THE NEUROLOGICAL IMPACT OF SPECIFIC NUTRIENTS ON FEMALE STRESS: A TWO-PHASE, SEQUENTIAL, MIXED METHODS STUDY

THIS THESIS IS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

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8 OCTOBER 2019

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McCabe D, Lisy K, Lockwood C, Colbeck M. The impact of essential fatty acid, B vitamins, vitamin C, magnesium and zinc supplementation on stress levels in women: a systematic review. Joanna Briggs Institute: Database of Systematic Reviews and Implementation Reports. 2017 March 1;15(2):402-53. Available from: DOI: 10.11124/JBISRIR-2016-002965.

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AA	Ascorbic acid (vitamin C)
ACT	Adrenocorticotrophin
ACTH	Adrenal corticotrophic hormone
AD	Antidepressant
ALA	Alpha linolenic acid
ANS	Autonomic nervous system
ACOG	The American College of Obstetricians and Gynaecologists
APA PMS	American Psychiatric Association Premenstrual Symptoms questionnaire
BDNF	Brain derived neurotrophic factor
CAM	Complimentary alternative medicine
CNS	Central Nervous System
CRH	Corticotrophin releasing hormone
DASS	Depression, Anxiety and Stress Scales
DBP	Diastolic blood pressure
DHA	Docosahexaenoic acid
DLCS	Difficult Life Circumstances Scale
DP	Dietary pattern
DPA	Docosapentaenoic acid
DQES	Dietary Questionnaire for Epidemiological Studies
DS	Dietary supplement
EFA	Essential fatty acid
EBM	Evidence based medicine
EBHC	Evidence based health care
ELS	Early life stress
EPA	Eicosapentaenoic acid
ETA	Eicosatetranoic acid
fMRI	Functional magnetic resonance imaging
FO	Fish oil
GABA	Gamma amino butyric acid
GAD-7	Generalised anxiety disorder scale
GC	Glucocorticoid
HADS-A	The Hospital Anxiety and Depression Scale - Anxiety

HPA	Hypothalamus pituitary adrenal
MD	Menstrual diary
MDE	Major depressive episode
MDQ	Moos Menstrual Distress Questionnaire
MgO	Magnesium oxide
MHQ	Menstrual Health Questionnaire
MTHFR	Methylenetetrahydrofolate reductase
MVM	Multi-vitamin and mineral
NE	Norepinephrine
PAR	Participatory Action Research
PCP	Primary care physician
PFC	Pre-frontal cortex
PGWBI	The psychological general well being index
PMS	Premenstrual syndrome
PNS	Parasympathetic Nervous System
POMS	Profile of Mood States
PSS	Perceived Stress Scale
ROS	Reactive oxygen species
SAM	Sympathetic-adrenal-medullary
SBP	Systolic blood pressure
SNS	Sympathetic Nervous System
SOS	Symptoms of Stress Inventory
STAI	State-Trait Anxiety Inventory
TSST	Trier Social Stress tests
VAS	Visual analogue scales

Abstract

A growing body of literature suggests that the intake of specific nutrients impact mental health, and recent findings support a causative relationship between dietary patterns and affective disorders, whilst also providing evidence of a robust relationship between affective disorders and chronic stress. There is however a paucity of evidence regarding the relationship between chronic stress and nutrition.

This thesis reports on research aimed to provide insight into the relationship between chronic stress and the intake of specific dietary nutrients among women. A systematic review examined the current evidence regarding nutrient intake and stress levels. A cross-sectional survey followed, in which a sample of women provided information regarding their perceived stress levels and nutrient intake via diet and dietary supplements. A participatory action research project concluded the study, wherein the lived experience of stress and the role of diet and/or dietary supplementation to manage stress was investigated.

The systematic review revealed that there was insufficient evidence to support the intake of specific dietary nutrients either via diet or supplementation to manage stress level. After adjustment, data from the cross-sectional survey revealed a further lack of evidence to support a robust relationship between stress and specific nutrients consumed via the diet or supplementation. The lived experience of stress and the use of specific nutrients to manage stress among a group of women in the PAR project was influenced by relationships and confusion and scepticism regarding the usefulness of nutrients to impact mental wellbeing, while early life stress and thinking patterns were further factors influencing the experience of stress.

Evidence regarding the neurobiological efficacy of nutrient intervention is required to support decision-making by clinicians and policy makers in light of the well-established role that chronic stress plays in the development of affective disorders among women globally. This study provides a first step towards the development of a knowledge base regarding the role that specific nutrients play in the stress response among women, although further research is warranted due to the far reaching neurobiological effects of chronic stress.

Acknowledgements

I would like to sincerely thank my supervisors, Associate Professor Craig Lockwood and Dr Tim Barker for their unwavering support during my candidature.

Craig, thank you for your ongoing support and guidance and your faith in my ability to complete from day one. Despite the 'life crises' and supervisory 'hiccoughs' that arose during the past few years you remained supportive and patient with my sometimes painfully slow progress. Your maxim, 'brevity is the hallmark of academic excellence,' and your approach to eating an elephant – one bite at a time – are both lessons that will guide me going forwards.

Tim, thank you for stepping in to become my supervisor at such a late stage in this project. Your practical guidance, support and insightful comments have been immensely appreciated.

Thank you Jana Bednarz, for also stepping in to support the statistical analysis and write-up at a late stage in this project. Your support and insight have also been immensely appreciated.

Thank you to an early supervisor, Karolina Lisy, who patiently supported me in writing and publishing my first paper and developing the SUQ questionnaire and to Catalin Tufanaru who encouraged wide reading on a variety of research and data analysis topics.

To my husband, who has been extremely supportive during this period and who has patiently listened to me thinking aloud about my research and encouraged me through the many challenges encountered during a project of this scale, often with wise advice. Words cannot express my appreciation for your belief in me.

To my children, thank you for your understanding and support. Being students, you know what it feels like to need to get something done before eating dinner. And in the early days, helping me with technical issues was appreciated immensely – now we can discuss Endnote!

To my mother, who has always believed in me and inspired me by tackling and overcoming seemingly impossible obstacles.

To friends and other family members, many far away – thank you for your support.

To all the participants of these research projects – a very big thank you for your interest in this research and for sharing your precious time with me. And for those who attended the PAR focus group evenings, your willingness to share your lived experience is appreciated more than I can adequately express. Every participant contributed to this project and my hope is that this research topic will gain the attention it needs so your contribution can be rewarded in a meaningful way.

"In the absence of science opinion prevails." Gorman JM, Nathan PE. A Guide to Treatments That Work, 4th ed. Oxford University Press; 2015, p. ix.

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I acknowledge the support I have received for my research through the provision of an Australian Government Research Training Program Scholarship.

Delia McCabe Date 8th October 2019

Statement of Authorship

Title of Paper	**The impact of essential fatty acid, B vitamins, vitamin C, magnesium and zinc supplementation on stress levels in women: a systematic review**
Publication Status	[X] Published [ ] Accepted for Publication [ ] Submitted for Publication [ ] Unpublished and Unsubmitted w ork w ritten in manuscript style
Publication Details	**Joanna Briggs Institute: Database of Systematic Reviews and Implementation Reports. 2017 March 1;15(2):402-53. Available from: DOI: 10.11124/JBISRIR-2016-002965.**

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Statement of Authorship

Title of Paper	**The impact of essential fatty acid, B vitamins, vitamin C, magnesium and zinc supplementation on stress levels in women: a systematic review**
Publication Status	[X] Published [ ] Accepted for Publication [ ] Submitted for Publication [ ] Unpublished and Unsubmitted w ork w ritten in manuscript style
Publication Details	**Joanna Briggs Institute: Database of Systematic Reviews and Implementation Reports. 2017 March 1;15(2):402-53. Available from: DOI: 10.11124/JBISRIR-2016-002965.**

Principal Author

Name of Principal Author (Candidate)	**Delia McCabe**		
Contribution to the Paper	**This author conceptualised the research question and accessed, selected, appraised and extracted research according to a published protocol; analysed raw data and wrote the narrative synthesis, including limitations, discussion, conclusion and implications for practice and future research.**		
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Name of Co-Author	**Associate Professor Craig Lockwood**		
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Statement of Authorship

Title of Paper	**The impact of essential fatty acid, B vitamins, vitamin C, magnesium and zinc supplementation on stress levels in women: a systematic review**
Publication Status	[X] Published [ ] Accepted for Publication [ ] Submitted for Publication [ ] Unpublished and Unsubmitted work written in manuscript style
Publication Details	**Joanna Briggs Institute: Database of Systematic Reviews and Implementation Reports. 2017 March 1;15(2):402-53. Available from: DOI: 10.11124/JBISRIR-2016-002965.**

Principal Author

Name of Principal Author (Candidate)	**Delia McCabe**		
Contribution to the Paper	**This author accessed, selected, appraised and extracted research according to a published protocol; analysed raw data and wrote the narrative synthesis, including limitations, discussion, conclusion and implications for practice and future research.**		
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Chapter 1

INTRODUCTION

1.1 Introduction

In modern society chronic psychological stress is a frequently experienced mental state that may contribute to the development of affective disorders such as anxiety and depression and associated depletion of a variety of nutrients critical for central nervous system (CNS) function. This in turn imposes increased metabolic demands on the brain.[1-3] Women may be particularly susceptible to chronic stress due to a variety of factors, and the use of dietary supplements (DSs) is commonly used to mitigate symptoms.[4-6] However, there is a paucity of evidence to support the intake of specific nutrients via diet or the use of DSs to manage chronic stress, although the global expenditure on stress-targeted DSs suggests the use of such products is prolific.[7-9]

The objective of this study was therefore to investigate the relationship between perceived stress and specific nutrients consumed in the daily diet and/or as DSs in a group of women. The first step in answering this research question was to establish the scope, quality and direction of evidence related to this relationship through a critical synthesis of research literature based on the Joanna Briggs Institute systematic review methodology. This systematic review (Chapter 2) investigated the impact of essential fatty acid (EFA), B vitamins, vitamin C, magnesium and zinc supplementation on stress levels in women.[10] As this review revealed a lack of evidence for the use of specific nutrients to manage perceived stress among women, a cross sectional study was performed which examined stress levels in relation to specific nutrient intake, consumed in the daily diet and/or via DSs (Chapter 3). The lived experience of stress among women, and the use of diet and/or DSs to manage stress, are similarly unexamined areas of research, so participatory action research (PAR) was used to investigate these concepts (Chapter 4).

This chapter contextualises the thesis by introducing the stress response, describing possible psychosocial, physiological and neurobiological mechanisms related to its proliferation including early life stress (ELS) and mechanisms whereby chronic stress may lead to nutrient deficiencies and stress management strategies including the use of DSs. Evidence-based medicine (EBM), including why this thesis is positioned within the JBI model of evidence-based healthcare (EBHC), is addressed.

1.2 Frequency and severity of stress and gender

The definition of stress, as originally coined by Hans Selye in 1936, has evolved past the understanding of being a natural physiological response to a life-threatening event.[11, 12] This theory was extensively revised in the 1970's and the definition used today has incorporated the recognition of, and challenges related to adapting to new stimuli.[13] Stress is understood informally as a specific psychological state of mind wherein individuals find their lives unpredictable and uncontrollable, leading to a sense of overload or overwhelm in relation to responsibilities, accompanied by a perception that they do not have the resources to cope with the situations they encounter.[14-16]

The extent to which this phenomena is experienced in the general population has been investigated and a number of studies have found that chronic psychological stress negatively impacts the lives of a significant number of women globally, with women consistently reporting higher levels of stress versus men.[17-25] For example, a 2014 national survey (n=1,602) found that Australian women reported significantly higher levels of stress (16.73) compared to men (14.97) as measured by the Perceived Stress Scale (PSS).[14, 17] Similarly, a survey conducted over 2014-2015 on Australian dwellings (n=14,700) revealed a higher percentage of women (13.5%) experienced high levels of psychological distress compared to men (9.9%).[26] Similar gender disparities and trends have been observed in surveys conducted in North America,[20, 21, 27] Canada,[22] the United Kingdom,[23-25] and Spain.[28] This evidence has suggested that globally women experience higher levels of stress and react more negatively to life events when compared to men. The stress response provides a physiological and neurobiological context for understanding the significant impact that chronic levels of stress can have on the health and mental wellbeing of women.

1.3 The stress response

The stress response includes the activation of the sympathetic nervous system (SNS), initiation of activity within the hypothalamus, pituitary and adrenal (HPA) axis and sympathetic-adrenal-medullary (SAM) systems, and de-activation of the parasympathetic nervous system (PNS), which is followed by a return to homeostasis when the threat passes.[12, 13, 29] The HPA axis is the primary stress control and regulatory system, connecting the CNS and the hormonal system.[30] This stress-responsive neuroendocrine system functions so as to allow adjustment to both physical and psychosocial changes perceived as being stressful in their environment.[30] This system works well unless stress becomes chronic, which impacts brain regions responsible for regulating emotion, such as the limbic system.[31-33]

The limbic system is a group of neurally interconnected structures allocated to linking 'visceral states and emotion to cognition and behaviour' (p. 172).[33] The amygdala forms part of this system and

is responsible for detecting danger and fear and preparing for the emergency response.[34, 35] In addition, the hippocampus is accessed to ascertain whether there is a memory for this perceived threat, which influences response options.[36] Fear and stress perception via these brain regions activate the release of corticotrophin releasing hormone (CRH) from the hypothalamus, adrenal corticotrophic hormone (ACTH) from the anterior pituitary, stimulating the release of adrenocorticotrophin (ACT) into the bloodstream and cortisol, a glucocorticoid (GC), from the adrenal cortex.[37] These rising cortisol levels inhibit the release of CRH and ACTH to stop further HPA activation in a negative feedback loop.[37, 38] The sympathetic-adrenal-medullary (SAM) system also plays a role in the stress response, by releasing catecholamines from the adrenal medulla, such as adrenalin (epinephrine), noradrenaline (norepinephrine (NE)) and dopamine.[31] When the SAM system is activated, these stress hormones interact with the autonomic nervous system (ANS) to regulate effects on the cardiovascular, pulmonary, hepatic, skeletal muscle and immune systems in the presence of stress.[31] A review of findings that examined the stress response, concluded that ongoing HPA activation and cortisol synthesis leads to the body becoming less responsive to its effects over time, whilst continued exposure to chronic stress results in HPA axis sensitisation to novel stressors.[30,39] The continued activation of the HPA and developed sensitisation may combine to prevent a return to homeostasis, which may in turn impact the limbic systems ability to regulate emotion. Figure 1.1 summarises the stress response.

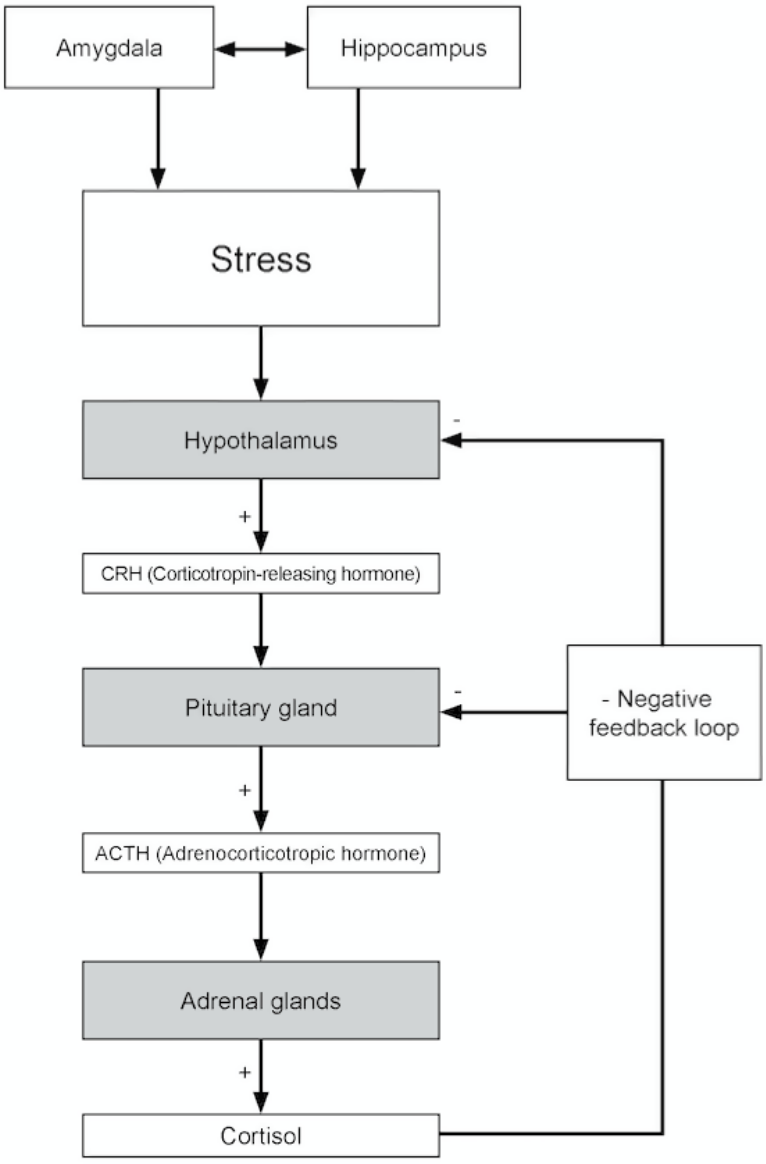


Figure 1.1: The stress response and the hypothalamus, pituitary and adrenal (HPA) axis feedback loops. Positive and negative feedback loops initiate and stop the stress response, respectively. The dysregulation of this system, during chronic stress when the negative feedback loop is unable to activate the parasympathetic nervous system (PNS), leads to a number of negative consequences.

The physiological and behavioural consequences of being psychologically stressed are similar to those following a fight for physical survival, with the aim of quickly returning to homeostasis.[1, 29, 40] However, psychologically stressful situations or psychosocial stressors are more prevalent in western society, last for longer, and involve higher brain processing.[1, 30, 40] Such stressors also require interpretation, in terms of meaning and context for the individual, and elicit processing activity in the hippocampus and the pre-frontal cortex (PFC), which engage memory, pattern-seeking and anticipatory thinking and behaviour.[30, 36, 41, 42] These stress-induced cognitive processes may lead to anticipatory, environment-specific habitual stress responses via maladaptive neuroplasticity, although, as it is ethically questionable to induce chronic stress in humans, there is insufficient human data to support

these findings. For example, Aschbacher et al. reported on a case-control study (n=48) that examined the effects of chronic stress exposure by matching post-menopausal women caring for spouses with dementia (a chronic stress model) and women with healthy spouses.[43] The Trier Social Stress test (TSST) was used to evoke stress responses, salivary cortisol and oxidative stress assays were administered and validated measurement instruments assessed perceived stress. The caregivers reported significantly greater levels of perceived stress and anticipatory cortisol reactivity ($p<0.01$) suggesting that the anticipation of a stressor may lead to anticipatory cortisol reactivity in a CNS that is poised for a heightened stress response.[43] Thus, chronic stress may predispose women to an 'anticipatory' state of stress making a return to homeostasis via PNS activation problematic.

Return to HPA homeostasis may also be inhibited through other cognitive strategies. Strauss et al. reported that women ruminate more than men.[44] Rumination being defined as the engagement of 'negative and unwanted past-centred thoughts,' by Gianferante et al. who reported that women exposed to the TSST engaged in more rumination than men, which was predictive of heightened cortisol responses to repeated stressors and was associated with a 'reduced ability to adapt to repeated stress that may interfere with healthy post-stress emotional and cognitive processing of the situation' (p. 245).[44, 45] These results have suggested that high levels of stress hormones may impact the brains ability to respond to stress adaptively via accurate emotion perception, processing and regulation, and may have a bi-directional relationship with rumination, possibly leading to an inability to return to homeostasis. Although the stress response provides insight into how chronic stress may lead to HPA dysregulation, it is necessary to examine the impact of chronic stress on women, as they have higher reported rates of affective disorders compared to men, and chronic stress plays a role in the development of these disorders.

1.4 Impact of Chronic Stress

Although the experience of stress is not a mental health disorder, when experienced chronically it may lead to the development of stress-associated affective disorders, such as depression and anxiety.[46-51] A secondary analysis of the American's Changing Lives (ACL) dataset (n=2,824) revealed that women were approximately three times more likely to experience depression in response to a stressful life event compared to men.[52] Similarly, a prospective female twin-study (n=1,898) found that stressful life-events over a one-year period had a marked causal relationship with depression onset.[53] In addition, a recent Swedish study (n=587) which examined the prevalence of perceived stress in patients from a working-age population who visited primary care physicians (PCPs), demonstrated that women reported higher stress levels compared to men, and that such stress was accompanied by symptoms indicative of

anxiety (64% of patients) and depression (33% of the patients).[54] Rojo-Moreno et al. reported similar results (n=100) from a study that examined depressed patients in relation to healthy controls and found that the risk of developing depression was 9.7 times greater in subjects who had experienced severe, stress-inducing events.[55] These findings support the view that chronic stress is related to the development of affective disorders in women.

Additionally, high rates of comorbid anxiety and depressive disorders have been reported in some studies, including population level studies.[56-58] Lamers, et al. reported that 75% of those with depression met criteria for an anxiety disorder and 79% of those with an anxiety disorder met criteria for depression.[58] These findings are consistent with those from Kessler et al. who reported that 45.7% of those who experienced depression in their lifetime also had one or more lifetime anxiety disorder events.[59] Surveys among community dwelling adults (n=72,933) investigating affective disorders in 15 countries in the World Health Organization Mental Health Survey Initiative (WHOMHSI), revealed that across all cohorts and countries women experienced higher rates of these disorders.[60, 61] These results have suggested that depression and anxiety are often experienced together and that stress may be the underlying causative mechanism linking them.

Affective disorders have also been associated with reported antecedent psychosocial factors such as symptomatic distress, diminished social support and more past-year distressing life events as revealed in a community sample of mid-life women (n=915).[62] These women also reported more childhood abuse or neglect, (classified as an ELS), compared to women with a history of either affective disorder alone.[62] Therefore, women may be at higher risk of developing stress-related affective disorders, which may partly explain the increased prevalence of such among women. However, there are further consequences to experiencing chronic stress.

An additional consequence of chronic stress includes Allostatic load. Defined as the 'wear and tear imposed upon the body' by physiological processes, Allostatic load has been previously associated with chronic stress exposure (p. 30).[40] The link between chronic stress and physiological disease states is well established and the explanation for such includes an increased vulnerability to inflammatory processes due to decreased tissue sensitivity to cortisol, an inflammatory-response regulator.[63] In support of Selye's early findings, a large meta-analysis of 293 studies examined 30 years of study (n=18,941), which concluded that there is an important link between chronic stress and inflammatory response dysregulation.[11, 12, 64] However, this physiological inflammatory response also impacts neurobiological functioning.

Heightened inflammatory responses have been linked to depression in both clinic- and community based samples as evidenced by three systematic reviews, which found inflammatory markers were positively associated with such.[65-67] In addition, Köhler-Forsberg et al. reported that

among 36 RCT's, that included approximately 10,000 patients, five out of six anti-inflammatory drugs improved depression scores compared to placebo.[68] Heightened inflammatory activity may signal neuronal activity changes in brain regions responsible for mood regulation, such as the limbic system.[69, 70] Therefore, high rates of comorbid depression and anxiety among women suggests that anxiety and inflammation may also be related.

Inflammation has also been associated with anxiety as revealed in a study that examined post-traumatic stress disorder (PTSD), in 12 women with childhood abuse related PTSD and 24 healthy controls.[71] Results revealed that PTSD sufferers demonstrated higher concentrations of inflammatory activity in blood samples versus controls.[71] Similarly, inflammatory markers in the blood of 48 individuals diagnosed with PTSD or panic disorder (PD) was compared to 48 healthy controls.[72] Eighty-seven percent of those with either anxiety disorder had six or more inflammatory markers compared to 25% of the controls ($p<0.0025$). In addition, the impact of chronic stress on inflammation may partly explain the mechanisms linking stress, anxiety and depression, as suggested in a review that examined mechanisms underlying these three phenomena, one of which includes poor dietary choices.[73] Figure 1.2 summarizes the relationship between the factors predisposing women to chronic stress and possible negative outcomes, including depression, anxiety and inflammatory responses.

Results from a year-long study in which subjects (n=516) were randomly assigned to three intervention dietary groups, one of which comprised a Mediterranean diet, suggests that diet and inflammation are related, as lowered inflammatory markers on the Mediterranean diet were noted.[74] It is suggested that multiple factors, including nutrient intake, may link chronic stress, inflammation, depression and anxiety.[75] For women, managing chronic stress via diet may contribute to preventing the onset of affective disorders and accompanying inflammation. However, it is presently unknown whether it is possible to ameliorate the effects of chronic stress via the consumption of specific nutrients.

1.5 Factors related to the proliferation of chronic female stress:

A number of different mechanisms may contribute to women's increased experience of stress globally in comparison to men, including psychosocial, neurobiological and hormonal mechanisms and ELS. Figure 1.2 lists these mechanisms.

1.5.1 Psychosocial factors

Psychosocial factors, such as ongoing time constraints imposed by increasingly complex and competing societal roles coupled with unchanging traditional roles and culture-specific stressors may contribute to the discrepancy between men and women in relation to reported levels of stress.[61, 76-81] In relation to health generally, Leineweber et al. reported on a Swedish study (n=6,580), which found that women

who experienced work-family conflict were at greater risk for self-reporting diminished health.[82] Diminished health may thus be related to stress, as results from another Swedish study (n=60) revealed that women's levels of stress remain high after work, as evidenced by cortisol levels remaining high, compared to men's levels which decreased.[79] This result is possibly due to traditional domestic and childcare responsibilities following daily paid work outside the home for women, which has been called their 'second shift' (p. 4).[83] Indeed, household labour studies a decade apart reveal that women still perform more housework and childcare activities compared to men.[84-86] It is estimated that women on average do 1.6 times the amount of housework as men and married mothers average 1.9 times the housework of married fathers.[85]

Other comparable data further highlights gender differences. Regarding paid work, household duties and childcare, a Swedish population survey (n=1,180) found that the presence of children impacted the workload for women, but not men.[81] The total workloads of men and women with no children were approximately 60 hours per week, whereas in families with three or more children, women's workload increased to almost 90 hours per week compared to men's 70 hour per week.[81] This amounts to a mean gender difference of 2.5 hours per day.[81] Therefore, married, working mothers may be more vulnerable to experiencing chronic stress compared to married, working women without children. In support of this hypothesis, Luecken et al. reported that working mothers had higher cortisol levels than childless women and women with at least one child at home excreted significantly more cortisol over a 24-hour period ($p<0.01$).[87] In addition, levels of strain at home were also significantly higher among mothers with live-in children ($p<.001$).[87] Similarly, the risk of developing affective disorders was higher among working mothers, as revealed by a Netherlands study (n=3,857).[88]

However, non-traditional family structures may also impact mother's psychological health. For example, in a German cross-sectional study that analysed questionnaires about psychological problems from similar groups of mothers, Franz et al.[89] reported that single mothers (n=531), when compared to a control group of married mothers (n=278), experienced higher rates of psychological distress. Similarly, Canadian single mothers (n=236) reported experiencing higher rates of psychological distress compared to partnered mothers (n=438), while a survey in the same country over 1996-97 found depressive symptoms were higher among single (n=3,030) versus married mothers (n=10,195).[90, 91] In support of this data, two American surveys reported that the prevalence of anxiety disorders was higher in single compared to married mothers (n=1,534 and n=2,956).[92, 93] In further support of these results, mood disorders were significantly more prevalent among single mothers ($p<0.01$) in a Singaporean survey.[94] A systematic review of 11 studies from six countries, which used secondary data from household surveys in which sample sizes ranged from 375 – 53,292 participants, also revealed that single mothers experienced lower levels of health compared to partnered mothers.[95] Single mothers

may therefore be more vulnerable to chronic stress, stress-related affective disorders and diminished health than married mothers.

Moreover, because of the 'unequal status of women in most societies across the world,' cultural stressors such as sex-specific socialisation and victimization, subordinate social rank and violence may also contribute to increased stress vulnerability among women (p. 40).[96] Therefore, female-specific psychosocial factors such as competing work and family roles, ongoing and unequal distribution of domestic tasks, single motherhood and women's social position may all contribute in varying ways to women experiencing more stress than men.

1.5.2 Neurobiological factors

A number of neurobiological factors may contribute to women experiencing increased levels of stress compared to men. A growing body of evidence supported by advances in the development of imaging technologies reveals gender differences in relation to brain function, neurochemistry and hormonal influences.[97-101] A review that examined neurological sex differences using magnetic resonance imaging (MRI), functional magnetic resonance imaging (fMRI), positron emission tomography (PET), and single photon emission computed tomography (SPECT), found important differences that distinguish male and female brains.[97, 98] These differences may combine and underlie specific mechanisms that contribute to increased levels of stress among women in the presence of psychosocial stressors.

A review of neurological sex differences concluded that a lower cortisol response to psychological stress in women compared to men may be a reflection of HPA hyporeactivity, wherein there is a reduced reaction to the stressor.[30] Viewed in this light, the consistently higher rates of female stress reported globally may be as a result of less-adaptive HPA activity in the presence of stress. This suggestion is supported by an in vivo study using fMRI to investigate cognitive down-regulation of stressful, negative sensations in both genders (n= 20men and n=23 women), which found that women reported higher subjective stress ratings versus men and higher limbic system activity was observed in women compared to men.[102] These results are indicative of the women in the study engaging in emotional responses via the limbic system more strongly than men when exposed to stress.[102] Wang et al. also reported heightened limbic system activation in response to mild to moderate stress in women (n=16) compared to men (n=16) in a similar fMRI study.[103] Therefore, ongoing limbic system activation, present during chronic stress, coupled with dysregulated cortisol release via HPA hyporeactivity, may contribute to women's heightened response to stress versus men. Specific mechanisms within the limbic system may contribute to this female-specific response.

The limbic system contains high levels of the neurotransmitter serotonin, which is involved in a number of critical processes within the brain, including the regulation of mood by facilitating flexible

responses and adaptations to environmental challenges, with 'distinct responses mediated by distinct serotonergic pathways' (p. 1092).[104, 105] Serotonergic pathways, or systems, are groups of neurons that collectively respond to this neurotransmitter and are found in the hippocampus, amygdala and pre-frontal cortex.[51] These systems all have roles to play in stress responsivity, with serotonin playing an important role in regulating GC feedback to maintain homeostasis within the HPA axis.[46] Furthermore, animal and human studies demonstrate that high levels of GCs are linked to decreased serotonin-mediated responses, leading to a hypofunctional serotonin state.[106-108] Anomalies in serotonin status and functioning, either in the presence of stress or not, may thus lead to both an increased susceptibility to stress and an inability to return to homeostasis after stress exposure.[107, 109, 110] Although specific nutrients are required for serotonin synthesis, it is unknown at present whether increasing the consumption of such may improve stress-responsivity.

1.5.3 Hormonal factors

Sex hormones impact neurotransmitters and steroid receptors, and large fluctuations in levels of sex hormones, such as progesterone and estrogen, which occur throughout women's reproductive life span, may impact receptors for these hormones which are highly expressed in the limbic system, making it sensitive to these fluctuations.[111, 112] In addition, such fluctuations may increase the potential for neurochemical system dysfunction in some women due to hormonal effects on serotonin, glutamate and gamma amino butyric acid (GABA) as suggested by animal and small human studies and reviews.[46, 112] For example, Altemus et al. reported that during the luteal phase of the menstrual cycle HPA responsiveness increased in relation to stress in a small study (n=9).[113] Another small study, using proton magnetic resonance spectroscopy (H-MRS), compared brain GABA levels in healthy women (n=14) with those experiencing pre-menstrual dysphoric disorder (PMDD) (n = 9), and found decreased GABA levels in the PMDD group during the follicular menstrual phase and an increase from this phase to mid- and late luteal phases, while in the healthy group it declined across the cycle.[114] These results suggested that hormone fluctuations may influence HPA responsiveness and that the GABAergic system may be modulated by hormone fluctuations in both healthy women and those with PMDD. Therefore, a subgroup of women experiencing normal physiological hormone changes may be vulnerable to neurotransmitter fluctuation anomalies, which being expressed in the limbic system, may account for mood dysregulation and possibly also influence stress coping mechanisms.[51, 115]

A number of studies suggest a relationship between hormone fluctuations and stress. For example, De Weerth et al. reported that during pregnancy, cortisol and HPA axis stress responses were supressed, as identified using salivary cortisol data.[116] The study compared healthy pregnant women (n=120) exposed to psychological stressors using the TSST to a control group of healthy women (n=31)

who were not pregnant.[116] In addition, lactation also produces a change in hormonal state, and using similar stressors, Heinrichs et al. reported that the stress response, identified using blood and salvia sampling, was suppressed in lactating women (n=43) who were randomly assigned to either breast-feed or hold their infants for 15 minutes before stress exposure.[117] Furthermore, two small PET studies revealed that estrogen and progesterone levels impacted serotonin receptor density in the cortex in postmenopausal women (n=5) and similar results were found in similar women (n=10) following estrogen replacement therapy.[118, 119] Although small, these studies suggested that recurring hormone fluxes across the lifespan may impact brain function and mood modulation via hormone-neurotransmitter interaction and may, in as yet unexamined ways, contribute to an increased vulnerability to experiencing chronic stress.[30, 46, 51, 98, 109]

1.5.4 Early Life Stress (ELS)

Although not specific to females, the neurological effects of ELS may also be a risk factor for increased sensitivity to stress later in life.[120-126] A review of recent longitudinal human studies revealed that exposure to ELS, comprised of one or a variety of negative events, which may include events in utero, childhood abuse, parental loss or prolonged separation and/or severe poverty or neglect, are psychosocial risk factors for the development of mental health disorders in later life.[122] Neurobiological changes during specific stages of brain development may partly explain this phenomenon, as some researchers suggest, with increased amygdala volume resulting from ELS leading to limbic system changes and heightened stress sensitivity.[122, 123] When exposed to the TSST, in the early 2000s, subjects that were separated from both caregivers as children during World War 2 (WW2) evacuations (n=68) all had higher average salivary cortisol and plasma ACTH concentrations versus the non-separated group (n=85).[127] Women also responded with higher baselines of both compounds compared to men.[127] Increased pituitary-adrenal reactivity, possibly related to altered HPA axis responsiveness initiated during childhood, 60 years earlier, may partly explain these results.[127]

However, ELS may include stressful events prior to birth, as stated by Babenko et al.: 'Being exposed to stress in utero and in early life may increase the risk of neurological and psychiatric disorders via altered epigenetic regulation' because 'epigenetic mechanisms are prone to changes in response to stressful experiences and environments perceived as hostile,' (p. 70).[128] Researchers suggest that ELS induces changes in the functional development of the amygdala and striatum, which impact the ability of animal and human offspring to evaluate and respond appropriately to a variety of stimuli.[129, 130] The amygdala reaches an earlier peak volume in women compared to men, so women may be more vulnerable to heightened stress responses in adulthood as a result of the effects of ELS on amygdala development.[122] These factors may partly explain how ELS contributes to stress sensitivity

in adulthood and why women may be more vulnerable to ELS, as their subordinate social rank and sex-specific socialisation and victimization may expose them to ELS more frequently than men.[96]

Figure 1.2 summarises the predisposing factors discussed thus far, some of which may interact and heighten women's susceptibility to experiencing chronic stress.[51, 97, 109, 131] A combination of these factors may also partly explain the 50% greater prevalence of affective disorders in women versus men.[47-51]

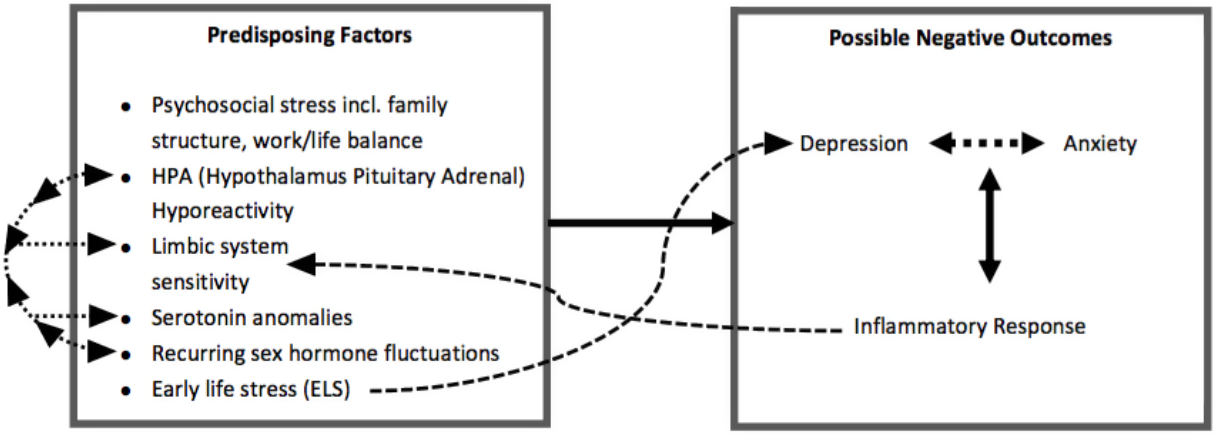


Figure 1.2: Predisposing factors that may contribute to chronic stress and possible negative outcomes. Predisposing factors (or moderators) can combine and lead to negative outcomes, such as depression and anxiety, which are often co-morbid, and have a bi-directional relationship with increased inflammatory responses. HPA hyporeactivity, limbic system sensitivity, serotonin anomalies and recurring sex hormone fluctuations may combine, possibly compounding their effects in the presence of psychosocial stress. ELS may also be a predisposing factor to a heightened stress response in the presence of psychosocial stress, and inflammatory responses may contribute to limbic system sensitivity.

1.6 Metabolic or Physiological processes whereby chronic stress may lead to nutrient deficiencies

The previous sections have identified a variety of predisposing factors that may impact the experience of and ability to manage chronic stress (Figure 1.2.). In addition, a variety of pathways to chronic stress have also been discussed, which include ongoing psychosocial stress, anticipatory cortisol reactivity leading to ongoing HPA and SNS activation and post stress rumination (Figure 1.3). However, nutrient deficiencies may be an additional pathway to chronic stress via ongoing stress hormone synthesis. For example, high nutritional needs are imposed due to increased metabolic activity required to generate energy aimed at survival strategies, including mobilizing energy from storage and diverting energy away from non-essential processes.[1, 2, 30] In addition, gastro intestinal (GI) challenges, sleep deprivation and appetite changes may also contribute to nutrient status. Possible mechanisms underpinning how nutrient deficiencies manifest are now addressed. Figure 1.3 summarizes possible pathways to chronic stress including these mechanisms.

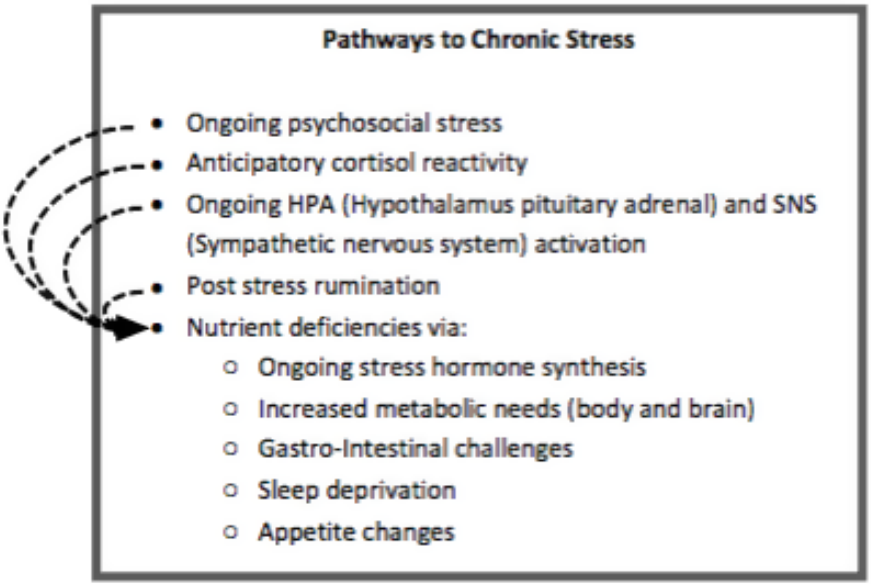


Figure 1.3: Pathways that may contribute to chronic stress. Pathways to chronic stress may combine in a number of ways that could lead to an inability to return to homeostasis. For example, ongoing psychosocial stress, anticipatory cortisol reactivity, ongoing HPA and SNS activation and post stress rumination may contribute to nutrient deficiencies via either one of the five, or any combination of the five mechanisms listed. Nutrient deficiencies may contribute to an inability to return to homeostasis because specific neurotransmitters, possibly lacking, are required for such to occur.

Amygdala activation via sensory perception, and hippocampal input, triggers the flight-or-flight response, prompting a biochemical cascade of activity that includes the synthesis of stress hormones (Figure 1.1).[1, 30, 36] In addition, the synthesis of neurotransmitters, including serotonin, which facilitates emotional equilibrium, requires many of the same nutrients as those required for stress hormone synthesis.[132, 133] Being a survival mechanism, the synthesis of stress hormones may take precedence over the synthesis of neurotransmitters that regulate mood because, as Ames stated: 'natural selection favours short-term survival at the expense of long-term health' (p. 17589).[2] Chronic stress may therefore negatively impact the synthesis and equilibrium between excitatory and inhibitory neurotransmitters and contribute to the development of affective disorders. In support of this hypothesis, Bitterlich et al. reported chronically stressed subjects stress states improved after supplementing with an amino acid-containing DS.[134] Therefore, the disequilibrium between neurotransmitters may be partly explained by a lack of nutrients with which to sustain neurobiological equilibrium in the presence of chronic stress, which may also prevent a return to HPA homeostasis.[2] Indeed, chronic stress, which triggers the survival response, may increase the already high metabolic needs of the brain.

The brain competes with the body for energy resources due to the critical role it plays in survival.[135] For example, Tomasi et al. assessed brain energy requirements in a human study of glucose metabolism using PET and MRI in healthy volunteers at rest (n=54) and found that basal metabolism, in the absence of connectivity, accounted for 30% of brain glucose utilization.[136] This led the researchers

to suggest that spontaneous brain activity accounted for 70% of the brains energy consumption and that higher energy demands of brain activity, which hinge on higher connectivity, may lead to vulnerability in specific areas that are vulnerable to energy deficits.[136] Reporting on such higher cognitive functions, Lennie reported that over 40% of whole-brain energy utilization accounts for such functions, as evidenced by electrophysiological recordings of neuronal neo-cortex activity which analysed energy demands and resources.[137] Therefore, spontaneous brain activity, which includes higher cognitive functioning and increased brain processing and increased connectivity between brain regions, may, in the presence of chronic stress, demand higher energy and nutrient usage.

As the brain and gut have an ongoing, bi-directional exchange which impacts digestion and absorption, the availability of nutrients may impact capacity for increased brain activity. For example, chronic stress has been previously related to digestive challenges via inflammatory mechanisms.[138] Bennett et al. have also reported that chronic life stress threat was a predictor of irritable bowel syndrome (IBS) symptom severity and no patient exposed to a high stressor improved clinically, while all the patients who improved did so in the absence of such.[139] Similarly, a prospective study which assessed perceived stress, depressive symptoms and stressful life events over 45 months in patients with ulcerative colitis found that acute stress did not trigger symptoms but long term perceived stress increased the risk of symptom exacerbation.[140] Yin et al. reported that stress inhibited normal gastric myoelectrical activity (GMA) in healthy human subjects (n=10).[141] Finally, GI motility was altered in the presence of chronic stress, as evidenced by a review that summarized the literature on this topic from animal and human studies.[142] Therefore, in the presence of chronic stress, optimal digestion and nutrient absorption may be hampered, which will consequently impact the availability of nutrients for brain function.

Behavioural factors associated with stress may also negatively impact nutrient availability, one such being sleep deprivation, which is associated with chronic stress in women. Hall et al. reported on a nine-year prospective, four-site, cohort study (n=330), which examined stress-inducing life events and sleep.[143] Furthermore, Vgontzas et al. reported a stimulatory effect on the HPA axis with sleep disturbance in a small group of men (n=10).[144] However, apart from being related to chronic stress and stimulating the HPA axis, sleep deprivation has other effects, including appetite changes, weight gain, and comfort, or emotional eating, defined as eating with the aim of relieving negative emotions or affect, such as anxiety or depression.[145-148] These sleep-deprivation induced physiological and behavioural changes occur via adaptations in energy and appetite regulation, which are influenced via activation of the SNS and HPA axis.[145, 146, 149] These adaptive changes have a number of consequences that may lead to nutrient deficiencies.

A systematic review of 17 studies (n=496) revealed that partial sleep deprivation led to increased energy intake, while a 14-day laboratory sleep study linked sleep deprivation to an increased intake of calories from snacks versus meals.[150, 151] Similarly, but specific to women (n=27,983), Kim et al. found that shorter sleep duration was associated with a greater propensity for high-fat and sweet snacks versus meals.[152] In terms of weight gain, a small controlled intervention with women (n=14) revealed that sleep deprivation led to an increase in energy intake of 20% and a body weight increase of 0.4 kg over eight days.[149] Supportive of these results, data from the Nurses Health Study (n=80,000) revealed that the lowest body mass index (BMI) was observed in participants sleeping seven to eight hours per night.[153] The interaction between emotional eating and short sleep duration was investigated in a one-year follow-up mixed gender study (n=911 men and n=553 women).[146] Results revealed that emotional eating and weight gain were significantly higher among women compared to men ($p=0.005$).[146] In addition to supporting the evidence that women have higher levels of stress than men, these studies suggest a relationship exists between chronic stress, sleep deprivation, appetite shifts and emotional eating.

In an attempt to understand the cognitive processes underlying appetite shifts, a mixed gender study assessed brain mechanisms responsible for the increased desire of calorie-dense foods in states of sleep-deprivation. Greer et al. reported on data gathered via fMRI for twenty three participants (n=10 men and n=13 women) which suggested that sleep deprivation results in reduced activity in cortical regions responsible for food stimulus evaluation, thus increasing the appetite shift towards, and desirability of, high-calorie foods.[154] However, ongoing sleep deprivation, unlike induced sleep restriction, may have a greater impact on food choices and appetite, and consequently nutrient deficiencies, over time, via the establishment of new neural pathways supporting habitual nutrient-deficient food choices. In addition, the intake of calorie-dense, nutrient-deficient foods, in the presence of chronic stress, may provide emotional comfort via the release of opioids that temporarily lower the stress response via dampened HPA activity.[155] Indeed, in support of this explanation, apart from being influenced by sleep deprivation, research suggests appetite is influenced directly by chronic stress.

A cross-sectional, longitudinal analysis of self-reported data from a population-level survey of Australian women (n=1,382), found an association between stress and frequent fast food consumption.[156] Similar trends and gender disparities have been observed in American surveys conducted between 2007 and 2015.[20, 27] Similarly, Kandiah et al. reported that in the presence of stress, 81% of female college students experienced a change in appetite and 62% experienced an increase in appetite, coupled with an increase in the consumption of sweet food and fast food.[157] Furthermore, Epel et al. found that pre-menopausal women exposed to the TSST who had high cortisol levels consumed more calories and sweet foods than low cortisol reactors and increases in negative mood were also

associated with greater food consumption.[158] Another laboratory stress study found similar results about which the researcher commented: 'stress may compromise the health of susceptible individuals through deleterious stress-related changes in food choices' (p. 853).[159] Therefore, regardless of how stress is induced, either via a laboratory stressor or via other pathways (see Figure 1.3), consequent appetite changes lead to women favouring high calorie, low nutrient foods.

Appetite changes may also be related to serotonin, a neurotransmitter that plays a role in regulating appetite.[160] In addition, serotonin is also the precursor to melatonin, which is necessary for deep, restorative sleep.[161] Both these compounds are reliant on a number of nutrients for synthesis.[132, 133, 161, 162] Therefore, a lack of nutrients may hamper serotonin's appetite-regulating function and melatonin's sleep inducing function, both of which may lead to the over-consumption of calorie dense, quick energy release, nutrient deficient foods.[145, 146, 149] A complex relationship may therefore exist between serotonin, sleep deprivation, and nutrient deficiencies.

Thus far, evidence suggests that appetite changes in the presence of chronic stress and sleep deprivation may over time lead to poor dietary choices, and possibly nutrient deficiencies, further contributing to the inflammatory responses associated with stress, depression and anxiety.[74, 75] (See Figures 1.2 and 1.3.) These factors may combine with the previously described pathways to chronic stress, thus preventing a return to homeostasis and the amelioration of chronic stress. Whether specific nutrients consumed via food and/or DSs can improve the experience of chronic stress for women is presently unknown although a growing body of research suggests that specific nutrients are important for mental wellbeing and their absence is linked to stress-associated disorders.[163-172]

1.7 The neurobiological role of specific nutrients in relation to chronic stress

The absence of specific nutrients have been associated with the presence of affective disorders, however, despite the relationship between stress and affective disorders, there is a dearth of evidence to support the importance of specific nutrients in relation to stress.[163-167] Due to the roles nutrients play in neurobiological functioning, a number of nutrients may indeed impact the stress response.[162, 172] For example, many vitamins function as cofactors of key enzymes in a variety of pathways including amino acid metabolism and neurotransmitter synthesis.[132] Some minerals also have important roles to play in maintaining normal brain function including neurotransmitter synthesis, and are involved as co-factors in energy metabolism and in the synthesis of stress hormones.[132, 133, 172] Inadequate dietary intake of nutrients may therefore impact the brains ability to manage chronic stress effectively due to the

association with higher metabolic activity and the increased demands imposed by chronic stress. Table 1.1 summarizes the neurobiological role of specific nutrients.

The brains metabolic requirements suggests that it may be 'sensitive to fluctuations in the nutrient state of the rest of the body' (p. 271).[173] Brozek et al. reported that thiamine (B1) and riboflavin (B2)-induced deficiency over 161 days resulted in psychological symptoms, such as depression, in a group of healthy men (n=10), who used self-rated questionnaires to report sensory and motor functions along with intelligence and personality and in whom irritability and depression appeared after anorexia and muscle weakness.[174] Similarly, Sterner et al. investigated B2-induced deficiency in a group of healthy male conscientious-objectors (n=6) over 56 days, which resulted in depression and hysteria among other physical and behaviour-specific effects.[175] Other mental wellbeing and B vitamin interventions have revealed similar results.

Aybak et al. reported that 300mg of pyridoxine (B6) supplemented daily over four weeks was effective as an anti-stress strategy leading to significant decreases in systolic ($p<0.01$) and diastolic ($p<0.005$) blood pressure measurements for hypertensive patients along with reductions in noradrenaline (18%) and adrenalin (16%).[176] McCarty hypothesized that this effect was likely due to a reduction in SNS activity along with serotonin modulation in the presence of this nutrient, which may dampen corticosteroid response and therefore be a useful clinical approach in the presence of psychological stress.[177] Indeed, as vitamin B6 is also a co-factor in serotonin synthesis, a deficiency may negatively impact stress levels.

In addition, folate (B9) deficiency may predispose or exacerbate depression in humans.[178] Furthermore, early studies of copabalamin (B12), found that a wide range of psychiatric symptoms, including anxiety, depression, confusion, psychosis and mania were reversed with B12 supplementation.[179] A more recent randomized double-blind, placebo-controlled (RCT) study which examined the effect of a-90 day, high dose, vitamin B-complex administration on work stress in sixty participants (n=19 men and n=41 women) reported significantly lower personal strain ($p=0.02$) from week 4 to week 12 and a reduction in depression.[180] Thus, evidence has suggested that either a deficiency, or supplementing with B vitamins, impact mood states, although whether these nutrients can ameliorate chronic stress levels is as yet unknown. (See Table 1.1 for a summary of B vitamin roles in the brain.)

In addition to the B vitamins, vitamin C also has a significant role to play in healthy brain function, with the concentration of such (ascorbic acid (AA)) being higher in the brain than in any other organ (besides the adrenal glands).[181] This suggests that vitamin C also has a role to play in the stress response. Furthermore, vitamin C plays a number of other roles in the brain (see Table 1.1), some of which are of particular importance due to the high oxidative metabolic rate of neurons (10-fold over

supporting glia (animal studies)).[182, 183] Brody et al. examined stress, cortisol and AA levels in relation to the administration of the TSST using a high dose sustained-release vitamin C supplement (1000 mg) on a mixed gender group of young adults (n=60) over 14 days.[184] A statistically significant reduction in anxiety in the supplement compared to the placebo group was noted at 14 days (p=0.0096) along with the same reduction in stress between the supplement group at baseline compared to 14 days (p≤0.0004).[184] The researchers suggested that supplementing with this nutrient rather than using dietary sources yielded anxiety and stress-lowering effects.[184] Reporting on a similar RCT, de Oliveira et al. found that the effects of 14 days of vitamin C supplementation (500mg) on anxiety scores in a mixed gender group of students (n=42) yielded significantly lower scores and plasma vitamin C status after the intervention period versus the placebo group (p = 0.010; p=0.001) and the heart rate of the supplement group was also significantly reduced compared to the placebo group (p=0.032).[185] These results have suggested that supplementing with either forms of this nutrient may be effective at reducing psychological stress. However, minerals also play a role in mental health. (See Table 1.1.)

In addition to the vitamins addressed, magnesium and zinc also impact affect states. A review examining the role of magnesium in affective disorders suggests this nutrient influences several systems related to such disorders which include a reduction in HPA axis activity.[186] In support of this review, Jacka et al. reported on a Norwegian survey (n=5,708) that found higher magnesium intake via food was associated with significantly lowered depression (ß=-0.11, 95%CI=-0.16 to -0.05) and lowered anxiety scores.[187]

Amani et al. reported on a controlled study that examined depression in relation to serum zinc levels in young women with moderate-to-severe depression (n=23) and healthy matched controls (n=23).[188] Significantly lower zinc intake and serum zinc concentrations were noted in the depressed group (p<0.01).[188] Similarly, an American survey revealed that women (n=2,163) with a low food-derived zinc intake were more likely to have depressive symptoms, whereas the same association was not found in relation to men (n=1,545).[189] This result suggested a gender-specific relationship between zinc and depression. In addition, some research suggests that zinc has a non-uniform distribution in the brain with high concentrations in specific areas, such as the cerebral cortex, which is associated with emotion and learning.[190] Zinc deficiency may therefore negatively impact the ability to cope with stress, as stress assessment and emotion regulation occur in these areas. However, there is no evidence to suggest that either magnesium or zinc supplementation alone, or combined, reduces anxiety or chronic stress.

Unlike B vitamins, vitamin C, magnesium and zinc, EFAs are not directly involved in the synthesis of neurotransmitters or the stress regulation process, although they are critically important nutrients required for optimal neurological functioning, (see Table 1.1) which suggests they may have a

role to play in ameliorating the stress response.[191, 192] Research has suggested that this may be the case in both genders. For example, Kiecolt Glaser et al. reported on a 12-week, RCT trial that examined omega 3 supplementation (2.5g/day) versus placebo in medical students, via blood samples that measured inflammatory markers, during low stress and high stress (exam days).[193] Validated measurement instruments assessed anxiety and food intake and a significant supplementation effect on anxiety was reported (p=0.04).[193] A similar RCT assessed responsiveness to mental stress (mental arithmetic) before and after eight weeks of omega 3 supplementation compared to placebo, and found a significant reduction in heart rate (HR) (p=0.002) in the supplement group versus the placebo group (p=0.756).[194] The dietary intake of the omega 3, docosahexaenoic acid (DHA), has also been associated with lower anxiety scores.[195] In addition, a lowering of test anxiety in men was found in a small three-week study using EFAs.[196] Conversely, Bradbury et al. reported on a RCT (n=90) that found no effect on chronic psychological stress using another omega 3, eicosapentaenoic acid (EPA).[197] Combined, these results suggest a gender response to anxiety, detected via physiological markers or psychological measurement instruments that are ameliorated in the presence of omega 3, in contrast to using the same for chronic stress.

Table 1.1: A summary of the neurobiological roles of specific vitamins, minerals and EFAs

Nutrient	Function
Vitamin B1 (Thiamin)	*Enzyme cofactor for acetylcholine synthesis;[132, 133] facilitates nerve conduction functioning; facilitates glucose metabolism.[133]*
Vitamin B2 (Riboflavin)	*Facilitates glucose metabolism.[133]*
Vitamin B3 (Nicotinamide)	*Enzyme cofactor for serotonin synthesis;[132, 133] facilitates glucose metabolism.[133]*
Vitamin B5 (Pantothenic acid)	*Facilitates glucose metabolism.[133]*
Vitamin B6 (Pyridoxine)	*Enzyme cofactor for noradrenaline, dopamine, serotonin, GABA, adrenaline synthesis;[132, 133] modulates binding of neurotransmitters at postsynaptic receptors;[198] protects cerebral blood flow.[199]*
Vitamin B9 (Folic acid)	*Enzyme cofactor for Noradrenaline, dopamine, adrenaline synthesis;[132, 133] maintains myelin sheath integrity; protects cerebral blood flow.[199]*
Vitamin B12 (copabalamin)	*Enzyme cofactor for noradrenaline, dopamine, adrenaline synthesis;[133] maintains myelin sheath integrity; protects cerebral blood flow.[199]*
Vitamin C (Ascorbic acid)	*Enzyme cofactor for Noradrenaline, adrenaline, dopamine synthesis; antioxidant.[133, 181, 182]*

Nutrient	Function
Magnesium	*Cofactor for over 325 enzymes; inhibits excitatory neurotransmitter release; facilitates glucose metabolism;[133] increases brain-derived neurotrophic factor (BDNF) expression;[200] enzyme cofactor for Glutamate, Serotonin synthesis.[39, 133, 201]*
Zinc	*Cofactor for over 300 enzymes; inhibits excitatory neurotransmitter release; cellular metabolism; increases brain-derived neurotrophic factor (BDNF) expression;[200] enzyme cofactor for glutamate and serotonin synthesis.[2, 133, 188, 202, 203]*
EFAs	*Involved in all neurotransmitter synthesis and release; improves oxygen and nutrient assimilation facilitating energy production and neuronal functioning; improves membrane and synaptic flexibility and permeability; optimal cognitive functioning relies on EFAs to compose 20% of brain dry weight; eicosanoid precursors.[191, 192]*

Overall, there is a paucity of evidence to support the role that B-vitamins, vitamin C, magnesium, zinc and EFAs play in CNS functioning specifically related to chronic stress among women. However, deficiencies in certain B vitamins can lead to poor mental health, while others suggest supplementing with them can ameliorate stress.[174-176, 178-180] Vitamin C supplementation has been associated with a reduction in physiological and psychological determinants of stress and magnesium has been associated with lowered depression and anxiety scores.[184-187] Zinc meanwhile has been associated with lowered depression scores and omega 3s with lowered anxiety scores.[188, 189, 193-195] Although few studies have examined the role of these nutrients in relation to chronic stress among women, the myriad of roles they play in both general metabolic and CNS functioning, and because chronic stress may pre-date the development of affective disorders, these nutrients may play a role in ameliorating chronic stress. Increased neural metabolic activity, gastrointestinal challenges, and changes in sleep and appetite as induced by chronic stress, coupled with an insufficient supply of these nutrients, may lead to an inability to concurrently synthesize stress hormones and neurotransmitters required for the maintenance of emotional equilibrium. Furthermore, the presence of these health challenges may lead to women using DSs in the belief that they can ameliorate the experience of chronic stress.

1.8 Stress management strategies including the use of DSs

As discussed, appetite is influenced by feelings of stress, but research has also suggested that eating may be used as a stress-management strategy among women. For example, an Australian survey (n=1,537) found that significantly more women than men reported 'turning to food' to manage stress (p. 8).[204] Although alcohol was also reported as being a support mechanism, gender differences are unclear.[17, 204] Similar support strategies were reported in a 2010 American survey (n=530 men and

n=604 women).[27] Appetite changes due to stress may therefore not be entirely due to changes in energy and appetite regulation systems but may also be due to women using food to manage stress. However, it is not known whether this strategy is used along with others, for example, visiting PCPs.

Although not gender segregated, a twenty-year survey which concluded in the 1980's, undertaken by an American managed-health care company, found that an estimated 60%-90% of visits to PCPs are related to stress.[205] In addition, more recent data (1997 to 2004) from the American National Health Interview Survey (NHIS), the samples of which represent 25,618,369 adults, found that healthcare expenditures and outpatient visits increased significantly as psychological distress increased.[206] Furthermore, more women than men tend to visit hospitals and PCPs in the US and the UK.[207, 208] Australian data supports this trend with a survey over 2017-18 that revealed women (14%) were twice as likely to be admitted to a hospital versus men (6%).[209] A Canadian survey also reported that chronic health conditions were 40% higher for women than for men as reported over a ten-year period (1994-2003; n=3,522).[210] This data suggests that, similar to stress levels, more women experience chronic ill health globally than men, which may be related to these high stress levels.

A Danish survey found women accounted for 69% of stress-related cases reported by a general practitioner (GP).[211] A 2018 Australian online survey of women (n=15,262) found that close to half of the respondents (46.1%) had been diagnosed with anxiety or depression by a doctor or psychologist.[212] Furthermore, this sample size increased by 49% from 2017 (n=10,377), suggestive of increased awareness among women regarding health and mental wellbeing.[212] These results suggested increased stress may drive more women to visit PCPs and be diagnosed with anxiety and/or depression. Given the relationship between stress and affective disorders and the increased prevalence of such among women, differences in health-seeking behaviour between genders may be expected. A population level UK survey (n=15,222) investigated such behaviour among men and women with common mental–health problems with women more likely to report that they would (and did) seek help.[213]

Help seeking behaviour may result in the use of psychotropic medications, such as anxiolytics, which are prescribed by PCPs to reduce symptoms of anxiety, which accompany feelings of stress.[214] An early study by Baker et al. reported on the use of anxiolytic/sedative medication over a 12 month period from the USA and 10 Western European countries wherein past year prevalence rates were much higher for women than for men in every country surveyed, with a ratio close to 2:1 in favour of female anxiolytic use.[215] In support of these findings, Ashton et al. revealed that throughout the USA and Europe, women were prescribed approximately twice as many psychotropic drugs as men.[214] In addition, Agarwal et al. reported on a recent large USA cross-sectional study (n=386,457) which examined data over 2003 – 2015 regarding benzodiazepam outpatient prescribing, and found that the

use of such anxiolytics had increased over this time period and that women were more frequently prescribed compared to men.[216] Data regarding antidepressant (AD) use also suggested higher use among women versus men. For example, Pratt et al. (n=13,951), reported that over 2011-2014, women were twice as likely to use such medication versus men.[217] Similarly, Mars et al. reported on a retrospective UK study over 1995-2011 that found that AD prescribing was about two times higher for women.[218] Similarly, Australian women were more likely to use a mental-health related treatment (18.2%) than men (11.7%) and that women (9.9%) were more likely than men (5.6%) to have an AD prescription filled.[219] In addition, the self-reported prevalence of taking either ADs or anxiolytics among Australian women (n=48,359) increased from 9.1% in 2001 to 10.3% between 2004-2005.[220] These results have suggested that women use more psychotropic medications than men globally, although whether this is the preferred method of managing affective mood states is unknown.

Research investigating stress management counselling in primary care, versus non-pharmacological interventions, is sparse. For example, O'Donnell et al. reported on a Canadian survey (n=2,916) that investigated mood and anxiety disorders and revealed that 47.6% of patients took medication, 6.9% received counselling and 27.3% were taking medication and had received counselling.[221] No significant differences were found between treatment type and gender.[221] In addition, Nerurkar et al. reported on an American survey that only 3% of office visits included stress management counselling from PCPs despite 34,065 visits to 1,263 physicians being related to stress, although this survey did not separate data by gender.[222] These results have suggested that either patients prefer medication or that few PCPs offer alternate stress-management strategies. Furthermore, research is also sparse regarding people who do not choose medical help for mental ill health challenges and may instead turn to what they believe are effective, alternative sources of support, such as DSs and complimentary alternative medicine (CAM), which includes DSs and dietary changes.

Three American surveys, totalling over 107,000 participants, reporting on data over 2007-2011, found that women used more DSs than men, with the difference ranging from between 10% to 16% more.[223-225] In addition, two recent Australian studies (n=14,000) reported a similar trend.[226, 227] Vatanparast et al. reported similar findings from a Canadian survey (n=35,107), with higher reported DS use among women (47%) compared to men (34%).[228] Overall, these findings have suggested that the use of DSs globally have been more prevalent among women than men. Although it is not possible to ascertain what percentage of such DS purchases are aimed specifically at stress-reduction among women, a number of studies have reported on the use of CAM and DSs for some mental health challenges.

Kessler et al. reported on a population level American survey (n=2,055) about the use of CAM wherein 9.4% of respondents reported suffering from anxiety attacks in the last 12 months and 7.2%

reported suffering from depression.[229] Results revealed that over half of these respondents used CAM and that over 60% of those who had visited a conventional provider also used CAM.[229] Similarly, reporting on a cross-sectional study in Switzerland (n=2,937 men and n=3,249 women) Marques-Vidal et al. found that the prevalence of multi-vitamin and mineral (MVM) and DS use increased in subjects who reported a personal history of anxiety and/or depression, among other health challenges.[230] Although these studies were not gender segregated and did not report on the use of MVMs and DSs in relation to stress, some gender segregated studies did report on such.

Two mixed gender studies have reported on stress and DS use. Firstly, a Japanese study (n=375) revealed that as daily stressors increased so did the consumption of DSs.[4] An Italian study (n=1,723) additionally revealed that the use, or non-use, of DSs was associated with self-assessed stress levels, wherein 48% of users reported no stress, 63% of users reported low stress levels and 57% of users reported high stress levels.[5] In addition, specifically related to women, data from a French study (n=83,058) found an association between DS use in 57.7% of those who reported work-related stress, and 26.4% among those who reported work being very stressful, although 59% of non-users also reported work-related stress.[6] These mixed results have suggested that increased stress does not necessarily accompany the increased use of DSs. However, regardless of motivations for use, DS use is more prevalent among women compared to men and is used by some women in the presence of stress, although what prompts the use of DSs requires examination.

The Pew Internet and American Life Project examined the extent of Internet use for health-related matters in 2000 using data from a representative population sample (n=521) wherein 26% reported looking for information about mental health including anxiety or depression while data from another Pew survey (n=12,751) revealed that 73% of users were women.[231] More recent Pew Research findings (n=3,014) found that 72% of American adults used the Internet for health information and distinguished between Internet health-seekers, revealing that women are more likely (40%) to look online to diagnose a health condition than men (30%).[232] Similar results from an Australian online poll (n=1,000) found that 78% of adults used the Internet for health-related information in 2015, with women reporting more Internet use than men on each of five criteria, including using social media (SM) for health related information.[233] Thus, Internet health seeking is a global behaviour that more women engage in than men. However, how this behaviour relates to DS purchasing is presently unknown.

Self-directed use of DSs occurs widely, as evidenced by the money spent on them.[9] For example, Nahin et al. reported on data for 2012 from the American National Institute of Health (NHIS; n=44,743), which revealed total out-of-pocket expenditures for DSs was US$30.2 billion.[9] Specifically related to DS use prevalence in the Australian market for 2018, expenditure was calculated at AU$2.77 billion.[234] However, global expenditure is expected to reach US$278.02 billion by 2024 for general DSs

and US$16.7 billion by 2025 for stress-targeted DSs despite a dearth of evidence to support their use as a health promotion or stress management strategy, respectively.[8, 235]

Thus far, the evidence has suggested that more women experience chronic stress than men, more women investigate health matters online and more use DSs, including to manage stress. The methods used by women to manage stress also include emotional eating, PCP visits, the use of psychotropic medication and DSs may be the result of female-specific pathways to chronic stress. (See Figure 1.4.) Although emotional eating (and PCP visits, resulting in psychotropic use), may provide improvements in mental health, the evidence to support measurable improvements in chronic stress from DS use is sparse.[10] Women's increased use of online health resources may expose them to convincing DS marketing strategies that may contribute to the prolific use of such for stress management. Figure 1.4 provides a conceptual framework, of the interaction between factors and pathways that women may use to manage stress including DS use.

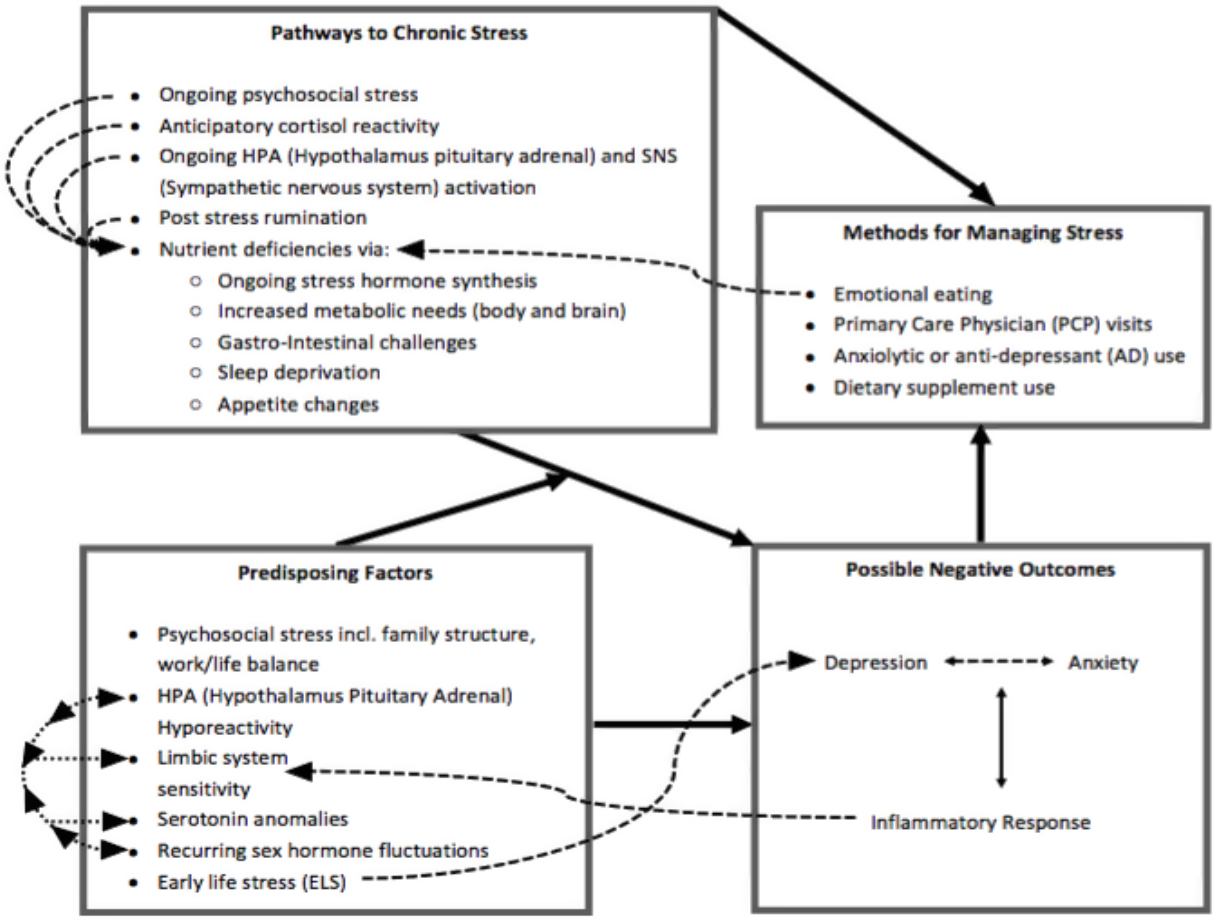


Figure 1.4: The conceptual framework linking predisposing factors and pathways to chronic stress, possible negative outcomes and methods for managing stress among stress. This conceptual framework links factors that may contribute to, and combine in a variety of ways to lead to intractable chronic stress. Predisposing factors may alone lead to negative outcomes, but may combine with pathways to lead to negative outcomes, because over time, in the presence of chronic stress, predisposing factors may lead to habitual or entrenched behaviours and thus support these pathways. Bi-directional relationships may exist between HPA hyporeactivity, limbic system

sensitivity, serotonin anomalies and recurring sex hormone fluctuations. Depression and anxiety have a bi-directional relationship with inflammatory responses and may contribute to limbic system sensitivity while ELS may lead to depression and anxiety. Pathways and possible negative outcomes may both lead to any of the methods for managing stress. Emotional eating may be used as a way to manage stress and be a consequence of chronic stress in the form of appetite changes and both may lead to nutrient deficiencies. Within pathways, ongoing psychosocial stress, anticipatory cortisol reactivity, ongoing HPA and SNA activation and post stress rumination may either alone, or in a variety of combinations, contribute to nutrient deficiencies via ongoing stress hormone synthesis, increased metabolic needs, gastro-intestinal challenges, sleep deprivation and appetite changes.

Despite women experiencing high levels of stress;[17, 20-26, 28] suffering more from stress-associated affective disorders than men;[47, 52, 60, 61] and consuming more DSs than men,[223, 225, 226, 228, 236, 237] there is a dearth of research investigating whether such interventions produce a measurable outcome.[10] Using an evidence-based approach to investigate this phenomenon may provide clarity for clinicians and prevent the ongoing use of ineffectual interventions among women. With this in mind, the following section discusses the origin, and the strengths and weaknesses of the evidence based medicine (EBM) approach to clinical care. A variety of initiatives that have arisen from this approach are considered along with a brief discussion regarding evidence based mental health and reasons for positioning this thesis within the Joanna Briggs Institute (JBI) Model of Evidence Based Health Care (EBHC).

1.9 The strengths and weaknesses of the EBM model and reasons for situating this thesis in the JBI model of EBHC

The concept of 'evidence-based medicine' (EBM) was introduced in 1991 when Guyatt coined the term in an editorial where he suggested that this new idea was a necessary and important advance required for the future practice of medicine.[238, 239] This approach to EBM proposed a hierarchy of knowledge in which randomised trials and meta-analyses were placed at the top of a linear ordering system and the 'pathophysiological understanding of disease processes and clinical experience occupied successively lower positions' (p. 892).[239] In this form EBM focused primarily on critical appraisal and the development of systematic reviews and clinical practice guidelines (CPGs). However, a comprehensive definition of EBM was not yet proposed.

Sackett, an original member of The EBM Working Group, proposed such a definition later in the same decade: 'Evidence-based medicine is the conscientious, explicit and judicious use of current best evidence in making decisions about the care of individual patients' (p. 71).[240] The use of the word 'current' in this definition implies that the clinical approach will be modified according to increased empirical knowledge, so that recent research will take precedence over older evidence.[241]

This approach to clinical care included a number of important features, including combining 'individual clinical expertise with the best available external evidence from systematic research' (p. 71).[240] This perspective placed a value on the judgement and proficiency that individual clinicians acquire through both clinical experience and practice. More effective and efficient diagnoses, coupled with the recognition of, and empathetic use of individual patients' preferences and rights, when making clinical decisions about their care were additional features. The use of clinically relevant research from medical science and patient centred clinical research that focused on the accuracy and precision of diagnostic tests, including clinical examinations, prognostic markers and the efficacious and safe use of preventive, rehabilitative and therapeutic regimens were key expectations of working to an EBM approach. The use of external clinical evidence, which will at times invalidate previously accepted diagnostic tests and treatments, leading to their replacement by new tests that are more accurate, powerful and safer, is another feature of this approach.[240] These features combine the use of individual clinical expertise with the best available external evidence as neither is considered individually sufficient for the practice of EBM.[240]

The EBM approach has also made a marked contribution to improvement in the quality of research via the documentation of challenges in current research, such as risks of bias, which has driven the development of improved research design and publication standards.[242] Indeed, more than two decades ago, Altman suggested that most medical research was flawed for a number of reasons, which included 'inappropriate designs, unrepresentative, and small, samples, incorrect methods of analysis and faulty interpretation' (p. 283).[243] Schulz et al. added to this criticism by highlighting the effects of systematic bias on research outcomes.[244] These realisations led to the suggestion that specific principles rather than categories established by research design should serve as the foundation for assessing the quality of evidence because researchers realised that assessing the quality of the research was more important than categorising research design.[244, 245] Therefore, the hierarchies of evidence used when EBM was in its infancy were replaced by robust classification systems with which to categorise evidence by quality (risk of bias) rather than design.

The Grading of Recommendations, Assessment, Development and Evaluation (GRADE) methodology is an example of an EBM informed classification system that addresses important concepts related to the credibility of guidelines and systematic research and evidence, such as consistency of results across studies, strengths and limitations of studies, publication bias, applicability of research, study design, significance of effect and dose-response gradients, all of which provide guidance for clinicians in assessing evidence.[242] The pursuit of EBM has also encouraged the development of checklists and other tools to guide the design, conduct and reporting of research, such as the Consolidated Standards of Reporting Trials (CONSORT) and the Preferred Reporting Items for

Systematic Reviews and Meta-Analyses (PRISMA) checklists, which guide researchers on the reporting of RCTs and Systematic Reviews and Meta-Analyses, respectively. [242, 246, 247]

Despite these contributions and initiatives, this approach has been criticised for a number of reasons. Holmes' polemic against the EBHC approach focused on its reliance on a specific method of knowledge production, based on privileging RCT's, and was therefore dismissive of other designs, leading to an exclusionary and rigid paradigm. Holmes suggested that EBHC had become 'a regimented and institutionalised version of the 'truth' (p. 181).[248] Although less extreme, others have argued that EBM may foster a 'formulaic' perspective to clinical practice, which dissuades deliberation and clinical reasoning, possibly leading to 'cook book' or robotic decision-making, (p. 1141).[249-252] Further to this criticism, other researchers have suggested this approach may encourage a rule-based reasoning technique, which may discourage intuitive, experience-based, person-centred care, which is the essence of expert opinion and judgement.[251]

Given the reliance on external evidence in EBHC, failure to publish research results, or suppression of negative results, is a practical problem that continues to challenge the EBM movement.[242, 253] The exclusion of negative results is a form of systematic bias which can lead to clinicians making uninformed decisions because positive results are more frequently published than negative or equivocal results. The initiative 'Alltrials,' is an attempt by a group of highly motivated EBM researchers and practitioners to address the suppression of, and failure to publish negative results.[254] This group advocates for the registration of all past and present clinical trials and the full reporting of all their methods and summary results, regardless of research outcome.[254] However, whether researchers or clinicians access this registry to guide evidence based clinical decision making is unclear.[253]

Another practical challenge to the use of EBM by clinicians relates to the time available to practitioners to access, select, appraise, extract and inform practice with the best available evidence. Timely access has become a barrier to best practice. This challenge is evidenced by the results of a 2014 cross-sectional study which analysed a sample of systematic reviews and found that approximately 8,000 systematic reviews were indexed in MEDLINE annually, which was a three-fold increase over a sample taken the previous decade when the total was 2,500.[255] In an attempt to address this challenge, electronic platforms such as BMJ Best Practice, Dynamed Plus and JBI-COnNECT+ were created to provide pre-processed information which clinicians can use to become informed about current best clinical practice in their fields of interest.[256-258] However, an evaluation of 26 existing point-of-care information sources found that they were not all equally reliable, suggesting that the onus remains on individuals and organisations to assess the quality of EBM information.[259]

A further ongoing challenge that may limit the practice of EBM is the need for more efficient research production, not just improved quality.[242, 253] Besides using reporting guidelines manually while

designing and writing up research, such as those provided by CONSORT and PRISMA, technology-driven solutions may facilitate increased rigor and efficiency of research generation. A number of options are already existent, and include software programs that encourage comprehensive research reporting and more recently, two that check manuscripts for missing information, one of which is the result of an EQUATOR network initiative.[260-263]

Sackett et al. once stated that over time the EBM movement would evolve and adapt, suggesting that the EBM model envisaged by the original team had the capacity to be responsive to medical advances, expanding human knowledge and understanding.[240] The EBM model has increased in scope and complexity and is now broadly known as evidence-based health care (EBHC), having expanded to include other disciplines such as nursing, dentistry, health policy and public and mental health.[242, 264] Furthermore, as evidenced by the initiatives above, this movement has also grown to encompass checks and balances that support its evolution and primary goal of providing the best evidence for clinical care.

However, it is challenging to generate evidence in complex settings, such as those in settings aimed at improving mental health, and the general EBM model falls short of providing an overarching framework within which to generate and assess diverse forms of evidence. Therefore, the primary studies undertaken as part of this thesis are better positioned within the Joanna Briggs Institute (JBI) model for EBHC (Figure 1.5) due to this models ability to support various forms of evidence. Furthermore, the flexibility and sensitivity inherent in this model is also supportive of a body of work that calls upon research from multiple disciplines.

Figure 1.5: The JBI model for evidence-based healthcare (EBHC). Jordan Z, Lockwood C, Aromataris E, Munn Z. The updated JBI model for evidence-based healthcare. Adelaide: The Joanna Briggs Institute; 2016, p. 4

1.10 The role of the JBI Model of EBHC in this thesis

The JBI model of EBHC represents the complexity of EBHC and provides a clear and succinct conceptualisation of the major components related to EBHC, namely evidence generation, evidence synthesis, evidence transfer and evidence implementation.[265] Each of these 'inner' components represent the higher-order, conceptual steps involved in 'the process of achieving an evidence-based approach to clinical decision-making' while each of the actionable components are represented by three linked outer sections, (p. 3).[265] Arrows depict a natural 'flow' between the components along with smaller arrows which acknowledge that the process of moving between components is not necessarily always a linear process.[265] This illustration of 'iterative, bi-directional movement' between the components showcases the models flexibility and adaptability, constructs especially important when researching complex topics.

The centre of the model, the 'pebble of knowledge,' summarises the foundation of evidence and how it informs practice via the FAME scale: Feasibility, Appropriateness and Meaningfulness of the activity or intervention and the Effectiveness of the intervention. The broad conceptualisation of FAME reflects the variety of research approaches undertaken by health researchers, such as the degree to which an activity or intervention is practical within a specific context (Feasibility), the degree to which an activity or intervention fits within a specific context (Appropriateness), the degree to which an intervention is meaningful and experienced positively by individuals or groups (Meaningfulness), and the degree to which an intervention achieves the intended result (Effectiveness).[265] The FAME scale takes diverse research and clinical practice into account by supporting a variety of research approaches undertaken by health researchers, which are informed by the specifics of the research question.[265]

Due to the complexity inherent in social interventions, evidence based on both experimental and non-experimental methods can be useful.[266] Indeed, the FAME scale acknowledges that answering complex research questions may require diverse research approaches. The JBI model therefore provided a conceptual framework that guided the design and implementation of the projects within this thesis and has the ability to further guide the research results in relation to evidence transfer and implementation. This program of research started with a systematic review of effectiveness that clearly identified a knowledge gap in the field under investigation, and was followed by a cross-sectional research project. A qualitative project followed, which used focus groups within a methodology of participatory action research (PAR) to explore the lived experience of stress and the use of DSs to manage stress in a group of women.

Using multiple research methodologies to investigate the overarching research questions in these three projects provided a broad and deep perspective to this thesis. This mixed-model approach is well situated within the framework of the JBI model, due to the complex nature of mental health, because 'evidence that addresses the multifarious questions, concerns and interests of clinicians ... will need to utilize a wide range of knowledge development approaches and varied research traditions' (p. 86).[267, 268]

The position of the 'Global Health' component at the top of the model underpins the primary reason and motivation for EBHC as suggested by the researchers when they originally developed the model, stating that 'the achievement of improved global health is conceptualised as both the goal and the endpoint of any or all of the model components, (p. 209).[269] Including mental health improvement in this conceptualization situates research aimed to this end comfortably within this model because similar variables apply to its pursuit, which can be operationalized in comparable ways.

1.11 Conclusions

As previously examined, few studies have explored nutritional factors in relation to women's experience of, and management of chronic stress, and these have predominantly focused on stress accompanying a hormonal state.[10] Some studies have investigated stress in relation to DSs in both genders. For example, Schlebusch et al. reported on a double blind, multi-centre, placebo-controlled intervention study (n=300) in the general population of two South African cities where stress is recognised as being high.[270] Across 30 days, participants used either a MVM or a placebo and experienced a significant reduction in stress as measured by all four self-report rating scales (p=0.0344; p=0.0148; p=0.0001; p= 0.0136).[270] Rucklidge et al. reported on results from an RCT (n=116) that investigated stress levels over a one-month period in relation to the consumption of MVM's, after an earthquake.[271] Results revealed that MVM users had better outcomes compared to controls. Another study found that a year after MVM supplementation was initiated using a double blind study (n=129) an improvement in mood in female students was noted.[272] These researchers suggested that providing higher levels of vitamins promoted long-term changes because short-term marginal deficiencies, addressed at three months, did not result in mood changes.[272] Furthermore, as discussed in section 1.7, 'The neurobiological role of specific nutrients in relation to chronic stress,' a few studies have investigated the effectiveness of vitamin C, and EFA supplementation on anxiety or stress in both genders, with mixed results, over shorter periods of time. [184, 185, 193-197] Examining the impact of dietary nutrients reveals similar results.

Indeed, results from a cross-sectional study of adult Iranians (n=3,846) who completed a FFQ and validated anxiety measurement instruments revealed that psychological distress increased significantly in women as their consumption of fast food increased (p=0.02).[273] Regardless of gender, but in support of these findings Banta et al. reported on a recent American population level study (n=245,891) that found moderate and serious psychological distress was associated with lower intakes of healthy foods and higher intakes of the opposite.[274] Mujcic et al. reported on data from a longitudinal study over 2007 – 2009 (n=5,949) and revealed that the probability of being diagnosed with anxiety or depression in 2009 was inversely related to the consumption of fruit and vegetables in 2007.[275] Conversely, Jacka et al. found no correlation between women's diets and their anxiety levels (n=1,494), assessed via a validated FFQ and a clinical interview.[276] However, Jacka et al. later reported on an Australian 12-week, parallel group, single-blind, RCT (n=67) which examined whether adherence to a specific diet (ModiMedDiet) impacted this mood state, and found a significant improvement between baseline and endpoint in the intervention group (p=0.001).[277] However, none of these studies compared the nutrient intakes of men and women in relation to chronic stress.

An interesting anomaly was revealed between men (n=272) and women (n=291) in relation to mental health and dietary patterns in a recent American cross sectional, Internet based survey.[278] Begdache et al. reported that men were more likely to experience mental wellbeing until severe nutrient deficiencies arose while women were less likely to experience the same until a balanced diet and healthy lifestyle were followed.[278] The authors propose that dietary sufficiency may potentiate heightened limbic system regulation in women, which is suggestive of nutrients impacting mental wellbeing more in women than men.[278] In support of this result, Firth et al. reported on a recent systematic review and meta-analysis of 16 RCTs (n=45,826) that examined the effects of dietary interventions on symptoms of depression and anxiety.[279] The results showed female subjects with significantly greater benefits from dietary interventions for symptoms of these affective disorders.[279] Combined, these results suggested that women may benefit uniquely from improved nutrient intake via food and as per the Benton et al. study, in which women alone benefited from a year-long MVM intervention, this may be the case for DSs too.[272] Although most of these studies suggested that specific nutrients in the form of DSs or food impact mental wellbeing, none of them examined women's experience of chronic stress in relation to specific nutrients.

As discussed, globally women report experiencing higher levels of stress and also experience higher rates of stress-related affective disorders than men. [17, 20-26, 28, 47, 52, 60, 61] A combination of factors may be responsible for this disparity including specific psychosocial, physiological and neurobiological differences. A variety of mechanisms related to the experience of chronic stress may lead to nutrient deficiencies due to the role that specific nutrients play in the stress response and the maintenance of emotional equilibrium. In addition, the consequences of chronic stress, including appetite changes, may further lead to nutrient deficiencies. In an attempt to manage chronic stress, women may use a number of approaches including the consumption of DSs, which they consume in greater quantities than men, despite insufficient evidence to support this behaviour. Figure 1.4 provides the conceptual framework linking the predisposing factors and pathways to chronic stress, possible negative outcomes and methods for managing stress.

This thesis is positioned within the JBI model of EBHC because the complex nature of this line of enquiry fits a model that supports diverse forms of evidence and takes into account that a number of factors contribute to the experience of health which include the role that society, culture and personal values play in such.[265, 269] As the objective of this study was to investigate the relationship between perceived stress and specific nutrients consumed in the daily diet and/or in DSs in a group of women, the first step in answering this research question was to investigate the present state of evidence. The author accessed, selected, appraised and extracted research that examined this relationship in a systematic review that clearly identified a knowledge gap.[10]

Chapter 2

THE IMPACT OF ESSENTIAL FATTY ACID, B VITAMINS, VITAMIN C, MAGNESIUM AND ZINC SUPPLEMENTATION ON STRESS LEVELS IN WOMEN: A SYSTEMATIC REVIEW

2.1 Introduction

This chapter is a modified version of the Systematic Review that was published (February 2017) as part of my PhD qualification, investigating stress and nutrient intake in women.[10] This review was conducted according to an *a priori* published protocol.[280]

2.2 Introduction to Systematic Review

The use of dietary supplements (DSs) fuels a global multibillion-dollar industry, with the figure estimated to reach $60 billion by 2020.[281] Furthermore, according to Goldstein Research, the global stress management DS market is expected to reach USD16.7 billion by 2025.[8] General DS usage is widespread, although some studies suggest use of vitamin and mineral supplements increases in people with a history of anxiety and/or depression.[4-6, 229, 230] Complementary or alternative therapies, which include DSs, were used by over half of the individuals diagnosed with an anxiety or mood disorder in a population level survey (n=2,055).[229] Prevalence of supplement usage has also been found to be higher among women compared to men.[223-228, 236, 237] Furthermore, evidence suggests women are 50% more susceptible to depression, generalized anxiety disorder, panic disorder, phobias and insomnia compared to men and this may influence the uptake of supplement usage among women and contribute to the global rise of DSs aimed at reducing stress.[47-51, 96]

Stress and anxiety not only appear to influence supplement usage, they are associated with nutritional deficiencies, which are thought to compound anxiety and stress.[2, 172] Biochemical evidence indicates that nutrients play a critically important role in CNS functioning.[133, 172, 282] In addition, subclinical nutrient deficiencies may influence psychological wellbeing and potentially precede physical or emotional/affective disorders.[172] Chronic stress leads to a cascade of negative physical and mental

sequelae such as cardiovascular disease, lifestyle-related diabetes, metabolic and immune dysfunction along with cognitive decline and depression.[2, 283, 284]

2.3 Objectives

The objective of this systematic review was to identify the impact of essential fatty acids (EFAs), B vitamins, vitamin C, magnesium and zinc, consumed as supplements to the daily diet, on stress levels in women.

2.4 Inclusion criteria

2.4.1 Types of participants

The systematic review considered studies that included all women over the age of 18 years, who had been included in a study where stress and/or anxiety was being assessed.

- Studies that included pregnant women were also considered.
- Studies that included both genders were considered where it was possible to extract data for female participants.
- Studies that included women taking prescription medication for anxiety or depression were excluded, as were hospitalized women or women suffering from eating disorders.

2.4.2 Types of intervention(s)/phenomena of interest

This review considered studies that evaluated the impact of supplementation with EFAs, B vitamins, vitamin C, magnesium and zinc at any dose/frequency on stress or anxiety being experienced by women. Supplements delivered in any form, including capsules, tablets, caplets, chewables, powders and liquids, at any dosage and for a duration of 14 days or longer were considered. The supplementation of these nutrients was investigated whether used individually, or in any combination.

2.4.3 Types of studies

This review considered experimental studies, including randomized controlled trials (RCTs), non-randomized controlled trials, and quasi-experimental studies. The review also considered observational studies, case series and case control studies and descriptive cross sectional studies.

2.4.4 Types of outcomes

The systematic review considered studies that included the following outcomes:

- Stress, as measured by any validated tool or measure, for example the Perceived Stress Scale (PSS) or the Perceived Stress Questionnaire (PSQ). Self- or observer reports were considered.
- Anxiety, as measured by any validated tool or measure, for example, the Beck Anxiety Inventory (BAI) or the State-Trait Anxiety Inventory (STAI). Self- or observer reports were considered.
- Physiological measures of stress, such as increased blood pressure or cortisol levels in blood or saliva were considered. The presence of stress-related symptoms, including, but not limited to, insomnia, irritability, headaches, fatigue, tense muscles, frequent upper respiratory tract and other infections, weight gain, weight loss, gastrointestinal disorders, such as irritable bowel syndrome (IBS) and loss of sexual desire were considered. Self- or observer reports were considered.

2.5 Search strategy

The search strategy aimed to find both published and unpublished studies. The review author conducted a preliminary search of the JBI COnNECT+ database, Embase, Prospero, CINAHL and PubMed, using the keywords below, and no prior or progressing systematic reviews addressing the effectiveness of EFAs, B vitamins, vitamin C, magnesium and zinc on stress levels in women were found. The search strategy aimed to find both published and unpublished studies. A three-step search strategy was utilized in this review. An initial limited search of MEDLINE via PubMed and Embase was undertaken, followed by an analysis of the text words contained in the title and abstract, and of the index terms used to describe the article. A second search using all identified keywords and index terms was then undertaken across all included databases. Thirdly, the reference list of all identified studies and articles was searched for additional studies.

Studies published in English were considered for inclusion in this review.

No date limits were stipulated in this review.

The following databases were searched:

MEDLINE (via PubMed)

Embase

Scopus

CINAHL

PsycINFO

In addition, the search for unpublished studies included the following databases:

MedNar

PsycARTICLES

National Institute of Mental Health

International Association for Women's Mental Health.

One unpublished paper was found in the CINAHL database.

Initial keywords used were:

Women, stress, psychological stress, mental fatigue, resilience, burnout, anxiety, omega 3 fatty acids, polyunsaturated fatty acids, micronutrients, dietary supplements, vitamins, B vitamins, Vitamin C, magnesium, zinc.

The full search strategy is provided in Appendix A.

2.6 Method of the review

Quantitative papers selected for retrieval were assessed by two independent reviewers for methodological quality prior to inclusion in the review using standardized critical appraisal instruments from the Joanna Briggs Institute Meta Analysis of Statistics Assessment and Review Instrument (JBI-MAStARI) (Appendix B). Any disagreements that arose between the reviewers were resolved through discussion, or with a third reviewer.

2.7 Data collection

Quantitative data were extracted from papers included in the review using the standardized data extraction tool from JBI-MAStARI (Appendix C). All included papers were RCTs/pseudo-RCTs, and therefore only the JBI data Extraction Form for Randomized and Pseudo-randomized Controlled Trials was used. The data extracted included specific details about the interventions, such as the populations, study methods and outcomes of significance to the review question, such as anxiety or stress scores and the interaction of specific nutrients with such. The authors of four of the selected studies that included both male and female participants provided the reviewer with gender-disaggregated data upon request, which facilitated data analysis and commentary.[184, 285-287]

2.8 Data synthesis

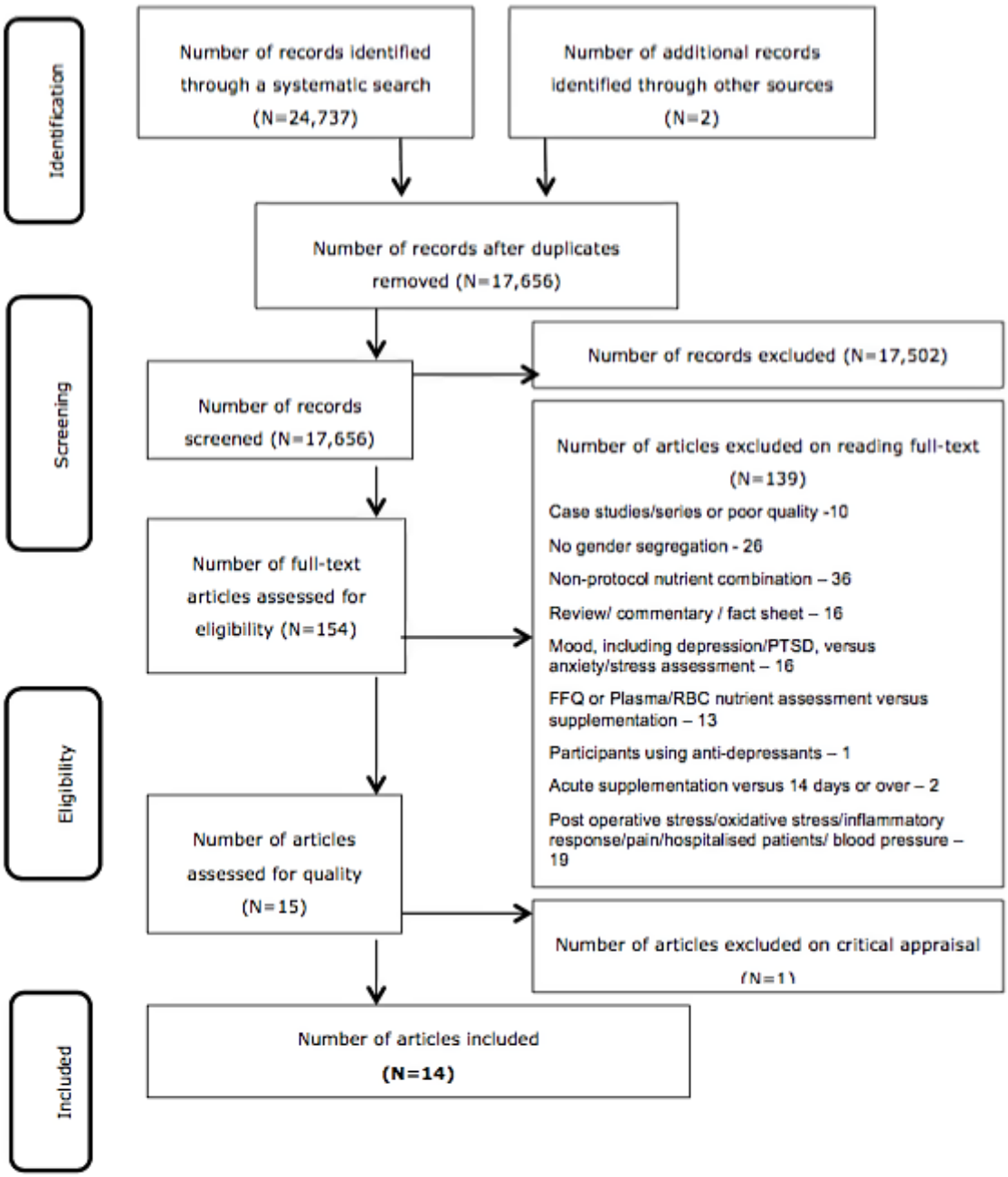
Statistical pooling was not possible due to the heterogeneity of the papers included. The data is therefore presented narratively, inclusive of tables, organised according to the outcomes of interest, stress, anxiety and physiological measures of stress. Means and standard deviations provided in

studies were used to compare treatment and placebo groups at baseline and endpoints using the independent t-test, with the online statistics calculator, graphpad (http://graphpad.com/quickcalcs/ttest2/) as raw data was not present to perform paired t-tests. Independent t-test results are more conservative than the results from paired t-tests. The disaggregated data provided for some studies was used in the same way. One study author provided raw disaggregated data, which was analysed in the statistical software program SPSS 23 (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.), where means and standard deviations were extracted allowing comparisons between groups using independent and paired t-tests and analysis of variance (ANOVA) statistical tests.[286]

2.9 Results

2.9.1 Study selection

A total of 24,739 papers were identified through database searching, including a further two from other sources. A total of 154 papers were considered relevant to the review based on assessment of the title and abstract and were retrieved in full for further evaluation. From these, 15 papers were selected and included for final assessment and methodological evaluation, although one of these papers was excluded when final methodological analysis was performed (Appendix D). References of the 14 papers were analysed but no papers met the protocol requirements for inclusion. Of the 14 research studies included for the review, 13 were published papers and one was an unpublished thesis. See the study selection flow chart for details.



From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). *P*referred *R*eporting *I*tems for *S*ystematic Reviews and *M*eta-*A*nalyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097

Figure 2.1: PRISMA Flow Diagram of selection and filtering process

All the included papers measured levels of stress or anxiety in females, using a variety of measurement tools, at specific points in time, related to hormonal phase or regardless of it. A total of 2,193 participants were assessed, and participant age ranged from 18 – 70 years. Out of the 14 selected papers, 13 mentioned the recruitment process utilised, with university students and/or graduates being volunteers in five of the papers,[286, 288-291] one paper using volunteers from a medical practice, the surrounding urban centre and local university,[285] one using respondents from an

advertisement in a local newspaper,[292] and one from the local university and urban area.[184] One used volunteers from the local metropolitan electoral roll,[293] while four mentioned the use of volunteers but provided no further detail,[287, 294-296] and one did not mention recruitment.[297] The participants represented a diverse range of socio-economic and cultural backgrounds.

All 14 included studies were RCTs or pseudo-RCTs. Four studies investigated the use of specific nutrients, namely magnesium, vitamin B6, or omega 3 fatty acids for the management of a variety of premenstrual symptoms, including anxiety.[290-292, 297] Three studies investigated the relationship between EFA supplementation and either peri- and post-menopausal symptoms, including anxiety and psychological distress during menopause,[295, 296] and prenatal stress,[294] while one investigated the role of vitamin B6 alone, or in combination with magnesium, in feelings of stress accompanying dysmenorrhea.[289] Of the six remaining papers, two investigated the relationship between the supplementation of the EFA (DHA) and cognitive and psychological functioning in young adults,[286, 288] one assessed mental wellbeing in older subjects who were taking a fish oil supplement,[287] and one investigated cognitive functioning and mood with DHA and eicosapentaenoic acid (EPA) supplementation.[285] The final two studies investigated the effects of vitamins and minerals, with the first one investigating the possible role of folate, vitamin B6 and vitamin B12 on memory and mood, which included anxiety, in women of various ages[293] and the final paper investigated the effect of high dose sustained-release vitamin C on subjective responses to psychological stress in young women.[184]

Two of the included studies used the TSST to induce stress in participants.[184, 294] The TSST is a standardised psychological laboratory-based stress induction procedure, which consists of specific instructions starting with the directive that participants will give a 5-minute speech to an unknown panel consisting of both genders, on their personal suitability for a job in the participants' field of interest. This is followed by a 5-minute mental arithmetic task, which consists of serial subtractions of 17, starting with 2023, with errors resulting in the participant being directed to start at the beginning of the arithmetic task. This test is performed in front of an audience and may also be recorded using a video camera.

Sample size varied between these 14 papers, with the smallest sample sizes being present in the hormonal phase studies, and the larger sample sizes being present in the studies that examined the outcome of interest regardless of hormonal phase. The participants in nine of the studies provided written consent.[184, 285-288, 290, 295-297] Four studies did not mention consent,[291-294] and although it was mentioned in another, no detail was provided.[289]

Although vitamin E was not one of the stipulated nutrients for study inclusion in the protocol, it is a commonly used antioxidant that acts as a preservative in EFA supplements, due to the sensitivity of the fatty acids to light, heat and oxygen.[298] Therefore, studies where it was included in the EFA supplement were included in this review.

The included studies were published between 1987 and 2015, with the majority being conducted during the last decade. The included studies were conducted from across a wide international range, namely Australia (1), Britain (5), Canada (1), Germany (1), Iran (2), the Netherlands (1) and the United States of America (3). A summary of each of the studies, including the participants, population, interventions and results can be found in Appendix E.

2.9.2 Methodological quality

All the papers selected for retrieval were critically appraised using the JBI Critical Appraisal Tool for RCTs or pseudo-RCTs (Appendix C). Table 2.1 is a summary of the methodological quality of all the included studies, where U=unclear, Y=yes, N=no and N/A =not applicable.

As summarised in Table 2.1, question one, eight of the 14 selected papers specified the specific randomization process used, with four of these studies using computer-generated stratified randomization lists to assign women to either an active treatment, or an identical placebo,[285-287, 296] and the other four used either a dynamic randomized algorithm,[295] a random number list,[184] a Latin square design to ensure randomization to four groups,[292] and a two-to-one ratio to randomly assign women to receive either active treatment or placebo, as the researchers expected greater variability in the dependant stress assessment.[294] Therefore, to optimise power, they chose to enrol a higher number of participants in the experimental group versus the placebo group. Although this randomization procedure has been criticised, it may have offered some benefits, as the women participating were pregnant and this randomization procedure does confer some benefits in statistical power for adverse event monitoring.[299] The other six papers stated that randomization was used to allocate participants to active and control groups but did not provide any detail regarding how this occurred.[288-291, 293, 297] The possible lack of randomization in these studies introduces the risk of allocation bias, which limits the internal validity of the studies and the conclusions that can be drawn from the interventions.

Participants were blinded to treatment allocation in 13 of the studies (Q2),[184, 285-291, 293-297] leading to the potential for performance bias in the paper where participant blinding did not occur.[292] Ten of those studies (Q3)[184, 285-287, 289, 293-297] concealed allocation to treatment groups from the allocator, and from those assessing outcomes (Q5), which introduced the potential for observation bias in the four papers where this did not occur.[288, 290-292]

Intention to treat (ITT) analysis was only adhered to in four of the studies (Q4),[285, 287, 293, 294] which introduces the potential for attrition bias in the 10 studies where ITT was not followed,[184, 286, 290-292, 295-297] as groups being measured at outcome may no longer be similar to the groups measured at baseline. The control and treatment groups were comparable at entry in 11 of the studies (Q6),[184, 285-289, 291, 292, 294, 296, 297] leaving the remaining three studies[290, 293, 295] open to potential risk of selection bias. All

of the studies, bar one, treated the groups identically apart from the named intervention, where participants were supplied with intervention on different days, where different instructions and participant differences in terms of hormone phase calculations may have impacted outcome measurement (Q7)[297] and only one study measured the outcomes differently for the two groups (Q8).[288] The potential for measurement bias is therefore high in these two studies.

All of the papers selected for this review used specific outcome measures to measure the stress or anxiety of the participants, with a number of the papers using a combination of measures to assess these psychological states. The outcome measures used ranged from high to low in terms of reliability, with 10 studies using reliable outcome measures (Q9),[184, 285-289, 293-296] while four used self-assessment methods based on diagnostic criteria in diaries, possibly introducing recall or recording bias.[290-292, 297] A full list of these 14 outcome measures can be found in Appendix 4 with references pertaining to test reliability.

All of the included papers used appropriate statistical analysis (Q10) and the disaggregated data was analysed by the reviewer using appropriate statistical tests, although a lack of raw data led to the use of independent t-tests in a number of studies where paired t-tests would have been more appropriate.[184, 285, 287, 289, 290, 294, 296, 297]

Table 2.1: Assessment of methodological quality

Citation	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10
Benton D et al.[288]	N	Y	U	N	U	Y	Y	N	Y	Y
Brody S et al.[184]	Y	Y	Y	N	Y	Y	Y	Y	Y	Y
Bryan J et al.[293]	U	Y	Y	Y	Y	U	Y	Y	Y	Y
Cohen LS et al.[295]	Y	Y	Y	N	Y	N	Y	Y	Y	Y
Davis LS.[289]	U	Y	Y	N	Y	Y	Y	Y	Y	Y
De Souza MC et al.[292]	Y	U	U	U	U	Y	Y	Y	U	Y
Jackson PA et al.[286]	Y	Y	Y	U	Y	Y	Y	Y	Y	Y
Kashanian M et al.[291]	U	Y	U	N	U	Y	Y	Y	U	Y
Keenan K et al.[294]	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Lucas M et al.[296]	Y	Y	Y	N	Y	Y	Y	Y	Y	Y
Rogers PJ et al.[285]	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Sohrabi N et al.[297]	U	Y	Y	N	Y	Y	N	Y	U	Y
van de Rest O et al.[287]	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Walker AF et al.[290]	U	Y	U	N	U	U	Y	Y	U	Y
%	64.29	92.86	71.43	28.57	71.43	78.57	92.86	92.86	71.43	100

Q1: Was the assignment to treatment groups truly random?
Q2: Were participants blinded to treatment allocation?
Q3: Was allocation to treatment groups concealed from the allocator?
Q4: Were the outcomes of people who withdrew described and included in the analysis?
Q5: Were those assessing outcomes blind to the treatment allocation?
Q6: Were the control and treatment groups comparable at entry?
Q7: Were groups treated identically other than for the named interventions?
Q8: Were outcomes measured in the same way for all groups?
Q9: Were outcomes measured in a reliable way?
Q10: Was appropriate statistical analysis used?

2.10 Findings of the review

The review findings are presented according to the outcomes stress or anxiety, or a combination of stress and anxiety, and physiological measures of stress assessed. Due to the heterogeneity of the included papers, which employed a wide variety of outcome measures (14 in total), it was not possible to perform a meta-analysis. (See Appendix F for details regarding the stress and anxiety outcome measures used in the studies.) The participants of the included studies also differed significantly in terms of age, as well as hormone cycle, and the nutrients used for supplementation differed in terms of

type, dosage, specific ingredients and nutrient combination. (See Appendix G for supplementary statistical data extracted for included studies not displayed in narrative or tables.)

2.10.1 Key findings related to the outcome stress

Both of the studies that examined the use of specific nutrients to reduce stress levels did so for women during a specific hormonal phase. Keenan et al. examined the association between EFA supplementation, perceived stress and cortisol response to a laboratory stressor, the TSST, during pregnancy in 64 African American women living in low-income environments.[294] The study ran for a maximum of 14 weeks with women being recruited from between 16 and 21 weeks of gestation. The treatment and placebo comprised two gel capsules to be taken daily, with treatment containing 450 mg DHA, 40 mg docosapentaenoic acid (DPA), eicosatetranoic acid (ETA) and EPA, with 15 international units of vitamin E. The placebo was matched in size, colour and smell and contained corn and soya oils. The ITT protocol was adhered to in this study. Cortisol response via saliva was collected at three time points during baseline, and at 24 and 30 weeks of gestation, 20 minutes after arrival at the laboratory, and 20 and 45 minutes post-TSST. Participants completed two outcome measures at these times, the Difficult Life Circumstances Scale (DLCS) which requires 28 either yes or no responses to present life challenges, with a score of 6 or less associated with increased stress and poor outcome, and the PSS-14 with a possible range of 0 – 56, with higher scores reflecting higher levels of stress.[300, 301] The reviewer used independent t-tests, due to a lack of raw data, to compare PSS scores at baseline, 24 and 30 weeks in the treatment group. There was no statistically significant difference in perceived stress in the EFA group at either 24 or 30 weeks gestation. In addition, statistical significance was not reached when comparing the stress scores of the EFA group at 24 weeks gestation or 30 weeks gestation with placebo stress scores (Appendix G). However, when controlling for negative life events and depression scores using ANOVA, perceived stress scores were significantly lower among the EFA group (mean 27.4) compared to placebo group (mean 29.5) at 30 weeks (F[1,47} 5.06, p=0.029, with Cohen's d=0.65). The reviewer used independent t-tests to compare circumstance stress at baseline and 24 weeks gestation for the EFA group. There was no statistically significant reduction in circumstance stress in the EFA group at baseline compared to 24 weeks. There was a statistically significant reduction in circumstance stress between the EFA group at baseline (5.16±3.4) and 30 weeks (3.91±0.6; (t(52)=2.1, p≤0.040) (Table 2.2). There was no statistically significant reduction in circumstance stress in the EFA group compared to the placebo group at follow up (Appendix G). The effect of EFA supplementation only had a significant effect on perceived stress and circumstance stress scores after 30 weeks gestation, suggesting that the length of time the participants were on the treatment influenced their subjective responses to stress and their challenging life circumstances.

Davis investigated the effects of vitamin B6 and magnesium supplementation on symptoms of stress and dysmenorrhea (very painful menstrual cramps).[289] Forty-seven women with dysmenorrhea were assigned to one of four dietary supplement groups: group 1) 500 mg magnesium per day; 2) 200 mg vitamin B6 per day; 3) 500 mg magnesium + 200 mg vitamin B6 or placebo. The supplements were to be consumed daily for four consecutive menstrual cycles, and stress response was measured at 10 and 14 days after commencement of study and during each of the four menstrual periods. The Symptoms of Stress Inventory (SOS) was used to assess participant stress levels, with higher scores reflecting higher levels of stress.[302] The reviewer used independent t-tests to compare SOS scores at baseline and endpoint for magnesium and vitamin B6 alone, and in combination, and to compare treatment against placebo. There was a trend towards decreased stress between baseline magnesium treatment (98.8±36.3) and endpoint (70.2±35.0, $t(24)=2.04$, $p\leq0.052$) and between baseline vitamin B6 treatment (67.7±26.6) and endpoint 45.1±21.9, $t(18)=2.07$, $p\leq0.052$). In addition, there was a downward trend with the combination of vitamin B6 and magnesium, between baseline (99.5±45) and endpoint (66.8±29.7; $t(20)=2.01$, $p\leq0.057$) suggesting that there may have been a synergistic effect of combining magnesium and vitamin B6. In addition, there was a downward trend with the use of vitamin B6, between endpoint (45.1±21.9) compared to placebo (73.6±39.5; $t(20)=2.03$,$p=0.055$) (Table 2.2). However, the use of magnesium alone and the combination of magnesium and vitamin B6 did not lead to a statistically significant reduction in stress at endpoint compared to placebo (Appendix G).

In summary, over time, the EFA treatment significantly lowered the pregnant participants' perceived stress levels and improved their perception of their difficult life circumstances.[294] Although there was a downward trend towards decreased stress levels using magnesium and vitamin B6 in isolation, and in combination, with women suffering from dysmenorrhea, statistical significance was not reached.[289]

Table 2.2: Summary of studies reporting stress as an outcome

Study	Intervention A	Intervention B	Intervention C	Intervention D	Results (Mean±SD)
Keenan K et al.[294]	DHA + DPA + ETA + EPA	Placebo	None	None	DLCS Scores Baseline treatment (N=20) 5.16±3.4 versus endpoint (N=34) 3.91±0.6 (t(52)=2.1;p=0.040*) PSS Scores ANOVA: controlling depression scores and negative life events At 30 weeks treatment (mean 27.4) versus placebo (mean 29.5) (F[1,47} 5.06, p=0.29, Cohen's d=0.65) Outcome measures: DLCS + PSS + cortisol (saliva)
Davis LS.[289]	Magnesium	Vitamin B6	Magnesium + vitamin B6	Placebo	SOS Scores Baseline (Mg) treatment (N=13) 98.8±36.3 versus endpoint 70.2±35.0 (t(24)=2.04, p=0.052*) Baseline (vitamin B6) treatment (N=10) 67.7±26.6 versus endpoint 45.1±21.9 (t(18)=2.07, p=0.052*) Baseline (Mg + vitamin B6) treatment (N=11) 99.5±45 versus endpoint 66.8±29.7 (t(20)=2.01, p=0.057*) Endpoint vitamin B6 (N=10) (45.1±21.9) versus placebo (N=12) (73.6±39.5; t(20)=2.03,p=0.055*) No statistically significant differences between magnesium alone or in combination with vitamin B6 at endpoint versus placebo Outcome measure: SOS

* p≤0.05

2.10.2 Key findings related to the outcome anxiety

Ten studies examined the effect of specific nutrients on anxiety levels, six of which did so for women experiencing a specific hormonal phase. Van de Rest et al. assessed the impact of omega 3 fish oil (DHA and EPA) supplementation on mental well-being in 139 women aged 65 ≥ years, over 26 weeks.[287] The EFA treatment was provided in six soft gelatine capsules daily, at either a high or low

dose, or placebo, all indistinguishable in appearance. The high dose active supplement contained a mean daily intake of 1093 mg EPA and 847mg DHA, and the low dose contained a mean daily intake of 226mg EPA and 176mg DHA, while the placebo group took oil capsules that contained oleic acid (olive oil). A subscale of the Hospital Anxiety Depression Scale - Anxiety (HADS-A) was used to identify anxiety symptoms, with higher scores reflecting higher levels of anxiety.[303] The reviewer used the means and standard deviations to perform independent t-tests on the disaggregated data and compared the HADS-A scores at baseline and three months for both the 400 mg and the 1800 mg groups. There was no statistically significant difference in anxiety in either the 400 mg or the 1800 mg EFA groups at baseline compared to three months or six months. There was no statistically significant difference in anxiety between the 400 mg group at three months compared to six months and no statistically significant difference in anxiety between the 1800 mg group at three months compared to six months. There was also no significant difference in anxiety comparing the 400 mg groups and the 1800 mg groups at six months. In addition, there was no statistically significant difference in anxiety between either the 400 mg or the 1800 mg groups compared to placebo at six months (Appendix G).

Benton et al. examined the effects of a DHA supplemented high oleic acid (omega 9) sunflower seed oil supplement over 50 days on the psychological functioning of a group of young female university students, aged between 21 and 28 years, with anxiety being a subscale of interest.[288] There was no detectable EPA, or other EFA in the mixture. Four 250mg capsules per day provided 400mg of DHA, while the placebo provided maize-soya oil, and was identical in colour, size and smell to the active capsules. The study was conducted in two phases, with two groups of 150 women each being recruited, three months apart. The data generated by these two groups of women was combined and statistics were employed to rule out the possibility that the time of the year could have influenced the results. Baseline testing used visual analogue scales (VAS) based on the Profile of Mood States (POMS) questionnaire, with higher scores related to greater frequency of distressed mood.[304] Benton et al. used a repeated measure ANOVA to assess the interaction between the outcome variable (composed-anxious dimension), the treatment (DHA versus placebo) and the study time period. Controlling for time, the authors examined the association between the anxiety score and DHA intake and found no significant association (F=2,566, p=0.38) (Table 2.3). Therefore, in this study DHA had no effect on anxiety among participants. Although the researchers mention that the dose of omega 3 may have been too small to introduce change, the presence of omega 6 in the placebo may have influenced this result.

Sohrabi et al. examined the impact of EFA supplementation over a three-month period on anxiety during premenstrual syndrome (PMS), according to the American College of Obstetricians and Gynecologists (ACOG) diagnostic criteria for PMS.[297, 305] The researchers prescribed 2g of omega 3 (comprised of 12% DHA and 18% EPA), found in two 1 g pearls, as a single daily dose, to be used for a

total of 30 days, while the placebo group were prescribed identical pearls containing no oil. Over the next two months the participants consumed their stipulated dose only on the 8 days preceding, and the 2 days after the onset of menstruation. A visual analogue score (VAS) was used to assess the severity of the symptoms, both at the start of the study, at 45 days and at 90 days from intervention, which were made up of 6 mood subscales, including the outcome of interest, anxiety. As the researchers commented on the significance of the results of a t-test, but did not specify these results, the reviewer used independent t-tests to calculate the mean differences between treatment and placebo groups using the means and standard deviations of the results at 45 and 90 days. There was a statistically significant reduction in anxiety severity in the EFA group (1.53±1.04) compared to the placebo group (4.07±0.91) at 45 days ($t(122)=14.45;p=0.0001$). There was however a statistically significant increase in the duration (days) of anxiety in the EFA group (9.81±1.43) compared to the placebo group (8.23±2.12) at 45 days ($t(122)=4.87;p=0.0001$). There was a statistically significant reduction in anxiety severity in the EFA group (0.79±1.04) compared to the placebo group (3.89±0.91at 90 days ($t(122)=17.64;p=0.0001$). There was a statistically significant reduction in duration (days) of anxiety in the EFA group (4.45±1.02) compared to the placebo group (8.23±1.94) at 90 days ($t(122)=13.64;p=0.0001$). There was a statistically significant reduction in anxiety in the EFA group at 45 days (1.53±1.04) compared to 90 days (0.79±1.04; $t(122)=3.96;p=0.0001$) (Table 2.3). Using a Chi-Square test, the authors examined the association between two binary variables, namely treatment or placebo and sedative use, either yes or no. On day 90, sedative use in the treatment group (15 cases 25%) compared to the placebo group (44 cases 73.3%) was significantly lower ($p=0.001$), suggesting that reduced sedative use may have been a result of reduced anxiety. The use of EFAs in this study reduced the severity of anxiety symptoms at 45 and 90 days, although anxiety duration was only reduced at 90 days, suggesting that this treatment became effective at reducing the duration of anxiety over time.

Cohen et al. used a multi-centre 12-week trial to determine the efficacy of EFAs in reducing anxiety, as a secondary outcome, in peri- and post-menopausal women.[295] Anxiety was measured using the Generalised Anxiety Disorder-7 (GAD-7) outcome measure, with higher scores related to higher levels of anxiety (range 0 – 21).[306] Either a 1.8 g/day EFA supplement (three pills per day), composed of 425 mg EPA, 100 mg DHA and 90 mg other EFAs, was used for treatment, or a matching olive oil-containing placebo. The reviewer used the means provided and the standard deviations calculated from the 95% Confidence Intervals provided in the paper to compare the mean differences of GAD-7 scores at 12 weeks treatment with the placebo group using an independent t-test. There was not a statistically significant difference in anxiety in the EFA group (-0.2±3.32) compared to the placebo group (-0.85±3.64) at 12 weeks ($t(335)=1.71;p=0.087$) (Table 2.3) although there was a downward trend

towards decreased anxiety. The use of EFAs in this study did not reduce anxiety significantly in the peri- and post-menopausal women, although there was a downward trend.

Lucas et al. compared the use of enriched ethyl-EPA with placebo for the treatment of psychological distress (PD) associated with menopause in 120 middle-aged women over an eight week period.[296] Twenty four % of the participants (29) experienced major depressive episodes (MDE). The Psychological General Well-Being (PGWB) outcome measure, which included anxiety among 22 items was used to assess PD, with lower scores related to higher rates of anxiety (range: 0 – 110).[307] Treatment and placebo consisted of a 500 mg capsule to be consumed three times a day for the study period, containing either 350 mg EPA and 50 mg DHA in the form of ethyl esters (the other 100mg is not mentioned) while placebo consisted of sunflower oil combined with 0.2% regular FO of which 18% was EPA and 12% was DHA for better matching of possible fish-oil taste, which was present in the treatment capsules. Assessing the data using independent t-tests, the reviewer calculated the difference in anxiety scores between the ethyl-EPA group at baseline and at eight weeks for all women. There was a statistically significant reduction in anxiety in the ethyl-EPA group between baseline (55.9±12.1) and eight weeks (68.3±15.9, $t(112)=4.7;p=0.0001$) regardless of the presence of MDE or not. There was a statistically significant reduction in anxiety in the ethyl-EPA group between baseline (42.5±13.0) and eight weeks (52.3±10.4; $t(23)=2.06;p=0.049$) for women who experienced MDE. There was a statistically significant reduction in anxiety in the ethyl-EPA group between baseline (59.8±8.8) and eight weeks (72.7±14.2; $t(87)=5.18;p=0.0001$) for women without MDE. There was a statistically significant reduction in anxiety in the ethyl-EPA group at eight weeks (72.7±14.2) compared to the placebo group (63.3±18.1) in women without MDE ($t(78)=2.6;p=0.011$) (Table 2.3) but there was no statistically significant difference in anxiety in the ethyl-EPA group at eight weeks compared to the placebo group in the presence of MDE (Appendix G). These results suggest that the effect of EFAS on anxiety in menopausal women may be reduced in the absence of MDE.

Walker et al. used the Menstrual Health Questionnaire (MHQ) and a self-report menstrual diary (MD) based on the Moos Menstrual Distress Questionnaire (MDQ) to investigate the effect of a daily supplement of 200 mg of magnesium oxide (MgO), or placebo, for two menstrual cycles on PMS symptom severity, including anxiety, among 41 women.[290, 308, 309] The menstrual diary symptoms had a range of 0 – 66, with higher scores reflecting more severe symptoms. Volunteers were assigned to two groups and for the first two cycles the first group took the supplement, and the second group took the placebo, and at the beginning of the third cycle, the groups crossed over for two cycles, thereby eliminating between group variance. The reviewer used independent t-tests to compare the mean anxiety scores at baseline treatment and endpoint, and treatment and placebo. There was no statistically significant difference in anxiety between the magnesium group at baseline compared to

endpoint and there was no statistically significant difference in anxiety between the magnesium group at endpoint compared to placebo (Appendix G). However, the scores were lower for anxiety after one month of magnesium treatment and the MHQ symptom scores indicated that the most prevalent symptom among the women was anxiety at 76%.

De Souza et al. examined the effect of 200 mg of magnesium and 50 mg vitamin B6, either alone or in combination or placebo, for the relief of anxiety-related PMS symptoms over a five-month period.[292] The authors used the MHQ to assess baseline pre-menstrual symptoms and a MD comprised of 30 symptoms, including anxiety, to assess anxiety over the study time period.[308] Participants were instructed to consume one of the three types of supplements or placebo, for one menstrual cycle, commencing on the first day of the second cycle, which meant that each participant changed to a different supplement according to this design on day one of her next cycle, until each of the four options had been completed, thereby eliminating between group variance. The reviewer was unable to perform any statistical tests. The researchers used a multivariate ANOVA to assess differences among the treatment group related to the dependant variable, anxiety, and the covariates, namely magnesium and vitamin B6. Using ANOVA, the authors state that there was a statistically significant interaction effect of magnesium and vitamin B6 for reducing PMS-related anxiety symptoms (p=0.040; mean scores of magnesium (22.0), vitamin B6 (21.8), magnesium + vitamin B6 (16.3), and placebo (19.8) (Table 2.3). Anxiety-related symptoms were the most prevalent symptom reported by women in both the MHQ and the MD, with 64% of the women reporting having stressful lives and 66% of them scoring the maximum scores for anxiety on the MHQ, which is a similar trend noted in participants in Walker et al's paper, above.[83] These results suggest that the combination of magnesium and vitamin B6 may be useful for reducing PMS related anxiety.

Kashanian et al. examined the effects of vitamin B6 on anxiety experienced during PMS. Forty-six women took 80 mg of vitamin B6 daily for two menstrual cycles, while 48 women took an identical looking and tasting placebo.[291] A recording sheet based on the American Psychiatric Association (APA) questionnaire was used to assess the presence of PMS (including anxiety) during the first three menstrual cycles without treatment.[310] The symptoms recorded during the first three cycles were compared to the symptoms recorded after the second cycle of treatment. The reviewer used an independent t-test to calculate the mean differences between the treatment and placebo groups. There was a reduction in anxiety in the vitamin B6 group (-0.22±0.35) compared to the placebo group at endpoint (-0.15±0.35) but the difference did not reach statistical significance (t(92)=0.969;p=0.3349) (Table 2.3).

Bryan et al. examined the effects of 35 days of using folate (vitamin B9), vitamin B12, vitamin B6 or placebo, on cognition and mood, including anxiety, in 211 healthy younger (20 – 30 years),

middle-aged (45 – 55 years) and older women (65 – 92 years).[293] Anxiety was measured using the POMS outcome measure at baseline and endpoint, with higher scores reflecting higher levels of anxiety.[304] The women were allocated to one of the four treatment groups, and consumed either 750 µg of folate (vitamin B9), 15 µg of vitamin B12, or 75 mg of vitamin B6, or a placebo, daily, for 5 weeks. All treatment groups' capsules were identical in shape and colour and the placebo contained calcium and magnesium. Each quartile constituted a sub-population within each age group, based on the intake of nutrients assessed by a food frequency questionnaire (FFQ), which was administrated to both the treatment and placebo groups prior to treatment. The researchers used either a 2-way ANOVA or repeated measures ANOVA to assess anxiety scores. No effect of treatment on anxiety scores over time within the three age groups was reported. However, independent t-tests performed by the reviewer to assess the differences between the endpoint treatment groups, for all ages, and the placebo groups, found a statistically significant reduction in anxiety at 35 days for the older age group using vitamin B6 (12.33±2.23) compared to placebo (14.70±3.42, $t(36)=2.53$;$p=0.015$). This result suggests that vitamin B6 may be effective at reducing anxiety in older women. The authors suggest that the general lack of effects of short-term B vitamin supplementation may be due to the short duration of supplementation.

In summary, EFA treatment was effective at reducing anxiety in one group of women experiencing premenstrual tension,[297] and one menopausal group in the absence of depression.[296] EFA treatment was ineffective at reducing anxiety in two groups of women experiencing no specific hormonal phase[287, 288] and one menopausal group.[295] A combination of magnesium and vitamin B6 was shown to be effective at reducing premenstrual anxiety in one group,[292] although the use of vitamin B6 alone was not shown to be effective at reducing anxiety in another premenstrual group,[291] and the use of magnesium in another premenstrual group was ineffective.[290] Regardless of hormonal phase, older women benefited with a significant reduction in anxiety from using vitamin B6, but not folate or vitamin B12, while folate, vitamin B12 or vitamin B6 used alone was ineffective at reducing anxiety in younger or middle aged women.[293]

Table 2.3: Summary of studies reporting anxiety as an outcome

Study	Intervention A	Intervention B	Intervention C	Intervention D	Results (Mean±SD)
van de Rest O et al.[287]	High dose EPA + DHA	Low dose EPA + DHA	Placebo	None	No statistically significant differences reported Outcome measure: HADS-A

Study	Intervention A	Intervention B	Intervention C	Intervention D	Results (Mean±SD)
Benton D et al.[288]	DHA	Placebo	None	None	No statistically significant interaction (p=0.38) between treatment and time; 3-way ANOVA (F=2,566), p=0.38) Outcome measure: VAS dimensions based on the POMS
Sohrabi N et al.[297]	DHA + EPA	Placebo	None	None	VAS scores of ACOG criteria for PMS 45 days treatment - severity of anxiety (N=63) 1.53±1.04 versus placebo (N=61) 4.07±0.91 (t(122)=14.4;p=0.0001*) 45 days treatment - duration of anxiety (N=63) 9.81±1.43 versus placebo (N=61) 8.23±2.12 (t(122)=4.8;p=0.0001*) 90 days treatment - severity of anxiety (N=63) 0.79±1.04 versus placebo (N=61) 3.89±0.91 (t(122)=17.64;p=0.0001*) 90 days treatment - duration of anxiety (N=63) 4.45±1.02 versus placebo (N=61) 8.23±1.94 (t(122)=13.64; p=0.0001*) Mean difference between treatment at 45 days versus 90 days (t(124) = 3.99, p=0.0001*) Sedative use in treatment versus placebo groups: Chi-Square test (p=0.0001*) Outcome measure: VAS of ACOG PMS diagnostic criteria
Cohen LS et al.[295]	EPA + DHA + other omega 3's	Placebo	None	None	GAD-7 Scores Endpoint treatment (N=169) - 0.2±3.32 versus placebo (N=168) - 0.85±3.64 (t(335)=1.71;p=0.087) Outcome measure (secondary outcomes - including anxiety): GAD-7

Study	Intervention A	Intervention B	Intervention C	Intervention D	Results (Mean±SD)
Lucas M et al.[296]	EPA + DHA (ethyl esters)	Placebo	None	None	PGWB Scores Baseline treatment (Combined MDE and no MDE) (N=59) 55.9±12.1 versus endpoint (N=55) 68.3±15.9 (t(112)=4.7;p=0.0001*) Baseline treatment (MDE) (N=13) 42.5±13.0 versus endpoint (N=12) 52.3±10.4 (t(23)=2.06;p=0.049*) Baseline treatment (no MDE) (N=46) 59.8±8.8 versus endpoint (N=43) 72.7±14.2 (t(87)=5.18;p=0.0001*) Endpoint treatment (no MDE) (N=43) 72.7±14.2 versus placebo (N=37) 63.3±18.1 (t(78)=2.6;p=0.011*) Outcome measure: PGWB
Walker AF et al.[290]	Magnesium	Placebo Crossover: after two cycles	None	None	No statistically significant difference between treatment baseline and endpoint or treatment endpoint and placebo group Outcome measures: MHQ; MDQ
De Souza MC et al.[292]	Magnesium	Vitamin B6	Magnesium + vitamin B6	Placebo Crossover: each participant crossed over to the next treatment until all 4 treatments were completed	MD anxiety scores MANOVA Statistically significant (p=0.040*) effect of combination Mg + vitamin B6 Outcome measure: MD

Study	Intervention A	Intervention B	Intervention C	Intervention D	Results (Mean±SD)
Kashanian M et al.[291]	Vitamin B6	Placebo	None	None	Anxiety scores Endpoint treatment (N=46) - 0.22±0.35 versus placebo (N=48) - 0.15±0.35 (t(92)=0.969;p=0.3349*) No statistically significant difference in anxiety scores between treatment baseline and placebo (p=0.3349*) Outcome measure: APA questionnaire
Bryan J et al.[293]	Folate	Vitamin B12	Vitamin B6	Placebo	POMS anxiety scores Endpoint treatment (Vitamin B6) 12.33±2.23 versus placebo 14.70±3.42 (t(36)=2.53; p=0.015*) Authors note: No effect of treatment on anxiety scores in any groups over time Outcome measure: POMS

* p≤0.05

2.10.3 Key findings related to the outcomes of stress and anxiety

Rogers et al. evaluated the effects of EPA and DHA supplementation on stress and anxiety as secondary outcomes in a group of mild to moderately depressed individuals over a 12-week period, using the Depression, Anxiety and Stress Scales (DASS) outcome measure, which is a 42-item instrument.[285, 311] Low scores indicate normal or mild levels of stress or anxiety and higher scores indicate moderate to severe levels. With the aim of maximising the participation of individuals with low mood who hadn't been labelled as such in a clinical setting, this study recruited people from a wide variety of places in an urban centre. Both the treatment and placebo groups were instructed to consume three capsules per day, which in the treatment group contained 630 mg EPA, 850 mg DHA, 870 mg olive oil, 7.5 mg mixed tocopherols and 12 mg orange oil, while the placebo group consumed 2360 mg olive oil, with an equal amount of tocopherols and orange oil. Using the disaggregated data, the reviewer calculated the differences between the baseline and endpoint scores, as well as the differences between treatment and placebo scores for the same time period using independent t-tests as raw data was not available with which to perform paired t-tests. There was a statistically significant reduction in stress in the EFA group between baseline (15.6±8.1) compared to 12 weeks (12.3±7.8; t(168)=2.70;p=0.0075). However, the comparative reduction in stress between the EFA and placebo groups at 12 weeks was not significant. There was a statistically significant reduction in anxiety in the

EFA group between baseline (5.9±5.6) compared to 12 weeks (4.2±4.6; t(168) =2.16, p=0.032) (Table 2.4). However, the comparative reduction in anxiety between EFA and placebo groups at 12 weeks was not significant (Appendix G). These results indicate that the EFAS used in this study may have had an effect on both the stress and anxiety levels of the participants in the treatment group over the 12-week period. However, there was no reduction in stress or anxiety when comparing EFA treatment to placebo group, as the placebo group also showed a reduction in stress and anxiety levels. Apart from the treatment, there may therefore have been another unknown variable that impacted both groups.

Jackson et al. aimed to examine the effects of two types of fish oil (FO) on healthy young adults using the DASS outcome measure, over 12 weeks.[286, 311] Each of the treatment groups were assigned a 1g daily dose, which was provided by 2 X 500 mg capsules, comprising 497.5 mg of deodorised FO with 2.5 mg mixed tocopherols (vitamin E). The first treatment group would be consuming DHA-rich FO, made up of 450 mg of DHA combined with 90 mg of EPA in a ratio of 5:1 DHA:EPA. The second group would be consuming EPA-rich FO, made up of 300 mg EPA and 200 mg DHA in a ratio of 3:2 EPA:DHA and the third group received 1 g of olive oil as placebo. The ratio of the DHA and EPA contained in the active treatments are a reflection of the range that exists naturally in fish consumed by the general population. Outcomes were measured at baseline and endpoint, using the raw disaggregated stress and anxiety DASS scores supplied to the reviewer. The means and standard deviations for the three groups were calculated and independent and paired t-tests were used to compare the DHA, EPA and placebo groups at baseline and after treatment. There was no statistically significant difference in stress or anxiety scores in either the DHA or the EPA groups between baseline and at 12 weeks. There was no statistically significant difference in stress or anxiety between either the DHA or the EPA groups compared to the placebo group at 12 weeks. Using ANOVA, there was no statistically significant difference found in stress (P value=0.686) or anxiety scores (P value=0.747) between the DHA, the EPA or the placebo groups (Appendix G). The use of EFAS for 12 weeks had no effect on the stress or anxiety scores of the female participants in this study.

Brody et al. examined the impact of supplementation on stress and anxiety by assessing the effects of a high dose sustained-release vitamin C (1000 mg) supplement, made up of two capsules, three times per day, in a group of 60 young adults over 14 days.[184] The treatment formula has a half-life of 19h unlike the conventional formula, which has a 2h half-life. Psychological stress was assessed using the German version of the Spielberger State Anxiety Scale (SSAS), also known as the State-Trait Anxiety Inventory (STAI) with higher scores reflecting higher levels of anxiety.[312] The researchers also assessed subjective stress responses with another outcome measure developed by the primary researcher, which measured stress on a scale from 0 (the least stress imaginable) to 10 (the worst

stress imaginable), although the details of this outcome measure were not provided in the research paper. During the pre-trial session, a brief medical examination was followed by a cannula being inserted into participants right forearm vein to enable assessment of cortisol and vitamin C levels in blood plasma. In addition, plasma cortisol response to an injection of 1μg of adrenocorticotrophic hormone (ACTH) was assessed after 45 and 60 minutes. During this time, participants completed the questionnaires and were then provided with the vitamin C supplements, and advised to consume them with ample fluid, which included two doses on the day of the post-trial session. During the endpoint laboratory session, participants again completed the questionnaires, and participated in the TSST. Subjective stress ratings were taken, at -10, -1, +10, +20, +30 and 40 minutes relative to the TSST. The reviewer performed independent t-tests using the disaggregated data to compare STAI scores at baseline and at endpoint after the TSST, and between treatment and placebo groups. There was a statistically significant reduction in anxiety in the vitamin C group at baseline (36.55±7.32) compared to 14 days (43.63±9.23), t(74)=3.7, p≤0.0004). There was also a statistically significant reduction in anxiety in the vitamin C group (43.63±9.23) compared to the placebo group (50.34±11.95) at 14 days (t(69)=2.66;p=0.0096). There was a statistically significant reduction in stress between the vitamin C group at baseline (3.71±2.02) compared to 14 days (2.25±1.35; t(74)=3.70, p≤0.0004) (Table 2.4). However the reduction in stress between the vitamin C and placebo groups at 14 days was not significant (Appendix G). These results suggest that vitamin C may be more effective at lowering anxiety than stress. Plasma vitamin C levels increased significantly over the course of the trial (F=3.9, p=0.002) for the treatment group (from mean of 1.55 to 2.65 mg/dl [88.0 to 150.5 mol/l]) but not for the placebo group (from a mean of 1.36 to 1.40 mg/dl [77.2 to 79.5 mol/l]). The researchers suggest that the use of high dose sustained-release vitamin C supplementation, rather than vitamin C from normal dietary sources, yields anxiety and stress-lowering effects. These results suggest that high dose sustained-release vitamin C may be effective at reducing induced psychological stress.

In summary, although stress and anxiety levels decreased significantly between baseline and endpoint for the EFA treatment groups in the Rogers et al. study,[285] they did not do so between treatment and placebo groups and there was no statistically significant difference between the treatment groups and placebo for stress and anxiety scores in the Jackson et al. study.[286] The use of high dose sustained- release vitamin C was effective at reducing anxiety when comparing treatment with placebo, but only reduced stress levels in the treatment group in the Brody et al. study.[184]

Table 2.4: Summary of studies reporting stress and anxiety as outcomes

Study	Intervention A	Intervention B	Intervention C	Results (Mean±SD)
Rogers PJ et al.[285]	EPA + DHA	Placebo	None	DASS stress scores Baseline treatment (N= 85) 15.6±8.1 versus endpoint 12.3±7.8 (t(168)=2.70;p=0.0075*) DASS anxiety scores Baseline treatment (N=85) 5.9±5.6 versus endpoint 4.2±4.6 (t(168)=2.16;p=0.032*) Outcome measure: DASS
Jackson PA et al.[286]	High dose DHA + low dose EPA	Low dose DHA + high dose EPA	Placebo	No statistically significant difference for any t-test or ANOVA Outcome measures: Bond Lader VAS and the DASS
Brody S et al.[184]	High dose, sustained-release vitamin C	Placebo	None	STAI Scores Baseline treatment (N=38) 36.55±7.32 versus endpoint (N=38) 43.63±9.23 (t(74)=3.7;p=0.0004*) Endpoint treatment versus placebo (50.34±11.95), t(69)= 2.66;p=0.0096*) Subjective Stress Scores Baseline treatment (N=38) 3.71±2.02 versus endpoint 2.25±1.35 (t(74)=3.70; p=0.0004*) Outcome measures: STAI

* p≤0.05

2.10.4 Key findings related to the physiological stress measurement outcomes

Keenan et al. examined the cortisol levels of pregnant women both before and after the TSST.[294] (Please see section 2.9, 'Results,' for details regarding the TSST.) Cortisol levels did not change as a function of EFA treatment at 24 weeks gestation, but at 30 weeks gestation, cortisol levels upon arrival at the laboratory were significantly higher in the placebo group. The authors state that they differed significantly as a function of EFA supplementation (mean 0.35 compared with 0.27; F [1.74, 74.63] 3.51,p=0.041) (Table 2.5). The placebo group had cortisol levels that were 20% higher than the EFA group at 30 weeks gestation following TSST; (active: placebo 1.86:2.24). The cortisol lowering effect of EFA supplementation was greater than its stress lowering effect over the 30 weeks of treatment, suggesting that EFA treatment may elicit physiological benefits but may not produce any noticeable psychological improvements in stress perception. The researchers suggest that EFA treatment may

influence the HPA axis by modulating the response to a social stressor as indicated by the significant differences in cortisol output over time.

Changes in systolic and diastolic blood pressure (SBP and DBP) in the presence of an acute psychological stressor, the TSST, were examined in relation to high dose sustained-release vitamin C treatment by Brody et al.[184] At baseline, participants supplied blood to assess cortisol levels, and saliva cortisol samples, blood pressure readings, and subjective stress ratings were taken, at -10, -1, +10, +20, +30 and 40 minutes relative to the TSST at endpoint. Upon completion of the last measurement, participants' blood was drawn to assess vitamin C and plasma cortisol levels, and the ACTH provocation procedure was repeated. There was no statistically significant difference in mean SBP measurements for the vitamin C group between baseline compared to 14 days, although there was a significant reduction in SBP measurements in the vitamin C group (111.28±11.41) compared to the placebo group at 14 days (118.72±9.44; t(69)=2.96;p=0.0041). There was a statistically significant increase in DBP in the vitamin C group at baseline (67.15±6.71) compared to 14 days (88.64±9.53; t(74)=11.36;p=0.0001). There was a statistically significant decrease in DBP in the vitamin C group (88.64±9.53) compared to the placebo group (95.21±11.64) at 14 days (t(69)=2.6;p=0.0011) (Table 2.5). These conflicting results suggest that there may have been an unknown variable impacting the vitamin C group, which led to increased DBP measurements between baseline and 14 days.

In summary, EFA treatment significantly reduced saliva cortisol over time[294] and SBP and DBP reduced significantly using high dose sustained-release vitamin C treatment in the presence of laboratory-induced stress.[184]

Table 2.5: Summary of studies reporting on stress using physiological outcomes

Study	Intervention A	Intervention B	Results (Mean±SD)
Keenan K et al.[294]	DHA + DPA + ETA + EPA	Placebo	Cortisol (saliva) (Mean 0.35 compared with 0.27; F [1.74, 74.63] 3.51,p=0.041*)

Study	Intervention A	Intervention B	Results (Mean±SD)
Brody S et al.[184]	High dose, sustained-release vitamin C	Placebo	Plasma vitamin C levels increased significantly during trial (F=3.9, p=0.002*) for treatment group (from mean of 1.55 to 2.65 mg/dl [88.0 to 150.5 mol/l]) but not for placebo (from a mean of 1.36 to 1.40 mg/dl [77.2 to 79.5 mol/l]) Blood Pressure Measurements SBP: Endpoint treatment (N=38) (111.28±11.41) versus placebo (N=33) (118.72±9.44) (t(69)=2.96;p=0.0041*) DBP: Baseline treatment (N=38) 67.15±6.71 versus endpoint 88.64±9.53 (t(74)=11.36;p=0.0001*) Endpoint treatment versus placebo 95.21±11.64 (t(69)=2.6;p=0.011*)

* p≤0.05

2.11 Discussion

The findings of this review have demonstrated that EFA treatment was effective at reducing stress in pregnant women[294] and a combination of vitamin B6 and magnesium may lower stress in women suffering from dysmenorrhea.[289] High dose sustained-release vitamin C was effective at reducing anxiety regardless of hormonal phase, but only reduced stress levels in the treatment group.[184] EFA treatment was effective at reducing anxiety in one group of women experiencing premenstrual tension,[297] and one menopausal group in the absence of depression[296] but was ineffective at reducing anxiety in another menopausal group,[295] and in two groups of women experiencing no specific hormonal phase.[287, 288] The use of vitamin B6 alone was ineffective at reducing anxiety in a group of premenstrual women,[291] and the use of magnesium in another group of anxious premenstrual women was also ineffective.[290] However, in combination, magnesium and vitamin B6 was shown to be effective at reducing anxiety in one group of premenstrual women.[292] Regardless of hormonal phase, older women benefited with a reduction in anxiety from using vitamin B6, but not folate or vitamin B12, while the same nutrients used alone were ineffective at reducing anxiety in younger or middle aged women.[293] Two studies that investigated both stress and anxiety found no significant reduction in either mental state from EFA treatment.[285, 286] Pregnant women's salivary cortisol was reduced with EFA treatment[294] and high dose sustained-release vitamin C treatment reduced the blood pressure of stressed women.[184]

A large proportion of the research conducted on supplementation and mental well being thus far has focussed on the relationship between nutrient supplementation and mood state, such as depression, cognitive functioning, memory or mental well being during old age, or during various stages of female hormone fluctuation, such as prenatal, pre-menstrual or menstrual phases, as clearly evidenced by the studies in this review. In addition, a small group of male and mixed gender studies

have investigated the effects of EFA or vitamin B or vitamin C or magnesium or multivitamin and mineral supplementation on stress and anxiety.[185, 193, 194, 196, 270, 313-318]

Studies wherein EFAs have been used primarily to treat female psychological stress and anxiety, regardless of hormonal phase, are not available for comparison with these review findings. However, there are some mixed gender and male studies that have investigated the use of these nutrients to treat stress and anxiety. A lowering of test anxiety in men was found in a small three-week study using EFAs[196] and anxiety was also reduced in a mixed gender study in which EFA treatment was used over a period of 12 weeks.[193, 297] These results are in agreement with the findings of this review where anxiety was reduced in PMS using EFAs over a 3-month period.[297] Another small male study conducted over three weeks using EFAs also resulted in reduced mental stress, possibly due to impacts on the central nervous system (CNS) according to the researchers.[313] Using physiological outcome measures of stress in an eight-week mixed gender study, EFAs significantly reduced heart rate and muscle sympathetic nerve activity (MSNA) reactivity to mental stress, supporting the hypothesis that EFA treatment may impact the CNS and thereby reduce mental stress.[194] This is in agreement with the findings of this review where pregnant women experienced a reduction in saliva cortisol after EFA treatment.[294] These studies were conducted over a significantly shorter period of time than any of the studies in this review that addressed stress or anxiety in women regardless of hormonal phase,[285-288] suggesting that gender may play an important, but currently overlooked role, in addressing the effectiveness of EFA treatment for stress and anxiety. A longer, two-month trial, analysing EFA treatment effects in a mixed gender study resulted in reduced plasma adrenaline and noradrenaline levels following treatment.[314] The researchers suggest that decreased HPA axis activation produced an anxiolytic effect, supporting Delarue et al.[313] and Carter et al's.[194] hypotheses relating to EFA activity in the CNS, and supporting the findings of this review. Biochemical, physiological and clinical findings, although sparse, support the hypothesis that EFAs may be useful in the treatment of stress and anxiety, although gender and length of study need to be taken into account when analysing results.[319-321]

Studies are not available wherein the use of the specific vitamins and minerals of interest to this review have been used to address female stress and anxiety regardless of hormonal phase. However, there are a few studies wherein the use of multivitamins (which include the review nutrients of interest) was examined in relation to stress and anxiety in both genders. In a mixed gender study that investigated the effect of a multivitamin on stress levels in a sample of people experiencing on-going social stress, a statistically significant difference was found between the treatment and placebo groups after 30 days.[270] Furthermore, two studies that examined subjective stress experienced by men found that a reduction in stress followed four weeks of multivitamin supplementation.[315, 316] Haskell et al. investigated the effect of multivitamin supplementation on cognitive functioning, mental fatigue and

mood in women, over a nine-week period and although supplementation improved cognitive function, it did not improve mental wellbeing, including anxiety.[317] A possible suggestion for the lack of treatment effectiveness is a gender-specific response distinction and the short duration of the study, a finding which is supported by a year long supplementation research project that yielded results in women's mood after 12 months, and supports the study comments in this review where a 35-day B vitamin supplementation program did not produce significant differences in all three age groups involved.[272, 293] In addition, the study in this review that examined the effect of vitamin B6 and magnesium on anxiety related to PMS found a significant difference after five months of treatment, again supporting the idea that the length of time that the nutrient is consumed impacts effectiveness.[292] Two studies wherein vitamin C was used to treat anxiety are available, and in agreement with the findings of this review, anxiety was effectively reduced using this form of treatment. In a 14-day study conducted by de Oliveira et al. anxiety was significantly reduced using vitamin C with a mixed-gender group, and anxiety was also reduced using the same nutrient, compared to vitamin E, in a study with mixed gender Type-2 diabetic patients conduced by Mazloom et al. over six weeks.[185, 318] These study comparisons suggest that the effectiveness of vitamin C in reducing anxiety is realized over a shorter period of time compared to possible B vitamin or EFA treatment effectiveness.

2.12 Limitations of the studies

Six studies used physiological measures of compliance to the interventions, leading to increased reliability in assessing treatment compared to placebo. To assess magnesium intake the magnesium-creatinine urine ratio analysis was used in two studies[290, 292] and EFA blood plasma assessment was used in three studies.[285-287] One study assessed levels of vitamin C in blood plasma.[184] The other eight studies used either capsule count,[289, 296] or self report,[293, 294] or a combination of self-report and capsule count,[295] while three of the papers did not mention compliance.[288, 291, 297] A lack of compliance evidence introduces the possibility that the placebo effect was responsible for any observed changes, leading to a lack of confidence in assessing outcomes related to treatment.

The use of olive oil alone in four studies,[285-287, 295] or in combination with maize and soya oil[288] may have acted as a confounding variable in these studies, rather than as placebos, which should be inert substances, due to a possible active effect of these oils. Sunflower oil alone,[296] and a combination of corn and soybean oil[294] were used for the same purpose in two other studies, and may have acted as confounding variables in these studies too.

The presence of antioxidants and plant sterols in olive and sunflower oil, and pro-inflammatory eicosanoids that are derivatives of the omega 6 EFAs used in these studies, found in sunflower oil, may

have acted as treatment confounders. Antioxidants inhibit damage caused by reactive oxygen species (ROS), which may improve neuronal membrane integrity, function, and neurotransmitter synthesis[322, 323] and plant sterols may lower high cholesterol and improve fat digestion,[324, 325] influencing the efficacy of essential fats being consumed as part of the participant's diet, consequently influencing membrane function and neurotransmitter synthesis.[191, 193, 325, 326] This may have been beneficial for the participants in the placebo group, thereby limiting the reliability of conclusions that can be drawn between the treatment and placebo groups in these studies, none of which reported significance between treatment and placebo.[285-287, 295] Inflammatory prostaglandins promote neuronal membrane inflammation and cytokine production which has been linked to increased anxiety[322, 327] and may have limited the reliability of the conclusions drawn between the treatment and placebo groups in these studies,[294, 296] with the longest running study showing no significant difference between treatment and placebo.[288] Only one study used an inert substance as a placebo, where significance was reached over time.[297]

The EFA manufacturing process, which introduces light, heat and oxygen to the fats being processed, leads to fatty acid degradation and the transformation of EFAs into a variety of peroxidation products, such as trans, polymerized, cyclised and cross-linked fats. [298, 325, 328-331] The presence of these damaged fats in neuronal cell membranes may lead to disturbances in membrane and neurotransmitter synthesis and function,[298, 325, 332] but was not mentioned in any of the studies. Two of the three studies that mentioned the form of magnesium used,[290, 292] made use of magnesium oxide, which has a limited range of bioavailability (0 – 4%) and may therefore have limited treatment efficacy.[39, 333] The use of a variety of compounds in placebos such as pressed lactose[289] with possible effects on lactose-intolerant participants, or calcium and magnesium[293] with magnesium's anxiolytic effects[39] or amino acids[289] or sedative use among participants[297] may have acted as confounding variables in these studies, limiting the reliability of their results. The presence of a variety of genetic polymorphisms may influence folate, (vitamin B9), vitamin B6 and vitamin B12 synthesis[334] and may have influenced the efficacy of these nutrients in the participants in the Davis, Kashanian et al., De Souza et al., and Bryan et al. studies.[289, 291-293] A polymorphism on the methylenetetrahydrofolate reductase gene (MTHFR) which influences the breakdown of vitamin B9 (folic acid) into methionine, an important amino acid which facilities protein synthesis and antioxidant utilization, and which has been implicated in a number of mental health challenges may have been present in an unknown number of participants that took part in the Bryan et al. study, and participants were not tested for this genetic variation.[293, 334, 335] The presence of this polymorphism may have influenced treatment outcome, as 10-20% of the worldwide population may possess this gene variant, although these percentages do vary according to ethnicity and geographical location.[334-337] These confounding variables introduce variance between the treatment and placebo groups that is not due to the treatment, thereby limiting the reliability of the results. In other words, the

active placebo ingredients act as extra independent variables, possibly having a hidden effect on the dependant variable, stress or anxiety, or, genetic variations inhibit the efficacy of the nutrient, also limiting result reliability.

The choice of participants as well as the size of the sample, can lead to limited generalizability of results. Five of the 14 papers recruited student volunteers as participants, introducing selection bias, and limiting the generalisability of the results.[286, 288-291] Recruiting stressed or depressed volunteers may have led to confounding due to very high levels of stress influencing treatment outcome or effectiveness[294] and depressed people may experience less stress or anxiety reduction from treatment than people who are not depressed.[285] Wide variation between study participants as indicated by the inter-quartile variation within age groups[293] does not facilitate a meaningful comparison between the groups, and variations in baseline nutrient intake as measured by FFQ analysis, may have introduced further confounding, although this phenomena was likely not limited to this study alone.

Sample size varied from just over 40 to 355 participants, leading to uncertainty regarding the generalizability of the results from the small samples. Four of the researchers reimbursed participants, leading to possible confounding,[184, 288, 295] with one using an accelerated schedule of reimbursement, where an increase in payments occurred for each subsequent visit, which may have influenced compliance reporting, especially as participants were drawn from a low income population. [184, 288, 294, 295]

Eight of the selected papers assessed the use of specific nutrients during a particular hormonal phase, limiting the generalizability of the results.[289-292, 294-297] The longest study that investigated the impact of EFA treatment on anxiety lasted for 26 weeks,[287] while the longest time period for any of the other nutrients investigated was five months.[292] Long-term studies may be required to adequately assess the impact of nutrients on mental wellbeing, with a study by Benton et al. showing a beneficial response in terms of mental composure only after 12 months, for female participants consuming a multivitamin and mineral supplement.[272] De Souza et al's. study supports this suggestion, as it was the longest running included study and significance was reached at five months.[292]

The wide variety of outcome measures used to assess outcomes, notwithstanding the fact that the majority possessed reasonable reliability, makes it challenging to compare results between studies. The use of outcome measures that have not been tested for reliability, such as a menstrual diary in four of the studies, within which participants recorded feelings of anxiety and stress, may introduce recall bias and a lack of consistency over time.[290-292, 297] Recording bias may also be a challenge in this type of measurement, with participants or researchers inaccurately deciphering responses.[338] Repeat testing bias may have been introduced when participants were exposed to the same outcome measure on both training and testing day, possibly limiting the results that can be drawn from this study.[286]

2.13 Limitations of this review

A significant challenge in attempting to compare the studies that used EFAs as treatment was that the actual amounts and combinations of EFAs consumed by the participants varied significantly. Clearly, comparing the use of these EFAs in differing doses, coupled with placebo's that contain substances with potentially confounding effects leads to an inability to adequately comment on the impact of treatment. In addition, none of the studies assessed the impact of any of the nutrients on participants' levels of perceived stress, but instead induced stress via the TSST.[184, 294] Eight of the 14 studies investigated stress or anxiety during specific hormonal phases,[289-292, 294-297] reducing generalizability, with six of these only investigating anxiety or stress as a secondary outcome, or as a small sub-scale of an outcome measure or self-report diary.[290-292, 295-297] The large range of outcome measures used to measure stress and anxiety, with varying rates of reliability, also make comparisons between these studies challenging. Due to a lack of raw data for the majority of the studies it was not possible for the reviewer to use any other statistical tests besides the independent t-test, which limited conclusions to be drawn from study results. No studies examining the impact of vitamin B1, B2, B3, B5 or zinc on female stress were identified during the systematic literature search. Due to the possible population variability in nutrient intake, as evidenced by Bryan et al. it may not be possible to assess the effect of supplementation on levels of stress or anxiety unless the baseline nutrient status is comparable, which was only established in the afore mentioned study.[293] Individual differences in age, body weight, nutrient and specific fat content of diet, metabolic rate, nutrient absorption, hormone phase, and/or the presence, absence or balance of specific hormones at the time of treatment, as well as general mental health, including the ability to manage stress and the perception of stress, and inborn errors of metabolism, such as gene polymorphisms, may all influence the impact of nutrient treatment on participants. The gathered evidence in this review is therefore conflicting, as there is no consensus as to whether the treatments were effective for reducing stress and/or anxiety in either non-hormonal or hormonal phases.

2.14 Conclusion

Stress and anxiety are common mental health challenges that affect women regardless of age. Within the specific nutrient treatments examined, no consensus was reached as to the effectiveness of the nutrients at reducing perceived levels of stress or anxiety. Using the best available evidence, this review suggests that EFA treatment may be effective at reducing stress during pregnancy and reducing anxiety during premenstrual syndrome, and during menopause in the absence of depression. Magnesium and vitamin B6 may be effective in combination at reducing stress in premenstrual women and vitamin B6

may lower anxiety in older women. Salivary cortisol may be reduced using EFA treatment and anxiety and blood pressure may be reduced using high dose sustained-release vitamin C.

2.14.1 Implications for practice

The use of EFAs may be useful at reducing prenatal stress levels and cortisol levels, as well as premenstrual anxiety or menopausal anxiety in the absence of depression (Grade B).[339] Magnesium and vitamin B6 in combination may be useful in reducing premenstrual stress (Grade B).[339] Vitamin B6 may be effective at reducing anxiety in older women (Grade B).[339] High dose sustained-release vitamin C may be effective at reducing anxiety and high blood pressure (Grade B).[339]

2.14.2 Implications for research

This review highlights the lack of research into the role that specific nutrients may play in reducing stress and anxiety in women regardless of hormonal phase. The use of EFA, B vitamin, vitamin C, magnesium and zinc treatment to address female stress requires further investigation using long-term studies, regardless of hormonal phase. Future research should take into account the complex interplay of daily life with its ongoing inherent stressors, coupled with individual differences, including nutrient status at baseline.

2.15 Conflict of interest

The authors declare no competing interests in relation to this systematic review.

A dearth of evidence to support the use of specific nutrients to reduce stress in the general female population was evident from the results of this systematic review. The knowledge gap thus identified led to the design and implementation of two primary research projects, a quantitative and a qualitative project. Firstly, a cross-sectional survey was conducted that examined perceived stress and nutrient intake via diet and/or DSs among a group of mixed-age women on the Gold Coast, in Australia.

Chapter 3

THE RELATIONSHIP BETWEEN SPECIFIC NUTRIENTS CONSUMED VIA THE DIET AND/OR DSS AND STRESS LEVELS AMONG A GROUP OF WOMEN: A CROSS SECTIONAL PROJECT

3.1 Introduction

The purpose of this chapter and research project was examine the relationship between perceived stress scores (PSS) and specific nutrients consumed in the diet and/or via DSs among a group of women in Queensland (Gold Coast), Australia. This project included three questionnaires, which gathered data about the perceived stress of the participants, the types of DSs used and associated variables, and food choices. The data was analysed to ascertain whether there was a relationship between any of these variables. The position of this project within the JBI EBHC model is also addressed in this chapter.

Although a growing body of evidence suggests that nutrients have an important role to play in mental health and specific dietary patterns (DPs) have been associated with such, the impact of nutrition and nutrient deficiencies on the experience of stress in women is presently underexamined.[163, 164, 170] The use of stress-targeted DSs is prolific, evidenced by the global expenditure on these products, which is estimated to reach USD16.7 billion by 2025.[8] However, the efficacy of these products, particularly in relation to women is currently unknown. The few experimental studies that have investigated this phenomenon used a combination of nutrients despite the prolific use of stress-targeted, nutrient-specific DSs.[180, 270, 317]

Stress hormone synthesis takes precedence over the synthesis of neurotransmitters that regulate mood, such as dopamine and serotonin, and the synthesis of all these compounds require many of the same nutrients.[2, 132, 133, 340] Whether a lack of specific nutrients has a role to play in the development of affective disorders is unknown at present, however, it is well recognized that chronic stress has the potential to lead to the development of depression and anxiety.[46-51] Nutrient sufficiency may therefore provide neurobiological support in the prevention of affective disorders. As women report higher levels of stress than men,[17, 18, 20-26, 28] are more likely than men to become depressed following

stressful life events,[52, 53, 284, 341-343] and use more DSs than men, research into whether the consumption of specific nutrients is related to lower stress levels among women is warranted.[223-225, 229]

3.2 Situating Quantitative Research Evidence Within The JBI Model of EBHC

As discussed in Chapter one, the JBI Model of EBHC takes into account that a wide variety of methodological approaches to health research may be useful because different approaches answer different questions (Figure 1.5, Section 1.9). The JBI model comprehensively facilitates the discovery of knowledge gaps, supports the generation of new knowledge and provides expertise in sharing knowledge by providing a useful framework to support researchers focused on producing EBHC.[344] This model therefore incorporates and supports a variety of approaches to evidence generation, synthesis, transfer and implementation, which in the case of this exploratory research project and as a first step, led to the identification of a knowledge gap, which resulted in a systematic review. (Chapter 2)[10, 265]

The centre of the JBI model, the 'pebble of knowledge,' summarises the foundation of evidence and how it informs practice via the FAME scale.[265] The broad conceptualisation of JBI-FAME clearly and simply reflects the variety of research approaches that health researchers may use, such as the degree to which an activity or intervention is practical within a specific context (Feasibility), the degree to which an activity or intervention fits within a particular context (Appropriateness), the degree to which an intervention is meaningful and experienced positively by individuals or groups (Meaningfulness), and the degree to which an intervention achieves intended results (Effectiveness).[265] JBI-FAME acknowledges that there is a need for diverse forms of evidence to support complex knowledge needs when investigating complex clinical questions including mental health challenges. This framework is therefore supportive of research that is informed by the specifics of the research question, such as is the case with this cross-sectional survey aimed at investigating the unexamined relationship between a psychological construct and a specific behaviour.[265]

3.3 Methods

3.3.1 Introduction

Questionnaires are a frequently used form of data collection, as they can collect large volumes of data about participants, including their knowledge, opinions, values, behaviour and attitudes, and can be either specific and narrow in scope, or more global and generalized.[345, 346] Three questionnaires were used in this cross sectional survey. The PSS is a validated measurement instrument that is used globally.[14] The dietary questionnaire for epidemiological studies (DQES) is an Australian validated food

frequency questionnaire.[347] The supplement use questionnaire (SUQ) was developed by the researcher and details regarding the piloting and testing of face validity for this instrument are addressed in section 3.3.12.2.

Combining the quantitative results from this survey with those from the qualitative project (Chapter 4) revealed a deeper understanding of the phenomena under investigation.[348]

3.3.2 Ethics approval

Ethics approval for this project was received on the 6th of May 2016 (H-2016-090) (Appendix H).

Participants provided consent on two occasions. SurveyMonkey, an online tool that allows for the design of surveys and the gathering of data was used for such, wherein the first page of the online survey form advised participants that by clicking the 'continue' button they were providing consent to the use of their data for the research project.[349] Secondly, an email was sent to participants with a unique, numerical Internet-based (URL) identification (ID) code providing access to the online DQES survey developed by the Cancer Council of Victoria (CCV) and advised them that by linking to the DQES they were providing consent to the use of their data. Appendix I contains the survey introductory page from the SurveyMonkey online form.

Participant data was anonymized after data cleaning when all the data from all three survey questionnaires were merged into an excel spreadsheet ready for upload and data analysis in Stata (version 15, Stata Corp, College Station, Texas, USA).

Appendix J contains the study timeline.

3.3.3 Population of Interest

Women living on the Gold Coast, in Queensland, Australia.

3.3.3.1 Sample size

Eighty-seven women were recruited to the project. A total of 74 women completed all three surveys.

3.3.3.2 Sampling and Recruitment Procedure

Recruitment was initially scheduled to occur over a 12 week period, from the 25th May 2016 to the 10th August 2016, and was initiated via information flyers at three locations on the Gold Coast, a medical practice, a pharmacy and a health store, with the aim of investigating whether women visiting these locations differed in their experience of stress and nutrient and DS use. The uptake of information flyers from the three locations was poor and it was therefore decided to change the recruitment strategy at week ten, as only four women contacted the researcher to take part in the research project and only from the health store flyers. All the flyers were removed from all the centers during week 12.

As a result of these poor recruitment results and with approval from the ethics board, it was decided to implement a combination of convenience and snowball recruiting, starting from the 12th of August, to run for four weeks, until the 9th of September 2016. The researcher wrote a blog post for a radio station inviting participation, sent an invitation to council workers via the council e-newsletter and invited female friends to participate and share details about the project with other interested women face-to-face and via Facebook. This is therefore a non-probabilistic sample.

All recruitment channels provided the researcher's email address, a telephone number dedicated to this project and a link to a Facebook page. Respondents expressed their interest to the researcher via any of these methods. (See Appendix K and L for details regarding the blog post and Facebook page respectively.)

Participants were aged between 21 and 65 and representativeness of age in this sample was assessed using the census data from the Australian Bureau of Statistics (ABS). The only available age related data for people living on the Gold Coast, Australia, related to both genders and reported that the median age was 38 as at 2016.[350]

A dedicated Facebook page, 'Female Stress and Nutrition – Research Project,' was created prior to recruitment to provide respondents with information about the project and as a means to contact the researcher via private message, email or telephone (Appendix L). The same options were available to women who did not use Facebook. Women were offered a free e-book about skin care via the Facebook page as an incentive for completion of all three surveys.

3.3.4 Response Rate

A total of 87 women responded to the invitation to participate in the study, 13 of which failed to complete all three surveys, for an 85% completion rate.

3.3.5 Eligibility and exclusion criteria of participants

Participants were deemed to be eligible to participate in the research project if they were female residents of the Gold Coast at the time of recruitment, aged from 18 years, and could provide informed consent. Participants were deemed ineligible if they did not live on the Gold Coast, did not fall within the age range, or were unable to provide informed consent.

3.3.6 Power Analysis

This research project was an exploratory research project aimed at gathering preliminary data to inform hypothesis generation and support further research.[346, 351] No formal sample size calculations were performed.

3.3.7 Procedures

SurveyMonkey was used to deliver the questionnaires to, and collect the data from participants. Delivering the surveys via SurveyMonkey enabled the participants to complete them when convenient and communication via the Internet allowed the researcher to send email reminders about completing the surveys and allowed for the verification of supplement brands, which was necessary on occasion, prior to data anonymization.

3.3.8 Contacting participants

Respondents provided their email address to the researcher who sent them two emails, one containing the ID link to the DQES and the second email was sent via SurveyMonkey where the PSS and SUQ were available for completion. See Appendix M for an example of these emails.

3.3.8.1 Participant information sheets and recruitment cover letter

The first email contained two documents: a recruitment cover letter, which was a two-page information document that explained in detail the nature of the project, including data anonymization and the contact details of all involved researchers, and a one-page participant and survey information sheet which briefly explained the aim of the research project with instructions to complete the questionnaires. See Appendix N and O for examples of these documents.

3.3.8.2 Researcher follow-up and reminders

The researcher sent a reminder email or Facebook message a week after the respondent received the initial emails. The respondent was reminded of the surveys and asked whether they had any questions related to the survey. On occasion a respondent required the survey links again, which the researcher supplied. Two weeks after sending the previous reminder email the researcher again sent a reminder about the survey. A final reminder was sent two weeks prior to the conclusion of the study, 12 weeks after the initial email was sent. Completes were thanked via email or Facebook and were sent the skin care e-book.

3.3.9 Data Collection

Data from the PSS and the SUQ was captured online via a SurveyMonkey form and exported into a spread sheet. Anonymous data from the DQES was captured online and analysed by the CCV after which it was sent to the researcher. All the data was held on the researchers private computer with two separate backups taking place every day, which the researcher alone had access to. The de-identified data was shared with a statistician for data analysis.

3.3.9.1 Data Cleaning

Data cleaning was undertaken by checking all the data to ensure no data was missing, incomplete or unusable and data quality was improved where necessary by correcting detected errors and omissions.

3.3.9.2 Merging Data

A spread sheet containing data for each participant was updated daily as completion of the surveys occurred, which allowed for follow up with participants who had not completed all three surveys. The DQES ID link was the code used to link participant data from all three questionnaires and simplified de-identification after the recruitment for the PAR project (Chapter 4) was complete. Nutrients not of interest to this project were removed from the data set supplied by the CCV before a final merged spread sheet was ready for upload into Stata (version 15, Stata Corp, College Station, Texas, USA).

3.3.9.3 Data Analysis

Participant characteristics and nutrient intakes were reported using frequencies and percentages for categorical items, and descriptively summarized using means and standard deviations for continuously measured items. Associations between categorical variables of interest were assessed using Fisher's Exact tests, and differences between continuously-measured outcome variables between participant sub-groups of interest were explored using the Wilcoxon Rank-Sum test and the student t-test. The relationships between specific nutrients and PSS scores were examined using Spearman's correlations and multivariable linear regression. In light of previous research the effect of particular nutrient combinations was analysed.[292, 352]

Analyses were carried out using Stata (version 15, Stata Corp, College Station, Texas, USA). The two-tailed level of significance was set to 0.05. Corrections for multiplicity were made using the Sidak-adjustment.

3.3.9.4 Instrumentation

Three questionnaires were used to gather data in this project, the PSS, the DQES, and the SUQ, the development of which was necessitated prior to data collection.[14, 347] (Appendix P and Q.)

3.3.10 Assessment of Perceived Stress using the Perceived Stress Scale (PSS)

Cohen et al. designed a self-report perceived stress measurement tool, the PSS-14, in 1983 as a means to measure the degree to which life situations were perceived as being stressful and how they may impact physical health.[14] Items were designed to account for how uncontrollable, unpredictable and overwhelming respondents experience their lives over a one-month time period, all central elements related to the experience of stress.[14]

In 1988 Cohen et al. shortened the PSS to a 10-item measurement tool, which was used in this survey.[353] The PSS-10 has been validated and used widely in a variety of English speaking, European and Asian countries, and in a number of fields, including business and health, to assess the degree of global stress being perceived in a given situation, the effectiveness of an intervention on psychological stress and the effect of perceived stress on quality of life.[354]

The PSS is not a diagnostic or screening tool but rather a self-administered tool to measure changes in perceived stress over time. There are no categories and only comparisons are made of total scores between people within the sample of interest.[14, 353] The minimum score representing no perceived stress is 0 and the maximum score representing the highest perceived stress is 40. See Appendix P for an example of the PSS-10.

The tool is self-administered and uses a matrix-style answering grid, with five response options for each of the ten questions, namely 'Never,' 'Almost never,' Sometimes,' Fairly often,' and 'Very often.' The scaling responses for each response option are 0, 1, 2, 3, and 4 respectively. Questions four, five, seven and eight are positively stated items, and scores are obtained by reversing these scores and then summing across all 10 items. This was added to a SurveyMonkey template and software logic made it possible to automatically score the responses yet keep them hidden from the participant which provided the researcher with the total PSS-10 score for each participant.

The PSS does not tie the appraisal of questions to specific situations and is therefore sensitive to the non-occurrence of events, which may also be perceived as stressful. This is of importance to this project because chronic stress may not be related to specific events. In addition, the PSS-10 is a short, one-page questionnaire that is simple to administer and score. Furthermore, the PSS-10 was designed for use in community samples with at least a high school education and the questions are of a general nature and therefore free of content that is specific to any subpopulation group.[353]

3.3.11 Assessment of Nutrient Intake via Food using the Dietary Questionnaire for Epidemiological Studies (DQES)

The DQES was originally developed by the CCV to measure dietary intake of people participating in the Melbourne Collaborative Cohort Study (MCCS).[347] The DQES version 3.2 (DQES v3.2) is an updated version of the DQES v2 designed to cover dietary intake over a period of 12 months.[347]

This FFQ reports on participant's usual intake of 152 foods and six alcoholic beverages using 37 questions; responses are guided by food portion images and beverage consumption guides. A combination of food frequency, multiple responses to a variety of foods, portion size, seasonal fruit intake and alcoholic beverage intake are used to calculate the approximate daily equivalent of nutrient intake for each participant. Nutrient intakes are computed using the Nutrient Tables for use in Australia

(NUTTAB) and Australian Food, Supplement and Nutrient (AUSNUT) reference and survey databases respectivly.[355-357] Intakes are expressed in gram/serves or g/mg of nutrients per day. Most food item questions use a 10-point scale and questions move from simple to complex.

This FFQ is a comprehensive, computer-based tool that can be self- or interviewer administered and was validated for assessing habitual dietary intake in the Australian population.[358] The first page of the DQES provides instructions about completing the survey along with details about the survey aims. Three options are present in the form of 'buttons' at the bottom of each screen 'page' facilitating a move to the previous page, to stop and save the survey, or move to the next page.

The researcher received 100 ID links from the CCV and sent a link to each participant via email. For this project, the CCV checked for FFQ completes each week, which allowed the researcher to prompt completion via an email.

The CCV generated four excel output reports for all the completes when data collection was complete (23 September 2016), and included raw data, nutrients from food excluding alcoholic beverages, nutrients from alcoholic beverages and food intakes in grams per day.

The FFQ made provision for all the nutrients of interest to this project and may yield data more valid to this project versus a 24-hour recall questionnaire because long-term, not short-term, nutrient intake or deficiency may be associated with mental health and a wide combination of nutrients may impact mood.[172, 272] In addition, as this tool was self-administered, participants could save their answers if unable to complete the FFQ in one sitting, which made it ideal for the sample of interest

3.3.12 Supplement Use Questionnaire (SUQ)

3.3.12.1 Introduction

A comprehensive search of relevant studies for an instrument to assess DS use was conducted. Three questionnaires were found, the Dietary Supplement Questionnaire (DSQ), the Supplement Frequency Questionnaire (SFQ) and the VITAL questionnaire.[359-361] None of these questionnaires were suitable for this project as none provided for the reporting of specific quantities of nutrient per supplement, which precluded examining a dose-response and only commonly used, American DSs were listed. In addition, motives for supplementation, perceptions of effectiveness, who/what prompted use and where DSs were purchased was also not addressed. Furthermore, the majority of the questions provided close-ended response options, limiting the possibility of collecting a wider range of associated data. Due to the nature of these limitations and the requirement for gathering specific data in line with the research questions, the design of a SUQ was completed in April 2016, prior to recruitment commencement (Appendix Q).

3.3.12.2 Design, Reliability and Validity of the SUQ (Methods)

The questions and design format of the DSQ, SFQ and VITAL questionnaires were examined, which provided examples of questions that did provide the scope required for the SUQ. Next, the researcher examined the labels of approximately 100 DSs, comprising MVMs and single nutrient DSs, which, combined with appropriate questions from the three questionnaires, resulted in a comprehensive list of the contents, form, dosage and frequency instructions for DSs. Questions were developed to provide data on perceptions and behaviour associated with DS use, for example, whether the DS was perceived as being useful and who/what prompted use. Using this data, a list of possible draft questions was compiled with which to answer the research question. These questions were separated into groups that informed specific data end points such that all the questions related to type of DS and nutrients were grouped together, as were those related to dosage, frequency and duration of use. Establishing these groups supported a simple design format and logical question flow from the beginning to the end of the SUQ. Next, comprehensive response options that made provision for a variety of possible responses were developed. A screening question, with which to establish respondent eligibility was developed, as was an introduction to the questionnaire and instructions to guide completion. Overall, specific characteristics, underpinned by questionnaire design research, were utilised throughout the questionnaire development process. For example, clear and direct phrasing in the introduction and instruction sections and clearly articulated questions and response options were used, as was design format aimed at increasing completion, such as logical question and group flow, and aesthetic appeal. Finally, two forms of the questionnaire were designed for dissemination; a pen-and-paper and an online form using SurveyMonkey, and the relevant logic and parameters. However, regardless of questionnaire dissemination, the presence of specific characteristics can improve validity and reliability, and hence, the quality of the data gathered.[362] Appendix R contains a list of questionnaire design characteristics that supported the development of the SUQ.

Three tests were performed to assess face and content validity and administration techniques. Firstly, the authors pretested the questionnaire among their peers to ensure the questions were clearly articulated, appropriate and relevant to the research question. Feedback suggested that these characteristics were present, which was indicative of both face and content validity. In addition, administration of the pre-test questionnaire was undertaken using both forms of the questionnaire, using the same techniques that were to be used for the survey.[363]

Secondly, the authors conducted a pilot test (feasibility study) wherein ten women were purposively sampled, provided with the SUQ, asked to complete it and provide feedback. The subjects ranged in age from 18 – 60 years and included a dietician and a pharmacist. Subjects completed the SUQ online after receiving the survey link via email, after which they completed a pen-and-paper form,

and then provided feedback on both forms. Two changes were made to the SUQ after feedback following this test, which included adding a question about multiple uses of DS per day and a clearer DS definition in the introduction. In addition, none of the respondents consumed more than 10 DSs, so this number was not increased in either forms of the questionnaire. Responses from this pilot test were suggestive of both face and content validity, which was further supported by the expert analysis of the instrument by the dietician and pharmacist, who have experience in DS use, dosage, recommendations, motivations for use and purchasing.[364] Test-retest reliability was informally assessed by using two different forms of the questionnaire, the results of which suggested a high degree of agreement with these two administrations of the SUQ. Furthermore, the possibility of systematic error due to the learning effect was reduced as different forms of the questionnaire were completed and compared.[365] Although informally assessed, these results suggest that reliable data can be generated using either forms of the SUQ.[366] Inclusion in this pilot study precluded participation in the research project.

The pilot test also provided an opportunity for the authors to test the administration and data collection procedure and the response rate, as the same administration procedure was to be used in the study. Some of the respondents required a reminder follow-up email to complete the survey. The response rate was 100%. Thirdly, a test to ascertain face validity was administered, using two groups of purposively sampled women, one of who had seen the SUQ before (n=20) and another who had not seen it previously (n=20). Both groups were provided with the questionnaire online, accompanied with one question and four possible response options. The question was: 'In your opinion does the Supplement Use Questionnaire (SUQ) look like it is assessing your supplementary use of dietary nutrients?' The four response option were: 'Yes, definitely;' 'Yes, I think so;' 'No, I don't think so' and 'No, definitely not.' The results of both respondent groups are in Table 3.1, which reflect a high percentage of positive responses, suggestive of face validity. The response rate was 100%.

Table 3.1: Face Validity percentage results of new and previous questionnaire respondents

	Yes, definitely	Yes, I think so	No, I don't think so	No, definitely not
New respondents	55	35	10	0
Previous respondents	50	45	5	0

Although confirming face and content validity is not possible using a statistical test because these concepts are primarily subjective in nature, the pre-test, the pilot test and the SurveyMonkey test all confirmed that the SUQ seemed to be measuring the concepts it was aimed at measuring and included the right sort of content, which included expert opinions in the field of dietary supplementation. Both

forms of the SUQ were made available for dissemination, a pen-and-paper form and an electronic form in SurveyMonkey, although only the electronic form was used in the survey (Appendix Q).

3.3.12.3 Calculation of nutrient intake via supplementation

The ingredients of each reported DS were examined to establish the amount of the nutrients of interest, then the total amount of the nutrient consumed at each serving was determined and used in data analysis. This resulted in the development of a DS database that totalled 96 items (Appendix S).

Effect estimates were presented as meaningful increments of increase, according to the recommended daily allowances (RDAs) for each nutrient of interest. For example, the RDA for plant-sourced Omega 3 (ALA) is 1000mg, while that of B12 is 2.4mg.[367, 368]

3.4 Results

3.4.1 Participant Characteristics

The participant cohort comprised 74 women who ranged from 21 to 70 years of age with a mean age of 42.7 years (SD: 13.8 years). Fifty-eight of the participants reported using DSs (78.4% of the sample), 16 reported no use of DS (16/74; 21.6%) and five (5/74; 6.76%) reported the use of DSs that were not of interest to this study. The total number of DSs containing nutrients of interest reported per participant ranged from one (n=22 participants) to five (n=3 participants). (See Appendix T for a summary of the DS combinations cited by participants.) PSS scores ranged from 10 to 36 with a mean score of 19.66 (SD: 5.90) and a median score of 19. Table 3.2 summarizes this data.

Table 3.2: Description of survey participants, total number of DS used and summary statistics for PSS scores

Participant characteristics		
N	74	
Age in years (mean ± SD)	42.7 ± 13.8	
Supplement use		
Yes (n, %)	58	(78.4)
Total number of supplements used (n, %)		
1	22	(29.7)
2	16	(21.6)
3	10	(13.5)
4	2	(2.7)
5	3	(4.1)
N/A (No DS used or nutrients not of interest)	21	(28.4)
Summary statistics for PSS scores		
Mean ± SD	19.66 ± 5.90	

Participant characteristics	
Median	19
Min	10
Max	36

PSS scores ranged from 10 – 36. The mean score was 19.66 (standard deviation of 5.90 units) and the median score was 19. Mean PSS scores were slightly lower among women who used DSs (M=19.2, SD=5.8) compared to women who did not use DSs (M=21.4, SD=6.3). Differences in PSS scores between groups was assessed using the Mann-Whitney U test. There was no statistically significant difference between groups (Z=1.20, *p*=0.231). Table 3.3 summarizes this data.

Table 3.3: Summary statistics for PSS scores by DS use

		Perceived Stress Scale score				
Supplement use	**Number of subjects**	**Mean**	**(SD)**	**Median**	**Min**	**Max**
No	16	21.4	(6.3)	21	12	36
Yes	58	19.2	(5.8)	18	10	33

3.4.2 Supplement characteristics

The total number of DSs cited among the 53 participants who reported using at least one DS was 107. The most frequently-cited types of DSs were essential fatty acids (EFAs) which accounted for approximately 23% of the 107 DSs reported, followed by multi-vitamins and minerals (MVMs), which comprised 16%, followed by vitamin C (13.1%), Magnesium (9.4%), and vitamin B12 (6.5%) (Appendix U).

Frequency and duration of use was recorded for each DS. The majority of the DSs were reported as being consumed once daily (65/107=61%). More than 60% of the reported supplements were reported as being taken for a duration of over six months, with 25.5% reported as being taken for a period of 6-12 months and 38.7% reported as being used for a duration of more than one year (Appendix V).

Of the cited supplements, 59% were perceived to be useful by their user. For 42% of supplements, the participant was 'unsure' of its efficacy, and the remaining 6% of supplements were perceived as *not* being useful. The most commonly reported motivation for DS use was to 'increase energy (around 35% of cited supplements) and the least commonly reported motivation was 'lose weight' (<10% of cited supplements).

A doctor prompted the use of 29% of the DSs cited while the prompt for use for 55% of the DSs was reported to be a source other than those listed. The primary place of purchase for the DSs were health stores (35.5%) (Appendix W).

3.4.3 Nutrient Intake via DSs

Each of the DSs contained one or more nutrients of interest. The most common nutrient of interest consumed via DSs was vitamin C, with about 64% of supplement takers (n=53) obtaining vitamin C through supplementation. Close to 60% of users supplemented with magnesium, vitamin B6 and B12 and half of DS users supplemented with vitamin B1, B2 and B3 (Appendix X).

Mean daily nutrient intake from DSs was estimated based on the dosage and frequency information provided by participants in relation to each DS they reported. For example, a participant reporting the use of 500mg of vitamin C per week would have an estimated daily intake of vitamin C of 71.4mg/ day (500mg/7days). If the frequency of use was not specified, an estimate for daily intake was not able to be determined. *Daily* nutrient intakes were calculated to be consistent with the unit of measure for estimated nutrient intakes derived from the FFQ (daily intake) (Appendix Y).

Nutrient intake via food was ascertained via the FFQ, which was completed by the entire sample (N=74 participants). Among the nutrients of interest, Omega 6 had the highest intake (mean=13,250mg/day), followed by Omega 3 (mean=1,543mg/day), magnesium (mean=492mg/day), vitamin C (mean=9.5mg/day) and folic acid (mean=103.96mg/day) (Appendix Z).

3.4.3.1 The effects of DS usage

There was little indication for a linear relationship between PSS scores and participant age (Pearson's r=-0.202, p=0.0844 and Figure 3.1). However previous research has indicated an association between age and PSS scores and analyses will be controlled for age to minimize potential confounding.[353]

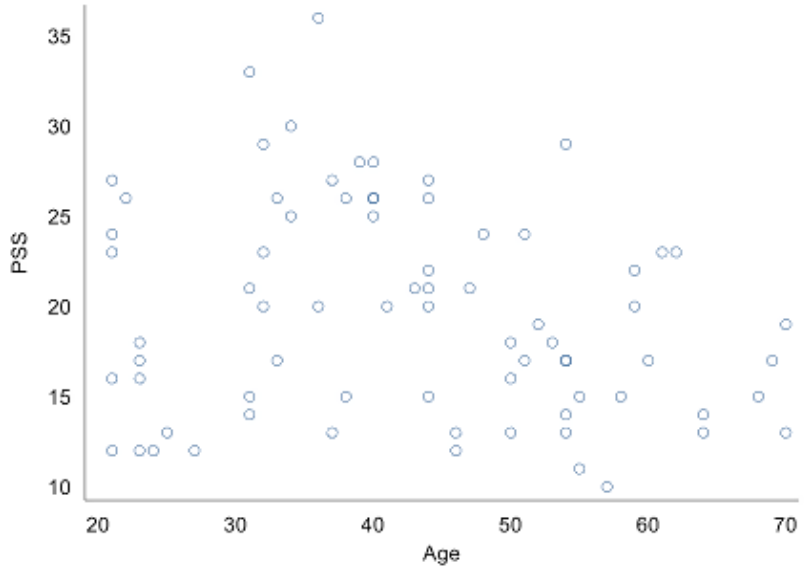


Figure 3.1 PSS Scatterplot of PSS scores against age for N=74 participants.

Supplement users were on average older than non-supplement users, with mean ages of 44.8 and 35.3 years for the two groups, respectively. The relationship between age and DS use was examined using logistic regression. The odds of being a DS user were estimated to increase by 32% for every 5 year increase in age from the mean age of the sample (OR=1.32, 95% CI 1.05, 1.67, p=0.019). Summary statistics for age in relation to supplement status are reported in table 3.4 and Figure 3.2.

Table 3.4: Summary statistics for age in relation to supplement status

	Supplement user (n=58)	**Supplement non-user (n=16)**
Mean (SD)	44.8 years (13.7 years)	35.3 years (11.8 years)
Median	45 years	36 years
Min	21	21
Max	70	61

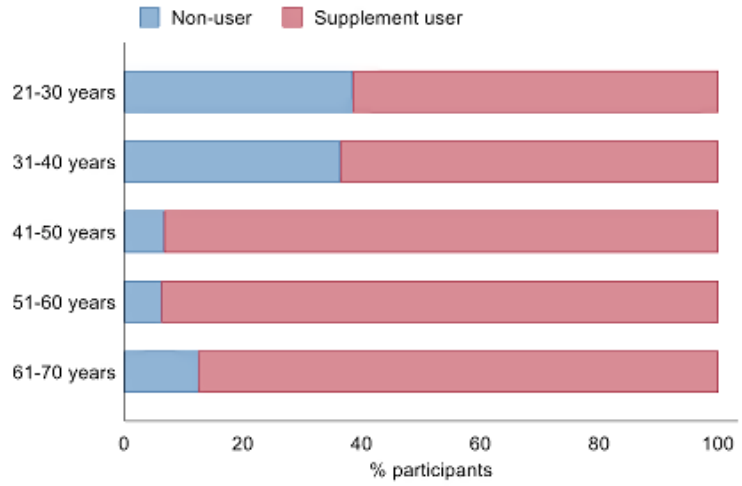


Figure 3.2: Supplement use by age group.

The relationship between age and the number of DSs used per participant was assessed using Poisson regression. Rate of DS use was estimated to increase by an average 2% for every 5 year increase in age from the mean age of the sample, although this increase was not statistically significant. (IRR=1.02, 95% CI 0.95, 1.09, p=0.570). Summary statistics for age and number of DSs used are presented in Figure 3.3.

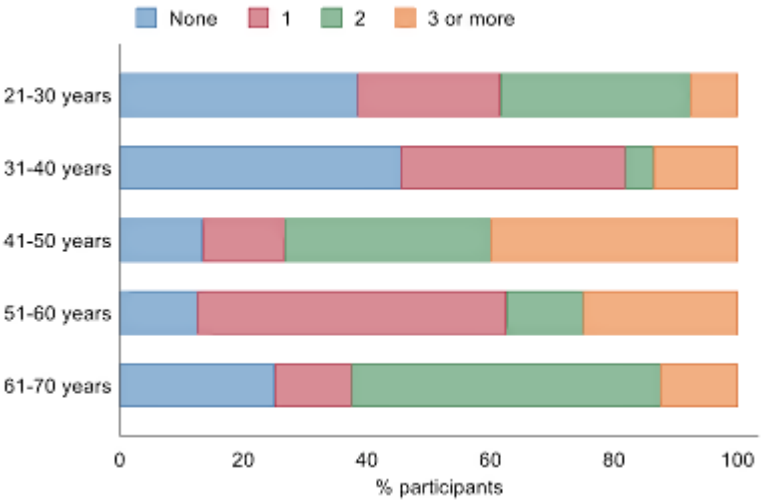


Figure 3.3: Number of supplements used by age group.

The relationship between the number of DSs used and PSS scores was examined using linear regression, controlling for age. Participants who did not use DSs (n=16) and those who used DS, which did not contain nutrients of interest (n=5), were excluded. Participants were grouped according to the number of DSs they reported using (1 DS, 2 DSs and 3 or more DSs). Summary statistics for PSS scores by DS use group are reported in Table 3.5.

Table 3.5: Summary statistics for PSS scores for DS use groups

Number of cited supplements	N	PSS Score				
		Mean	(SD)	Median	Min	Max
1	22	19.09	(5.55)	17	10	28
2	16	16.75	(4.22)	15	12	24
3 or more	15	20.67	(6.33)	20	11	30

Participants who reported using two DSs were estimated to have, on average, PSS scores that were 2.07 units lower than those reporting the use of one DS (95% CI -1.43, 5.57 *p*=0.240). Participants using three or more DSs had PSS scores that were on average 3.96 units higher compared to participants using 2 DSs (95% CI 0.14, 7.77 p=0.043). There was no statistically significant difference between PSS scores between these groups (global p-value=0.125).

3.4.3.2 Effects of nutrient intake via DSs on PSS score

The effect of supplementation for each nutrient of interest on PSS scores was examined using linear regression, controlling for age. For each nutrient, participants were classified according to whether they had consumed that nutrient via supplementation based on their reported intake details. Model adequacy was assessed via visual examination of residuals plots.

The age-adjusted coefficient for each supplemented nutrient gives the estimated average change in PSS score associated with a change in supplementation status, providing all other factors are held constant. The Sidak correction was applied to the p-values to account for multiple testing to control the Type I error rate. Inspection of the point estimates and 95% CIs indicates that potentially substantive reductions in PSS scores associated with the use of specific nutrients cannot be ruled out. These nutrients include ALA, where PSS scores were an average of 3.3 units lower among supplementers and LA where PSS scores were an average of 4.1 units lower among supplementers. However, there is no consensus on what constitutes a clinically meaningful change in PSS scores. Vitamin C and B12 were associated with a small increase in PSS scores, however, after adjusting for multiple testing, there were no statistically significant relationships between the use of a particular nutrient and PSS scores. Table 3.6 provides a summary of the results.

Table 3.6: Results of linear regression models for PSS scores according to supplementation status for nutrients of interest*

Nutrient	Coefficient (adjusted for age)	Standard Error	95% CI Lower	95% CI Upper	Unadjusted p-value	Sidak-adjusted p-value
ALA	-3.34	2.32	-7.97	1.29	0.154	0.886
LA	-4.08	2.45	-8.97	0.82	0.101	0.775
EPO	-2.23	2.49	-7.20	2.74	0.375	0.996
EPA/DHA[a]	-4.05	2.02	-8.07	-0.03	0.048	0.522
Undefined Omega-3	-1.56	2.19	-5.92	2.80	0.478	0.997
Vitamin C	0.05	1.37	-2.68	2.77	0.973	0.998
Magnesium	-0.85	1.40	-3.64	1.94	0.545	0.997
Zinc	-0.85	1.45	-3.75	2.05	0.559	0.997
Vitamin B1	-1.27	1.41	-4.07	1.54	0.372	0.996
Vitamin B2/B3[b]	-1.14	1.42	-3.97	1.69	0.426	0.996
Vitamin B5	-0.63	1.51	-3.65	2.38	0.678	0.997
Vitamin B6	-0.90	1.38	-3.65	1.85	0.517	0.997
Folic Acid	-0.40	1.46	-3.30	2.51	0.786	0.998
MTHFR[c]	-0.81	3.46	-7.70	6.08	0.816	0.998
Vitamin B12	0.24	1.38	-2.51	3.00	0.860	0.998

ALA, Alpha linolenic acid; LA, Linoleic acid; EPO, Evening Primrose oil; EPA, Eicosapentaenoic acid; DHA, Docosahexaenoic acid; MTHFR, methylenetetrahydrofolate reductase

* The coefficient for ALA is -3.34, which means that the PSS scores are 3.34 units lower, on average, for those who supplement with ALA compared to those who do not supplement with this nutrient. The 95% CI provides the range of plausible values for the 'true' effect of ALA supplementation on PSS scores that are compatible given the observed data. We therefore cannot rule out that the 'true' effect of ALA supplementation is as extreme as a 7.97 unit decrease in PSS scores and we cannot rule out that the 'true' effect of such is a 1.29 unit increase in PSS scores (95% CI -7.97, 1.29) and the 'true' effect may lie anywhere between these two values.

[a] Model estimates for EPA and DHA use are identical, since every person who was an EPA user was also a DHA user.

[b] Model estimates for B2 and B3 use are identical, since every person who was a B2 user was also a B3 user.

[c] Activated folic acid supplement

3.4.3.3 Correlation between nutrient intake from DSs and PSS scores

Spearman rank correlation coefficients were calculated between intakes for each nutrient and total PSS score. It has been suggested that the minimum sample size for a Spearman correlation is n=10 observations. Correlations were therefore carried out only for those supplemented nutrients for which there were at least 10 users with daily intake data available. The results indicated a strong positive correlation between EPA and DHA intake and PSS scores (ρ=0.79), and a weak negative correlation

between vitamin C intake and PSS score (ρ=-0.35) and vitamin B6 intake and PSS scores (ρ=-0.50). The Sidak correction was applied to account for multiple testing. The results are reported in Table 3.7.

Table 3.7: Spearman's Rank Correlations for daily nutrient intake via DS and PSS scores for selected nutrients of interest (for n≥10)

Nutrient	No. observations	Rho (ρ)	Unadjusted p-value	Sidak Adjusted P-value
EPA (milligrams/day)	10	0.7903	0.0065	0.0753
DHA (milligrams/day)	10	0.7903	0.0065	0.0753
Omega-3 (milligrams/day)	23	0.1157	0.5991	0.9947
Omega-6 (milligrams/day)	11	0.0780	0.8197	0.9947
Vitamin C (milligrams/day)	33	-0.3453	0.0491	0.3956
Magnesium (milligrams/day)	29	-0.1035	0.5930	0.9947
Zinc (milligrams/day)	22	-0.2444	0.2730	0.9220
Vitamin B1 (milligrams/day)	25	-0.0296	0.8885	0.9947
Vitamin B2 (milligrams/day)	23	-0.3533	0.0982	0.6055
Vitamin B3 (milligrams/day)	24	-0.0931	0.6654	0.9947
Vitamin B5 (milligrams/day)	20	-0.1504	0.5268	0.9947
Vitamin B6 (milligrams/day)	29	-0.4971	0.0060	0.0750
Vitamin B12 (micrograms/day)	27	-0.1249	0.5347	0.9947

3.4.3.4 The effect on PSS scores of supplementation with specific nutrient combinations

Table 3.8 summarizes the use of magnesium and vitamin B6 among supplement users. Twenty-seven participants obtained both magnesium and vitamin B6 from DSs. Most magnesium users were also vitamin B6 users (27/31=87%). Many of the supplements cited by participants had magnesium in their composition, with more than half of all supplementers (31/58) obtaining magnesium through DSs.

Table 3.8: Distribution of magnesium and Vitamin B6 use among N=74 supplement users

Magnesium User	Vitamin B6 User			
	Yes Freq.	(%)	**No** Freq.	(%)
Yes (n=31)	27	(87.1)	4	(12.9)
No (n=43)	4	(9.3)	39	(90.7)

The effect of concurrent magnesium and vitamin B6 supplementation on PSS scores was examined using linear regression. All effect estimates were adjusted for age. PSS scores among participants who used magnesium *without* concurrent vitamin B6 had PSS scores that were on average 1.92 units lower compared to those who did not supplement with either nutrient although this difference was not statistically significant (95% CI -8.68, 4.83, p=0.571). PSS scores among participants using vitamin B6 *without* concurrent magnesium were estimated to be an average of 1.98 units lower compared to those who do not supplement with either nutrient. Again, this difference was not statistically significant (95% CI -8.20, 4.23, p=0.526). When *both* magnesium and vitamin B6 were consumed via DSs, PSS scores were estimated to be on average 0.92 units lower compared to participants who did not supplement with either nutrient. The difference was not statistically significant (95% CI -3.88, 2.03; p=0.536).

3.4.4 Association between nutrient intake from food and PSS scores

3.4.4.1 Correlation between nutrient intake from food and PSS scores

The relationship between the intake of nutrients of interest from food (generated via the FFQ) and PSS scores was examined using Spearman's Rank Correlations. The Sidak correction was applied to account for multiple testing. The results suggest that there is limited evidence for a monotonic relationship between the level of intake of any of the nutrients consumed via food and PSS scores (Appendix AA).

Linear regression was used to investigate the relationship between each specific nutrient of interest consumed via food and PSS scores adjusted for age. The results are presented in Table 3.9. After adjusting for age, a 10mg increase in vitamin B6 from food was associated with an average decrease of approximately 3.5 units in PSS score, although inspection of the 95% CI indicate a high level of uncertainty for this effect (95% CI -28.43, 21.47). The point estimates and associated confidence intervals for the other nutrients of interest reveal relatively small effects of nutrient intake on PSS.

Table 3.9: Results of linear regression models for Perceived Stress Scale (PSS) scores for intakes of selected nutrients of interest from food.

Nutrient	Coefficient (adjusted for age)	Standard Error	95% CI Lower	95% CI Upper	Unadjusted p-value	Sidak-adjusted P-value
ALA (per 1000 mg)	0.298	1.187	-2.070	2.666	0.803	1.000
LA (per 1000 mg)	-0.072	0.138	-0.346	0.202	0.602	1.000
EPA (per 100 mg)	0.352	0.571	-0.787	1.490	0.540	1.000

Nutrient	Coefficient (adjusted for age)	Standard Error	95% CI Lower	95% CI Upper	Unadjusted p-value	Sidak-adjusted P-value
DHA (per 100 mg)	0.172	0.291	-0.409	0.753	0.558	1.000
Total Omega-3 (per 500 mg)	0.243	0.482	-0.719	1.205	0.616	1.000
Total Omega-6 (per 500 mg)	-0.034	0.069	-0.172	0.103	0.619	1.000
Vitamin C (per 25 mg)	-0.273	0.230	-0.732	0.187	0.241	0.988
Magnesium (per 10 mg)	-0.022	0.039	-0.010	0.056	0.582	1.000
Zinc (per 10 mg)	2.004	2.161	-2.304	6.313	0.357	0.999
Vitamin B1 (per 1 mg)	0.051	0.830	-1.602	1.705	0.951	1.000
Vitamin B2 (per 1 mg)	0.202	0.762	-1.319	1.722	0.792	1.000
Vitamin B3 (per 10 mg)	0.500	0.844	-1.183	2.182	0.556	1.000
Vitamin B5 (per 1 mg)	0.355	0.562	-0.765	1.478	0.529	1.000
Vitamin B6 (per 10 mg)	-3.481	12.513	-28.432	21.470	0.782	1.000
Vitamin B7 (per 1 mg)	-0.036	0.040	-0.115	0.044	0.378	0.999
Vitamin B12 (per 1 microgram)	0.646	0.390	-0.132	1.423	0.102	0.839
Total folates (per 100 micrograms)	0.069	0.347	-0.623	0.761	0.842	1.000

3.4.5 Association between reported nutrient intakes from food and DS status

Binary logistic regression was used to investigate whether intake of nutrients of interest via food was associated with the supplementation status for that nutrient. The effect estimates are presented in the form of odds ratios (ORs). The OR for a nutrient describes the change in the odds of being a DS user of that nutrient associated with a 1-unit increase in intake of that nutrient from food. All estimates were adjusted for age. With the exception of vitamin C and vitamin B6, all of the ORs are greater than 1, which suggests that on average, as the intake of the nutrient from food increased so too did the odds of supplementing with that nutrient. However, after adjustment for multiple comparisons, there was no statistically significant difference on the effect of nutrient intake from food on supplementation status for any of the nutrients. The results are presented in Table 3.10.

Table 3.10: Results of logistic regression models for supplementation status for selected nutrients according to recorded intake from food*

Nutrient (obtained through food)	Odds Ratio (adjusted for age)	Standard Error	95% CI Lower	95% CI Upper	Unadjusted p-value	Sidak-adjusted P-value
ALA (per 1000 mg)	1.310	0.866	0.359	4.789	0.683	0.943
LA (per 1000 mg)	1.105	0.090	0.943	1.296	0.217	0.851

Nutrient (obtained through food)	Odds Ratio (adjusted for age)	Standard Error	95% CI Lower	95% CI Upper	Unadjusted p-value	Sidak-adjusted P-value
EPA (per 100 mg)	2.196	0.855	0.996	4.839	0.051	0.466
DHA (per 100 mg)	1.368	0.231	0.983	1.904	0.063	0.511
Total Omega-3 (per 500 mg)	1.130	0.275	0.701	1.821	0.616	0.943
Vitamin C (per 25 mg)	0.932	0.077	0.793	1.095	0.391	0.918
Magnesium (per 10 mg)	1.030	0.015	1.001	1.060	0.043	0.435
Zinc (per 1 mg)	1.253	0.116	1.046	1.502	0.014	0.191
Vitamin B1 (per 1 mg)	1.319	0.384	0.746	2.335	0.341	0.918
Vitamin B2 (per 1 mg)	1.553	0.432	0.900	2.680	0.114	0.664
Vitamin B3 (per 10 mg)	1.699	0.532	0.920	3.139	0.090	0.611
Vitamin B5 (per 1 mg)	1.094	0.230	0.724	1.653	0.671	0.943
Vitamin B6 (per 1 mg)	0.728	0.341	0.291	1.824	0.499	0.937
Vitamin B12 (per 1 microgram)	1.461	0.230	1.073	1.989	0.016	0.202
Total folates (per 100 micrograms)	1.173	0.150	0.913	1.506	0.212	0.851

* For example, for every 1 microgram increase in vitamin B12 intake from food, the odds of supplementing with B12 increased by 46.1% (OR:1.46; 95% CI 1.07, 1.99; adjusted p=0.202).

For each participant, total intake of each nutrient was calculated by summing the reported intake from food, and the reported intake for DSs (if the participant consumed this nutrient via DSs). Linear regression was used to investigate associations between total nutrient intake from the combined nutrient sources and PSS scores, adjusting for age. The results are presented in Table 3.11. The point estimates and 95% CIs for the effect of increased intake of any nutrients of interest on PSS are very small, with most in the order of less than 1 unit in PSS.

Table 3.11: Results of linear regression models for Perceived Stress Scale (PSS) scores by nutrient intake from food and supplementation combined.

Nutrient	Coefficient (adjusted for age)	Standard Error	95% CI Lower	95% CI Upper	Unadjusted p-value	Sidak-adjusted p-value
ALA (Per 1000 mg)	0.294	1.187	-2.073	2.661	0.805	0.999
LA (Per 1000mg)	-0.072	0.138	-0.346	0.202	0.602	0.997
EPA (Per 100 mg)	-0.012	0.187	-0.385	0.360	0.948	0.999
DHA (Per 100 mg)	0.042	0.174	-0.305	0.390	0.808	0.999
Total Omega-3 (Per 500 mg)	-0.093	0.145	-0.381	0.195	0.523	0.997

Nutrient	Coefficient (adjusted for age)	Standard Error	95% CI Lower	95% CI Upper	Unadjusted p-value	Sidak-adjusted p-value
Total Omega-6 (Per 500 mg)	-0.049	0.065	-0.179	0.081	0.456	0.997
Vitamin C (Per 25mg)	-0.085	0.042	-0.168	-0.002	0.045	0.499
Magnesium (Per 10 mg)	-0.021	0.026	-0.073	0.030	0.408	0.997
Zinc (Per 10 mg)	-0.372	0.301	-0.972	0.229	0.222	0.962
Vitamin B1 (Per 1 mg)	-0.002	0.012	-0.026	0.022	0.882	0.999
Vitamin B2 (Per 1 mg)	-0.046	0.026	-0.097	0.005	0.079	0.684
Vitamin B3 (Per 10 mg)	-0.119	0.177	-0.472	0.235	0.505	0.997
Vitamin B5 (Per 1 mg)	-0.029	0.028	-0.085	0.027	0.299	0.986
Vitamin B6 (Per 10 mg)	-0.027	0.127	-0.524	-0.019	0.036	0.444
Vitamin B12 (Per 1 microgram)	-0.004	0.007	-0.018	0.009	0.535	0.997
Folic acid (Per 100 micrograms)	-0.245	0.323	-0.889	0.398	0.450	0.997

3.5 Discussion

Chapter 1 cited population level studies that revealed a relationship between some mood states and DS use, with the presence of anxiety, depression and stress being associated with an increased intake of MVMs.[4-6, 230] However, as evidenced in the systematic review (Chapter 2), very few randomized controlled or quasi-randomized studies have investigated the effectiveness of specific nutrients in the diet or DSs on stress levels among women without also addressing a specific stage of the reproductive cycle.[10] Due to the lack of evidence to support dietary intervention and/or DS use to manage stress in women clinically, this exploratory study aimed to explore this relationship. To the knowledge of the researcher, this represents the first cross-sectional survey to examine such.

Although mean PSS scores were lower among participants who used DSs compared to those who did not, the difference was not statistically significant. In addition, among supplementers, average PSS scores differed according to level of DS use but again, the overall effect of level of DS use on PSS scores was not significant. For the majority of the specific nutrients of interest, besides vitamin C and B12, PSS scores were on average lower among supplementers compared to non-supplementers. In addition, the 95% CIs demonstrate that potential differences in stress scores between supplementers and non-supplementers may be as large as approximately 9 units (LA), 8 units (ALA) and 7 units (EPO). Although the clinical importance of changes in PSS scores of these magnitudes has not been empirically determined, such differences may represent considerable change in perceived stress, despite differences not being statistically significant after adjustment.

Correlational results suggest that DHA and EPA use was associated with increased PSS scores and vitamin C and B6 with lower PSS scores, although after adjustment these correlations were not statistically significant. The effect of the combination of magnesium and vitamin B6 supplementation on PSS scores revealed that PSS scores were estimated to be an average of 0.92 units lower compared to no supplementation with either nutrient, but this effect was not significant. Furthermore, the point estimates for the average differences in PSS scores for magnesium supplementers in the present study are consistent with some literature that suggest this nutrient has stress-lowering effects.[352, 369] In addition, in the present study it was found that magnesium was a common ingredient in the supplements cited by participants. Participants were therefore frequently obtaining magnesium in varying dosage from multiple supplements, which may partly account for the lower stress levels observed among supplementers relative to non-supplementers. However, the relative duration of use of a single nutrient was unable to be validly determined.

Some research has suggested specific single-nutrient DSs, such as vitamin C or EFAs, had stress-lowering effects in both genders.[184, 194] However, in the present study, use of DHA and EPA was associated with higher PSS scores. This may be a reflection of high stress scores being present when participants started using the DS, which may also reflect their motivation for use. Being a cross-sectional study a causative relationship between these variables cannot be established.

Previous studies suggested that a B complex reduced stress among women over 30 and 90 days respectively.[180, 270] In contrast, the effect of vitamin B supplementation on stress in the present study was largely inconclusive. This is likely due to confounding by dose and duration of supplementation, which could not be controlled in a cross-sectional setting, and by low study power to detect anything other than large differences. Furthermore, this study investigated the effects of each individual B vitamin rather than a vitamin complex. However, Haskell et al. reported a reduction in self-reported stress over 9 weeks using a MVM and Benton et al. reported mood improvements among women taking a MVM after one year of continued supplementation.[272, 317] The duration of supplementation may be an important factor influencing effectiveness.

The effect of intake of specific nutrients via food on PSS scores was examined using linear regression. There was little evidence for an effect of intake of a particular nutrient on PSS score. In addition, the total intake of each nutrient from food and DSs combined was examined in relation to PSS scores using linear regression. The results indicated an effect of vitamin C intake and vitamin B6 intake on stress. However, given the large number of tests performed and limited sample size, caution is required when drawing conclusions from these findings. To constrain Type I error rate, a Sidak correction was applied and after this adjustment, no effects were found to be statistically significant.

The findings from the present study are contrary to those from a number of similar cross sectional studies that investigated dietary influences on mental health. Bhattacharyya et al.[370] investigated the association between dietary factors and psychological distress, and reported that increased fish intake was associated with lower psychological distress, suggestive of an impact via EFAs. In addition, Jacka et al. investigated the association between 'Western' and 'traditional' diets and depression and anxiety in a sample of women in Australia. It was found that less healthy, 'Western' foods were associated with higher scores of psychological symptoms, including anxiety, compared to more traditional diets comprising fresh produce, grains, fish and meat.[164] In the following year, Jacka et al. investigated the association between habitual diet and mental health in elderly Norwegian men and women, and found that a healthy dietary pattern (DP), similar to the 'traditional' diet in the 2010 study, was associated with reduced anxiety only in women.[163] Conversely, in another study, the impact of magnesium intake via food on depression and anxiety revealed no association between magnesium intake and anxiety scores.[187] In addition, Begdache et al. reported on a mixed-gender cross-sectional study that men were more likely to experience mental wellbeing until severe nutrient deficiencies arose while women were less likely to experience the same until a balanced diet and healthy lifestyle were followed.[278] The authors proposed that dietary sufficiency may potentiate heightened limbic system regulation in women, which is suggestive of nutrients, in the form of different DPs, impacting mental wellbeing more in women than men. It should be noted that most of these studies examined food groups and DPs rather than individual nutrient intakes. The effect of individual nutrients may not be able to be isolated from the context of an individuals broader DP. In addition, the small size of the present sample meant that only very large effects would have resulted in significance at the 5% level. Indeed, inspection of the estimated effects and CIs for the specific nutrients reveal that further research is warranted before concluding that no relationship exists between specific nutrients and PSS scores.[371]

The association between reported nutrient intakes from food and supplementation status was examined. With the exception of vitamin C and B6, on average, the odds of supplementing with a particular nutrient increased as intake of that nutrient via food increased, although these effects were not statistically significant after adjusting for multiplicity. This suggests that in this sample, supplementers were also consuming more of the examined nutrients in their regular diets compared to non-supplementers, which leads to the assumption that the former group are at lower risk of micronutrient deficiencies compared to the latter group. (Note that examining the nutritional adequacy of participants based on their reported food frequency intakes was beyond the scope of this study.) This result is broadly compatible with a 2013 NHANES survey, which found that DS use was greater among healthier respondents compared to less-healthy respondents.[372] Motivations driving supplementation behaviour among the healthy remain unexamined but may include a few factors. Firstly, DS users may

be unsure of effectiveness (as DS use is unrelated to measurable health outcomes) yet decide to use DSs as a form of 'insurance' against possible illness. Secondly, the use of DSs in the presence of an adequate diet may be a form of health anxiety and may be associated with the reported increase of DS consumption in anxious populations.[4-6, 230, 373] Thirdly, whether DS users are aware of which nutrients they are deficient in, which foods contain those nutrients, and whether they choose to eat more of, and supplement with those nutrients, is presently unexamined. Therefore, in the presence of health, and possibly related to a preoccupation with health or illness, and/or knowledge about which nutrients they may be deficient in and where they are found, a portion of the population may use DSs regardless of a lack of evidence to support use. Indeed, a population level survey that examined the influence of government statements regarding efficacy claims from DS manufacturers revealed that only 25% of users would cease DS use if they were shown to be ineffective.[374] Clearly, motivations driving this behaviour and reasoning require continued investigation.

Further, the association between DPs, specific nutrients and chronic stress remains unexamined among women. In addition, the prevalence of chronic stress among the present sample is not known. Chronic stress imposes an additional nutritional burden on the CNS that may only be ameliorated by significant increases in nutrients via supplementation. As examined in Chapter 1, research suggests that people experiencing chronic stress may change their diet, with the majority choosing to eat more nutrient-deficient food.[158, 375-379] The previously mentioned studies that reported on nutrient intervention leading to stress-reduction may have provided nutrients lost via appetitive changes and increased metabolic demands.[180, 270, 317]

Apart from nutrient deficiencies, the form of nutrients may influence their impact. For example, polymorphisms on the MTHFR gene, which influence the breakdown of vitamin B6, B9 (folic acid), B12 into their active, co-enzyme forms and into methionine, an amino acid that facilities protein synthesis and antioxidant utilization, may have a role to play in chronic stress, due to its presence in a number of mental health challenges.[334-337] These polymorphisms also negatively impact neurotransmitter synthesis, including serotonin and GABA, via a number of mechanistic pathways.[334] As 10-20% of the population worldwide may possess this gene variant, activated forms of these nutrients may provide benefit to women experiencing chronic stress.[172, 334-337] Indeed, stress levels may not be reduced unless these nutrients are available in their activated form. This may further predispose vulnerable women to the development of affective disorders, such as depression, via neurobiological and neurochemical mechanisms not yet fully understood.[380, 381]

Six other factors related to DS consumption were examined, including motivations for use and perceived efficacy. The results revealed a variety of motivations, which included reducing stress (27%), however the most commonly cited reason for using a DS was to increase energy (35%). In addition, the

use of 31% of the DSs were cited as being prompted by a doctor whereas a mixed-gender NHANES survey reported that 23% of the use of such products were based on health care provider recommendations.[372] This discrepancy may reflect increased PCP visits by women or may be naturally higher in the surveyed population.[207, 208] Perceived efficacy of DS use was examined and results revealed that 59% of cited DSs were perceived as being useful, however, for 42% of DSs used, participants were unsure of efficacy, and 6% of cited DSs were perceived as not being helpful. These results may be related to the increased odds of using DSs as nutrient intake increases, where despite uncertainty of effectiveness, women may use DSs as an 'insurance' against illness. (Appendix BB contains the other four reported factors related to DS use.)

3.5.1 Limitations

There are a number of limitations in this study. Similar to all cross-sectional surveys, the methods do not enable inference of a causal relationship between the independent and dependent variables, therefore, whether the amount of nutrient consumed (the exposure), preceded or followed the amount of stress (outcome) experienced by participants remains unknown. Therefore, DS use may not only influence stress but stress may drive DS use. In addition, the study size and non-random sampling methods preclude generalizing the results to women in general, and the sampling procedure may have introduced selection bias. Chronically stressed women may have been unaware of the study, or been unable to participate, and would therefore be underrepresented in this sample. Furthermore, many more women used DSs in this study versus non-users, so users may have been disproportionally represented. In addition, the small sample size limited the precision of results. A larger sample would have increased the precision of results and provided greater power to detect true effects.

In relation to the PSS, many of the validations were done on college students not representative of the population of interest in this project. In addition, this tool is not a diagnostic or screening tool so no normative data exists with which to compare study results, so researchers make comparisons between the total scores within the sample of interest. The PSS is also not a measurement tool that reflects chronic stress levels. In relation to the DQES, multiple weeks of recording dietary intake is the ideal method of assessing nutrient intake versus a once-off assessment. In addition, over- and under-reporting are challenges inherent in all FFQs, possibly including the DQES.[382, 383] In relation to the SUQ, this tool was not formally tested for intra- or inter-rater reliability prior to use. In addition, due to the possible sporadic nature of DS use it is not possible to obtain an accurate intake of nutrients supplied via DSs for each participant although this would be the case with any self-administered DS use questionnaire.

Further, the SUQ gathered data on DS intake over differing time periods compared to the FFQ, which gathered dietary nutrient intake over a 12-month period, and the PSS investigated the perception of stress over the last month. In addition, it is likely that recall bias of varying degrees was associated with each instrument.[384]

The dose-response and the duration of specific-nutrient consumption in relation to PSS scores could not be assessed as most DSs contain more than one nutrient, as evidenced by the inclusion of magnesium in many used DSs, which may have led to confounding. Finally, due to the small sample size, multivariate analysis was not possible, so analysis was restricted to evaluating the effect of single nutrients only and the intake of other nutrients was not controlled for.

3.5.2 Conclusion

Although previous research has suggested that a broad spectrum of nutrients are supportive of brain health, this study sought to examine the effect of specific nutrients, given that a variety of specific-nutrient DSs and stress-management formulations are marketed and consumed globally. Indeed, according to Goldstein Research, the global stress management DS market, which includes magnesium, B vitamins and zinc, is expected to reach USD16.7 billion by 2025.[8]

As previously discussed, a number of studies have examined the role that some of these purportedly stress-ameliorating nutrients play in mental health, either in various combinations or alone, and in various contexts, and a number of reviews have summarised such studies.[172, 385, 386] However, there is a lack of evidence to support the clinical use of specific-nutrient DSs in the presence of chronic stress among women despite the acknowledged role that chronic stress plays in both physiological disease states and diminished mental health.[31, 108, 381, 387-390] When experienced chronically, stress becomes a catalyst for the development of stress-associated affective disorders, such as anxiety and depression, possibly due to lack of nutrients required to maintain emotional stability and resilience against such.[46-51] As depression is now the leading cause of disability globally, it is timely to address one of its possible antecedents to examine whether the impact of nutrients on chronic stress is similar to the reported effects of such on depression, and whether genetic polymorphisms impact nutrient bioavailability and thus stress levels.[163, 276, 277, 334, 391-395]

In the present study, DS users were significantly older than non-DS users, but the relationship between PSS scores and age was not significant. Prior to adjustment for multiple testing there was evidence for a number of significant relationships between nutrients consumed via DSs and PSS scores. The effect estimates and 95% CIs indicated that there were potentially substantive reductions in PSS scores related to intake of ALA, LA and EPO via supplements compared to those not supplementing with these nutrients. There was a strong positive correlation between PSS scores and

intakes of EPA and DHA among those participants who used these supplements, and a weak correlation between PSS scores and intakes of vitamin C and B6. Contrary to previous research, the combination of B6 and magnesium was not found to be significantly associated with lower stress levels either before or after adjustment for multiplicity. When assessing the relationship between nutrient intake from food and PSS scores, a 10mg increase in B6 was associated with an average decrease of about 3.5 units in PSS scores. Similarly, it was found that for most nutrients, as the intake from food increased so too did the odds of supplementation with that nutrient. However, after adjustment for multiple comparisons there was little evidence for significant relationships between any of these variables. Finally, analysis of the relationships between total intake of each nutrient (from food and DS combined) and PSS scores yielded estimates of effect considered to be very small in magnitude. Although there is a substantial body of research that suggests that such nutrients have critical roles to play in the CNS, there was inconclusive evidence from the present study for the effect of these nutrients on stress, regardless of whether they were obtained from food or dietary supplements. However, as discussed, the limitations of this study, particularly the limited statistical power due to the small sample size, are likely to have affected the results obtained.

This study has suggested that DS use to ameliorate stress among women warrants further research attention, ideally in the form of a prospective cohort study or RCT. A longitudinal study will allow for the investigation of DPs and nutrient intake via diet and DSs and associated behaviour and perceived stress and affective disorders over time. This will inform on any causal relationships between these variables, for example, whether DSs are consumed in response to high stress levels and whether cessation occurs when stress levels decrease, or whether they are used as a form of nutrient 'insurance' in the presence of stress. Furthermore, such studies will be better able to control for cyclical reproductive hormones compared to cross-sectional surveys wherein they become confounding variables. In addition, as current research suggests, overall DPs may be a better indicator and predictor of mental health. Knowledge is also required about the overall balance and intake of micro- and macronutrients, along with the ratio of specific nutrients, such as omega 3 and omega 6, in relation to chronic stress and depression over time. Furthermore, including the use of biological markers of nutrient intake will further inform on the relationships between nutrient intake, physiological stress markers and genetic polymorphisms. A well designed RCT will allow for the examination of an effect of specific nutrient intervention on perceived stress in a defined population under controlled conditions, such as duration, dosage, timing etc. This will inform on the efficacy and safety of specific nutrients with the aim of informing clinical practice.

Results from studies such as these may provide evidence to either support dietary changes and/or dietary supplementation to ameliorate chronic stress, or prevent the use of such ineffectual

solutions to these ends. In addition, they may inform on the relationship between chronic stress and nutrient intake and the development of affective disorders. However, such studies lack the ability to inform on variables that are challenging to quantify, such as the psychological experience of stress among women, how diet and/or DSs are used to manage stress, and other factors related to the lived experience of stress. Qualitative research, using action research methods, is better at informing on subjective variables.

Chapter 4

THE LIVED EXPERIENCE OF STRESS AND THE USE OF DIET AND/OR DIETARY SUPPLEMENTS TO MANAGE STRESS: A PARTICIPATORY ACTION RESEARCH (PAR) STUDY

4.1 Introduction

The purpose of this chapter is to report on the paradigm, methodology, methods and results of the participatory action research (PAR) project that investigated the lived experience of stress and the role of diet and/or DSs in this experience among eight women, over an eight-week period. This chapter will briefly address the philosophical considerations underlying the choice of a PAR approach and will report the methodology and methods used, including the quality control measures taken to ensure credibility, dependability and data transferability. A description of how the in-depth data analysis, coding and categorisation occurred and how the resultant themes were developed follows. The position of this project within the JBI EBHC model is examined, along with a discussion about the results, limitations and implications.

The aim of this PAR project was to answer two research questions: what is the lived experience of stress among women and what role do diet and/or DSs play in this experience? Investigating these questions within a PAR framework was a logical progression from investigating the relationship between perceived stress and nutrient intake in the cross sectional project (Chapter 3). Employing a qualitative approach to these research questions provided depth and insight into these lived experiences that the cross-sectional project (Chapter 3) was unable to elicit.

4.2 Philosophical Considerations

Qualitative research is grounded in a particular worldview, or paradigm, which significantly influences how the research question is investigated and answered. Therefore, defining and situating the paradigm is an important component of providing the context in reporting qualitative research. A paradigm can be defined as a 'loose collection of logically related assumptions, concepts or propositions that orient thinking and research' (p. 22).[396] This research project investigated a specific psychological construct which is at present not well understood. How a phenomena is investigated and how knowledge is

gained, or 'how we know what we know,' is aligned with the paradigm within which researchers situate their research (p. 8).[397, 398] Paradigms are underpinned by a particular ontology, defined as being 'concerned with the nature of the social world and what can be known about it' and a particular epistemology, defined as being 'concerned with the nature of knowledge and how it can be acquired' (p. 22-23).[399] Therefore, it was incumbent on the researcher to choose a paradigm that was capable of supporting an exploratory and flexible examination of this construct.

The ontological framework underpinning the positivist approach is the assumption that there is a single, objective, external reality or truth, which can be observed and measured.[398, 400] Research undertaken within this theoretical framework (such as the study in the previous chapter) aims to keep the researchers from influencing the outcomes or results by stipulating methods of gathering and analysing data to maintain objectivity. Quantitative research is aligned with positivism and post-positivism (where it is held that accurate knowledge about the world can be identified through sensory experience because the world exists independently of our knowledge of it) and because knowledge is objectively gathered, represented and quantified.[401] However, it has been argued that the positivist and post-positivist paradigms fail to address the complexity of unobservable variables that underpin the human experience, such as emotions, feeling and thinking.[402]

The ontological framework underpinning the interpretive paradigm is the assumption that no single objective truth exists; instead, social meaning is created through engagement between individuals within groups.[398, 403] Reality is thus understood 'in the form of multiple, intangible mental constructions, that are socially and experientially based' and rely on the meanings that individuals and groups ascribe to their experiences (p. 110).[404] Thus, if reality is subjective, interpreted by people and differs from person to person and within groups, it is socially constructed and is therefore capable of changing.[398, 403-405] 'Meanings and interpretations' are therefore of primary importance in the interpretative paradigm, while 'observed phenomenon' are the focus in the positivist paradigm (p. 26).[398]

The ontological framework underpinning the critical paradigm is the assumption that 'reality is shaped by social, political, cultural, economic, ethnic and gender values' (p. 109).[404] Critical theory has its origins in the philosophy of Karl Marx and its development has been attributed to the Frankfurt school of social theory and philosophy in Germany, which was associated with the Institute for Social Research at the Goethe University in Frankfurt.[406] These researchers suggested that reality was socially constructed and influenced by groups of powerful people within society who wield power over less powerful people. Critical theory and research within this paradigm is concerned with emancipation from these powerful and dominating forces, which often occur within a political context.[398, 407] This approach to research was therefore the most suitable one in which to position this study as it takes into account

that reality is subjective, and thus changeable, and makes provision for examining complex human experiences that are shaped by social, political, economic, ethnic, and gender values.

Critical theory's leading proponent today, Jurgen Habermas, proposes that there are three human interests or orientations related to the human species and our interaction and the work that we engage in, including an 'emancipatory' interest, which focuses on emancipation from domination and leads to 'being in control of the conditions under which one lives' (p. 27).[398, 407, 408] This theory of emancipatory interest is underpinned by Habermas's belief that when people use 'communicative action' purposefully, their situations can improve. However, although 'theory is a powerful resource for developing insight and understanding' it is necessary to bridge the gap between theory and practice so as to allow research to impact on, or improve practice (p. 104).[409] Therefore, a method was required to bridge the gap between Habermas's theoretical framework and practice, and 'Action Research (AR)' met this need.[408] AR has been described as a 'family of practices of living enquiry that aims, in a great variety of ways, to link practice and ideas in the service of human flourishing' (p. 1).[410]

Kurt Lewin, a Prussian born, pioneering social psychologist, has been called the 'Father of AR' and coined the term in a 1946 article.[411, 412] Critical theory generally, and Habermas more specifically, provides a theoretical framework for Lewin's formative work on AR.[410] AR bridges the gap between theory and practice by encouraging 'collective self-reflective' and self-imposed enquiry undertaken by participants who aim to improve their situations via critically examining their activities (p. 5).[413] AR accomplishes this by combining the identification of a problem with action aimed at improvement and reflection on action.[398] Lewin's focus on action within social settings and the use of what he termed a 'cycle of enquiry,' leads to research proceeding in a 'spiral of steps, each of which is composed of a circle of planning, action and fact-finding about the result of the action' (p. 206).[414] This cyclical process is one in which 'problem identification, planning, action and evaluation are interlinked' (p. 11).[415]

Two adult educators, Freire and Fals-Borda, put AR into practice in Latin America in the 1950's, where they gave it an 'emancipatory orientation,' and encouraged participation between all group members investigating a research question (p. 131-2).[416] Social liberation and the transformation of communities via participation are both cornerstones of PAR methodology today and it is recognised as a methodology that can be used to 'solve problems and transform consciousness and ultimately society' (p. 11).[417-420] AR is different to PAR in a number of ways, for example, AR does not necessarily engage participants directly in an emancipatory research process, whereas PAR does so, collaboratively, albeit at different levels of engagement.[421]

McTaggart suggested that instead of viewing emancipation in PAR as needing to be 'revolutionary,' the process of change can also be evaluated according to whether it has resulted in improvement that is valued by all the participants (p. 205).[422] McTaggart goes on to suggest that

participatory action researchers regularly ask themselves whether things are more 'reasonable, coherent, just, humane and satisfying for participants than they were' (p. 205).[422] McTaggart's line of thought was deeply influential in this work as change was not anticipated to occur within a social or organisational context, but rather in a personal context, so change was measured according to shifts in individual perspective, possibly viewed by participants as being 'emancipatory' in nature.

4.3 Methodology

Methodology is defined as the 'strategy, plan of action, process or design lying behind the choice and use of particular methods of data collection and links the choice and use of methods to the desired outcomes' (p. 3).[397] Macdonald states that 'the purpose of qualitative methodology is to describe and understand rather than to predict and control' (p. 34).[423] The methodology underpinning PAR goes a step further and aims to study 'disenfranchised and vulnerable populations' and in so doing 'address racial, ethnic, socio-economic and health disparities' with the aim of emancipation for such groups (p. 9).[412] Thus, the individuality of participants impacts how the research progresses and influences what actions are taken because participants are partners, or collaborators with the researcher and have a high level of involvement and influence.[410]

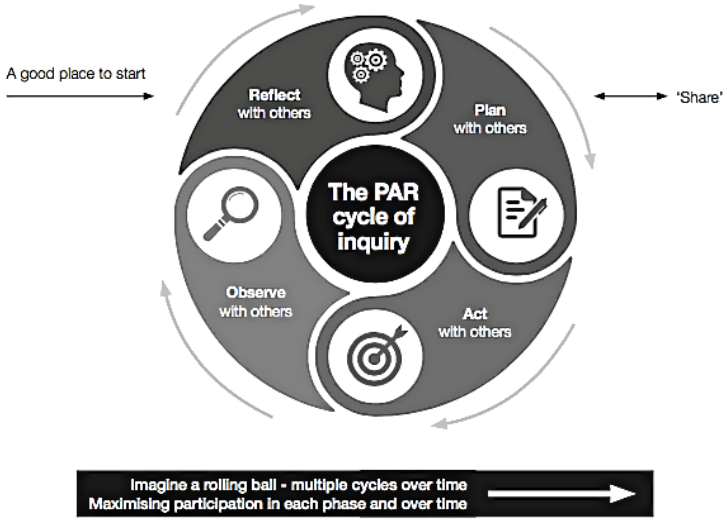


Figure 4.1: The traditional PAR cycle of enquiry. Australian Government: Action Research Induction Kit. Department of Families, Housing, Community Services and Indigenous Affairs. Commonwealth of Australia, p. 11

Many authors have advocated for diversity in methodology and method using AR and PAR approaches. Green et al. extends the concept of 'there's no one size fits all' PAR methodology by stating that 'the key to PAR lies not in any given method, but, rather, in the attitudes of the researchers, which in turn affect how and for whom the research is constructed and conducted' (p. 28).[424] Other

authors have proposed that 'No study conforms exactly to a standard methodology: each one calls for the researcher to bend the methodology to the uniqueness of the setting or case' (p. 7.)[425]

The unique contexts within which PAR is undertaken provide for the 'self-determination of research partners in the PAR process,' as Bergold et al. suggests, and demonstrates why PAR cannot be canonised as a single methodological approach (p. 192).[426] Each project therefore provides researchers and participants with 'many opportunities to construct knowledge and integrate theory and practice in ways that are unique and practical to a particular group' suggests McIntyre (p. 67).[427] However, a broad conceptualisation of this methodological approach is possible, and represented in Figure 4.2, where participation (life in society), action (experience) and research (mind, knowledge) combine to support research in which participants can find solutions to their problems using action and knowledge relevant to their experience.[428] Within PAR, people insert themselves into the research process as participants of their own life story.[419] For these reasons, and due to the flexibility inherent in this methodology, this research project was positioned within a PAR methodology.

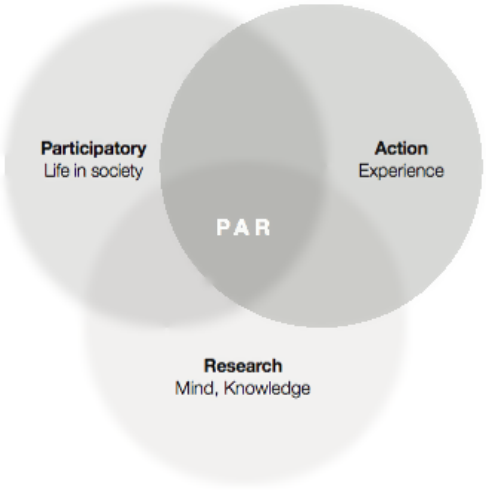


Figure 4.2: Intersection between participation, action and research. Chevalier JM, Buckles DJ. Participatory Action Research: Theory and Methods for Engaged Inquiry. United Kingdom: Routledge; 2013, p. 10

4.4 Situating Qualitative Research Evidence Within The JBI Model of EBHC

As discussed in Chapter one and three, the JBI Model of EBHC (Figure 1.5, Section 1.9) takes into account that a wide variety of methodological approaches to health research may be useful because different approaches answer different questions. Therefore, although evidence of effectiveness is valuable, it is not the only type of evidence that can inform clinical decisions. The centre of the model, the 'pebble of knowledge' summarises the foundation of evidence and how it informs practice via the FAME scale.[265] JBI-FAME acknowledges that there is a need for diverse forms of evidence to support

complex knowledge needs when investigating complex clinical questions including mental health challenges. This framework therefore supports the use of alternative research approaches that require flexibility and sensitivity informed by the specifics of the research question, such as was the case with this PAR project.[265]

The JBI Model of EBHC does not rank results of qualitative research below those of quantitative research, but considers all research methods as contributing to evidence generation and able to be synthesised and contribute to EBHC. This model therefore acknowledges that healthcare 'needs to take a patients perspective into account and understand their experiences in the context of their everyday life' (p. 386-7).[429]

4.5 Methods

4.5.1 Background to the PAR approach

As stated by Green et al. 'PAR seeks to ensure community representation and ownership of questions to be asked, the methods to be used and, ultimately, the interpretation and application of research results' (p. 2).[424] The foundation of this approach to research rests therefore on the researchers attitudes, rather than any given method. Cornwall et al. suggests that 'methods are seen less as a means to an end … the emphasis not on outcomes but on processes' (p. 1667).[430] However, there is still a need to be able to draw valid and trustworthy meaning from data generated qualitatively, so that the knowledge gained can be relied upon.[425] To this end, the cycle of inquiry proposed by Lewin is commonly used within a PAR methodological approach, because it takes into account that investigating lived experiences and the understanding that individuals give them will diverge from the planned research path.[423] Lewin's methods account for changes within the cycle of enquiry by enabling 'overlapping' of action and reflection (p. 170).[431] Thus, knowledge gained while researching, leads to reflection and informs action and this iterative process led Lewin to suggest that a system could not be understood until an attempt was made to change it, and takes into account that a system under investigation will change, possibly in unexpected ways, demanding a flexible and responsive approach that is unable to be anticipated or prepared for (p. 19).[432] As such, iterative processes have the potential to lead to a deep understanding of the phenomenon under investigation and facilitate change within and between participants in a natural process. Therefore, 'actual research will continue to evolve and be shaped by the realties of the context' (p. 90).[433]

Due to the contextual differences outlined below, and as per Green et al's.[424] position that no single method will suit each PAR study, and as this study was not entirely 'co-planned' and the researcher was dealing with a new area of enquiry, including social phenomena not yet investigated, an

exploratory PAR approach was taken (p. 25).[434, 435] Figure 4.3 illustrates the process of exploration and action, which has an unknown endpoint within this PAR project.

This project was not aligned with the goals or aims of any organisation or specific community group. Instead, the context within which the participants engaged with each other was informal and guided by their personal desire to explore the research topic after participating in the cross sectional study (Chapter 3). In addition, their shared experience of living with stress can be seen as an overarching, shared context. Co-planning commenced when the research process was underway (stage one, Figure 4.3) and included participants exploring, analysing and reflecting on the research questions in ways they chose to do so. Participants observed and reflected on action taken in the second stage by sharing their actions and personal progress. It was not possible to cycle back to re-examine and reflect on the questions and action taken and observe changes in a pre-determined or planned manner due to the informal context and nature of this exploratory project. However, the 'overlapping' of action and reflection, as per Lewin's methods, did occur at the start of each week's session, as participants discussed the transcripts from the previous week and shared their journal notes, including how they had used the knowledge gained and what action was taken using this knowledge. Transcript reading, or 'member checking,' became a form of 'cyclical reckoning,' which also prompted journaling among participants, thus providing another natural cycle of personal reflection within this project.

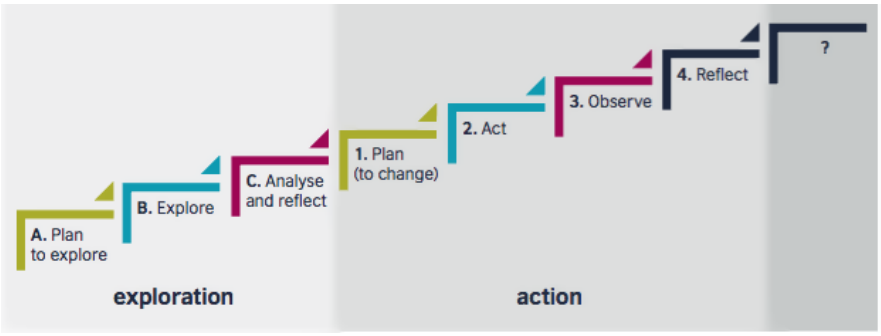


Figure 4.3: Process of Exploratory Action Research. Smith, R. and Rebolledo, P. (2018) A Handbook for Exploratory Action Research. British Council.

4.5.2 Sampling and recruitment (Access to the sample)

On the 1st of September 2016, as recruitment and data collection for the cross sectional project (Chapter 3) was concluding, recruitment for this project was initiated via an email and Facebook message invitation (Appendix CC). All 74 participants who had completed all three cross-sectional surveys (reported in Chapter 3) were invited to participate in the eight-week study; 22 indicated their interest by responding and eight took part in total. One participant dropped out after the second session, and another only attended two sessions due to work commitments. A total of six women completed all

eight sessions. Five of the participants were aged over 50 and three were aged between 31 and 41, two having no immediate family commitments.

This group was relatively homogenous as the sample originated from the same geographical area and had personal Internet access, suggestive of similar socio-economic status and had self-selected from the previous study (Chapter 3). Some authors advocate for homogeneity within focus groups as large gaps between social background and life style can hinder participants desire to speak freely.[436]

4.5.3 Ethics

The Office of Research Ethics, Compliance and Integrity at the University of Adelaide provided ethical approval for this project on the 6th of May 2016 (Appendix H).

Upon confirmation of attendance all participants received a plain language information sheet (Appendix DD) explaining the aims and the processes of study, the identity and contact details of the researchers and a telephone number for psychological support if required during the study. Consent forms (Appendix EE) were signed at the first session, participants were also advised that the sessions would be recorded and that confidentiality and anonymity would be maintained throughout the project and any subsequent reports and articles for publication. Participants chose their own pseudonyms that were used in transcripts.

All electronic data from the study in the form of audio recordings, transcripts, and journal entries are held on a private password protected computer with two backup systems in place.

4.5.4 Focus groups

Focus groups 'offer unique insights into the possibilities of critical inquiry as deliberative, dialogic, democratic practice' suggest Kamberelis et al. (p. 547).[437] The use of focus groups was therefore a natural methodological fit with the PAR process because, as suggested by McTaggart, 'PAR is in principle a group activity' (p. 170).[431] In addition, the concept of 'the whole is greater than the parts,' as suggested by Morgan, is clearly evident in focus group interaction, revealing subtleties and depth while addressing the research question, which other data generating approaches, such as interviews or participant observation, are unable to elicit.[436] The natural affinity that people have to others who have similar experiences to them further supported the use of focus groups within a PAR process of enquiry.[438]

Using email or Facebook, the researcher provided interested women with five possible time options for group sessions: four options during the day or at night and a weekend option. The meeting place was chosen as being equidistant for each of the interested women. A safe, neutral, quiet and spacious room, with a large table and comfortable chairs was hired from the local Gold Coast

community centre, with easy access to bathrooms and a kitchen where refreshments were made prior to each session. Using the same venue for all eight sessions established a feeling of familiarity and comfort for the participants, which was important toward the aims of this project and increased incentives for attending.[439] Prior to the commencement of the first session the use of Chatham House rules was explained to the participants to encourage safe psychological sharing as was the cyclical nature of PAR. The two research questions guided the discussions, with the first question being addressed during the first four sessions and the next four sessions addressing the second question. Deviations from these questions were accommodated although the researcher guided the discussion back to the question when necessary.

Eight focus groups took place between 10 October 2016 and 28 November 2016 consisting of between one hour and one and a half hours at each session. A final follow up, or PAR reunion, took place on 23 September 2017, requested by the participants, and was used as a data-checking opportunity. (Appendix FF contains the study timeline.)

4.5.5 Planning the analysis

An interactive model of data analysis was used which combined four components during the analytic process: data collection, data condensation or analysis, data display or results and conclusions (discussion) as advocated in Miles et al. and illustrated in Figure 4.4.[440] An additional component, quality control, was included, using Lincoln and Guba's criteria for assessing the 'trustworthiness' of qualitative data (p. 218).[441] (See Section 4.5.5.3, 'Quality Control.)

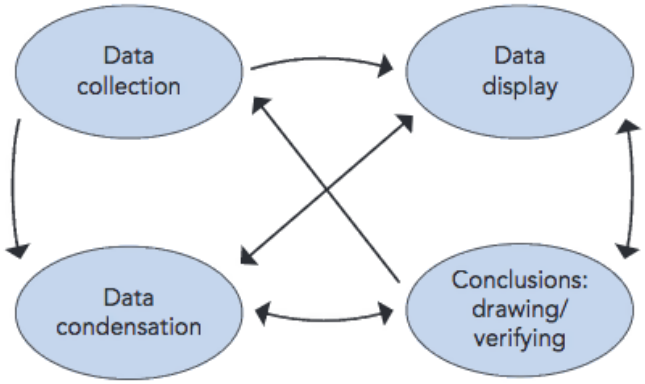


Figure 4.4: Components of Data Analysis: Interactive Model. Miles, MB. and Huberman, AM. (1994) Qualitative data analysis: An expanded sourcebook (2nd Ed.). Thousand Oaks, CA: Sage Publications.

4.5.5.1 Data collection

Data collection took place using focus groups, journal entries, a follow-up (reunion) session to check data, and via emails after the reunion session. Data recording, transcription and storage was initiated

from the first focus group session. Focus group discussions were recorded using an electronic application and transcribed verbatim by the researcher. Participants were provided with a copy of the transcripts within two days after each session via email, a process known as 'member checks' (p. 247).[442] Data from participant reflective journals was collected after the last session and data from the researchers own reflective journal was collected after each session. Emails from participants after the last reunion session became an unexpected source of data. See Table 4.1 for a detailed explanation of collection methods.

Table 4.1: Outline of data collection methods

Stage	Description	Date
Focus group sessions	Eight focus groups lasting between 60 and 90 minutes were conducted, recorded and transcribed verbatim.	10 Oct – 28 Nov 2016
During and after sessions and reunion	Voice Memos (Apple iPhone Application) recorded all group sessions and were converted into MP3 audio files after each session; transcripts were generated in Microsoft Word, totalling 218 A4 typed pages (average 27 pages/ session).[443, 444] The researcher typed up researcher reflective journal notes and participant journal notes that resulted in 11 A4 typed pages. Final data codes and categories were recorded in Excel spread sheets and imported into NVivo where final data analysis was confirmed.[445, 446]	10 Oct – Dec 2017
After session eight and reunion	Participants shared additional detail about their early life with the researcher via email, which the researcher copied into a word document.	Aug – Nov 2017

4.5.5.2 Data analysis (condensation)

Data analysis (condensation) was accomplished in phases, firstly by analysing the data semantically, which involved looking at the surface, or explicit meanings of the data.[447] Subsequent analysis extended to an interpretation of patterns within the data, using thematic analysis.[447] In phase 1, the researcher analysed focus group transcripts, journal entries line by line to find data pertinent to answering the two research questions, including any reference to reflection, action and change, all of which were highlighted in the text. Codes were ascribed to highlighted illustrations that related to similar concepts using a variety of coding strategies, including etic, emic and a priori coding. Highlighted illustrations and codes were imported into Excel. In phase 2, 3 and 4, the researcher compared the data between each new session with the previous sessions, noting differences and similarities across time. A number of codes addressed both research questions and were therefore grouped together to denote this. Codes were analysed and grouped together to form categories if they related to similar concepts as the data set evaluation progressed. Third-party code and category verification occurred when the researcher discussed the codes with her supervisors. In phase 5 the full range of codes and categories were

positioned on large pieces of cardboard (Appendix GG), to support the visual comparison of codes and categories between the sessions. As the emails were received after the reunion session, data generated from them was not available to add to this verification process, however this data was included in the next phase, phase 6, when the data was moved from Excel into NVivo. Examining the data in NVivo confirmed the patterns noted between all data sources, sessions and participants in the spread sheets and visually on the cardboard. This final stage of data analysis led to the development of 16 categories and six themes, three of which addressed both research questions. Table 4.2 provides details of the analytic process and Table 4.4 provides details of the categories and themes.

A specific number of cycles, or coding phases are not stipulated for qualitative research using thematic analysis.[447] Six coding phases occurred in this project as the researcher aimed to ensure all the nuances within the data were addressed and aimed to compare data between different data sources. The six coding cycles led to a deep understanding of the data, as it allowed for a process of 'abstracting, focusing, selecting and simplifying the data,' also referred to as 'data condensation', which is preferable to data summarization which reduces, versus distilling, the meaning within the data (p. 12).[425] As suggested by Padgett, 'a constant comparative analysis stays close to the data' and is, in practice, cyclical, which may be 'repeated many times over,' and 'subsequent cycles of coding help to narrow the analyses to ever important findings' (p. 177).[448] Data saturation or 'informational redundancy' was reached when no new information was being shared or uncovered within sessions (p. 875).[449]

The participants were not involved in data analysis because of their time limitations and in practice, 'many tasks associated with the PAR process may be burdensome, impractical, or even infeasible for some participants' (p. 84).[450] However, 'member checks' across eight weeks provided participants with the emerging analysis and at the reunion session, the three attendees reviewed the codes, categories and patterns that had been developed during data analysis for authenticity and representativeness. Table 4.2 provides details about the six phases of data analysis as per Miles et al. and Padgett's approach.[425, 448] Appendix HH contains an image representing the files containing raw and analysed data on the researchers computer.

Table 4.2: Phase, description and dates related to data analysis

Phase	Description	Date
1 – Initial coding	Transcriptions were typed in Microsoft Word and analysed by the researcher semantically, line by line with illustrations highlighted when they referred to a feeling, belief, experience, discovery, personal revelation, action or replies to such related to the research questions. Highlighted illustrations were linked to a researcher-generated short phrase or a single word, termed descriptive coding. Codes were generated based on a variety of concepts including the research questions and researcher experiences (etic codes); 'emic' or 'in vivo' codes based on participant's words and concepts; 'a priori' codes based on existing literature; codes based on the conceptual framework established prior to analysis (process coding), which uses gerunds ('ing' words) to signify observable and conceptual action within the illustrations; emotion coding which labels emotions experienced or being recalled by participants; values coding which uses the prefix 'V' to code a value, 'A' to code an attitude or 'B' to code a belief.[348] Initial codes were 'generic' as they did not convey personal insights, but rather general comments about experiences, feelings and ideas. Comparing the data and codes between sessions in Word documents revealed that codes became more personal as the sessions progressed. Some codes were applicable to more than one session and were used as such, and constant comparison between data and codes revealed patterns within the data. Journal notes and emails were analysed similarly after the last session. Transcriptions were shared with all participants after each session (member checks). Pertinent illustrations were imported into Excel spread sheets after initial coding, with a separate spread sheet created for each session.	10 Oct 2016 – 23 Sept 2017
Phase 2, 3 and 4 – Second phase coding, third party verification and development of categories and themes	Codes were distilled into more succinct codes after each new transcription was analysed, as a result of a deeper understanding of the data and between-session data comparison. Some codes addressed both research questions and were grouped together. Third-party code verification occurred via sharing of Excel spread sheets with the researchers' supervisors. Categorisation arose by grouping codes that related to similar concepts together. Analysing categories led to the researcher becoming aware of patterns appearing across categories, resulting in the development of themes. Themes thus identified function as a way to group together data that comprises a topic made up of repeating ideas, and 'a clear and identifiable distinction between themes' occurred (Braun et al. (p. 91).[348, 447] Researcher reflective notes and participant journal notes verified this data with the emerging categories and themes across sessions. Ongoing reflection about shared insights, interaction between participants and similarities and differences in their experiences informed these cycles of data analysis.	10 Oct 2016 – 1 Aug 2017
Phase 5 – Visual data analysis	All codes were positioned on large pieces of cardboard with a column for each session and a ninth column for the journal entries to ensure the emergent categories were true to the data and codes that had been created. Codes were grouped within columns, using coloured sticky notes, which facilitated the observation of code, category and theme development over the eight weeks via visual examination. Participants checked and verified the data in this form. (Appendix GG.)	30 Aug – 23 Sept 2017

Phase	Description	Date
Phase 6 – NVivo confirmatory data analysis	Data was imported into NVivo from Excel where final analysis and confirmatory coding, categorisation and theme development occurred, with the aim of examining word frequencies and creating a digital visual network of emergent themes. Using NVivo facilitated data comparison between participants, between and within each session and across all eight sessions, journal entries, and emails, which resulted in both highlighting similarities between, and differences among, participants. Data comparison revealed changes in the depth of sharing over time and supported the visual comparison of codes, categories and themes. By examining 250 of the most frequently used words (minimum length of six letters and including stemmed words) a table was created (Appendix II) which verified the qualitative data.[440] The relationships between codes and categories was confirmed and sixteen categories were verified. Ten categories (10/16) related to the role of relationships in both the lived experience of stress and in managing physical health and stress, two (2/16) related to confusion and scepticism about diet and dietary supplements, two (2/16) related to thinking patterns, and one (1/16) related to causes and effects of stress and one (1/16) to strategies aimed at managing stress. A total of six themes were developed from the repeated reading of these categories, four of which had more data driving their emergence than the others, as evidenced in the summary of codes and categories in Section 4.6 (Results of the PAR study), Table 4.4.	23 Sept – 15 Dec 2017

4.5.5.3 Quality control

Quality control involved, 'member checking' of transcripts and codes and categories. Mertens et al. suggests the standards for quality in qualitative research necessitates careful documentation regarding how the research and data were conducted and analysed, along with how the interpretative and thinking process of the researcher unfolded.[402] This study was based on careful transcribing, reflective note taking, analysing journal data and emails and data comparison in the development of codes, categories and themes that reflected the data.[425, 451] These practices aim to increase the 'trustworthiness' of the data so 'one can establish confidence in the truth of the findings' suggest Lincoln and Guba (p. 218).[441] Several criteria have been identified for judging whether such can be established in qualitative research, including 'dependability' and 'credibility.'[442]

Lincoln and Guba have advocated for the use of a number of techniques to establish 'credibility,' a number of which were present in this study.[441] Firstly, 'prolonged engagement,' which requires the researcher to immerse themselves in the participants experience, which was accomplished in this study over eight weeks of data collection, transcription and reflective journaling. Secondly, 'persistent observation,' which required in-depth, detailed investigation, gathering detail about the context and the participants, which in this study was evidenced by the richness and depth of description in the data collected, specific to the phenomena of interest, which lent itself to the analytic depth indicative of this technique being adhered to, according to Miles et al.[440] Thirdly, 'member checking,'

which in this study included the checking of data, interpretations and conclusions by those from whom the data was gathered (p. 247).[442] Within this study, the data and interpretations were checked each week via transcripts, and participant's commented on them at the next session, which also provided a 'member check' among group members. A final 'member-check' occurred ten months after the eighth recorded session, at the reunion (23 September 2017), to which all the participants were invited to review the final data analysis in poster form. The three participants present at the reunion agreed that their ideas, experiences and stress-management strategies had resulted in codes, categories and themes that resonated with them, their experience and the data they had provided. This result supports these authors opinion that, 'credibility' is enhanced when the 'data sources (most often humans) find the inquirer's analysis, formulation and interpretations to be credible (believable)' (p. 246).[442] Thus, there was a 'fit' between the authors interpretation of the data and the original data source.[452]

These three techniques included the study participants, whereas the next one included a form of 'peer debriefing', wherein the researchers' supervisors checked the developing codes and categories in the third and fourth phases of data analyses, which was helpful in ensuring no 'biases, motivations, interests, and perspectives …' were existent on the researchers part (p. 218).[441] (See Table 4.2.) In addition, the researcher maintained a reflective journal for critical self-reflection about herself and the research relationship, which Lincoln and Guba propose increases the quality of the research findings.[441]

These authors also advocate using a variety of 'data sources, different perspectives … to cross-check data and interpretation' (p. 247).[442] In this study, transcriptions, journal entries and emails were used. The transcriptions were shared with all group members via email, and journal entries from the participants and the researcher were shared by reading aloud to the group each week, which provided data crosschecking and interpretation.[442] Emails were not shared with participants due to the personal nature of such but were included in data analysis.

Finally, the research steps were transparently described from the start of the project through to data analysis and the reporting of the results. This depth of reporting, reflective journaling and the use of the techniques as proposed by Lincoln and Guba described above, combined to increase the quality of the research findings.[441, 453]

Figure 4.5 provides a visual summary of data sources and verification procedures that occurred during and after the project, which supported quality control.

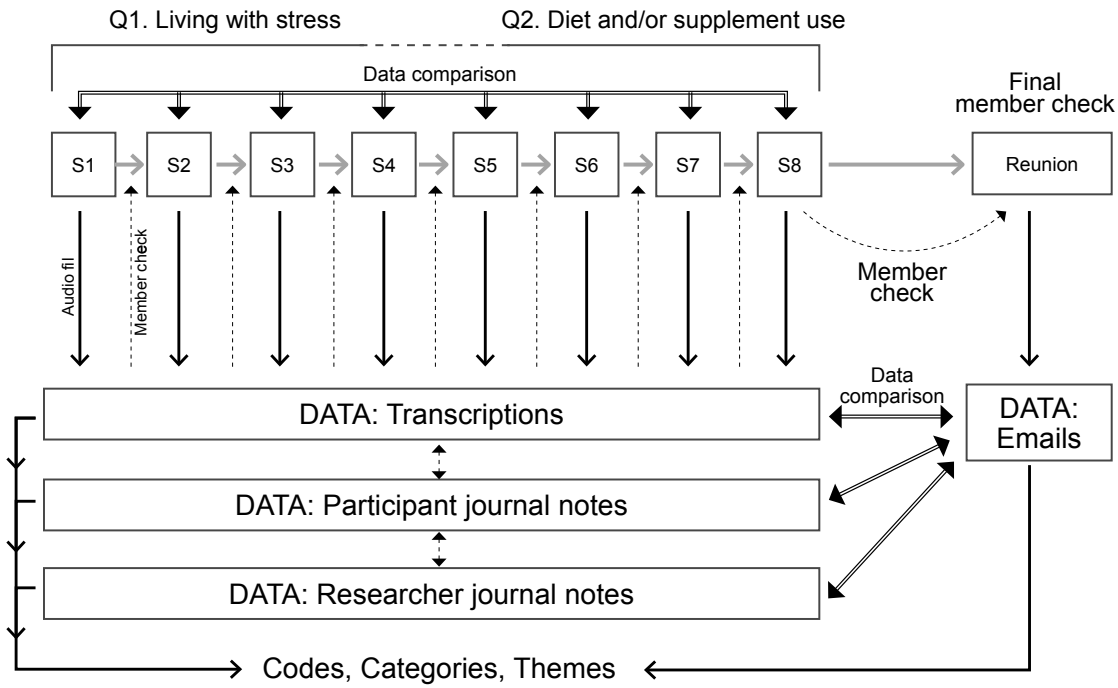


Figure 4.5: A Model of Data Sources and Verification

Solid black arrows link the data generation, collection and comparison processes and the generation of codes, categories and themes from such. The light grey solid arrows link the eight consecutive sessions with each other including the final reunion session. A broken line between Q1 and Q2 represents the 'overlap' of discourse between sessions four and five that occurred when moving between research questions. Broken arrows between data transcriptions and each of the sessions represent 'member checking' of such and similar arrows between transcriptions and journal notes represent data checking between these sources.

4.6 Results of the PAR study

The two key questions this study sought to address were: what is the lived experience of stress among women and; how do diet and/or DSs play a role in this experience? Data generated from eight focus groups, participant and researcher journal notes and email correspondence were analysed to investigate these research questions using thematic analysis via an exploratory PAR enquiry.

The participants were aged between 31 and 70, three were full-time employees, two were semi-retired, one was fully retired, one was a full time student and one was a part time student and full time carer for her husband. One woman was single, one was widowed, two were married, one was living with a partner and five women had been divorced. Only one woman had children still living at home, three women were child-less and the rest had adult children. Table 4.3 presents a visual summary of the participants personal and relationship demographics.

Table 4.3: Group composition and summary of personal and relationship demographics (pseudonyms used)

Names	Age	Occupation	Marital Status	Children
Amy	31	Full-time student	Single	None
Cindy	38	Full-time employee	Married	2 at home
Jan	41	Full-time employee	Divorced	None
Poppy	51	Full-time employee	Divorced	None
Sandy	51	Full-time carer and part-time student	Divorced and re-married	3 grown
Mandy	62	Semi-retired	Married	2 grown
Tanya	64	Semi-retired	Divorced and living with partner	2 grown
Danni	70	Retired and volunteer	Widow; first marriage ended in divorce	3 grown: 1 from 1st marriage 2 from 2nd marriage

During data analysis 438 codes and then 16 categories were generated using data from the eight focus groups, journal entries and emails. Comparing the data between sessions and sources led to the detection of patterns across the data set, which resulted in the development of six themes and illustrations were chosen that clearly articulated the theme. Three themes related to both research questions: 'role of relationships in the lived experience of stress,' 'role of relationships in managing physical health and stress' and 'strategies aimed at managing stress.' Two themes, 'thinking patterns' and 'causes and effects of stress' related to the question addressing the lived experience of stress, and the theme 'confusion and scepticism about using diet and/or DSs to manage stress' related to the second research question about the role that such may play in managing stress. Table 4.4 provides a summary of these themes and categories.

Table 4.4: Summary of categories and themes

Themes	**Causes and Effects of Stress**	**Thinking Patterns and stress**	**Confusion and Scepticism about Using DSs**	**The Role of Relation-ships in the Lived Experience of Stress**	**The Role of Relationships in Managing Physical Health and Stress**	**Strategies Aimed at Managing Stress**
Categories	Causes and effects of stress - physical and psychological	Self-awareness about stress	Alternatives to pharmaceuticals	Empathizing with participants	Family of origin - health influences	Strategies aimed at managing stress – including reflecting on diet and exercise choices

Themes	Causes and Effects of Stress	Thinking Patterns and stress	Confusion and Scepticism about Using DSs	The Role of Relation-ships in the Lived Experience of Stress	The Role of Relationships in Managing Physical Health and Stress	Strategies Aimed at Managing Stress
		Role of thinking patterns in stress	Supplementation and diet	Accepting and offering help	Comparisons between participants – diets	
				Family of origin - psychological influences	Social media and Internet health advisors	
				Offering participants suggestions to manage stress	Role of relationships in health	
				Comparisons between participants – stress	Doctors advice	

A network summary of the six themes and their relationship to the two research questions, including where themes addressed both questions is presented in Figure 4.6 below. The data driving each theme has been proportionally represented by the size of the theme's ovals.

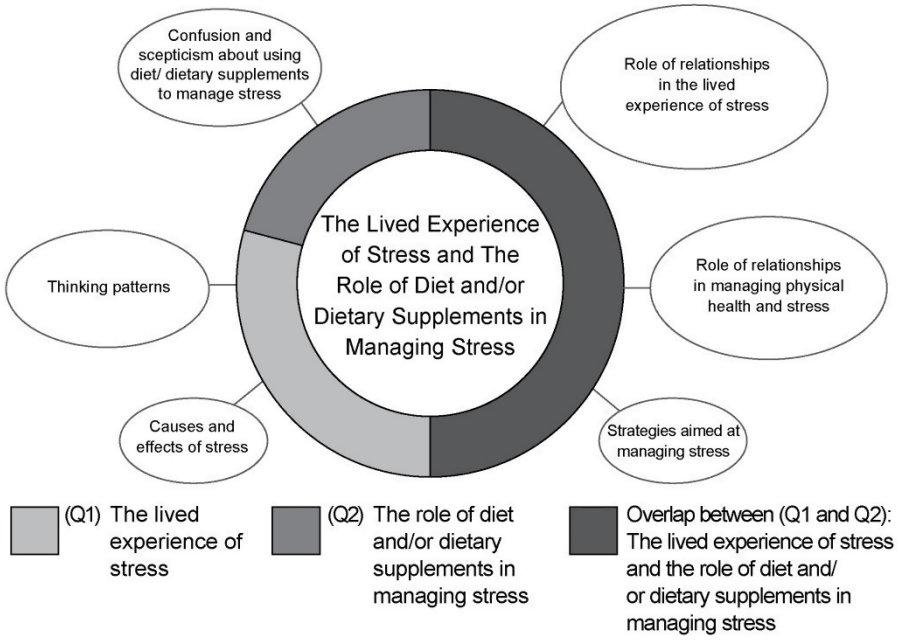


Figure 4.6: A network summary of themes in relation to the research questions

The themes were informed by illustrations of the action and change that occurred across the eight weeks. In a number of cases it became evident that the change process had escalated from the first focus group to the last email as participants increasingly reported and recorded more examples of

changed thinking and behaviour. The themes and illustrations are discussed in more detail below. The illustrations are presented in narrative format, and summarised in tables organised according to participant name, source of data (sequentially across the eight sessions, including journal entries and emails after the reunion) and theme name. The themes are:

- Causes and effects of stress
- Thinking patterns and stress
- Confusion and scepticism about using diet and/or dietary supplements to manage stress
- The role of relationships in the lived experience of stress
- The role of relationships in managing physical health and stress
- Strategies aimed at managing stress

4.6.1 Theme 1: Causes and effects of stress

This theme contributed to answering the research question about the lived experience of stress. The researcher was surprised by the confusion in participants' minds about the differences between anxiety and stress, as this group of women were volunteers from the cross-sectional survey (Chapter 3). Participants wanted clarity about what we were investigating, so the pursuit of this topic became a group activity via discussion and became a common springboard from which we participated in and engaged with the research questions across the sessions.

This shared, exploratory activity was useful to the participants as it clarified their personal experience of stress and/or anxiety, which helped them examine its causes and effects. A number of participants stated that their psychological experience of stress often accompanied a physical sensation. One of the participants acknowledged that she might play a role in creating her own stress, an idea that has the potential to improve her quality of life, and which was acknowledged, albeit from a different perspective, by other participants and is reported in the next theme. Illustrations from the discussion about the definitions of stress and anxiety and the causes and effects of stress are presented below.

Table 4.5: Causes and effects of stress (illustrations)

Names	Source	Causes and effects of stress (illustrations)
Sandy	S2	*'They're hand in hand (referring to stress and anxiety) aren't they?' … 'Stressed, unhappy, pressure on my chest is constant.'*
Poppy	S2	*'In relation to reading last weeks transcript and thinking about 'butterflies' in stomach 'I think (what I feel) it's more anxiety than stress.'*

Names	Source	Causes and effects of stress (illustrations)
Poppy	S3	*Reading from an Internet post: ' … stress is a state of mental or emotional strain, or tension resulting from adverse or demanding circumstances. Anxiety is a feeling of worry, nervousness, or unease about something with an uncertain outcome … it manifests itself physically as panic attacks, hot and cold flashes, racing heart, tightening of the chest, quick breathing, restlessness or feeling tense, wound up and edgy. Psychologically, excessive fear, worry, catastrophizing or obsessive thinking, and it's avoidance of situations that make you feel anxious, which can impact on study, work, or social life.'*
Tanya	S3	*(Discussing the difference in Sessions 1 and 2) 'I know now, anxiety is something I have a lot of, heaps… but stress, it's different. It's 50,000 things to do at once. That's my version of it.' 'Anxiety's different, it's just as you read; you're stricken. That's how I had the ah-ha moment that was the difference between stress and anxiety.'*
Cindy	S4	*'But yeah, I've noticed that I've created all of it … So I've just been thinking, how much do I create unnecessarily? How much stress in my life do I create unnecessarily?'*
Cindy	S6	*'Your body starts to seize up when you can't control how many things are coming at you.'*
Mandy	Journal Entry	*'I used to experience an almost constant 'fight or flight' feeling when I was working.'*

4.6.2 Theme 2: Thinking patterns and stress

This theme contributed to answering the research question about the lived experience of stress. A number of illustrations recorded in participant's journals as their 'conversations with self' suggest an increased propensity to reflect on their thinking related to stress, which was driven by their involvement in this project. Some participants acknowledged that thinking patterns are a learned response and can be habitual, and a tentative idea emerged that they may also be learned behaviour. Apart from one participant, it was recognized that thoughts and thinking patterns play a role in their experience of stress and these participants alluded to having some control, or desiring some control over such. Two categories supported this theme: 'self-awareness about stress' and 'role of thinking patterns in stress.' The following illustrations demonstrate participants' thoughts about their thinking patterns and how they changed over the eight weeks.

Table 4.6: Thinking patterns and stress (Illustrations)

Names	Source	Thinking patterns and stress (illustrations)
Danni	S2	*'Today … I was having a fantastic day until I allowed something to stress me. I really shouldn't have gotten tensed up and angry about it, because there was nothing I could do.'*
Mandy	S2	*'So, I had to then acknowledge that a lot of that stress is the person. It's managing the thoughts that go on in my head.'* *'I've been working on trying to be mindful when driving, so that I'm not gripping the wheel tight and I'm not clenching my jaw about people's behaviour.'*

Names	Source	Thinking patterns and stress (illustrations)
	S4	*'I had a triumph. I have turned around a behaviour, I think, that has had me in its grip for many many, years, and that's road rage … So now when I get in the car I go through a process. I make sure everything is done when I back out. I'm mindful. Then I say "I'm going to have a safe, calm journey. I've left plenty of time." I'll do this little speech. And it's working.'* *'My driving attitude has been completely transformed as I accept that I don't have to 'police' every driver who behaves badly. This has resulted in my driving becoming a much more pleasant experience. It is part of recognising "the things that I cannot change.'*
Jan	S4	*(Referring to what another participant was sharing about pausing before reacting.) '… to just pause like you did, because that's such a brilliant example... just to pause sometimes before responding.'*
Tanya	S2	*Referring to thinking: 'I think it's a learned response. You've learnt it all your life, and you go back to it again whenever your body does or whatever, until you become mindful … Step back a bit and see what you're doing …'* *'I have actually made a change, I downloaded Andy Puddicombe, I don't know if anybody's heard of it. Headspace, it is. (Referring to an app for meditation) It's all about just letting the thoughts flow through.'*
	S3	*' … I thought, this is bullshit. That's exactly what I thought. You've got to start thinking in here, change your thoughts.'*
	S8	*(Referring to a quote that this participant sent to the group relating to not having the power to change circumstances, but having the power to choose how you respond) 'Things we can't change. A bit like serenity... That really resonated with me. Because we don't have control.'*
Sandy	S2	*'And yes, it's very stressful, but I can't change who I am.' [Exception]*

4.6.3 Theme 3: Confusion and scepticism about using diet/ dietary supplements to manage stress

This theme contributed to answering the research question about the lived experience of using diet and/or DSs to manage stress. A general sense of scepticism about using DSs was shared by all but one participant but did not prohibit purchasing them by some of the sceptics. All the participants shared dissatisfaction with the evidence available for the effectiveness of DS consumption and diet in relation to health generally. None of the participant's were positive about using prescription pharmaceuticals to manage anxiety or stress, regardless of whether they had used them previously or were still using them. Only one participant shared a positive experience using an activated form of folate and vitamin B12. Participants who used DSs brought them to the last three sessions and shared their usage habits. Two categories supported this theme: 'alternatives to pharmaceuticals' and 'supplementation and diet.' The following illustrations demonstrate participants' thoughts about their confusion and scepticism with regards to the use of diet and/or DSs to manage stress.

Table 4.7: Confusion and scepticism about using diet and/or dietary supplements to manage stress (Illustrations)

Names	Source	Confusion and scepticism about using diet/ dietary supplements to manage stress (illustrations)
Cindy	S2	*'… how to fix it (stress) without going to the doctor and getting the doctor to fix it'*
	S4	*'I'm so conflicted with all these different ideas of how to eat.'*
	S5	*'(Referring to the supplements on display) 'I am really bad with supplements. I'll take them for a month, and then I just forget about them. I just found that, and I think I'm going to take that because that's good for stress, so I thought that might just be good for now. And that. And I'm going to start taking that. And I should be taking...They're just 'sometimes ones,' so that's how I go. But that will change. In a month that will look different.'*
	S6	*'I was very confused over what I was investing in.'* *'Is it really making a difference? There was just no real markers to see, to give me the evidence. So I think that's why I do get over it, and just don't do it anymore.'*
Mandy	S4	*'… he put me on antidepressants you see, because none of this was discussed. I've been on antidepressants for 20 years, so trying it is not the issue. Ever being not on antidepressants is the issue. I'm not on a high dosage. But I wish in some ways that I had got the cognitive therapy along with the antidepressant.'*
	S5	*'I've always been caught between skepticism of anything that hasn't been researched in the traditional scientific way, and given the seal of approval.'*
Tanya	S4	*'When I was younger I was always on antidepressants. But then I wouldn't do it again, because I thought this is not the right way to go …'* *(Referring to a MTHFR gene polymorphism) 'Through a blood test it was discovered that I had two gene mutations, one from my mother, and one from my father. Part of those mutations also gives you anxiety, or makes your anxiety worse. So I thought, 'oh for god's sake, this all sort of makes sense to me.' My body hadn't been absorbing these B vitamins that I'd been taking for years.'*
	S7	*Referring to the use of dietary supplements to manage anxiety and stress: 'This is working for me. This is the best I've ever been in years. I'm not obsessing as much' [Exception]*
Poppy	S7	*(Referring to using dietary supplements) 'You know the stuff that scientists say, all you're doing is making expensive urine.'*
Amy	S7	*'Why would any of us, and I don't know because I'm not qualified to say this … if we maintained a healthy diet, and a healthy physical regimen would we need supplements forever and a day?'* *'So I wouldn't know how long to take something, unless I'm told so yeah. But with education I guess. Education needs to be done about all the supplements.'*

4.6.4 Theme 4: The role of relationships in the lived experience of stress

This theme contributed to both research questions. Relationships were experienced as being stressful in many of the participant's lives, including at work, within marriages and relationships with adult children, and during specific life stages when children were younger. In addition, some participants revealed via

email after the reunion, that childhood family relationships had been extremely stressful. The data revealed that change occurred sequentially. Five categories supported this theme: 'empathizing with participants,' 'accepting and offering help,' 'family of origin – psychological influences,' 'offering participants suggestions to manage stress,' and 'comparisons between participants – stress.' The following illustrations demonstrate participants' thoughts about the role of relationships in their experience of stress.

Table 4.8: The role of relationships in the lived experience of stress (illustrations)

Names	Source	The role of relationships in the lived experience of stress (illustrations)
Poppy	Journal Entry	*'Why am I so worried about what people will think?'*
	S4	*'I have a problem with speaking up for myself. I'll do anything for a quiet life.'*
	S4	*(Referring to a situation acted on at work after writing about it in her journal) 'That was just me standing up for myself, which is a first.'*
Poppy	Email	*'I'd never have met 'Assertive Poppy if not for these PAR sessions. She's given me, or shown me I have, what it took to endure the last 12 months. What would it have been like if hadn't learned I can say no, or yes less often, or speak up for myself and people would still like me? … One notices when I revert to 'Little Miss Sunshine' and asks "where's Assertive Poppy?"'*
Poppy	Email	*'My mother got pregnant at 20 while she was still at home … She became pregnant again when I was 4 … We were sent away until my sister was born … She was bad with money and all that entails. Won't go into detail but it wasn't fun. Missed Nanie so much I'd cry myself to sleep.'*
Tanya	S2	*'I have been stressed all my life. Alcoholic father, mother left us when we were young. He was very abusive. Uncle in our family was very abusive. So, I never had a normal life.'*
	S7	*(Referring to a habitual response to a colleague and action taken to overcome such) ' ... I got off the phone and I went, 'now this is my chance, how I react to this, because this is where my buttons do push.' So every time I sort of thought about it again I thought 'don't speak to me like this. I know why you are, and I understand your reasons.' I haven't really thought about it since. Yes. That's a hell of an accomplishment.'*
Cindy	S5	*(Referring to her family) '… and so I'm starting to get into that victim mentality of 'why do I have to do it at all, and can't someone help me?'*
	S8	*(Referring to her family) 'I felt like, okay, maybe they don't understand, or they don't notice what is ticking me off, so maybe I just need to be more obvious.'*
	Email	*'… been quite busy really, promotion at work has stretched me mentally but I seem to be in calm waters … have been running more meditation groups lately and had the chance to practice formulating a relaxation session … getting good feedback … '*
Mandy	Journal Entry	*Alcoholic father, brothers and daughter…'*

Names	Source	The role of relationships in the lived experience of stress (illustrations)
	S5	*'I was working four jobs at four different locations … I was painting the house … doing gardening. I was coaching … athletics, and taking them (children) to swimming, and piano lessons ... So it was a typical 40 something then, crazy time.'*
	S8	*(Referring to her daughters alcoholism) '… my job is not to take on internally the burden of her stress, for my health…. she is the only one who can fix ... make the offer, and then step back. That's all I feel I can do.'*
	Email	*In relation to her mother, who later divorced her father: 'Her pregnancies were not planned ... At that time they had two rooms in an old tenement building.*
Danni	S1	*'Years ago, I was going through a very despairing time, felt highly stressed because I mean I had a successful career, some would say, happily married with three children and juggling tasks and …'*
	S2	*'I think my experience is that women are almost expected to be able to cope under any circumstance and that they're always there for other people …'*

4.6.5 Theme 5: The role of relationships in managing physical health and stress

This theme contributed to answering both research questions, about the lived experience of stress and using diet and/or DSs to manage such. Results revealed that some of the participants use diet and/or dietary supplements to manage stress and that close relationships or the lack of them, did impact food choices. Family and friends may have influenced participant's choices of dietary supplements, through 'word-of-mouth', and the Internet and Social Media also played a role in nutritional and dietary supplement education. During the eight weeks, some of the participants initiated changes that they felt were supportive of their well being, which included hiring a personal trainer and using vitamin D supplements. Four categories supported this theme: 'family of origin – health influences,' 'comparisons between participants – diets,' 'social media and Internet health advisors,' 'role of relationships in health' and 'doctors advice.' The following illustrations demonstrate participants' thoughts about the role that relationships play in managing physical health and stress.

Table 4.9: The role of relationships in managing physical health and stress (Illustrations)

Names	Source	The role of relationships in managing physical health and stress (illustrations)
Cindy	S3	*'Then, he had the hide to say, "well, you spent $200 at the supermarket the other day … you've been buying too many blueberries. You've been buying heaps of health food, and it's costing a lot … " '… But it's to help me manage the stress.'*

Names	Source	The role of relationships in managing physical health and stress (illustrations)
	S6	*'I have invested in a personal trainer. And she's on me, and it is really good. It's a good reminder, it's in my face … I just thought support is actually really big. Just like our emotions need support of others, I can see how our physical body would require support, and how much better we feel when we get that input from others. So we're not meant to do it alone. Support gives you confidence.'* *'I don't know. I've just heard they're good for stress.'*
Poppy	S6	*' … I would always go for the healthy. But I just can't be bothered making it. I think it's because I live on my own. So what I have toast, there's no one here to see me … I made a food diary, and it's shocking.'*
Danni	S7	*'I've done it, and I've heard you all say it … Good old Dr. Google has told us something. Because some of these you look at, and you go, you start reading it and then it's sort of advertising something. You sort of think, what value has this got. What qualifications do the people have who are saying this?'*
Mandy	Journal	*'Bought Magnesium tablets on the suggestion of my mother who claimed that taking them had reduced her leg cramps and was helping her sleep.'*
	Email	*'I am still very much a work in progress needing to take more responsibility for dietary choices; but I have been taking large doses of Vitamin D and have been on a program for improving my hormone balance. The natural progesterone, in combination with magnesium seem to have improved my sleep pattern. I think the Vitamin D has given me more energy. I no longer need to lie down in the afternoon for a rest.'*

4.6.6 Theme 6: Strategies aimed at managing stress

This theme contributed to answering both research questions, about the lived experience of stress and using diet and/or DSs to manage such. Results revealed that participants used different strategies to manage stress, some of which related to diet and exercise. One participant had already established practices in her life related to stress, aspects of which she shared with the group. Another participant believed a specific DS helped her manage stress and another believed that being healthy and taking responsibility would have a similar effect. Some participants believed that supportive relationships were helpful in managing stress. There were no changes noted in relation to these strategies. One category supported this theme: 'strategies aimed at managing stress – including reflecting on diet and exercise choices.' The following illustrations demonstrate participants' thoughts about strategies they use to manage stress.

Table 4.10: Strategies aimed at managing stress (Illustrations)

Names	Source	Strategies aimed at managing stress (illustrations)
Danni	S1	*'I went to a seminar where I was given all sorts of tips on how to manage my stress levels. And I can say, 15 years later, I'm still practicing most of those and as a result, I very rarely feel super stressed anymore.'*
	S8	*(Referring to the eight sessions). 'I've gone full circle. I understand when I become stressed it has a detrimental effect on every aspect of my life. It is within my power to use strategies learned over the years to cope as best I can. Occasionally I believe supplements can be beneficial, especially if they are natural, taken for the correct duration, and are necessary. Diet and exercise play an enormous role in our mental and physical well-being. That's how I summed it up.'*
	Journal Entry	*'On my return home, I rang one of my friends and as soon as she answered … I said: "Can I vent?" I told her about my day, then had a lovely catch up chat … Immediately felt less stressed! Have definitely realized how important support can be in helping to relieve stress.'* *'Last night's discussion really provoked a lot of deep thought for me. Not only is the relationship between anxiety and stress interesting, but how different personality types react to stress. Also realized for myself that verbalizing my anxieties/stress to others immediately helps me to feel better.'*
Tanya	S6	*(Referring to the supplements she brought with her to the session) 'These are the MTHFR tablets that I take … When she (Doctor) found out I had these gene abnormalities she got me onto these, so I know that the Bs are working, I don't get so stressed.'*
Poppy	S2	*'I've got a friend … we get together for hugs. He'll pull me in at the coffee shop, and the hug has to last at least five seconds or it's not a hug … At least three or four times a week. And he's been away for six weeks, so I'm going through withdrawal from hugs.'*
Amy	S5	*'The best thing is taking responsibility. Because once you take the responsibility then you know you have the power to take control over what's happening. As much control as what you can do. But if you don't take any of the responsibility then you're left at the whim of what's happening to you.'*

4.6.7 Planning for action

Participants were more open in their introspective journaling and more likely to anticipate action in the service of managing their stress in this medium versus what they communicated with each other within the group setting. Furthermore, it was noticeable to all present that reflective journaling occurred less regularly as the sessions continued, with a number of the participants commenting on this phenomena and interpreting it as progress in terms of their understanding of themselves, their response to stress and their ability to manage it more effectively. For example, as Danni stated in the last session:

> *'Interesting to note that initially I made copious notes but the more I understood how and when I was stressed, plus how I could manage it better, I began to make less notes!'*

The table below contains illustrations reflecting 'plans for action' that appeared in the participant's journals and which were vocalized differently in the group sessions.

Table 4.11: Plans for action (Illustrations)

Names	Source	Plans for action (illustrations)
Poppy	Journal Entry	*'Have noticed over these weeks that I work myself up and I just stop and say 'no' or 'disagree' it's not the end of the world.'*
	Journal Entry	*'Decide I will say something at union meeting: feel sick, cold, shaking.'*
	Journal Entry	*'In other stressful situations, I find myself recognizing and examining the cause of the symptoms when I feel anxious. My self-talk has become more positive, though a lifetime of being negative and 'down on myself' will not disappear overnight.'*
Mandy	Journal Entry	*'I have been reading a lot about diet and nutrition and am committed to improving my habits … and discussed lifestyle changes with my husband. If I can persevere with this mental and physical health focus, I am hopeful I will be able to suffer less anxiety and feel less fatigued.'*
	Journal Entry	*'Today I made 'laid back' driving a focus, being mindful of not allowing other road users to trigger angry responses in me. Really enjoyed the exercise and felt quite pleased with myself.'*
	Journal Entry	*(Referring to feeling anxious and stressed) 'Working on strategies to recognize and defuse the feelings…'*
	Journal Entry	*'In an attempt to reduce caffeine consumption and aid relaxation: 'I bought Chai tea to replace evening cuppa and then discovered that it also has caffeine.'*

4.6.8 Sharing progress and action

Although participants continued to comment on and discuss their experiences in relation to both research questions towards the end of session eight, all the participants articulated the feeling that the group had circled back and completed our investigation. As Tanya stated at the final session:

> *'I don't know where we can go from here. We can listen, and be supportive of one another when problems arise, but I don't feel that we can achieve much more regarding our original topic.'*

One participant suggested that the group get together again, for a reunion, to which all the participants were amenable, and which did lead to a reunion ten months after the final session, which became the final 'member check' of the study:

'But what I would like to see, and I don't know if this can be arranged, is in six months if we did meet, whether we met for a lunch and just said what have we done, what have we changed, has it worked.' (Mandy)

This suggestion provided a data verification opportunity and initiated a discussion about stress in childhood, which unexpectedly led to another source of data, and to a final cycling back to the first research question, the lived experience of stress. However, 'cycles don't just mean returning to the past; cycles mean moving forward in the present, recalling past experiences …. for references as comparable situations in the future' according to Saldana (p. 43).[454]This participant was planning on moving forward within her cycle of new knowledge, referencing the past to ascertain whether what she and other participants had put into action, was still working.

4.7 Discussion

This exploratory PAR project examined an under investigated phenomenon that is both deeply personal, yet shared by many women globally; the experience of stress and the role that diet and/or DSs may play in attempting to manage stress. The PAR methods provided an opportunity for flexibility by allowing the participants to respond as they saw fit, to develop insight and possibly improve their lives.[398, 422, 435]

Six themes were developed from the analysis of focus group data, the journal notes and the emails received after the reunion session. The six themes related to action and change in relation to the two research questions. (See Figure 4.6, Section 4.6, 'Results of the PAR study').

4.7.1 Causes and effects of stress

A general lack of clarity regarding the difference between stress and anxiety was pervasive among the participants, which led to a group activity to examine the differences leading to descriptions of the physical sensations that accompanied the experience. Some of the causes related to time pressure and a lack of control, as illustrated by Tanya and Cindy's comments: ' *… stress, it's different. It's 50,000 things to do at once … you're stricken*' and '*Your body starts to seize up when you can't control how many things are coming at you.*' Mandy confirmed the physical sensation by stating: '*I used to experience an almost constant 'fight or flight' feeling when I was working …*'

4.7.2 Thinking patterns

Thinking patterns were recognized among participants as influencing their lived experience of stress and behaviour and were acknowledged as possibly becoming habitual via introspective comments shared in sessions and journal entries. Attempts to change such were noted among many of the

participants. For example, Tanya stated that: '*I thought, this is bullshit. That's exactly what I thought. You've got to start thinking in here, change your thoughts.*' Tanya also stated that: '*You've learnt it all your life, and you go back to it again … until you become mindful … Step back a bit and see what you're doing …*' Mandy changed an habitual, thinking pattern: '*I had a triumph. I have turned around a behaviour, I think, that has had me in its grip for many, many, years, and that's road rage … So now when I get in the car I go through a process. I make sure everything is done when I back out. I'm mindful. Then I say "I'm going to have a safe, calm journey. I've left plenty of time." I'll do this little speech. And it's working.*' In relation to introspection Danni stated: '*Today … I was having a fantastic day until I allowed something to stress me. I really shouldn't have gotten tensed up and angry about it, because there was nothing I could do.*' There is little research to support whether thinking patterns are recognized as contributing to the experience of stress among women or whether chronic stress contributes to them becoming habitual. However, one mixed gender study which allocated student volunteers (n=44 (11 men and 33 women)) to either an experimental group (n=21) that repeated positive thoughts in the presence of feelings of anxiety, depression or stress, or a control group (n=23) which did not, revealed that positive thinkers experienced a reduction in such mental states including a reduction in stress.[455] In addition, a study that investigated women (n=98) with fibromyalgia (FM), found that high levels of rumination, previously defined as 'the engagement of negative and unwanted past-centred thoughts,' were correlated with stress as measured using the PSS (p. 245).[45, 456] Rumination may thus lead to poor problem solving capacity and inflexible thinking.[456] Although not directly related to stressed women these results suggest that, as in this study, chronic stress was related to ruminative thinking which may become entrenched over time, and is reflected in Tanya's comment about her thinking patterns and stress: '*I think it's a learned response.*'

4.7.3 Confusion and scepticism about using diet and/or DSs to manage stress

Confusion and scepticism regarding the use of diet/and or DSs to manage stress was pervasive among participants with Cindy stating '*Is it really making a difference? There was just no real markers to see, to give me the evidence.*' The same participant admitted she was '*really bad with supplements. I'll take them for a month, and then I just forget about them.*' However, a desire to use alternatives to pharmaceuticals to manage stress and anxiety may drive purchasing, as stated by the same participant: ' *… how to fix it (stress) without going to the doctor and getting the doctor to fix it.*' Conversely, Tanya stated, in relation to a gene-specific nutrient: '*This is working for me. This is the best I've ever been in years. I'm not obsessing as much.*' However, for the rest of the participants, purchasing behaviour and the use of DSs may be based more on 'Hope and Hype,' than confidence as to their efficacy (p. 188).[457]

In addition, all the participants agreed with Amy who stated: '*Education needs to be done about all the supplements.*' There is no research examining whether confusion and scepticism is related to the use of diet/ DSs to manage health or stress, despite growth of the stress-management DS market being expected to reach USD16.7 billion by 2025.[8]

4.7.4 The role of relationships in the lived experience of stress

A variety of relationships played a role in the experience of stress among the participants. In relation to the multiple roles that women play in society, Danni stated: '*I think my experience is that women are almost expected to be able to cope under any circumstance and that they're always there for other people, so they're there for their husband or their children, their parents … you take it on as if you have to.*' Although there is scant research that has focused on female anxiety and stress generally, or whether being expected to cope just by virtue of being a women leads to anxiety or stress, a Swedish study (n=60) that investigated work-life challenges, time scarcity and the physiological response to such, revealed that female cortisol levels stayed high after work in relation to men's levels.[79] In addition, Luecken et al. reported that mothers have higher levels of stress than women without children as revealed by a survey where levels of strain at work and at home were measured in women (n=109) over a 24-hour period using a validated Strain Scale measurement tool.[87] Furthermore, a study investigating parents' perceptions of parenting in the UK via interviews found that work-home conflict did generate stress in female participants (n=17 (1 male and 16 women)).[458]

Personal insight into what stress means personally, change and increased control within and in relation to relationships, occurred in a myriad of ways. For example, Poppy stated in an email after the reunion session that: '*I'd never have met 'Assertive Poppy if not for these PAR sessions. She's given me, or shown me I have, what it took to endure the last 12 months. What would it have been like if hadn't learned I can say no, or yes less often, or speak up for myself and people would still like me? …* In relation to being more direct with her family in terms of what she needed, Cindy stated that: '*I felt like, okay, maybe they don't understand, or they don't notice what is ticking me off, so maybe I just need to be more obvious.*' In relation to her daughter's alcoholism, Mandy stated that: *my job is not to take on internally the burden of her stress, for my health…. she is the only one who can fix ... make the offer, and then step back. That's all I feel I can do.*' There is a paucity of research examining a personal change process that occurs as a result of insight into stress and challenging relationships. However, the link between close relationships and quality of life has been established in light of the basic human desire to belong, be accepted and maintain close relationships.[459-461]

Although present relationships were primarily reported on during the focus group sessions, emails after the reunion session reported on relationships during childhood. This change in the depth of

sharing became an unexpected and additional source of data and revealed that the three participants in the project who reported experiencing the most stress and anxiety in adulthood also shared the experience of childhood trauma, also known as early life stress (ELS). Mounting evidence supports a causal relationship between the stress they experienced as children and their adult stress-responses. For example, results from the Helsinki Birth Cohort Study 1934-44 (n=13,345) in which approximately 14% of the children were temporarily separated from both parents during child evacuations in WWII, revealed that these individuals later experienced increased stress reactivity among other psychiatric effects.[125, 127] Partial explanations are the disruption of optimal HPA development and functioning and the possible disruption of optimal PFC development with the concomitant inability to manage self and environment optimally in the face of adulthood stressors.[42, 128, 130, 462-467] In addition, ELS in the form of caregiver deprivation impacts amygdala development negatively in humans, which reduces the ability to assess and respond to affective stimuli, which may explain higher rates of anxiety noted in such populations.[129] Indeed, a positive habitual response to stress exposure may be acquired when ELS is not present.[468] However, there is no research examining how women respond to discovering that ELS may impact their ability to manage stress nor whether using specific nutrients can ameliorate the experience of such when ELS was present.

4.7.5 Role of relationships in managing physical health and stress

Whether relationships directly supported their health or not was discussed among the participants and one of the women decided to invest in a personal trainer because she felt that: ' *… support is actually really big. Just like our emotions need support of others, I can see how our physical body would require support, and how much better we feel when we get that input from others. So we're not meant to do it alone. Support gives you confidence.*' Positive personal relationships do impact health and happiness levels as evidenced by a review that investigated such.[469] Furthermore, the presence or absence of family members may also impact food choices as suggested by Poppy: '*But I just can't be bothered making it. I think it's because I live on my own.*'

With regards to 'word of mouth' DS use prompting, Cindy stated that: '*I don't know. I've just heard they're good for stress.* As a result of the discussion about DSs in the focus groups, Mandy investigated whether she was deficient in any nutrients with the help of her medical doctor and stated: '*I am still very much a work in progress needing to take more responsibility for dietary choices; but I have been taking large doses of Vitamin D and have been on a program for improving my hormone balance. The natural progesterone, in combination with magnesium seem to have improved my sleep pattern. I think the Vitamin D has given me more energy. I no longer need to lie down in the afternoon for a rest.* There is limited research evidence examining how and why relationships impact the use of diet and/or

DSs as stress-management strategies despite large multi-level marketing companies using relationships to encourage the purchase of DSs generally.[470] One Italian survey of students (n=770) reported that some of the respondents took advice about food supplements while nearly half of them reported that their advice came from medical doctors.[471] Similarly, results from an Indian survey (n=120) reported that 12% of the respondents relied on family and friends for advice about MVM supplementation, while close to 70% relied on the advice of a medical practitioner.[472] The role of relationships in the purchasing and use of DSs was articulated by Mandy: '*Bought Magnesium tablets on the suggestion of my mother.*'

4.7.6 Strategies aimed at managing stress

Participants commented on strategies already used to manage stress levels, for example, as stated by Poppy: '*I've got a friend … we get together for hugs. He'll pull me in at the coffee shop, and the hug has to last at least five seconds or it's not a hug …* Danni agreed by stating: ' *… realized for myself that verbalizing my anxieties/stress to others immediately helps me to feel better …*' A review of 81 studies that investigated the relationship between social support and physiological processes found that relationships may act as a buffer against stress and that familial sources of support may impact health.[473] The researchers suggest that secure and supportive family relationships are critical determinants of the capacity for regulation of emotional and physiological processes.[473] Similarly, Burns et al. reported on a study (n=364) that examined psychological functioning and the quality of interpersonal relationships, which found that interpersonal relationships moderated the effect of life events on subjective wellbeing more than number of life events.[460] However, there is limited research available that has investigated self-imposed stress-management strategies among women, although a small study was undertaken to investigate such via interviews with 18 women who juggled a number of roles.[474] The results revealed that the participants employed up to six strategies, including managing time and energy, releasing responsibility and maintaining health and wellbeing to find balance between their roles. In support of these findings, some participants believed that managing stress should include a focus on health and taking responsibility, as Danni stated: '*Diet and exercise play an enormous role in our mental and physical well-being*' while Amy stated that: '*The best thing is taking responsibility. Because once you take the responsibility then you know you have the power to take control over what's happening.*'

The difference between the depth of detail of participant sharing between data sources may suggest a desire by the participants to avoid verbalising intentions. Participants may have felt comfortable writing about an anticipated action before expressing their intention in the group setting. In their minds, thinking and journal writing may have represented planning for action. They may thus have avoided establishing expectations or accountability in the group, so as to avoid disappointing

themselves or others. The decreased journal writing over the eight-weeks suggests that as participants became more active in controlling and self-managing their stress, it became less important for them to document their behaviour, their stressful experiences and how they managed such. As stated by Danni: '*Interesting to note that initially I made copious notes but the more I understood how and when I was stressed, plus how I could manage it better, I began to make less notes!*' There is no research to support either the difference between planning for action privately and sharing such plans, or decreased journaling as personal control increased.

Many of these introspective and reflective theme-based illustrations reflect changes in behaviour and thinking patterns that occurred during and after the eight weeks, with illustrations from journals reflecting sentiments shared in the sessions related to change and thinking patterns. For example, Poppy stated in her journal: ''*Have noticed over these weeks that I work myself up and I just stop and say 'no' or 'disagree' it's not the end of the world.*' While Mandy, referring to feeling anxious and stressed, stated in her journal: '*Working on strategies to recognize and defuse the feelings…*' These changes signify emancipatory change and action from specific thinking patterns and habitual responses that participant's became aware of and acted on to change.

Greater personal change was noted in a number of the participants as the project progressed, with significant changes in participants lives reported via email after the reunion, as evidenced by the illustrations in the tables reflecting such. For example, Poppy stated: ''*I'd never have met 'Assertive Poppy if not for these PAR sessions …*' As expected, emancipatory change and action was unique to each participant and did not follow a pre-planned or anticipated format because progress evolved iteratively across the project, with 'action' and 'reflection' overlapping as per Lewin's proviso.[431, 475] In addition, emancipation, as per McTaggart's definition, was not 'revolutionary' but instead resulted in value driven changes (including in perspective) that the participants felt improved their lives compared to what they experienced prior to this project (p. 205).[422]

4.7.7 Limitations

There are a number of methodological limitations to this study. There are a large number of variations in method and methodology for PAR studies, including that participants should ideally be co-planners and analyse the data, both of which did not occur in this study.[426] In addition, this project was not conducted in a specific political or organisational context aimed at emancipation from external domination or control, another goal that many critical social theory PAR methods share. Instead, this was an exploratory study to investigate a psychological phenomenon, initiated by the researcher, who also decided on the research approach in the context of a PhD candidature. This required the researcher to develop and lead the inquiry in a suitable timeframe.

The written word, via transcription, is not a completely reliable data source because it cannot accurately reflect all 'the mental, social, affective and cultural components of both the individual and group performance' (p. 65).[476] Subtle nuances may be missed despite careful transcribing. However, obtaining feedback from the participants via transcription reading between sessions somewhat mitigated this limitation, as did using journal entries to validate transcription data and final data checking at the reunion. In addition, the informal structure and relaxed atmosphere within which the focus groups took place, led to laughter and humorous comments between participants, and these moments were recorded in transcriptions, and considered in contextualizing the analysis.

The difficulty recruiting time-poor women, the over-representation of older women, and only eight of 22 interested women taking part in the project could be perceived as limitations. However, it may also be a confirmatory factor within the developing theory that relationships, including family responsibilities during certain life stages, play an integral part in women's lived experience of stress. In addition, this limitation would exist for any research project attempting a deeper understanding of this topic.

That participants were equally confused and sceptical regarding this topic is further evidence of a knowledge gap in this area of women's health. In addition, although the positive impact of healthy social relationships on psychological wellbeing is well documented, the impact of challenging personal relationships, and/or ELS, on the experience of female stress is an area that warrants further investigation. It is recommended that evidence regarding the lived experience of stress and the impact on chronic stress of diet and/or DSs should be made available via online resources in light of their prolific use globally and women's use of the Internet for health-related information.[8, 232] In light of the growing body of evidence linking ELS to heightened stress-reactivity, the conceptualisation and development of intervention strategies targeting pregnant women and mothers, with the aim of mitigating ELS for children, so as to improve anxiety and stress reactivity in adulthood, is warranted.

4.7.8 Conclusion

Many PAR projects, including this one, aim to create positive change on a small scale, with a view to further research that can support change on a larger scale. Engagement with a small community or group is as important as large-scale projects because any attempts made by people to increase their ability to make sense of their world and act effectively within it improve their quality of life and the lives of those around them. This in turn creates positive change in the world.[410] The benefit to participant's within this project were an increased understanding of what living with stress feels like for other women, being exposed to positive strategies used by others to manage their feelings of stress, insight into ways of being, including thinking patterns and habitual behaviour that may increase stress levels, and

information about whether diet and/or DSs were useful in managing stress. The findings suggest that the participant's benefited from being in this project as evidenced by positive action and behaviour change, which continued beyond the eight weeks of active group participation.

Chapter 5

CONCLUSION

5.1 Conclusion

Despite a growing body of evidence suggesting that diet may be a modifiable risk factor in relation to mental health and that a specific DP can prevent depression, the impact of nutrition on chronic stress remains unexamined.[163, 164, 170, 277] As this thesis has shown, women report higher rates of stress than men globally and consume more DSs, and due to the growing expenditure on stress-targeted DSs, the objective of this thesis was to investigate the relationship between perceived stress and specific nutrients consumed in the daily diet and/or via DSs in among women.[4-6, 8, 17-28, 230]

To investigate this issue, three studies were conducted, a systematic review (Chapter 2), a cross-sectional survey (Chapter 3) and a PAR project (Chapter 4). How these projects formed a cohesive whole with which to answer the research question is addressed in this chapter, along with limitations specific to these projects and those related to broader ones in EBM and mental health. Implications for practice and future research into the use of nutrients for female stress management are also discussed.

The JBI Model of EBHC (Figure 1.5, Section 1.9) introduced in chapter 1 provided the framework for evidence synthesis and generation to investigate the high levels of stress reported by women globally and the prolific use of DSs to manage stress.[265] This model visually presents generation of knowledge as being iterative in nature and illustrates that although global health improvement is 'both the goal and the endpoint of any or all of the model components …' this process may not be linear (p. 209).[269] Therefore, the relationship between the 'inner wedges' may at times be bi-directional, as occurred within this study, where evidence synthesis preceded evidence generation. Thus, the systematic review (Chapter 2), which identified a knowledge gap, led to the generation of evidence that informed the following two research projects. These projects were underpinned by the models broad conceptualisation of evidence, which aligns the type of evidence generated to the particular knowledge need.[265] The 'evidence generation' wedge therefore provided a framework for both research projects: the cross sectional survey (Chapter 3) which generated quantitative evidence about the phenomena of interest and the PAR project (Chapter 4) which generated qualitative evidence using a participatory

group process and focus groups. Thus, combined, evidence was generated in relation to the research topic that provided empirical knowledge in the form of frequencies and quantities and also a deep, personal perspective about the experience under investigation. In addition, the centre of the model, the 'FAME' scale, broadly conceptualises the different types of evidence that can be generated and which clinicians are concerned with when using evidence in point of care practice.[265] Combined, all three of the thesis projects provided evidence that informed on the practical application of best practices (feasibility), on the specific context (appropriateness), on the individual experience (meaningfulness) and on which interventions were beneficial (effectiveness). The JBI model recognises that both robust quantitative and qualitative evidence may be required to inform complex healthcare challenges and practices, which was required for this research topic.[265] This model thus provides a conceptual framework within which such evidence can be generated, synthesised, transferred and implemented, providing healthcare researchers with a comprehensive EBHC model aimed at improved global health.[265]

5.2 Systematic review

The use of stress-targeted DSs fuels a growing global multibillion-dollar industry, with expenditure expected to reach USD16.7 billion by 2025, despite very little evidence to support the effectiveness of such to reduce stress levels.[8] Studies have suggested the use of vitamin and mineral supplements increases in people with a history of anxiety and/or depression. As women experience higher rates of these disorders than men, and consume more DSs than men, it is likely women also use more stress-targeted DSs.[4-6, 229, 230] The systematic review results in chapter two revealed a dearth of evidence to support the use of such DSs in the general female population, although the use of some specific DSs provided anxiolytic and stress-reduction benefits to some women during specific reproductive life stages. However, disregarding a specific hormonal stage, only vitamin B6 reduced anxiety in a group of older women and vitamin C reduced anxiety and mitigated increased blood pressure in response to stress among another group.[184, 293]

Although how hormones play a role in emotional modulation and impact the ability to manage stress was discussed in chapter 1, they do not account entirely for the high rates of stress reported by women globally. However, as evidenced by the Systematic review, current research that has examined stress in relation to hormonal disequilibrium and nutrient intake excludes women who are experiencing chronic stress regardless of reproductive life stage. The gap in the evidence base identified through the systematic review informed both the design of the cross-sectional project (to generate descriptive data) and the PAR project for evidence of the impact of stress on the lives of study participants.

5.3 Cross Sectional Project

The cross sectional study was undertaken to examine the topic of chronic stress and nutrient intake among women regardless of hormonal stage. This study revealed that the relationship between PSS scores and age was not significant, however, DS users were significantly older than non-DS users. In addition, prior to adjustment for multiple testing, there was evidence for a number of significant relationships between nutrients consumed via DSs and PSS scores. There were substantive reductions in PSS scores related to intake of ALA, LA and EPO via supplements compared to non-supplementers. There was also a strong positive correlation between PSS scores and intakes of EPA and DHA among supplementers of these nutrients. This may be an indication that as stress levels rose so did the intake of DHA and EPA. In addition, there was a weak correlation between PSS scores and intakes of vitamin C and B6, and contrary to previous research, the combination of B6 and magnesium was not found to be significantly associated with lower stress levels either before or after adjustment for multiplicity. When assessing the relationship between nutrient intake from food and PSS scores, an increase in B6 was associated with a decrease in PSS scores. In relation to most nutrients consumed via food, as the intake of such nutrients increased so too did the odds of supplementation with that nutrient. However, after adjustment for multiple comparisons there was little evidence for significant relationships between any of these variables. Finally, analysis of the relationships between total intake of each nutrient (from food and DS combined) and PSS scores yielded estimates of effect considered to be very small in magnitude. Therefore, although there is a substantial body of research that suggests that such nutrients have critical roles to play in the CNS, evidence from the present study was inconclusive for the self-reported effect of these nutrients on stress, regardless of whether they were obtained from food or DSs.

Apart from the relationships between nutrients and PSS scores, behaviour associated with DS use revealed that the two main reasons for using DSs was to increase energy (35%) and reduce stress (22%). In addition, the use of 31% of the DSs were cited as being prompted by health care provider recommendations, although for 42% of DSs used, participants were unsure of whether the DSs were useful. Whether there is a relationship between these variables is uncertain, although it may be that participants used DSs as directed and as ‘health insurance’ but a lack of measurable health outcomes led to uncertainty in self-reporting usefulness. Furthermore, as the nutrients of interest increased in the participant’s diets, so too did the odds of them using DSs. The concept of ‘health insurance’ (Discussion, Section 3.5) may partly explain the use of DSs despite the lack of self-reported benefits.

5.4 PAR Project

The PAR project was undertaken to gain a deeper understanding of the experience of living with stress and the role that diet and/or DSs play in this experience. Relationships emerged as playing the most significant role in both the experience of stress and the decisions made about DS use. Stress was understood by participants as being related to the multiple roles women play at specific life stages, such as being mothers and working outside the home. In addition, relationships were also understood as having the capacity to provide support during times of stress, and the use of DSs was prompted by suggestions from trusted others who had experienced positive results. Furthermore, the three participants who reported experiencing the most stress and anxiety in adulthood also shared the experience of ELS. Other research suggests the quality of personal relationships contribute to a sense of wellbeing, that ELS impacts the ability to modulate stress neurobiologically in adulthood and that relationships impact food choices, yet there has been no research about how relationships impact DS choice and use in female stress, nor whether DS use impacts the effect of ELS on the adult stress response.[128-130, 465-467, 477] In addition, similar to previous research that has revealed that women use the Internet prolifically to gather health related information, which included the use of SM, the PAR participants acknowledged their use of such for the same purpose.

Although the use of the Internet for health related information is prolific among women and the use of DSs to manage stress is also prolific globally, and a number of participants did use DSs to manage stress, this study showed participants all shared a general sense of confusion and scepticism about the use of diet and/or DSs to this end. Furthermore, they all shared a desire for education about DSs to be made available.

Although relationships were related to the experience of stress, and DS use, and their confidence in DS efficacy was poor, some participants also revealed a rudimentary understanding about the neuropsychological processes underpinning long-established, habitual thinking patterns, which they described as learned responses. During this project, participants self-awareness increased and led to positive changes in their thinking patterns and behavioural responses, and changes in DS use, all of which they described as helpful experiences resulting from participating in this project.

5.5 Recent research

There is limited evidence linking the amelioration of chronic stress experienced by women to specific nutrients, consumed either via the diet and/or DSs. This is despite evidence linking the same nutrients to stress hormone and neurotransmitter synthesis, and the possibility that stress hormone synthesis may take precedence over the synthesis of neurotransmitters that modulate emotion and mood.[2, 172, 282,

[340, 478] Similarly, although evidence links chronic stress to the development of affective disorders, and research suggests that the first stress-induced episode of depression increases the likelihood of subsequent episodes, there is no evidence to suggest that ameliorating chronic stress via the consumption of specific nutrients in any form may prevent the development of affective disorders among women.[46-55, 479, 480] For example, a systematic review that investigated the effectiveness of MVM supplementation in reducing stress levels only included one exclusively female study that examined psychological distress.[172, 317] In addition, although studies have reported a reduction in stress among women using a B-complex DS, impact on chronic stress is under-reported.[180, 270]

Nevertheless, more recent research suggests a gender-influence; nutrients may impact female versus male neurobiology differently, which in turn may impact subsequent experiences of chronic stress. Men are more likely to experience mental wellbeing until severe nutrient deficiencies arise while following a western-type diet, while women are less likely to experience the same until a balanced diet and healthy lifestyle are attained, such as that obtained using the Mediterranean diet and lifestyle.[278] The authors suggest dietary sufficiency potentiates heightened limbic system regulation in women, which is suggestive of nutrients impacting mental wellbeing differently in women than men.[278] In addition, a recent systematic review and meta-analysis revealed female subjects noted significantly greater benefits from dietary interventions for symptoms of depression and anxiety.[279]

Therefore, by the time depression and/or anxiety manifest, due to the neurobiological impact of chronic stress, nutrient deficiencies may have become severe and been present for a considerable period of time. However, the evidence is that benefits in relation to female mood take up to a year of MVM supplementation.[272] In addition, individual variation in relation to HPA activation and a possible inability to return to homeostasis, nutrient-specific genetic polymorphisms and the presence of ELS, may further influence nutrient requirements in that specific nutrients may be more useful to stress-reduction than others. Therefore, as per the conceptual framework developed in chapter 1, women may be both uniquely predisposed to experiencing chronic stress and uniquely experience benefits from nutrient interventions in relation to chronic stress. However, the paucity of evidence examining these variables prevents the clinical recommendation of specific nutrients to reduce stress levels.

The lack of research examining the neurobiological effect of specific nutrients on female stress is compounded by the lack of evidence for the use of specific forms of nutrients by women who have MTHFR gene polymorphisms. Research suggests that individuals with these genetic variations may be more susceptible to mood disorders, although no research has examined whether this may impact the ability to manage stress; similar mechanisms may underpin such.[336] As mental wellbeing relies on a wide variety of biochemical activities, minor dietary deficiencies and genetic variations in enzyme efficiency may combine to impact emotional modulation and cognitive functioning and impact stress

management abilities.[172, 334, 481] (See Sections 2.12 (Limitations of the studies), 3.5 (Discussion) and 4.6.3 and 4.6.6 (Results), for information about the use of activated DSs.)

ELS significantly increases the likelihood of nutritional deficiencies due to the circumstances within which it exists, (including deficiencies of specific nutrients in utero and childhood that influence CNS structural development and function and the development of neurotransmitter systems), all of which predispose adults to less adaptive coping strategies in the face of stressors.[482, 483] Animal studies have suggested early nutritional interventions provided clinical benefits to offspring in relation to brain structure and function by offsetting the neurobiological effects of ELS during early brain development.[484] Whether this is the case in relation to human neurological development in the presence of ELS remains unexamined.

Similarly, despite the prolific use of the Internet by women to gather health related information, there is limited evidence about what percentage of such behaviour is aimed at managing chronic stress and whether it results in the use of stress-targeted DSs. There is also limited evidence about whether, and how often, advice from friends and family inform stress-management DS use, although some studies suggest friends and family do impact DS use, although use is more influenced by advice from medical doctors.[471, 472] However, none of these studies report on whether DS use was perceived as being beneficial. Lack of evidence does not correlate with belief, as a 2015 online survey (n=2,016), initiated by the Council for Responsible Nutrition, reported that 85% of participants were confident in their use of MVMs.[485] Therefore, the use of the Internet for gathering information about managing stress using DSs, whether advice from friends and family inform DS use for the same end and whether there is confidence in the use of DSs for stress-management purposes all remain poorly examined areas of research.

5.6 Limitations

The lack of standardisation in the amounts, combinations, and confounding substances in DSs used in the EFA studies in the systematic review was a limitation, as was the lack of baseline data on chronic stress in relation to daily life regardless of reproductive life stage. In addition, the heterogeneity and varying rates of reliability of the outcome measures made study comparisons challenging, as did the variations in study length. Overall, the evidence identified through the systematic review was conflicting and no consensus was able to be established on the effectiveness of using any nutrient for stress and/or anxiety reduction, regardless of life stage.

The directionality of the relationship between perceived stress and nutrient intake and/or DS use cannot be ascertained from cross sectional studies. In addition, the sample was small and not

randomly selected although it is likely that recruiting stressed women is a research challenge inherent to this field of research. In relation to the measurement instruments, the SUQ was not fully tested for construct validity or reliability. Assessing chronic stress presents diagnostic challenges due to individual perception and response to stress. Within the cross sectional survey perceived stress scores could only be compared across the sample using the PSS instrument, which although being a well-validated self-assessment measurement tool, is not a diagnostic or screening tool. Therefore, using the PSS in conjunction with biological stress markers would go some way to addressing this challenge.

In relation to the PAR project, stressed women may have been unable to participate in the focus groups, which would have contributed to selection bias. The nature of action research does not lend itself to generalizability, therefore the results are best considered as applicable to participants, and transferability of these findings to other women in similar circumstances may not lead to the same results.

5.7 Within EBM/H

Due to the complexity inherent in social interventions, there may be a need for clinicians to consider evidence based on both experimental and non-experimental methods.[266] Therefore, the JBI model not only provided a framework for the research approaches within this thesis, it also allowed for the examination of a research topic that included components from the disciplines of psychology, nutrition and neurology.

5.8 Contributions and implications for practice and future research

The projects that comprise this thesis contribute to the scholarly research and literature in the field of human nutritional neuroscience and chronic female stress in a number of ways. Firstly, despite the prolific consumption of stress-targeted DSs in the form of isolated nutrients, no research has examined the relationship to chronic stress among women. Secondly, the lived experience of stress among women and the use of nutrients to cope has been identified as a research gap, as is the effect of DSs on women who experienced ELS and as adults have experienced stress-related affective disorders.

Despite robust evidence linking chronic stress to the development of affective disorders, and research linking depression to specific DPs, there is no evidence to suggest that the consumption of specific nutrients, in any form, can prevent the development of these disorders among chronically stressed women.[46-55, 277, 480] Whilst previous research has causatively linked nutrition and depression, these results cannot be generalised to stressed women, regardless of the fact that many of the women

in these studies may have developed depression as a result of chronic stress.[277] Furthermore, polymorphisms on the MTHFR gene have not been examined in relation to female stress. Although some research has suggested that specific nutrients may be effective at reducing some forms of stress in both genders, until now, no research has examined the relationship between specific nutrients, consumed in any form, and chronic stress among women.

Being the first study to examine this relationship, the knowledge gained speaks to a number of health professionals in relation to improving clinical practice. For example, until evidence is available, PCPs, psychiatrists and psychologists who treat chronically stressed female patients (with or without ELS) using DSs to manage stress, possibly under their, or the guidance of other clinicians, may propose other evidence-based stress-management strategies.

There are three areas where future research is warranted, the results of which may inform clinical practice. Firstly, as depression is now the leading cause of disability worldwide and the role of chronic stress in this disorder is widely recognised, preventative strategies are sorely needed. Research examining nutrient interventions prospectively via RCTs in the presence of chronic stress is therefore warranted. In addition, such studies are able to control for cyclical reproductive hormones unlike cross sectional studies. Secondly, although the role that genetic polymorphisms play in the experience of mental health is acknowledged, a similar role in relation to stress vulnerability remains unexamined, and such research is therefore warranted. Thirdly, it is well established that mental wellbeing in later life is largely determined by the first few years of life, and nutrition plays a critical role in brain development, research examining ELS interventions that target nutrition in parenting is warranted. Although animal research has suggested that micronutrient supplementation may be useful to offset the neurobiological effects of ELS during early brain development, similar research is lacking among human subjects.[484] Due to the potential for long-reaching effects, research examining preventative strategies in all these areas is warranted. Well-designed prospective cohort studies and RCTs can provide evidence regarding the interaction of variables identified in the projects undertaken in this thesis and in affiliated research.

Turning to preventative strategies, Australian clinical guidelines for the treatment of affective disorders recommend changes in behaviour related to diet, exercise, smoking and sleep before other forms of treatment are considered.[486] Although chronic stress is acknowledged as a predisposing factor for the development of such disorders, and nutrition likely plays a role, there is as yet no evidence to support the development of similar guidelines. The results of future research examining the relationship between stress and nutrient intake should be included in such guidelines.

In the general population, due to a lack of guidelines, pregnant women and mothers may benefit from knowledge about the relationship between ELS and later mental wellbeing being translated and implemented via PCP's, schools or the Internet, considering its prolific use by women. In light of its

potential to advise women about health generally, evidence about nutrition and chronic stress should also be made available online to counter the lack of evidence-based information available, hence preventing the pursuance of ineffective stress-management strategies. Considering the prolific global use of stress-targeted DSs this behaviour may be challenging to address, therefore more effective evidence translation and implementation strategies via this medium are required.

5.9 Conclusion

Although the use of specific nutrients in DSs have been examined in relation to stress or anxiety during specific reproductive life stages and some studies have addressed the use of a combination of nutrients in relation to stress among women regardless of life stage, no studies to date have examined the relationship between specific nutrients consumed in the diet and/or via DSs and chronic stress generally among women. This study investigated the relationship between chronic stress and the use of diet and/or DSs among a group of women with the aim of generating evidence about this relationship. Although the two primary research projects were exploratory in nature and the results of the survey were based on a small sample, initial observations suggest that there may be a relationship between specific nutrients and stress levels and that personal relationships, thinking patterns and confusion and scepticism about using DSs may contribute to the lived experience of stress.

The relationship between the consumption of specific nutrients among women and stress levels, and the lived experience of stress, including how other factors are related to this experience, are thoroughly under-examined. The combined results of these projects provide the most up to date, quantitative and qualitative evidence about a previously unexamined facet of the lived experience of stress and stress management among women.

Applying this knowledge and other accumulating evidence linking neurobiological functioning and nutrition in well-designed studies will further elucidate the relationship between nutrition and chronic stress among women. Considering the high levels of stress reported by women globally, the growing use of stress-targeted DSs, the recognized neurobiological role that chronic stress plays in the development of affective disorders, the costs associated with such disorders and the long-reaching implications for individuals, families and society, this research topic has been neglected for too long. The growing body of research regarding how nutrition impacts brain function, which is informing the field of psychology and psychiatry, suggests a role for nutrition in chronic stress that clearly warrants further exploration.

Appendix A: Search strategy

PubMed (pubmed.gov)

Search on 15 October 2015

Search	Query
#1	Female[tw] OR Female[mh] OR Female*[tiab] OR Women[tw] OR Women[mh] OR Woman[tw] OR Woman[mh]
#2	Anxiety/psychology[mh] OR Anxiety[tw] OR anxiet*[tiab] OR Anxiety/etiology[mh] OR Anxiety disorders/etiology[mh] OR Anxiety/prevention and control[mh] OR Burnout, professional[mh] OR Burnout[tw] OR Mental Fatigue/psychology[mh] OR mental fatigue[tiab] OR mood disorders[tw] OR mood[tiab] OR Stress*[tw] OR Stress, psychological[mh] OR Stress, physiological/drug effects[mh]
#3	Flax[mh] OR flax seed*[tiab] OR linseed[tw] OR linseed*[tiab] OR Omega-3 Fatty Acids[mh] OR Omega-3 Fatty acids[tw] OR n-3 PUFA[tw] OR n-3 Fatty Acid*[tw] OR n-3 Polyunsaturated Fatty Acid*[tw] OR essential fatty acid[mh] OR essential fatty acid[tw] essential fatty acid*[tiab] OR Unsaturated fatty acids[mh] OR Unsaturated fatty acids[tw] OR Unsaturated fatty acid*[tiab] OR alpha linolenic acid[tiab] OR alpha linoleic acid[tiab] OR Eicosapentaenoic Acid[tw] OR Docosahexaenoic Acid[tw] OR fish oil[mh] OR fish oil[tw] OR fish oil*[tiab] OR Omega-6 Fatty acids[tw] OR n-6 PUFA[tw] OR n-6 Fatty Acid*[tw] OR n-6 Polyunsaturated Fatty Acid*[tw] OR Micronutrient[tw] OR Micronutrients[mh] OR micronutrient*[tiab] OR Dietary supplements[mh] OR dietary supplements[tw] OR dietary supplement*[tiab] OR Food supplement*[tw] OR Vitamins[mh] OR vitamins[tw] OR vitamin*[tiab] OR multivitamin*[tw] OR Thiamine[mh] OR Thiamine[tw] OR Vitamin B1[tw] OR Vitamin B 1[tw] OR Riboflavin[mh] OR Riboflavin[tw] OR vitamin b2[tw] OR vitamin b 2[tw] OR niacin[tw] OR nicotinic acid[tw] OR Niacinamide[tw] OR Nicotinamide[tw] OR vitamin b3[tw] OR vitamin b 3[tw] OR Pantothenic Acid[mh] OR Pantothenic Acid[tw] OR vitamin b5[tw] OR vitamin b 5[tw] OR Pyridoxine[mh] OR Pyridoxine[tw] OR vitamin b6[tw] OR vitamin b 6[tw] OR Folic acid[mh] OR folic acid[tw] OR Folate[tw] OR Folacin[tw] OR vitamin B9[tw] OR vitamin B 9[tw] OR vitamin b12[mh] OR Cyanocobalamin[tw] OR vitamin b 12[tw] OR Ascorbic Acid[mh] OR ascorbic acid[tw] OR L-ascorbic acid[tw] OR vitamin C[tw] OR magnesium[mh] OR Magnesium[tw] OR Zinc[tw]
#4	#1 AND #2 AND #3
Limit to English	

Embase (embase.com)

Search on 28 October 2015

Search	Query
#1	Female*/syn OR female*:ab,ti OR women:ab,ti OR woman:ab,ti
#2	anxiety/de OR anxiety:ab,ti OR 'anxiety disorders'/de OR 'anxiety disorders':ab,ti OR burnout/de OR burnout:ab,ti OR Stress/de OR stress:ab,ti OR ‘emotional stress’/de OR ‘emotional stress’:ab,ti OR ‘mental fatigue’:ab,ti OR ‘mood change’/de OR ‘mood change’:ab,ti OR mood/de OR ‘mood’:ab,ti

Search	Query
#3	'flax'/de OR flax:ab,ti OR 'flax seed':ab,ti OR 'flax seeds':ab,ti OR 'linseed oil'/de OR 'linseed oil':ab,ti OR 'omega 3 fatty acid'/de OR 'omega 3 fatty acid':ab,ti OR 'omega 3 fatty acids':ab,ti OR 'linolenic acid'/de OR 'linolenic acid':ab,ti OR 'omega 6 fatty acid'/de OR 'omega 6 fatty acid':ab,ti OR 'omega 6 fatty acids':ab,ti OR 'linoleic acid'/de OR 'linoleic acid':ab,ti OR 'fatty acids':ab,ti OR 'essential fatty acid'/syn OR 'essential fatty acids':ab,ti OR 'polyunsaturated fatty acid'/syn OR 'polyunsaturated fatty acids':ab,ti OR 'docosahexaenoic acid'/syn OR dha:ab,ti OR 'icosapentaenoic acid'/syn OR 'eicosapentaenoic acid':ab,ti OR epa:ab,ti OR 'fish oil'/de OR 'fish oil':ab,ti OR 'fish oils':ab,ti OR 'micronutrient'/de OR micronutrient:ab,ti OR micronutrient*:ab,ti OR 'diet supplementation'/syn OR 'vitamin'/de OR vitamin:ab,ti OR vitamins:ab,ti OR 'vitamin b complex'/de OR 'vitamin b complex':ab,ti OR 'multivitamin'/de OR multivitamin*:ab,ti OR 'thiamine'/de OR thiamine:ab,ti OR 'riboflavin'/de OR riboflavin:ab,ti OR 'nicotinic acid'/de OR 'nicotinic acid':ab,ti OR nicotinamide:ab,ti OR 'pantothenic acid'/de OR 'pantothenic acid':ab,ti OR 'cyanocobalamin'/de OR cyanocobalamin:ab,ti OR 'l ascorbic acid'/de OR 'l-ascorbic acid':ab,ti OR 'l ascorbic acid':ab,ti OR 'magnesium'/de OR magnesium:ab,ti OR 'zinc'/de OR zinc:ab,ti
#4	#1 AND #2 AND #3
Limit to English AND Embase	

SCOPUS (scopus.com)

Search on 31 October 2015

Search	Query
#1	female OR women OR woman
#2	anxiety OR “anxiety disorder*” OR "mental fatigue" OR mood OR ‘psychological stress’ OR stress OR burnout
#3	“Flax seed*” OR linseed* OR “omega-3 fatty acid*” OR “n-3 PUFA” OR “n-3 Fatty Acid*” OR “n-3 polyunsaturated fatty acid*” OR “essential fatty acid*” OR “polyunsaturated fatty acid*” OR “unsaturated fatty acid*” OR “omega 6 fatty acid*” OR “omega-6 fatty acid*” OR “n-6 PUFA” OR “n-6 Fatty Acid*” OR “n-6 polyunsaturated fatty acid*” OR "alpha linolenic acid" OR "alpha linoleic acid" OR DHA OR EPA OR “docosahexaenoic acid*” OR “eicosapentaenoic acid*” OR “fish oil*” OR micronutrient* OR “dietary supplement*” OR “food supplement*” OR vitamin* OR multivitamin* OR “vitamin B complex” OR “vitamin B group” OR Thiamine OR “vitamin b1” OR Riboflavin OR “vitamin b2” OR Niacin OR Niacinamide OR “vitamin B3” OR “Pantothenic acid” OR “vitamin B5” OR Pyridoxine OR “vitamin B6” OR “Folic acid” OR folate OR “vitamin B9” OR “vitamin B 12” OR “vitamin B12” OR Cyanocobalamin OR “ascorbic acid” OR “L-Ascorbic acid” OR “Vitamin C” OR magnesium OR Zinc
#4	rat OR rats OR poultry OR cattle OR hen* OR mice
#5	#1 AND #2 AND #3 NOT #4
Limit to English AND article	

CINAHL (health.ebsco.com/products/cinahl-with-full-text)

Search on 30 October 2015

Search	Query
#1	MH female OR MH women OR MH woman OR AB female* or TI female* OR AB wom?n OR TI wom?n
#2	MH stress OR MH stress, psychological OR AB "psychological stress" OR TI "psychological stress" OR MH stress, occupational OR AB "occupational stress" OR TI "occupational stress" OR MH burnout, professional OR AB Burnout OR TI Burnout OR MH Anxiety OR AB Anxiety OR TI Anxiety OR AB "anxiety disorder*" OR TI "anxiety disorder*" OR AB "life stress*" OR TI "life stress*" OR AB "chronic stress" OR TI "chronic stress" OR AB "emotional stress" OR TI "emotional stress" OR AB "mental fatigue" OR TI "mental fatigue" OR AB mood OR TI mood
#3	MH flaxseed OR AB flax OR TI Flax OR AB flaxseed* OR TI flaxseed* OR AB "flax seed*" OR TI "flax seed*" OR MH fatty acids, omega-3+ OR AB "omega-3 fatty acids" OR TI "omega-3 fatty acids" OR AB "omega 3 fatty acids" OR TI "omega 3 fatty acids" OR AB "n-3 PUFA" OR TI "n-3 PUFA" OR AB "n-3 Fatty Acid*" OR TI "n-3 Fatty Acid*" OR AB "n-3 polyunsaturated fatty acid*" OR TI "n-3 polyunsaturated fatty acid*" OR MH fatty acids, essential+ OR AB "essential fatty acid*" OR TI "essential fatty acid*" OR AB "docosahexaenoic acid*" OR TI "docosahexaenoic acid*" OR AB "eicosapentaenoic acid*" OR TI "eicosapentaenoic acid*" OR AB "alpha linolenic acid" OR TI "alpha linolenic acid" OR MH fatty acids, omega-6+ OR AB "omega 6 fatty acid*" OR TI "omega 6 fatty acid*" OR AB "omega-6 fatty acids" OR TI "omega-6 fatty acids" OR AB "n-6 PUFA" OR TI "n-6 PUFA" OR AB "n-6 Fatty Acid*" OR TI "n-6 Fatty Acid*" OR AB "n-6 polyunsaturated fatty acid*" OR TI "n-6 polyunsaturated fatty acid*" OR AB "n-6 polyunsaturated fatty acid*" OR TI "n-6 polyunsaturated fatty acid*" OR MH fish oils OR AB "fish oil*" OR TI "fish oil*" OR MH dietary supplements OR AB "dietary supplement*" OR TI "dietary supplement*" OR AB "food supplement*"OR TI "food supplement*"OR AB "dietary supplementation" OR TI "dietary supplementation" OR AB micronutrient* OR TI micronutrient* OR MH vitamins OR AB vitamin* or TI vitamin* OR MH vitamin B complex OR AB "vitamin B complex" OR TI "vitamin B complex" OR MH thiamine OR AB thiamine OR TI thiamine OR MH riboflavin OR AB riboflavin OR TI riboflavin OR MH niacin OR AB niacin OR TI niacin OR AB Niacinamide OR TI Niacinamide OR MH pantothenic acid OR AB "pantothenic acid" OR TI "pantothenic acid" OR MH folic acid OR AB "folic acid" OR TI "folic acid" OR AB "folate" OR TI folate OR MH vitamin B12 OR AB "vitamin B12" OR TI "vitamin B12 OR AB "vitamin b 12" OR TI "vitamin b 12" OR AB Cyanocobalamin OR TI Cyanocobalamin OR MH ascorbic acid OR AB "ascorbic acid" OR TI "ascorbic acid" OR AB "vitamin C" OR TI "vitamin C" OR AB "L-Ascorbic acid" OR TI "L-Ascorbic acid" OR MH magnesium OR AB magnesium OR TI magnesium OR MH zinc OR AB zinc OR TI zinc
#4	#1 AND #2 AND #3
Limit to English AND exclude MEDLINE	

PsycINFO and PsycARTICLES (apa.org/pubs/databases/psycinfo/)

Search on 28 October 2015

Search	Query
#1	Human females.sh OR women.tw OR woman.tw
#2	anxiety.tw OR anxiety disorder*.tw OR life stress.tw OR emotional states.sh OR mood.tw OR chronic stress.tw OR occupational stress.tw OR psychological stress.tw
#3	Fatty acids.sh OR Flax seed*.tw OR linseed*.tw OR omega 3 fatty acid*.tw OR n-3 PUFA.tw OR n-3 Fatty Acid*.tw OR n-3 polyunsaturated fatty acid*.tw OR omega 6 fatty acid*.tw OR n-6 Fatty acid*.tw OR n-6 polyunsaturated fatty acids.tw OR essential fatty acid*.tw OR polyunsaturated fatty acid*.tw OR unsaturated fatty acid*.tw OR omega 6 fatty acid*.tw OR docosahexaenoic acid.tw OR DHA.tw OR eicosapentaenoic acid.tw OR EPA.tw OR micronutrient*.tw OR dietary supplement*.sh OR dietary supplement*.tw OR vitamin*.tw OR food supplement*.tw OR multivitamin*.tw OR vitamin B complex.tw OR vitamin B.tw OR Thiamine.tw OR Riboflavin.tw OR Niacin.sh OR Niacin.tw OR Niacinamide.tw OR nicotinamide.sh OR Pantothenic acid OR Pyridoxine.tw OR Folic acid.sh OR folic acid.tw OR folate.tw OR vitamin B 12 OR vitamin B12.tw OR Cyanocobalamin.tw OR ascorbic acid.sh or ascorbic acid.tw OR Vitamin* C.sh or vitamin C*.tw OR magnesium.sh OR magnesium.tw OR Zinc.sh or zinc.tw
#4	#1 AND #2 AND #3
Limit to English	

MedNar (mednar.com), National Institute of Mental Health (https://www.nimh.nih.gov) and International Association for Women's Mental Health (www.iawmh.org)

Search on 20 October 2015

Search	Query
#1	women
#2	psychological stress
#3	Nutrient*
#4	#1 AND #2 AND #3

Appendix B: Appraisal instruments

MAStARI appraisal instrument

JBI Critical Appraisal Checklist for Randomised Control / Pseudo-randomised Trial

Reviewer _____ Date _____

Author _____ Year _____ Record Number _____

	Yes	No	Unclear	Not Applicable
1. Was the assignment to treatment groups truly random?	☐	☐	☐	☐
2. Were participants blinded to treatment allocation?	☐	☐	☐	☐
3. Was allocation to treatment groups concealed from the allocator?	☐	☐	☐	☐
4. Were the outcomes of people who withdrew described and included in the analysis?	☐	☐	☐	☐
5. Were those assessing outcomes blind to the treatment allocation?	☐	☐	☐	☐
6. Were the control and treatment groups comparable at entry?	☐	☐	☐	☐
7. Were groups treated identically other than for the named interventions	☐	☐	☐	☐
8. Were outcomes measured in the same way for all groups?	☐	☐	☐	☐
9. Were outcomes measured in a reliable way?	☐	☐	☐	☐
10. Was appropriate statistical analysis used?	☐	☐	☐	☐
	☐	☐	☐	☐

Overall appraisal: Include ☐ Exclude ☐ Seek further info. ☐

Comments (Including reason for exclusion)

Appendix C: Appraisal instruments

MAStARI data extraction instrument

JBI Data Extraction Form for Experimental / Observational Studies

Reviewer _____ Date _____

Author _____ Year _____

Journal_____ Record Number_____

Study Method

RCT ☐ Quasi-RCT ☐ Longitudinal ☐

Retrospective ☐ Observational ☐ Other ☐

Participants

Setting

Population

Sample size

Group A _____ Group B _____

Interventions

Intervention A

Intervention B

Authors Conclusions:

Reviewers Conclusions:

Appendix D: Excluded studies

Callender K, McGregor M, Kirk P, Thomas CS. A double-blind trial of evening primrose oil in the premenstrual syndrome: Nervous symptom subgroup. Human Psychopharmacology: Clinical and Experimental. 1988 Mar;3(1):57-61.

Reason for exclusion: Methodology lacked rigor including very large drop out rate after treatment commencement.

Appendix E: List of study findings/conclusions/Characteristics of included studies (MAStARI)

Study	Methods	Participants	Intervention A	Intervention B	Intervention C	Intervention D	Results and Notes
Keenan K et al.[294]	A randomized, double-blind, placebo controlled 14 week study to investigate the association between omega 3 (DHA) supplementation on perceived stress and cortisol response to the TSST	64 pregnant African American women aged between 20-30 years living under stressful life circumstances	2 X gel capsules daily providing 450 mg DHA + 40 mg DPA and ETA + 90 mg EPA + 15 IU vitamin E	Placebo: 2 X gel capsules – no details regarding contents	None	None	Over time, EFA treatment reached significance; with treatment, saliva cortisol levels reduced significantly between treatment and placebo Oleic and omega 6 EFAs (in corn + soya oil) in placebo; participation reimbursed; participants stressed at baseline
Davis LS.[289]	A 4 month comparison and intervention study to investigate the effect of vitamin B6 and magnesium on stress in women with or without dysmenorrhea	47 women who experienced dysmenorrhea aged between 18-35 years of age	500 mg Magnesium tablet per day	200 mg Vitamin B6 tablet per day	500mg Magnesium + 200 mg vitamin B6 tablets per day	Placebo: daily tablet of pressed lactose	Magnesium and vitamin B6 alone and in combination reduced stress, but did not reach significance Pressed lactose In placebo

Study	Methods	Participants	Intervention A	Intervention B	Intervention C	Intervention D	Results and Notes
van de Rest O et al.[287]	A randomized, double-blind, placebo controlled 26 week study to investigate the effects of FO supplementation on mental well-being in older adults	Independently living adults aged greater than or equal to 65 years of age (169 men and 133 women)	6 X capsules FO daily with 900 mg high dose EPA + DHA (approx. 1093 mg EPA + 847 mg DHA + 2.7 mg tocopherol	6 X capsules daily with 900 mg low dose EPA + DHA FO (approx. 226 mg EPA + 176 mg DHA + 2.7 mg tocopherol	Placebo: 6 X 900 mg high oleic acid sunflower seed oil capsules	None	No significant differences between anxiety for either of the treatments Oleic and omega 6 EFAs (in sunflower seed oil) in placebo
Benton D et al.[288]	A double-blind, placebo-controlled 50 day trial investigating the effects of EFAs on cognition and mood	285 Adult female university students aged between 21-8 years	4 X 250mg capsules per day providing 400mg of DHA from microalgae	Placebo: 4 X 250 mg maize-soya oil capsules	None	None	No significant interaction between treatment and time Oleic and omega 6 EFAs (in maize/soya oil) in placebo; participation reimbursed
Sohrabi N et al.[297]	A randomized, double-blind, placebo controlled 2 month pilot trial to investigate the effect of EFAs on PMS	139 women between the age of 20-45 years experiencing PMS	2 X 1 g soft gel per day providing 12% DHA + 18% EPA	Placebo: 2 X 1 g soft gel per day containing no oil	None	None	Treatment reduced anxiety severity at 45 days and 90 days and anxiety duration at 90 days. Sedative use; unknown ingredient used in placebo and treatment combined with EFAs

Study	Methods	Participants	Intervention A	Intervention B	Intervention C	Intervention D	Results and Notes
Cohen LS et al.[295]	A randomized, double-blind, placebo-controlled 12-week trial to investigate the effectiveness of EFAs or placebo on anxiety (one of secondary outcomes)	355 women experiencing VMS and bother in peri- and post menopause	3 X capsules per day providing EPA 425 mg + DHA 100 mg FO + other omega 3's 90 mg + 15 IU Vitamin E + lemon + rosemary oil	Placebo: 3 X capsules containing olive oil + 15 IU Vitamin E + lemon + rosemary oil	None	None	No significant difference in anxiety due to treatment Olive oil in placebo; more white women in treatment group vs. African-American women; participation remuneration
Lucas M et al.[296]	A randomized, double-blind, placebo-controlled 8 week clinical trial to investigate the effectiveness of EFAs for the treatment of psychological distress and depressive symptoms	120 middle-aged women between the ages of 40 and 55 years of age in pre-menopausal, menopausal and post-menopausal phase	1 X 500 mg FO capsules 3 X per day providing 350 mg EPA + 50 mg DHA in ethyl esters	Placebo: 1 X 500 mg capsule 3 X per day providing 0.2% FO (18% EPA + 12% DHA) in sunflower seed oil	None	None	Significant difference in anxiety due to treatment versus placebo in women without MDE Sunflower seed oil in placebo
Walker AF, et al.[290]	A randomized, double-blind, placebo controlled, 4 month crossover study to investigate the effects of magnesium on premenstrual symptoms	38 female university students experiencing premenstrual symptoms including anxiety	1 X 200 mg MgO + 100 mg amino acids tablet per day	Placebo: 1 X identical tablet per day containing microcrystalline cellulose Crossover: after two cycles (2 months) the groups crossed over for 2 cycles	None	None	No significant difference between treatment and placebo Presence of amino acids in treatment supplement

Study	Methods	Participants	Intervention A	Intervention B	Intervention C	Intervention D	Results and Notes
De Souza MC et al.[292]	A random, double-blind, placebo controlled, 5 month cross-over study investigating the single or combined effects of vitamin B6 and magnesium	44 women experiencing premenstrual symptoms including anxiety	1 X 200 mg MgO tablet per day	1 X 50 mg vitamin B6 tablet per day	1 X 200 mg MgO + 1 X 50 mg vitamin B6 per day	Placebo: no detail Crossover: on day 1 of next cycle each participant crossed over to the next treatment until all 4 treatments were completed	Significance reached when combining magnesium and vitamin B6 No detail provided regarding placebo
Kashanian M et al.[291]	A double-blind, randomized, controlled 2 month trial to investigate the effect of vitamin B6 on symptoms of PMS	94 female university students experiencing PMS	1 X 80 mg vitamin B6 (pyridoxine) tablet per day for two menstrual cycles	Placebo: identical tablet	None	None	No significant difference between treatment baseline and placebo
Bryan J et al.[293]	A randomized, double-blind, 35 day placebo-controlled experiment to investigate into the effects of either short-term supplementation with folate, vitamin B12 or vitamin B6 or placebo on cognition and mood	211 young, middle-aged and older women randomized to each of the intervention groups	1 X capsule daily providing 750 µg folate	1 X capsule daily providing15 µg vitamin B12	1 X capsule daily providing 75 mg vitamin B6	Placebo: contained microcrystalline cellulose + calcium + soy polysaccharide + magnesium	Vitamin B6 treatment reached significance versus placebo for the older age group Magnesium and calcium used in placebo; large variation between and within groups at baseline

Study	Methods	Participants	Intervention A	Intervention B	Intervention C	Intervention D	Results and Notes
Rogers PJ et al.[285]	A randomized, double-blind, placebo controlled 12 week trial to investigate the effect of EFAs on depressed mood and cognitive function	Mild to moderately depressed adults aged between 18 - 70 years of age (50 men and 168 women)	3 X capsules per day which contained 630 mg EPA, 850 mg DHA, 870 mg olive oil, 7.5 mg mixed tocopherols and 12 mg orange oil	Placebo: 3 X capsules per day which containing 2360 mg olive oil, 7.5 mg tocopherols and 12 mg orange oil	None	None	No significance reached between treatment and placebo for stress or anxiety scores Olive oil in placebo; depressed participants
Jackson PA et al.[286]	A randomized, double-blind, placebo controlled 12 week study to investigate the effect of EFAs on cognitive function or mood	Healthy young adults aged 18-35 years (47 men and 93 women)	2 X 500 mg DHA-rich FO capsules per day: 450 mg DHA + 90 mg EPA + 2.5 mg mixed tocopherols	2 X 500 mg EPA-rich FO capsules per day: 300 mg EPA + 200 mg DHA	Placebo: 2 X 500 mg olive oil capsules	None	No significance reached for any treatment versus placebo Olive oil in placebo; training day testing acclimatisation
Brody S et al.[184]	Randomized, double-blind, placebo controlled 14-day trial to assess the impact of high dose sustained-release vitamin C on induced stress (TSST) symptoms	Healthy young adults (49 men and 71 women)	2 tablets 3 X per day providing1000 mg high dose sustained-release vitamin C	Placebo: identical in shape and taste	None	None	Significance reached for anxiety scores between treatment versus placebo; SBP and DBP showed significance between treatment versus placebo Participant remuneration

Appendix F: Stress and anxiety outcome measures

Outcome measure	Dimensions, Items and comments	Remarks	Reliability
Difficult Life Circumstances scale (DLC) Used in paper: Keenan K et al.[294]	Examines the severity of life stressors experienced by a primary caregiver either at work or at home in a set of 28 yes or no questions.	Designed to be applicable to socio-economically disadvantaged people, with a score of 6 or less being associated with less than optimal outcomes.	Unknown[300]
Perceived Stress Scale (PSS) Used in paper: Keenan K et al.[294]	Designed to measure the degree to which present life situations are appraised as being stressful during the past month; 14-point instrument scale (also available as a 10-point scale).	Higher scores suggest greater levels of perceived stress; Range of 14pt scale: 0 – 56; 10pt scale: 0-40.	Good[14, 487]
Symptoms of Stress Inventory (SOS) Used in paper: Davis LS.[289]	Measures behavioural, physical and psychological responses to stress; 118 items aim to measure the frequency of a variety of physical and emotional symptoms reported by individuals experiencing stress; rating occurs on a 5-point Likert scale, ranging from 0 (never) to 4 (very frequently) on a 5 point scale (94 of these items were used in this study).	Measures frequency with which respondents have experienced a specific symptom of stress over the past week; higher scores are indicative of higher levels of stress.	Adequate[338]
The Hospital Anxiety and Depression Scale - Anxiety (HADS-A) Used in paper: van de Rest O et al.[287]	Used to assess anxiety specifically, and consists of 7 items; a 4 point Likert scale which ranges from 0 (not at all) to 3 (very often indeed) is used to assess anxiety levels.	Responses based on symptom frequency over the last week; possible total scores range from 0 – 21, with increasing scores indicating increased levels of anxiety. Constructed nearly 3 decades ago it been criticised for a number of reasons, one of them which may be of relevance to this study, which was conducted in the Netherlands, and where the results of the translation from British colloquial expressions into a variety of translations may not have offered a reliable scale of assessment.	Controversial[488-490]

Outcome measure	Dimensions, Items and comments	Remarks	Reliability
Profile of mood states (POMS) Used in papers: Benton D et al.[293]	The scale assesses 6 primary domains of mood: tension: anxiety, depression-dejection, anger-hostility, vigour-activity, fatigue-inertia and confusion-bewilderment; mood states are reflected in 65 adjectives describing a variety of both positive and negative mood states, and are based on a 5-point scale, ranging from 1 (not at all) to 5 (extremely).	Participants are requested to report on the frequency with which they experienced these 65 mood states over the last week; scores range from 20 – 80, with higher scores related to greater frequency of depressed mood; This measurement instrument has been used extensively to assess transient and fluctuating affective mood states, with consistent reliability over a 1- week period, although test-retest reliability decreased for the day-day interval in a test review. As the participants were assessed at trial initiation, and then 3 months later, this may have influenced the outcomes.	Good[304]
The American College of Obstetricians and Gynaecologists (ACOG) diagnostic criteria for PMS Used in paper: Sohrabi N et al.[297]	Affective symptoms based on the ACOG : depression, nervousness, jitteriness, anxiety, low concentration, loss of social activity, and 4 somatic symptoms; A VAS used to evaluate symptom severity.	Patient reports at least one of the affective and somatic symptoms in three consecutive menstrual cycles to be diagnosed with PMS.	Unknown[305, 491]
Generalised anxiety disorder (GAD-7) scale Used in paper: Cohen LS et al.[295]	Used to assess 7 symptoms of anxiety; one of 4 scales within each of the 7 items are selected, namely 0 (not at all), 1 (several days), 2 (more than half the days) or 3 (nearly every day).	Assesses the severity of symptoms that a respondent has experienced over the last two weeks; scores of 5, 10 and 15 taken as cut off points for mild, moderate or severe anxiety; Higher scores related to higher levels of anxiety.	Good[306, 492]
The psychological general well being index (PGWBI) Used in paper: Lucas M et al.[296]	Used to measure individuals subjective well being and psychological distress (PD) symptoms, which consists of 22 items that are used as indicators of 6 affective states; responses range from 5 (excellent) to 0 (poor), with total scores ranging from 0 (extreme distress) to 110 (optimal well-being).	Total scores that fall between 0 and 60 are representative of severe PD, between 61 and 72 represent moderate PD, while ≥73 represent positive well being. This measure is a dimensional measure of non-specific PD rather than a specific disorder diagnostic measure, which suited the non-specificity of possible menopausal symptoms; range: 0 – 110.	Good[307]

Outcome measure	Dimensions, Items and comments	Remarks	Reliability
Menstrual Health Questionnaire (MHQ) Used in papers: Walker AF et al.[292]	27 items used to establish menstrual health and Pre-Menstrual (PMS) symptoms ranked according to 1 (very mild, 2 (mild), 3 (moderate), 4 (severe) or 5 (very severe).		Unknown[308]
Moos Menstrual Distress Questionnaire (MDQ) Used in paper: Walker AF et al.[290]	Dimensions relate to both psychological and somatic symptoms, consisting of 47 items, each rated on a six point scale, with 1 (no experience of the symptom) to 6 (acute or partially disabling experience of the symptom); Menstrual Diary used a 22-item daily symptom 4-point scale: 0, none; 1, mild-present but does not interfere with activities; 2, moderate-present and interferes with activities but not disabling; 3, severe-disabling, unable to function.	Items can be used as the basis for a daily diary recording system for study participants.	Good[309]
APA (American Psychiatric Association) PMS Symptoms Used in paper: Kashanian M et al.[291]	11 items composed of both behavioural and somatic symptoms, including anxiety.	Based on the Diagnostic Statistical Measure (DSM) criteria for Pre-Menstrual (PM) dysphoric disorder.	Unknown[310]
Depression, anxiety and stress scales (DASS) Used in papers: Rogers PJ et al.[286]	Depression, anxiety and stress; Each scale is composed of 14 primary symptoms or subscales; items are divided subscales of 2-5 items with similar content and responses from 0 – 4, with 0 = 'did not apply to me at all,' 1 = 'applied to me to some degree,' or 'applied some of the time,' 2 = 'applied to me to a considerable degree,' or 'a good part of the time' and 3 = 'applied to me very much,' or 'most of the time.'	Items are rated on their severity during the past week; Low scores indicates normal – moderate levels of stress or anxiety and high scores indicate severe to very severe levels.	Good[311]
Bond and Lader Visual Analogue Scale (VAS) Used in paper: Jackson PA et al.[286]	Originally used to assess drug sedative effects: mental sedation or intellectual impairment, physical sedation or bodily impairment, tranquilization or calming effects and other types of feelings or attitudes.[493]	Researcher combined these dimensions to form three mood factors: alert, calm and content.	Some challenges[494]
Spielberger State-Trait Anxiety Inventory (STAI) Used in paper: Brody S et al.[184]	40 item measurement instrument wherein the first 20 items measure state anxiety, which is a reflection of the way a respondent feels at present, while the second 20 items measure trait anxiety, which refers to the way the respondent feels generally; each of the 40 items has a range of four possible responses.	It is a useful measure to distinguish anxiety from depression; Higher scores suggest higher levels of anxiety.	Good[312]

Appendix G: Supplementary statistical data extracted for included studies

Study	Outcome measures (Mean±SD)	Results
Keenan K et al.[294]	PSS Scores	
	Baseline treatment (N=20) 27.77±2.2 versus 24 weeks treatment (N=35) 28.06±3.5	t(53)=0.33;p=0.739*
	Baseline treatment versus endpoint treatment (N=34) 27.47±3.4	t(52)=0.35;p=0.725*
	24 weeks treatment (N=35) 28.06±3.5 versus placebo (N=18) 27.72±2.0	t(51)=0.38;p=0.705*
	Endpoint treatment versus placebo (N=17) 29.29±3.1	t(49)=1.85;p=0.069*
	DLCS Scores	
	Baseline treatment (N=20) 5.16±3.4 versus 24 weeks treatment (N=43) 4.57±3.9	t(53)=0.56:p=0.574*
	24 weeks treatment versus placebo (N=18) 3.11±2.4	t(59 =1.47;p=0.146*
	Endpoint treatment (N=34) 3.91±0.6 versus placebo (N=17) 3.59±0.9	t(49)=1.45;p=0.136*
Davis LS [289]	SOS Scores	
	Endpoint (Mg + vitamin B6) treatment (N=11) 66.8±29.7 versus placebo (N=12) 73.6±39.5	t(21)=0.463;p=0.648*
	Endpoint (Mg) (N=13) 70.2±35.0 versus placebo	t(23)=0.228;p=0.821*
van de Rest O et al.[287]	HADS-A Scores	
	Baseline 400 mg (N=45) 2.67±2.27 versus 3 months (N=45) 3.22±2.60	t(88)=1.06;p=0.288*
	Baseline 400mg versus endpoint (N=45) 3.49±3.5	t(88)=1.31;p=0.190*
	3 months 400mg versus endpoint	t(88)=0.41;p=0.678*
	Baseline 1800 mg (N=42) 2.67±2.74 versus 3 months (N=42) 2.43±2.09	t(82)=0.45;p=0.652*
	Baseline 1800 mg versus endpoint (N=43) 2.67±3.19	t(82)=0.00;p=1.000*
	3 months 1800 mg versus endpoint	t(83)=0.40;p=0.683*
	Endpoint 400mg versus 1800 mg endpoint	t(86)=1.14; p=0.255*
	Endpoint 400 mg versus placebo endpoint (N=46) 2.35±2.10	t(89)=1.88; p=0.062*
	Endpoint 1800 mg versus placebo endpoint	t(87)=0.56; p=0.575*
Benton D et al.[293]	In narrative	
Sohrabi N et al.[297]	In narrative	
Cohen LS et al.[295]	In narrative	
Lucas M et al.[296]	Endpoint treatment (MDE) (N=12) 52.3±10.4 versus placebo (N=14) 65.2±20.7	t(24)=1.95;p=0.062*
Walker AF et al.[290]	Anxiety Scores	
	Baseline treatment (N=35) 10.74±7.81 versus endpoint (N=30) 8.30±8.11	t(63)=1.23; p=0.221*
	Endpoint treatment (N=30) 8.30±8.11 versus placebo (N=32) 12.43±11.93	t(60)=1.58; p=0.118*
De Souza MC et al.[292]	In narrative	

Study	Outcome measures (Mean±SD)	Results
Kashanian M et al.[291]	In narrative	
Bryan J et al.[293]	POMS (Tension/Anxiety subscale)	
	Young age group	
	Endpoint treatment (Folate) (N=14) 17.54±3.67 versus placebo (N=14) 16.71±6.24	t(26)=0.42;p=0.671*
	Endpoint treatment (Vitamin B12) 17.22±3.99 versus placebo	t(26)=0.25;p=0.798*
	Endpoint treatment (Vitamin B6) 16.90±2.69 versus placebo	t(26)=0.10;p=0.917*
	Middle aged group	
	Endpoint treatment (Folate) (N=20) 17.17±5.49 versus placebo (N=20) 16.61±7.49	t(38)=0.26;p=0.788*
	Endpoint treatment (Vitamin B12) 17.55±6.84 versus placebo	t(38)=0.41;p=0.680*
	Endpoint treatment (Vitamin B6) 16.48±6.56 versus placebo	t(38)=0.05;p=0.953*
	Older aged group	
	Endpoint treatment (Folate) (N=19) 14.76±4.96 versus placebo (N=19) 14.70±3.42	t(36)=0.04;p=0.965*
	Endpoint treatment (Vitamin B12) 15.71±6.28 versus placebo	t(36) = 0.61;p=0.542*
Rogers PJ, et al.[286]	DASS stress scores	
	Treatment endpoint (N=85) 12.3±7.8 versus placebo endpoint (N=83)12.6±7.4	t(166)=0.25;p=0.798*
	DASS anxiety scores	
	Treatment endpoint 4.2±4.6 versus placebo endpoint 5.7±5.9	t(166)=1.84;p=0.067*
Jackson PA et al.[286]	Treatment DHA	
	DASS stress scores	
	Baseline treatment (N=29) 8.79±5.63 versus endpoint treatment 8.93±6.6	t(28)=-0.183;p=0.856*
	Endpoint treatment versus endpoint placebo (N=37) 7.51±6.73	t(64)=0.851; p=0.398*
	DASS anxiety scores	
	Baseline treatment (N=29) 3.55±3.99 versus endpoint treatment 3.10±3.26	t(28)=0.585;p=0.563*
	Endpoint treatment versus endpoint placebo (N=37) 3.32±4.39	t(64)=-0.226;p=0.822*
	Treatment EPA	
	DASS stress scores	
	Baseline treatment (N=28) 8.68±7.44 versus endpoint treatment 8.5±7.08	t(27)=0.116;p=0.908*
	Endpoint treatment versus endpoint placebo (N=37) 7.51±6.73	t(63)=0.572;p=0.570*
	DASS anxiety scores	
	Baseline treatment (N=28) 3.11±4.36 versus endpoint treatment 3.93±4.83	t(27)=-0.966;p=0.342*
	Endpoint treatment versus endpoint placebo (N=37) 3.32±4.39	t(63)= 0.529;p=0.601*
	1 way ANOVA DASS stress (DHA, EPA and Placebo)	F=0.378, p=0.686*
	1 way ANOVA DASS anxiety (DHA, EPA and Placebo)	F=0.293, p=0.747*
Brody S et al.[184]	Subjective stress scores	
	Endpoint treatment (N=38) 2.25±1.35 versus placebo (N=33) 2.54±1.71	t(69)=0.79;p=0.427*
	Blood Pressure Measurements	
	SBP Baseline (N=38)107.82±11.05 versus endpoint 111.28±11.41	t(74)=1.34;p=0.183*

* p≤0.05

Appendix H: Ethics approval letter

THE UNIVERSITY of ADELAIDE

RESEARCH BRANCH
OFFICE OF RESEARCH ETHICS, COMPLIANCE AND INTEGRITY
THE UNIVERSITY OF ADELAIDE

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CRICOS Provider Number 00123M

6 May 2016

Associate Professor C Lockwood
School: School of Translational Health Sciences

Dear Associate Professor Lockwood

ETHICS APPROVAL No: **H-2016-090**

PROJECT TITLE: **A mixed methods study investigating stress and dietary supplementation among women: evidence of effectiveness and lifestyle impact**

The ethics application for the above project has been reviewed by the Low Risk Human Research Ethics Review Group (Faculty of Health Sciences) and is deemed to meet the requirements of the *National Statement on Ethical Conduct in Human Research (2007)* involving no more than low risk for research participants. You are authorised to commence your research on **06 May 2016**.

Ethics approval is granted for three years and is subject to satisfactory annual reporting. The form titled *Annual Report on Project Status* is to be used when reporting annual progress and project completion and can be downloaded at http://www.adelaide.edu.au/ethics/human/guidelines/reporting. Prior to expiry, ethics approval may be extended for a further period.

Participants in the study are to be given a copy of the Information Sheet and the signed Consent Form to retain. It is also a condition of approval that you **immediately report** anything which might warrant review of ethical approval including:

- serious or unexpected adverse effects on participants,
- previously unforeseen events which might affect continued ethical acceptability of the project,
- proposed changes to the protocol; and
- the project is discontinued before the expected date of completion.

Please refer to the following ethics approval document for any additional conditions that may apply to this project.

Yours sincerely,

Sabine Schreiber
Secretary, Human Research Ethics Committee
Office of Research Ethics, Compliance and Integrity

Appendix I: SurveyMonkey questionnaire introduction and consent request

Female Stress and Supplement Use

Introduction and Permissions

Thank you for your time.

The aim of this research is to understand how women report experiencing stress and their dietary intake and supplementary use of nutrients, specifically essential fatty acids (EFA's, for example, flax or fish oil), B vitamins, vitamin C, magnesium and zinc to manage stress.

This research project is about women and specific nutrients they may or not be eating. It's also about whether there is an association between stress and nutrients.
There is very little research that has looked at this issue.

Please remember that your own, personal responses are what I am looking for, not what someone else thinks you may or should be feeling. For example, when you are filling in the questionnaire about your stress level, do not ask for anyone else's ideas or opinions. I want and need YOUR replies only.

It will take you between 10 and 20 minutes to fill these questionnaires in.

This is a two-part survey.
Part 1 is about perceived stress and there are 10 questions for you to answer.
Part 2 is about your supplementary use of nutrients, including dietary supplements and the length of this section depends on how many supplements you take.

As discussed, your participation in this survey is voluntary, your identity is not linked to your answers, and your answers will become anonymous when you submit them.

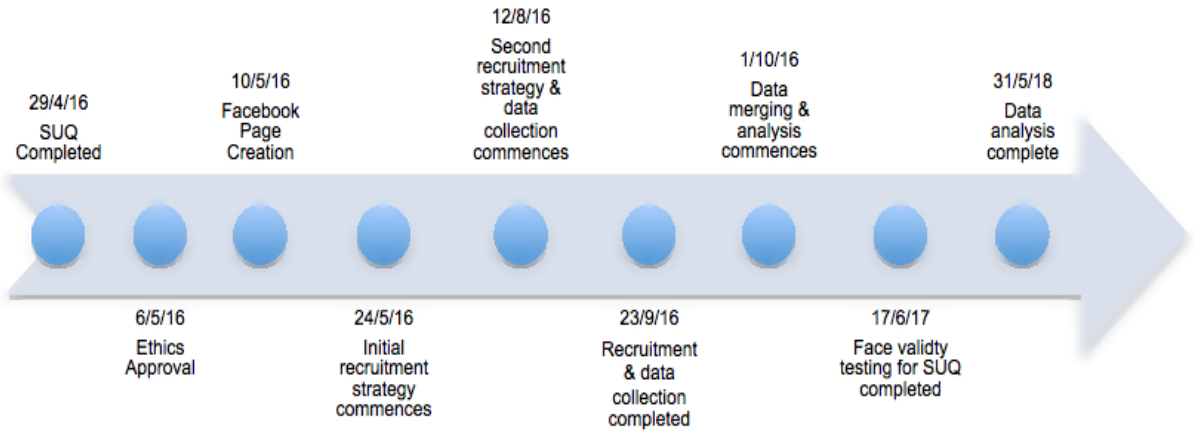
If you have any questions while filling in the questionnaires, please call me on: [redacted]

Your time is very precious and I thank you in advance for completing these questionnaires.
It will be very helpful to have you complete these questionnaires by [redacted] the latest.

By clicking the 'next' button below you are providing consent to the use of this data in a research project.

Sincerely
Delia

Appendix J: Cross-sectional Survey Timeline: March 2016 – May 2018



Appendix K: Recruitment blog post

Gold Coast women needed for stress and nutrition study

August 12, 2016 8:22 am in Health by Carla Tooma

A Gold Coast mum is on a mission to help females manage stress more effectively.

As part of her PhD with the University of Adelaide, local Delia McCabe is invesitgating the role nutrition plays on female stress."I decided to start my PhD last year when I started feeling like a super stressed wife and mother," Delia told myGC.

"My research is an investigation into whether there is an association between specific nutrients and the level of stress that women experience. If there are nutrients that can help us manage stress more effectively, I want to share this with women who can benefit and help improve the quality of all our lives."

Delia said told myGC she chose to focus on women in her research project as they are more likely to experience depression due to ongoing or chronic stress. "Compared to men, women are 50% more susceptible to depression, generalized anxiety disorder, panic disorder, phobias and insomnia," she said.

Delia McCabe PHD – Mental Health and Nutrition Researcher

"To add insult to injury, when females are stressed we crave processed foods, containing fats and sugar, or salt, that offer a quick energy and pleasure 'hit' to the brain, leading to weight gain and increased blood sugar ups and down, leading to further mood swings and anxiety.

"Our busy, complex lives are not going to suddenly calm down and change – we need to find ways to manage our stress more effectively so that we can enjoy the quality of life that we deserve with the effort we put in."

To get the ball rolling on her project, Delia is on the lookout for local women to fill-out a short online questionnaire. "I need 150 women to fill in three surveys each – they are all online surveys with links that I send to them via email addresses. I want to know whether they feel stressed or not, what supplements (if any) they take and what a general daily food intake looks like for them," she said. "The data will be anonymous so everyone's privacy is protected.

"I am interested in the experiences of women on the Gold Coast because this is where I live. This is where I see women rushing, working, parenting, shopping, being active, multi-tasking and performing all the roles that can exhaust us. Although this happens all over the world, this is my home, where my friends and I live, and so it feels more personal to me," she said.

Gold Coast women wanting to participate in the study or find out more information are encouraged to visit the project's Facebook page – fb.com/FemaleStressandNutrition/ – and send a private message to Delia.

Appendix L: Female stress and nutrition – research project Facebook page

Our Story

FEMALE STRESS AND NUTRITION - RESEARCH PROJECT

This research project is being undertaken by Delia McCabe, towards a PhD degree through The School of Translational Health Science, The Joanna Briggs Institute, Faculty of Health Sciences - The University of Adelaide. The Ethics Approval number is: H-2016-090.

The aim of this research project is to understand how women report experiencing stress and their dietary and supplementary intake of EFAs (for example, flax or fish oil), B vitamins, vitamin C, magnesium and zinc to manage stress. This study will get a quick 'snap shot' of a group of women's food choices, supplements and their stress levels, and may uncover an association between specific nutrients which may be useful for further research.

The aim of this research is to understand how women report experiencing stress and their dietary and supplementary intake of essential fatty acids (EFAs,) B vitamins, vitamin C, magnesium and zinc to manage stress.

Appendix M: Example of email sent to all cross-sectional survey participants for FFQ link

Delia McCabe 15 September 2016 at 10:47 AM

To [redacted]

Female Stress and Nutrition Research Project (3)

Hi [redacted]

Thanks very much for getting back to me re' this research project!

I really appreciate your time.

This is the link to the 3rd (and last) survey which is all about your food intake and will take you about 10 minutes to complete.

https://www.iviewsurveys.com.au/mrlWeb/mrlWeb.dll?I.Project=O15Y0855&ID=106_0094&Password=sqnbrj95

Work through this survey, reading the questions carefully, until you get to the last page and 'Submit' it to save your information.

If you need to stop the survey and go and do something else, please be sure to save it before you leave, so that you can come back to it and continue!

In a separate email I will send you the link to Survey 1 and Survey 2.

Please complete ALL THREE of the surveys.

Also, please find attached some more information about the research project, which I have to send to each person that takes part.

Please feel free to share information about this research project with any female friends or family members that you feel may be interested - if they can get back to me in the next few days and fill the surveys in by Saturday.
I have attached the URL for the Facebook page below.

Thanks again for your help - I look forward to your responses and will send you the free eBook when I receive them :)

Have a lovely day!
Delia

[redacted] Recruitment Cover letter.docx

PARTICIPANT INFORMAT…ection.docx

Delia McCabe, MA (Psych), PhD(c)
delia.mccabe@adelaide.edu.au
Mob [redacted]
https://www.fb.com/FemaleStressandNutrition/

Appendix N: Recruitment cover letter

[Date]

Dear [Name]

Thank you for your time.

The aim of this research is to understand how women report experiencing stress and their dietary and supplementary intake of essential fatty acids (EFAs,) B vitamins, vitamin C, magnesium and zinc to manage stress.

This research project is about women and specific nutrients they may or not be eating. It's also about whether there is an association between stress and nutrient supplements. There is very little research that has looked at this issue.

Please remember that your own, personal responses are what I am looking for, not what someone else thinks you may or should be feeling. For example, when you are filling in the questionnaire about your stress level, do not ask for anyone else's ideas or opinions. I want and need YOUR replies only.

It will take you between 30 and 40 minutes to fill all the questionnaires in.

As we have discussed, your participation in this survey is voluntary, your identity is not linked to your answers, and your answers will become anonymous when you submit them. If you have any questions while filling in the questionnaires, please call me on: [redacted]

Your time is very precious and I thank you in advance for completing these questionnaires and sending them back to me. It will be very helpful to have your completed questionnaires returned to me by the [date].

Sincerely, Delia

Appendix O: Participant information sheet

PARTICIPANT INFORMATION SHEET

PROJECT TITLE: The association between the dietary and supplementary intake of essential fatty acids (EFAs,) B vitamins, vitamin C, magnesium and zinc to manage stress: a cross sectional study
HUMAN RESEARCH ETHICS COMMITTEE APPROVAL NUMBER: H-2015-090
PRINCIPAL INVESTIGATOR: Associate Professor Craig Lockwood
STUDENT RESEARCHER: Delia McCabe
STUDENT'S DEGREE: PhD candidate

Dear Participant,

You are invited to participate in the research project described below.

What is the project about?
The aim of this research is to understand how women report experiencing stress and their dietary and supplementary intake of EFAs, B vitamins, vitamin C, magnesium and zinc to manage stress. This study will get a quick 'snap shot' of a group of women's food choices, supplements and their stress levels, so it won't be able to discuss whether food choices are influenced by stress levels, whether specific nutrients can reduce stress levels or whether a lack of specific nutrients leads to experiencing more stress, but it may uncover an association between specific nutrients which may be useful for further research and to inform the next stage of the research project.

Who is undertaking the project?
This project is being conducted by Delia McCabe.
This research will form part of the degree of Doctor of Philosophy (PhD) at the University of Adelaide under the supervision of [redacted].
There are no commercial sponsors, external partners or funding being provided for this research.

Why am I being invited to participate?
You are being invited to participate in this study because you are a woman aged between 18 and 65, live on the Gold Coast, can provide informed consent, and have expressed an interest in being involved in this project.

What will I be asked to do?
If you have chosen to receive the questionnaires via post you will receive two questionnaires in the post, along with a reply paid envelope. You should set aside 20 to 30 minutes to fill in the two questionnaires. You will not need to put your name on any of the questionnaires, as you will be allocated a number to protect your privacy. You will be asked to post the questionnaires back to the researcher, in the reply paid envelope provided. Your envelope will contain a batch of food photographs that will be used as examples of portion sizes that you will use to fill in the Food Frequency Questionnaire online with the researcher, via a telephone call, which will take between 10 and 15 minutes to complete. If you have chosen to receive the questionnaires via email, you will be sent a link to 'click' on, and you should set aside 30 – 45 minutes to fill in all the online questionnaires.

How much time will the project take?
The questionnaires will take between 30 and 45 minutes to fill in, depending on whether you are completing the questionnaires on paper and via a telephone call or online.

Are there any risks associated with participating in this project?
There are no foreseeable risks in completing these questionnaires. You may feel uncomfortable for a few minutes while you fill in the questionnaire about your feelings of stress. If you feel very uncomfortable it is suggested that you make an appointment with your medical practitioner and discuss your feelings with them. If you would like the name of medical practitioner then please contact the researcher and she will provide you with a medical practitioners contact details. Alternatively, please call The Mental Health

Association Queensland on (07) 5519 2550 if you feel the need to talk to someone who is qualified to help you about your feelings of stress.

What are the benefits of the research project?
There are no immediate benefits to the participants of this research. Women all over the world have many different roles to play in society, for example as mothers, carers of elderly parents, workers, community and school volunteers and domestic workers in their homes. This research has the potential in increase what we know about stress and how specific nutrients may influence stress. It therefore has the potential to inform other researchers and may in time result in other women being able to benefit from other research.

Can I withdraw from the project?
Participation in this project is completely voluntary. If you agree to participate, you can withdraw from the study at any time. Once the researcher has received your questionnaires and submitted your data you will not be able to withdraw the data, as it is anonymous.

What will happen to my information?
The information that you provide on the questionnaires will be provided anonymously, and the completed questionnaires will be stored in a locked cabinet within a locked archive room in The School of Translational Health Science, at the University of Adelaide. Data will be kept for seven years prior to secure destruction, as per the current research policy guidelines. The results of the study, will be presented using combined rather than individual data, and will be used in journal articles and publications, conferences and in the researcher's PhD thesis. The results will be made available to participants via a letter posted to their address or via a personal email if they so wish.

Who do I contact if I have questions about the project?
Should you have any questions about the research, please contact the researcher on [redacted]
The other researchers involved in this research project can also be contacted via telephone:
[redacted]

What if I have a complaint or any concerns?
The study has been approved by the Human Research Ethics Committee at the University of Adelaide (approval number H-2016-090). If you have questions or problems associated with the practical aspects of your participation in the project, or wish to raise a concern or complaint about the project, then you should consult the Principal Investigator. Contact the Human Research Ethics Committee's Secretariat on phone [redacted] if you wish to speak with an independent person regarding concerns or a complaint, the University's policy on research involving human participants, or your rights as a participant. Any complaint or concern will be treated in confidence and fully investigated. You will be informed of the outcome.

If I want to participate, what do I do?
If you would like to participate in this research project please fill out the questionnaires included in the envelope and then put all the questionnaires into the reply paid envelope and post it back to the researcher. By completing and returning the questionnaires you are consenting to participation in this project. If you are completing the questionnaires online, your consent is provided when you complete the questionnaires and click the 'done' button.

The second phase of this research project involves a group of interested women discussing their experience of stress and dietary and supplementary intake of essential fatty acids (EFAs,) B vitamins, vitamin C, magnesium and zinc to manage stress. The group will meet every week for between eight and ten weeks. Each session will run for an hour to an hour and a half. If you are interested in taking part in this next phase of the project, please contact the researcher on [redacted]

Yours sincerely,
[redacted]
Student researcher – Delia McCabe – [redacted] **delia.mccabe@adelaide.edu.au**

Appendix P: The PSS-10

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

Appendix Q: Pen and paper option of the Supplement Use Questionnaire (SUQ)

Supplement Use Questionnaire (SUQ)

Thank you for your time. This survey is about your supplementary use of dietary nutrients. A dietary supplement is a **nutritional compound that contains a nutrient/s aimed at adding extra nutritional value** to the diet, in the form of one or any combination of the following substances: a vitamin, a mineral, a herb or other botanical, an amino acid, a concentrate or an extract. This research project is specifically interested in essential fatty acids (EFAs, for example, flax or fish oil), B vitamins, vitamin C, magnesium and zinc.

Dietary supplements come in a wide range of forms, such as tablets, capsules, chewables, lozenges, gelcaps, powders, liquids, granules or others (for example, 'pearls') and injections, such as vitamin B or B12 injections. They do not include prescription medicine prescribed by your doctor or over the counter pain medication.

Please read each question carefully and answer what is true for you now in your life.

Section 1 instructions: Please read the question and tick the box that holds your answer.

Q1: Do you take any supplements or have you had any vitamin injections in the last 2 years?' ☐ Yes ☐ No

If you answered 'NO' to question 1, you have completed the questionnaire and you can ignore the rest of the questions.

Section 2 instructions: Please complete each of the sub-sections below, by writing your answers in the boxes or circling your response. Please provide details if you choose the 'other' option. This section is about the brand, type and form of the supplement/s you use, along with the regularity, quantity, and longevity of use. It also questions the reason you take the supplement/s and whether you think it/they are helping you. Each column focuses on one supplement. Please complete one column for every supplement taken.

	SUPPLEMENT 1	SUPPLEMENT 2
Supplement brand/manufacturer name - *e.g. Blackmores* *Or if it is an injection, write 'injection'*		
Type of supplement - *e.g. Magnesium* *Or if it is an injection, write 'B/B12 vitamin'*		
What form does the supplement come in?	Tablets Capsules Powder Liquid Granules Chewables Lozenges Pearls Injection Other: _____	Tablets Capsules Powder Liquid Granules Chewables Lozenges Pearls Injection Other: _____
How often do you take this supplement?	Every day 4-5 times per week 2-3 times per week Once per week Once per month Other: _____	Every day 4-5 times per week 2-3 times per week Once per week Once per month Other: _____
If you take this supplement every day, how often do you take it every day?	1 x per day 2 x per day 3 x per day Other: _____	1 x per day 2 x per day 3 x per day Other: _____
How many/how much of this supplement do you take EACH time *e.g. 1 tablet/capsule OR 1 tsp OR 1 Tbsp OR 1 injection*		
For how long have you taken this supplement?	Less than 1 month 1-3 months 3-6 months 6-12 months More than 1 year	Less than 1 month 1-3 months 3-6 months 6-12 months More than 1 year
Why do you take this supplement?	Increase energy levels Lose weight Prevent disease Reduce stress Improve memory & concentration Other: _____	Increase energy levels Lose weight Prevent disease Reduce stress Improve memory & concentration Other: _____
Is this supplement helping you achieve this goal/these goals?	Yes No Unsure	Yes No Unsure
What prompted you to start taking this supplement?	Doctor Naturopath Magazine article Newspaper article Advertisement Other: _____	Doctor Naturopath Magazine article Newspaper article Magazine advertisement Other: _____
Where do you purchase this supplement?	Health Store Supermarket Naturopath Online Doctor Other: _____	Health Store Supermarket Naturopath Online Doctor Other: _____

Please turn the page over to continue adding supplement details.

Appendix R: Questionnaire design characteristics

Criteria	Rationale
Length	Use short questionnaires to sustain engagement and support completion.[495, 496]
Aesthetic appeal	Create an attractive, colourful layout to increase enjoyment, which may support completion.[363]
Clarity, simplicity and specificity	Avoid complexity and confusion so as to foster understanding and accurate completion.[495, 496] Questions need to be understood in the same way by people who may differ in many ways, as the researcher is unavailable to paraphrase or explain the intention behind questions.[363] Avoid confusing phrasing, double negatives and double-barrelled questions, which ask two questions in the same sentence.[363]
Questioning style	Use direct questions to avoid confusion and only ask one question at a time.[495, 496]
Word choice	Use broadly understood, unambiguous, familiar words and avoid technical terms to foster understanding and completion.[495] Avoid vague qualifiers, such as 'Often, sometimes, rarely or never' as individual interpretation can vary significantly.[495]
Relevance of questions	Select good and relevant questions that flow from research question/s and do not include any irrelevant questions or comments.[363] A combination of factual and value questions can be used which can be relevant to either closed- or open-response options.[495]
Sequence of questions	Place broad questions first, which serve as 'filter or screening' questions which clarify respondent eligibility; direct respondents to whom the research is not directed to a different section of the questionnaire to prevent input and possible misinterpretation.[363, 495]
Bias	Avoid words or sentences that are loaded, or biased, such as words or sentences that suggest some answers are better or worse than others, which produce misleading answers. For example, in relation to personal questions about health behaviours, respondents may feel that some answers are more desirable than others.[363] Avoid asking questions related to emotions of feelings unless the research aims to investigate such as some people prefer to 'agree' with a statement, so as to avoid being seen as disagreeable, introducing 'agreement bias.'[363]
Context effects	Avoid unintentional influences or context effects on responses by presenting alternative answers with equal emphasis, and by ensuring no questions influence how subsequent ones are interpreted.[363]
Close-ended response options	Collect quantitative data via close-ended response options which can be coded and analysed, but response options should include every possible response, including one for those respondents who do not want any of the provided options.[363, 495]
Open-ended responses options	Add open-ended response options when it is not possible to provide for every alternative, or when qualitative data is required.[363, 495]
Coherence and flow	Ensure the questions are sorted into broad thematic categories to maximise overall questionnaire coherence and flow, which maximises engagement and supports completion.[363]
Managing recall challenges	Use relatively short reference points for regular events, whereas longer reference periods may be better for uncommon or irregular events because they decrease the risk of telescoping bias.[495]

Appendix S: Dietary supplement database

Supplement Brand Name	Ingredient name/ Ingredient quantity	Dose	Supplement Brand Name	Ingredient name/ Ingredient quantity	Dose	Supplement Brand Name	Ingredient name/ Ingredient quantity	Dose	Supplement Brand Name	Ingredient name/ Ingredient quantity	Dose
AgeLoc Nu Skin Y Span	TG's – fish oil: EPA 150mg; DHA 100mg	2 capsules	Odorless 1000 mg	Omega 3 300mg	1 capsule	Ethical Nutrients mega magnesium powder	Mag 300mg; Vitamin C 300mg; Vit B6 50mg	1 dose (8.7 g)	Poliquin zinc	Zinc 30mg	1 capsule
Amcal Lysine	Vitamin C 500mg; Zinc 5mg	1 tablet	Blackmores Multi	Sustained: B1 30mg; B2 10 mg; Nicotinamide (B3) 30mg; B5 28.9mg; B6 30mg; B12 50mcg; Vit C 150mg	1 tablet	Ethical nutrients magnesium (tablets)	Magnesium 200mg; zinc 7mg; Vit b6 16.6mg	1 tablet	Poliquin Arginine	Activated folate 400mcg	2 tablets
Australian Organic Flaxseed oil (Planet organic) – 500ml	ALA and LA: 4: 1 in favor of omega 3	1 tsp[1]	Blackmores Women's Multi	B1 25mg; B2 12mg; Nicotinamide 50mg; Vit b5 30mg; B6 25mg; B12 50mcg; Vit C 100mg; Folic Acid 300mcg; Mg 67.5mg; Zinc 20mg	1 tab per day	Ethical Nutrients Zinc fix	VIt C 1g; Zinc 23mg; Mag 5mg; B6 5mg	1 dose (1.9g)	Poliquin Multi	Vitamin C 900 mg (150 /tab); Thiamin 22.5 mg (3.75 /tab); Riboflavin 25.5 mg (4.25 /tab); Niacin 315 mg (52.5 /tab); Vitamin B6 30 mg (5 /tab); Folate (as folic acid and calcium L-5-methyl tetrahydrofolate) 600 mcg (100mcg /tab); Vitamin B12 (as cyanocobalamin) 150 mcg (25 /tab); Magnesium (as magnesium bis-glycinate and magnesium citrate) 187.5 mg (31.25 /tab); Zinc (as zinc glycinate) 15.5 mg (2.5 /tab)	6 tablets
Thompson's B12	B12 50mcg	1 tablet	Blackmores Omega Triple Concentrate FO	Omega 3 900mg (ie undefined Omega 3)	1 capsule	Ferrograd C with iron	Vit C 500mg	1 tablet	Poliquin FO	EPA-DHA 720 Blend; EPA 860mg; DHA 580mg	2 softgels
Berocca Performance	B1 15mg; B2 15mg; B3 50mg; B5 23 mg; B6 10mg; B12 10mcg; Folic acid 400mcg; Vit C 500mg;	1 tablet	Blackmores Pregnancy Gold	Per 2 capsule: Omega 3 300mg; B3 18mg; Vit C 60mg; Zinc 13.64mg; Mg 116.2mg; B1 1400mcg	2 caps/day	Herbs of gold - organic iron max (also contain iron)	B1 10mg; B2 10mg; B6 10mg; B12 250mcg; Folic acid 250mcg; Vit C 250mg	1 capsule	Poliquin Bliss Tryptophan and Folate	Folate (as activated MTHF Metafilin 2000mcg)	2 capsules

[1] Calculation: Flaxseed oil @ 1 tsp = 4.5gms; EFA composition ALA:LA in ratio of 1:4; 4.5/5 = 0.9g of ALA and 3.6g of LA

Supplement Brand Name	Ingredient name/ Ingredient quantity	Dose	Supplement Brand Name	Ingredient name/ Ingredient quantity	Dose	Supplement Brand Name	Ingredient name/ Ingredient quantity	Dose	Supplement Brand Name	Ingredient name/ Ingredient quantity	Dose
	Magnesium 100mg; Zinc 10mg			(1.4mg); B2 1400mcg (1.4mg); B6 2.32mg; B12 2.6mcg; Folic Acid 500mcg Per 1 capsule: Omega 3 TG 150mg; B3 9mg; Vit C 30mg; Zinc 6.82 mg; Magnesium 58.1mg; B1 700mcg; B2 700mcg; B6 1.16mg; B12 1.3mcg; Folic Acid 250mcg							
Berocca with Ginseng for over 50s	B1 15mg; B2 15mg; B3 50mg; B5 23mg; B6 10mg; B12 10mcg; Vit C 500mg; Folic Acid 400mcg; Mg 100mg; Zinc 10mg	1 tablet	Blackmores Vit B (exec stress)	B1 75mg; B2 10mg; B3 100mg; B5 75mg; B6 25mg; B12 30mcg; Folic acid 150mcg; Vit C 250mg; Vit E; Inositol; Choline; Herbs	1 tablet	Isagenix FO	EPA 600mg; DHA 480mg; Omega blend 220mg with GLA 8%[2]; No amount given for flax seed (ALA) oil present in supplement	2 softgels	Poliquin magnesium	Mg 240mg	2 capsules
Biospark Vit C (NTS health)	Vit C 800mg	½ teas	Blackmores Mega B complex	B1 50mg; B2 30mg; B3 100mg; B5 50mg; B6 50mg; B12 50mcg; Folic acid 300mcg; Inositol; Choline; Biotin (Vit H)	1 tablet	Mediherb	Iron; Zinc; B		Quality Health FO 2000mg	EPA 360mg; DHA 240mg	1 capsule
Bioglan FO Odorless 1500mg	TG as: EPA 270mg; DHA 180mg	1 cap	Blackmore EPO	GLA 100mg	1 capsule	Metagenics Calm X	350mg mg; Vit C 500mg; Zinc 10mg; B1 25mg; B2 25mg; B3 25mg; B5 25mg; B6 25mg	2 scoops = 1 dose	Quality Health High Strength Krill oil		1 capsule (1000mg)
Bioglan FO Odorless 1000mg	TG as: EPA 180mg; DHA 120mg	1 cap	Cenovis Echinacea, Garlic, Zinc and C	1 tablet: Vit C 500mg; Zinc 2mg	1 tablet three times daily	NaturesOwn Magnesium Chelate	Magnesium 100mg	1 capsule twice daily with food	Quality Health EPO 1000mg		1 capsule (100mg GLA)
Bioglan FO Superfish oil (concentrated)	TG as: EPA 180mg; DHA 120mg	1 cap	Cenovis womens multi	Vitamin B12 (Cyanocobalamin) 10mcg; Vitamin B2 (Riboflavine) 4mg; Vitamin B6 (Pyridoxine Hydrochloride) 60.8mg; Vitamin B1 (Thiamine Nitrate) 4mg; Vitamin B3 (Nicotinamide) 36mg;	1 capsule per day	NaturesOwn Mega B 150	Vitamin B1 (thiamine nitrate) 150mg; Vitamin B2 (riboflavine) 150mg; Vitamin B3 (nicotinamide) 150mg; Vitamin B5 (calcium pantothenate)150mg; Vitamin B6 (pyridoxine hydrochlori	1 tablet daily	Sanofi aventis betamine	Vit B1 100mg	1 tablet

[2] Calculation: 220mg X 8% (GLA) = 17.6 mg GLA

Supplement Brand Name	Ingredient name/ Ingredient quantity	Dose	Supplement Brand Name	Ingredient name/ Ingredient quantity	Dose	Supplement Brand Name	Ingredient name/ Ingredient quantity	Dose	Supplement Brand Name	Ingredient name/ Ingredient quantity	Dose
				Vitamin B5 (Calcium Pantothenate) 13mg; Vitamin C (Ascorbic Acid) 120mg; Zinc (as Oxide) 5mg; Folic Acid 300mcg; Magnesium (as Oxide - heavy) 50mg; Evening Primrose Oil (EPO) 200mg; D3; A; Iron			de)150mg; Vitamin B12(cyanocobalamin)150mcg; Folic acid 99mcg; also contains herbs and choline				
Bioglan FO Superfish oil 2000 (concentrated)	TG as: EPA 360mg; DHA 240mg	1 cap	Cenovis Magnesium	Mg 325mg; Vit B6 60mg; Manganese	1 capsule per day	NaturesOwn Odorless FO 2000mg	EPA 360 mg; DHA 240mg	1 tablet	Potassium and magnesium aspartate (GNC)	Monomagnesium Di-L-Aspartate 250 mg (equivalent 20mg elemental magnesium)[3]; Monopatassium aspartae 250mg	1 capsule
Bioglan FO Red Krill oil		500mg per capsule 1000mg (Double strength)	Cenovis Mega B	Vitamin B1 (Thiamine Nitrate) 50mg; Vitamin B2 (Riboflavine) 25mg; Vitamin B3 (Nicotinamide) 50mg; Vitamin B5 (Calcium Pantothenate) 50mg; Vitamin B6 (Pyridoxine Hydrochloride) 50mg; Vitamin B12 (Cyanocobalamin) 50mcg; Vitamin C (Ascorbic Acid) 100mg; Folic Acid 200mcg; Inositol; Choline; Biotin	1 capsule per day	NaturesOwn Triple strength Garlic + C, Horseradish	Vit C 500mg	1 tablet	Swisse Multi (women's)	B1 50mg; B2 50mg; B3 50mg; B5 68.7mg; B6 41.1mg; B12 50mcg; Folic acid 500mcg; Vit C 165.2mg; Mg 47.16mg; Zinc 5mg; also contains D, E, calcium, iron, manganese, copper, chromium	1 tablet
BioCeuticals Ultra Muscleze Magnesium	1 scoop: Magnesium 244 + 36.2mg; B1 25mg; B3 20mg; B6 41.1mg; B9 (folate) 150mcg; B12 – 20mcg 2 scoops:	1 scoop powder (5g) 2 scoops powder	Cenovis Vitamin C Sugarless C 500mg	500mg Vit C	2 tablets per day	Natures way FO	EPA 200mg; DHA 150mg	1 softgel	Swisse zinc	Zinc 25mg; B6 30mg; Mg 35mg	1 tablet

[3] Calculation: Magnesium aspartate is 288 molecular mass and magnesium is 24; 24/288=8%;250 mag-aspartate X 8% = 20mg magnesium

Supplement Brand Name	Ingredient name/ Ingredient quantity	Dose	Supplement Brand Name	Ingredient name/ Ingredient quantity	Dose	Supplement Brand Name	Ingredient name/ Ingredient quantity	Dose	Supplement Brand Name	Ingredient name/ Ingredient quantity	Dose
	Mag 280.2mg = 560.4; B1 50mg; B3 40mg; B6 82.2mg; B9 (folate) 300mcg; B12 40mcg										
BioCeuticals Armaforce (Andrographis)	Vit C - 250mg ; Zinc – 5mg	1 tablet	Cenovis Mega (Bio) Vitamin C 1000mg	1 tablet: Vitamin C 1000mg	1 – 3 tablets per day	NOW Krill Oil	EPA 120mg; DHA 70mg	1 softgel (500mg)	Swisse hair skin nails – liquid	Vit C 200mg	10ml
BioCeuticals B+ (BioCeuticals Mega B Q10)	Vit C 100mg; B1 50mg; B2 50mg; B3 100mg (2 forms); B5 45mg; B6 50mg (2 forms); Folate (activated) 400mcg; B12 400mcg; Incl Co Enzyme Q10 and Vit E among others	1 capsule	Cenovis Multivitamins and Minerals	Vitamin B1 (Thiamine Nitrate) 10mg; Vitamin B2 (Riboflavine) 10mg; Vitamin B3 (Nicotinamide) 25mg; Vitamin B6 (Pyridoxine Hydrochloride) 2mg; Vitamin B12 (Cyanocobalamin) 2mcg; Vitamin C (Ascorbic acid) 75mg; Vitamin B5 (Calcium Pantothenate) 8mg; Magnesium (as Oxide) 36mg; Zinc (as Oxide) 1.5mg	2 tablets per day	NOW Zinc Picolinate 6a		1 capsule (50mg)	Swisse Ultiboost hair skin nails – tablets	Zinc 30mg; Vit C 50mg; also contains iron, silicon, herbs biotin	1 tablet
BIOmega USANA	EPA 580mg; DHA 470mg	2 capsules	Centrum Complete A – Zinc	Vit C 100mg; Vitamin B1 1.4mg; Vitamin B2 1.75mg; Vitamin B6 2mg; Vitamin B12 2.5mcg; Folic Acid 200mcg; Niacin (NE) 20mg; Pantothenic Acid 7.5mg; Mg 100mg; Zinc 5mg	1 tablet daily	Nutralife Magnesium Complete		1 tablet (300mg)	Swisse FO ultiboost 4X strength wild fish	EPA 720mg; DHA 480mg	1 capsule
Blackmores Bio C Chewable 500mg	Citrus bioflavonoids	2 tablets per day	Centrum Adults	Vit C 60mg; Thaimin 1.5mg; Riboflavin 1.7mg; Niacin 20mg; B6 2mg; Folic Acid 400mcg; Vit B12 6mcg; Mg 50mg	1 tablet daily	Nutra-life B Vitamin	Thiamine (Vit B1) 40.5mg; Riboflavin (Vit B2) 20mg; Nicotinamide 50mg; Pantothenic acid (Vit B5) 45mg; Folic Acid 300µg; Pyridoxal 5-phosphate monohydrate 6mg, Equiv. Pyridoxine (Vit B6) 3.83mg;		Ultiboost odourless wild fish oil	EPA 180mg; DHA 120mg	1 capsule (1000mg)

Supplement Brand Name	Ingredient name/ Ingredient quantity	Dose	Supplement Brand Name	Ingredient name/ Ingredient quantity	Dose	Supplement Brand Name	Ingredient name/ Ingredient quantity	Dose	Supplement Brand Name	Ingredient name/ Ingredient quantity	Dose
							Cyanocobalamin (Vit B12) 50µg				
Blackmores Vit C	Bio C 1000mg: 1000mg vit C; hesperidin and rutin and rosehips and acerola	1 tablet	Centrum Multi Gummies	1 gummy: 9mg Vit C; 1mg B6; 80mcg Folic Acid; 4.5mcg B12; 1.25mg zinc; Vit D; E 2 gummies: Vit C 18mg; Vit B6 2mg; Folic Acid 160mcg; Vit B12 9mcg; Zinc 2.5mg	2 gummies	Nutralife Ester C+ Bio	Vitamin C 1000mg	1 tablet	Ultiboost odourless high strength wild fish oil	EPA 270mg; DHA 180mg	1 capsule (1500mg)
Blackmores Bio Magnesium	Vit B6 50mg; Vit C 50mg	1 tablet (301.5mg)	Dr Wilsons Adrenal C Formula	Vitamin C 580mg; Mag 42mg; Zinc 7.5mg	1 tablet	Nutralife Ester C powder	Vitamin C 1000mg	1 dose(1g)	Ultiboost wild fish oil	EPA 139.2mg; DHA 87mg (per 870mg)	1 ml per day
Blackmores Muscle magnesium(powder)	Magesnium 2.07g	1 tsp (5g)	Dr Wilsons Super Adrenal Stress Formula	Vitamin C 308mg; B1 5mg; B2 5mg; B3 26mg; B6 30mg; Folate (B9) 200mcg; Magnesium 40mg;Zinc 3mg	1 tablet	Bioceuticals B12 spray	B12 500mcg	1 spray	Swisse Immune	Zinc 25mg; Vit C 500mg; Magnesium 18.75mg	1 tablet
Blackmores Super magnesium	Magnesium 300mg; Vit C 50mg; Vit B6 50mg	1 tablet	Compounded Activated B's	5-MTHF 500mcg; Pyridoxal 5P 25mg; Riboflavin 5P 100mg; Methylcobalamin (B12) 400mcg	1 capsule per day	Nutriway Multi	Thiamin Nitrate (Vitamin B1) 2.2 mg; Riboflavin (Vitamin B2) 2.6mg; Nicotinamide 20mg; Pantothenic Acid 12mg; Pyridoxine (Vitamin B6) 1.5mg; Cyanocobalamin (Vitamin B12) 10mcg; Folic Acid 400mcg; Vitamin C 85mg; Magnesium (as Magnesium Oxide) 111mg; Zinc (as Zinc Oxide) 15mg		Swisse Ultiboost Magnesium	Mg 150mg	1 tablet
Blackmores B12	B12 100umcg	1 tablet	Eagle B Plus	Magnesium aspartate (equiv. magnesium 800mcg); Magnesium phosphate (equiv. magnesium 7mg); Zinc gluconate (equiv. zinc 14.4mg); Nicotinamide 220 mg;	1 tablet	Nutriway EPO	Oenothera biennis(Evening primrose) seed oil 128.53mg; (equivalent to oenothera bienis fresh seed 642.65mg); Borago officinalis (borage)		Udo's Oil	ALA 6.5g; LA 2.95 ALA 13g; LA 5.9g ALA 2.2g; LA 0.98g	1 tablespoon 2 tablespoons 1 teaspoon

Supplement Brand Name	Ingredient name/ Ingredient quantity	Dose	Supplement Brand Name	Ingredient name/ Ingredient quantity	Dose	Supplement Brand Name	Ingredient name/ Ingredient quantity	Dose	Supplement Brand Name	Ingredient name/ Ingredient quantity	Dose
				Nicotinic acid (B3)[4] 5 mg; Ascorbic acid 50 mg; Pyridoxine hydrochloride (B6) 50 mg; Thiamine hydrochloride (B1) 50 mg; Riboflavin (B2) 20 mg; Folic acid (B9) 150 mcg; Cyanocobalamin (B12) 100 mcg 3 tablets equal and 6 tablets provide: Mag 23.4 mg (46.8mg); Zinc 43.2mg (86.4mg); B3 675mg (1350mg); Ascorbic Acid (Vit C) 150mg (300mg); B6 150mg (300mg); B1 150mg (300mg); B2 60mg (120mg); B9 (folic Acid) 450mcg (900mcg); B12 300mcg (600mcg)			seed oil 129.13mg;(equilivalent to borago officinale fresh seed 516.52mg); Zingiber officinale (Ginger) root extract 39.15mg; (equivalent to zingiber officinale dry root 665.55mg); Vitex agnus castus (Chasteberry) extract 15mg; (equivalent to vitex agnus castus fresh fruit 150mg); Angelica polymorpha (Dong Quai) extract 17.41mg; (equivalent to agelica polymorpha fresh fruit 121.87mg); Bioflavonoids from citrus sinensis fruit 8.62mg				
Blackmores FO 1000mg	Omega 3 300mg	1 capsule	Elektra magnesium oil as spray	1 spray: magnesium 57.14mg 7 sprays: magnesium 400mg	1 spray 7 sprays	Nutrilite (Amway) Bio C Plus	Vit C 500mg; also contains bioflavonoids	1 tablet	Vitamore Magnesium 600mg	Mg 320mg; B6 50mg; also contains Vit D and manganese	1 capsule
Vitamore Omega-3 1000mg	EPA 180mg; DHA120mg	1 capsule	Wagner Magnesium Sleep	Mg 200mg ; Zinc 2mg; Includes ziziphus, corydalis, passionflower	1 capsule	Injection B12		1 shot (100ug)	Hemp Foods Australia	Hemp seeds (EFAs)[5]	1 tsp

[4] Calculation: Combining Nicotinamide 220 mg and Nicotinic acid 5 mg totals 225mg B3

[5] Calculation: Hemp seeds @ 3 Tablespoons = 25g (3 tablespoons) with 7.5g = LA and 3g = ALA and 0.6g GLA; 1 tablespoon = 2.5g LA and 1g ALA and 0.2 g GLA; to get 1 teaspoon divide 1/3 = 0.3g ALA and 0.83g LA and 0.06g GLA

Appendix T: Summary of the distribution of DS combinations cited by participants who reported using between 1 and 5 DSs

One DS (n=22)

DS Type	Frequency	Percentage
B12	3	(13.6)
B6+Magnesium+ Zinc	1	(4.5)
B vitamins	2	(9.1)
B vitamins+Magnesium	1	(4.5)
EFAs	6	(27.3)
Magnesium	1	(4.5)
Multi-vitamin	4	(18.2)
Vitamin C	4	(18.2)

Two DSs (n=16)

DS Type 1	DS Type 2	Frequency	Percentage
Multi-vitamin	Vitamin C	2	(12.5)
EFAs	Vitamin C	2	(12.5)
EFAs	B vitamins	1	(6.3)
EFAs	Vitamin B1	1	(6.3)
EFAs	Vitamin B12	1	(6.3)
EFAs	B vitamins+Magnesium	1	(6.3)
EFAs	Magnesium	1	(6.3)
EFAs	Vitamin B12	1	(6.3)
Multi-vitamin	Magnesium	1	(6.3)
Multi-vitamin	B vitamins+Magnesium	1	(6.3)
Vitamin C	B12	1	(6.3)
Vitamin C	B6+Magnesium	1	(6.3)
EFAs	Multivitamin	1	(6.3)
Vitamin B+Magnesium	Vitamin B+Magnesium	1	(6.3)

Three DSs (n=10)

DS Type 1	DS Type 2	DS Type 3	Frequency	Percentage
EFAs	Magnesium	Magnesium	1	(10.0)
EFAs	Magnesium+Zinc	Vitamin C+Zinc	1	(10.0)
EFAS	Multi-vitamin	Vitamin C+Zinc	1	(10.0)
EFAs	EFAs	Vitamin B6+Magnesium	1	(10.0)
Vitamin C	B-vitamins+Vitamin C	Magnesium+Vitamin C	1	(10.0)
Vitamin C	Multi-vitamin	Vitamin B6+Magnesium	1	(10.0)
Multi-vitamin	EFAs	Vitamin C	1	(10.0)
Multi-vitamin	Vitamin B12	Magnesium	1	(10.0)
B vitamins	Magnesium	Magnesium	1	(10.0)
B vitamins	Magnesium	B vitamins+Magnesium	1	(10.0)

Four DSs (n=2)

DS Type 1	DS Type 2	DS Type 3	DS Type 4	Frequency	Percentage
B6+Mag+VitC	EPO	VitC+Zinc	VitC+Zinc	1	(50.0)
Vit B1	Multi	Vit B12	EPO	1	(50.0)

Five DSs (n=3)

DS Type 1	DS Type 2	DS Type 3	DS Type 4	DS Type 5	Frequency	Percentage
EFAs	Multi	VitC	Vit B6+Mag	EPO	1	(33.3)
EFAs	Multi	Mag	Zinc	Folate	1	(33.3)
EFAs	EFAs	B Vits	Vit B6+Mag	VitC+Zinc	1	(33.3)

Appendix U: Distribution of DS types for N = 107 cited DSs

Supplement Type	**Frequency**	**Percentage** (as a percentage of *all supplements* cited)
EFAs	25	(23.4)
Multi-vitamin	17	(15.9)
Vitamin C	14	(13.1)
Magnesium	10	(9.4)
Vitamin B12	7	(6.5)
B Vitamins	6	(5.6)
B Vitamins+Magnesium	6	(5.6)
Vitamin B6+Magnesium	5	(4.7)
Vitamin C+Zinc	5	(4.7)
EPO	3	(2.8)
Vitamin B1	2	(1.9)
Vitamin B6+Magnesium+Vitamin C	1	(0.9)
Vitamin B6+Magnesium+Zinc	1	(0.9)
B Vitamins+Vitamin C	1	(0.9)
Folate	1	(0.9)
Magnesium+Vitamin C	1	(0.9)
Magnesium+Zinc	1	(0.9)
Zinc	1	(0.9)
TOTAL	**107**	**(100.0)**

Appendix V: General DS usage patterns (all reported DS, N = 107)

Characteristic	Frequency	Percentage
Frequency of Use (n, %)		
Daily	65	(60.8)
4-5 times per week	11	(10.3)
2-3 times per week	8	(7.5)
Once per week	8	(7.5)
Once per month	7	(6.5)
Other	8	(7.5)
Daily Dosage (n, %)		
1 time daily	70	(65.4)
2 times daily	11	(10.3)
3 times daily	4	(3.7)
Other	22	(20.6)
Duration of Use (n, %)		
Less than 1 month	3	(2.8)
1-3 months	14	(13.2)
3-6 months	18	(17.0)
6-12 months	27	(25.5)
More than 1 year	41	(38.7)
Other	3	(2.8)

Appendix W: Summary of associated DS use variables

Characteristic	Frequency	Percentage
Is the supplement helping achieve goals? (n, %)		
Yes	59	(55.1)
No	6	(5.6)
Unsure	42	(39.3)
Reason for taking supplement		
Increase energy (n, %)	37	(34.6)
Lose weight (n, %)	9	(8.4)
Prevent disease (n, %)	31	(29.0)
Reduce stress (n, %)	29	(27.1)
Improve memory/concentration (n, %)	20	(18.7)
Other (n, %)	61	(57.0)
What prompted use of the supplement?		
Doctor (n, %)	31	(29.0)
Naturopath (n, %)	15	(14.0)
Magazine (n, %)	13	(12.2)
Newspaper (n, %)	1	(0.9)
Ad (n, %)	0	(0.0)
Other (n, %)	59	(55.1)
Where is the supplement purchased?		
Health store (n, %)	38	(35.5)
Supermarket (n, %)	25	(23.4)
Naturopath (n, %)	5	(4.7)
Online (n, %)	8	(7.5)
Doctor (n, %)	5	(4.7)
Other (n, %)	37	(34.6)

Appendix X: Nutrients of interest obtained through DS

Nutrient	Frequency	% of total number of supplement-takers (n=53)
Vitamin C	34	64.2
Magnesium	31	58.5
Vitamin B6	31	58.5
Vitamin B12	31	58.5
Vitamin B1	27	50.9
Vitamin B2	26	49.1
Vitamin B3	26	49.1
Zinc	24	45.3
Folic Acid	24	45.3
Vitamin B5	21	39.6
EPA	10	18.9
DHA	10	18.9
Omega-3 undefined	8	15.1
ALA	7	13.2
LA	6	11.3
EPO	6	11.3
MTHFR	3	5.7

Appendix Y: Summary statistics for daily nutrient intake from supplementation for selected nutrients

		Amount consumed per day				
Nutrient	**No. of consumers**	Mean	(SD)	Median	Min	Max
ALA (grams)	7	4.83	(5.74)	2.2	0.25	13
LA (grams)	6	3.18	(2.32)	2.75	0.83	5.90
EPO (milligrams)	6	65.91	(76.00)	46.43	0.06	200
EPA (milligrams)	10	635.57	(583.92)	420	71.43	1740
DHA (milligrams)	10	447.29	(445.15)	260	68.57	1410
Omega-3 undefined (milligrams)	8	428.57	(345.55)	300	107.14	1000
Omega-3 (milligrams)	23	2090.68	(3649.07)	760	107.14	13300
Omega-6 (milligrams)	11	1778.35	(2306.43)	890	2.57	5900
Vitamin C (milligrams)	33	395.77	(506.76)	250	5.45	2500
Magnesium (milligrams)	29	187.28	(192.65)	114.3	1.56	845
Zinc (milligrams)	22	20.26	(35.61)	5	0.17	140
Vitamin B1 (milligrams)	25	54.56	(89.66)	25	0.90	350
Vitamin B2 (milligrams)	23	28.67	(41.17)	12	1.16	150
Vitamin B3 (milligrams)	24	45.06	(50.20)	35.86	1.29	210
Vitamin B5 (milligrams)	20	36.10	(35.43)	25.50	2.27	150
Vitamin B6 (milligrams)	29	49.05	(75.36)	25	1.00	382.2
Vitamin B12 (micrograms)	27	82.08	(152.91)	17.86	1.61	640
Folic Acid (micrograms)	23	260.21	(257.68)	200	16.50	1200
MTHFR	3	966.67	(896.29)	500	400	2000

Appendix Z: Descriptive statistics for daily nutrient intake for selected nutrients derived from the FFQ (N=74)

Nutrient	Mean	(SD)	Median	Min	Max
Total omega-6, mg/day	13250.27	(5176.38)	11975.88	4035.27	28768.18
Total omega-3, mg/day	1542.99	(719.63)	1370.42	505.45	4185.67
Magnesium, mg/day	492.15	(180.60)	496.61	179.67	944.79
Vitamin C, mg/day	128.26	(75.13)	111.23	30.47	365.47
Folic acid, mg/day	103.96	(104.50)	70.16	0	472.18
Niacin (Vitamin B3+tryptophan-derived), mg/day	40.71	(13.22)	38.89	16.16	75.61
Biotin (B7), mg/day	36.75	(17.28)	33.13	10.96	102.09
Niacin (B3) pre-formed, mg/day	23.3	(8.25)	22.66	8.11	48.05
Zinc, mg/day	9.54	(3.21)	9.72	3.97	20.49
Pantothenic acid (B5), mg/day	3.35	(1.27)	3.09	1.16	7.93
Cobalamin (B12), mg/day	2.97	(1.78)	2.76	0.02	9.01
Riboflavin (B2) mg/day	2.13	(0.93)	2.02	0.64	5.30
Thiamin (B1) mg/day	1.50	(0.83)	1.27	0.39	4.46
Pyridoxine (B6), mg/day	1.14	(0.55)	0.99	0.43	3.95
Folate, microgram/day	319.37	(141.16)	282.13	94.49	660.32
Total folates, microgram/day	423.43	(200.24)	415.46	112.86	1007.0
Dietary folate (combined with estimate of higher bioavailability of folic acid), microgram/day	493.10	(256.59)	468.7	119.11	1239.18

Appendix AA: Correlation between daily nutrient intake from food and PSS scores (Spearman's Rank Correlations (N=74))

Nutrient	Rho (ρ))	Sidak Adjusted P-value
Magnesium, mg/day	-0.0749	1.000
Zinc, mg/day	0.1390	0.997
Vitamin C, mg/day	-0.1891	0.925
Thiamin (B1) mg/day	-0.0206	1.000
Riboflavin (B2) mg/day	-0.0542	1.000
Niacin (B3) pre-formed, mg/day	0.0222	1.000
Niacin (Vitamin B3+tryptophan-derived), mg/day	0.0508	1.000
Pantothenic acid (B5), mg/day	-0.0889	1.000
Pyridoxine (B6), mg/day	-0.1335	0.998
Biotin (B7), mg/day	-0.2020	0.889
Cobalamin (B12), mg/day	0.1989	0.894
Folic acid, mg/day	0.0519	1.000
Folate, microgram/day	-0.0234	1.000
Total folates, microgram/day	0.0126	1.000
Dietary folate (combined with estimate of higher bioavailability of folic acid), microgram/day	0.0085	1.000
LC w3 PUFA	0.0765	1.000
Total omega-3, mg/day	0.0291	1.000
Total omega-6, mg/day	-0.0963	1.000

Appendix BB: Factors related to DS use

Factor Related to Use	Results
Types of DS used	Supplements labelled as EFAs and MVMs were the two most commonly-cited DSs. However, when considering individual nutrients, vitamin C was the most frequently consumed nutrient, with 64% of supplementers consuming this nutrient. Inspection of the products cited among the sample revealed that most DSs contained multiple micronutrients which suggests that some supplementers may not always be aware of the range of micronutrients present in their DSs and highlights the importance of inspecting the ingredients list to determine the appropriateness of a DS rather than using the product name alone to guide use.
DS use and age	Age may be associated with DS use, as increasing age may be associated with increased health literacy, increased stress and increased health challenges, among other factors. Among the sample, the odds of being a DS user were estimated to increase by 32% for every 5 year increase in age from the mean age of the sample and the rate of DS use was estimated to increase by 2% for every 5 year increase in age from the same. These results suggest that age may be related to DS use among women in as yet unexamined ways.
Duration of DS use	The majority of DSs were reported to be consumed once daily (61%) and more than 60% of the reported DSs had been consumed for a duration of over six months, and 38.7% over one year. The duration of DS consumption may impact its effectiveness, as previously noted, where women reported mood improvements only one year after supplementation was initiated.[272] However, research is scant on the duration of DS use and stress among women, and controlling for duration of use in this study was not possible.
DS purchasing behaviour	The primary place of DS purchase was health stores (36%), and around a quarter of supplements were purchased in a supermarket. There is no evidence with which to compare these results, and no relationship between such and stress was noted in this study.

Appendix CC: Example of recruitment email sent to all cross-sectional survey participants

To:

Cc:

Subject: Female Stress and Nutrition Research Project

From: Delia McCabe – a1689774@adelaide.edu.au Signature: None

Hi [Name]

Here's hoping this message finds you well.

Thanks again for your participation in the first phase of this research project 'Female Stress and Nutrition.'

An Invitation – Female Stress and Nutrition Research Project – Participatory Action Research (PAR) [Phase 2]

As part of the second (and final) phase of this project, a group of women who participated in the first phase, AND who are interested in furthering their understanding of stress and nutrition are invited to get together every week, for approximately an hour to an hour and a half, for a maximum of 10 weeks.

This will be an intimate and focused discussion about what stress means to us, how it impacts our lives, what we are doing to help manage this stress and what role nutrition and supplements play in our lives.

If you are interested in taking part, please reply to this message to let me know and include where you live on the Gold Coast, so that we can find a place that is suitably convenient for all the participants.

At this point in time, I'm looking at starting this group at the beginning – middle of October.

Thanks again for your interest in this project and for your valuable time.
Delia

Delia McCabe, MA (Psych), PhD(c)
delia.mccabe@adelaide.edu.au
Mob 0450451443
https://www.fb.com/FemaleStressandNutrition/

Appendix DD: Example of participant information sheet sent to all participants

PARTICIPANT INFORMATION SHEET

PROJECT TITLE: The association between the dietary and supplementary intake of essential fatty acids (EFAs,) B vitamins, vitamin C, magnesium and zinc to manage stress: a cross sectional study
HUMAN RESEARCH ETHICS COMMITTEE APPROVAL NUMBER: H-2015-090
PRINCIPAL INVESTIGATOR: Associate Professor Craig Lockwood
STUDENT RESEARCHER: Delia McCabe
STUDENT'S DEGREE: PhD candidate

Dear Participant,

You are invited to participate in the research project described below.

What is the project about?
The aim of this research is to understand how women report experiencing stress and their dietary and supplementary intake of EFAs, B vitamins, vitamin C, magnesium and zinc to manage stress. This study will get a quick 'snap shot' of a group of women's food choices, supplements and their stress levels, so it won't be able to discuss whether food choices are influenced by stress levels, whether specific nutrients can reduce stress levels or whether a lack of specific nutrients leads to experiencing more stress, but it may uncover an association between specific nutrients which may be useful for further research and to inform the next stage of the research project.

Who is undertaking the project?
This project is being conducted by Delia McCabe.
This research will form part of the degree of Doctor of Philosophy (PhD) at the University of Adelaide under the supervision of [redacted].
There are no commercial sponsors, external partners or funding being provided for this research.

Why am I being invited to participate?
You are being invited to participate in this study because you are a woman aged between 18 and 65, live on the Gold Coast, can provide informed consent, and have expressed an interest in being involved in this project.

What will I be asked to do?
If you have chosen to receive the questionnaires via post you will receive two questionnaires in the post, along with a reply paid envelope. You should set aside 20 to 30 minutes to fill in the two questionnaires. You will not need to put your name on any of the questionnaires, as you will be allocated a number to protect your privacy. You will be asked to post the questionnaires back to the researcher, in the reply paid envelope provided. Your envelope will contain a batch of food photographs that will be used as examples of portion sizes that you will use to fill in the Food Frequency Questionnaire online with the researcher, via a telephone call, which will take between 10 and 15 minutes to complete. If you have chosen to receive the questionnaires via email, you will be sent a link to 'click' on, and you should set aside 30 – 45 minutes to fill in all the online questionnaires.

How much time will the project take?
The questionnaires will take between 30 and 45 minutes to fill in, depending on whether you are completing the questionnaires on paper and via a telephone call or online.

Are there any risks associated with participating in this project?
There are no foreseeable risks in completing these questionnaires. You may feel uncomfortable for a few minutes while you fill in the questionnaire about your feelings of stress. If you feel very uncomfortable it is suggested that you make an appointment with your medical practitioner and discuss your feelings with them. If you would like the name of medical practitioner then please contact the researcher and she will

provide you with a medical practitioners contact details. Alternatively, please call The Mental Health Association Queensland on (07) 5519 2550 if you feel the need to talk to someone who is qualified to help you about your feelings of stress.

What are the benefits of the research project?
There are no immediate benefits to the participants of this research. Women all over the world have many different roles to play in society, for example as mothers, carers of elderly parents, workers, community and school volunteers and domestic workers in their homes. This research has the potential in increase what we know about stress and how specific nutrients may influence stress. It therefore has the potential to inform other researchers and may in time result in other women being able to benefit from other research.

Can I withdraw from the project?
Participation in this project is completely voluntary. If you agree to participate, you can withdraw from the study at any time. Once the researcher has received your questionnaires and submitted your data you will not be able to withdraw the data, as it is anonymous.

What will happen to my information?
The information that you provide on the questionnaires will be provided anonymously, and the completed questionnaires will be stored in a locked cabinet within a locked archive room in The School of Translational Health Science, at the University of Adelaide. Data will be kept for seven years prior to secure destruction, as per the current research policy guidelines. The results of the study, will be presented using combined rather than individual data, and will be used in journal articles and publications, conferences and in the researcher's PhD thesis. The results will be made available to participants via a letter posted to their address or via a personal email if they so wish.

Who do I contact if I have questions about the project?
Should you have any questions about the research, please contact the researcher on [redacted]
The other researchers involved in this research project can also be contacted via telephone:
[redacted]

What if I have a complaint or any concerns?
The study has been approved by the Human Research Ethics Committee at the University of Adelaide (approval number H-2016-090). If you have questions or problems associated with the practical aspects of your participation in the project, or wish to raise a concern or complaint about the project, then you should consult the Principal Investigator. Contact the Human Research Ethics Committee's Secretariat on phone [redacted] if you wish to speak with an independent person regarding concerns or a complaint, the University's policy on research involving human participants, or your rights as a participant. Any complaint or concern will be treated in confidence and fully investigated. You will be informed of the outcome.

If I want to participate, what do I do?
If you would like to participate in this research project please fill out the questionnaires included in the envelope and then put all the questionnaires into the reply paid envelope and post it back to the researcher. By completing and returning the questionnaires you are consenting to participation in this project. If you are completing the questionnaires online, your consent is provided when you complete the questionnaires and click the 'done' button.

The second phase of this research project involves a group of interested women discussing their experience of stress and dietary and supplementary intake of essential fatty acids (EFAs,) B vitamins, vitamin C, magnesium and zinc to manage stress. The group will meet every week for between eight and ten weeks. Each session will run for an hour to an hour and a half. If you are interested in taking part in this next phase of the project, please contact the researcher on [redacted]

Yours sincerely,
[redacted]
Student researcher – Delia McCabe – [redacted] **delia.mccabe@adelaide.edu.au**

Appendix EE: Example of consent form

The University of Adelaide

Human Research Ethics Committee (HREC)

CONSENT FORM

1. I have read the attached Information Sheet and agree to take part in the following research project:

Title:	**The experience of the dietary and supplementary intake of essential fatty acids (EFAs,) B vitamins, vitamin C, magnesium and zinc to manage stress: A Participatory Action Research (PAR) project**
Ethics Approval Number:	**H-2016-090**

2. I have had the project, so far as it affects me, fully explained to my satisfaction by the research worker. My consent is given freely.
3. I have been given the opportunity to have a member of my family or a friend present while the project was explained to me.
4. Although I understand that the purpose of this research project is to improve the quality of medical care, it has also been explained that my involvement may not be of any benefit to me.
5. I have been informed that, while information gained during the study may be published, I will not be identified and my personal results will not be divulged.
6. I understand that I am free to withdraw from the project at any time and that this will not affect medical advice in the management of my health, now or in the future.
7. I agree to the sessions being audio recorded. Yes ☐ No ☐
8. I am aware that I should keep a copy of this Consent Form, when completed, and the attached Information Sheet.

Participant to complete:

Name: _____ Signature: _____

Date: _____

Researcher/Witness to complete:

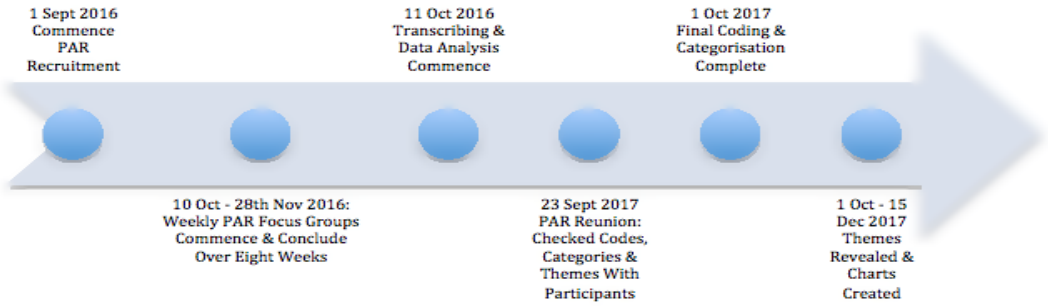
I have described the nature of the research to _____
(print name of participant)

and in my opinion she/he understood the explanation.

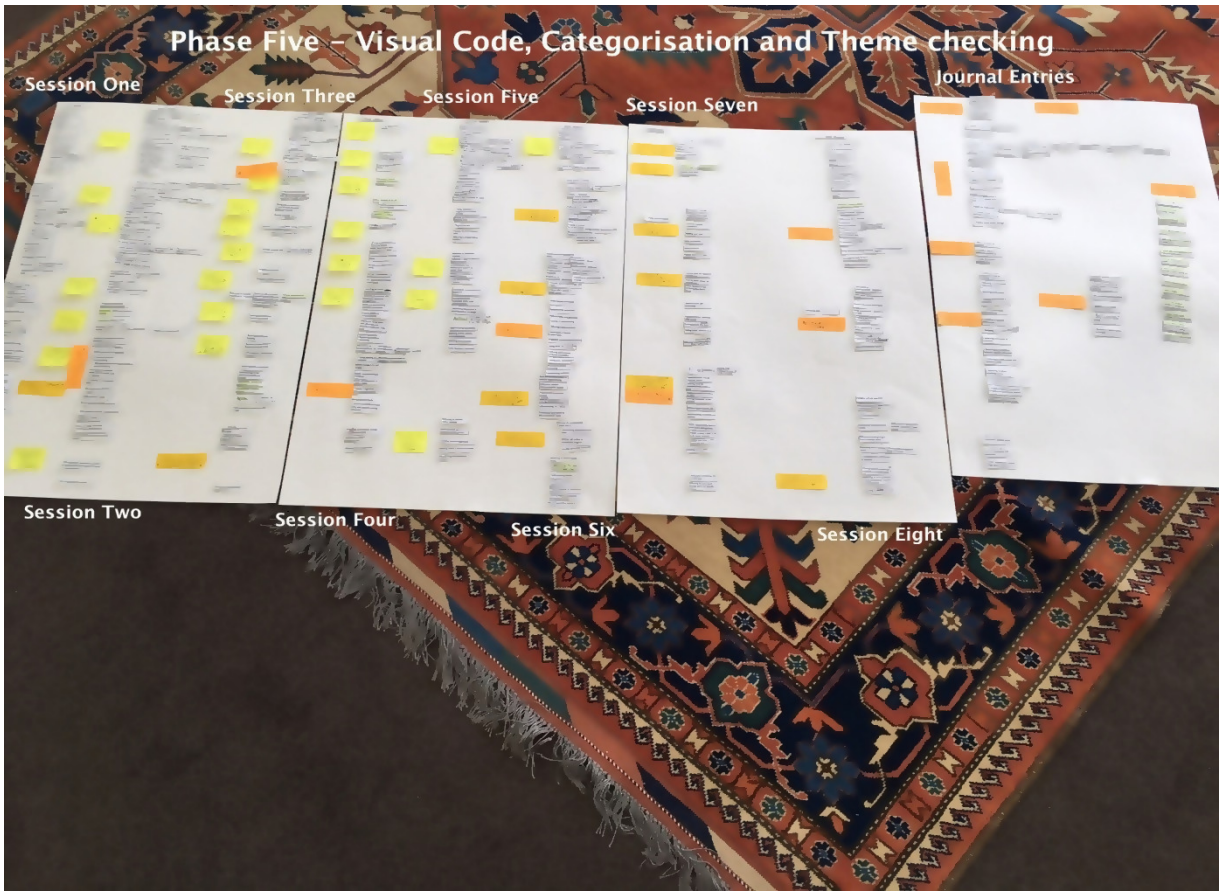
Signature: _____ Position: _____

Date: _____

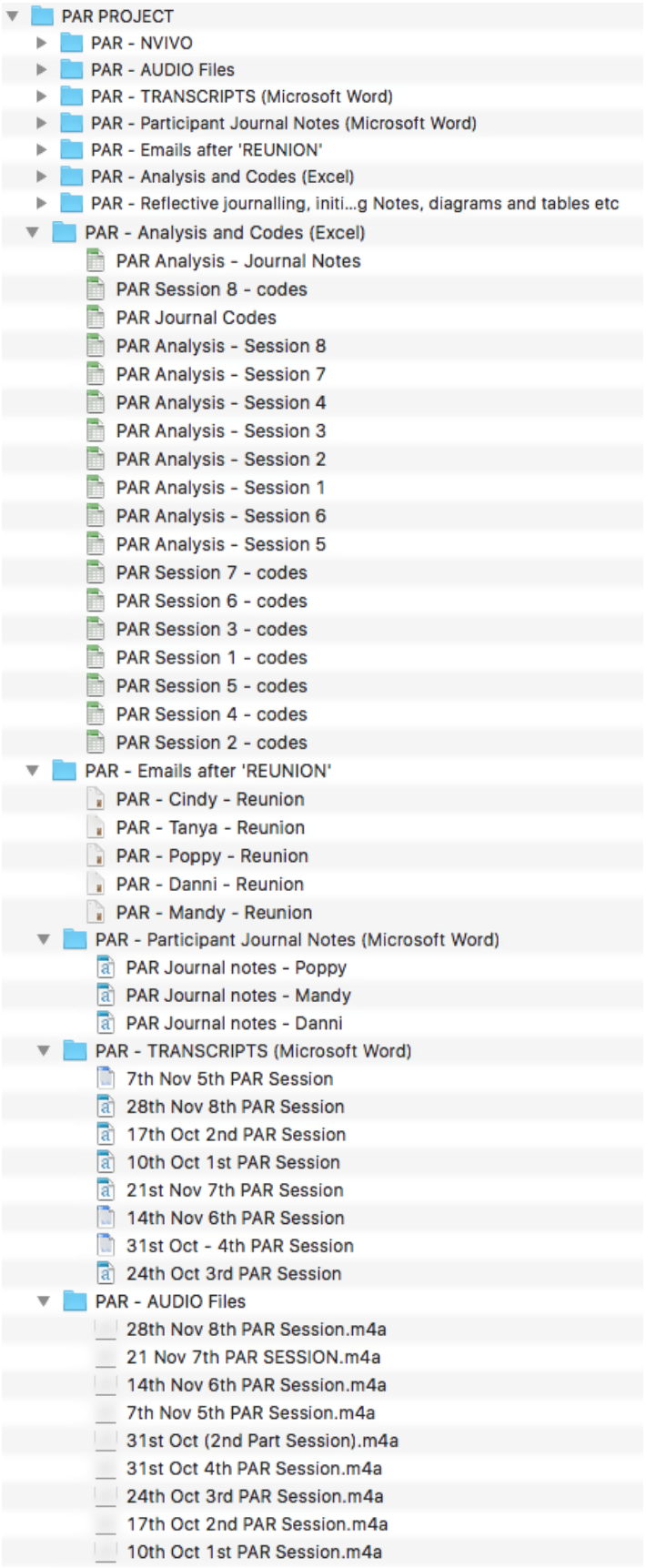
Appendix FF: PAR Project Timeline: 1 September 2016 – 29 September 2017



Appendix GG: Phase Five Data Analysis code, categorisation and pattern (theme) development – visual checking



Appendix HH: PAR raw and analysed data in files



Appendix II: Meaningful Word Frequencies

Meaningful Word Frequencies (including stemmed words)	Frequency	Relative Frequency (n=250)
Stressful including stress, stressed and stresses	475	1.9
Relationships including people, family, others, children, daughter, husband, friends, marriage, partner, parents, sharing, childhood, mother and driving	358	1.4
Thoughts, including thinking, introspecting and reflection	271	1.1
Supplements including alternatives, vitamins, magnesium, nutrients and conflicting information and scepticism	217	0.9
Anxiety including anxious and worried	112	0.4
Health, including eating, exercise, healthy and cooking	109	0.4
Actions including meditation, relaxation and therapy	89	0.4
Pharmaceuticals including medication, prescribing and anti-depressants	76	0.3
Empathy including empathizing and suggestions	69	0.3
Beliefs and believing	67	0.3
Depression, exhaustion and fatigue	49	0.2
Expectations and expected	34	0.1
Habitual and habits	25	0.1
Internet, Facebook, Google	25	0.1

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