrated well-being decreased in the yoga group following post-treatment, which was hypothesised as related to ceasing yoga practice. As the promotion of well-being is being developed as a focus in mental health services, there is potential that yoga may provide an important affective intervention beyond treating psychological distress and stress (Slade, 2010). However, mixed findings at follow-up (i.e., maintained positive affect and decreased well-being as a cognitive construct) require further investigation to disentangle, particularly in light of the potentially negative effect of ceasing an intervention.

In regards to psychological distress, results presented in this series of papers demonstrated clear benefit with regular yoga practice. While these papers focused on a community population, demonstration of decreased distress has clinical relevance as women were recruited for participation in this study if their Kessler Psychological Distress Scale (K10) score suggested they may be experiencing moderate symptoms of, or some form of, depression and/or anxiety which may be a cause of some distress in their daily life (Australian Bureau of Statistics, 2003). Although the studies are limited in their ability to infer causality (as discussed in more detail below), the findings are generally consistent with

previous studies reporting the antidepressant and anxiolytic effects of yoga (Büssing et al., 2012; Cramer et al., 2013; Field, 2011; Li & Goldsmith, 2012; Pilkington et al., 2005).

The reliable and clinically significant change in levels of distress reported in Study 1 (Chapter 2) indicates that yoga has a role in addressing the increasing levels of distress reported in the community (Cassey & Ling, 2014), and may prevent development of clinical conditions that require further healthcare services. Further, the decrease in distress is beyond that of the control group when attending an average of one yoga class per week (Study 2; Chapter 3), indicating that contact with the intervention was associated with enhanced benefit. However, these benefits were not maintained upon conclusion of the intervention (Study 3; Chapter 4), further highlighting the need for regular engagement. While these studies have limitations, particularly the lack of an active control group as highlighted below, they present results of a large clinical trial, thus meeting the demand for high-quality literature contribution to this growing field of investigation. As a whole, this series of papers supported the literature on yoga and distress; most specifically supporting studies that investigated distress in community populations of women (Michalsen et al., 2005; Michalsen et al., 2012; Smith et al., 2007). Research in other populations appears warranted, as it is demonstrated that at least weekly yoga practice may reduce perceived distress. The administration of yoga should be explored as a preventative intervention for women at risk of developing clinical levels of distress and for use as an adjunct therapy when distress is clinical.

In addition to addressing psychological distress, this series of papers demonstrates the potential of yoga as a stress management intervention. These findings are generally consistent with the literature which supports yoga as a tool for stress reduction despite the methodological caveats (Sharma, 2014). Previous research suggested that a similar style of yoga to that administered (detailed in Study 1; Chapter 2) is more effective than physical

exercise (Chattha et al., 2008). The style of yoga administered included the dynamic sun salutations, and previous research has demonstrated that dynamic styles are more clearly associated with decreased stress than gentle styles (Cowen & Adams, 2005). Nonetheless, non-actively controlled trials using a less dynamic style of yoga in a community population of women have also demonstrated stress reduction (Michalsen et al., 2005; Michalsen et al., 2012), this indicates that multiple mechanisms are likely involved in yoga eliciting this perceptual change. Insofar as modern lifestyles are associated with increased stress, regular yoga practice should be further explored for effectiveness at reducing stress in some populations.

6.3.2 The Need for Continuing Research on Yoga's Mind-Body Connection

The work presented in this thesis supports the proposition that yoga elicits bidirectional communication between the mind and body. Communication between the mind, nervous system, and immune system was simplified and presented in Figure 3 (Chapter 1). Study 2 (Chapter 3) and Study 4 (Chapter 5) tested physiological and biological outcomes, in addition to psychological outcomes, which were the focus of Study 3 (Chapter 4). Results presented in this thesis indicate that the communication pathways between mind, nervous system and immune system (depicted in Figure 3, Chapter 1) were supported to some extent, although a clear association between biomarkers of the immune system and the mind (as captured by psychological outcome variables) was less clearly supported. A minimisation of inflammatory response has been associated with yoga practice in previous trials (Kiecolt-Glaser et al., 2014; Kiecolt-Glaser et al., 2010; Pullen et al., 2008; Pullen et al., 2010); however, the results of Study 4 (Chapter 5) demonstrate serum levels of cytokines are not remarkably different between groups as a non-significant, though a moderate association between a higher IL-6 score and yoga was demonstrated. This may have been due, at least in

part, to the community population used, with the number of cytokine observations below the limit of detection indicating the population was generally experiencing low levels of inflammation. In contrast, the population in the Pullen et al. (2008, 2010) trials were heart failure patients which is a condition associated with elevated cytokine levels (Matsumori, 2000), and cytokines were stimulated in the Kiecolt-Glasser et al. (2010, 2014) trials enhancing detectability. Thus, it could be that enhanced methods are necessary to utilise these biomarkers in health populations, although as previously discussed this subtly alters the immune phenotype. Further, it is also the case that moderate effects did not show significance and that this study was limited by power. At the same time, positive association demonstrated between IL-6 and yoga is directionally different to results reported in previous trials where IL-6 was negatively associated with yoga. The role of IL-6 in rejuvenation was contrasted with its inflammatory functions (Scheller et al., 2011) in Study 4 which highlights difficulties in interpretation and the need for further investigation of these mixed findings.

In contrast to the unclear associations between yoga and serum measures of inflammation, DNA methylation patterns of the *TNF* region demonstrated robust effects, which may indicate demethylation was an early response to the yoga intervention. While this is supported by the non-controlled longitudinal data of the waitlist control group, there is no baseline data to confirm this proposition and further longitudinal research is vital. While it is recognised that regulation of *TNF* expression is complex, it is theoretically possible that this association may be linked with the anti-inflammatory role of the vagus nerve (Tracey, 2002). The vagus nerve responds to cytokines and then communicates with the HPA axis via acetylcholine helping to turn off the production of proteins which fuel inflammation (Bonaz et al., 2016a). Low 'vagal tone' inhibits this regulation, while activities, such as exercise and experience of positive emotions, increase vagal tone (Bonaz, Sinniger, & Pellissier, 2016b; Kok et al., 2013; Kok & Fredrickson, 2010). Thus, these methylation differences may be

connected with HPA axis activity, ultimately connecting them with mental health and the body's allostatic load. This theory is consistent with the proposition that one mechanism for the positive effect of yoga is through stimulation of the vagus nerve (Innes et al., 2005), thus eliciting a relaxation response and potentially improving vagal tone.

Inflammation is also associated with visceral fat (Fontana, Eagon, Trujillo, Scherer, & Klein, 2007), and it has been suggested that 'stress', as measured by activation of the HPA axis and sympathetic nervous systems, may cause abdominal obesity through elevated cortisol levels (Björntorp, 2001). Larger waist-to-height ratios demonstrated in the waitlist control group following yoga intervention indicate that yoga may be associated with decreased allostatic load, particularly in light of equal between group physical activity, heart rates, and blood pressure (i.e., physiological measures of activity levels and sympathetic nervous system activation). This supports findings from a yoga trial where changes in waist circumference, but not weight, were demonstrated in a population of breast cancer patients (Littman et al., 2012), indicating the neuroendocrine system may have been affected. Future research would benefit by including more precise measures of adipose tissues in trials, as opposed to the more general waist-to-height ratio, and exploration of associations of adipose tissue and cortisol. In light of increased positive affect being negatively correlated with salivary cortisol (West et al., 2004), an association between vagal tone and positive affect (Kok & Fredrickson, 2010), and increased positive affect reported following each yoga class and overall (Study 1; Chapter 2), it would be interesting to explore how adipose tissue may be a useful measure of the mind-body relationship.

The associations between mental health outcomes and biological outcomes presented in Study 4 (Chapter 5) did not show a pattern of results consistent with group comparisons. This highlights the complexity involved in investigating the relationships various systems have with mental health, alongside exploration of the underlying mechanisms of the positive

effects of yoga practice. Strong positive correlations were demonstrated between perceived stress, serum hsCRP and global methylation (*LINE-1*) highlighting the effect of psychological stress on inflammatory markers and DNA methylation. Thus, while hsCRP and global methylation were not associated with yoga practice, it seems they capture a relationship between psychological stress and the immune system, as proposed by the field of psychoneuroimmunology (PNI; Ader, 2000; Ader & Cohen, 2001). As regular yoga practice was linked with decreased perceived stress, yoga practice may buffer against the negative consequences of repeated stress on the body, although a larger dose may be needed before changes are reflected biologically. Interestingly, positive affect was negatively associated with methylation of *CRP* and global methylation, indicating another possible mechanism of mood. In addition to the possibility that the minimum intervention necessary for change (MINC) of some biological markers may not have been provided, it also appears that yoga, like exercise, exerts maximal benefits to mental health only so long as it is engaged with regularly (Babyak et al., 2000), as opposed to cognitive-based interventions (e.g., cognitive behaviour therapy [CBT]: Durham et al., 2005; mindfulness-based stress reduction [MBSR]: Miller et al., 1995) and relaxation (Smith et al., 2007).

The theory that other psychological interventions are better maintained following treatment needs to be explored further. One of Smith et al.'s (2007) suggestions as to why relaxation was associated with improved mental health at follow-up, when yoga had demonstrated a stronger association at post-treatment, was that it was easier for women to practice relaxation following cessation of intervention. Also, tools taught in a cognitive-based intervention help individuals develop more helpful thinking styles and behaviours, such that when the intervention ceases the practice can continue. This is similar in MBSR, as one of the outcomes Miller et al. (1995) explored was ongoing compliance with meditation, which these authors reported to be high. Thus, regular practice of skills is necessary for psychological

benefits to be maintained. If it is the case that populations are less likely to engage with yoga outside of a formal class (Penman et al., 2012), consideration of the costs and benefits of offering longer interventions should be explored. In light of the possibility that yoga may treat clusters of symptoms (i.e., psychological, physiological, immunological, neuroendocrinal) and provide physical activity, as regular engagement is known to reduce costs on the healthcare system, health services and public policy changes to foster participation in yoga classes should be considered, such as re-imbursement or subsidisation of attendance cost at accredited classes.

6.3.3 The Biopsychosocial Model of Healthcare and Public Policy

Historically, infection epidemics and other communicable illnesses were the most concerning threats to well-being; however, the current global burden of disease is increasingly composed of non-communicable diseases, such as stress, anxiety and depression (Vos et al., 2012). It is known that mental health and physical health are intrinsically related, as proposed by the field of PNI. Accordingly, health services must undergo change that reflects health challenges faced by the population, and must help facilitate promotion of good health and disease prevention, while reducing demand on the healthcare system. These studies indicates that an alternative intervention is yoga. It is clear that the longstanding benefits of psychotherapy to effect thinking styles, and of pharmacology to adjust chemical imbalances, may not be replaced in clinical populations; however, yoga may provide a useful adjunct therapy or 'preventative treatment' for individuals not currently presenting with clinical symptoms of a mental health disorder. Yoga may be particularly relevant for those with high stress levels vulnerable to developing clinical ailments. As current treatment moves from a biomedical model of care to a biopsychosocial approach, a systems oriented approach must be considered requiring the uniting of different approaches to address the various

components involved. Thus, if yoga is associated with immediate benefit to positive affect, described previously as the 'hallmark of well-being' (Lyubomirsky et al., 2005), it likely follows that yoga is associated with improved immunity (Cohen et al., 2003), which may be maintained during periods of chronic stress (Folkman & Moskowitz, 2000). Plausible mechanisms highlighted in this research support the potential of yoga as a contributing factor in an individual's achievement of their best physical and mental health.

Interestingly, mind-body interventions are often held to higher standards than traditional medicine, needing to demonstrate both quality of care and cost-savings (Mayer & Saper, 2000). There is an increasing need to consider the monetary value of population based prevention programs and interventions as evidence suggests communities are on "the verge of a stress-induced public health crisis" (American Psychological Association, 2016; p. 5), which likely stems from the physical consequences of stress (i.e., increased 'wear and tear' on the body) and from the unhealthy behaviours in which individuals engage when stressed (e.g., drinking, smoking, decreased exercise, dietary changes). Yoga is reported as acceptable by a community population of middle-aged women (Study 1; Chapter 2), and supported by adherence to intervention (i.e., *Iyengar*, *hatha* yoga) by similar populations in previous literature (Michalsen et al., 2005; Michalsen et al., 2012; Smith et al., 2007). Australia's guidelines for physical activity, which helps to decrease the risk of chronic diseases and improve psychological well-being (Department of Health, 2014), are directly supported by offering community classes. Yoga may also provide additional psychological and biological benefits when compared to some other forms of exercise (Ross & Thomas, 2010). If a single strategy can treat clusters of stress and distress symptoms, yoga seems a good investment, even when administered regularly. However, in order for the Australian Government to provide funding for such initiatives, yoga must withstand rigorous scientific methodology, such as the conduct of large-scale clinical trials and detailed evaluation of outcomes, to which

this series of papers contributes. Further, should yoga move into the realm of public health interventions, more consideration will need to be given to the precise classification of 'yoga' based on demonstrated efficacy, risk of participation (e.g., one adverse event was reported in this series of papers, and at yoga intervention cessation a potential decrease in well-being and a positive association between therapeutic alliance and psychological distress were noted) and the professional accreditation of instructors.

6.4 Limitations

The generalisability and conclusions of the studies presented in this research is limited by a number of aspects of study designs, sampling framework, and outcome measures. These limitations are discussed in each of the papers of this thesis and are summarised here.

6.4.1 Study Design

As a general problem in exercise interventions, full blinding was not possible and participants were aware of the condition they were allocated. Consequently, both placebo and attentional effects limit interpretation of results. The nature of the timeframe in this series of papers is such that the intervention commenced within six weeks of recruitment, thus participants may have been recruited at a time of particularly high distress (crisis) and a steeper regression to the non-clinical range would be more likely (Evans et al., 1998). While eight weeks is the median duration of yoga interventions administered in Western countries and reflects popular MBIs (e.g., MBSR), it may be the case that the MINC was not provided for some outcome variables. A brief follow-up period explored residual effects of intervention. However, follow-up at one month post-treatment limited direct comparison with another community study that conducted follow-up at six weeks (Smith et al., 2007), although one month appears to have been long enough to ascertain limited duration of some

psychological outcome measures following intervention cessation. It is also noted that cessation of the first intervention and commencement of the second (for the waitlist control) may have had an additional effect on participant mood and cognitive appraisals of their situations. As discussed in Study 4, DNA methylation was not originally anticipated as part of the study. Thus, there are no baseline measures which means that the only between-group analysis possible for these outcomes was cross-sectional analysis.

6.4.2 Population

The participants in this study were middle-aged women in an educated population. While this population is reflective of those who engage in community yoga classes, results may be less generalisable to populations with different resources and values. Additionally, these women were self-selected and motivated to engage in the intervention offered as a means to manage psychological distress. As they were a functional population there was less room for clinically meaningful change.

6.4.3 Sample Size

The original power analysis calculated the necessary sample size for detecting moderate-to-large effects (depending on the type of analysis planned), meaning the above studies were underpowered to detect small effects (less meaningful). Post hoc power analysis conducted in Study 2 indicated that .80 power was not demonstrated in the analysis conducted. As Study 4 was composed of a sub-set of the population of the other studies, it was further limited by power, as indicated by the number of non-significant meaningful effect sizes demonstrated. Only a small number of participants attended yoga classes twice-weekly, limiting comparison and investigation of dosage with those who attended once-weekly.

6.4.4 Measurement Issues

The research presented here encountered a number of measurement issues which highlight challenges of self-report and 'objective' outcomes. Energy expenditure was measured using the International Physical Activity Questionnaire (IPAQ), which has been well validated (Craig et al., 2003) but is prone to bias in reporting. The use of daily activity diaries was trialled due to their more rigorous nature, but this approach was reported as burdensome by participants and only a limited number partially completed them. Consequently, activity diaries were not included in analysis. This was not unexpected given the major concern for this population was reported as 'limited time'. Like the IPAQ, all self-report measures are susceptible to social desirability bias, which is particularly relevant in non-blinded trials, such as those reported here. It would have been useful to have measured psychological distress regularly and conducted class-by-class survival analyses on psychological distress to provide more information on the relative impact of cumulating yoga practice.

In regards to biophysiological outcomes, waist-to-height ratio is known to be predictive of disease risk (Savva et al., 2013). However, this ratio does not directly measure adipose tissue, which is known to be associated with cortisol (Björntorp, 2001). IL-6 was included as an outcome measure of inflammation in the design of Study 4. However, through familiarisation with the literature, it became apparent that IL-6 is involved in both pro- and anti-inflammatory pathways (Scheller et al., 2011; Wolf, Rose-John, & Garbers, 2014), which prohibits clear interpretation without further markers of the various systems involved in its signalling. A number of participants' cytokine measures were below the detection limit, thereby limiting sensitivity of the outcome analysis. It is possible that using lipopolysaccharide-simulated production of these measures, as other studies have done (Kiecolt-Glaser et al., 2014; Kiecolt-Glaser et al., 2010), would have been more informative.

In light of the way biological outcomes are measured as having effect, it should also be highlighted that DNA methylation was explored using whole blood and cannot be used to infer methylation of organs, such as the brain and spleen which are linked to the 'inflammatory reflex' of the vagus nerve.

6.5 Future Research Directions

Findings reported in this thesis indicate that practicing yoga regularly is associated with improved elements of mental health, supporting findings of several previous studies (for reviews see: Cramer et al., 2013; Li & Goldsmith, 2012; Pilkington et al., 2005; Sharma, 2014; Woodyard, 2011). Given that yoga has been established as a potentially efficacious intervention for mental health when practiced regularly, future trials should include active control groups. Park et al. (2014) noted that comparison conditions should be selected to help isolate specific mechanisms of effect. Thus, this may include exploring psychological and biophysiological outcomes, and comparing exercise and yoga interventions of varying intensities, as intensity of exercise is reported have differential impacts on affect and anxiety (Hall, Ekkekakis, & Petruzzello, 2002; Landers & Petruzzello, 1994). Further, standardised interventions using different styles of yoga should be compared against varying outcomes in differing populations (e.g., health-status, gender, age, socioeconomic status) as, like exercise, they are likely to have different underlying mechanisms that warrant application in different situations.

In exploring underlying biological mechanisms, it is recommended that a theoretical framework is applied to guide exploration and interpretation. For example, Figure 3 (Chapter 1) presented in the literature review was developed based on PNI and epigenetic principles, and, while simple, it offers guidance on the bidirectional effect that should be considered in exploring the effects of stress and mechanisms of mind-body interventions. Perhaps this

model will be further developed, or perhaps a more encompassing model will be presented in literature to come. This may involve exploring the role of the vagus nerve and markers of neuroendocrine function. As the first study to explore yoga and DNA methylation was presented (Study 4; Chapter 5), it is highly recommended that further research explores the utility of epigenetic measures to develop understanding of the biological effects of yoga. This would involve further exploration of markers of immunity in blood, but also extend to other mechanisms, and methylation patterns in other tissues. Further, the relationship of DNA methylation and gene-expression warrants exploration, as does research into the potential role of yoga in regulation of gene-expression (Saatcioglu, 2013). The trials that explore this area need to consider current limitations in the field, and ensure development of a credible evidence base by documenting a rigorous design and delivery process that would enable replication and clear interpretation of results, while implementing high quality methodology. Finally, in addition to evaluating styles of yoga and outcomes for which yoga may be efficacious, future research should explore situations where yoga does not follow best practice guidelines and document risks involved in implementation of yoga intervention.

6.6 Final Comments

The series of papers presented in this thesis improve understanding of the implementation and outcomes of a yoga intervention for middle-aged Australian women reporting chronic stress and psychological distress. The aims of this thesis were to:

- conduct a process evaluation that would facilitate understanding of intervention development and interpretation, and aid in replication,
- conduct a clinical trial with sufficient power to detect meaningful effects of yoga on mental health measures in a community population, and

- explore the effect of yoga on biological measures, which might help develop understanding of underlying mechanisms of effect.

Results suggest that a secular yoga intervention is acceptable and associated with improved affect and decreased psychological distress and perceived stress. The research undertaken contributes to the literature by having conducted the first process evaluation in a community population which details development and implementation of a standardised protocol. In addition, this research presents one of the largest longitudinal studies of yoga in a community population, utilising high quality methodology, thus overcoming the main concerns with the literature previously reported. Correspondingly, a pilot study was conducted to explore 'gold-standard' biological outcomes, also reported as lacking in the literature, and presents the first investigation of the association between yoga and DNA methylation.

APPENDIX A

Demographics Questionnaire

What is your first name?

What is your surname?

Contact number:

Email address:

What suburb do you live in?

How many hours have you worked in the past week?

Birth year:

What is your highest level of education achieved?

- ☐ Primary school
- ☐ High school (no degree)
- ☐ High school degree
- ☐ Vocational school (e.g. TAFE)
- ☐ Bachelor's degree
- ☐ Master degree
- ☐ Doctorate degree

Do you have children? Yes ☐ No ☐

What is your marital status?

- ☐ Single, Never Married
- ☐ Married
- ☐ Living with Partner
- ☐ Separated
- ☐ Divorced
- ☐ Widowed
- ☐ Prefer Not to Answer

Do you take any medications (satins) to control your cholesterol levels? Yes ☐ No ☐

Do you take any β- blockers?

These are medications often used to treat high blood pressure, glaucoma, overactive thyroid, or migraines – see the following examples:

Acebutolol (Sectral) Atenolol (Tenormin) Betaxolol (Betoptic)Bisoprolol (Cardicor, Emcor, Zebeta) Carteolol (Teoptic)Carvedilol (Coreg, Eucardic)Celiprolol (Celectol)Labetalol (Trandate) Levobunolol (Betagan) Metipranolol (Metipranolol Minims) Metoprolol (Betaloc, Lopresor, Lopressor, Toprol XL) Nadolol (Corgard) Nebivolol (Bystolic, Nebilet) Oxprenolol (Trasicor) Pindolol (Visken)Propranolol (Inderal LA) Sotalol (Beta-Cardone, Sotacor) Timolol (Betim, Nyogel, Timoptol)

Yes ☐ No ☐

Have you been diagnosed with a convulsive disorder? Yes ☐ No ☐

Do you take an oral anti-contraceptive? Yes ☐ No ☐

When was your last menstrual period?

If over a year ago please just note >1 year.

☐

Have you, or are you, experiencing any medical symptoms that your General Practitioner has suggested are menopausal changes? Yes ☐ No ☐

Have you, or are you, undergoing hormone replacement therapy for menopause?

Yes ☐ No ☐

If you answered "Yes" to the above question, what form of therapy are you undergoing?

- ○ Combined Hormone Therapy (Estrogen – Progesterone)
- ○ Estrogen Therapy

How many times in the last month have you been extremely stressed? ☐

What experiences or difficulties have you had to cope with in the past month that have caused you significant distress? Please note how long each of these experiences has been causing you difficulty. ☐

e.g. Serious illness or injury to you or a close relative; death of a first-degree relative; death of a close family friend or second-degree relative; separation due to marital difficulties; break-up of steady relationship; serious problem with a close friend, neighbour or relative; unemployment/seeking work for more than a month; sacked from your job; major financial crisis, problems with police and court appearance; something valuable lost or stolen.
Please use the following rating scale to quantify the amount of distress you currently experience for each of the experiences you have noted above.

100	Highest anxiety/distress that you have ever felt
90	Extremely anxious/distressed
80	Very anxious/distressed, unable to concentrate
70	Quite anxious/distressed, interfering with performance
60	
50	Moderate anxiety/distress, uncomfortable but can continue to perform
40	
30	Mild anxiety/distress, no interference on performance
20	Minimal anxiety/distress
10	Alert and awake, concentrating well
0	Totally relaxed

Do you have any of the following conditions?

Pregnancy Yes ☐ No ☐

Anaemia Yes ☐ No ☐

Diabetes Yes ☐ No ☐

Cardiovascular disease such as coronary heart disease Yes ☐ No ☐

Cancer (including leukaemia and lymphoma) Yes ☐ No ☐

Inflammatory bowel disease such as Crohn's disease Yes ☐ No ☐

Autoimmune diseases Yes ☐ No ☐

(e.g. autoimmune thyroiditis and lupus, rheumatoid arthritis, pernicious anaemia)

Asthma Yes ☐ No ☐

Immunodeficiency diseases, such as HIV and infectious mononucleosis Yes ☐ No ☐

Have you been healthy and free from acute infections for at least 2 weeks Yes ☐ No ☐

Do you currently smoke cigarettes? Yes ☐ No ☐

Do you regularly take any medication? Yes ☐ No ☐

Please provide a list: ☐

If you answered "Yes" to the above question, please list the medication you use (e.g. low dose aspirin, Aleve, Valium): ☐

Do you currently take a multi-vitamin? Yes ☐ No ☐

If yes, how many do you take per week? 2 or less ☐ 3-5 ☐ 6-9 ☐ 10 or more ☐

What brand do you take? ☐

Have you taken any of the following supplements in the past week?

Vitamin C	Yes ☐	No ☐
Vitamin E	Yes ☐	No ☐
Vitamin D	Yes ☐	No ☐
Omega-3 Fatty Acid	Yes ☐	No ☐

Do you take any other supplements? Yes ☐ No ☐

If you answered yes to the above question, please list the supplements you take (e.g. Vitamin A, Zinc, Fish Oil), and the dose per day (e.g. 400 mg, 1200 IU, "Don't know"): ☐

Drinking Scale Guide

How often do you have a drink containing alcohol?

- ☐ Never
- ☐ Monthly or less
- ☐ 2 to 4 times a month
- ☐ 2 to 3 times a week
- ☐ 4 or more times a week

How many drinks containing alcohol do you have on a typical day when you are drinking? ☐ 1 or 2

- ☐ 3 or 4
- ☐ 5 or 6
- ☐ 7, 8 or 9
- ☐ 10 or more

How often do you have six or more drinks on one occasion?

- ☐ Never
- ☐ Monthly or less
- ☐ 2 to 4 times a month
- ☐ 2 to 3 times a week
- ☐ 4 or more times a week

In the last 3 months, have you used any of the following substances?

Marijuana/Cannabis	Yes ☐	No ☐
Cocaine	Yes ☐	No ☐
Ecstasy	Yes ☐	No ☐
Amphetamines	Yes ☐	No ☐
Opioids	Yes ☐	No ☐
Hallucinogens	Yes ☐	No ☐

APPENDIX B

The Yoga for Stress Class Protocol

Session 1

Introduction (8 minutes)

- Names
- Introduction to class philosophy and safety

Meditation & Breathing (4 minutes)

- Introduction to the concept of mediation
    - Talk about how thoughts will flow
- Breath as the meditation
- Watching breath move in and out

Initial warming (5 minutes)

- Neck and shoulder stretches
- Wrist stretches
- Cat/Dog tilts
- Extended Cat/Dog
- Puppy Dog
- Balasana (Child's Pose)
    - Talk about how this is where one can safely return to through class

Move to standing

- Knee stretches
- Hamstring stretches
- Ankle stretches

Sun Salutation A (3 - 5 sets – 15 minutes)

- Introduce the arms for the 'Dogs' while in standing position (so students can learn how the shoulder girdle works)
- Make sure students bending knees and moving slowly in postures
- Demonstrate modifications for all postures

Sun Salutation B (3 sets – 15 minutes)

- Start with warrior legs and hands positioned on the floor – moving slowly into the full version

Cooling postures (3 minutes)

- Paschmottanasana (Seated forward bend)
- Dandasana (Staff Pose)
- Final spine twist

Savasana (5 minutes)

- Poem: *Our Greatest Fear* by Marianne Williamson

Session 2

Meditation & Breathing (6 minutes)

- Breath meditation
- Breathing into different body parts

Initial Warming (5 minutes)

- Neck and shoulder stretches
- Wrist stretches
- Cat/Dog tilts
- Extended Cat/Dog
- Puppy Dog
- Balasana (Child's Pose)

Move to standing

- Knee stretches
- Hamstring stretches
- Ankle stretches

Sun Salutation A (3 times – 7 minutes)

Sun Salutation B (3 times – 12 minutes)

Standing Postures (2 times – 12 minutes)

- Virbhadrasana I
- Virbhadrasana II
- Virbhadrasana III

Begin with demonstration of postures and explicate their meaning, then students perform with focus on alignment

Backbends (3 minutes)

- Salabashana A (3 times)
- Setu Bandha Sarvangasana (3 times)

Cooling postures (5 minutes)

- Paschmottanasana (Seated forward bend)
- Dandasana (Staff Pose)
- Jathara Parivartanasana with both knees bent (Revolving Twist)
- Viparita Karani (Legs up wall)

Savasana (5 minutes)

- Poem: *Desiderata* by Max Erhman

Session 3

Meditation & Breathing (6 minutes)

- Chakra meditation

Initial Warming (5 minutes)

- Neck and shoulder stretches
- Wrist stretches
- Cat/Dog tilts
- Extended Cat/Dog
- Puppy Dog
- Balasana (Child's Pose)

Move to standing

- Knee stretches
- Hamstring stretches
- Ankle stretches

Sun Salutation A (3 times – 7 minutes)

Sun Salutation B (3 times – 12 minutes)

Standing Postures (2 times – 12 minutes)

- Virbhadrasana III (Warrior III)
- Virabhadrasana II (Warrior II)
- Utthita Trikonasana (Extended Triangle)
- Vrkasana (Tree)
- Parsvottanasana (intense extended side angle pose – standing separate leg head to knee)

Floor Postures (10 minutes)

- Wind removing
- Bhujangasana (Cobra)
- Balasana (Child's Pose)
- Bhujangasana (Cobra)
- Bhujangasana (Cobra)
- Salabasana A (Locust)

Cooling postures (5 minutes)

- Half-Tortoise
- Baddha Konasana (Bound ankle pose)
- Dandasana (Staff Pose)
- Final spine twist

Savasana (5 minutes)

- Poem: *Joy and Sorrow* by Kalilh Gilbran (The Prophet)

Session 4

Meditation & Breathing (6 minutes)

- Counting meditation (1-10, count each inhale and exhale)

Sun Salutation A (5 times – 12 minutes)

Sun Salutation B (3 times – 12 minutes)

Standing Postures (2 times – 15 minutes)

- Virbhandrasana III
- Virbhandrasana II

Demo Triangle postures (particular focus on alignment of shoulders/chest)

- Utthita Trikonasana (Extended Triangle)
- Parivrtta Trikonasana (Revolved Triangle)
- Utthita Parsvokanasana (Extended Side Angle)
  (*repeat Triangle sequence)

Triangle sequence is from Ashtanga series

Cooling postures (5 minutes)

- Half-Tortoise
- Bridge
- Paschmottanasana (Seated forward bend)
- Dandasana (Staff Pose)
- Jathara Parivartanasana with both knees bent (Revolving Twist)

Savasana (5 minutes)

- Poem: *That Lives In Us* by Rumi

Session 5

Meditation & Breathing (6 minutes)

- Counting meditation (1-10, count each inhale and exhale)

Sun Salutation A (5 times – 12 minutes)

Sun Salutation B (3 times – 12 minutes)

Standing Postures (2 times – 12 minutes)

- Virbhadrasana III (Warrior III)
- Virabhadrasana II (Warrior II)
- Utthita Trikonasana (Extended Triangle)
- Vrkasana (Tree)
- Parsvottanasana (intense extended side angle pose – standing separate leg head to knee)

Floor Postures (12 minutes)

- Wind removing
- Bhujangasana (Cobra)
- Virasana (Hero Pose)
- Baddha Konasana (Bound Angle Pose)
- Dhanurasana
- Setu Bandha Sarvangasana (Bridge) x3
- Matsyasana (Fish Pose)
- Ardha Matsyendrasana (Half Lord of the Fishes Pose)

Savasana (5 minutes)

- Poem: *Teaching* by Kalilh Gilbran (The Prophet)

Session 6

Meditation & Breathing (6 minutes)

- Counting meditation (1-10, count each exhale)

Sun Salutation A (5 times – 12 minutes)

Sun Salutation B (3 times – 12 minutes)

Standing Postures (2 times – 12 minutes)

- Virabhadrasana II (Warrior II)
- Dancing Warrior
- Utthita Parsvakonasna (Extended Side Angle)
- Utthita Trikonasana (Extended Triangle)
- Parsvottanasana (intense extended side angle pose – standing separate leg head to knee)
- Vrkasana (Tree)

Floor Postures (12 minutes)

- Wind removing
- Salabasana (Locust)
- Bhujangasana (Cobra) x 3
- Dhanurasana (Bow Pose)
- Virasana (Hero Pose)
- Balasana (Child's Pose)
- Ustrasana (Camel Pose)
- Balasana (Child's Pose)
- Baddha Konasana (Bound Angle Pose)
- Setu Bandha Sarvangasana (Bridge) x3
- Jathara Parivartanasana with both knees bent (Revolving Twist)
- Dhanurasana

Savasana (5 minutes)

- Poem: *A Creed to Live By* by Nancy Sims

Session 7

Meditation & Breathing (6 minutes)

- Counting meditation (1-10, count each exhale – breathing down to lower abdomen)

Sun Salutation A (5 times – 12 minutes)

Sun Salutation B (3 times – 12 minutes)

Standing Postures (5-8 breaths – 12 minutes)

Go over Pada bandha (grounding through the feet)

- Virabhadrasana II (Warrior II)
- Utthita Trikonasana (Extended Triangle)
- Parsvottanasana (intense extended side angle pose – standing separate leg head to knee)
- Vrksasana (Tree)
- Guillotine warm-up (squat)

Floor Postures (12 minutes)

- Balasana (Child's pose)
- Pavanamuktasana (Wind removing)
- Bhujangasana (Cobra)
- Eka Pada Rajakapotasana (Pigeon)
- Setu Bandha Sarvangasana (Bridge) x3
- Dandasana (Staff Pose)
- Jathara Parivartanasana with both knees bent (Revolving Twist)

Savasana (5 minutes)

- Poem: *The Invitation* by Oriah Mountain Dreamer

Session 8

Meditation & Breathing (6 minutes)

- Chakra Body Scan

Sun Salutation A (3 times – 12 minutes)

Sun Salutation B (3 times – 12 minutes)

Standing Postures (5-8 breaths – 7 minutes)

Go over Mula bandha

- Virabhadrasana II (Warrior II)
- Utthita Trikonasana (Extended Triangle)
- Dancing Warrior Posture (with right leg bent stretch right arm overhead towards back, looking up to the right hand, slide left arm down left leg to help arch the spine)
- Utthita Parsvakonasna (Extended Side Angle)

Floor Postures (18 minutes)

- Butterfly
- Sleeping Swan
- Seal or Sphinx
- Cat's Breath (cat/cow)
- Child's Pose
- Reclining spine twist

All poses to be held for 3-4 minutes

Savasana (5 minutes)

- Poem: *Pleasure* by Kalilh Gilbran (The Prophet)

Session 9

Meditation & Breathing (6 minutes)

- Choice

Sun Salutation A (5 times – 12 minutes)

Sun Salutation B (3 times – 12 minutes)

Standing Postures (5-8 breaths – 12 minutes)

Go over Uddiyana bandha

- Tadasana (Mountain Pose)
- Vrksanaa (Tree Pose)
- Garudasana (Eagle Pose)
- Virabhadrasana II (Warrior II)
- Utthita Trikonasana (Extended Triangle)
- Parsarita Padottanasana (Wide Leg Forward Bend)
- Parsvottanasana (Intense Extended Side-Angle Pose; Standing separate head to knee)

Floor Postures (12 minutes)

- Pavanamuktasana (Wind removing)
- Paripurna Navasana Prep (Boat Posture) x3
- Yogic bicycles (1 minute)
- Salabhasana A (Locust) x3
- Set Bandha Sarvangasana (Bridge) x3
- Dandasana (Staff Pose)
- Jathara Parivartanasana with both knees bent (Revolving Twist)

Savasana (5 minutes)

- Poem: *Self Knowledge* by Kalilh Gilbran (The Prophet)

Session 10

Meditation & Breathing (6 minutes)

- Heart Centred Breathing

Sun Salutation A (5 times – 12 minutes)

Sun Salutation B (5 times – 12 minutes)

Hold Virbhadrasana I for 10 breaths on last round

Standing Postures (5-8 breaths – 12 minutes)

- Virabhadrasana II (Warrior II)
- Utthita Parsvakonasna (Extended Side Angle)
- Utthita Trikonasana (Extended Triangle)
- Parsarita Padottanasana (Wide Leg Forward Bend)
- Garudasana (Eagle Pose)
- Vrksanaa (Tree Pose)
-

Floor Postures (12 minutes)

- Pavanamuktasana (Wind removing)
- Dhanurasana (Bow Posture)
- Virasana / Supta Virasana (Hero's Posture/ reclining)
- Ardha Kurmasana (Half Tortoise Pose)
- Ardha Matsyendrasana (Half Lord of the Fishes Pose, Spine Twist)
- Baddha Konasana (Bound Angle Pose)
- Jathara Parivartanasana with both knees bent (Revolving Twist)

Savasana (5 minutes)

- Poem: *Love* by Kalilh Gilbran (The Prophet)

Session 11

Meditation & Breathing (6 minutes)

- Chanting Meditation

Sun Salutation A (5 times – 12 minutes)

Sun Salutation B (3 times – 12 minutes)

Standing Postures (5-8 breaths – 12 minutes)

Go over Jalandhara bandha

- Tadasana (Mountain Pose)
- Virabhadrasana II (Warrior II)
- Utthita Parsvakonasna (Extended Side Angle)
- Utthita Trikonasana (Extended Triangle)
- Parsarita Padottanasana (Wide Leg Forward Bend)
- Parsvottanasana (intense extended side angle pose – standing separate leg head to knee)
- Vrksasana (Tree)

Floor Postures (12 minutes)

- Pavanamuktasana (Wind removing)
- Setu Bandha Sarvangasana (Bridge Posture) x3
- Virasana / Supta Virasana (Hero's Posture/ reclining)
- Ustrasana (Camel Posture)
- Pursvottanasana (Upward Facing Plank)
- Balasana (Child's Posture)
- Jathara Parivartanasana with both knees bent (Revolving Twist)

Savasana (5 minutes)

- Poem: *Talking* by Kalilh Gilbran (The Prophet)

Session 12

Meditation & Breathing (6 minutes)

- Counting meditation (1-10, count each exhale)

Sun Salutation A (5 times – 12 minutes)

Sun Salutation B (3 times – 12 minutes)

Standing Postures (5-8 breaths – 12 minutes)

Go over Jalandhara bandha

- Tadasana (Mountain Pose)
- Garjunasana (Eagle Pose)
- Virabhadrasana II (Warrior II)
- Utthita Parsvakonasna (Extended Side Angle)
- Utthita Trikonasana (Extended Triangle)
- Parsarita Padottanasana (Wide Leg Forward Bend)
- Parsvottanasana (intense extended side angle pose – standing separate leg head to knee)
- Vrksasana (Tree)

Floor Postures (12 minutes)

- Balasana (Child's pose)
- Shoelase
- Eka Pada Rajakapotasana (Pigeon)
- Bhujangasana (Cobra)
- Setu Bandha Sarvangasana (Bridge) x3
- Janu Sirasana (Head on Knee Pose)
- Dandasana (Staff Pose)
- Jathara Parivartanasana with both knees bent (Revolving Twist)

Savasana (5 minutes)

Poem: *The Call* by Oriah Mountain Dreamer

Session 13

Meditation & Breathing (6 minutes)

- Silence – expand awareness of senses

Sun Salutation A (5 times – 12 minutes)

Sun Salutation B (5 times – 12 minutes)

Hold Virbhadrasana I for 10 breaths on last round

Standing Postures (5-8 breaths – 12 minutes)

- Virabhadrasana II (Warrior II)
- Utthita Parsvakonasna (Extended Side Angle)
- Utthita Trikonasana (Extended Triangle)
- Parsarita Padottanasana (Wide Leg Forward Bend)
- Garudasana (Eagle Pose)
- Vrksanaa (Tree Pose)

Floor Postures (12 minutes)

- Balasana (Child's pose)
- Pavanamuktasana (Wind removing)
- Shoelace
- Eka Pada Rajakapotasana (Pigeon)
- Boat x3 (then lie down straight)
- Setu Bandha Sarvangasana (Bridge) x3
- Final spine twist

Savasana (5 minutes)

Poem: *Silence* by Hafiz

Session 14

Inspired by Mindsight (Dr. Dan Siegel)

Meditation & Breathing (6 minutes)

- Focus on what you are feeling, practice accepting it, to then let it go and transform the experience ("I feel [sad]" vs "I am [sad]")

Sun Salutation A (5 times – 12 minutes)

Sun Salutation B (3 times – 12 minutes)

Standing Postures (2 times – 12 minutes)

- Virbhadrasana III (Warrior III)
- Virabhadrasana II (Warrior II)
- Utthita Trikonasana (Extended Triangle)
- Vrkasana (Tree)
- Parsvottanasana (intense extended side angle pose – standing separate leg head to knee)

Floor Postures (12 minutes)

- Wind removing
- Bhujangasana (Cobra)
- Virasana (Hero Pose)
- Baddha Konasana (Bound Angle Pose)
- Boat x3
- Dhanurasana
- Setu Bandha Sarvangasana (Bridge) x3
- Ardha Matsyendrasana (Half Lord of the Fishes Pose)

Savasana (5 minutes)

- Poem: *The Guest House* by Rumi

Session 15

Meditation & Breathing (6 minutes)

- Chakra meditation

Sun Salutation A (5 times – 12 minutes)

Sun Salutation B (3 times – 12 minutes)

Standing Postures (2 times – 15 minutes)

- Virbhandrasana III
- Virbhandrasana II
- Garudasana (Eagle)
- Utthita Trikonasana (Extended Triangle)
- Parivrtta Trikonasana (Revolved Triangle)
- Utthita Parsvokanasana (Extended Side Angle)

Cooling postures (5 minutes)

- Salabasana A (Locust)
- Half-Tortoise
- Shoelace
- Eka Pada Rajakapotasana (Pigeon)
- Dandasana (Staff Pose)
- Jathara Parivartanasana with both knees bent (Revolving Twist)

Savasana (5 minutes)

- Poem: *Mystery* by Nirmala (Gifts with No Giver, a love affair with truth)

Session 16

Meditation & Breathing (6 minutes)

- Focus on something you are grateful for

Sun Salutation A (5 times – 12 minutes)

Sun Salutation B (3 times – 12 minutes)

Standing Postures (5-8 breaths – 12 minutes)

- Virabhadrasana II (Warrior II)
- Virbadhrasana III (Warrior III)
- Utthita Parsvakonasna (Extended Side Angle)
- Utthita Trikonasana (Extended Triangle)
- Parsarita Padottanasana (Wide Leg Forward Bend)
- Garudasana (Eagle Pose)
- Vrksanaa (Tree Pose)

Floor Postures (12 minutes)

- Pavanamuktasana (Wind removing)
- Bhujangasana (Cobra)
- Virasana (Hero Pose)
- Shoelace
- Boat x3 (then lie down straight)
- Setu Bandha Sarvangasana (Bridge) x3
- Dandasana (Staff Pose)
- Jathara Parivartanasana with both knees bent (Revolving Twist)

Savasana (5 minutes)

- Poem: *Be Thankful* by Author Unknown

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