Subjective Wellbeing in Healthy, Community-Dwelling, Older Adults: Measurement Operationalisations and Examination of Folate, Vitamin B12, Homocysteine and Omega-3 Polyunsaturated Fatty Acids as Potential Predictors

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A thesis submitted in fulfilment of the requirements for the degree of

Doctor of Philosophy

December, 2013
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Laura Catherine Edney
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<td>5-HIAA</td>
<td>5-hydroxy-indoleacetic acid</td>
</tr>
<tr>
<td>5-MTHF</td>
<td>5-methyltetrahyrofolate</td>
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<td>5, 10-MTHFR</td>
<td>5,10-methylenetetrahydrofolate reductase</td>
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<td>A</td>
<td>Agreeableness</td>
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<td>AA</td>
<td>arichidonic acid</td>
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<td>ABS</td>
<td>Australian Bureau of Statistics</td>
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<td>AD</td>
<td>Alzheimer’s Disease</td>
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<td>AIHW</td>
<td>Australian Institute of Health and Welfare</td>
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<td>AL</td>
<td>Adaptation Level</td>
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<td>ALA</td>
<td>α-linolenic acid</td>
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<td>Analysis of Covariance</td>
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<td>ANOVA</td>
<td>Analysis of Variance</td>
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<td>Average Variance Extracted</td>
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<td>Beck Anxiety Inventory</td>
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<td>Beck Depression Inventory</td>
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<td>Bond-Lader Mood Scales</td>
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<td>BPAQ</td>
<td>Buss-Perry Aggression Questionnaire</td>
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<td>BSI</td>
<td>Berocca Stress Index</td>
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<td>C</td>
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<td>CCVFFQ</td>
<td>Cancer Council of Victoria Food Frequency Questionnaire</td>
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<td>CDI</td>
<td>Children’s Depression Inventory (self-rated)</td>
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<td>CDRS</td>
<td>Children’s Depression Rating Scale (clinician rated)</td>
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<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>CES-D</td>
<td>Centre for Epidemiological Studies Depression Scale</td>
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<td>CFA</td>
<td>Confirmatory Factor Analysis</td>
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<td>CSIRO</td>
<td>Commonwealth Scientific Industrial Research Organisation</td>
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<td>CSM</td>
<td>Complete State Model</td>
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<td>CVD</td>
<td>Cardiovascular Disease</td>
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<td>Depression Anxiety and Stress Scale</td>
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<tr>
<td>d.f.</td>
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<td>DHA</td>
<td>docosahexaenoic acid</td>
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<td>DHUS</td>
<td>Daily Hassles and Uplifts Scale</td>
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<td>DPA</td>
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<td>DSM</td>
<td>Diagnostic and statistical manual of mental disorders</td>
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<tr>
<td>E</td>
<td>Extraversion</td>
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<tr>
<td>EAR</td>
<td>Estimated Average Requirement</td>
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<td>E-DHA</td>
<td>ethyl- docosahexaenoic acid</td>
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<td>E-EPA</td>
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<td>Folic Acid</td>
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<td>FFQ</td>
<td>Food Frequency Questionnaire</td>
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<td>Acronym</td>
<td>Description</td>
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<tr>
<td>GAFS</td>
<td>Global Assessment of Functioning Scale</td>
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<td>Global Assessment Scale</td>
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<td>GDP</td>
<td>Gross Domestic Product</td>
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<td>Geriatric Depression Scale</td>
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<td>General Health Questionnaire</td>
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<td>Gross National Product</td>
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<td>Inventory for Depressive Symptomology</td>
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<td>ISSFAL</td>
<td>International Society for the Study of Fatty Acids and Lipids</td>
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<tr>
<td>LA</td>
<td>linoleic acid</td>
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<td>Long Chain</td>
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<td>LGCM</td>
<td>Latent Growth Curve Modelling</td>
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<td>Life Satisfaction</td>
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<td>Montgomery-Åsberg Depression Rating Scale</td>
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<td>Mcg</td>
<td>Micrograms</td>
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<td>MCI</td>
<td>Mild Cognitive Impairment</td>
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<td>MDD</td>
<td>Major Depressive Disorder</td>
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<td>Abbreviation</td>
<td>Description</td>
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<td>Mg</td>
<td>Milligrams</td>
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<td>MI</td>
<td>Modification Indices</td>
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<td>MMSE</td>
<td>Mini–mental state examination</td>
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<td>MMSQ</td>
<td>Multi-Modal Strain Questionnaire</td>
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<td>MTHF</td>
<td>methyltetrahydrofolate</td>
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<td>N</td>
<td>Neuroticism</td>
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<tr>
<td>n-3</td>
<td>Omega-3</td>
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<td>n-6</td>
<td>Omega-6</td>
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<tr>
<td>NA</td>
<td>Negative Affect</td>
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<td>NHMRC</td>
<td>National Health and Medical Research Council</td>
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<td>nmol</td>
<td>NanoMole</td>
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<td>NPI</td>
<td>Neuropsychiatric Inventory</td>
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<td>O</td>
<td>Openness</td>
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<td>OAS-M</td>
<td>Overt Aggression Scale, Modified</td>
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<td>Obsessive-Compulsive Disorder</td>
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<td>OCEANIC</td>
<td>Openness Conscientiousness Extraversion Agreeableness Neuroticism Index Condensed</td>
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<td>Positive Affect</td>
</tr>
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<td>PANAS</td>
<td>Positive and Negative Affect Schedule</td>
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<td>Parkinson’s Disease</td>
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<td>P-F Study</td>
<td>Aggression-estimating test</td>
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<td>PFT</td>
<td>Picture-Frustration Test</td>
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<td>PGE2</td>
<td>prostaglandin E2</td>
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<td>PGWB</td>
<td>General Psychological Wellbeing</td>
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<td>PHQ-9</td>
<td>Patient Health Questionnaire – 9</td>
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<td>Abbreviation</td>
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<tr>
<td>pmol</td>
<td>PicoMole</td>
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<td>POMS</td>
<td>Profile of Mood States</td>
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<td>PRIME-MD</td>
<td>Primary Care Evaluation of Mental Disorders</td>
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<td>PSQ</td>
<td>Personal Strain Questionnaire</td>
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<td>PSS</td>
<td>Perceived Stress Scale</td>
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<td>PUFA</td>
<td>Polyunsaturated Fatty Acid</td>
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<td>QoL</td>
<td>Quality of Life</td>
</tr>
<tr>
<td>RC</td>
<td>Reliability of the Construct</td>
</tr>
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<td>RCT</td>
<td>Randomised Control Trial</td>
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<td>RDI</td>
<td>Recommended Dietary Intake</td>
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<td>RMSEA</td>
<td>Root Mean Square Error of Approximation</td>
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<td>SAHOS</td>
<td>South Australian Health Omnibus Survey</td>
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<td>SAMe</td>
<td>S-adenosylmethionine</td>
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<td>SC</td>
<td>Short Chain</td>
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<td>Socio-Economic Indexes for Areas</td>
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<td>SHI</td>
<td>Steen Happiness Index</td>
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<td>SKIP</td>
<td>Single Key Impulsivity Paradigm</td>
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<td>SLT</td>
<td>Schmid-Leiman Transformation</td>
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<td>SMD</td>
<td>Standardised Mean Difference</td>
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<td>SSRI</td>
<td>Selective Serotonin Reuptake Inhibitors</td>
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<td>STAI</td>
<td>State-Trait Anxiety Inventory</td>
</tr>
<tr>
<td>STAXI</td>
<td>State-Trait Anger Expression Inventory</td>
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</tbody>
</table>
SWB  Subjective Wellbeing
SWLS  Satisfaction with Life Scale
TLI  Tucker Lewis Index
Trt  treatment group
µmol  micromoles
µg  micrograms
VAMS  Visual Analogue Mood Scale
VAS  Visual Analogue Scales
WD  withdrawn
WHO  World Health Organisation
WLSMV  Weighted Least Squares Mean- and Variance-Adjusted
WRMR  Weighted Root Mean Square Residual
WVS  World Views Survey
YBOCS  Yale-Brown Obsessive-Compulsive Scale
YMRS  Young Mania Rating Scale
YPAS  Yale Physical Activity Survey
Summary

Traditional psychological research is frequently preoccupied with disability and treatment; Positive Psychology seeks to complement this approach to help form whole model conceptualisations of mental health. This thesis sought to assess (1) the empirical measurement of one measure of positive functioning – Subjective Wellbeing, (2) whether certain nutritional components commonly associated with mental illness were also associated with positive mental health, and (3) whether we could alter the normative trajectory of positive mental health with a nutritional intervention. Nutrition offers a potentially preventative measure to ill-health and was therefore investigated as a departure from the traditional focus on treatment of disability. If nutrition can be identified as a risk factor for sub-optimal positive mental health then it offers a preventative measure that is modifiable, easy to implement at the population-level, relatively cheap, and available to all.

Subjective Wellbeing (SWB) is a well-defined measure of positive mental health that has been studied extensively. It is composed of three components: Positive Affect (PA), Negative Affect (NA), and Life Satisfaction (LS). Despite this consensus on what the construct is, there is little uniformity in its measurement. Paper 1 sought to review theory and empirical evidence on the definition and measurement of SWB, compare and contrast four common methods used to measure SWB, and provide an example of how these different methods may influence study results. Results favoured one method of measuring SWB and we demonstrated that each of the four methods had the potential to differentially impact any subsequent conclusions drawn regarding SWB and its relationship to an external variable of interest.
Papers 2 and 3 sought to assess the influence of aspects of nutrition on SWB, using the most appropriate method for measuring SWB as identified in Paper 1. In Paper 2 we examined the relationships between folate, vitamin B$_{12}$, homocysteine, and SWB. Folate and vitamin B$_{12}$ are two B-vitamins that have been consistently implicated in mental illness, either directly, or via their influence on homocysteine levels. Folate, vitamin B$_{12}$, and their interaction significantly predicted levels of PA 18 months later but had no impact on levels of NA, or LS. Cross-sectionally, homocysteine was related to PA but this relationship was completely attenuated in longitudinal analyses suggesting that homocysteine is merely a marker for folate and vitamin B$_{12}$ status. This is the first study to demonstrate a potential causal link between levels of folate and vitamin B$_{12}$ to PA in a large, non-clinical population.

Paper 3 involved results from a double-blind placebo-controlled RCT to investigate whether omega-3 long-chain polyunsaturated fatty acid (n-3 LC PUFA) supplementation was able to predict any observed change in the trajectory of SWB over 18-months in older people. n-3 LC PUFAs have been implicated in several mood disorders; deficient levels have been found in psychiatric patients and several randomised controlled trials have observed an improvement in depression with n-3 LC PUFA supplement alone, or as an adjuvant treatment to existing therapies. Our results demonstrated little change in SWB across 18 months in either the treatment or placebo groups. Treatment group did not predict change in PA, NA or LS; however, initial levels of n-3 LC PUFAs (EPA+DHA) were associated with initial levels of PA, but were not associated with initial levels of NA or LS or with change in PA, NA or LS. Initial levels of n-3 PUFAs (EPA+DHA+DPA+ALA) did not predict initial levels of PA, NA, or LS; however, they were able to predict rate of change over 18 months in NA and LS. These
results were consistent when gender was controlled for. Results thus provided some
evidence for a cross-sectional association between \( n-3 \) LC PUFAs with PA, and suggest a
potential role of \( n-3 \) PUFAs in reducing rate of increase in NA and of decline in LS in
otherwise healthy, older individuals.

Clarification of the theoretical and empirical differences between models of SWB
used and the application of SWB to an area often dominated by investigation of disorder
constitute the two main areas of original contribution provided by this thesis. Results of
the three papers suggest that choice of measurement of SWB has an impact on
conclusions drawn, that one model provides a superior measurement to the other three
commonly reported in the literature, that folate and Vitamin B\(_{12}\) may play a role in
Positive Affect independently of homocysteine, and that \( n-3 \) PUFAs are associated with
change in NA and LS over 18 months.
Chapter 1. Introduction and Literature Review

Overview

The aim of this research was to assess whether nutrition plays a role in positive mental health. Upon review of the literature, it became clear that to investigate this relationship thoroughly we would need to investigate how positive mental health should be most appropriately measured. We here conceptualised positive mental health as Subjective Wellbeing (SWB) and tested four competing but commonly employed empirical models. We found support for one measurement model of SWB which was then adopted in our subsequent assessment of whether this construct is related to certain nutritional components. Thus, the three papers presented here are related in that the second and third were heavily reliant on the first, and in that all three used the same sample of participants. These three papers form the foundation of this thesis and are presented as papers to be submitted for publication; each paper is set in the broader context of both this thesis and of the literature prior to presentation.

The current chapter provides a summary of previous literature relevant to conceptualisations of positive mental health, specifically SWB, an overview of the nutrition factors considered, and concludes with the role of these nutrition factors in mental health.

Chapter 2 provides an exegesis for the broad thesis. The aim is to provide a theoretical rationale for each of the papers based on the overall aims of this thesis. This
chapter also includes an overview of the study in its broader context, methodological information regarding the study design, and descriptive statistics of the sample.

The three papers submitted for publication are presented in Chapters 3, 4, and 5, with statements outlining the relative contributions made by myself and my supervisors appearing in Appendix 1. Preceding each paper, additional information is presented that includes an overview of the rationale, how the aims fit into the broader research context of this thesis, and a justification and explanation of statistical methods employed, where required. A summary of the results and an overall discussion are presented in Chapter 6.
Chapter 1.1 Mental Health

We hold these truths to be self-evident, that all men are created equal, that they are endowed by their Creator with certain unalienable Rights, that among these are Life, Liberty and the pursuit of Happiness.

(Thomas Jefferson, United States Declaration of Independence, 1776)

Mental health is more than just the absence of mental illness (Keyes, 2007; WHO, 1948); there are individual differences in good mental health and those without mental illness are not all functioning well (Keyes & Lopez, 2002; Keyes, 2005b). This is captured in the definition of mental health provided by the World Health Organisation (WHO) as “a state of wellbeing in which the individual realises his or her own abilities, can cope with the normal stresses of life, can work productively and fruitfully, and is able to make a contribution to his or her community” (WHO, 2001, p. 1). Despite this, good mental health has been neglected for a focus on treatment of disorder and mental health is often presumed in the absence of mental illness (Keyes, 2005b). It is increasingly recognised that a more holistic approach to mental health is required, one in which a healthy individual is so defined due to the presence of emotional, psychological and social wellbeing, in addition to the absence of psychopathology (Keyes, 2002, 2005a, 2005b).

The Complete State Model (CSM) of mental health (Keyes & Lopez, 2002) formalises this view and promotes a dual framework to reduce mental illness: to foster and
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protect mental health, in addition to the prevention and treatment of mental illness (Keyes & Michalec, 2010). This research falls within the area of Positive Psychology which broadly seeks to understand what constitutes mental health (e.g., Keyes, 2005b).

**Positive Psychology**

Positive Psychology is concerned with the study and promotion of good mental health, rather than with mental illness. It is the scientific study of what makes life worthwhile, and of what enables individuals and communities to thrive (Seligman & Csikszentmihalyi, 2000). It does not seek to replace, ignore, or undermine the importance of mental illness, but rather it seeks to complement and extend this approach to provide a more complete understanding of mental health (Seligman & Csikszentmihalyi, 2000).

Positive Psychology has focused on happiness (Seligman & Csikszentmihalyi, 2000), which can mean many things to many different people; with different approaches based on religion, philosophy, and psychology. Psychological approaches motivated by Positive Psychology seek to apply the scientific method to the study of happiness; however, this approach has necessarily been influenced by philosophical and religious perspectives on what it means to be happy. As a result, there are several lines of psychological enquiry on concepts broadly related to the study of happiness. Here we provide a brief overview of these historical philosophical arguments. These form two distinct schools which still divide wellbeing research today and therefore provide context for discussion of scientific enquiry into wellbeing.

From philosophy, Aristotle believed that happiness was the *virtuous activity of the soul in accordance with reason*, that it is an activity rather than an emotion, and that it is
the only thing that we desire for its own sake (Aristotle. The Nicomachean Ethics, 350 BCE). Similarly, happiness was to be found in self-knowledge according to Socrates, and through the discovery of deeper meaning by Plato. Conversely, Aristippus (4th century BCE) proposed that the aim of life is to experience the maximum amount of pleasure, similar to Hobbes (1588-1679) who believed happiness was the successful pursuit of individual desires and appetites, and to Bentham (1748-1832) who considered happiness as seeking pleasure and avoiding pain. Notable religious views include those from the Muslim, Hindu, Catholic, and Buddhist faiths. Al-Ghazali wrote the ‘Alchemy of Happiness’, Patanjali wrote ‘Yoga Sutras’ discussing the psychological and ontological roots of bliss, Catholics believe that the ultimate end of human existence is felicity, “blessed happiness”, to be attained in the next life, and the Noble Eightfold Path in Buddhist teaching leads to Nirvana, where ultimate happiness is only achieved by overcoming craving in all forms.

These perspectives have resulted in many different conceptualisations of what it means to function well; these can be broadly categorised as belonging to either a hedonic or eudaimonic tradition of positive functioning (Ryan and Deci, 2001). The hedonic tradition of wellbeing (e.g., Kahneman, Diener & Schwarz, 1999) equates happiness to seeking pleasure, to the presence of positive affect and the absence of negative affect. It is therefore considered as externally derived, and can be seen as reflected in the ideas of Aristippus, Hobbes, and Bentham. Conversely, the eudaimonic tradition (e.g., Waterman, 1993) equates happiness to the actualisation of human potential, it is seen as a process rather than an endpoint. It is therefore considered to be an internal factor and is reflected in the philosophies of Aristotle, Socrates, and Plato.
Early psychological discussion of Positive Psychology came from the humanistic psychologists, such as Maslow and Rogers, but it was Seligman who formalised this as an area of psychology in 1998 when he chose it as the theme for his presidency of the American Psychological Association. He and Csikszentmihalyi (Seligman & Csikszentmihalyi, 2000) state that the “aim of Positive Psychology is to begin to catalyse a change in the focus of psychology from preoccupation only with repairing the worst things in life to also building positive qualities” (p. 5). It is logically intuitive that the absence of mental disorder does not automatically equate to the presence of positive mental health. Positive Psychology formalises this view and has applied the scientific method to investigate what, if not the absence of mental illness, are the individual and contextual factors that do promote positive mental health?

The psychological study of happiness includes the study of happiness as hedonism, and happiness as eudaimonia, in line with the distinction drawn in philosophy. One of the most commonly used measures of happiness in the literature is Subjective Wellbeing (SWB) which aligns with the hedonic tradition of happiness. SWB is empirically derived, subjective and defined as the presence of Positive Affect (PA), the absence of Negative Affect (NA), and the level of satisfaction with life (Life Satisfaction; LS). PA is the frequency of positive emotions and is characterised by pleasurable engagement, enthusiasm, activity and alertness, whereas NA, the frequency of negative emotions, is characterised by unpleasurable engagement, anger, contempt, disgust, guilt, fear and nervousness (Watson, Clark & Tellegen, 1988), thus these align with the hedonic tradition of happiness. LS is the cognitive component of SWB, evaluative self-judgements are made based on an individual’s life as a whole (LS), or within particular domains such as health (Diener, Suh, Lucas & Smith, 1999). Conversely, the psychological study of
eudaimonic happiness has focused on what it means, theoretically, to be well functioning. Measures of eudaimonic happiness have therefore necessarily included a broader range of dimensions such as self-acceptance, positive relations (e.g., PWB scale; Ryff, 1989), meaning, and accomplishment (e.g., PERMA scale, Seligman, 2011). The hedonic and eudaimonic traditions are often presented as competing ideas, but they are not mutually exclusive. Although eudaimonic wellbeing can lead to hedonic wellbeing, the reverse is not true. Studies have shown that both hedonic and eudaimonic measures of happiness are moderately correlated with each other (Compton et al., 1996; Huta & Ryan, 2010; Waterman, Schwartz, & Conti, 2008), suggesting that wellbeing, or happiness, may be best considered as multidimensional constructs that include both hedonic and eudaimonic measures of happiness (Ryan & Deci, 2001). Therefore, despite their different perspectives they both have an important and often complementary role to play in the broad area of Positive Psychological enquiry. Ultimately, the research question of interest will dictate which measure of happiness is most appropriate – questions concerning the impact of life events on wellbeing will best employ the measure of hedonistic wellbeing (SWB); similarly, those where wellbeing is one of many outcome variables of interest will best use SWB due to its relative brevity. Conversely, questions concerning the nature of happiness, or of what it means to be psychologically well functioning will necessarily employ measures of eudaimonic wellbeing (e.g., PWB, PERMA) to provide a more detailed picture. This thesis sought to assess whether nutrition influences wellbeing and for this reason SWB was employed throughout.

**Subjective Wellbeing**

Subjective Wellbeing (SWB) is a multidimensional construct (e.g., Davern & Cummins, 2006) that includes both an affective and a cognitive component. It is the
subjective evaluation of current life according to the experience of the individual (e.g., Diener, 1994; Schwartz & Strack, 1999), the central defining feature of this construct is that it is an individual’s self-assessment. Further definition is implied by the measures used. The affective component consists of the presence of positive affect (PA) and the absence of negative affect (NA), whilst the cognitive component relates to cognitive evaluations of life, known as life satisfaction (LS). Despite this agreement on the substantive components of SWB, there is no single agreed upon explanation of how these constructs are related to each other (Busseri, Sadava & Decourville, 2007; Davern & Cummins, 2006; Davern, Cummins & Stokes, 2007; Schmitt & Jüchtern, 2001; Vittersø & Nilsen, 2002; Wiesmann & Hannich, 2008), or to any understanding of their individual relationship to the overall construct of SWB (Davern, Cummins & Stokes, 2007; Vittersø & Nilsen, 2002; Wiesmann & Hannich, 2008) – two critical issues for the valid definition and conceptualisation of SWB (Schmitt & Jüchtern, 2001).

One of the first issues to be considered sought to establish whether PA and NA were reflections of opposite ends of the same continuum, or whether they formed two distinct constructs (Ryff et al., 2006). Russell (1980) proposed that PA and NA were located on a bipolar continuum; however, Watson and Tellegen (1985), in their two factor theory of affect proposed that they were independent constructs. To date, the majority of research has been in support of the latter authors; that is, PA and NA are separate dimensions (Bostic & Ptacek, 2001; Bradburn, 1969; Headey, Holmström & Wearing, 1984; Kahneman, 1999; Ryff, et al., 2006). Reported correlations between PA and NA range between -0.12 and -0.23 (Watson, Clark & Tellegen, 1988). Similar results have been reported more recently based on the relationships between PA and NA as latent constructs. Confirmatory Factor Analysis has indicated that they share 9% of variance
(e.g., $r=-0.297$; Crawford & Henry, 2004). This suggests that they are not entirely independent constructs; moreover, Exploratory Factor Analysis (Brown, Jose, Ng, & Guo, 2002; Diener, Smith, & Fujita, 1995) and Confirmatory Factor Analysis (Crawford & Henry, 2004) have supported the presence of two factors of affect. Further evidence of their separability comes from their differential relationships with factors such as health and personality (Bradburn, 1969; Costa & McCrae, 1980; Diener & Emmons, 1984; Watson & Clark, 1992). Research has additionally established that both PA and NA are distinct from LS (Davern, et al., 2007; Lucas et al., 1996; Ryff, Singer & Love, 2004), though some debate does exist regarding the degree of relationship between each affective component and LS. The assumption that both are similarly related to LS (Davern, et al., 2007; Lucas et al., 1996) has not been unequivocally supported (see Vittersø & Nilsen, 2002).

Given this lack of consensus, further investigation is needed regarding the relationships between PA, NA and LS to facilitate better understanding of their relationship to SWB and to how they form the overall construct of SWB (Davern, et al., 2007; Schmitt & Jüchtern 2001). Four main possibilities to emerge from the literature have been recently identified by Busseri and Sadava (2011): SWB can be conceptualised as the inter-correlations between PA, NA, and LS, as a higher-order construct with PA, NA, and LS as indicators; as an additive composite of PA, NA, and LS; or, as a causal system whereby PA and NA are thought to influence LS. Previous research has been unable to provide conclusive support for any one structural model; thus, whilst a strong general SWB factor is often identified this has been confounded by significant unique variance found in both the affective and cognitive components (Davern, et al., 2007). Provision of an agreed upon structural representation of the relationships among PA, NA,
and LS will facilitate formation of an overall model of SWB. This will enhance both the theoretical and conceptual validity of the construct by eliminating the existing ambiguity associated with its definition and measurement, and by guiding expectations and analyses involving its use.

**Theories of Subjective Wellbeing**

As a construct SWB has primarily been empirically derived, with theories of SWB being retrospectively formulated to account for observed data, rather than being explanatory in nature (Headey, 2006). The dominant theory of SWB claims that individuals have a set-point of SWB which they are unlikely to deviate from (Headey & Wearing, 1992; Lykken & Tellegen, 1996), with several others postulating similar theories under different names. Brickman and Campbell (1971) were the first to propose this in their Adaptation Level (AL) theory; they suggested that any changes to happiness in response to positive or negative life events are followed by a period of adaptation back to that individual’s set-point and were therefore only transitory. This theory is similar to Headey and Wearing’s (1989, 1992) dynamic equilibrium theory that posited stable personality traits maintain an equilibrium or normal level of SWB for each individual and are responsible for returning SWB to this equilibrium following any gains or losses. They extended the theory by incorporating life events; rather than being thought of as purely external, they suggested that they are partly internal, being driven by an individual’s stable personality traits. The Easterlin Paradox (Easterlin, 1974) similarly states that economic growth has no impact on happiness and the Personality Theory of SWB (Costa & McCrae, 1980) was formulated based on the proportion of variance in PA and NA scores accounted for by the personality traits of Extraversion (E) and Neuroticism (N). Multiple Discrepancies Theory (Michalos, 1985) and Homeostatic Theory (Cummins, 1995) are
both more concerned with population-level data, and attempt to explain why most people report that they are satisfied most of the time.

It is increasingly recognised that these adaptation level theories fail to adequately explain all research outcomes, particularly those from longitudinal investigations. Diener, Lucas and Scollon (2006) highlight five key pieces of evidence that suggest that while adaptation occurs, the theory does require major revision. Most evidence suggests that set-points are greater than neutral (e.g., Diener & Diener, 1996), that there are individual differences in the rate and extent of adaptation (e.g., Lucas et al., 2003), and that there are individual differences in set-point both between and within individuals – that is, set-points can vary between individuals (e.g., Diener & Lucas, 1999), and different aspects of SWB can have different set-points for one individual (e.g., Scollon & Diener, 2005). Perhaps the most convincing argument for revising adaptation level theories comes from intervention studies designed to improve wellbeing with a recent meta-analysis (Sin & Lyubomirsky, 2009) reporting an overall significant effect for enhanced wellbeing based on 49 studies that employed Positive Psychology interventions. Diener, Lucas and Scollon (2006) concluded that research may be best focused on attempting to understand the processes that underlie adaptation to life events.

**Individual Differences in Subjective Wellbeing**

Since the early 1960s, research has focused heavily on examining potential correlates of SWB including demographics, contextual factors and personality. Demographics such as gender, age, income, socio-economic status (SES; Bishop, Martin & Poon, 2006; Headey et al., 1984) and education (Ryff et al., 2004) have been found to have consistent but weak associations with SWB. It appears that such demographic factors
are far more likely to negatively affect ill-being when unfavourable, than they are to positively affect wellbeing when favourable (Headey, et al., 1984). Individual contextual factors such as social relationships, meaningful marriage (Headey et al., 1984; Headey, 2007), physical activity (e.g., Fox, 1999) and health (e.g., Bishop et al., 2006; Windle & Woods, 2004) have also demonstrated consistent relationships with SWB, though the direction of causality is not firmly established due to their bi-directional nature (e.g., Feist, Bodner, Jacobs, Miles & Tan, 1995). In 1946 the World Health Organisation (WHO) redefined health to include more than simply the absence of disease or infirmity, instead regarding it as a complete state of mental and social wellbeing (WHO, 1948). This positive change in focus of mental health as including something more than just good health, has been empirically supported (e.g., Abbott, et al., 2008) even in people with terminal health conditions. This focus on a more positive definition of mental health, however, does not imply that we can ignore the fact that health and SWB are two strongly related constructs. Previous research has suggested that although health does not account for all of the variance in SWB, it does account for a significant proportion, particularly among older populations (e.g., Windle & Woods, 2004), where greater variability in health and mobility is apparent. Several studies have concluded that health and perhaps more importantly, the maintenance of mobility, are the most important and the strongest predictors of SWB (Bishop, et al., 2006) in older people. Research by Keyes (2004, 2005) has also established that mentally healthy adults have a lower risk of cardiovascular disease (Keyes, 2004), fewer chronic physical diseases with age (Keyes, 2005a), and lower health care utilisation (Keyes & Grzywacz, 2005). The direction of causality between health and SWB, however, is less certain, with most attempts finding a bi-directional relationship between the two (Feist, Bodner, Jacobs, Miles & Tan, 1995). Further investigations into normative age-related changes in physical health and SWB,
and the relation between the two are important for the understanding of SWB and of the relationship between mental and physical health.

Personality has emerged as the strongest and most reliable predictor of variance in SWB (e.g., Costa & McCrae, 1992). Consistent relationships have been found between SWB and the personality traits of extraversion (E), defined by warmth, gregariousness, assertiveness, activity, excitement seeking and positive emotions (Costa & McCrae, 1992), and neuroticism (N), defined by anxiety, angry hostility, depression, self-consciousness, impulsiveness and vulnerability (Costa & McCrae, 1992). E has been found to predict SWB (Argyle, 1987), and to explain a significant amount of unique variance in PA (Bostic & Ptacek, 2001; Headey, et al., 1984; Headey, 2007; Ryff, et al., 2004). This has been supported by longitudinal research which demonstrated that extraverts reported larger gains in LS over a 20 year period (Headey, 2007). Similarly, N has been found to predict levels of NA (Bostic & Ptacek, 2001; Headey, et al., 1984; Headey, 2007; Ryff, et al., 2004), again supported by longitudinal research showing that those with higher levels of neuroticism demonstrated larger declines in LS (Headey, 2007). This pattern of results has been largely supported, with the conclusion being that E explains more variance in PA and LS, whereas N explains the most variance in NA (Headey, 2007; Headey, Holmström & Wearing, 1985). From such results it has been argued that personality acts as the mediator between external experiences and SWB (Cummins, Gullone & Lau, 2002). Given that personality plays an important role in psychiatric disorders, it would seem essential to further investigate its role in SWB. Although research suggests that the largest proportion of variance in SWB is accounted for by genetically determined traits such as personality, there is still evidence that suggests
considerable variance is accounted for by individual, contextual, and situational factors, therefore plausibly suggesting that SWB can be modified via these variables.

Subjective Wellbeing in Context

In addition to assessing individual differences in SWB others have sought to investigate cross-country comparisons of SWB levels. This evidence is presented here because it constitutes another important use of SWB measurement, therefore highlighting the significance of accurate measurement. Cross-country comparisons of SWB levels were initially motivated by the desire to investigate potentially differential predictors of SWB cross-culturally. More recently, investigation of SWB levels across the world has been motivated by a desire to complement and enhance existing measures of ‘success’, or how well a country is doing. Currently, progress is defined predominantly by Gross Domestic Product (GDP) or Gross National Product (GNP). GDP and GNP are measures of the value of goods and services produced by a country. GDP estimates this value based on goods and services produced within the territorial boundary of a country by both nationals and foreigners, whereas GNP estimates this value based on goods and services produced by its nationals on any land. However, the ultimate goal of collecting these measures is to have some quantitative, objective measure of how well a country is performing. Measures of GDP and GNP measure economic growth well and provide some indication of how successful certain government policies may be. However, they do not measure whether policies are successful in improving the wellbeing of a country’s citizens.

There are several international surveys that contain at least one question on happiness or wellbeing. Figure 1 and 2 display summary data compiled from five
international surveys\(^1\) (World Values Survey, 1999-2009; European Values Study, 1999 [Halman, 2001]; Latinobarómetro 2008; International Social Survey Program [ISSP], 2001, Social Networks II; and ISSP, 2007, Leisure and Sports) that have all included a question on happiness, in the general format: “In general, would you say you are… (1) Very Happy, (2) Quite Happy, (3) Not Very Happy, (4) Not At All Happy. The level of happiness of a country has been reported via a ‘Happiness Index’ which is calculated as the number of people who report being ‘very’ or ‘quite’ happy ([1] or [2]), minus the number who report being ‘not very’ or ‘not at all’ happy ([3] or [4]), plus 100. The index therefore ranges from 0 to 200; happier countries score closer to 200, unhappier countries closer to 0 and countries with an index of 100 have an equal number of people who are happy (responded [1] or [2]) and unhappy (responded [3] or [4]). From this we can see that the world population is, in general, quite happy. As shown in Figure 1, the top 10 happiest countries according to the Happiness Index are Iceland, New Zealand, Norway, Sweden, Ireland, Canada, Singapore, Malaysia, Puerto Rico, and the Netherlands, all with a Happiness Index greater than 188. As shown in Figure 2, Zambia, Armenia, Romania, Latvia, Zimbabwe, Ecuador, Iraq, Moldova, Peru, and Bolivia have the ten lowest Happiness Indices, all under 115, with Peru and Bolivia having a Happiness Index less than 100 which means that more individuals classify themselves as unhappy, than there are individuals who classify themselves as happy.

\(^1\) Aggregate File Producer: ASEP/JDS, Madrid (http://www.jdsurvey.net/jds/jdsurvey.jsp)
Figure 1. Top 50 ranked countries on the Happiness Index.
Figure 2. Bottom 50 ranked countries on the Happiness Index.
Life Satisfaction (LS) has also been assessed cross-nationally. Figure 3 is based on data obtained from the World Views Survey (WVS, 2005-2008), in response to “All things considered, how satisfied are you with your life as a whole these days?” (Q. V22) where responses are coded from (1) dissatisfied, to (10) satisfied. The means have been weighted such that higher values indicate less satisfaction with life. As shown in Figure 3, the 10 countries with the highest rates of LS were identified here as the Netherlands, Spain, Norway, Switzerland, Great Britain, Sweden, Andorra, Canada, the United States, and Italy. Lowest rates of life satisfaction were reported for South Africa, China, Iran, Iraq, the Russian Federation, Zambia, Mali, Ghana, Egypt, and Jordan.
Figure 3. Countries ranked on Life Satisfaction from the World Views Survey.
Comparison of the ranking order of countries on the Happiness Index and Life Satisfaction revealed a few differences. Because only 57 countries were surveyed for the Life Satisfaction Question, we reduced the Happiness Index comparison to those same 57 countries.

The top 20 countries were remarkably similar on both indices, with the Netherlands, Norway, Switzerland, Great Britain, Sweden, Canada, the United States, Finland, Japan, Thailand, Australia, and France appearing in both. For the remaining sixteen countries that did not appear in the top 20 for both scales, the largest differences of more than 20 ranking places was for Indonesia which was ranked 10 for Happiness, but in 36\textsuperscript{th} position for Life Satisfaction, and for Morocco and Uruguay who conversely ranked much higher on Life Satisfaction (13\textsuperscript{th} and 18\textsuperscript{th} position) than on Happiness (33\textsuperscript{rd} and 39\textsuperscript{th} position); Uruguay was the only country to appear in the top 20 for Life Satisfaction, and the bottom 20 for Happiness. Comparison of the similarities between the bottom 20 countries in both scales revealed several differences. Countries that appeared in the bottom 20 on both measures were Iraq, Russia, Zambia, India, Peru, Georgia, Moldova, Bulgaria, Ukraine, Romania, China, South Africa and Ghana. For the remaining fourteen countries that did not appear in the bottom 20 for both scales five had a difference of more than 20 ranks; Jordan, Egypt, Mali, Ethiopia and Uruguay.

**Australia in Context**

Australia fares well in international surveys of happiness and life satisfaction, placing 16\textsuperscript{th} in terms of Life Satisfaction, just under Thailand, but just above New Zealand. Including all 103 surveyed countries for the Happiness Index, Australia ranks 24\textsuperscript{th}, just below Nigeria, and just above Saudi Arabia, the estimated Happiness Index of
183.6 indicating that rates of people who identify as very or quite happy, are considerably higher than those who respond as not very, or not at all happy.

National Surveys in Australia confirm these results; on average, Australians seem to evaluate their life closer to satisfactory than unsatisfactory. Figure 4 displays the results from the 26th Australian Unity Wellbeing Survey, conducted in September 2011 (Cummins et al., 2011). Participants were 2000 Australians from a sample that is demographically equivalent to the Australian population. The Personal Wellbeing Index (Cummins et al., 2011), the National Wellbeing Index, and a question regarding perceptions of likelihood of a terrorist attack were included in each survey. Here we focus on one question that asks “Thinking about your own life and personal circumstances, how satisfied are you with your life as a whole?”, responses range from 0 to 10, and are then scaled to achieve a range of possible scores from 0 (completely dissatisfied) to 100 (completely satisfied). Normative data suggest that scores generally range from between 70 to 80 (e.g., Cummins, 2003). Figure 4 shows the data from 2011, by age and for the total sample. Mean levels of satisfaction with life as a whole are highest for the youngest (18-25 years), and oldest (>66 years) participants. Mean levels that are below the mean of the total are for the age brackets from 26 to 65 years; the range of scores for each age range are remarkably similar for all age groups above 36 years (range from $SD=15.91$ for 66-75 to $SD=17.68$ for 46-55), and are similar to the variability in the total sample mean ($SD=17.41$). The youngest age group were the most uniform ($SD=13.52$), and the second youngest age group were the least uniform ($SD=20.27$).
Figure 4. Life Satisfaction of Australian adults by age groups and the total sample from the 26th Australian Unity Wellbeing Survey.

Population mean SWB data is useful for cross-country comparisons, and allows us to assess macro-level correlates of SWB. Variables that have been found to have an influence at this level include direct democracy (Frey & Stutzer, 2000), ideological complexion (Radcliff, 2001), welfare state (Radcliff, 2001), political and private freedom (Veenhoven, 2000), climate change (Rehdanz & Maddison, 2005), inflation, and the effects of unemployment on the economy (Becchetti, Stefano & Giuntella, 2006). This information was reviewed here because it highlights the increased use of SWB data. Not only is it increasingly employed as an important outcome variable across a range of research disciplines but it is also employed as a measure of national success along with more traditional measures such as GDP and GNP.
Conclusion

This section has provided a general background to the study of mental health going beyond mental illness and has highlighted the historical influences on the broad study of happiness. Following this we provided a background of the scientific study of Subjective Wellbeing (SWB), including its measurement, issues related to its measurement, and some of the theories that have guided research in this area. This section concluded with a discussion of the individual, and the cross-national differences in SWB in order to explicitly highlight the growing use of, and therefore importance of the measurement of, SWB. In addition to providing a more holistic measure by which interventions can be assessed, SWB can also be employed to assess the impact of national policies. For these outcomes to be fully realised, we need an accurate measurement model of SWB, with both empirical and theoretical support.
Chapter 1.2 Nutrition

One of the biggest tragedies of human civilization is the precedence of chemical therapy over nutrition. It's a substitution of artificial therapy over nature, of poisons over food, in which we are feeding people poisons trying to correct the reactions of starvation.

(R. Lee, 1951)

Nutrition is an important behavioural determinant of human health that provides primary prevention for a range of chronic non-communicable diseases. Cardiovascular diseases, certain cancers, type 2 diabetes, hypertension, metabolic syndrome, musculoskeletal conditions, respiratory conditions, rheumatoid arthritis, chronic obstructive pulmonary diseases, depression and other mental health disorders can all be influenced by nutrition (see e.g., Boeing et al., 2012) either directly, or via overweight or obesity. Overweight and obesity estimates for Australia provide a proxy for how poor nutrition affects us nationally. In 2007/08 it was estimated that 61% of the Australian population were overweight or obese (AIHW, 2010). High body mass contributed to 7.5% of the total burden of disease in 2003 (Begg et al., 2007) and estimates place the direct cost of overweight and obesity for Australia in 2005 at $21billion per year (Colagiuri et al., 2010). Trends suggest this is to get worse: Adoption of good nutrition is therefore of significant public health interest.
Nutrition and Aging

Nutrition is particularly important to maintain in later life. Energy intake requirements decline with age due to reduced physical activity and due to decreases in fat-free mass (by 15% between 30 and 80 years; NHMRC, 2013) which results in a lowered metabolic rate (Glick, 1990). This can result in reduced dietary intakes with aging (Morley, 2001), which can lead to malnutrition through elimination of food groups. Other biological factors such as gastrointestinal diseases, maldigestion and malabsorption of nutrients can also lead to poor nutritional status in older age (Pirlich & Lochs, 2001). Physical and social factors associated with aging can also result in malnutrition such as poor dentition, dementia, social isolation and poverty (Morley, 1997). Therefore, maintenance of good nutrition is of particular importance for older adults.

What is Good Nutrition?

Food-Based Dietary Guidelines have been developed by reference to both population based dietary patterns and to health, clinical and experimental research so as to provide practical advice to enable individuals to achieve good nutrition. Quantification of good nutrition is provided by two main sources in Australia: the Nutrient Reference Values for Australia and New Zealand (NHMRC, 2006) which provides specific nutrient levels required to prevent chronic illness, and the Australian Dietary Guidelines (NHMRC, 2013) which include practical food type based information. The Australian Dietary Guidelines (NHMRC, 2013, pV) include five key guidelines that Australians should follow for good nutrition. These are:
**Guideline 1:** To achieve and maintain a healthy weight, be physically active and choose amounts of nutritious food and drinks to meet your energy needs.

**Guideline 2:** Enjoy a wide variety of nutritious foods from these five food groups every day:

1. Plenty of vegetables of different types and colours, and legumes/beans
2. Fruit
3. Grain (cereal) foods, mostly wholegrain and/or high cereal fibre varieties, such as breads, cereals, rice, pasta, noodles, polenta, couscous, oats, quinoa and barley
4. Lean meats and poultry, fish, eggs, tofu, nuts and seeds, and legumes/beans
5. Milk, yoghurt, cheese and/or their alternatives, mostly reduced fat

And drink plenty of water.

**Guideline 3:** Limit intake of food containing saturated fat, added salt, added sugars and alcohol.

**Guideline 4:** Encourage, support and promote breastfeeding.

**Guideline 5:** Care for your food; prepare and store it safely.

There are also State-specific programmes aimed at improving healthy living in general, such as the *Eat Well Be Active* Strategy for South Australia 2011-2016 (South Australia, Department of Health, Public Health and Clinical Systems Division, 2011). These guidelines are all aimed at providing practical advice in order to achieve the Recommended Dietary Intakes (RDIs) for all nutrients. The Nutrient Reference Values for Australia: Executive Summary (2005) provides information regarding optimal amounts of nutrients required for avoidance of nutritional deficiency states.
Good nutrition is essential because it delivers the specific dietary factors essential for optimal functioning. Our focus here was on assessment of folate and vitamin B\textsubscript{12} within a previously well-detailed biological pathway between folate, vitamin B\textsubscript{12} and the amino-acid homocysteine. Secondly, we sought to assess the impact of \textit{n}-3 long-chain polyunsaturated fatty-acids (\textit{n}-3 LC PUFAs) on positive mental health, given the recent mixed evidence regarding their role in depressed mood (e.g., Antypa et al., 2012; Makrides et al., 2010). What follows is a brief discussion regarding current guidelines (NHMRC, 2006) for folate, vitamin B\textsubscript{12}, and \textit{n}-3 LC PUFA consumption in Australia.

**Folate**

Folate is a water-soluble B-group vitamin essential for the synthesis, methylation, and repair of DNA. Natural folates, found in foods such as leafy green vegetables and meat organs, are approximately 50\% less bioactive than its synthetic form, folic acid which is more stable in foods and better able to be absorbed. Dietary recommendations for folate in Australia are the same for men and women over 19 years: the Estimated Average Requirement\textsuperscript{2} (EAR) is 320µg/day and the Recommended Dietary Intake\textsuperscript{3} (RDI) is 400µg/day (Nutrient Reference Values for Australia and New Zealand; NHMRC, 2006). Individuals are classified as being at risk for folate deficiency with values less than 5nmol/L. Folate deficiency is marked by macrocytic anaemia and has been associated with atherosclerosis (Robinson et al., 1998), and some cancers, though whether folate is protective of, or promotes certain cancers is still unclear (Kim, 2004). Folate deficiency can also lead to neural tube birth defects (Fairfield & Fletcher, 2002); evidence regarding high deficiency in the population and neural tube birth defects led to folate fortification

\textsuperscript{2} Estimated daily nutrient levels to meet the requirements of half the healthy individuals in a particular life stage and gender group
\textsuperscript{3} Average daily dietary intake to meet the requirements of 97-98\% of healthy individuals in a particular life stage and gender group
programmes in many countries including the US (in 1998), Canada (in 1998), Chile (in 2000) and Australia (in 2009). Mandatory folic acid fortification of wheat flour used in bread-making was introduced in Australia in September 2009 (Australia New Zealand Food Standards Code, Standard 2.1.1 Clause 4(2), 2009) following voluntary fortification since 1995. A recent study (Brown et al., 2011) found that folic acid fortification significantly reduced prevalence of folate deficiency in Australia; low serum folate was reduced by 77% and RBC folate by 85% between April 2009 and April 2010 (Brown et al., 2011).

**Vitamin B\textsubscript{12}**

Vitamin B\textsubscript{12}, also known as cobalamin, is essential for normal functioning of the brain and nervous system; it is involved in the myelination of nerves and synthesis of nucleic acids. Vitamin B\textsubscript{12} is found in shellfish, meat, fermented cheeses, other dairy products, and egg yolks. Dietary recommendations for Vitamin B\textsubscript{12} in Australia are the same for men and women over 19 years: the Estimated Average Requirement (EAR) is 2.0µg/day and the RDI is 2.4µg/day (Nutrient Reference Values for Australia and New Zealand; NHMRC, 2006). Individuals with vitamin B12 less than 150pmol/L can be classified as at risk for deficiency, and less than 120pmol/L as deficient.

**Polyunsaturated Fatty Acids**

Polyunsaturated Fatty Acids (PUFAs) are essential for normal development and brain functioning across the lifespan (Arterburn, Hall & Oken, 2006; Simopoulos, 1991). The two types of PUFAs – omega-6 (\textit{n}-6) and omega-3 (\textit{n}-3), are both essential fatty acids – they must be obtained through dietary sources as they cannot be produced by the body.
These PUFAs should be consumed in equal parts; however, Western diets are generally deficient in $n$-3 PUFAs and contain excess amounts of $n$-6 PUFAs (Simopoulos, 1991). A recent estimate indicated that Australian adults consume these PUFAs in a ratio of 8:1 (Meyer et al., 2003), though other estimates have suggested it may be as high as 20:1 (Simopoulos, 1991; 2002). This is problematic because the parent $n$-6 PUFA – linoleic acid (LA), and the parent $n$-3 PUFA – α-linolenic acid (ALA), compete for conversion into their longer-chain equivalents. ALA, found in vegetable oils such as canola and soybean, can metabolise into the longer-chain (LC) $n$-3 PUFAs: eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and docosapentaenoic acid (DPA). However, the \textit{in vivo} conversion is poor; in men ≤8% of ALA is converted to EPA (Burdge, Jones & Wootton, 2002), and ≤4% to DHA (Burdge et al., 2002; Emken, Adlof & Gulley, 1994), and in women ≤21% to EPA and ≤9% to DHA (Burdge & Wootton, 2002). Direct dietary sources of EPA and DHA such as fatty fish are therefore required. The dietary recommendations for $n$-3 LC PUFAs in Australia are 160mg/day for adult males (>19 years) and 90mg/day for adult females (>19 years) (Nutrient Reference Values for Australia and New Zealand; NHMRC, 2006). These values are based on Adequate Intakes, and it has been recommended that a minimum of 500mg/day of EPA and DHA should be consumed for cardiovascular health (International Society for the Study of Fatty Acids and Lipids, 2004). Dietary data from 10,851 Australian adults collected in 1995-6 reported average $n$-3 LC PUFAs consumption of 189mg/day (Meyer et al., 2003), with 220mg/day for adult men, and 162mg/day for adult women (Meyer et al., 2003). This exceeds the Australian Dietary Recommendations based on Adequate Intakes, but is less than half of the recommended intake for cardiovascular health (ISSFAL, 2004). Increased dietary consumption of $n$-3 LC PUFAs is essential due to their implication in numerous health outcomes such as arthritis (e.g., Goldberg & Katz, 2007), Alzheimer’s disease (e.g.,
Söderberg et al., 1991), dementia (e.g., Kalmijn et al., 1997) CVD (e.g., Albert et al., 2002) and depression (e.g., Tanskanen et al., 2001). This can be achieved through greater consumption of fatty fish such as salmon and mackerel, or through n-3 LC PUFA supplements.
Chapter 1.3 Nutrition and Mental Health

If we could give every individual the right amount of nourishment and exercise, not too little and not too much, we would have found the safest way to health.

(Hippocrates c. 460 BC – c. 370 BC)

Traditionally, Western investigations of physical and mental illness have operated within the biomedical model of medicine. The premise of this model is that all deviations from normative functioning (physical and mental) can be traced to an organic cause such as a pathogen, an injury, or a genetic or developmental abnormality. Although still a dominant view, it is increasingly recognised that other factors can impact a disease process; whilst disease processes may have an organic, biological cause, they can be exacerbated by psychological, environmental, and social factors. Health psychology has formalised this view with the biopsychosocial model of health, which emphasises the interaction between biological, psychological, and social processes as integral to physical health and illness (Suls & Rothman, 2004). Health can be seen as a complex interaction of these factors: Broadening research beyond purely biological explanations of health thus provides a greater understanding for public health prevention and treatment (Lowenberg & Davis, 1994). Nutrition is one environmental factor that has been implicated in the aetiology of many physical and mental disease processes. It can offer a holistic approach to prevention and treatment, is easily modifiable, and, in principle, available to all. Nutrition is well established as an important determinant of physical health (WHO, 2002) due to its association with many non-communicable diseases that have a large public
health burden such as obesity, diabetes, cardiovascular diseases, cancers, osteoporosis, and dental diseases (WHO, 2003). Research has also suggested that adequate overall nutrition plays an important role in the physical health outcomes of hospitalised patient populations. For example, six week treatment with a nutritional supplement that provided 995 kcal of energy and 100% of the Reference Nutrient Intakes for vitamins and minerals for a healthy older person significantly reduced non-elective readmissions in patients with acute illness (Gariballa et al., 2006). This idea has been supported by a meta-analysis which concluded that complications and mortality were reduced with supplementation of undernourished patients in short term care hospitals, though no benefit was found for supplement use at home (Milne, Avenell & Potter, 2006).

Evidence is growing, but is less well established, regarding the role that nutrition may play in mental health. Evidence regarding the associations between dietary patterns and mental health has been promising. A growing body of research suggests mental health benefits associated with the Mediterranean diet (Sanchez-Villegas et al., 2009), traditional Japanese diets (Nanri et al., 2010), healthy dietary patterns (Jacka et al., 2010; Kuczmarski et al., 2010; Chatzi et al., 2011; Tangney et al., 2002), and consumption of fruits and vegetables (Konttinen et al., 2010; Rooney et al., 2012; Sarlio-Lahteenkorva, Lahelma & Roos, 2004). However, evidence remains equivocal, with others reporting no relationships (Okubo et al., 2011; Samieri et al., 2008). A recent systematic review (Quirk et al., 2013) suggested that there is limited and conflicting evidence to support associations between dietary patterns and depression. Furthermore, interpretation is often confounded by the presence of bi-directional causality: depression and psychotropic medications can affect appetite and therefore weight (e.g., Fernstrom, Krowinski, & Kupfer, 1981); and alcohol, caffeine and consumption of certain foods can directly affect
mood. Others have sought to assess the role of nutrition in the mental health outcomes of hospitalised patients. It has been shown that Quality of Life (QoL), which encompasses functional status, emotional wellbeing, social wellbeing and general health, is lower in malnourished patients (Norman et al., 2006) and nutritional risk has been associated with Health Related QoL in frail older people (Keller, 2004), and with global QoL in severely malnourished patients (Laws, Tapsell & Kelly, 2000). Furthermore, a randomised, double-blind, placebo controlled trial reported that six month treatment with nutritional supplements in acutely ill, hospitalised older adults resulted in a significant difference in the physical functioning and in the physical and social role functioning domains of the SF-36, though not in the mental health domain (Gariballa & Forster, 2007). However, others have reported no effect of a nutritional intervention on SF-36 scores in patients at risk of poor nutrition (Johansen et al., 2004).

These results are potentially confounded by many other factors in patient populations such as oral and dentition complications (e.g., Van Lancker et al., 2012). However, this relationship has also been observed in physically healthy participants, suggesting that the observed relationships are not solely due to factors secondary to poor physical health. Clinical trials have reported some benefit of multivitamins on mood in children: multivitamin treatment resulted in significant improvement in mood and anxiety in children (8-15 years) with mood and behavioural problems after 13.6 weeks in a small ($n=11$), open-label trial (Kaplan et al., 2004), and reduced anti-social behaviour in school children (6-12 years; $n=80$) following four month low-dose vitamin-mineral supplement compared to placebo (Schoenthaler & Bier, 2000). Clinical trials have also reported improvements in the antisocial behaviour of incarcerated young adults; multivitamin treatment significantly reduced the number of offences committed by incarcerated young
adult prisoners by 26.3% (Gesch et al., 2002) and 34% (Zaalberg et al., 2010), both significantly different from placebo.

In the following we review the evidence for folate, vitamin B\textsubscript{12} and omega-3 Polyunsaturated Fatty Acids ($n$-3 PUFAs) for depressed mood and mental health. This chapter is divided into two sections; the first section reviews folate and vitamin B\textsubscript{12} for depressed mood and mental health. We begin this section with a discussion of the evidence regarding the role of multivitamins for mood in healthy adults, to establish that vitamin supplementation can have an impact on mood in healthy adults. Following this we review evidence for folate, vitamin B\textsubscript{12} and homocysteine for mood, this includes discussion of prevalence rates, associational research and intervention trials, and concludes with mechanistic accounts proposed to explain this link. The second section reviews the evidence regarding the role of $n$-3 PUFAs for mood including discussion of prevalence rates, associational research and intervention trials, and again concluding with the mechanistic accounts proposed to explain this link.

**Multivitamin Supplementation for mood in Healthy Adults**

Clinical trials have sought to assess the efficacy of multivitamin supplementation in otherwise healthy adults. This has been partially motivated by the increased adoption of multivitamins and minerals by the general population. For example; a large ($N=3027$) South Australian study found that 36.4% of respondents had used non-prescribed vitamins in the past year (MacLennan, Wilson & Taylor, 2002), similar to data from the US National Health and Nutrition Examination Survey that found 35% of adults reported using multivitamin-multimineral supplements in the previous month (Radimer et al., 2004). Thirteen randomised, double-blind, placebo controlled intervention trials were
identified here that assessed the efficacy of vitamins/mineral supplements in non-clinical populations (see Table 1); of these only one reported a treatment effect for all measures – anxiety (HARS), general psychological wellbeing (PGWB), and stress (VAS & BSI; Schlebusch et al., 2000). This trial compared supplementation with a B-vitamin (Berocca Calmag®) which included vitamin B₁₂ but not folic acid, compared to placebo in healthy adults with high stress levels. A further four studies (Carroll et al., 2000; Harris et al., 2011; Kennedy et al., 2010; Long & Benton, 2013) reported a significant treatment effect on a total scale score – all for stress, measured with both the Perceived Stress Scale (PSS; Carroll et al., 2000; Kennedy et al., 2010; Long & Benton, 2013) and the Depression Anxiety and Stress Scale (DASS; Harris et al., 2011). These trials were conducted with healthy adult males who received supplements including both vitamin B₁₂ and folic acid in a multivitamin/mineral supplement. Taken together, these trials suggest a potential role for multivitamins in reducing stress.
Table 1

Randomised, double-blind, placebo-controlled trials assessing efficacy of multivitamin/mineral supplementation on mood outcomes in healthy adults.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Year</th>
<th>N</th>
<th>Trial</th>
<th>Participants</th>
<th>Study Length</th>
<th>Mood Measures</th>
<th>Treatment (µg)</th>
<th>Results</th>
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</thead>
<tbody>
<tr>
<td>Carroll et al.</td>
<td>2000</td>
<td>80</td>
<td>Randomised; double-blind; placebo-</td>
<td>Healthy male students 18-42 years</td>
<td>28d</td>
<td>GHQ-28; HADS; PSS</td>
<td>Berocca® (B₁₂ 10µg; FA 400µg)</td>
<td>Total GHQ, somatic symptoms (GHQ) improved in treatment, but no main treatment effect compared to placebo</td>
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<td></td>
<td></td>
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<td>controlled</td>
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<td>Both groups improved on depression scores (HADS), but no significant treatment effect compared to placebo</td>
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<td>Significant effect of treatment on insomnia (GHQ), anxiety (HADS), &amp; PSS compared to placebo</td>
</tr>
<tr>
<td>Schlebusch et al.</td>
<td>2000</td>
<td>300</td>
<td>Randomised; double-blind; placebo-</td>
<td>Healthy adults with high stress, 18-65 years, 68% female</td>
<td>30d</td>
<td>HARS; PGWB; VAS</td>
<td>Berocca Calmag® (B₁₂ 10µg; FA 0)</td>
<td>Significant improvements in both groups (HARS, PGWS, VAS, BSI)</td>
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<td></td>
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<td></td>
<td>controlled</td>
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<td>(stress); BSI</td>
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<td>Treatment group significantly greater improvement (HARS, PGWS, VAS, BSI) compared to placebo</td>
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<tr>
<td>Authors</td>
<td>Year</td>
<td>N</td>
<td>Trial</td>
<td>Participants</td>
<td>Study Length</td>
<td>Mood Measures</td>
<td>Treatment (µg)</td>
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<tr>
<td>Ussher &amp; Swann</td>
<td>2000</td>
<td>313</td>
<td>Randomised; double-blind;</td>
<td>Healthy adults 35-65 years; 55% female</td>
<td>56d</td>
<td>MMSQ; POMS;</td>
<td>Multivitamin/mineral/trace element capsule (B_{12} 1.0µg; FA 0)</td>
<td>Significantly greater change in confusion-bewilderment (POMS) &amp; behavioural strain (MMSQ) for treatment compared to placebo. No significant difference between groups in total POMS or SF-36. Subgroup analysis of highly restrained eaters found significantly greater improvement in tension-anxiety (POMS), confusion-bewilderment (POMS), total mood disturbance (POMS), behavioural strain (MMSQ), cognitive strain (MMSQ), total strain (MMSQ), mental health (SF-36) in treatment compared to placebo.</td>
</tr>
<tr>
<td>Bryan, Calvaresi &amp; Hughes</td>
<td>2002</td>
<td>211</td>
<td>Randomised; double-blind;</td>
<td>Healthy females; three age groups (20-30; 45-55; 65-92 years)</td>
<td>35d</td>
<td>CES-D; POMS</td>
<td>Three interventions (FA 750µg; B_{12} 15µg; B_{6} 75mg)</td>
<td>No significant treatment effects on CES-D or POMS. Significant improvements were found in all groups on CES-D, vigour/activity (POMS), fatigue/inertia (POMS), &amp; confusion/bewilderment (POMS).</td>
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<td>Authors</td>
<td>Year</td>
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<td>Trial</td>
<td>Participants</td>
<td>Study Length</td>
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<tr>
<td>Hvas et al., 2003</td>
<td>140</td>
<td></td>
<td>Randomised; double-blind; placebo-controlled</td>
<td>Healthy adults with modestly elevated plasma methylmalonic acid; median age=70 years; 70% female</td>
<td>28d</td>
<td>SF-36</td>
<td>Vitamin B₁₂ (1000µg) injection weekly</td>
<td>Significant improvement for the general health dimension of the SF-36 compared to placebo (non-significant for seven remaining dimensions)</td>
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<tr>
<td>Williams et al. 2005</td>
<td>23</td>
<td></td>
<td>Randomised; double-blind; placebo-controlled</td>
<td>Healthy males; 21-39 years</td>
<td>84d</td>
<td>PANAS</td>
<td>FA Supplementation (100µg/d d1-42; 200µg/d d43-84)</td>
<td>No differences in PANAS between groups post-intervention</td>
</tr>
<tr>
<td>Gosney et al. 2008</td>
<td>73</td>
<td></td>
<td>Randomised; double-blind; placebo-controlled</td>
<td>Frail elderly nursing home residents; &gt;60 years; no info on gender provided</td>
<td>56d</td>
<td>HADS; MADRS</td>
<td>Micronutrient supplement (B₁₂ 200µg; FA 600µg)</td>
<td>No statistically significant treatment effect for HADS or MADRS</td>
</tr>
<tr>
<td>Authors</td>
<td>Year</td>
<td>N</td>
<td>Trial</td>
<td>Participants</td>
<td>Study Length</td>
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<td>Treatment (µg)</td>
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<tr>
<td>Haskell et al.</td>
<td>2010</td>
<td>216</td>
<td>Randomised; double-blind; placebo-controlled</td>
<td>Healthy females; 25-50 years</td>
<td>62d</td>
<td>POMS; SF-36; CFS; STAI; BLMS</td>
<td>Supradyn® (B₁₂ 3µg; FA 600µg)</td>
<td>No treatment effects on SF-36, CFS, POMS, BLMS</td>
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<td></td>
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<td></td>
<td>Treatment group significantly reduced physical tiredness (STAI) associated with a multi-tasking battery compared to placebo</td>
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<tr>
<td>Kennedy et al.</td>
<td>2010</td>
<td>215</td>
<td>Randomised; double-blind; placebo-controlled</td>
<td>Healthy males; 30-55 years</td>
<td>33d</td>
<td>GHQ-12; PSS; POMS; BLMS; VAS (energy)</td>
<td>Berocca® (B₁₂ 10µ; FA 400µg)</td>
<td>Significant improvement in PSS, GHQ, vigour (POMS) &amp; energy (VAS) compared to placebo</td>
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<td></td>
<td>Treatment group demonstrated increased alertness (BLMS), concentration (VAS), mental stamina (VAS) &amp; physical stamina (VAS) compared to placebo</td>
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<tr>
<td>Harris et al.</td>
<td>2011</td>
<td>50</td>
<td>Randomised, double-blind, placebo-controlled</td>
<td>Healthy males; 50-69 years</td>
<td>56d</td>
<td>GHQ-60; DASS; PSS; POMS; VAMS</td>
<td>Swisse Men’s Utivite® (B₁₂ 30µg; FA 500µg)</td>
<td>Significant improvement in total DASS, alertness (VAMS) &amp; GHQ compared to placebo</td>
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<td></td>
<td>Treatment group demonstrated increased alertness (BLMS), concentration (VAS), mental stamina (VAS) &amp; physical stamina (VAS) compared to placebo</td>
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<tr>
<td>Kennedy et al.</td>
<td>2011</td>
<td>198</td>
<td>Randomised; double-blind; placebo-controlled</td>
<td>Healthy males; 30-55 years</td>
<td>33d</td>
<td>BLMS; VAS (state/energy)</td>
<td>Berocca® (B₁₂ 10µg; FA 400µg)</td>
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<td>Authors</td>
<td>Year</td>
<td>N</td>
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<tr>
<td>Stough et al.</td>
<td>2011</td>
<td>60</td>
<td>Randomised; double-blind; placebo-controlled</td>
<td>Healthy adults</td>
<td>84d</td>
<td>STAI; POMS; PSQ</td>
<td>1. Executive B-active sustained release (Both: B&lt;sub&gt;12&lt;/sub&gt; 30µg; FA 150µg)</td>
<td>Both treatments equally effective in personal strain (PSQ), but not significantly different from placebo. No effect of treatment on STAI. Treatment group showed significant reduction in depression-dejection &amp; confusion (POMS) across time compared to placebo.</td>
</tr>
<tr>
<td>Long &amp; Benton</td>
<td>2013</td>
<td>173</td>
<td>Randomised; double-blind; placebo-controlled</td>
<td>Healthy male adults</td>
<td>84d</td>
<td>PFT; BPAQ; PSS; GSIP; TIME; SKIP</td>
<td>1. Centrum Advance 50+® (B&lt;sub&gt;12&lt;/sub&gt; 2.5µg; FA 200µg) 2. DHA 3. Centrum + DHA</td>
<td>No effect of treatment with Centrum Advance 50+® alone compared to placebo for measures of frustration (PFT), aggression (BPAQ), and impulsivity (GSIP, TIME, SKIP). Treatment group demonstrated decreases in stress (PSS) compared to placebo.</td>
</tr>
</tbody>
</table>

*BLMS, Bond-Lader Mood Scales; BPAQ, Buss-Perry Aggression Questionnaire; BSI, Berocca Stress Index; CES-D, Centre for Epidemiological Studies Depression Scale; CFS, Chalder Fatigue Scale; DASS, Depression Anxiety and Stress Scale; FA, Folic Acid; GHQ, General Health Questionnaire; GSIP, GoStop Impulsivity Paradigm; HADS, Hospital Anxiety and Depression Scale; HARS, Hamilton Anxiety Rating Scale; MADRS, Montgomery-Åsberg Depression Rating Scale; MMSQ, Multi-Modal Strain Questionnaire; PANAS, Positive and Negative Affect Schedule; PFT,*
Picture-Frustration Test; PGWB, Psychological General Well-Being Schedule; POMS, Profile of Mood States; PSQ, Personal Strain Questionnaire; PSS, Perceived Stress Scale; SF-36, Short-Form 36-Item Health Survey; SKIP, Single Key Impulsivity Paradigm; STAI, State-Trait Anxiety Inventory; μg, micrograms; VAMS, Visual Analogue Mood Scale; VAS, Visual Analogue Scales.
Three studies reported no treatment effect when healthy adults were supplemented with either folic acid or vitamin B₁₂ (Bryan, Calvaresi & Hughes, 2002), with folic acid alone (Williams et al., 2005), or with a micronutrient supplement containing both vitamin B₁₂ and folic acid (Gosney et al., 2008). The remaining five studies (Haskell et al., 2010; Hvas et al., 2003; Kennedy et al., 2011; Stough et al., 2011; Ussher & Swann, 2000) all employed a multivitamin as treatment compared to placebo and reported some benefit of treatment over placebo on at least one measured subscale. No consistent differences were noted in regard to composition of vitamin B₁₂ and/or folic acid, length of treatment, or participant type; thus suggesting that these factors did not influence results. Vitamin B₁₂ ranged from 2.5-30µg/d, and folic acid from 0-500µg/d in the five trials that reported a treatment effect for at least one total scale; from 1.0-30µg/d vitamin B₁₂, and 0-600µg/d folic acid, with one study administering 1000µg vitamin B₁₂ alone per week, for those trials that reported some effect on a subscale, and from 0-200µg/d vitamin B₁₂ and 0-750µg/d folic acid in trials that found no treatment effect. Although there appears to be little difference in folic acid and vitamin B₁₂ composition, each multivitamin contained varied compositions of additional vitamins, minerals and even trace elements. Therefore, conclusions for active treatment ingredient cannot be made, though a recent meta-analysis reported a general tendency for trials with higher B vitamin doses to produce the largest effect sizes on mood outcomes (Long & Benton, 2013).

Comparisons of study length revealed no differences; all studies ranged from 28 to 84 days; with those that found no effect ranging from 35 to 84, those that found an effect on at least one total score ranging 28 to 84, and those that reported some effect on a subscale ranging from 28 to 84 days. Additionally, all trials were conducted with healthy adults; two minor deviations being healthy frail elderly where no treatment effect was
found (Gosney et al., 2008), and healthy adults with high stress where a treatment effect was reported (Schlebusch et al., 2000).

Analysis by constructs measured rather than scales employed revealed more consistent results. For mood, none of the four studies that employed the Bond-Lader Mood Scale (BLMS) reported an overall treatment effect for the total scale (Harris et al., 2011; Haskell et al., 2010; Kennedy et al., 2010; Kennedy et al., 2011), though two did find an effect for the subscale of alertness (Harris et al., 2011; Kennedy et al., 2011). Similar results were reported when mood was measured using the POMS. None of the six studies that employed the POMS (Bryan, Calvaresi & Hughes, 2002; Harris et al., 2011; Haskell et al., 2010; Kennedy et al., 2010; Stough et al., 2011; Ussher & Swann, 2000) found an overall treatment effect for the total scale; though it did approach significance ($p=0.054$) in one (Kennedy et al., 2010). Three trials reported a treatment effect for the POMS subscales of confusion-bewilderment (Stough et al., 2011; Ussher & Swann, 2000), vigour-activity (Kennedy et al., 2010) and depression-dejection (Stough et al., 2011). These results are partially supported by a recent meta-analysis by Long and Benton (2013) which analysed the pooled results from eight RCTs on multivitamin supplementation in healthy adults (all of which were included here). They reported significant treatment effects based on pooled samples for self-reported mood ratings of clear-headed–confused ($n=776$; SMD=0.23; 95%CI=0.38–0.07, $p<0.003$), energetic–tired ($n=992$; SMD=0.27; 95%CI=0.40–0.15, $p<0.001$), and elated–depressed ($n=776$; SMD=0.26; 95%CI=0.40–0.12, $p<0.001$). Further they reported a significant treatment effect for agreeable–hostile ($n=498$; SMD=0.23; 95%CI=0.41–0.05, $p<0.011$), but not for composed–anxious ($n=992$; SMD=0.05; 95%CI=0.15–0.14, $p=0.718$). Results suggest little potentiation of overall mood by multivitamin/mineral supplementation in healthy
adults when measured with the POMS or the BLMS. Where treatment effects are observed, they appear confined to specific aspects of mood, with most evidence for improvements in confusion, vigour and depressed mood.

Of four trials that measured the syndrome of depression, rather than depressed mood as with the POMS, only one reported a treatment effect (DASS, Harris et al., 2011) compared to three that did not (Center for Epidemiological Studies Depression Scale [CES-D], Bryan, Calvaresi & Hughes, 2002; HADS, Carroll et al., 2000; HADS & the Montgomery Åsberg Depression Scale [MADRS], Gosney et al., 2008). The DASS measures symptoms associated with dysphoric mood (Lovibond & Lovibond, 1995), the HADS measures anhedonic state (Zigmond & Snaith, 1983), and the CES-D is a measure of depressed affect, somatic symptoms, wellbeing and interpersonal relations (Radloff, 1977). This result is supported by the meta-analysis: no significant improvement was found in depression due to multivitamin supplementation in aggregated data from 303 participants (SMD=0.12; 95%CI 0.42–0.03, p<0.089; Long & Benton, 2013). Taken together, current evidence based on RCTs therefore suggests little potentiation of subclinical depression in healthy adults with multivitamin supplementation compared to placebo.

Results were mixed for anxiety, three reported a positive treatment effect (Carroll et al., 2000; Harris et al., 2011; Schlebusch et al., 2000) based on the HADS, HARS, and DASS, respectively, and two reported no effect (Gosney et al., 2008; Stough et al., 2011) based on the HADS, and the STAI. Long and Benton (2013), however, reported an overall benefit on anxiety based on a pooled sample of 603 participants (SMD=0.32;
95%CI=0.48–0.16, \( p<0.001 \)). More consistent results were found when looking at the constructs of general health and stress. Three of four studies that measured general mental health reported a significant treatment effect based on subscales from the SF-36 (Hvas et al., 2003) and the GHQ (Harris et al., 2011; Kennedy et al., 2010); however, another reported no benefit for the GHQ (Carroll et al., 2000). Again, pooled sample analyses suggested positive treatment effects on general mental health \( (n=1034; \text{SMD}=0.30; 95\%\text{CI}=0.43–0.18, \text{p}=0.001; \text{Long \\ & \text{Benton, 2013})}. \) Five studies reported a significant treatment effect on stress; this was for the entire scale for the PSS (Carroll et al., 2000; Kennedy et al., 2010; Long \& Benton, 2013), and for total scores on the DASS (Harris et al., 2011), the VAS and the BSI (Schlebusch et al., 2000). However, whilst Harris and colleagues (2011) reported a treatment effect for the DASS total score, they did not for the PSS scale, and the stress subscale from the DASS was not significant (Harris et al., 2011). The pooled estimate based on 1076 participants from Long and Benton (2013; SMD=0.35; 95%CI=0.47–0.22, \( p=0.001 \)) suggested overall improvements in stress with multivitamin/mineral supplementation in healthy adults compared to placebo.

Tentative conclusions can also be made regarding measures of mental and physical tiredness. Kennedy and colleagues (2011) found that supplementation with Berocca® for 33 days improved concentration, mental stamina and alertness compared to placebo in healthy adult men, a result that has been supported by Harris and colleagues (2011) who found significant improvements compared to placebo on alertness in healthy adult men supplemented with Swisse Men’s Utivite for 56 days. Three studies assessed measures of physical tiredness; significant treatment effects were reported for insomnia (Carroll et al., 2000) and physical stamina (Kennedy et al., 2011); however, another reported conflicting results with a significant treatment effect reported for physical tiredness, but not for
fatigue in healthy adult women (Haskell et al., 2010). Taken together, results suggest some potential for multivitamin/mineral supplementation to improve mental and physical tiredness in healthy older adults. This conclusion is also supported by Long and Benton (2013) who reported significant pooled treatment effects on self-reported energetic–tired mood \( n=992; \text{SMD}=0.27; 95\%\text{CI}=0.40–0.15, p<0.001 \), suggesting that multivitamin supplementation potentially increases physical and mental energy in healthy adults compared to placebo.

An additional eight trials assessed the impact of some multivitamin/mineral supplement on mood in healthy adults, though they did not employ strict randomised, double-blind, placebo-controlled designs. Gruenwald, Graubaum and Harde (2002) reported improvements in stress in 42 healthy adults (81% female) supplemented with a probiotic for 168 days, consistent with the previously reviewed research. However, no placebo group was employed and this is of particular importance here because improvement in mood outcomes has been seen in the placebo group of similar trials (e.g., Carroll et al., 2000; Schlebusch et al., 2000). Further, an analysis in a single-blind trial by America and Milling (2008) reported decreases in depression for healthy students who were supplemented with a multivitamin, a B-complex vitamin, or the placebo. There was no significant difference between these three groups, but the vitamin groups were significantly different from no-treatment controls. The authors concluded that there was a placebo effect that was partially mediated by response expectancies (America & Milling, 2008), thereby highlighting the importance of placebo-controlled trials in this area.

Another study that contributes to our understanding of the placebo effect employed supplementation with three different multivitamin/mineral formulas (Berocca®, low-dose CNE® & high-dose CNE®) in 91 healthy adults experiencing heightened stress or anxiety
2-3 months following a large earthquake in Christchurch, New Zealand (Rucklidge et al., 2012). All three groups reported significant improvements in depression, anxiety and stress (as measured with the DASS), avoid, intrusion, arousal (as measured by the IES-R), and stress (as measured with the PSS) across four week supplementation, though there were no significant differences in response between these three supplements. Pooled analyses of all three supplement types compared to non-randomised controls revealed significant differences for the anxiety and stress subscales of the DASS, the avoid subscale of the IES-R, but not for the depression subscale of the DASS, the intrusion or arousal subscales of the IES-R, and not for stress as measured with the PSS. These results suggest some benefit of supplementation on mood and add to our understanding of the placebo effect in multivitamin/mineral trials. It is possible that results are due to a placebo effect; however, the finding that subscales from the DASS and the IES-R, and the total PSS did not differ significantly from supplementation groups, suggests the observed effects cannot be solely explained by a placebo effect. Other studies that did employ a randomised placebo group reported significant treatment effects on the vigour-activity dimension of the POMS and in stress (Ussher, Dewberry, Malson & Noakes, 1995) but not for the total POMS scale in healthy older (Cockle et al., 2000), and young adults (Benton, Haller & Fordy, 1995), consistent with our previous conclusions. A further trial reported significant treatment effects for increased energy and mood with Swisse Ultivite F1® supplementation for 116 days in 112 healthy adults, using qualitative analysis with three open-ended questions as the outcome measure (Sarris et al., 2012), and Brown, Goldstein-Shirley, Robinson and Casey (2001) reported significant treatment effects on depression, mood, happiness, self-esteem and general wellbeing in 112 females with mild-to-moderate depression. Results from this trial are interpreted cautiously here as this was a tri-modal intervention comparing a daily 20-minute walk plus light therapy plus
multivitamin compared to placebo vitamin (Brown, Goldstein-Shirley, Robinson & Casey, 2001).

Taken together, results from RCTs and intervention trials suggest that vitamin/mineral supplementation, specifically those with higher doses of B-vitamins, has some effect on aspects of mood in healthy, non-clinical adults. There is evidence that supplementation influences stress, anxiety, general mental health, physical and mental tiredness, and the specific mood subscales of confusion, vigour and depression, though little evidence that it may affect overall mood or depression.

Evidence regarding treatment effects of multivitamin/mineral supplements on mood in healthy adults was reviewed here, despite our focus on specific vitamins, for several reasons. Firstly, our review of folate and vitamin $B_{12}$ trials presented subsequently includes research in clinical populations due to limited research in healthy adults. These studies provide evidence that folate and vitamin $B_{12}$ supplementation may improve mood in clinical populations, and provide some justification to assess these relationships in healthy adults. However, there are two main issues associated with using evidence from clinical populations to provide evidence for behaviour of healthy adults in this area. Clinical populations can often be vitamin and mineral deficient due to medications (e.g., Patenaude, 1996) and the established link between mental health and subsequently poor eating habits (e.g., Fernstrom, Krowinski, & Kupfer, 1981) and we know that mental health does not exist on a continuum from poor to good mental health (Keyes, 2002). Therefore, whilst these following studies provide a general rationale for investigation within healthy adults, their evidence may not be directly applicable. Current evidence on
multivitamin/mineral supplementation for mood in healthy adults was therefore reviewed first because this suggests that the positive effects of nutritional supplements in clinical populations are not due to an inherent characteristic of this specific population, such as poor nutrition. This body of evidence partially eliminates the hypothesis that supplementation can lead to positive outcomes in clinical populations solely due to rectification of a co-occurring problem, such as poor nutrition. Secondly, because mental health does not exist on a continuum, the correlates and predictors of mental illness can be unique. The above evidence suggests that vitamins and minerals are of importance for both. These trials are important in that they demonstrate that nutrients have the potential to influence positive mood in a range of population types; however, their interpretation is confounded by the use of large omnibus inclusion of vitamins.

Folate, Vitamin B_{12}, Homocysteine:
Levels in Psychiatric Patients and Healthy Controls

High prevalence rates of low folate and vitamin B_{12} have been observed in psychiatric patients. Low serum vitamin B_{12} concentrations were reported for 26% of newly admitted patients (Carney & Sheffield, 1978) and low serum folate for 21% (Carney & Sheffield, 1978) and low red blood cell (RBC) folate for 38% (Carney, 1990) of patients admitted to a psychiatric unit. In comparison to healthy controls, depressed patients have demonstrated significantly lower serum and RBC folate concentrations (Abou-Saleh & Coppen, 1989; Bottiglieri et al., 2000; Carney, 1967); however, mean serum vitamin B_{12} concentrations in depressed inpatients were not significantly different to healthy controls (Bottiglieri et al., 2000). These higher prevalence rates have been replicated in non-clinical, community populations. Compared to healthy, non-depressed participants, community-dwelling adults with depression had significantly lower folate
concentrations in both older (>60 years) Greek (Dimopoulos et al., 2007) and Chinese (>55 years) adults (Ng et al., 2009). Vitamin B\textsubscript{12} plasma concentrations were also significantly lower in the Greek sample (Dimopoulos et al., 2007), and although mean levels of vitamin B\textsubscript{12} did not differ significantly between the depressed and non-depressed participants in Chinese adults, vitamin B\textsubscript{12} deficiency was significantly higher in depressed participants \((n=178; 7.9\%)\) compared to non-depressed, healthy participants \((n=491; 3.5\%)\). These prevalence rates are further supported by a population-based study of the general US population. Controlling for sociodemographic factors, serum vitamin B\textsubscript{12} concentration, alcohol consumption, overweight, use of vitamins/minerals, cigarettes, and illegal drugs, those with a lifetime diagnosis of major depression had significantly lower serum and RBC folate concentrations than those who had never been depressed (Morris et al., 2003). There appears growing evidence that folate and vitamin B\textsubscript{12} are somehow implicated in mental health.

Homocysteine is an amino acid that, at high levels, has been associated with adverse health outcomes such as increased risk of cardiovascular disease (e.g., Zakai et al., 2007), and depression (e.g., Tiemeier et al., 2002). Homocysteine is intimately linked with folate and vitamin B\textsubscript{12} because deficiencies in these B-vitamins lead to increased homocysteine levels (Bottiglieri, 1996). Homocysteine has been associated with cardiovascular diseases (CVD), atherosclerosis and stroke, Alzheimer’s disease (AD), renal disorders and rheumatoid arthritis (e.g., Kumar, Jayaraman & Muralidharan, 2012; Miller & Kelly, 1997). Higher homocysteine concentrations have also been reported in inpatients with depression compared to patients with neurological disorders and to healthy controls (Bottiglieri et al., 2000) and in non-clinical populations, higher homocysteine
(Tiemeier et al., 2002; Dimopoulos et al., 2007; Almeida et al., 2004) has been reported in those with depressive symptoms compared to controls.

**Folate and Mental Health:**

**Cross-Sectional Relationships**

Cross-sectional studies have reported associations between folate, vitamin B$_{12}$ and homocysteine, and depressive symptoms. Depressive symptoms were associated with plasma folate concentrations in a small sample of older, community-dwelling, depressed Greek adults (Dimopoulos et al., 2007), with serum folate in non-depressed, older, community-dwelling Chinese adults (Ng et al., 2009) and with dietary folate intake in Japanese adults (Miyaki et al., 2012). This relationship was further supported in a nationally representative sample of US adults (Beydoun et al., 2010) and dietary folate intake was associated with depressive symptoms, though not anxiety, in an age-stratified, randomly-selected, population-based sample of women controlling for age, SES, health behaviours, BMI and energy intake (Jacka et al., 2012). Others have reported a relationship between the two, but only in subsets of either female (Bjelland et al., 2003; Ramos et al., 2004), or male participants (Murakami et al., 2008; Nanri et al., 2010; Sánchez-Villegas et al., 2006, 2009). However, others have reported a relationship in general population samples of only males (Tolmunen et al., 2003) and only females (Jacka et al., 2012). These observed relationships appear independent of several confounding factors; however, Sachdev and colleagues (2005) found that homocysteine reduced the strength of association, suggesting that the observed relationship may be driven by homocysteine. A relationship between folate status and depression has been further supported by a meta-analysis based on pooled estimates from 15,315 participants and this relationship was independent of study design, confounders, method of assessing folate
levels (serum, plasma, or dietary record) and of whether mandatory folate fortification was present (Gilbody, Lightfoot & Sheldon, 2007). Others have found no relationship between folate and depressive symptoms in individuals who had attempted suicide (Engström & Träskman-Bendz, 1999), in inpatients with major depression (Lee, Wing & Fong, 1998), in community-based samples of older adults (Hin et al., 2006; Lindeman et al., 2000; Moorthy et al., 2012; Robinson et al., 2011; Tiemeier et al., 2002) and with depressive symptoms, anxiety and Quality of Life in older women (Cassidy et al., 2004).

**Vitamin B\textsubscript{12} and Mental Health:**

**Cross-Sectional Relationships**

Several studies have reported a relationship between vitamin B\textsubscript{12} and depressive symptoms. Depressive symptoms were associated with vitamin B\textsubscript{12} in a small study of depressed, community-dwelling, elderly Greek adults (Dimopoulos et al., 2007), with serum vitamin B\textsubscript{12} deficiency in a sample of physically disabled, non-demented, community-dwelling older females (Penninx et al., 2000) and with serum vitamin B\textsubscript{12} deficiency, but not levels of vitamin B\textsubscript{12}, in older, community-dwelling Chinese adults (Ng et al., 2009). This relationship has also been reported in a larger ($n=2806$), population-based study of Finnish adults where serum vitamin B\textsubscript{12} was found to be associated with melancholic, but not non-melancholic, depressive symptoms controlling for age, use of antidepressant medications and number of chronic diseases (Sepälä et al., 2013). The relationship between depressive symptoms and low plasma vitamin B\textsubscript{12} concentration was replicated in a large, ethnically diverse population of adults, though no relationship was reported between plasma folate concentrations or homocysteine with depressive symptoms (Moorthy et al., 2012). This finding was replicated in a sample ($n=466$) of community-dwelling, non-demented elderly in inner-city Dublin; dietary
vitamin B\textsubscript{12} intake was associated with depressive symptoms controlling for age, gender, social class, cognitive functioning, and psychosocial and cardiovascular risk factors (Robinson et al., 2011). However, the association was attenuated to non-significance once folate and homocysteine were accounted for (Robinson et al., 2011), thus suggesting that the observed relationship between vitamin B\textsubscript{12} and depressive symptoms may be driven by either homocysteine and/or folate levels in support of the previously discussed findings (Sachdev et al., 2005). Others have reported a relationship between vitamin B\textsubscript{12} and depressive symptoms in subsets of their study populations. Tiemeier and colleagues (2002) reported that vitamin B\textsubscript{12} deficiency was associated with depressive symptoms only in those with depressive disorders, and Sánchez-Villegas and colleagues (2006, 2009) found that dietary vitamin B\textsubscript{12} was associated with prevalence of depression in only female volunteers. Similar studies have reported no association between vitamin B\textsubscript{12} and depressive symptoms in adults who had attempted suicide (Engström & Träskman-Bendz, 1999), in community-dwelling older adults (Hin et al., 2006; Lindeman et al., 2000; Sachdev et al., 2005), with depression and anxiety in older females (Cassidy et al., 2004), and in two population-based studies nationally representative of Norwegian (Bjelland et al., 2003) and American (Beydoun et al., 2010) adults.

**Homocysteine and Mental Health:**

**Cross-Sectional Relationships**

Homocysteine was related to depressive symptoms in older, community-dwelling, depressed adults (Dimopoulos et al., 2007) and to depression, but not anxiety, in a large population-based study after controlling for demographic variables and smoking status (Bjelland et al., 2003). Another population-based study reported that the relationship between hyperhomocysteinemia and depressive disorders was attenuated to non-
significance once functional disabilities and cardiovascular risk factors were considered (Tiemeier et al., 2002) suggesting that hyperhomocysteinemia may act as a proxy for these risk factors. However, another study has reported that elevated total serum homocysteine was associated with depressive symptoms in males independent of sociodemographic factors, health-related behaviours, biological risk factors and medication or vitamin use (Nabi et al., 2013) suggesting that homocysteine is associated with depression independently of these risk factors. This was also supported in another study that reported elevated homocysteine was associated with increased severity of depressive symptoms in adults aged 60-to-64 years after controlling for gender, physical health, smoking status, high creatinine, low folate and low vitamin B$_{12}$ (Sachdev et al., 2005). In addition to supporting a relationship between homocysteine and depression independent of health status, this also suggests that the relationship may not be solely driven by the dependence of homocysteine on these B-vitamins. Two studies reported that the relationship between serum homocysteine and depressive symptoms was present only in male respondents (Nanri et al., 2010; Tolmunen et al., 2004b) and in a large ($n=3752$), community-based sample of older men an association was found between homocysteine with both current and previous incidence of depression (Almeida et al., 2008). That the relationship is only present in males was further supported by a community-based study that reported no association between serum homocysteine and depression in a sample of older women (Penninx et al., 2000). Another study found that total plasma homocysteine was moderately correlated with depression, though not anxiety, in older, community-dwelling females (Almeida et al., 2004). Two larger studies reported no association between plasma homocysteine and depression in large samples of US adults (Beydoun et al., 2010; Moorthy et al., 2012), though Beydoun and colleagues (2010) did find an association in both sexes combined for older ($\geq50$years) adults only. One study of particular relevance
here assessed the relationship between positive mood and homocysteine in a sample of older (≈80 years) Swedish adults (Jensen et al., 1998). Mean plasma homocysteine concentrations higher than 15\(\mu\)mol/l were associated with decreased life satisfaction and subjective health and with decreased responses for the subscales of mood and zest for life (Jensen et al., 1998). There appears evidence to suggest some relationship between homocysteine and depression, though whether this relationship is independent from health risk factors is unclear. Although the relationship was found to be independent of health-related behaviours, biological factors (Nabi et al., 2013) and physical health (Sachdev et al., 2005), another study reported that it was not independent of functional disabilities and cardiovascular risk factors (Tiemeier et al., 2002). Gender also appears to have an influence on the outcome, with the relationship present only in male participants for two studies (Nanri et al., 2010; Tolmunen et al., 2004b).

**Folate, Vitamin B\(_{12}\), Homocysteine and Mental Health:**

**Longitudinal Relationships**

If a true relationship does exist between folate, vitamin B\(_{12}\) and homocysteine with depressive symptoms, then an alternative hypothesis is that depressive symptoms result in lower folate and vitamin B\(_{12}\) and therefore higher levels of homocysteine. This direction of reversed causality cannot be theoretically ruled out as an explanation for these cross-sectional relationships because the hypothesis is plausible. The DSM-IV-TR reports significant change in appetite or weight as one of the criteria of major depressive disorder (DSM-IV-TR, 2000) and empirical studies have shown that poor appetite is associated with depressive symptoms, low emotional wellbeing and low hardiness (Engel et al., 2011) and psychotropic drugs used to treat mood disorder can also affect appetite (e.g., Harris, 1986). Therefore, longitudinal and intervention trials are necessary to establish
whether low levels of folate and vitamin B\textsubscript{12}, which contribute to high homocysteine concentrations, can lead to depression.

Several longitudinal investigations have been conducted in large scale, population-based studies in an attempt to address this, though results have been mixed. Longitudinal relationships have been reported between baseline serum folate (Kim et al., 2008), dietary folate intake (Tolmunen et al., 2004a), serum vitamin B\textsubscript{12} (Kim et al., 2008) and plasma homocysteine (Forti et al., 2010; Kim et al., 2008) with subsequent measures of depressive symptoms up to 16 years later (Tolmunen et al., 2004a). An inverse association was also reported between serum vitamin B\textsubscript{12} and decline in depressive symptoms over six months in a sample of 115 outpatients with a diagnosis of major depressive disorder (DSM-III-R) at baseline (Hintikka et al., 2003). Furthermore, this relationship remained significant after controlling for age, sex, duration of illness, family history of depression, alcohol use, smoking status, BMI, weight loss, gastrointestinal symptoms, depression severity at baseline, adequate drug treatment, weekly psychotherapy and inpatient treatment, suggesting that the relationship is independent of these factors. Serum folate concentrations were also found to correlate with decline in depressive symptoms but this relationship was weak and reduced to non-significance once the above confounders were controlled for (Hintikka et al., 2003).

Three studies have assessed these relationships in community-dwelling samples of non-depressed adults. Kim and colleagues (2008) reported that low serum folate, low serum vitamin B\textsubscript{12} and high plasma homocysteine concentrations were associated with an increased risk of depression over 2-3 years follow-up in a sample of older (≥65 years) Korean adults (n=732), adjusting for cognitive functioning, disability and use of vitamin
supplements. The two relationships, serum folate with incident depression and serum vitamin B$_{12}$ with incident depression were also not attenuated once serum vitamin B$_{12}$ and plasma homocysteine and serum folate and plasma homocysteine, respectively, were additionally controlled for, suggesting that both folate and vitamin B$_{12}$ have effects on depressive symptoms independent of homocysteine and of each other. However, the relationship between plasma homocysteine concentrations and incident depression was significantly reduced once serum folate and vitamin B$_{12}$ were controlled for (Kim et al., 2008) suggesting that homocysteine may be associated with incident depression due to its dependence on folate and vitamin B$_{12}$. The authors, however, suggest that this reduction be interpreted cautiously because serum measures of folate and vitamin B$_{12}$ status are only a proxy for their cellular level functioning (Kim et al., 2008).

A larger ($n=2313$) prospective study of Finnish men aged between 42 and 60 years similarly reported an association between dietary folate intake and incident depression on 11-to-16 years follow-up, controlling for SES, baseline depression, and intakes of fibre, vitamin C, total fat and total energy; however, they did not find an association between dietary vitamin B$_{12}$ intake and incident depression (Tolmunen et al., 2004a). The study authors proposed that this could be due to the differences in intakes of these two vitamins: whereas only 25% of participants met the recommended daily intake of folate, almost all (99%) consumed the recommended daily intake of vitamin B$_{12}$. In contrast, a large ($n=3503$), community-based study of older adults ($\geq 65$ years) reported an association between higher total dietary intake of vitamin B$_{12}$ and decreased likelihood of incident depression over 12 years follow-up, controlling for age, sex, race, education, income and antidepressant medications; however, they found no relationship between dietary folate intake and depressive symptoms over an average of 7.2 years (Skarupski et al., 2010),
though similarly one potential reason for this was due to relatively high initial levels of folate. Another population-based study of older (≥65 years) Italian adults reported that plasma homocysteine was associated with increased risk of depression over four years controlling for age, education, serum creatinine, general health, functional status, vascular risk factors and vascular conditions, though this was only observed in female participants (47.5% of the sample; Forti et al., 2010).

Others have reported no causal relationships. Kendrick and colleagues (2008) reported that RBC folate was not associated with subsequent incidence of depressive symptoms over the following two years in a large study of women aged between 20 and 34 years despite the presence of a relationship at baseline. The authors suggest that low RBC folate concentrations may therefore be the consequence of depressive symptoms rather than the cause (Kendrick et al., 2008), though this contradicts the findings reported above. Dietary folate and vitamin B$_{12}$ intake and serum homocysteine were not associated with depressive symptoms in a study of elderly men (70-90 years) without diabetes or any cardiovascular diseases at baseline (Kamphuis et al., 2008). Serum homocysteine was measured in 1985, depressive symptoms in 1990, and dietary folate and vitamin B$_{12}$ intake were assessed with the crosscheck dietary history method in 1985 and 1990. This method for assessing dietary history has been validated and the dietary folate intake was associated with serum homocysteine as expected, though dietary vitamin B$_{12}$ intake was not. The authors suggest that selective participation of healthier elderly participants may offer an explanation for the lack of associations found (Kamphuis et al., 2008). Finally, Williams and colleagues (2008) reported no relationship between subjective mood, plasma homocysteine and RBC and serum folate concentrations in healthy males aged between 19 and 47 years in the only longitudinal study identified that assessed a measure of positive
functioning, operationalised as positive affect (PA) and negative affect (NA), rather than depression. RBC and serum folate concentrations, and plasma homocysteine concentrations were assessed at baseline, following which the PANAS was administered twice daily for the subsequent week. However, due to lack of variation in PA and NA ratings by time, and by day, these responses were summed across each day, and then for the week, thus resulting in a cross-sectional analysis of longitudinal data. Serum folate and homocysteine concentrations were not associated with PA, NA, or their variability; and whilst RBC folate was not associated with PA, NA, or PA variability, it was associated with variability in NA; as RBC folate increased, the variability in NA decreased. This supports previous findings reported with depression outcomes because low NA is a feature of depression. As stated by the authors, this result also suggests that long-term folate status is more important for mood stability because RBC concentrations provide a more accurate measure of long-term folate than serum concentrations (Williams et al., 2008).

Evidence of a longitudinal relationship from folate, vitamin B\textsubscript{12} or homocysteine to depressed mood appears to be confounded by differential participant characteristics, particularly whether folate and vitamin B\textsubscript{12} levels are adequate or deficient in the sample. There also appears some evidence to suggest the folate and vitamin B\textsubscript{12} have an independent effect on depressed mood, whereas homocysteine does not, suggesting that observed relationships may be due to the dependence of homocysteine on folate and vitamin B\textsubscript{12}. 
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Folate, Vitamin B₁₂, Homocysteine and Mental Health:

**Intervention Trials**

Support for a role of folate, vitamin B₁₂ and homocysteine in depressed mood has been further provided by intervention trials where folate and vitamin B₁₂ alone (Almeida et al., 2010; Guaraldi et al., 1993; Hvas et al., 2003; Passeri et al., 1993), or as adjunct treatments (Alpert et al., 2002; Coppen & Bailey, 2000; Coppen, Chaudhry & Swade, 1986; Godfrey et al., 1990), have demonstrated some improvements in mood outcomes. This has been partially driven by research that suggests initial folate levels predict differential response to treatment of depression. Higher RBC folate concentrations have been found to increase response to treatment with sertraline, though not for treatment with nortriptyline, in older (≥60 years) patients with a major depressive disorder (Alpert, Silva & Pouget, 2003). This has been replicated in three trials of fluoxetine by Papakostas and colleagues (2004a, 2004b, 2005) in patients with major depressive disorder: all reported that low folate concentrations predicted delayed onset of clinical improvement, though neither vitamin B₁₂ nor homocysteine concentrations were associated with improvements (Papakostas et al., 2004a, 2004b, 2005).

Eight trials assessing folate and/or vitamin B₁₂ as mono-therapies for depressed mood, and four adjunct trials are summarised in Table 2. Treatment with methyltetrahydrofolate (MTHF) alone has demonstrated improvements in depressive symptoms in elderly patients with depression (Gauraldi et al., 1993), and in patients with mild to moderate dementia and depression (Passeri et al., 1993). A combination of folic acid, vitamin B₁₂, and vitamin B₆ for an average of 7.1 years (SD=2.1 years) was also associated with reduced risk of onset of major depression compared to placebo, in a population at risk for depression (Almeida et al., 2010) and some benefit has been
documented in healthy adults who received weekly vitamin B$_{12}$ injections over one month, though improvement was only observed in one of the eight domains from the SF-36 – the general health dimension (Hvas et al., 2003). Others have reported no effect of supplementation with folic acid (Williams et al., 2005), folic acid, vitamin B$_6$, and vitamin B$_{12}$ (Ford et al., 2008), and folic acid and vitamin B$_{12}$ (Christensen et al., 2011; Walker et al., 2010) in non-clinical populations of older men (Ford et al., 2008; Williams et al., 2005), and in older adults with elevated psychological distress (Walker et al., 2010), or with depressive symptoms (Christensen et al., 2011).
Clinical trials assessing efficacy of folic acid, vitamin $B_{12}$, and vitamin $B_6$ as mono- or adjunct therapy to improve mood outcomes in clinical and non-clinical populations.

<table>
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<th>Authors</th>
<th>Year</th>
<th>N</th>
<th>Trial</th>
<th>Clinical vs. non-clinical populations</th>
<th>Study Length</th>
<th>Mood Measures</th>
<th>Treatment</th>
<th>Results</th>
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<tbody>
<tr>
<td>Mono-therapy</td>
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<tr>
<td>Guaraldi et al.</td>
<td>1993</td>
<td>20</td>
<td>Open-label trial</td>
<td>Clinical (major depression)</td>
<td>42d</td>
<td>HAM-D</td>
<td>MTHF (50mg/d)</td>
<td>Decline of 50% or more on HAM-D</td>
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<td></td>
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<td>Older adults</td>
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<tr>
<td>Passeri et al.</td>
<td>1993</td>
<td>96</td>
<td>Double-blind, controlled trial</td>
<td>Clinical (cognitive impairment)</td>
<td>56d</td>
<td>HAM-D</td>
<td>MTHF (50mg/d) vs trazodone (50mg/d)</td>
<td>Significant decline in HAM-D in both groups</td>
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<td>Older adults</td>
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<tr>
<td>Hvas et al.,</td>
<td>2003</td>
<td>140</td>
<td>Randomised; double-blind; placebo- controlled</td>
<td>Non-clinical Adults</td>
<td>28d</td>
<td>SF-36</td>
<td>Vitamin $B_{12}$ (1000µg) injection weekly</td>
<td>Significant improvement for the general health dimension of the SF-36 compared to placebo (non-significant for seven remaining dimensions)</td>
</tr>
<tr>
<td>Authors</td>
<td>Year</td>
<td>N</td>
<td>Trial</td>
<td>Clinical vs. non-clinical populations</td>
<td>Study Length</td>
<td>Mood Measures</td>
<td>Treatment</td>
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<tr>
<td>Williams et al.</td>
<td>2005</td>
<td>23</td>
<td>Randomised; double-blind; placebo-controlled</td>
<td>Non-clinical Adult men</td>
<td>84d</td>
<td>PANAS</td>
<td>FA Supplementation (100µg/d d1-42; 200µg/d d43-84)</td>
<td>No differences in PANAS between groups post-intervention</td>
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<tr>
<td>Ford et al.</td>
<td>2008</td>
<td>299</td>
<td>Placebo-controlled, randomised, double-blind</td>
<td>Non-clinical Older men</td>
<td>2y</td>
<td>BDI</td>
<td>FA (2mg/d) + B₆ (25mg/d) + B₁₂ (400µg/d) vs. placebo</td>
<td>No difference between groups and no significant change over time</td>
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<tr>
<td>Almeida et al.</td>
<td>2010</td>
<td>273</td>
<td>Randomised; double-blind; placebo-controlled</td>
<td>Non-clinical (at risk for depression) Older adults</td>
<td>1-10.5y</td>
<td>Major depressive episode</td>
<td>FA (2mg/d) + B₆ (25mg/d) + B₁₂ (0.5mg/d) vs. placebo</td>
<td>Treatment associated with reduction in hazard of major depression</td>
</tr>
<tr>
<td>Walker et al.</td>
<td>2010</td>
<td>909</td>
<td>Randomised controlled trial</td>
<td>Non-clinical (elevated Psychological Distress) Older adults</td>
<td>2y</td>
<td>PHQ-9</td>
<td>FA (400mcg/d) + B₁₂ (100mcg/d) vs. placebo (+ physical activity &amp; mental health literacy)</td>
<td>FA + B₁₂ did not reduce depressive symptoms at any time point</td>
</tr>
<tr>
<td>Authors</td>
<td>Year</td>
<td>N</td>
<td>Trial</td>
<td>Clinical vs. non-clinical populations</td>
<td>Study Length</td>
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<tr>
<td>Christensen et al.</td>
<td>2011</td>
<td>900</td>
<td>Double-blind; placebo-controlled, randomised</td>
<td>Non-clinical (with depressive symptoms)</td>
<td>2y</td>
<td>PHQ-9; PRIME-MD</td>
<td>FA (400mcg/d) + B₁₂ (100mcg/d) vs. placebo</td>
<td>Little evidence for treatment of FA + B₁₂ on depressive symptomatology</td>
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<td><em>Adjunct Therapy</em></td>
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<tr>
<td>Coppen et al.</td>
<td>1986</td>
<td>75</td>
<td>Double-blind, placebo-controlled</td>
<td>Clinical (unipolar and bipolar disorder) Adults</td>
<td>352d</td>
<td>BDI</td>
<td>FA (200µg/d) + lithium</td>
<td>Greater reduction in BDI scores for those with unipolar depression in FA group</td>
</tr>
<tr>
<td>Godfrey et al.</td>
<td>1990</td>
<td>24</td>
<td>Double-Blind, placebo-controlled, randomised trial</td>
<td>Clinical (major depression) Adults</td>
<td>168d</td>
<td>HAM-D; COS</td>
<td>MTHF (15mg/d) + standard antidepressant treatment</td>
<td>Significant decline in COS, but not HAM-D in MTHF group at 3 and 6 months</td>
</tr>
<tr>
<td>Coppen &amp; Bailey</td>
<td>2000</td>
<td>127</td>
<td>Double-blind, placebo-controlled, randomised trial</td>
<td>Clinical (major depressive episode) Adults</td>
<td>70d</td>
<td>HAM-D</td>
<td>FA (500µg/d) + fluoxetine</td>
<td>Women benefitted from treatment with FA</td>
</tr>
<tr>
<td>Authors</td>
<td>Year</td>
<td>N</td>
<td>Trial</td>
<td>Clinical vs. non-clinical populations</td>
<td>Study Length</td>
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<td>Alpert et al.</td>
<td>2002</td>
<td>22</td>
<td>Open-label trial</td>
<td>Clinical (major depressive disorder)</td>
<td>56d</td>
<td>HAM-D</td>
<td>MTHF (15mg/d for 2wk; 30mg/d for 6wk) + treatment as usual</td>
<td>Subgroup had decline ≥50% on HAM-D</td>
</tr>
</tbody>
</table>

*BDI, Beck Depression Inventory; COS, Clinical Outcome Scale (clinical impression regarding clinical and social functioning); FA, Folic Acid; HAM-D, Hamilton Rating Scale for Depression; mcg, micrograms; mg, milligrams; MTHF, methyltetrahydrofolate; PANAS, Positive and Negative Affect Schedule; PHQ-9, Patient Health Questionnaire – 9; PRIME-MD, Primary Care Evaluation of Mental Disorders (diagnostic instrument for common psychiatric disorders); SF-36, Short-Form 36-Item Health Survey; µg, micrograms.*
Some studies have assessed the adequacy of folic acid as an adjunct treatment for depression. Of four such trials identified, all reported some benefit of folic acid as adjunct treatment. Patients with depression showed a greater reduction in affective morbidity with folic acid plus lithium treatment compared to placebo plus lithium (Coppen et al., 1986); and a significantly greater improvement was found with folic acid plus fluoxetine compared to placebo plus fluoxetine in patients with major depression, though this was only observed in female patients (Coppen & Bailey, 2000). The authors concluded that the improvement was related to homocysteine, which significantly decreased only in female, not male participants, rather than due to change in folate concentrations which significantly increased, though to a lesser amount for males than females (Coppen & Bailey, 2000). Two other trials reported significant treatment improvement with MTHF (Godfrey et al., 1990), or folic acid (Alpert et al., 2002), plus standard antidepressant treatment in patients with major depressive disorder. Significant improvements were reported for clinical and social recovery (Godfrey et al., 1990) and it was concluded that folic acid as adjunct was able to moderately reduce depressive symptoms among SSRI-refractory patients (Alpert et al., 2002).

None of these studies have specifically addressed whether homocysteine mediates the proposed causal relationship from folate and vitamin B_{12} to depressed mood. Homocysteine may directly affect depressive symptoms, or may simply serve as a marker for low folate and vitamin B_{12} which affect depressive symptoms (e.g., Bottiglieri et al., 2005). This proposed mediational relationship has been investigated within research into cardiovascular outcomes. Results have suggested no impact on cardiovascular outcomes with folate and/or vitamin B_{12}, despite successfully lowering homocysteine (Albert et al.,
2008; Armitage, 2010; Ebbing et al., 2008), though some do suggest that folate may have a direct effect on cardiovascular outcomes (Verhaar, Stroes & Rabelink, 2002).

**Folate, Vitamin B\textsubscript{12}, Homocysteine and Mental Health:**

**Mechanist Accounts**

Homocysteine is metabolised in the body in the methionine cycle via the two pathways displayed in Figure 5, re-methylation and trans-sulfuration. The re-methylation of homocysteine to methionine, an amino acid essential for protein synthesis, requires the methyl group 5-methyltetrahydrofolate (5-MTHF) which is synthesised by 5,10-methylenetetrahydrofolate reductase (5, 10-MTHFR). After re-methylation, methionine can be re-used to generate S-adenosylmethionine (SAMe; Crellin, Bottiglieri, & Reynolds, 1993; Reynolds, Carney & Toone, 1984). This biochemical process requires the presence of folate and vitamin B\textsubscript{12} as co-factors necessary for the methylation of homocysteine to methionine and in the synthesis of SAMe (Bottiglieri, 1996; Bottiglieri & Hyland, 1994; Moretti et al., 2004), therefore dietary deficiencies of either can lead to a break in the methionine cycle, resulting in increased homocysteine levels and reduced SAMe synthesis. A relationship between folate, vitamin B\textsubscript{12}, and homocysteine with neuropsychiatric disorders such as depression, may be biologically explained by this reduction in the synthesis of SAMe. SAMe is a methyl donor with antidepressant properties due to its involvement in the metabolism of neurotransmitters such as norepinephrine, dopamine, melatonin and serotonin (Moretti et al., 2004; Reynolds, Carney & Toone, 1984). Therefore, reduced synthesis of SAMe may be an underlying mechanism for the association between low folate, low vitamin B\textsubscript{12}, high homocysteine and depressed mood. Alternatively, homocysteine can be degraded through the trans-sulfuration pathway, under the presence of vitamin B\textsubscript{6} as a co-factor, into cysteine and
then taurine, an amino acid that has a role in the development of the central nervous system (Schuller-Levis & Park, 2003) where it may function as an inhibitory neuromodulator and neurotransmitter (Oja & Saransaari, 1996; Olive, 2002); it may also have a role in coronary heart disease (Özkan, Özkan & Şimşek, 2002; Yanagita, 2008).

**Figure 5.** The methionine cycle: the trans-sulfuration and the re-methylation pathway (simplified).

*Note: 5-MTHF, 5-methyltetrahydrofolate; 5,10-MTHFR, 5,10-methyleneetetrahydrofolate reductase; SAH, S-adenosyl-homocysteine; SAMe, S-adenosylmethionine; THF, Tetrahydrofolate.*

Functional deficiencies of folate or vitamin B₁₂ may therefore result in disturbed mood either directly or indirectly via elevated homocysteine (Bottiglieri, 1996; Bottiglieri et al., 2000; Lindenbaum et al., 1988) and reduced SAMe concentrations (Penninx et al.,
Several recent B-vitamin intervention trials have reported no impact on cardiovascular outcomes despite significantly lowering Homocysteine levels (Albert et al., 2008; Armitage, 2010; Ebbing et al., 2008; Lonn et al., 2006), suggesting that high homocysteine may be a marker for, rather than a cause of CVD risk. This is of particular relevance here given that consistent reports confirm a bi-directional relationship between CVD and depression (e.g., Freedland et al., 2003; Van der Kooy et al., 2007), with prevalence of depression ranging from 16-to-23% in patients with CVD (Musselman, Evans & Nemeroff, 1998).

**Folate, Vitamin B\(_{12}\), Homocysteine and Mental Health:**

**Concluding Comments**

Early research observed low concentrations of folate and vitamin B\(_{12}\) and high concentrations of homocysteine in those with depression. Relationships between these have been subsequently supported in cross-sectional studies, though interpretation has been confounded due to the bi-directional relationship between diet and mood. Longitudinal evidence and intervention trials have supported the temporal order from folate, vitamin B12 and homocysteine to depressed mood, though evidence suggests that the relationship between homocysteine and depressed mood may be due to the dependence of homocysteine on folate and vitamin B12 concentrations. Conclusions are still confounded by differential participant characteristics.

A large proportion of this evidence has also been derived from clinical samples. Review of the literature relevant to multivitamins and minerals for mood in healthy adults established that the effects are not only relevant to poor mental health, nor are they solely
due to rectification of poor nutritional intake. In the following section we now review the evidence for the role of omega-3 Polyunsaturated Fatty Acids (n-3 PUFAs) in mental health.

**Omega-3 Long-Chain Polyunsaturated Fatty Acids:**

**Levels in Psychiatric Patients and Healthy Controls**

Prevalence rates of mental disorders are low in countries with high fish consumption (Noaghiul & Hibbeln, 2003) and n-3 LC PUFA deficits have been observed in a range of patient populations including those with mild (Mamalakis et al., 2002) and major depression (Maes et al., 1999; Peet et al., 1998). Comparisons with non-depressed controls have shown that older participants with depressive symptoms have a lower percentage of n-3 LC PUFAs and a higher ratio of n-3 LC PUFAs to n-6 LC PUFAs compared to non-depressed controls (Tiemeier et al., 2003).

**Omega-3 Long-Chain Polyunsaturated Fatty Acids and Mental Health:**

**Cross-Sectional Relationships**

Associations have been reported between fish consumption with both depressive symptoms (Tanskanen et al., 2001) and self-reported mental health (Silvers & Scott, 2002), and between n-3 LC PUFA intake calculated from Food Frequency Questionnaires (FFQs) and depressive symptoms (Colangelo et al., 2009; Golding et al., 2009; Yary & Aazami, 2011). An association has also been reported between n-3 LC PUFAs calculated solely from fish intake with depressed mood, though this relationship was attenuated once age and an index of multiple deprivation were controlled for (Appleton et al., 2007). Others have reported associations between depressive symptoms with adipose tissue DHA
levels (Mamalakis et al., 2002) and with erythrocyte EPA levels, the ratio of AA to EPA, and the ratio of $n$-6 LC to $n$-3 LC PUFAs (Adams et al., 1996). Others have not found an association. Jacka and colleagues (2004) reported no difference in $n$-3 PUFA intake calculated from seafood, fish and cod liver oil consumption between self-reported depressed ($n=97$, 12.85%) and non-depressed ($n=658$) participants in a population-based, community sample of women. Though $n$-3 PUFA intake was low, and rates of self-reported depression were high (Jacka et al., 2004). Similarly, another large-scale, cross-sectional, follow-up study reported no association between either fish or $n$-3 LC PUFA intake calculated from dietary history questionnaires with either depressed mood, depressive episodes, or suicide in the following nine years (Hakkarainen et al., 2004). Taken together, these results suggest that $n$-3 LC PUFAs may have a role in mental health.

Different methods of measurement of $n$-3 LC PUFAs, from fish intake to erythrocyte membrane concentrations; differences in outcome measurement, from mental health to depression; varied study populations employed, from depressed to non-clinical; may all partially account for equivocal findings. Potential bi-directional causality may further impede general conclusions here.

**Omega-3 Long-Chain Polyunsaturated Fatty Acids and Mental Health:**

**Longitudinal Relationships**

Several longitudinal studies have investigated associations between $n$-3 LC PUFAs and depressed mood. High fish intake and high $n$-3 LC PUFA intakes calculated from FFQs were associated with lower prevalence of psychotic-like symptoms in an 11-year follow-up study (Hedelin et al., 2010). Fatty fish intake greater than 0.1% of total
energy intake was associated with decreased risk of depressive episode, and recurrent depressive episodes six years later (Astorg et al., 2008). But a nested case-control analysis of the same cohort revealed that $n$-3 LC PUFAs in baseline serum phospholipids were not associated with recurrent depression eight years later (Astorg et al., 2009). Three recent meta-analyses reported that $n$-3 LC PUFAs demonstrated some beneficial role for those diagnosed with depressive mood (Appleton et al., 2006, 2010; Lin & Su, 2007) but that heterogeneity and publication bias were responsible for some of the positive results (Appleton et al., 2006, 2010; Lin & Su, 2007). Appleton and colleagues (2006, 2010) suggested that the benefits of $n$-3 LC PUFAs may be confined to those with a diagnosis of depression.

**Omega-3 Long-Chain Polyunsaturated Fatty Acids and Mental Health: Randomised Control Trials**

Numerous RCTs have now been conducted in an attempt to better determine what affect $n$-3 LC PUFAs have on depressed mood; however, conclusions are still mixed. Results from 39 placebo-controlled trials published between 1990 (Behan et al., 1990) and 2012 (Antypa et al., 2012) are presented in Table 3. Of the 39 identified trials, 44% (17 of 39) reported a significant difference between the $n$-3 LC PUFA treatment and placebo groups on measures of mood, in trials ranging from four to 16 weeks (mode=12wks, median=12wks). Of these, 94% (16 of 17) employed an EPA-rich treatment, with 12 trials containing higher EPA than DHA concentrations, four with EPA alone, and only one with a DHA-rich treatment. The mean EPA dose used for trials that reported a significant treatment effect was 1.77g/d ($SD=1.53$), with a mean DHA dose for these trials of 0.75g/d ($SD=0.92$). A greater number of trials (56%; 22 of 39) reported no significant differences between treatment with $n$-3 LC PUFA and placebo groups in trials.
of comparable length (4 to 45 weeks; mode= 12, median=12). Approximately half of these used an EPA-rich treatment (10 of 22), and half a DHA-rich treatment (11 of 22).

The mean EPA dose used for trials that reported no significant treatment effect was 1.05g/d ($SD=1.56$), with a mean DHA dose for these trials of 0.89g/d ($SD=0.91$). The one consistent result here is that most trials that reported a significant effect of treatment employed an EPA-rich supplement. However, 46% of studies that reported no benefit of treatment also used an EPA-rich supplement. The mean EPA dose for trials that reported a significant treatment effect was higher (1.77g/d) than those that did not report an effect (1.05g/d), though the difference between dosages was not significant, and DHA-dosages were similar in both those that reported an effect ($M=0.78$), and those that did not ($M=0.89$).
Table 3

*Results from placebo-controlled trials of the effects of omega-3 polyunsaturated fatty acid supplementation on depressed mood.*

<table>
<thead>
<tr>
<th>Authors</th>
<th>Year</th>
<th>N</th>
<th>Patients</th>
<th>Study Length</th>
<th>Assessment of depression</th>
<th>Dose/day</th>
<th>Depression/Mood Outcomes</th>
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<tbody>
<tr>
<td>Behan et al.</td>
<td>1990</td>
<td>63</td>
<td>Postviral Fatigue Syndrome</td>
<td>12wks</td>
<td>4-pt Likert scale (fatigue, myalgia, dizziness, concentration, &amp; depression)</td>
<td>EPA (0.14g/d) + DHA (0.09g/d) Placebo=liquid paraffin + LA (0.4g/d)</td>
<td>Significant difference between groups</td>
</tr>
<tr>
<td>Hamazaki et al.</td>
<td>1996</td>
<td>41</td>
<td>University students</td>
<td>12wks</td>
<td>P-F Study</td>
<td>DHA-rich (49.3% DHA + 6.7% EPA)= 1.5-1.8g/d DHA Placebo=97% soybean oil + 3% fish oil</td>
<td>Aggression significantly increased in placebo group across study period but did not change in treatment group</td>
</tr>
<tr>
<td>Hamazaki et al.</td>
<td>1998</td>
<td>46</td>
<td>University students</td>
<td>12wks</td>
<td>P-F Study</td>
<td>1.5g/d DHA Placebo=97% soybean oil + 3% fish oil</td>
<td>Aggression significantly decreased in the control group, but remained stable in the treatment group</td>
</tr>
<tr>
<td>Authors</td>
<td>Year</td>
<td>N</td>
<td>Patients</td>
<td>Study Length</td>
<td>Assessment of depression</td>
<td>Dose/day</td>
<td>Depression/Mood Outcomes</td>
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<td>Stoll et al.</td>
<td>1999</td>
<td>30</td>
<td>Patients with bipolar</td>
<td>16wks</td>
<td>YMRS, HDRS, CGI, GAS</td>
<td>EPA (6.2g/d) + DHA (3.4g/d) Placebo= olive oil</td>
<td>Treatment group had a significantly longer period of remission and significantly lower HRSD, CGI and GAS scores compared to placebo group; no difference between groups on YMRS scores</td>
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<td></td>
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<td>depression</td>
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<td>ethyl esters Adjunct to antidepressant treatment</td>
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<tr>
<td>Warren et al.</td>
<td>1999</td>
<td>50</td>
<td>Chronic Fatigue Syndrome</td>
<td>12wks</td>
<td>BDI</td>
<td>EPA (0.14g/d) + DHA (0.09g/d) Placebo=sunflower oil</td>
<td>No significant difference between groups</td>
</tr>
<tr>
<td>Nemets et al.</td>
<td>2002</td>
<td>20</td>
<td>Patients with MDD</td>
<td>4wks</td>
<td>HDRS</td>
<td>ethyl-EPA (2g/d) Placebo=2g/d Adjunct to antidepressant treatment</td>
<td>Significant effects at weeks 2, 3, and 4 of treatment</td>
</tr>
<tr>
<td>Peet &amp; Horrobin</td>
<td>2002</td>
<td>70</td>
<td>Depressed patients</td>
<td>12wks</td>
<td>HDRS, MADRS, BDI</td>
<td>Ethyl-EPA (1g/d, 2g/d or 4g/d) Placebo= liquid paraffin Adjunct to antidepressant treatment</td>
<td>1g/d group= significant better outcome than placebo on HDRS, MADRS, BDI; 2g/d group showed little evidence of efficacy; 4g/d group showed non-significant trends toward improvement</td>
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</table>

Overall benefit: Y
<table>
<thead>
<tr>
<th>Authors</th>
<th>Year</th>
<th>N</th>
<th>Patients</th>
<th>Study Length</th>
<th>Assessment of depression</th>
<th>Dose/day</th>
<th>Depression/Mood Outcomes</th>
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<tbody>
<tr>
<td>Llorente et al.</td>
<td>2003</td>
<td>138</td>
<td>Breast-feeding mothers</td>
<td>16wks Postpartum depression</td>
<td>BDI, EPDS, clinical interview</td>
<td>DHA (≈0.2g/d) Placebo (undefined)</td>
<td>No difference between groups in self-rated or diagnostic measures of depression Overall benefit: N</td>
</tr>
<tr>
<td>Marangell et al.</td>
<td>2003</td>
<td>36</td>
<td>Patients with MDD</td>
<td>6wks</td>
<td>MADRS, HDRS, GAFS</td>
<td>DHA (2g/d DHA) Placebo (undefined)</td>
<td>No difference between groups Overall benefit: N</td>
</tr>
<tr>
<td>Su et al.</td>
<td>2003</td>
<td>22</td>
<td>Outpatients with MDD</td>
<td>8wks</td>
<td>HDRS</td>
<td>EPA (4.4g/d) + DHA (2.2g/d) Placebo= olive oil ethyl esters Adjunct to antidepressant treatment</td>
<td>Treatment group significantly greater reduction in HDRS scores compared to placebo Overall benefit: Y</td>
</tr>
<tr>
<td>Bradbury et al.</td>
<td>2004</td>
<td>30</td>
<td>Stressed University staff</td>
<td>6wks</td>
<td>PSS</td>
<td>EPA (0.06g/d)+DHA (0.25g/d) Placebo= 1000mg olive oil – predominantly monounsaturated fatty acids</td>
<td>Significant reduction in perceived stress but in both experimental and placebo groups; significant difference between experimental group and no-treatment controls in rate of stress reduction (but not compared to placebo) – study was inadequately powered, mainly looking at trends Overall benefit: N</td>
</tr>
<tr>
<td>Authors</td>
<td>Year</td>
<td>N</td>
<td>Patients</td>
<td>Study Length</td>
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<tr>
<td>Fux et al.</td>
<td>2004</td>
<td>11</td>
<td>Patients with OCD; cross-over design</td>
<td>6wks</td>
<td>YBOCS, HDRS, HRSA</td>
<td>EPA (2g/d) Placebo=liquid paraffin (2g/d)</td>
<td>No significant differences between groups; both groups improved on YBOCS, but not on HRSD or HRSA</td>
</tr>
<tr>
<td>Hirashima et al.</td>
<td>2004</td>
<td>21</td>
<td>Women with bipolar disorder</td>
<td>4wks</td>
<td>HDRS, YMRS</td>
<td>EPA (5-5.2g/d) + DHA (3-3.4g/d) EPA (1.3g/d) + DHA (0.7g/d) Placebo (undefined + adjunct treatment)</td>
<td>No significant differences between groups; no significant change in HDRS or YMRS in the three groups across time</td>
</tr>
<tr>
<td>Fontani et al.</td>
<td>2005</td>
<td>49</td>
<td>Healthy Adults</td>
<td>5wks</td>
<td>POMS</td>
<td>EPA (1.6g/d) + DHA (0.8g/d) Placebo=4g olive oil</td>
<td>Increase in vigour and decreases in anger, anxiety, depression and fatigue from baseline to 5 weeks in treatment group</td>
</tr>
<tr>
<td>Silvers et al.</td>
<td>2005</td>
<td>77</td>
<td>Depressed participants</td>
<td>12wks</td>
<td>HDRS-SF, BDI</td>
<td>EPA (0.6g/d) + DHA (2.4g/d) Placebo=8g/d olive oil + concurrent depression treatment</td>
<td>No difference between groups; mood improved significantly in both groups</td>
</tr>
</tbody>
</table>

Overall benefit: N

Overall benefit: N

Overall benefit: Y

Overall benefit: N
<table>
<thead>
<tr>
<th>Authors</th>
<th>Year</th>
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<th>Patients</th>
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<th>Assessment of depression</th>
<th>Dose/day</th>
<th>Depression/Mood Outcomes</th>
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<tbody>
<tr>
<td>Buydens-Branchey &amp; Branchey</td>
<td>2006; 2008</td>
<td>24</td>
<td>Non-clinical substance abusers</td>
<td>12wks</td>
<td>Tension and anger scales from POMS</td>
<td>EPA (2.25g/d) + DHA (0.5g/d) + other n-3 PUFAs (0.25g/d) Placebo=soybean oil</td>
<td>Treatment group showed significant difference in tension and anger scores compared to placebo</td>
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<td>Overall benefit: Y</td>
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<tr>
<td>Frangou et al.</td>
<td>2006</td>
<td>75</td>
<td>Out-patients with bipolar depression</td>
<td>12wks</td>
<td>HDRS, YMRS, CGI</td>
<td>Ethyl-EPA (1g/d, 2g/d) Placebo= paraffin oil</td>
<td>No benefit of 2g over 1g; significant improvement with ethyl-EPA treatment compared to placebo on HRSD and CGI; no significant differences between treatment and placebo on YMRS</td>
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<td>Overall benefit: Y</td>
</tr>
<tr>
<td>Keck et al.</td>
<td>2006</td>
<td>116</td>
<td>Patients with bipolar depression or rapid cycling bipolar disorder</td>
<td>16wks</td>
<td>IDS, YMRS, CGI-Bipolar Disorder</td>
<td>EPA (6g/d) Placebo=liquid paraffin + adjunct treatment</td>
<td>No difference between groups</td>
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<td></td>
<td>Overall benefit: N</td>
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<tr>
<td>Nemets et al.</td>
<td>2006</td>
<td>20</td>
<td>Children with major depression</td>
<td>16wks</td>
<td>CDRS, CDI, CGI</td>
<td>EPA (0.4g/d) + DHA (0.2g/d)</td>
<td>Highly significant effects of treatment on symptoms</td>
</tr>
<tr>
<td>Grenyer et al.</td>
<td>2007</td>
<td>83</td>
<td>Outpatients with MDD</td>
<td>16wks</td>
<td>HDRS, BDI, GAFS</td>
<td>EPA (0.6g/d) + DHA (2.2g/d) + concurrent depression treatment</td>
<td>No benefit compared to placebo; improvements in both groups</td>
</tr>
<tr>
<td>Hallahan et al.</td>
<td>2007</td>
<td>49</td>
<td>Repeated self-harm</td>
<td>12wks</td>
<td>BDI, HDRS, OAS-R, PSS, DHUS</td>
<td>EPA (1.2g/d) + DHA (0.9g/d) + corn oil + 1% EPA/DHA</td>
<td>Significantly greater improvement in depression, suicidality, and daily stresses; no difference on impulsivity, aggression and hostility</td>
</tr>
<tr>
<td>da Silva et al.</td>
<td>2008</td>
<td>29</td>
<td>Patients with PD and major depression</td>
<td>12wks</td>
<td>MADRS, CGI, BDI</td>
<td>EPA (0.64g/d) + DHA (0.48g/d)</td>
<td>Significant decrease in MADRS and CGI-depression scores compared to placebo; no difference in BDI</td>
</tr>
<tr>
<td>Authors</td>
<td>Year</td>
<td>N</td>
<td>Patients</td>
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<tr>
<td>Freeman et al.</td>
<td>2008</td>
<td>59</td>
<td>Perinatal women with MDD</td>
<td>8wks</td>
<td>EPDS, HDRS; CGI</td>
<td>EPA (1.1g/d) + DHA (0.8g/d) Placebo= corn oil + 1% n-3 PUFAs</td>
<td>Both groups showed significant decreases in depression symptoms; no benefit of treatment over placebo</td>
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<tr>
<td>Freund-Levi et al.</td>
<td>2008</td>
<td>204</td>
<td>Patients with AD</td>
<td>24wks</td>
<td>NPI, MADRS</td>
<td>DHA (1.7g/d) + EPA (0.6g/d) Placebo= corn oil</td>
<td>No significant treatment effects; significant positive treatment effects on scores in the NPI agitation domain in APOE-4 carriers; and on MADRS scores in non-APOE-4 carriers</td>
</tr>
<tr>
<td>Jazayeri et al.</td>
<td>2008</td>
<td>60</td>
<td>Outpatients with MDD</td>
<td>8wks</td>
<td>HDRS</td>
<td>E-EPA (1.0g/d) + fluoxetine Placebo (rapeseed oil) Fluoxetine (20mg/d) + E-EPA Placebo (rapeseed oil) E-EPA (1.0g/d) + fluoxetine (20mg/d)</td>
<td>E-EPA+fluoxetine treatment group had significantly lower HDRS than other 2 groups</td>
</tr>
<tr>
<td>Rogers et al.</td>
<td>2008</td>
<td>190</td>
<td>Mild-to-moderate depression</td>
<td>12wks</td>
<td>DASS, BDI, GHQ, STAXI, Mood diary</td>
<td>EPA (0.63g/d) + DHA (0.85g/d) + olive oil (0.87g/d) Placebo=olive oil (2.36g/d)</td>
<td>No difference between groups on depression</td>
</tr>
</tbody>
</table>

Overall benefit: N
<table>
<thead>
<tr>
<th>Authors</th>
<th>Year</th>
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<th>Overall benefit</th>
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</thead>
<tbody>
<tr>
<td>Rees et al.</td>
<td>2008</td>
<td>26</td>
<td>Women with MDD during perinatal period</td>
<td>6wks</td>
<td>EPDS, HDRS, MADRS</td>
<td>DHA (1.64g/d) + EPA (0.42g/d) Placebo= sunola oil</td>
<td>No significant difference in depression scores</td>
<td>N</td>
</tr>
<tr>
<td>Su et al.</td>
<td>2008</td>
<td>24</td>
<td>Pregnant women with MDD</td>
<td>8wks</td>
<td>HDRS, BDI, EPDS</td>
<td>EPA (2.2g/d) + DHA (1.2g/d) Placebo= olive oil ethyl esters</td>
<td>Treatment group significantly lower depressive symptoms and significantly higher remission rate compared to placebo</td>
<td>Y</td>
</tr>
<tr>
<td>van de Rest et al.,</td>
<td>2008</td>
<td>302</td>
<td>Non-depressed, community-dwelling older adults</td>
<td>26wks</td>
<td>CES-D, MADRS, GDS, HADS-A, POMS</td>
<td>EPA (1.09g/d) + DHA (0.85g/d); EPA (0.23g/d) + DHA (0.18g/d) Placebo= sunflower oil high in oleic acid</td>
<td>Treatment had no impact on measures of depression or mood</td>
<td>N</td>
</tr>
<tr>
<td>Antypa et al.</td>
<td>2009</td>
<td>54</td>
<td>Healthy, university students</td>
<td>4wks</td>
<td>POMS</td>
<td>EPA (1.74g/d) + DHA (0.25g/d) Placebo=3g olive oil</td>
<td>No impact on self-reported mood, except for small effect on fatigue</td>
<td>N</td>
</tr>
<tr>
<td>Authors</td>
<td>Year</td>
<td>N</td>
<td>Patients</td>
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<tr>
<td>Carney et al.</td>
<td>2009</td>
<td>122</td>
<td>Patients with CHD and major depression</td>
<td>10wks</td>
<td>BDI, HDRS</td>
<td>EPA (0.93g/d) + DHA (0.75g/d) Placebo=corn oil Both + sertraline (0.05g/d)</td>
<td>No significant differences between groups Overall benefit: N</td>
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<tr>
<td>Doornbos et al.</td>
<td>2009</td>
<td>119</td>
<td>Healthy, pregnant women</td>
<td>Wk16 of pregnancy to 3 months postpartum</td>
<td>EPDS, blues questionnaire</td>
<td>DHA (0.22g/d), DHA (0.22g/g) + AA (0.22g/d) Placebo= soy bean oil</td>
<td>No mean, or change score differences between groups; no mean difference in incidence of severity of postpartum blues; RBC DHA, AA and DHA/AA ratio did not correlate with EPDS or blues scores Overall benefit: N</td>
<td></td>
</tr>
<tr>
<td>Bot et al.</td>
<td>2010</td>
<td>25</td>
<td>Diabetic patients with MDD</td>
<td>12wks</td>
<td>MADRS</td>
<td>Ethyl-EPA (1g/d) Placebo=rapeseed oil</td>
<td>No significant differences between groups; significant improvement in both groups Overall benefit: N</td>
<td></td>
</tr>
<tr>
<td>Gracious et al.</td>
<td>2010</td>
<td>51</td>
<td>Children with bipolar depression</td>
<td>16wks</td>
<td>YMRS, CDRS-R, CGI</td>
<td>ALA (0.55g/d) Placebo=olive oil</td>
<td>No significant difference between groups; correlation between n-3 PUFAs with CGI scores Overall benefit: N</td>
<td></td>
</tr>
<tr>
<td>Authors</td>
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<td>Makrides et al.</td>
<td>2010</td>
<td>2399</td>
<td>Women with singleton pregnancies &lt;23 wks</td>
<td>&lt;21wks gestation – 24wks postpartum</td>
<td>EPDS</td>
<td>DHA (0.8g/d) + EPA (0.1g/d) Placebo= vegetable oil</td>
<td>No significant difference in % of women reporting high levels of depressive symptoms at 6wks or 6months postpartum</td>
<td>Overall benefit: N</td>
</tr>
<tr>
<td>Rondanelli et al.</td>
<td>2010</td>
<td>46</td>
<td>Depressed elderly female patients</td>
<td>8wks</td>
<td>GDS, SF-36</td>
<td>EPA (1.67g/d) + DHA (0.83g/d) Placebo= paraffin oil</td>
<td>Treatment group showed significant improvements in GDS and SF-36 (physical and mental scales) compared to placebo</td>
<td>Overall benefit: Y</td>
</tr>
<tr>
<td>Kiecolt-Glaser et al.</td>
<td>2011</td>
<td>68</td>
<td>Healthy medical students</td>
<td>12wks</td>
<td>CES-D, BAI</td>
<td>EPA (2.09g/d)+DHA (0.35g/d) Placebo= mixture of palm, olive, soy, canola and coco butter oils</td>
<td>Significant difference between treatment and placebo on anxiety, but not depression Correlation between n-6:n-3 ratio and anxiety</td>
<td>Overall benefit: Y</td>
</tr>
<tr>
<td>Authors</td>
<td>Year</td>
<td>N</td>
<td>Patients</td>
<td>Study Length</td>
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<tr>
<td>Antypa et al.</td>
<td>2012</td>
<td>71</td>
<td>Recovered Depressed Adults</td>
<td>16wks</td>
<td>POMS, BDI</td>
<td>EPA (1.74g/d)+DHA(0.25g/d) Placebo=olive oil</td>
<td>Significant difference between groups on depression and tension subscales of the POMS; no significant difference on the BDI</td>
<td></td>
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</tbody>
</table>

Overall benefit: Y

AD, Alzheimer’s Disease; ALA, alpha linolenic acid; BAI, Beck Anxiety Inventory; BDI, Beck Depression Inventory; CDI, Children’s Depression Inventory (self-rated); CDRS, Children’s Depression Rating Scale (clinician rated); CDRS-R, Child Depression Rating Scale-Revised; CES-D, Center for Epidemiological Studies Depression Scale; CGI, Clinical Global Impression Scale; CHD, Coronary Heart Disease; DHA, docosahexaenoic acid; E-DHA, ethyl-docosahexaenoic acid; E-EPA, ethyl-eicosapentaenoic acid; EPA, eicosapentaenoic acid; EPDS, Edinburgh Postnatal Depression Scale; GAFS, Global Assessment of Functioning Scale; GAS, Global Assessment Scale; GDS, Geriatric Depression Scale; GHQ, General Health Questionnaire; HADS-A, Hospital Anxiety and Depression Scale; HDRS-SF, Short Form of the Hamilton Rating Scale for Depression; HRSA, Hamilton Rating Scale for Anxiety; HDRS, Hamilton Depression Rating Scale; HSCL-D-20, Hopkins Symptom Checklist Depression Scale; IDS, Inventory for Depressive Symptomology; LA, linoleic acid; MADRS, Montgomery Åsberg Depression Scale; MDD, major depressive disorder; NPI, Neuropsychiatric Inventory; OAS-M, Overt Aggression Scale, Modified; OCD, Obsessive-Compulsive Disorder; PD, Parkinson’s Disease; P-F Study, an aggression-estimating test; PGWB, Psychological General Well-Being Schedule; POMS, Profile of Mood States; PSS, Perceived Stress Scale;
DHUS, Daily Hassles and Uplifts Scale; SF-36, Short-form 36-Item Health Survey; STAXI, State-Trait Anger Expression Inventory; YBOCS, Yale-Brown Obsessive-Compulsive Scale; YMRS, Young Mania Rating Scale.
The placebo-controlled trials presented in Table 3 were all designed to assess the
effect of $n$-3 LC PUFA treatment compared to placebo on outcomes ranging from
depression (e.g., the Hamilton Depression Rating Scale) to mood (e.g., the Profile of
Mood States) and aggression (e.g., the Overt Aggression Scale). Additionally, they have
employed a range of population types from those diagnosed with major depressive
disorder (MDD; e.g., Nemets, Stahl & Belmaker, 2002) to healthy older adults (e.g., van
de Rest et al., 2008). The effect of $n$-3 LC PUFA on mood does not appear to be mediated
by either type of outcome variable used, or population type. Populations employed in the
placebo-controlled trials listed in Table 3 can be categorised into five broad classes:
individuals with depression ($N$ trials=15), individuals with depression and co-morbid
physical disease ($N$ trials=3), pregnant women ($N$ trials=6), individuals with a mental
illness other than depression ($N$ trials=7), and non-depressed healthy individuals ($N$
trials=7).

Approximately half (8/15) of $n$-3 LC PUFA trials in depressed individuals
reported a positive effect of treatment and, of these; all used an EPA-rich treatment. Only
two trials in depressed individuals that reported no effect of $n$-3 LC PUFA treatment used
an EPA-rich treatment; trial lengths ranged from 4-to-16 weeks in those that reported a
treatment effect and those that did not. There were only three reported trials with
depressed individuals with co-morbid illness – diabetes (Bot et al., 2010), coronary heart
disease (Carney et al., 2009), and Parkinson’s Disease (da Silva et al., 2008) and only one
reported a significant effect of treatment (da Silva et al., 2008) despite all using higher
EPA-to-DHA concentrations. The potential for $n$-3 LC PUFAs to alleviate depressed
mood during pregnancy has received much attention (e.g., Makrides, 2009) due to the
increased requirement for $n$-3 LC PUFAs during this time; however, five of six placebo-
controlled trials found no significant benefit of $n$-3 LC PUFA treatment over placebo. The trial to report a significant treatment effect was unique only in that it had the smallest sample size ($n=24$, Su et al., 2008; c.f. to $n=2399$, Makrides et al., 2010) and provided the largest dose of EPA (2.2g/d) of all these studies, with the second largest dose in these trials being 1.1g/d (Freeman et al., 2008). Trials conducted in patients with a mental disorder other than depression included those with repeated self-harm (Hallahan et al., 2007), patients with Alzheimer Disease (AD; Freund-Levi et al., 2008), postmenopausal women with psychological distress and depressive symptoms (Lucas et al., 2009), individuals with postviral chronic fatigue (Behan et al., 1990), chronic fatigue syndrome (Warren, McKendrick & Pea, 1999), individuals with obsessive-compulsive disorder (Fux, Benjamin & Nemets, 2004), and non-clinical substance abusers (Buydens-Branchey & Branchey, 2006, 2008). Again, no pattern emerged differentiating the three that reported a significant treatment effect (Behan et al., 1990; Buydens-Branchey & Branchey, 2006, 2008; Hallahan et al., 2007) from those that did not (Freund-Levi et al., 2008; Fux et al., 2004; Lucas et al., 2009; Warren, McKendrick & Pea, 1999). As a result of large methodological differences, conclusions regarding the overall efficacy of $n$-3 LC PUFAS for mood are premature. Length of trial, $n$-3 LC PUFA type and dosage, sample type and size, properties of the placebo employed, and assessment of depressed mood all vary across these published trials. The only consistent preliminary statement based on these trials is that higher EPA dosages appear to be linked to positive treatment effects.

**Omega-3 Long-Chain Polyunsaturated Fatty Acids and Mental Health:**

**Randomised Control Trials with Healthy Adults**

Mood may be improved with $n$-3 LC PUFA supplementation in healthy, non-depressed adults (e.g., Fontani et al., 2005), potentially acting as a protective factor
against depressed mood. Of eight identified trials conducted in non-depressed healthy adults, four reported a significant treatment effect (Antypa et al., 2012; Fontani et al., 2005; Hamazaki et al., 1996; Kiecolt-Glaser et al., 2011), and four reported no treatment effect (Antypa et al., 2009; Bradbury, Myers & Oliver, 2004; Hamazaki et al., 1998; van de Rest et al., 2008).

Hamazaki and colleagues (1996, 1998) conducted two early placebo-controlled trials to investigate the effect of n-3 LC PUFA treatment for mood in healthy participants. Both studies employed a small sample (n=41, n=46 respectively) of healthy university students supplemented with a DHA-rich treatment (1.5g/d) for 12 weeks. In their first trial, Hamazaki and colleagues (1996) proposed that treatment would influence extraggression (aggression against others) compared to placebo in University students (n=41; 71% female) during a period of stress. The trial began at the end of summer vacation and the final assessment occurred 12 weeks later during final examinations and theses submissions; aggression was measured using the P-F test, where reactions to 24 illustrations of frustration were recorded. Extraggression significantly increased from baseline to 12 weeks in the control group, but remained stable in the DHA group, therefore the authors concluded that during stress, DHA intake stabilised extraggression.

In a follow-up trial, Hamazaki and colleagues (1998) sought to replicate these results under non-stressful conditions. University students (n=46; 48% female) completed the same P-F test of aggression in addition to a measure of hostility. Contrary to expectation, extragression remained stable from baseline to 12 weeks in the DHA group, and significantly decreased in the control group, and there were no changes in hostility. Thus, extragression did not change over 12 weeks of DHA treatment either with (1996), or
without (1998), a stressor, and whilst, extragggression increased under stress in the placebo group, it actually decreased in the placebo group without a stressor.

Methodologically, the two trials (Hamazaki et al., 1996; Hamazaki et al., 1998) were very similar: treatment and placebo composition and dosages were almost identical, both required participants to maintain their regular weight, physical activity, and food intake, both reported no significant differences between groups in lipid intake based on Food Frequency Questionnaires, and both reported significant increases in DHA and EPA as a result of treatment. In their 1998 paper, Hamazaki and colleagues suggested that extragggression may have unexpectedly decreased in the control group due to testing habituation, but allow that this does not provide a wholly adequate account because no such decrease in extragggression was observed in the DHA group. It is also possible that DHA only has a beneficial effect of extragggression during stressful periods. Differences between the changes in other fatty acids within the treatment and placebo groups of both trials may offer another explanation: Palmitic acid (a saturated fatty acid) and vaccenic acid (a trans-fatty acid) both significantly decreased from baseline to 12 weeks in the DHA group only in the 1996 trial but remained stable in the 1998 trial, and linoleic acid (an unsaturated n-6 Fatty Acid) significantly increased in the control group from baseline to 12 weeks in 1998 but remained stable in the 1996 study. Palmitic acid has been associated with increased risk of depression (Astorg et al., 2009) and higher concentrations of vaccenic acid have been found in patients with major depressive disorder and bipolar disorder (McNamara et al., 2010), and in post-mortem orbitofrontal cortex of schizophrenia patients (McNamara et al., 2007) compared to controls. Thus, the decrease in palmitic and vaccenic acid in the DHA group in 1996 may account for the observed decrease in extragggression, rather than the increase in DHA concentrations.
Furthermore, it has been reported that linoleic acid is significantly associated with a decreased risk of depression (Astorg et al., 2009) and therefore the increase in linoleic acid observed in the control group in the 1998 study may account for the observed decrease in extraggression.

More recently, three RCTs (Antypa et al., 2012; Fontani et al., 2005; Kiecolt-Glaser et al., 2011;) have all reported positive effects of \( n-3 \) LC PUFA supplementation on mood in healthy adults (Fontani et al., 2005), healthy university students (Kiecolt-Glaser et al., 2011), and in adults who have recovered from depression (Antypa et al., 2012).

Fontani and colleagues (2005) reported significant improvements in the depression, anxiety, fatigue, anger, and vigour subscales from the POMS (Profile of Mood States) following five weeks of daily EPA-rich treatment (EPA:DHA, 2:1) in 49 healthy adults (age: \( M=33, \ SD=7.0 \) years). These results were partially replicated (Antypa et al., 2012) in a slightly younger sample of adults (age: treatment \( M=25.8, \ SD=11.8 \) years; placebo \( M=23.5, \ SD=6.0 \) years) who had recovered from depression. Significant treatment effects, again with an EPA-rich treatment (EPA:DHA, \( \approx 7:1 \)), were reported for the POMS subscales of depression and anxiety, but not for the subscales of fatigue, anger or vigour (Antypa et al., 2012). Although both these studies reported significant effects of treatment on the depression subscale of the POMS, no treatment effect was found for depression as measured by the BDI (Antypa et al., 2012). Although these constructs have been shown to be highly correlated in both student and adult samples (\( r=0.69, \ p<0.01; \ r=0.76, \ p<0.01 \), respectively; Watson et al., 1995), this still only equates to 48% and 58% shared variance between the two measures.
The depression subscale from the POMS and the BDI measure two slightly different conceptualisations of depression, this difference provides an account for the contrast in conclusions drawn. The BDI (Beck et al., 1979) is a measure of syndrome depression, designed to measure the severity of depressive symptoms. Participants respond to one of four statements for each of the 21-items that describes the way they have been feeling during the past two weeks, including today; for example, (0) “I do not feel sad” (1) “I feel sad much of the time” (2) “I am sad all the time” (3) “I am so sad or unhappy that I can’t stand it”. The POMS measures multiple dimensions of affect (e.g., anxiety and depression); it contains 65 mood adjectives (e.g., sad, gloomy) to which respondents rate the extent to which they have experienced each mood state on a 5-point scale from (0) “Not at all” to (4) “Extremely”. It is recommended for evaluating interventions as it provides a measure of fluctuating mood states (McNair, Lorr & Droppleman, 1971). The depression subscale contains 15 adjectives; for example, sad, gloomy, miserable, lonely, helpless, worthless). Therefore, whilst the BDI provides a measure of syndrome depression, the depression subscale of the POMS provides a measure of depression as an affective state, this could therefore account for why improvement in depression was only observed when measured with the POMS. Furthermore, scores less than 10 on the BDI indicate no, or minimal depression; the means for both treatment and placebo groups, pre- (M=5.7, SD=5.0; M=7.7, SD=5.9, respectively) and post- treatment (M=6.6, SD=7.3; M=6.5, SD=6.4, respectively) reported by Antypa and colleagues (2012) fell well below this cut-off.

Kiecolt-Glaser and colleagues (2011) reported a significant effect of a daily EPA-rich treatment (EPA:DHA, \( \approx 6:1 \)) on anxiety, as measured by the Beck Anxiety Inventory), but not on depression, as measured by the CES-D scale in 68 healthy university medical
students (age: $M=23.65$, $SD=1.87$ years). The CES-D is designed to estimate the presence of depressive symptoms, so similarly to the BDI, this could explain why no change was observed in a non-depressed sample. This sample did not have an anxiety disorder and baseline levels on the BAI, a measure of cognitive and physiological symptoms of anxiety designed to discriminate anxiety from depression (Beck et al., 1988), were low (median: treatment 3.5, placebo 2.5: total scale range 0-63), suggesting that $n$-3 LC PUFAs may benefit symptoms of anxiety in students without an anxiety disorder.

These RCTs with healthy participants all employed EPA-rich treatments (1.6g/d, 1.74g/d, 2.09g/d) in combination with lower DHA dosages (0.8g/d, 0.25g/d, 0.35g/d) and all reported significant benefit of treatment for improving anxiety – this was across two different measures of anxiety: the subscale of the POMS (Antypa et al., 2012; Fontani et al., 2005), and as measured by the BAI (Kiecolt-Glaser et al., 2011). The results for improving depression outcomes are less clear; positive results were reported using the depression subscale from the POMS (Antypa et al., 2012; Fontani et al., 2005) but no treatment effects for depression were reported when measured by the BDI (Antypa et al., 2012) or the CES-D (Kiecolt-Glaser et al., 2011), potentially due to the depression subscale of the POMS measuring depressed mood, compared to the BDI and the CES-D measuring depressive symptoms, as discussed.

Bradbury and colleagues (2004), van de Rest and colleagues (2008), and Antypa and colleagues (2009) all found no significant effects of treatment with $n$-3 LC PUFAs on measures of mood. Bradbury and colleagues (2004) assessed the utility of a low-dose, DHA-rich treatment (EPA:DHA, ≈0.25:1) to reduce stress over six weeks in 30 stressed university staff (placebo: $M=44.43$, $SD=9.39$ years; treatment: $M=40.69$, $SD=7.39$ years).
They reported improvements in stress, as measured by the Perceived Stress Scale (PSS; Cohen, Kamarck & Mermelstein, 1983), in both the treatment and placebo groups, though no significant differences between groups. The authors provided several reasons for these results such as sampling bias, power, the placebo effect, blinding and use of the olive oil as placebo. Blinding was found to be an issue in this trial. All participants in the treatment group correctly identified their group allocation reportedly due to the fishy after-taste, and half of the participants in the placebo group incorrectly identified that they were in the treatment group due to self-perceived trial benefits. This provides one explanation for the results, the use of olive oil as placebo was offered as another explanation. Although there are documented issues with the use of olive oil as a placebo in n-3 LC PUFA trials (e.g., Puri & Richardson, 2000), others have reported treatment effects with an olive oil placebo (Antypa et al., 2012; Fontani et al., 2005) suggesting that it can serve as an appropriate placebo.

The largest trial (N=302) conducted within a healthy population (van de Rest et al., 2008), reported no benefit of a 26 week n-3 LC PUFA treatment for mood or depression in community-dwelling, non-depressed, older (>65years) adults. Placebo was compared to daily treatment with either a high- (≈1800mg EPA+DHA), or low-dose (≈400mg EPA+DHA) treatment; neither group demonstrated improvement in mood or depression outcomes despite encompassing a range of measurements including the CES-D, MADRS, Geriatric Depression Scale (GDS), Hospital Anxiety and Depression Scale (HADS-A), and the POMS (van de Rest et al., 2008). These authors reported no issues with treatment blinding, there was no significant difference between groups on number of participants who thought they were in the treatment group and compliance was confirmed with EPA and DHA plasma cholesteryl esters.
In a trial of younger participants (treatment $M=22.2$, $SD=3.6$ years; placebo $M=22.6$, $SD=4.1$ years; Antypa et al., 2009), four week EPA-rich (EPA:DHA, $\approx 7:1$) treatment had no effect on self-reported mood states as measured by the POMS in 54 healthy university students. This result contrasts with Antypa and colleagues’ (2012) later study, discussed above, that reported a positive effect on the depression and anxiety subscales of the POMS in adults who had recovered from depression. Both of these studies employed the same treatment dose, the same placebo, and both had a similarly uneven ratio of females to males. The main differences were that the later study that reported a positive treatment effect had a slightly larger sample size ($n=71$ compared to $n=54$ in 2009), a longer treatment period (16 weeks compared to 4 weeks in 2009), and employed a different sample of participants. Other studies have reported positive treatment effects with smaller sample sizes (Fontani et al., 2005; Hamazaki et al., 1996), and with trial lengths of 5 weeks (Fontani et al., 2005). Both studies (Antypa et al., 2009; 2012) employed healthy adults; however, participants in the 2012 study had recovered from depression. Assessment of negative mood states within groups for both studies (see Table 4), revealed higher scores on negative mood states for this sample. It is possible that this accounts for the different results because this sample had more room for improvement.
Table 4

POMS subscale scores at baseline reported by Antypa and colleagues (2009, 2012) in two samples: one that reported a treatment effect for the POMS (n=71, 2012) and one that did not (n=54, 2009).

<table>
<thead>
<tr>
<th>POMS Subscales</th>
<th>Treatment Group</th>
<th>Placebo Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2009</td>
<td>2012</td>
</tr>
<tr>
<td>Depression</td>
<td>2.07 (2.97)</td>
<td>3.1 (4.6)</td>
</tr>
<tr>
<td>Anxiety</td>
<td>2.44 (2.22)</td>
<td>3.1 (4.6)</td>
</tr>
<tr>
<td>Anger</td>
<td>2.15 (2.21)</td>
<td>2.4 (2.2)</td>
</tr>
<tr>
<td>Fatigue</td>
<td>2.85 (2.48)</td>
<td>4.8 (4.1)</td>
</tr>
<tr>
<td>Vigour</td>
<td>8.63 (3.28)</td>
<td>9.0 (4.0)</td>
</tr>
</tbody>
</table>

POMS, Profile of Mood States.

Note: Although van de Rest et al., (2008) and Fontani et al., (2005) used the POMS with a healthy sample, this was not assessed at baseline (van de Rest et al, 2008), and mean values were not reported (Fontani et al., 2005).

Gender did also not appear to contribute to results obtained with healthy participants. There were three well-balanced studies, with regard to gender, in healthy participants: two reported no treatment effect (Hamazaki et al., 1998; van de Rest et al., 2008) while the other reported an effect (Kiecolt-Glaser et al., 2011). Three other trials reported an effect with relatively unbalanced gender ratios (Antypa et al., 2012; Fontani et al., 2005; Hamazaki et al., 1996), two others reported no effect with similarly unbalanced
designs (Antypa et al., 2009; Bradbury et al., 2004). As can be seen in Table 5, all unbalanced designs favoured females.

Table 5

*Male and female participants from RCTs with healthy participants.*

<table>
<thead>
<tr>
<th>Author</th>
<th>Treatment Group</th>
<th>Placebo Group</th>
<th>Total Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males</td>
<td>Females</td>
<td>Males</td>
</tr>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>Positive Treatment Effect</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hamazaki et al., 1996</td>
<td>5 (23)</td>
<td>17 (77)</td>
<td>7 (37)</td>
</tr>
<tr>
<td>Fontani et al., 2005</td>
<td>13 (39)</td>
<td>20 (61)</td>
<td>4 (25)</td>
</tr>
<tr>
<td>Kiecolt-Glaser et al., 2011</td>
<td>18 (53)</td>
<td>16 (47)</td>
<td>20 (59)</td>
</tr>
<tr>
<td>Antypa et al., 2012</td>
<td>6 (17)</td>
<td>30 (83)</td>
<td>27 (20)</td>
</tr>
<tr>
<td>No Treatment Effect</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hamazaki et al., 1998</td>
<td>13 (59)</td>
<td>9 (41)</td>
<td>11 (46)</td>
</tr>
<tr>
<td>Bradbury et al., 2004</td>
<td>6 (37.5)</td>
<td>10 (62.5)</td>
<td>3 (21)</td>
</tr>
<tr>
<td>van de Rest et al., 2008</td>
<td>53 (55)a</td>
<td>43 (45)b</td>
<td>59 (56)</td>
</tr>
<tr>
<td>Antypa et al., 2009</td>
<td>6 (22)</td>
<td>21 (78)</td>
<td>4 (15)</td>
</tr>
</tbody>
</table>

aHigh-dose treatment participants reported here; low-dose: males n=55 (56%)
bHigh-dose treatment participants reported here; low-dose: females n=45 (45%)
Omega-3 Long-Chain Polyunsaturated Fatty Acids and Mental Health:
Mechanistic Accounts

Several biological mechanisms have been proposed to account for how n-3 LC PUFAs may influence the pathophysiology of depressed mood. Two main pathways that have received most support concern the anti-inflammatory properties of n-3 LC PUFAs and their role in cellular structure and functioning. n-3 LC PUFAs inhibit production of pro-inflammatory eicosanoids (Browning, 2003) such as prostaglandin E2 (PGE2; Das, 2006; Farooqui, Ong & Horrocks, 2006; James, Gibson & Cleland, 2000; Su, 2009), thus having anti-inflammatory properties. Chronic inflammation can result in oxidative stress by increasing free radicals (Su, 2009) and it has been associated with chronic physical and mental disorders, including depression (Das, 2004; Su, 2009; Young & Conquer, 2005;)

n-3 LC PUFAs can also regulate signal transduction (Sinclair et al., 2007; Su, 2009) via their influence on neural plasticity (Morgan & Jorm, 2008; Sinclair et al., 2007). Deficiencies in n-3 LC PUFAs have been associated with decreases in dopamine and increases in serotonin (Berg, Maayani & Clarke, 1996; Chalon et al., 1998; Chalon et al., 2001; Delion et al., 1994; Delion et al., 1996; Farooqui, Hirashima & Horrocks, 1992; Su, 2009; Vaddadi, 2006). High n-3 LC PUFAs have been associated with high 5-hydroxy-indoleacetic acid (5-HIAA; Hibbeln et al., 1998) – an indicator of serotonin turnover in the brain – and increases in n-3 LC PUFAs have been shown to increase 5-HIAA (Nizzo et al., 1978). Dopaminergic and serotonergic functions are thought to play a role in depression (Maes & Meltzer, 1995; Su, 2009).
Omega-3 Long-Chain Polyunsaturated Fatty Acids and Mental Health:

Concluding Comments

National prevalence rates of depression and their apparent correlation with national rates of fatty acid consumption provided the initial justification for interest in this area. Early research reported lower concentrations of $n$-3 LC PUFAs in patient populations compared to healthy controls and whilst some cross-sectional studies reported an association, these were necessarily confounded by the bidirectional relationship present between diet and mood. There are now a substantial number of Randomised Control Trials in the area, supported by plausible potential biological accounts for a connection. Methodological heterogeneity has limited consensus positions at this stage.

Nutrition and Mental Health:

Overall Conclusion

Focused public health campaigns have been largely successful: for example, we are all aware that excessive alcohol is bad for your liver, that exercise is good for your heart and lungs, and that calcium is necessary for healthy bones and teeth. The commonality here is the focus on physical health. What do we know about maintaining good mental health? Public health messages concerned with mental health focus on what to do or how to seek help for mental health problems. There is little publicly available information to assist individuals to autonomously maintain their own good mental health. This can be seen as partly reflecting the belief that good mental health occurs by default in the absence of mental illness.

This chapter has summarised the literature relevant to Subjective Wellbeing (SWB), and has presented the current state of knowledge regarding the potential influence of omega-3 long-chain polyunsaturated fatty acids ($n$-3 LC PUFAs), folate, vitamin $B_{12}$,
and homocysteine on depressed mood, and when available, on wellbeing. We first sought to empirically compare and contrast four different models of SWB that are used interchangeably in the literature. This was done to highlight their differences and provide evidence to support one model to be recommended for use. This is essential for measurement consistency of relevance to a range of research disciplines that employ SWB as an important outcome variable, and of relevance nationally as SWB variables are increasingly employed alongside traditional measures of success, such as GDP and GNP.

Our second aims were to establish whether n-3 LC PUFAs alter the normative trajectory of SWB over 18 months, and whether folate and/or vitamin B_{12} impact SWB, and if they do, whether this is a direct relationship or mediated via the amino acid homocysteine. These nutritional components were investigated here as growing evidence suggests they are implicated in mental illnesses such as depression. Investigation of these factors with a measure of good mental health therefore served as a departure from the traditional focus on mental illness and treatment. Identification of nutrition as important for good mental health offers a potential preventative measure that could be easily disseminated via public health campaigns, and which is easily modifiable and available to all.

The following chapter contains the exegesis of this research program to provide additional background and rationale for each paper within the thesis, along with an overview of the study design and methodology.
Chapter 2. Exegesis

Preamble

This body of research sought to investigate Subjective Wellbeing (SWB), a measure of positive mental health, and the role that nutrition may play in SWB. This was motivated by the desire to address issues of interest to the majority of the population. Thus, the focus was on good mental health, rather than mental illness, and we were interested in whether this state of positive functioning was associated with a lifestyle factor freely available to all, namely nutrition.

Traditionally, research has focused on mental illness and its treatment with psychotropic medications such as Selective Serotonin Re-Uptake Inhibitors (SSRIs) or tricyclic antidepressants. However, in his role as President of the APA, Martin Seligman officially recognised the need for research focused on Positive Psychology; we therefore employed Subjective Wellbeing (SWB) as our outcome measure of interest throughout this thesis as opposed to the more traditionally used measures of ill health such as depression. Furthermore, it is also generally acknowledged that SSRIs and tricyclic antidepressant as treatment for depression are not suitable for long-term use, are effective for fewer than 50% of patients (e.g., Berton & Nestler, 2006) and are associated with side-effects for up to nearly 50% of patients (Snow, Lascher & Mottur-Pilson, 2000). Investigation of nutrition for the promotion of positive mental health, rather than medication as treatment for mental illness is an area of interest given that fostering psychological strengths may prevent subsequent occurrence of mental ill health (Seligman, 2008; Venning et al., 2011). Nutrition was investigated over other lifestyle
factors because it offers a readily modifiable, cheap, easily accessible, side-effect free, and more holistic approach to the possible prevention of low mood.

Current evidence supports the idea that nutrition may have a beneficial role as a modifiable risk factor for poor mental health. We sought to investigate whether the dietary components of folate, vitamin B\textsubscript{12}, and n-3 long chain polyunsaturated fatty acids (n-3 LC PUFAs) were associated with SWB in a sample of older, community living adults. We focused on a sample of older individuals because this population can be vulnerable to mental illness and reduced nutrient intake, which often interact to compound associated disability and can lead to early onset of diseases commonly associated with aging such as Mild Cognitive Impairment (MCI), dementia and Alzheimer’s Disease (AD).

Some Theoretical Issues

Two positions from the literature impact the broad research agenda presented in this thesis. The first relates to the argument that SWB is static (Headey & Wearing, 1992), and the second relates to the argument that research into individual nutrients lacks congruence with a realistic diet.

Theoretical Issue One:

Is Subjective Wellbeing Static?

Early research reported that SWB appeared remarkably stable, most individuals are happy most of the time (Diener & Diener, 1996), with most scores falling between 70 to 80 per cent of the maximum score based on population-level LS data (Cummins, 1995, 1998, 2003). This led to the concept of a set-point of SWB – the idea that individual variation around an existing set-point is minimal, and that even major life events will have
only temporary effects on this set-point (Brickman & Campbell, 1971; Brickman, Coates & Janoff-Bulman, 1978; Headey, 2007). This research was consistently replicated and appeared to be explained by the role of personality traits in maintaining SWB set-points (Headey, 2007). Costa and McCrae (1980) presented a model of happiness, displayed in Figure 6, to account for the observed correlations. This model specifies the two personality traits of extraversion and neuroticism as two independent sources that affect the two independent constructs of positive and negative affect, and that it is the balance between these that in turn influences overall SWB. Despite this, extraversion and neuroticism do not account for all the variance in positive and negative affect, Costa and McCrae’s (1980) own analyses put the shared variance between 3 and 7% between positive affect and extraversion, and between 8 and 18% between negative affect and neuroticism, thus suggesting that other factors are also important.

![Diagram](image_url)

*Figure 6. A model of the personality influences on positive and negative affect on subjective wellbeing, adapted from Costa and McCrae (1980, p. 675).*

Emerging results from population-based longitudinal studies over the past few decades, however, have provided enough accumulative evidence to suggest that permanent changes in SWB can arise (Fujita & Diener, 2005; Lucas, Clark, Georgellis &
Diener, 2003). The German Socio Economic Panel Survey (Headey, 2007) provided conclusive evidence against set-point theory; 20% of their national representative sample recorded substantial and more or less permanent changes in LS over a 20 year period (Headey, 2007). Whilst observed permanent changes in SWB were initially labelled as *exceptional* situations, which the theory should not have to account for, these have now accumulated to such an extent that it is clear that set-point theory needs either major revision or complete replacement (Headey, 2006, 2007). Furthermore, intervention trials have demonstrated changes in wellbeing.

In a randomised controlled trial assessing happiness exercises against a control, happiness was improved for up to six months (Seligman, Steen, Park, & Peterson, 2005). This trial was delivered entirely online and happiness was measured via the Steen Happiness Index (SHI) designed to measure (1) the pleasant life, (2) the engaged life, and (3) the meaningful life and to be sensitive to change (Seligman, Steen, Park, & Peterson, 2005). Similarly, a recent meta-analysis of 49 studies found that Positive Psychology interventions significantly enhanced wellbeing (mean $r=0.29$; Sin & Lyubomirsky, 2009). Taken together, this literature suggests that adaptation to a set-point is not inevitable or immutable (Seligman, Steen, Park, & Peterson, 2005) thus opening the potential for individual, community, and societal level interventions to improve wellbeing.

We therefore consider there to be enough evidence to investigate change in SWB in our sample, and its relationship to certain nutrients. However, in light of the evidence presented for the hedonic treadmill, we view small gains in SWB to be of import, therefore we have also deemed it necessary to investigate such change with statistical
methods more sensitive to smaller fluctuations in change, namely Latent Growth Curve Modelling (LGCM).

**Theoretical Issue Two:**

**Analysis of Single Nutrients versus Dietary Patterns**

Nutrition has long been studied in relation to both physical and mental health. Analysis of the relationship between single, isolated nutrients and disease states or health outcomes is common. However, this approach is increasingly criticised as lacking ecological validity; single nutrients do not always reflect *how* nutrients are consumed within a diet, nor does it consider their potential interactive and synergistic effects (National Research Council, 1989). Single nutrient analyses are also vulnerable to confounded results based on statistical issues. Any potential impact of a single nutrient on a health outcome may be too small to detect (Sacks, Obarzanek, & Windhauser, 1994) due to insufficient power, and single nutrients may be confounded by the effects of a dietary pattern which they may be characteristic of. Additionally, when large numbers of individual nutrients are analysed, results may be influenced by multicollinearity (e.g., Lee, et al., 1988) due to co-dependency between certain nutrients; and multiple comparisons can increase the Type 1 error rate producing potentially spurious results (Farchi, Mariotti, Menotti, et al., 1989).

These concerns need to be addressed because Papers 2 and 3 both focus on single nutrients. Despite the focus on two single nutrients, Paper 2 addresses these concerns in several ways. Although the analyses were focused on single nutrients, we were modelling a whole nutrient process that is known to occur in the body. Levels of folate and vitamin B₁₂ are known to influence levels of homocysteine and evidence links all three with
mental health. However, whether folate and vitamin B\textsubscript{12} influence mental health directly, or due to their influence on homocysteine, is unknown: this was the specific pathway assessed here. The interaction between folate and vitamin B\textsubscript{12} was also assessed here because it is known that these vitamins are intimately related. Folate and vitamin B\textsubscript{12} may further interact with other nutrients; however, our focus here was on their interaction and relationship to both homocysteine and mental health. This paper does not consider that levels of folate and vitamin B\textsubscript{12} may represent a particular dietary pattern, though we are not aware of any evidence to suggest this. Path analysis within a Structural Equation Modelling (SEM) framework was employed as this technique is more sensitive to small effects, should they be present. Finally, folate and vitamin B\textsubscript{12} were not highly correlated so multicollinearity was not an issue; we were not at risk of increasing the Type I error rate because multiple comparisons were not performed and the analyses were based on strong a priori hypotheses derived from the literature.

Paper 3 also assessed the relationship between –\textit{n-3} polyunsaturated fatty acids (\textit{n-3} PUFAs), and mental health. It is possible that the previously observed relationships between \textit{n-3} PUFAs and mental health are a reflection of the broader, and well documented, health benefits of the Mediterranean diet. This was outside the scope of the paper, but we suggest further research investigate this possibility if results continue to indicate a positive relationship between \textit{n-3} PUFAs and mental health. Furthermore, randomised controlled trials are necessary, particularly in the area of nutrition and mental health, as they are able to separate cause and effect. LGCM was used to assess whether \textit{n-3} PUFAs were associated with change in SWB across the intervention period as this technique is more powerful to detect a small slope of change than simply using pre- and post-test techniques. Multicollinearity was an issue between \textit{n-3} PUFA levels in
erythrocyte membranes and treatment group, which can result in issues with interpretation of results, to counter this we centred the variables of \( n \)-3 PUFA levels to aid in their interpretation. Type I error rates were controlled via all analyses being performed simultaneously within the one model based on strong \( a \ priori \) hypotheses.

Two further points on why we felt this was a valid approach are worth discussing. Firstly, there is considerable debate in the literature on the value of \( n \)-3 LC PUFAs for not only mental health but also physical health and development. For this reason we felt that to add to this body of research, and to compare our results meaningfully, we needed to use a similar randomised, placebo-controlled trial method; the gold standard for evaluating effects of nutrients. Secondly, analysis of \( n \)-3 PUFAs can be seen as consistent with consumption of \( n \)-3 PUFAs as dietary supplements. We were interested in whether a dietary supplement of \( n \)-3 LC PUFAs would predict the trajectory of SWB over 18 months, whilst dietary intake of nutrients remained unchanged via intervention. Thus we felt that this type of analysis was meaningful within this context.
Study Design

The three papers comprising this thesis were based upon data obtained as part of a larger randomised control trial investigating the effects of omega-3 PUFAs in older adults; a complete protocol can be found at Danthiir et al. (2011). Paper 1 employed baseline data to assess models of SWB, Paper 2 employed baseline and endpoint data on serum folate, serum vitamin B$_{12}$, plasma homocysteine and Subjective Wellbeing (SWB), whilst Paper 3 assessed the overall $n$-3 LC PUFA intervention pertaining to SWB.

Participants were recruited via public advertisement, media release, and through organisations for older people. Information sheets were provided upon request and were followed up with a questionnaire that was mailed out to assess their eligibility according to the inclusion criteria listed below.

- Aged 65 to 90 years
- Fluent in English
- Not currently taking and commit to not commence taking own $n$-3 fish oil (or algal) supplementation for trial length
- No history of brain injury or trauma
- No history of heart attack, stroke, or surgery
- No history of alcohol or drug abuse
- Absence of neurological diseases
- No significant medical conditions
- Absence of diagnosis of intellectual disability, clinically diagnosed major depression, diabetes, or dementia
Eligible adults were invited to attend an information session where the study protocol was detailed and individual requirements and potential outcomes and benefits were explained. Following this, examples of tasks and practice tasks were provided. Individuals who wished to participate then provided informed consent ($N=428$) and the Mini–Mental State Examination (MMSE) was administered by a trained research assistant. Following the information session, participants were booked to attend their first assessment visit which took place approximately two-to-three months later. Four weeks prior to this date, SWB questionnaires were mailed out with a reply paid envelope and a request that they be returned one-to-two weeks prior to their first assessment visit, to highlight and allow follow-up of any missing data. At the baseline assessment, after overnight fast, demographic information and objective measures of health including blood samples were obtained. Participants were then provided with a standardised breakfast and began the cognitive assessment session, conducted as the primary outcome for the randomised control trial. Table 6 displays the measures collected from participants, as detailed for each assessment session; this list is not exhaustive of the entire protocol, but rather reflects measures relevant to the design and analyses presented within this thesis.
Table 6

Summary of the measures collected across the 18 month study.

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>6 month assessment</th>
<th>12 month assessment</th>
<th>18 month assessment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height</td>
<td>(\checkmark)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Weight</td>
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</tr>
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<td>Demographic Information</td>
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<td>SWB Questionnaires</td>
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<td>(\checkmark)</td>
<td>(\checkmark)</td>
</tr>
<tr>
<td>Serum Folate</td>
<td>(\checkmark)</td>
<td>–</td>
<td>–</td>
<td>(\checkmark)</td>
</tr>
<tr>
<td>Serum vitamin B(_{12})</td>
<td>(\checkmark)</td>
<td>–</td>
<td>–</td>
<td>(\checkmark)</td>
</tr>
<tr>
<td>Plasma homocysteine</td>
<td>(\checkmark)</td>
<td>–</td>
<td>–</td>
<td>(\checkmark)</td>
</tr>
<tr>
<td>Erythrocyte Fatty Acids</td>
<td>(\checkmark)</td>
<td>(\checkmark)</td>
<td>(\checkmark)</td>
<td>(\checkmark)</td>
</tr>
</tbody>
</table>

Following the baseline assessment session, participants were randomised to receive either fish oil or placebo. This randomisation was conducted by an independent researcher with an age-stratified, one-to-one treatment-to-placebo allocation. The intervention was commenced immediately following baseline assessment. Participants
returned to the clinic every three months to collect their following three month supply of capsules (every alternate visit was solely for capsule collection; thus participants collected their alternate three month supply at their assessment session). Another three assessment sessions, six months apart, were conducted following the same procedure. The flow of participants through the study protocol, including attrition, can be seen in Figure 7.
Figure 7. Participant flow through the study from information session, baseline assessment, through to 18 month study end point.
Table 7

Summary of reasons for attrition.

<table>
<thead>
<tr>
<th>Reason for withdrawal</th>
<th>WD prior to baseline (n=37)</th>
<th>WD prior to 6mo (n=23)</th>
<th>WD prior to 12mo (n=10)</th>
<th>WD prior to 18mo (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>n</td>
<td>% in trt</td>
<td>n</td>
</tr>
<tr>
<td>Wants/needs to take n-3 supplements</td>
<td>2</td>
<td>3</td>
<td>66.7</td>
<td>0</td>
</tr>
<tr>
<td>Travel</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Too busy</td>
<td>13</td>
<td>1</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Family illness</td>
<td>5</td>
<td>1</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Deteriorating health</td>
<td>12</td>
<td>7</td>
<td>85.7</td>
<td>7</td>
</tr>
<tr>
<td>Deceased</td>
<td>0</td>
<td>2</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>Lost to follow-up</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Reaction to capsules</td>
<td>0</td>
<td>7</td>
<td>71.4</td>
<td>1</td>
</tr>
<tr>
<td>Other</td>
<td>1 (^a)</td>
<td>0</td>
<td>-</td>
<td>0</td>
</tr>
</tbody>
</table>

\(^a\)Does not believe in the study

n-3, omega-3; trt, treatment group; WD, withdrawal.

Reasons for withdrawal were coded into one of nine possible explanations, as shown in Table 7. Deteriorating health accounted for the majority of reasons given for withdrawal at any time: 32.4% for those who withdrew prior to baseline, 30.4% of those who withdrew following baseline, 70% of those who withdrew following 6 months
assessment, and 66.7% of those who withdrew following completion of their 12 month assessment; other time commitments also accounted for a large number of withdrawals (35.1%) prior to baseline assessment. For those who withdrew after completing baseline assessment but prior to their six month assessment, 30.4% (7 of 23) cited their explanation as a reaction to the capsules, which included complaints of gastric symptoms \((n=5)\), weight gain \((n=1)\), and reflux \((n=1)\), of which, five (71.4%) were in the treatment group. Only one other withdrawal occurred due to a reaction with the capsules, cited as gastric problems, which occurred prior to the 12 month assessment for a participant in the placebo group.

Treatment and placebo groups both received visually identical capsule containers identified with a unique code consecutively numbered for each person according to the randomisation schedule. The placebo capsule was matched to the treatment capsule for colour, size, and taste. Low odour fish oil was used in the treatment capsule, and the placebo capsule contained 1% fish oil. Four capsules were consumed by all participants each day; two in the morning, and two in the evening. This totalled 1720mg DHA and 600mg EPA per day for the treatment group, and 990mg olive oil, 1.2mg DHA and 1.8mg EPA for the placebo group.

Blood samples were collected from participants after overnight fasting. Serum folate, serum vitamin B_{12}, and plasma homocysteine concentrations were measured at an accredited laboratory. Erythrocyte membrane fatty acid concentrations were measured on site, based on a previously reported method (Ridges et al., 2001).
The study was approved by the Human Experimentation Ethics Committee of CSIRO Health Sciences and Nutrition, and followed the Good Clinical Research Practice guidelines. The trial can be found at the Australia and New Zealand Clinical Trials Register (ANZCTR): ACTRN12607000278437.

**General Descriptive Statistics**

Participants (n=391; n=227 female) were aged between 64 and 91 (M=72.32, SD=5.54 years) at time of enrolment. Figure 8 displays the distribution of the frequency of participants’ ages at enrolment. The highest level of education achieved for the majority of participants (32%) was Year 10, with 22% achieving Year 12, and 24% a Diploma or Certificate. Primary school was the highest level of education achieved for 4% and 17% had received a University degree or higher. Socio-Economic Indexes for Areas (SEIFA) percentiles (ABS, 2008) indicated that the majority (34.4%) of participants fell in the top quartile indicating relative advantage based on household qualifications, income and occupations; only 12.8% of participants were in the lowest quartile representing relative disadvantage. Nearly all participants (94%) were retired, as expected given that the retirement age in Australia is 65 years. The majority of the sample (45.8%) were in the overweight category for Body Mass Index\(^4\) (BMI 25-29.9), with 32.2% a normal weight (BMI 18.5-24.9), 21.5% obese (BMI ≥30), with only two participants classified as being underweight (BMI<18.5).

\(^4\) BMI is calculated as weight (kg) / (height (m))^2
Figure 8. Age (in years) of participants at study enrolment ($N=391$).

Compliance was assessed via the ratio of capsules consumed to the number of capsules expected to have been consumed. Capsules consumed were calculated based on the number of capsules returned every three months. Overall compliance was excellent with mean compliance at 97.5% (range 97.78% to 110.2%, $SD=4.6\%$). There was no difference in compliance between the treatment ($M=97.32\%$) and placebo ($M=97.71\%$) groups ($t(375)=0.810$, $p=0.418$). The excellent rate of compliance is further demonstrated in Figure 9; total $n$-3, DHA and EPA all increased from baseline to 6 months in the treatment group, and this increase was maintained through to 18 months; conversely levels remained stable in the placebo group.
Figure 9. Total n-3, EPA and DHA as per cent of total fatty acids at baseline and 6, 12, and 18 months in the treatment and placebo group.
At study completion, participants were asked to indicate whether they thought that had received the treatment or the placebo capsule, or whether they were unsure, to measure the success of masking the treatment and placebo capsules. Table 8 indicates judgements made by participants within each group; 32% correctly guessed they were in the treatment group, compared to 52.7% who correctly guessed they were in the placebo group. Guesses made by those in the treatment group were very similar for all three choices and did not differ from that expected by chance ($\chi^2(2)=0.151, p=0.927$). Just over half of the participants in the placebo group correctly guessed their condition, and only a small number thought they were in the treatment group, these guesses differed from what we would expect by chance ($\chi^2(2)=42.769, p<0.001$), suggesting that masking of the placebo condition was less successful than the masking of the treatment condition.

Table 8

*Accuracy of judgement on group allocation.*

<table>
<thead>
<tr>
<th>Judgement</th>
<th>Treatment</th>
<th>Placebo</th>
<th>Unsure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actual</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment (n=182)</td>
<td>32%</td>
<td>34.3%</td>
<td>33.7%</td>
</tr>
<tr>
<td>Placebo (n=172)</td>
<td>13.2%</td>
<td>52.7%</td>
<td>34.1%</td>
</tr>
</tbody>
</table>
Table 9 displays the means and standard deviations of serum vitamin B\textsubscript{12}, serum folate, and plasma homocysteine concentrations for males and females at baseline and 18 months. Independent samples \textit{t}-tests revealed significant differences for males and females in all concentrations apart from the serum folate concentration at baseline.

Table 9

\textit{Plasma homocysteine (μmol/L), serum vitamin B\textsubscript{12} (pmol/L) and serum folate (nmol/L) concentrations by gender at baseline and 18 months.}

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th></th>
<th>18 months</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>\textit{N}</td>
<td>\textit{M}</td>
<td>\textit{SD}</td>
<td>\textit{N}</td>
</tr>
<tr>
<td>Plasma Homocysteine (μmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>179</td>
<td>11.21</td>
<td>3.05</td>
<td>164</td>
</tr>
<tr>
<td>Female</td>
<td>206</td>
<td>10.00</td>
<td>3.10</td>
<td>184</td>
</tr>
<tr>
<td>Serum Vitamin B\textsubscript{12} (pmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>178</td>
<td>268.56</td>
<td>136.43</td>
<td>166</td>
</tr>
<tr>
<td>Female</td>
<td>205</td>
<td>322.30</td>
<td>216.52</td>
<td>184</td>
</tr>
<tr>
<td>Serum folate (nmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>179</td>
<td>24.69</td>
<td>9.10</td>
<td>166</td>
</tr>
<tr>
<td>Female</td>
<td>205</td>
<td>26.35</td>
<td>9.64</td>
<td>184</td>
</tr>
</tbody>
</table>
Given there was a significant difference between serum vitamin B\textsubscript{12}, serum folate and plasma homocysteine concentrations, rates of deficiency (serum vitamin B\textsubscript{12} <221.9pmol/L; serum folate <10.9nmol/L) and elevated homocysteine (hyper-homocysteinaemia >15\(\mu\)mol/L) are presented by males and females in Figure 10. Vitamin B\textsubscript{12} deficiency was higher than folate deficiency or hyper-homocysteinaemia in this sample. The difference in serum Vitamin B\textsubscript{12} concentrations between baseline and 18 months was not significant for the whole sample, or males or females separately. Plasma homocysteine concentrations were significantly higher at 18 months than they were at baseline \((t(343)=8.588, p<0.001)\), and this was true for males \((t(161)=7.181, p<0.001)\) and females \((t(181)=4.935, p<0.001)\) separately. Serum folate concentrations were found to increase slightly in females, but not males from baseline \((M=26.52, SD=9.36)\) to 18 months \((M=28.20, SD=9.58)\), though this was significant \((t(180)=2.555, p<0.05)\).
Figure 10. Percentage of male and female participants with folate and vitamin B\textsubscript{12} deficiency and hyper-homocysteinaemia at baseline and 18 months.
Chapter 3. Paper 1

Comparing Structural Models of Subjective Wellbeing: Choice of Model Affects Relationships with Personality

The rationale for Paper 1 came from what appeared to be relatively minor inconsistencies in the literature. Despite theoretical consensus that Subjective Wellbeing (SWB) includes three components – Life Satisfaction (LS), the presence of Positive Affect (PA), and the absence of Negative Affect (NA) – and clear agreement regarding how to empirically assess these three components, there was a lack of discussion for how to derive a measure of SWB from these or widespread recognition that different methods for deriving SWB may potentially impact conclusions drawn. The underlying empirical structure of SWB thus remains unclear. For this reason we felt that prior to using this as an outcome measure it was necessary to investigate the empirical structure of this construct in detail, and to establish whether empirical differences between models employed across studies would impact research outcomes. That is, would different results be observed based on differential empirical conceptions for how PA, NA, and LS should be used to derive the overarching construct of SWB?

SWB has been conceptualised as composed either of three separate research domains or some combination of the three (Busseri & Sadava, 2011). At the time, there were several papers that had sought to address this issue using confirmatory factor analysis (CFA) techniques, and in 2011 a review article was published (Busseri & Sadava, 2011) that summarised five different structural models of SWB and the theoretical implications of each. We considered two main areas where we could add to this literature: Firstly, we were aware that previous CFA techniques with SWB data were based on
Pearson’s correlation matrices. PA, NA and LS are measured using Likert scale questionnaires, therefore items are ordinal variables; Pearson’s correlation coefficients are known to underestimate relationships when categorical variables are used because they violate the underlying assumption that the variables are continuous (e.g., Flora & Curran, 2004). The first main area that we could contribute something new was therefore with the use of CFA specifically for categorical data which is available through the Mplus programme (Muthén & Muthén, 1998-2007). Estimation with categorical data here is based on a matrix of Polychoric correlation coefficients which provides a more accurate estimate for categorical variables. Therefore, we sought to use these superior methods to model each theoretical structure of SWB. Secondly, we felt that we needed to explicitly address the relevance of this question: that is, what, if any, are the practical implications of these differential theoretical operationalisations of SWB? Busseri and Sadava (2011) had accurately summarised the theoretical differences between models and the assumptions made by each, thus highlighting the theoretical differences between the models; however, we felt that this did not completely address the empirical similarities between them. We felt that a more tangible approach should necessarily include the application of each model to a research outcome to highlight any real differences involved in the utilisation of each model. To this end we used personality as the example. The results from this paper suggested preference for one particular model of SWB; this was then adopted as far as possible in the subsequent two papers which assessed the relationships between SWB and nutrition.
Comparing Structural Models of Subjective Wellbeing: Choice of Model Affects Relationships with Personality

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Nicholas R. Burns, University of Adelaide

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To be submitted for publication

Statement of Contributions

This chapter is a co-authored manuscript. Please refer to Appendix 1 for a statement of author contributions.
Abstract

Subjective Well-Being (SWB) is made up of three components: positive affect (PA), negative affect (NA), and life satisfaction (LS). Evidence for how these three components define a structural model of SWB is limited and the implications of adopting alternate models are unclear. PA, NA, LS, and personality were assessed in a sample (N=391) of community-dwelling, older (64-91 years; M=72.32, SD=5.55) adults. Confirmatory factor analysis (CFA) was used to compare four models of SWB reviewed by Busseri and Sadava (2011). We found support for SWB modelled reflectively as three inter-correlated factors. Differential correlations between personality and SWB across each of the four structural models of SWB highlight the practical implications of model choice.

Comparing Structural Models of Subjective Well-Being: Choice of Model Affects Relationships with Personality

Subjective Well-Being (SWB) research is not without controversy: As numerous self-help bestsellers can attest, people generally think they know what makes them happy. However, research from diverse fields has dispelled many commonly held beliefs about what it is that makes us happy. People are no better informed about what makes them happy (Wilson & Gilbert, 2003, 2005) than they are about the causes of mental illness and it has only been relatively recently that the study of positive mental health has been approached scientifically in the same way that mental illness has been (e.g., Seligman, Steen, Park & Peterson, 2005).
The key defining feature of SWB is that it is subjective – it is the multidimensional evaluation of current life according to the experience of the individual (e.g., Diener, 1994). Further definition is implied by the measures used. Thus, Diener (1994) argued that such evaluations of SWB must include a global component and capture positive as well as negative experiences and include cognitive evaluations of life. Empirical evidence supports the presence of these two distinct elements: a cognitive component, comprised of evaluative judgements of an individual’s satisfaction with their life as a whole (LS), or within particular life domains such as health; and an affective component concerned with the frequency of pleasant (PA) and unpleasant affect (NA; Diener & Lucas, 1999). The abundance of tests to measure these components (just over 1200 questionnaires designed to measure some aspect of quality of life or SWB are listed on the Australian Centre of Quality of Life website, http://www.deakin.edu.au/research/acqol/instruments/instrument.php) and the recommendation by Diener (1994) and others (Linley, Maltby, Wood, Osborne & Hurling, 2009) to measure all three components has led to an extensive body of research based on PA, NA, and LS measured separately, resulting in a lack of whole model conceptualisations of SWB (Busseri & Sadava, 2011). This has led to difficulties in synthesising research regarding both the theoretical development of SWB and its potential practical uses.

In the following we consider four alternative structural models of SWB. First, we review the independent relationships between PA, NA, and LS because these form the theoretical basis of the structural models. Theoretical evidence specific to each structural model is then presented and these models are compared using CFA techniques.
Relationship between positive and negative affect

Bradburn (1969) was the first to propose that PA and NA were independent constructs. Negative weak-to-moderate correlations, based on multiple measures of affect, have been reported between the two, with similar magnitudes reported for method-free correlations (e.g., \( r = -0.44 \); Diener, Smith and Fujita, 1995). Factor analytic evidence also supports the presence of two constructs: in exploratory models scale items load on two factors representing PA and NA; and two factors have demonstrated better fit over a single affect factor using CFA methods (Brown, Jose, Ng & Guo, 2002; Diener, Smith & Fujita, 1995). Furthermore, PA and NA have been shown to be differentially related to external factors such as health (Bradburn, 1969) and personality traits (Costa & McCrae, 1980; Diener & Emmons, 1984; Watson & Clark, 1992). Consistent with this empirical evidence, the four structural models of SWB to be assessed here assume PA and NA are correlated but largely independent constructs.

Relationship between affect and cognition

Affect (PA and NA) and cognition (LS) provide two qualitatively different sources of information to the overall conceptualisation of SWB. Weak-to-moderate correlations have been reported between the two domains and their discriminant validity has been established (Lucas, Diener & Suh, 1996; Schimmack, Radhakrishnan, Oishi, Dzokoto & Ahadi, 2002; Schimmack, Schupp & Wagner, 2008; Suh, Diener, Oishi & Triandis, 1998). Results are mixed regarding the strength of association between PA and LS compared to NA and LS; some have reported similar magnitudes (Suh et al., 1998, study 1), whereas others have found stronger relationships between PA and LS (Kuppens, Realo & Diener, 2008; Suh et al., 1998, study 2) consistent with the argument that positive
experiences contribute more to SWB than does the absence of negative experiences (Diener & Lucas 2000).

These conceptual and empirical differences between PA, NA, and LS have led to calls for researchers to employ measures of all three components in SWB research (e.g., Diener et al., 1999). However, to unify measurement of SWB requires some understanding of how these three components ‘fit together’ – the focus on independence of PA, NA, and LS is somewhat detrimental to this aim. Establishing how PA, NA, and LS are related to each other and to overall SWB is necessary for measurement consistency (Vittersø & Nilsen, 2002). The models of SWB recently summarised by Busseri and Sadava (2011) differ in the theoretical accounts they propose for the observed pattern of inter-correlations between PA, NA, and LS, in the treatment of data, the calculation of outcome variables and in how results are presented and discussed. We now consider these models.

**Structural models of subjective wellbeing**

1. **SWB as three inter-correlated factors**

   SWB is most commonly modelled as three separate constructs, PA, NA, and LS (Busseri & Sadava, 2011); consistent with the recommendation to measure all three components (e.g., Diener, 1994). Theoretically within this model, SWB should be inferred from the common variance reflected in inter-correlations between the components (Diener, 1994), in addition to the unique variance associated with PA, NA, and LS (Kim-Prieto et al., 2005). In practice, however, we generally see three separate analyses reported, thus ignoring both common variance and the additional unique variance that PA, NA, and LS add to explanation of external variables. Over-reliance on PA, NA, and LS as
separate constructs has resulted in literature detailing their individual correlates (e.g., DeNeve & Cooper, 1998); conclusions regarding overall SWB cannot be easily made.

2. SWB as a higher-order construct

The second structural model specifies SWB as a higher order latent factor with PA, NA, and LS as first-order reflective indicators. Direction of causality is from SWB to first-order factors, such that PA, NA, and LS can be seen as manifestations of the latent SWB construct (Jarvis, MacKenzie & Podsakoff, 2003; Petter, Straub & Raj, 2007). SWB as a higher order factor was initially proposed to account for the significant correlations observed between PA, NA, and LS (e.g., Diener, 1994; Diener, Suh, Lucas & Smith, 1999; Stones & Kozma, 1985) and is consistent with Diener’s (1994) hypothesis that these reflect individual differences in general life appraisals (see also Busseri, Sadava & DeCourville, 2007; Diener et al., 1999; Vittersø, 2004). However; the treatment of PA, NA, and LS as mere indicators of latent SWB is inconsistent with theoretical definitions and empirical evidence. Whilst a higher-order SWB is often inferred, definitions also highlight the unique and necessary contribution of each separate component (Lawrence & Liang, 1988).

This situation does not align with the requirements of a true reflective model – that is, that indicators are interchangeable without altering the underlying construct. Such a model also assumes that there is no significant unique variance associated with each component; moderate correlations consistently reported between PA, NA, and LS and their differential patterns of nomological validity are not compatible with this assumption; there is more to each component than simply what they have in common (Busseri, Sadava
& DeCourville, 2007; Diener, Suh, Lucas & Smith, 1999; Kim-Prieto, Diener, Tamir, Scollon & Diener, 2005; Stones & Kozma, 1985; Vittersø & Nilsen, 2002;). Thus, in a study of 264 Norwegian students, Vittersø (2004) found that a higher-order SWB factor accounted for only about half of the variance in the first-order factors. The amount of variance left unexplained by this higher-order SWB factor was substantial and different for each component, ranging from 37% in LS to 67% in NA. Similarly, Busseri, Sadava and DeCourville (2007), in their structural analysis of six studies (N=1682) that varied in the participant characteristics and measures used, found that approximately half of the variance in the first order factors was accounted for by a higher order latent SWB factor and unique variances unexplained by this latent SWB factor ranged from 10% in LS to 65% in NA. These results suggest that SWB is manifest most in LS and least in NA and that while a higher-order SWB factor is able to account for substantial variance in first-order factors, unique variance in PA, NA, and LS cannot be ignored.

### 3. SWB as a composite

This approach specifies SWB as a composite of PA, NA, and LS judgements; that is, SWB is conceptualised as a unidimensional construct caused by PA, NA, and LS and all three indicators must be included as they are defining features of SWB (Jarvis, MacKenzie & Podsakoff, 2003; Petter, Straub & Raj, 2007). Empirically, this composite can be produced in two ways: total scale scores for each construct can be used to calculate a composite SWB by subtracting NA from PA and summing the result with LS; alternatively, a formative CFA model can be specified to obtain a latent composite SWB factor. The main difference between these approaches concerns the weighting or relative importance of PA, NA, and LS in calculating SWB. The first approach assumes an equal contribution of all three constructs to SWB (Busseri, Sadava & DeCourville, 2007) whilst
the second provides unique weightings for each. The former approach is most often employed due to its relative simplicity; however, evidence suggests the second specification is more appropriate because it controls for measurement error and is consistent with reports that suggest PA, NA, and LS do not equally contribute to SWB (Busseri, Sadava & DeCourville, 2007; Vittersø & Nilsen, 2002).

4. SWB as a causal system

The final model to be considered specifies PA and NA as causal predictors of LS. While the balance between PA and NA has previously been offered as a definition of both happiness (Bradburn, 1969) and SWB (Costa & McCrae, 1980), a model wherein PA and NA independently predict LS has been proposed by others (e.g., Suh, Diener, Oishi & Triandis, 1998). Several potential mechanisms plausibly account for this causal process. Individuals may rely on affective information when making LS judgements (Kim-Prieto et al., 2005; Schimmack, Diener & Oishi, 2002; Schwarz & Clore, 1983; Wyer & Carlston, 1979); affective information may direct attention to understanding possible causes for feelings (Wyer & Carlston, 1979); or our experiences and reactions to life events may be influenced by affective information (Busseri & Sadava, 2011).

In two experimental studies, Schwarz and Clore (1983) found that current emotional experiences were used to form judgements of LS, thereby supporting the informational function of affect (Schimmack, Diener & Oishi, 2002; Schwarz & Clore, 1983, 2007, Wyer & Carlston, 1979). A more recent report (Messner & Wänke, 2011) has replicated these results by demonstrating that more positive judgements were made by those in a good mood than those in a bad mood. These studies show that current mood
influences subsequent judgements, including LS judgements. A more common approach employs survey design to assess the unique contribution of PA and NA to LS. Kuppens, Realo and Diener (2008) reported significant individual contributions of PA (β=0.31) and NA (β=-0.18) to LS in regression models based on cross-sectional data and it has been shown that affect balance explains individual differences in LS more so in individualistic (β =0.556) than in collectivist (β =0.34) cultures (Suh, Diener, Oishi & Triandis, 1998). Affect as a predictor of LS has also been assessed in mediation models; Schimmack, Diener and Oishi (2002) reported that the influence of personality on LS was mediated by hedonic balance, with a standardised regression coefficient from hedonic balance to LS of 0.46. A similar value from affect balance to LS (β=0.53) was obtained when affect balance was specified as mediating the relationship between psychological well-being and LS (Sanjuán, 2011). Such regression models are not evidence for unidirectional causality from affect to LS, but together with the experimental research of Schwarz and Clore (1983) and Messner and Wänke (2011) provide strong evidence for the order of causality and for SWB modelled as a causal system.

These four models offer different theoretical perspectives on the structure of SWB yet they are often not explicitly distinguished in the literature. In the following we assess whether PA, NA, and LS are best specified as three inter-correlated factors (Model 1), as three first-order indicators of a higher-order SWB factor (Model 2), as three indicators of a composite SWB (Model 3), or as a causal process from affect to LS (Model 4). We conclude that the correlates and predictors of SWB differ depending on the model employed and personality is used to demonstrate this. A brief review of SWB and personality research follows.
**Personality**

When predicting individual differences in SWB, external factors including demographics (Wilson, 1967) and more recently, situational factors such as life circumstances (Schimmack & Lucas, 2010) have been examined, though both have demonstrated relatively minimal influences on SWB (e.g., Diener et al., 1999). Personality has been examined as a within-individual factor that may account for the unexplained variance in SWB. Two traits – extraversion (E) and neuroticism (N) from the Five Factor model of personality (e.g., McCrae & Costa, 1997) have been shown to account for significant variance in SWB. Moderate relationships of E and N have been reported with affect balance and LS (Schimmack, Schupp & Wagner, 2008). Similar relationships were reported for SWB with E and N (DeNeve & Cooper, 1998), though recent evidence highlights N as the sole *unique* predictor (Heller, Watson & Ilies, 2004; Schimmack et al., 2004). Relationships from O, C, and A to overall SWB, LS and affect balance are weak-to-moderate (DeNeve & Cooper, 1998; Schimmack, Schupp & Wagner, 2008). Personality appears to correlate weak-to-moderate with affect balance, LS, and overall measures of SWB. Personality is included here to investigate whether its relationship with SWB is influenced by the model of SWB used. We hypothesise that personality-SWB correlations will differ between the four tested models, and will more closely reflect the true correlation in the best fitting model.
Method

Participants

Participants (N=391; female n=211, 54.1%) were community dwelling older adults aged between 64 and 91 (M=72.32, SD=5.55) from Adelaide, South Australia.

Materials

Life Satisfaction: Satisfaction with Life Scale (SWLS; Diener, Emmons, Larsen, & Griffin, 1985)

The SWLS is a five-item questionnaire designed to measure global LS, the cognitive component of SWB. Respondents indicate their level of agreement with each item on a seven-point Likert scale (1=Strongly Disagree, to 7= Strongly Agree). The SWLS has demonstrated adequate internal consistency reliability (average α=0.78 across 62 articles; Vassar, 2008) and has been validated for use in older adults (α=0.83; Pavot et al., 1991).

Positive and Negative Affect: Positive and Negative Affect Schedule (PANAS; Watson, Clark & Tellegen, 1988)

The PANAS contains 20 mood descriptors (10 positive; 10 negative) and requires respondents to rate ‘to what extent have you felt this way during the past week?’ from 1 (very slightly, or not at all) to 5 (extremely). The PANAS has demonstrated good internal consistency reliability for both PA (range α=0.86-0.90) and NA (range α=0.84-0.87) in undergraduate students (Watson, Clark & Tellegen, 1988), and has been validated in older adults (PA range: α=0.84-0.96 and NA range: α=0.64-0.91).
Personality: The Openness Conscientiousness Extraversion Agreeableness Neuroticism Index Condensed (OCEANIC 45; Schulze & Roberts, 2006)

Personality was measured using the OCEANIC, a 45-item questionnaire designed to assess personality using the Five Factor Model framework (e.g., Costa & McCrae, 1992). Respondents indicate whether each of the statements describes the way they think/feel from 1 (never) to 6 (always). Adequate internal consistency reliability has been reported ($\alpha$>0.77 for each trait; Schultz & Roberts, 2006).

Self-Reported Health Status: Short-Form-36 Health Survey, version 2 (SF-36v2; Ware, Kosinski & Dewey, 2000)

Perceived health status was measured with the SF-36v2, a 36 item questionnaire designed to measure physical and mental health using Likert scales to record responses. Both the physical ($\alpha$=0.95) and mental ($\alpha$=0.93) health components have high internal consistency reliability (Ware, Kosinski, Bjorner, Turner-Bowker, Gandek & Maruish, 2008). For the present sample, the physical and mental summary scales were calculated using factor score weights (Hawthorne, et al., 2007) derived from the 2004 South Australian Health Omnibus Survey dataset (SAHOS; Harrison Health Research, 2004). Norm-based scores were used; therefore scores below 50 are below the general South Australian population mean.
**Socio-Economic Status (SES)**

*Socio-Economic Indexes for Areas (SEIFA; ABS, 2008)*

SEIFA percentiles of relative disadvantage and advantage were used as a proxy measure of SES. The index measures qualifications, income, education and household type in 21 questions from the Australian 2006 census. Distribution of all SEIFA scores were divided into 100; the lowest scoring 1% (score of 1) represent relative disadvantage and the top 1% (score of 100) represent relative advantage. Our results are presented as proportion of our sample that had a SEIFA score that fell in the top or bottom quartile of the population.

*Income & Education*

Income was measured on a 15 point scale following the Australian Census (2006). Education was measured on a seven point scale in common educational categories from 1= finished primary school, to 7=postgraduate studies.

**Procedure**

Data presented here form part of a larger study on nutrition, health and cognition in older people (Danthiir et al., 2011). Participants were recruited into this study via local newspaper advertisements and South Australian agencies and organisations for older citizens. Paper versions of the SWLS and the PANAS were mailed to participants and returned by reply-paid mail. A paper version of the OCEANIC was administered 6 months later and the SWLS again 18 months later; these were also returned by participants via reply-paid post.
Results

Preliminary results

Less than 5% of data were missing on the PANAS and the SWLS. Listwise deletion was used for 1% of cases that were unable to be estimated (missing >50% of a scale); remaining missing values (<5% with responses <50%, MAR) were estimated using the Expectation-Maximisation (EM) algorithm (Dempster, Laird, and Rubin 1977). Listwise deletion for the PANAS and the SWLS was used for analyses that involved personality because 15% of participants did not complete the OCEANIC scale who had completed the PANAS and the SWLS.

The sample were normally functioning (MMSE>23) and physical ($M=43.62$ $SD=7.01$) and mental ($M=52.22$ $SD=9.00$) summary scales from the SF36v2 were almost identical to age matched (65+) South Australian norms (physical: $M=42.70$ $SD=11.83$; mental: $M=53.02$ $SD=8.87$) indicating average overall health. The majority (34.4%) of participants fell in the top quartile of all possible SEIFA scores indicating high relative advantage based on household qualifications, income and occupations; only 12.8% were in the lowest quartile representing relative disadvantage. Participants had an average of 13.5 years of education ($SD=6.07$) with 41.7% of participants achieving some level of education beyond high school, and just over half earned between $250 and $599 per week with few (~6%) earning less than $249 per week.
Confirmatory factor analysis model estimation

Models were estimated using Mplus Version 5.21 (Muthén & Muthén, 1998-2007). The robust Weighted Least Squares Mean- and Variance-adjusted (WLSMV) estimator was used due to both violations of normality (Kolmogorov-Smirnov statistic indicated significant deviations from normality for all data) and use of categorical (i.e., item-level) data. This estimator adjusts parameter estimates, standard errors and fit indices using diagonally weighted least squares estimation and the asymptotic covariance matrix. Models were assessed via three criteria: fit indices, model parameters, and model reliability. Fit statistics demonstrate the model provides a good fit to the data when there is little discrepancy between the estimated and the actual variance-covariance matrix. A non-significant chi-square ($\chi^2$) distribution and the Root Mean Square Error of Approximation (RMSEA) less than 0.06 indicate acceptable absolute fit (Hu & Bentler, 1999). The Comparative Fit Index (CFI) and the Tucker Lewis Index (TLI) provide measures of comparative fit, values greater than 0.95 indicate good fit (Hu & Bentler, 1999). The weighted Root Mean Square Residual (WRMR) is a residual based fit statistic for categorical data, values less than 0.9 indicate acceptable fit (Yu & Muthén, 2002). Model parameter estimates were also assessed for model integrity; factor loadings in the expected direction, significant and greater than 0.7 suggest a meaningful model; whilst reliability coefficients tell us the utility of the model; neither of these issues are addressed with fit statistics.

Statistical analyses

Data were analysed in four main steps: parallel and congeneric measurement models for PA, NA, and LS were compared to assess the relationships from scale items to the latent construct; reliability and validity of the individual constructs were assessed; the
four alternative models of SWB – SWB as three inter-correlated factors (Model 1), SWB as a higher order construct (Model 2), SWB as a composite (Model 3) and SWB as a causal process (Model 4) – were specified and compared and personality was then used to highlight the role that model choice has on subsequent results.

Main analyses

Step 1: individual measurement models

Item to construct loadings were held to equality (parallel model) and then freely estimated (congeneric model) to assess the best representation of PA, NA, and LS. Both models provided poor fit to the data for PA, NA, and LS, though fit indices were improved for the congeneric specification (see Table 10) and all factor loadings were significant and substantial ($\lambda > 0.587$). Modification Indices (MIs) suggested co-varied error terms between items would improve fit for all three constructs. For PA and NA error terms were allowed to co-vary where MIs were consistent with Zevon’s (1982) prior analysis of the PANAS, and for LS two co-variations were included based on their theoretical plausibility. Despite significant $\chi^2$ values for PA and NA, incremental fit indices suggested acceptable fit for all three models and RMSEA and WRMR values were adequate. The lowest factor estimates were still above acceptable cut offs for PA (lowest $\lambda = 0.518$, all others $> 0.646$), NA ($\lambda > 0.582$), and LS ($\lambda > 0.625$); all items were retained to replicate existing measurements as best possible.
Table 10

**Goodness-of-Fit statistics for parallel and congeneric individual measurement models of PA, NA and LS.**

<table>
<thead>
<tr>
<th></th>
<th>Absolute Fit Indices</th>
<th>Incremental Fit Indices</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chi-square (df*)</td>
<td>p-value</td>
</tr>
<tr>
<td><strong>PA</strong></td>
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<td></td>
</tr>
<tr>
<td>Parallel Model</td>
<td>143.848</td>
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</tr>
<tr>
<td>Congeneric Model</td>
<td>126.175</td>
<td>0.0000</td>
</tr>
<tr>
<td>Final Model</td>
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<td>0.0002</td>
</tr>
<tr>
<td><strong>NA</strong></td>
<td></td>
<td></td>
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<tr>
<td>Parallel Model</td>
<td>191.771</td>
<td>0.0000</td>
</tr>
<tr>
<td>Congeneric Model</td>
<td>185.294</td>
<td>0.0000</td>
</tr>
<tr>
<td>Final Model</td>
<td>81.182</td>
<td>0.0000</td>
</tr>
<tr>
<td><strong>LS</strong></td>
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<td></td>
</tr>
<tr>
<td>Parallel Model</td>
<td>255.009</td>
<td>0.0000</td>
</tr>
<tr>
<td>Congeneric Model</td>
<td>68.475</td>
<td>0.0000</td>
</tr>
<tr>
<td>Final Model</td>
<td>4.788</td>
<td>0.1880</td>
</tr>
</tbody>
</table>

*df* cannot be interpreted in the standard way; WLSMV estimates d.f. to approximate a \( \chi^2 \) distribution resulting in lower bound estimates (Muthén & Muthén, 2001).

*CFI, Comparative Fit Index; d.f., degrees of freedom; LS, Life Satisfaction; NA, Negative Affect; PA, Positive Affect; RMSEA, Root Mean Square Error of Approximation; TLI, Tucker Lewis Index; WRMR, Weighted Root Mean Square Residual.*
Step 2: reliability and validity

Internal consistency reliability

Internal consistency reliability was estimated using two methods appropriate within CFA frameworks. Fornell and Larcker’s (1981) reliability of the construct (RC) based on the ratio of variance explained by the construct to the total variance of that composite (Fornell & Larcker, 1981), suggested high internal consistency indicating that the observed variables in each measurement model were adequate indicators of PA (RC=0.899), NA (RC =0.912), and LS (RC =0.904). Coefficient $H$ (Hancock & Mueller, 2001) provided an additional measure of internal consistency not based on classical test theory; this coefficient is calculated as the squared correlation from the latent construct and the optimum linear combination from its indicators. Values were greater than 0.8 demonstrating excellent reliability for PA ($H = 0.908$), NA ($H = 0.927$), and LS ($H = 0.947$).

Discriminant validity

Covariance between each pair of latent variables provided some evidence for their discriminant validity (see Table ). This was further established via the paired constructs test (Jöreskog, 1971); Wald’s test of parameter constraints was used to test whether the covariance between each pair of latent constructs was significantly different from unity with results suggesting all pairs were best specified as two separate constructs. This method has been criticised because even high covariations can remain significantly different from 1.0 (Hair et al., 2006). Therefore, we also applied Fornell and Larcker’s (1981) method where discriminant validity is supported between two constructs if the
Average Variance Extracted (AVE) for each is greater than their shared variance (Farrell & Rudd, 2009; Hair et al., 2006). Discriminant validity between all pairs measured as individual reflective latent measurement models was again supported using this more stringent method (see Table 11).

Table 11

*Average Variance Extracted and Shared Variance Estimates for PA, NA and LS.*

<table>
<thead>
<tr>
<th>Latent Construct</th>
<th>Number of Items</th>
<th>Positive Affect</th>
<th>Negative Affect</th>
<th>Life Satisfaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive Affect</td>
<td>10</td>
<td><strong>0.474</strong></td>
<td>0.060</td>
<td>0.176</td>
</tr>
<tr>
<td>Negative Affect</td>
<td>10</td>
<td>-0.245</td>
<td><strong>0.513</strong></td>
<td>0.141</td>
</tr>
<tr>
<td>Life Satisfaction</td>
<td>5</td>
<td>0.419</td>
<td>-0.376</td>
<td><strong>0.658</strong></td>
</tr>
</tbody>
</table>

*Note.* Correlations are presented below the diagonal, all are significant at $p<0.001$; bold indicates AVE (Average Variance Extracted) estimates; squared correlations (i.e., $R^2$ values) above the diagonal.

*Convergent validity*

Estimated factor loadings from item indicators to latent constructs greater than 0.7 and AVE values greater than 0.5 provide evidence of convergent validity. Results here were mixed; the majority of factor loadings were greater than 0.6 for all constructs with
average loadings of 0.68 for PA, 0.71 for NA and 0.8 for LS. AVE values greater than 0.5 indicate there is more variance explained by the latent factor structure than error in each of the items on average, values (see Table 11) were greater than 0.5 for NA and LS, though fell just below this for PA.

**Step 3: alternative models (1-4)**

Parameter estimates for each SWB Model of three inter-correlated latent factors (Model 1), a higher-order Model (Model 2), a formative latent construct (Model 3) and as a causal system (Model 4) are presented in Figures 11 to 14.
\[ \chi^2(70) = 162.894, \ p < 0.001, \ CFI = 0.972, \ TLI = 0.985, \ RMSEA = 0.058, \ WRMR = 0.980 \]

Figure 11. PA, NA and LS as three inter-correlated factors (Model 1).

Models 1 and 2 are equivalent; they have the same degrees of freedom and goodness-of-fit statistics rendering traditional CFA methods such as the chi-square difference test unable to identify the more acceptable model. Equivalent models, however, are not necessarily equivalent in meaning (Schulze, 2005). The difference between Models 1 and 2 here concerns whether a higher-order latent SWB factor is able to explain relationships between the first-order factors. Both models provided acceptable fit to the
data (see Figures 11 and 12). Parameter estimates were also sensible for both models indicating that neither model could be immediately excluded on these grounds (Hancock & Mueller, 2006). Several alternative methods were used to assess whether the higher-order factor was more appropriate. Firstly, factor inter-correlations (Figure 11) were weak-to-moderate suggesting that a higher-order factor is not suitable. However, factor loadings from first-order factors to the higher-order SWB factor in Figure 12 are substantial and significant and MIs do not suggest covaried first-order disturbances would improve model fit, suggesting the higher-order SWB factor may be appropriate. The AVE (Fornell & Larcker, 1981) in the first-order factors by the second-order SWB factor relative to measurement error was calculated (Bagozzi & Heatherton, 1994) using standardised parameters from Model 2. This indicated that SWB explains 37.9% of the variance in the first-order factors, leaving the amount of variance unexplained by the higher-order SWB factor at 36% in LS, 73% in PA and 78% in NA. This can be interpreted as suggesting that co-variations among these first-order factors are not best explained by higher-order SWB.
$\chi^2(70)=162.894, \ p<0.001, \ CFI=0.972, \ TLI=0.985, \ RMSEA=0.058, \ WRMR=0.980$

Figure 12. Hierarchical reflective model with PA, NA and LS as first-order factors and a second-order SWB factor (Model 2).

The Schmid-Leiman transformation (SLT; Schmid & Leiman, 1957) was next applied to the standardised parameter estimates from Model 2 to establish the independent contribution of variance explained in each item due to the first- and second-order factors. Variance explained by the higher-order factor is maximised because this is extracted first, followed by the first-order factors modelled as uncorrelated both to each other and the
higher-order construct. First-order PA and NA factors explained approximately three times the variance in their indicators than did the higher-order SWB factor, so a larger proportion of the variance in these items was attributable to the first-order PA and NA factors, and a much lower proportion was attributable to the second-order SWB factor – thus providing support for Model 1. The first-order LS factor explained approximately half of the variance in LS items than the higher-order SWB factor was able to account for, so the larger proportion of the variance in these items was attributable to the second-order SWB factor, thus providing support for Model 2. Results support the use of Model 1 over Model 2. That the SWB factor was unable to account for more than 40% of the variance in PA, NA and LS, and therefore the large unique unexplained variance in PA, NA and LS, is compelling evidence in favour of Model 1.
Model is unidentified ($d.f. < 0$)

*Figure 13.* Hierarchical formative model with PA, NA and LS as first-order factors and a second-order SWB factor (Model 3).

Model 3 (Figure 13) specifies SWB as a formative model where the direction of causality is from the first-order factors to the general higher-order construct (Bollen, 1989); that is, the higher-order latent SWB factor is conceptualised as the unit weighted linear sum of its indicators, minus error. Such models are unidentified because there is insufficient information ($d.f. < 0$) for a unique solution (Loehlin, 2004); methods available
to identify such models (MacCallum & Browne, 1993) are rejected here because they alter
the substantive meaning of the construct. Parameter estimates and goodness-of-fit
statistics cannot be produced so we are unable to independently evaluate the model or to
assess comparative fit with reference to models 1, 2 or 4. We can however, comment on
whether such a model is consistent with theoretical expectations.

This model does not constrain the magnitude or direction of correlations between
indicators; they each provide a unique, independent contribution to the construct meaning
and are therefore vital – eliminating one indicator will alter the meaning of the construct.
Although PA, NA, and LS are usually found to be correlated to some degree, evidence
suggests that they are independent constructs, suggesting that a formative approach may
be appropriate. Further evidence in support of the formative model arises from numerous
authors who suggest that complete conceptualisations of SWB must necessarily include
PA, NA, and LS (i.e., eliminating one construct will alter the substantive meaning of the
SWB construct). However, this approach fails to account for the unique variances
associated with each component, and does therefore not accurately reflect what we know
about SWB.
\[ \chi(67)^2 = 162.797, \ p < 0.001, \ CFI = 0.969, \ TLI = 0.982, \ RMSEA = 0.060, \ WRMR = 1.025 \]

*Figure 14.* SWB as a causal process with PA and NA as causes of LS (Model 4).
Model 4 posits PA and NA as causes of LS (Figure 14), this model shows good fit to the data and standardised parameter estimates are all significant and in the expected direction. This model is based on LS data collected 18 months following collection of PA and NA data, and suggests that PA and NA exhibit a significant causal influence on LS. As this model is nested within Model 1\(^5\) we can statistically compare the two; results ($\chi^2(1) = 20.863, p < 0.001$) indicate that the restrictions imposed in Model 4 significantly worsen model fit, therefore Model 4 is rejected in favour of Model 1. However, the goodness-of-fit statistics were very similar to Models 1 and 2 and prior analyses suggested that LS was most strongly related to SWB, that the higher-order SWB construct explained the most variance in the first-order LS factor, and that this higher-order factor accounted for a greater proportion of the variance in the LS items than did the first-order LS factor. Therefore, we recommend that further conclusions regarding the appropriateness of this model should be based on theory.

**Step 4: Personality as an example**

Personality was used to highlight the role that model conceptualisation has on conclusions drawn; Table 12 shows the differential correlations obtained between personality and SWB measured from each model specification. Model 3 was unable to be estimated within a CFA framework, so traditional composite variables were created based on parallel and congeneric (using factor weights from CFA) measurement to act as a proxy for SWB as a formative model. Within the CFA models (1, 2 and 4), the Big Five personality factors – openness, conscientiousness, extraversion, agreeableness and

---

\(^5\) As models 1 and 2 are equivalent it is also correct to say Model 4 is nested within Model 2; however, the comparison is only conducted with Model 1 because this model was preferred over Model 2 based on the prior analyses
neuroticism are specified as continuous, inter-correlated observed variables correlated with PA, NA, LS, and SWB, whereas in Model 3 these personality factors were correlated with PA, NA, LS, and SWB, consecutively. Correlations between the same constructs across the different models demonstrated the same direction of association and pattern of significance for all apart from personality with LS from Model 4. However, absolute correlation values showed some degree of variation. Correlations from PA and NA to personality were reasonably similar across models 1, 3, and 4; though they were slightly higher with models 1 and 4. Similarly, LS and personality correlations in Model 1 were slightly larger than the values obtained for Model 3 but were much larger than those obtained for Model 4. Of additional importance is that the higher-order SWB factor from Model 2 correlates more strongly with personality than it does in Model 3.

The relationship between O and PA was consistent across the four models, though slightly weaker in both the parallel and congeneric versions of Model 3 compared to models 1 and 4; the same pattern was observed across the four models for the relationship between PA with each of C, E, A, and N in turn. O was not significantly correlated with NA, LS or SWB in any of the models; the remaining four personality factors of C, E, A, and N were significantly correlated with NA and again these relationships were consistent across the four models and slightly weaker for the parallel and congeneric versions of Model 3. We observed a significant correlation between C and LS that was consistent only across models 1 and 3, Model 4 demonstrated a very different pattern of results for LS; this pattern was also consistent for the relationships between LS with each of E, A, and N across models 1 and 3. Two further observations are of interest; the parallel and congeneric versions of Model 3 had almost identical estimates for PA, NA, and LS within each personality factor, the biggest difference was in the relationships between personality...
factors and SWB where the relationship was much stronger when using the parallel version; additionally, SWB to C, E, A, and N correlations were much stronger in Model 2 compared to both the parallel and congeneric versions of Model 3.
Table 12

*Correlations between personality with PA, NA, LS and SWB.*

<table>
<thead>
<tr>
<th></th>
<th>Model 1</th>
<th>Model 2&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Model 3</th>
<th>Model 4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Parallel&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Congeneric&lt;sup&gt;c&lt;/sup&gt;</td>
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</tr>
<tr>
<td><strong>Openness</strong></td>
<td></td>
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</tr>
<tr>
<td>PA</td>
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</tr>
<tr>
<td>NA</td>
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<td>x</td>
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<td>0.02</td>
</tr>
<tr>
<td>LS</td>
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<td>x</td>
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<tr>
<td>SWB</td>
<td>x</td>
<td>0.11</td>
<td>0.07</td>
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<tr>
<td><strong>Conscientiousness</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>PA</td>
<td>0.27</td>
<td>x</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>NA</td>
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<td>x</td>
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<td>x</td>
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<tr>
<td>SWB</td>
<td>x</td>
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<td><strong>Extraversion</strong></td>
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<td>Model 1</td>
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</tr>
<tr>
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<td>Parallel&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Congeneric&lt;sup&gt;c&lt;/sup&gt;</td>
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**Agreeableness**

<table>
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<th>SWB</th>
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<tbody>
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<td></td>
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<td>-0.29</td>
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**Neuroticism**

<table>
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<tbody>
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<td></td>
<td>-0.25</td>
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<tr>
<td></td>
<td>-0.25</td>
<td>0.51</td>
<td>-0.00</td>
<td>x</td>
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</tbody>
</table>

*Note.* Bold indicates significance at $p<0.05$ level

<sup>a</sup>PA, NA, LS correlations not reported as represent relationship with personality after controlling for the higher-order SWB factor

<sup>b</sup>Simple summation of indicator items for PA, NA, LS; SWB from SWB=LS+(PA-NA)

<sup>c</sup>Unit-weighted sum of indicator items for PA, NA, LS; SWB from SWB=LS+(PA-NA)

*LS, Life Satisfaction; NA, Negative Affect; PA, Positive Affect; SWB, Subjective Wellbeing.*
Discussion

Based on the evidence presented here we suggest that SWB is best represented by three inter-correlated first-order factors and that these first-order factors be modelled reflectively as congeneric constructs. We have also demonstrated that conclusions regarding the correlates and predictors of SWB are substantially dependent on the structural model used.

Equally weighted aggregate scores are most commonly used to measure PA, NA, and LS. This approach assumes that the underlying structure of the scales are parallel (Raykov, 1997), rather than congeneric. In practice this is rarely true (Bollen, 1989), as we have demonstrated here for PA, NA, and LS. Previous CFA analyses have also reported unequal item to factor weights for PA, NA, and LS (Arthaud-Day, Rode, Mooney & Near, 2005; Vittersø & Nilsen, 2002; Vittersø, 2004), although these authors did not explicitly test the difference between the two model specifications. This outcome questions the validity of using equally weighted aggregate scales when calculating summary scales for PA, NA, and LS and has further implications for the assessment of reliability. We found internal-consistency reliability coefficients (RC & H) suitable for non-parallel measurement (Graham, 2006; Raykov, 1997) to represent the upper bound of previously reported reliability values for PA, NA, and LS. Discriminant validity was established for all three constructs. Method free correlations were weak to moderate, suggesting independence of constructs, in line with previous estimates from equally-weighted aggregate scores (Kuppens, Realo & Diener, 2008; Suh, Diener, Oishi & Triandis, 1998; Vittersø & Nilsen, 2002) and method free non-parallel correlations (Arthaud-Day, Rode, Mooney & Near, 2005; Brown, Jose, Ng & Guo, 2002). Both the paired constructs test (Joreskog, 1971) and the more stringent AVE method (Fornell & Larcker, 1981)
established independence, consistent with previous studies (Brown, Jose, Ng & Guo, 2002). Differential correlations from PA, NA, and LS to personality traits also provided evidence for nomological validity, as previously established (Arthaud-Day, Rode, Mooney & Near, 2005; Lucas, Diener & Suh, 1996; Schimmack, Schupp & Wagner, 2008).

Of the four tested models, all had good fit to the data and parameter values consistent with theoretical expectations. Support was found for Model 1 – SWB as three inter-correlated first-order factors, over Model 2 – SWB as a higher-order factor with three first-order factors, based on the strength and relative strength of associations between first- and second-order factors and indicators. Weak-to-moderate factor correlations and discriminant validity between PA, NA, and LS support SWB modelled as three inter-correlated factors.

Factor loadings from Model 2 (from PA, NA and LS to SWB: 0.52, -0.47, 0.80) suggested that the higher-order SWB factor manifests most strongly in LS and least in NA, consistent with previously reported estimates (Vittersø, 2004). Studies that have used aggregated observed variables for PA, NA, and LS as indicators of SWB have reported similar factor loadings (0.46, -0.45, 0.73 (time 1) and 0.60, -0.50 and -0.77 (time 2), Busseri, Sadava & DeCourville, 2007; 0.63, -0.53, and 0.76, Molnar, Busseri, Perrier & Sadava, 2009). Variance explained by first- and second-order factors provided further mixed evidence; the second-order SWB factor explained less than half of the variance in first-order PA, NA, and LS factors supporting the importance of the unique variance in PA, NA, and LS and of SWB as three inter-correlated factors. Previous studies have found
SWB to account for approximately half of the variance in first-order PA, NA, and LS factors (Abbey & Andrews, 1986; Liang, 1985; Stones & Kozma, 1985; Vittersø, 2004) which has been cited as evidence for the validity of a general SWB component which may reflect overall ‘good versus bad’ evaluations.

This interpretation is consistent with Grosuch’s (1983) recommendation that higher-order factors are of use if they can explain 40-50% of the variance in the first-order factors, suggesting that the increase in generality of higher-order SWB will have little negative impact on the loss of accuracy from first-order factors. However, results from the Schmid-Leiman Transformation indicated that the larger proportion of variance in both PA and NA indicators was attributable to the first-order PA and NA factors rather than to the higher-order SWB factor; though conversely, the larger proportion of variance in LS indicators was attributable to the higher-order SWB factor rather than the first-order LS factor. Vittersø and Nilsen (2002) reported that a latent higher-order SWB factor accounted for more of the variance in LS and PA items than did the respective LS and PA first-order factors and that the first-order NA factor accounted for more of the variance in NA items than did the higher-order SWB factor. Busseri, Sadava and DeCourville (2007) reported the same pattern of results; the higher-order SWB factor explained most of the variance in LS items, but the second-order PA and NA factors explained more of the variance in their respective items than did the higher-order SWB factor.

Whether the relationships between first-order factors are best specified as correlations or as indicating the presence of a higher-order SWB factor needs further consideration. Based on evidence presented here we suggest that they are best specified as
correlations, and that LS may be the most important factor. Where CFA techniques are available for use, we recommend Busseri, Sadava and DeCourville’s (2007) hybrid model for analysis at both the higher-order and the first-order factors to further investigate the most appropriate representation.

SWB was next modelled as a formative model whereby PA, NA, and LS are combined to create SWB (Busseri, Sadava & Decourville, 2007). This model was unable to be estimated as formative latent models are unidentified. This model fails to account for the unique variance associated with PA, NA, and LS; summing responses will ignore this unique variance (Busseri, Sadava & Decourville, 2007) and so is recommended against. A formative approach is commonly employed as it is specified when a SWB variable is calculated by subtracting NA from PA and summing LS (e.g., Bostic & Ptacek, 2001; Costa et al., 1987; Emmons & Diener, 1985). As the model cannot be statistically estimated, the decision of whether SWB is reflected in, or is made up of, PA, NA, and LS needs to be based on theoretical expectations. Recognition that these two approaches differ based on the direction of causality they assign between first and second order factors is needed.

The final model considered SWB as a causal process from inter-correlated PA and NA to LS (Model 4). This model demonstrated adequate fit and linear regression values from PA to LS ($\beta=0.413$) and NA to LS ($\beta=-0.252$) indicate significant moderate casual paths from affect to LS measured 18 months later. This model suggests that the shared variance between affect and LS is due to the reliance on affective information in making LS judgments, rather than indicating presence of higher-order SWB. Evidence for this
affect-as-information hypothesis (Schwarz & Clore, 2007) has been provided in a series of experimental designs by Schwarz and colleagues (Schwarz & Clore, 1983; Schwarz, Strack, Kommer & Wagner, 1987). Others have focused on using regression and path analysis based on concurrent data to assess the influence of affect on LS; Kuppens, Realo and Diener (2008) found that while both positive and negative emotions independently contributed to LS, positive emotions accounted for twice as much of the variance in LS than did negative emotions. Our data also suggested that the presence of PA has a stronger influence on LS judgements than does the absence of NA, consistent with claims that the presence of PA contributes more to happiness than does the absence of NA (Diener & Lucas 2000). Other studies have looked at the influence of hedonic balance (PA minus NA) on LS reporting values closer to our PA-LS path; β=0.53 (Sanjuán, 2011) and β=0.76 in individualistic nations (Schimmack, Radhakrishnan, Oishi, Dzokoto and Ahadi, 2002). These estimates do not satisfy requirements for demonstrating causality as they are based on concurrent data. Our results suggest that PA and NA have a causal influence on LS judgements made 18 months later supporting the affect-as-information hypothesis (Schwarz & Clore, 2007). Comparisons indicated that this model provided a poorer fit to the data than did models 1 and 2, however; given that the fit statistics were very similar and that the SLT and factor loadings from Model 2 both suggested that LS was the most important factor, we recommend this model is further investigated.

Despite similar empirical solutions for these models there are clear theoretical and practical differences between them; Personality demonstrates that results are dependent on the particular model used. Similar patterns of significance and direction of association from Models 1, 2, and 3 with personality were found; the main difference can be seen in the magnitude of correlation values. Consistent with previous research (DeNeve &
Cooper, 1998; Schimmack, Schupp & Wagner, 2008), we found the strongest correlations from E to both PA and SWB and from N to both NA and SWB across all models apart from the smaller relationship between SWB and N in congeneric Model 3, potentially due to NA contributing least to SWB in this model. Results suggest that while the four models provide similar patterns of significance and direction, correlation magnitudes differ considerably. Therefore, conclusions drawn will vary; for example, parallel and congeneric versions of Model 3, which are typically employed in the literature, both demonstrate consistently weaker relationships with personality factors than models 1, 2, and 4, in some cases by as much as 0.5 (between N and SWB).

Limitations

Results presented are based on a sample of well-functioning, older individuals potentially limiting generalisability. However, whilst evidence suggests that mean-levels of SWB change across the lifespan (e.g., Baird, Lucas & Donnellan, 2010; Mroczek & Spiro, 2005; Schilling, 2006), there is no evidence to suggest that the underlying structure of SWB should alter. Another limitation common to SWB research concerns the over-reliance on self-report measures due to the subjective definition of the construct. Well documented issues with this include experience versus perception, social desirability and motivational biases (Schwarz & Strack, 1999). Reliance on a single method can also confound interpretation of observed relationships; the extent to which these represent shared method or construct variance is unknown. Measurement error specific to the use of common methods can be partialled out within CFA models; however, researchers should be aware that other statistical procedures are unable to do this. A final consideration concerns our use of composite variables as representations of the formative
conceptualisation of Model 3; whilst this method is commonly used and is theoretically consistent with a formative conceptualisation, calculation of a single score based on either parallel or unit-weighted composites is unable to model the construct-level error associated with the formative construct and is thus unable to entirely mimic the formative CFA model.

**Future Recommendations**

Where feasible, we recommend that future research conceptualise SWB as three, reflectively modelled, inter-correlated factors – PA, NA, and LS. Whilst we were able to determine that the formative measurement of these constructs was not inconsistent with the data, we suggest that reflective measurement best captures how SWB is theoretically conceptualised. Researchers should be aware of the differences between specifying measurement as formative or reflective and we strongly recommend that future work consider the theoretical implications of each specification. Replication of this model is needed across different age groups within individualistic cultures for meaningful comparisons to current results (Diener & Diener, 1995). A notable admission from structural models presented here is domain life satisfaction. Despite extensive research in the area and its inclusion in recent definitions of SWB, its absence was justified here due to a lack of theoretical expectation for how it is to be included. Of note is that domain LS should be modelled formatively – there is no underlying domain LS construct assumed to be represented by satisfaction within life domains, rather these are summed to create overall domain LS. Whether this construct is best specified as an indicator of LS as measured here, or whether it forms a first-order factor with our measure of LS under a higher-order factor of global LS needs investigation. We also recommend that future
research into causal models of SWB adopt longitudinal approaches to satisfy minimal conditions for causality; sufficient preliminary evidence exists based on concurrent data. Finally, we suggest there is value in presenting guidelines for the calculation of SWB; whether this is to advocate the use of CFA methods or to present standardised factor weights (*normed factor scores*) based on representative data, we believe this will aid in coherence of SWB research across the range of disciplines into which it is now commonly employed.

**Conclusion**

The aim of this paper was to detail the theoretical differences between four models of SWB employed in the literature and recently reviewed by Busseri and Sadava (2011), to provide evidence to support one model, and to demonstrate that model selection has an impact on further conclusions drawn. We conclude that SWB modelled as three reflectively measured, inter-correlated components of PA, NA, and LS provides the best representation of the data but that SWB modelled formatively cannot be entirely discounted based on current evidence. We recommend against the calculation of a single SWB score and suggest that future research account for the inter-correlations between the three factors when performing subsequent analyses. Using personality as the example we were able to demonstrate that results drawn are largely dependent on the measurement model used; that is, results based on one model of SWB do not necessarily generalise to other models of SWB. Careful consideration should be taken when selecting a model and some discussion of rationale should be included in subsequently published research.
Context

SWB is defined as the presence of PA, the absence of NA and levels of LS. How to operationalise SWB from these three components is less clear. Different operationalisations make different theoretical assumptions and impede coherence in the area.

Design

SWB was measured four times over 18 months with the PANAS and the SWLS in healthy, older adults (N=391). Structural equation modelling techniques allowed us to empirically compare four different operationalisations of SWB and assess their relationships to personality.

Contribution

Relationships between personality and SWB are dependent on how SWB is operationalised. SWB is best modelled as inter-correlations between PA, NA and LS.

Implications

Demonstration of the empirical differences between models of SWB employed highlights the need for model coherence in the area. This will facilitate research coherence and simplify efforts to employ SWB as an indicator of policy success cross-nationally.
Chapter 4. Paper 2

Folate, Vitamin B₁₂, Homocysteine and Subjective Wellbeing: Relationships in Older, Community-living Adults

The main aim of this thesis was to assess potential relationships between Subjective Wellbeing (SWB) and nutrition – an area traditionally dominated by investigations with mental illness. Therefore, after establishing the best measurement of SWB the next step involved assessing potential relationships with components of nutrition. Diet can be readily modified, it can be influenced at a population level, is relatively cheap and has no side effects within the normal range of consumption. Therefore, the potential that diet may regulate mental health offers a viable and attractive preventative alternative to medical intervention.

B-vitamins have been implicated in psychiatric disorders since the 1960’s when low levels of folate and vitamin B₁₂ were observed in psychiatric inpatients (e.g., Carney & Sheffield, 1978). Subsequent research over the following five decades has been unable to provide conclusive evidence for the role of folate and vitamin B₁₂ in depression. Associations, including longitudinal relationships between folate and vitamin B₁₂ with depression, have been reported in psychiatric patients (e.g., Hintikka et al., 2003) and in population-based studies (e.g., Kim et al., 2008). These have been based on varying measures of depression, with folate and vitamin B₁₂ measured via dietary intake (e.g., Tolmunen et al., 2004a) and blood analyses (e.g., Kim et al., 2008). However, similar studies have also reported no associations (e.g., Kendrick et al., 2008). Another area of interest has been in folate and vitamin B₁₂ as adjuvant to existing therapies such as SSRIs
(e.g., Godfrey et al., 1990); however, results have also been mixed in here. One common feature of this research is that the main outcome of interest is most often depression; those that have focused on investigations in the general population have assessed the relationships between folate and vitamin B_{12} with levels of depressive symptomology, with few assessing the relationship with measures of SWB. We address this gap in the literature by using SWB as the outcome measure, rather than depression thereby potentially offering greater applicability to the larger majority of the population.

Another important feature of this research is the inclusion of homocysteine. Homocysteine is an essential amino acid that is produced during the methylation of folate and vitamin B_{12}. High levels of homocysteine have been implicated in numerous adverse outcomes such as cardiovascular disease (e.g., Zakai et al., 2007). Low levels of folate and/or vitamin B_{12} impair the decline of homocysteine such that these are associated with high levels of homocysteine. Again, research has been mixed, and the question of whether folate and vitamin B_{12} have an independent influence on depression, or whether this relationship is mediated by homocysteine, remains unanswered. We investigate this mediation using SWB as the outcome.

**Analysis Justification for Paper 2**

Mediation occurs when one variable is responsible for an observed relationship between two other variables. Traditionally, mediation has been defined when the presence of one variable, the mediator, reduces the observed relationship between two other variables, the predictor and the outcome variable. Therefore, it has been used to further understand the mechanisms behind previously observed relationships (Baron & Kenny, 1986). However, this approach has been criticised because it has been demonstrated that
mediation (i.e., an indirect effect of the predictor variable on the outcome variable) may occur in the absence of a direct effect from the predictor variable to the outcome variable (Rucker, Preacher, Tormala & Petty, 2011). This research could be deemed more exploratory in nature, as we are attempting to elucidate whether there is more going on within an observed relationship. From a theoretical viewpoint, it is also possible that it is meaningful to test mediation without the presence of a direct effect from the predictor to the outcome variable, as was the case here. Rather than attempting to better understand an observed relationship, here we sought to assess a process that is known to occur biologically.

Mediation models must be assessed in the correct temporal order at which it is theoretically postulated that the predictor variable affects the mediator and the outcome variable, and the mediator variable may also affect the outcome variable. Thus necessitating that the predictor variable be measured at time point 1, the mediator variable at time point 2, and the outcome variable at time point 3 as demonstrated in Figure 15.

![Figure 15. Temporal requirements of a mediation model.](image)
In our study, folate, vitamin B_{12}, and homocysteine were only measured at baseline and 18 months, thereby providing only two measurement points rather than the necessary three. We were aware of the cross-lagged mediation approach which can be used to satisfy the temporal sequence of modelled relationships, when only two data points are available. We felt that this method had several advantages over a half-longitudinal design, most importantly, no paths were estimated between variables measured concurrently, which we know introduces significant bias into mediational models. We applied the cross-lagged mediation approach to our two-wave data by assuming stationarity between the mediator and the outcome variable, whilst accounting for autoregressive effects (Maxwell & Cole, 2007). That is, we assumed that the degree of change in PA, NA, and LS caused by homocysteine does not change, across time, from wave to wave, this approach also accounts for autoregressive effects when estimating all causal paths. Thus we assessed two models: one to assess the direct effects of the predictor on the outcome, and one to assesses the indirect effects of the predictor on the mediator, and of the mediator on the outcome, these two models are presented schematically in Figure 16.
Figure 16. Indirect (top model) and direct effects as estimated within a path analysis, cross-lagged design with two-wave data.

Figure 16 provides a simplified diagram of how cross-lagged path analysis can be used in a two-step process to infer causality, under the assumption of stationarity between the mediator and the outcome variable, with two-wave data. The mediated path cannot be directly observed but only calculated from the product of the path from the predictor to the mediator, and the path from the mediator to the outcome variable; therefore partial mediation was determined from the product of these two indirect paths.

Path analysis was used to estimate these models as it allowed us to model SWB as the latent constructs of PA, NA and LS. This added another level of complexity to our
models; however, it was performed due to three clear advantages. Firstly, we were able to estimate all our parameters simultaneously, we were able to allow the residuals from PA, NA and LS to co-vary across time, and finally we were able to model PA, NA, and LS as latent constructs, thereby removing measurement error from these constructs.
Folate, Vitamin B\textsubscript{12}, Homocysteine and Subjective Wellbeing:

Relationships in Older, Community-living Adults

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Statement of Contributions

This chapter is a co-authored manuscript. Please refer to Appendix 1 for a statement of author contributions.
Abstract

Primary prophylactic measures for depression, such as nutrition, are of interest due to the high individual and societal burden of mental illness. Cross-sectional and longitudinal relationships between folate, vitamin B\textsubscript{12}, homocysteine, and Subjective Wellbeing were assessed in a sample of 391 older, community living adults without clinically diagnosed depression. Levels of vitamin B\textsubscript{12}, but not folate, influenced homocysteine levels 18 months later. Folate, vitamin B\textsubscript{12}, and their interaction significantly predicted levels of positive affect 18 months later, but had no impact on levels of negative affect, or life satisfaction. Cross-sectional relationships between homocysteine and positive affect were completely attenuated in longitudinal analyses, suggesting that the cross-sectional relationship is driven by the dependence of homocysteine on folate and vitamin B\textsubscript{12}. This is the first study to offer some evidence of a causal link between levels of folate and vitamin B\textsubscript{12} on positive affect in a large, non-clinical population.

**Folate, Vitamin B\textsubscript{12}, Homocysteine and Subjective Wellbeing: Relationships in Older, Community-living Adults**

Depression is the fourth leading cause of disease burden worldwide (Üstün et al., 2004); it affects one in five Australian adults and treatment costs alone have been estimated at over AUD$600 million per annum in Australia (www.beyondblue.org.au). Depression is more prevalent in older (>65 years) adults (WHO, 2001), where social and physical risk factors (Colasanti et al., 2010; Rodda, Walker & Carter, 2011) are particularly prominent. The interactive effect of physical and mental illness can further compound implications for this age group (Unützer et al., 1997).
Current guidelines (APA, 2010) for the treatment of depression in older adults recommend psychosocial interventions such as social contact, psychological interventions such as cognitive behavioural therapy, or medication such as selective serotonin reuptake inhibitors. Treatment success, defined as a 50% improvement in symptoms, is approximately 66% and although most people eventually respond, 10-20% respond poorly, or not at all to treatment (McIntyre & O'Donovan, 2004). Relapse rates are also common – previous incidence of depression is one of the strongest predictors of possible future depression (ABS, 2007). There is a clear need to investigate lifestyle factors such as nutrition, as preventative strategies for depression.

Whilst the importance of nutrition for physical health is well established, its role in mental health is less clear. Growing evidence suggests that B-vitamins, such as folate (e.g., Gilbody et al., 2007) and vitamin B\textsubscript{12} (e.g., Robinson et al., 2011) are implicated in mental health. Both folate and vitamin B\textsubscript{12} are necessary for the methylation of homocysteine to methionine (Bottiglieri, 1996), which is the precursor of S-adenosylmethionine (SAMe; Crellin, Bottiglieri, & Reynolds, 1993; Reynolds, Carney & Toone, 1984). Homocysteine is an amino acid which, at high levels, is associated with adverse health outcomes such as cardiovascular disease (CVD; e.g., Zakai et al., 2007) and depression (e.g., Tiemeier et al., 2002); SAMe is a methyl donor with antidepressant properties due to its involvement in the metabolism of neurotransmitters such as norepinephrine, dopamine, melatonin and serotonin (Moretti et al., 2004; Reynolds, Carney & Toone, 1984). Functional deficiencies of folate or vitamin B\textsubscript{12} may therefore result in disturbed mood either directly or indirectly via elevated homocysteine (Bottiglieri, 1996; Bottiglieri et al., 2000; Lindenbaum et al., 1988), and reduced SAMe concentrations (Penninx et al., 2000). Several recent B-vitamin intervention trials have
reported no impact on cardiovascular outcomes despite significantly lowering homocysteine levels (Albert et al., 2008; Armitage, 2010; Ebbing et al., 2008; Lonn et al., 2006), suggesting that high homocysteine may be a marker for, rather than a cause of CVD risk. This is of relevance here given that consistent reports confirm a bi-directional relationship between CVD and depression (e.g., Freedland et al., 2003; Van der Kooy et al., 2007), with prevalence of depression ranging from 16-to-23% in patients with CVD (Musselman et al., 1998).

High prevalence rates of low folate (Carney, 1967; Carney & Sheffield, 1978; Carney, 1990) and vitamin B₁₂ concentrations (Carney & Shieffield, 1978) have been observed in psychiatric inpatients with depression. Inpatients with depression have demonstrated lower serum and red blood cell (RBC) folate concentrations (Abou-Saleh & Coppen, 1989; Bottiglieri et al., 2000), higher homocysteine concentrations (Bottiglieri et al., 2000), though no difference in serum vitamin B₁₂ levels (Bottiglieri et al., 2000), compared to health controls. In non-clinical populations, lower vitamin B₁₂ (Dimopoulos et al., 2007; Tiemeier et al., 2002), higher homocysteine (Almeida et al., 2004; Dimopoulos et al., 2007; Tiemeier et al., 2002), and lower folate (Dimopoulos et al., 2007; Morris et al., 2003; Ng et al., 2009) have all been reported in those with depressive symptoms compared to controls.

**Cross-sectional relationships: folate**

Cross-sectional studies support associations between depression with both plasma folate concentrations (Dimopoulos et al., 2007), serum folate (Ng et al., 2009), dietary folate intake (Miyaki et al., 2012) and in a nationally representative sample of US adults (Beydoun et al., 2010). Others have only observed this in subgroups of female (Bjelland et
al., 2003; Ramos et al., 2004) or male participants (Murakami et al., 2008; Nanri et al., 2010; Sánchez-Villegas et al., 2006, 2009), and others have reported a relationship in general population samples that have included only males (Tolmunen et al., 2003) or females (Jacka et al., 2012). This relationship has been supported in a meta-analysis that further concluded that the relationship was independent of study design, confounders, method of assessing folate, and presence of mandatory folate fortification (Gilbody, Lightfoot & Sheldon, 2007). This was supported by Sachdev and colleagues (2005) who found the relationship to be independent of sex, physical activity, smoking status, creatinine and vitamin B_{12} levels. However, the relationship was not independent of homocysteine (Sachdev et al., 2005), suggesting that homocysteine may mediate this observed relationship. Others have reported either a very weak relationship (Hintikka et al., 2003), or no association between depression and low serum folate in suicide attempters (Engström & Träskman-Bendz, 1999), in inpatients with major depression (Lee et al., 1998), in community-based samples of older adults (Hin et al., 2006; Lindeman et al., 2000; Moorthy et al., 2012; Robinson et al., 2011; Tiemeier et al., 2002) and with depressive symptoms, anxiety and Quality of Life in older women (Cassidy et al., 2004).

**Cross-sectional relationships: vitamin B_{12}**

Lower levels of vitamin B12 have been associated with higher levels of depressive symptoms in community-dwelling older adults (Dimopoulos et al., 2007), physically disabled, community-dwelling older females independent of health status and socio-demographic characteristics (Penninx et al., 2000) and in a large, ethnically diverse population of adults (Moorthy et al., 2012). Others have reported these associations independent of age, use of antidepressant medication and number of chronic diseases (Sepälä et al., 2013), and of age, gender, social class, cognitive functioning and
psychosocial and cardiovascular risk factors in community-dwelling elderly (Robinson et al., 2011). However, these authors found that inclusion of folate and homocysteine reduced the association to non-significance, suggesting that the relationship may be driven by folate and/or homocysteine. Others have reported no relationship in adults who had attempted suicide (Engström & Träskman-Bendz, 1999), in community-dwelling older adults (Hin et al., 2006; Lindeman et al., 2000; Sachdev et al., 2005), older females (Cassidy et al., 2004) and in nationally representative population-based samples (Beydoun et al., 2010; Bjelland et al., 2003).

**Cross-sectional relationships: homocysteine**

Elevated homocysteine has been associated with depressive symptoms in community-dwelling older adults (Dimopoulos et al., 2007), in the general population controlling for demographic variables and smoking status (Bjelland et al., 2003), in only males (Almeida et al., 2008; Nanri et al., 2010; Tolmunen et al., 2004b) and in community-dwelling older women (Almeida et al., 2004). These associations have been found when controlling for sociodemographic factors and medication or vitamin use in men (Nabi et al., 2013), controlling for gender, physical health, smoking status, high creatinine, low folate and low vitamin B_{12} (Sachdev et al., 2005). However, Tiemeier and colleagues (2002) found the relationship was attenuated to non-significance once functional disabilities and cardiovascular risk factors were considered. Others have found no relationship in older women (Penninx et al., 2000), or in the general population (Beydoun et al., 2010; Moorthy et al., 2012).
Longitudinal relationships

Reduced appetite can be a symptom of depression (Engel et al., 2011) that may account for observed cross-sectional relationships; longitudinal studies are necessary to establish causal direction. Kim and colleagues (2008) reported that low serum folate, low serum vitamin B₁₂, and high plasma homocysteine were associated with an increased risk of depression at 2-3 years follow-up in non-depressed adults controlling for cognitive functioning, disability and use of vitamin supplements. The relationship between folate and incident depression was independent of vitamin B₁₂ and homocysteine and the relationship between vitamin B₁₂ and incident depression was independent of folate and homocysteine; however, the relationship between homocysteine and incident depression was significantly reduced controlling for folate and vitamin B₁₂, suggesting that the relationships from folate and vitamin B₁₂ to incident depression were independent of homocysteine. Tolmunen and colleagues (2004a) reported an inverse association between energy-adjusted dietary folate, though not vitamin B₁₂ intake, and hospital discharge diagnosis of depression in men at 11-16 years follow-up controlling for socio-economic status, baseline depression, and fibre, vitamin C, total fat and energy intakes. One potential reason for the lack of association for vitamin B₁₂ was due to the higher levels of vitamin B₁₂ intake. A population-based study of older adults reported that high plasma homocysteine concentrations were associated with an increased risk of depression over four years, though this was only observed in women (47.5% of the sample; Forti et al., 2010). Hintikka and colleagues (2003) reported an inverse association between serum vitamin B₁₂ and depression after six months, controlling for demographics and health status including history of depression and treatment. A community-based study of older adults reported an associated between dietary intake of vitamin B₁₂, but not between dietary folate intake and depressive symptoms over an average of 7.2 years (Skarupski et
al., 2010), though again, one potential reason for this was due to relatively high initial levels of folate. Similar results were reported by Kendrick and colleagues (2008) who found that RBC folate was not associated with subsequent incidence of depressive symptoms over the following two years despite the presence of a relationship at baseline, suggesting that low folate was the consequence rather than the cause of depression (Kendrick et al., 2008). Williams and colleagues (2008) reported a relationship between variability in negative affect (NA) and RBC folate concentrations, though not with plasma homocysteine in healthy males. As RBC folate increased, variability in NA decreased, suggesting that long-term folate status may be important for mood stability.

**Intervention trials**

Intervention trials provide further support for the influence of nutrition on mental health. Initial folate levels have been shown to predict differential response to treatment of depressive symptoms (Alpert, Silva & Pouget, 2003; Papakostas et al., 2004a, 2004b, 2005), though initial levels of vitamin B12 and homocysteine have not (Papakostas et al., 2004a, 2004b, 2005). Treatment with methylenetetrahydrofolate (MTHFR) improved depressive symptoms in a six-week open-label trial for depressed elderly patients (Gauraldi et al., 1993) and in an eight-week trial for elderly patients with mild-to-moderate dementia and depression (Passeri et al., 1993). Almeida and colleagues (2010) reported that folic acid + vitamin B6 + vitamin B12 supplementation for 1-to-10.5 years was associated with a reduced risk of onset of major depression compared to placebo in a population at risk for depression (Almeida et al., 2010). However, others have reported no benefit of folic acid or vitamin B12 as treatment for depressive symptoms. A large study of older community living adults found that daily supplementation with folic acid (400mcg/d) + vitamin B12 (100mcg/d) had no impact on depressive symptoms over two
years (Walker et al., 2010), though homocysteine did not decline but remained stable in the intervention group, compared to increasing levels in the placebo group, potentially due to insufficient folate and/or vitamin B\textsubscript{12} dose (Walker et al., 2010). Additionally, no treatment effect was reported for folic acid (Williams et al., 2005), folic acid, vitamin B\text{6} and vitamin B\text{12} (Ford et al., 2008), or folic acid and vitamin B\text{12} (Christensen et al., 2011).

Others have found evidence that folate potentiates the effects of standard antidepressant treatment. Improved outcomes in patients with depression have been reported for folate plus standard treatment compared to placebo plus standard treatment (Alpert et al., 2002; Coppen et al., 1986; Godfrey et al., 1990). Coppen and Bailey (2000) also reported this pattern but only observed it in female participants, and found that improvement was related to homocysteine, which decreased in the intervention group, rather than folate. None of these studies directly investigated homocysteine as a mediator of the folate/ vitamin B\textsubscript{12} – depression relationships. This hypothesis has been investigated within CVD research: results suggest no impact on cardiovascular outcomes with folate and/or vitamin B\textsubscript{12}, despite successfully lowering homocysteine (Albert et al., 2008; Armitage, 2011; Ebbing et al., 2008), though some do suggest that folate may have a direct effect on cardiovascular outcomes (Verhaar, Stroes & Rabelink, 2002).

**Positive mood**

Cross-sectional relationships have been assessed between positive mood and homocysteine (Jensen et al., 1998), and folate and vitamin B\textsubscript{12} (Cassidy et al., 2004): Jensen and colleagues (1998) reported an inverse association between high homocysteine concentrations and Life Satisfaction, zest for life and subjective health in older people.
 (>80yrs), and Cassidy and colleagues (2004) reported no association between folate and vitamin B₁₂ deficiencies with mood in community dwelling older women (>70yrs), though this study had low levels of folate and vitamin B₁₂ deficiencies.

Several longitudinal trials have compared various multi-combinations of vitamins, minerals, amino acids, anti-oxidants, and essential fatty acids to placebo and have reported some benefit on mood in children (Kaplan et al., 2004) and antisocial behaviour in prisoners (Gesch et al., 2002). Several have also assessed a commonly available multivitamin (Berocca®) and reported positive effects after ~1 month on anxiety and perceived stress (Carroll et al., 2000), stress-related symptoms (Schlebusch et al., 2000), and on stress, mental health and vigour (Kennedy et al., 2010). Two others reported positive effects on stress with supplementation for one month with Centrum® in healthy male adults (Long & Benton, 2013), and on alertness, concentration and mental and physical stamina with three month supplementation with Swisse Men’s Utivite® in healthy males (Harris et al., 2011). The multi-combination capsules used in these trials all included folate and vitamin B₁₂, apart from one that only included vitamin B₁₂ (Schlebusch et al., 2000). These trials are important in that they demonstrate that nutrients have the potential to influence positive mood in a range of population types; however, their interpretation is confounded by the use of large omnibus inclusion of vitamins. One placebo-controlled trial that has assessed the sole use of folic acid for mood over 12 weeks in healthy males, found no difference on measures of positive or negative affect despite increased serum and erythrocyte folate and decreased plasma homocysteine in response to treatment (Williams et al., 2005).
The exact nature of a relationship between folate, vitamin B\textsubscript{12}, homocysteine and depression is confounded by different initial levels of nutrients and mental health, and whether covariates such as age, gender, alcohol, and BMI are considered. Here, we assess the relationships between folate, vitamin B\textsubscript{12}, homocysteine, and Subjective Wellbeing (SWB) in a sample of well-functioning, community-living, older Australian adults with consideration of potential covariates including age, gender, BMI, smoking status, education, use of cardiovascular medications, alcohol intake, energy intake, energy expenditure, physical health, treatment group, and omega-3 levels. The aims are to assess (1) the direct effects of folate, vitamin B\textsubscript{12}, and their interaction on SWB, and (2) the indirect effects of these nutrients and their interaction on SWB as mediated via homocysteine.

**Method**

**Participants**

Participants ($N=391$; 53.7\% female) were community living older adults aged between 64 and 91 ($M=72.3$, $SD=5.54$yrs) who provided written informed consent prior to participation. Participants with current major clinically diagnosed depression, or history of drug or alcohol abuse, were excluded from the study; further exclusion criteria were applied but are not relevant here, see Danthiir et al. (2011).
Materials

**Positive and Negative Affect: Positive and Negative Affect Schedule (PANAS; Watson, Clark & Tellegen, 1988)**

The PANAS contains 20 mood descriptors (10 positive; 10 negative) and requires respondents to rate ‘to what extent have you felt this way during the past week?’ from 1 (very slightly, or not at all) to 5 (extremely), higher scores indicate greater Positive Affect (PA) or Negative Affect (NA). The PANAS has demonstrated acceptable internal consistency reliability for both PA (range $\alpha=0.86$-$0.90$) and NA (range $\alpha=0.84$-$0.87$) in undergraduate students (Watson, Clark & Tellegen, 1988), and has been validated in a sample of older adults (PA range: $\alpha=0.84$-$0.96$ and NA range: $\alpha=0.64$-$0.91$).

**Life Satisfaction: Satisfaction with Life Scale (SWLS; Diener, Emmons, Larsen, & Griffin, 1985)**

The SWLS is a five-item questionnaire designed to measure global Life Satisfaction (LS), the cognitive component of SWB. Respondents indicate their level of agreement with each item (e.g., item 1; “In most ways, my life is close to my ideal”) on a seven-point Likert scale (1=Strongly Disagree, to 7= Strongly Agree), with higher scores indicating greater satisfaction. The SWLS has demonstrated adequate internal consistency reliability (average $\alpha=0.78$ across 62 articles; Vassar, 2008) and has been validated for use in aged populations ($\alpha=0.83$; Pavot et al., 1991).
Biochemical assays: folate, vitamin B\textsubscript{12} and homocysteine

Overnight (≈12-hour) fasted blood samples were forwarded to an accredited clinical pathology laboratory (IMVS, Adelaide, Australia) for analysis. Serum folate (nmol/L), serum vitamin B\textsubscript{12} (pmol/L) and plasma homocysteine (umol/L) concentrations were tested and reference ranges (serum folate: range 5.0–45.0 fasted; serum vitamin B\textsubscript{12}: range 100–700; plasma homocysteine: range 4.0–140.0 fasted) were established in the clinical pathology laboratory in accordance with the Australian National guidelines.

Covariates

Socio-Economic Status (SES)

SEIFA percentiles (ABS, 2008) of relative disadvantage and advantage were used as a proxy measure of SES. The index measures qualifications, income, education, and household type in 21 questions from the Australian 2006 census.

Body Mass Index (BMI)

Body Mass Index was calculated as weight (kg)/height (m)\textsuperscript{2}; weight was measured using a calibrated precision scale (UC-321PBT; A&D Medical, Sydney, Australia), and height using a wall-mounted stadiometer (SECA; Hamburg, Germany).
Self-Reported Health Status: Short-Form-36 Health Survey, version 2 (SF-36v2; Ware, Kosinski & Dewey, 2000)

Perceived health status was measured with the SF-36v2, a 36 item questionnaire designed to measure physical and mental health using Likert scales to record responses. Both the physical ($\alpha=0.95$) and mental ($\alpha=0.93$) health components have high internal consistency reliability (Ware, Kosinski, Bjorner, Turner-Bowker, Gandek & Maruish, 2008).

Cancer Council of Victoria Food Frequency Questionnaire (CCVFFQ; Giles & Ireland, 1996; Hodge et al., 2000)

Self-reported daily dietary intake was measured with the CCVFFQ, a validated (Hodge et al., 2000) 74-item questionnaire designed to measure food and nutrient intakes in epidemiological studies of Australian adults. Responses are optically scanned and assessed for usual daily nutrient intake and grams consumed at the Cancer Council (Carlton, Victoria) using data from the Australian nutrient database (NUTTAB95). Total energy intake (kJ/day) calculated from food and alcohol, and alcohol intakes (grams per day) were used herein.

Omega-3 Polyunsaturated Fatty Acid status

Erythrocyte membrane PUFAs were analysed according to Ridges et al. (2001) from fasted blood samples at baseline and at 18 months intervention. The $n$-3 index, a sum of EPA and DHA expressed as percentage of total fatty acids, was used herein.
Yale Physical Activity Survey (YPAS) for Older Adults (Dipietro, et al., 1993)

The YPAS was used to measure self-reported physical activity because it was specifically developed to accurately reflect the lower intensity activities performed by older adults (Dipietro et al., 1993). Levels of activity were assessed during a typical week in the last month, and minor modifications of instructions facilitated self-administration. Time spent participating in specific activities were summed to create an energy expenditure index, expressed as kilocalories per week.

Procedure

Data presented here form part of a larger clinical study on nutrition, health and cognition in older people, a more detailed methodology of the study protocol can be found at Danthiir et al. (2011). Information sheets were distributed in response to expressions of interest from potential participants who became aware of the study from either local advertisements, media releases, or through organisations for older people. Participants who met initial inclusion criteria were screened for dementia using a modified for telephone version of the MMSE (Newkirk et al., 2004) and informed, written consent was obtained. Four assessment sessions (baseline, 6mo, 12mo & 18mo) were conducted, during which fasted blood samples were collected for fatty acid profile (each assessment), plasma homocysteine, serum folate, and serum vitamin B₁₂ (first and final assessments). Paper versions of the questionnaires were mailed to participants approximately four weeks before each assessment and were returned via reply-paid post prior to their assessment visits, the CCVFFQ was completed at baseline and study completion; all other questionnaires were completed at each of the four assessments. Height was assessed at baseline only and weight at each assessment.
Results

Cross-lagged path analyses were used to assess the direct effects of folate, vitamin \( B_{12} \), and their interaction on SWB – measured as the inter-correlation between PA, NA, and LS – and the indirect effects of these nutrients on SWB via homocysteine as the mediator. Two cross-lagged regression models were thus specified to assess: (1) the direct effects of folate, vitamin \( B_{12} \), and their interaction, measured at baseline, on SWB 18 months later, controlling for baseline levels of SWB; and (2) the indirect effects of folate, vitamin \( B_{12} \), and their interaction on SWB via the mediator homocysteine, controlling for baseline levels of both homocysteine and SWB.

Preliminary analyses

Listwise deletion was applied to less than 1% of cases that were unable to be estimated (missing >50% of a scale); remaining missing values (<5% with responses <50% and MAR) were estimated with the Expectation-Maximisation (EM) algorithm (Dempster, Laird & Rubin, 1977). Study attrition was minimal; 90 per cent of participants completed baseline and 18 month assessment (\( n=391 \) at baseline, \( n=355 \) at 18mo). Independent samples \( t \)-tests confirmed there were no significant differences at baseline between those that completed the 18 month assessment compared to those that dropped out subsequent to baseline assessment on nutrition (vitamin \( B_{12} \), folate, and homocysteine) and SWB (PA, NA, and LS) measures.

Descriptive statistics

The majority (34.4%) of participants fell in the top quartile of all possible SEIFA scores indicating high relative advantage based on household qualifications, income and occupations; only 12.8% were in the lowest quartile representing relative disadvantage.
The majority (45.8%) of the sample were overweight (BMI 25-29.9; $M=27.3$, $SD=4.23$), and physical and mental summary scales from the SF36v2 were almost identical to age matched (65+) South Australian norms (physical: $M=42.7$ $SD=11.83$; mental: $M=53.0$ $SD=8.87$) indicating average overall health.

Means, standard deviations, and zero-order correlations for measures of SWB, folate, vitamin B$_{12}$, and homocysteine are presented in Table 13. Mean levels of folate, vitamin B$_{12}$, and homocysteine were all within the normal range; 6.8% were marginally folate deficient (6.8-11nmol/L), 7.5% were hyperhomocysteinic (homocysteine>15µmol/L), and 8.4% were vitamin B$_{12}$ deficient (B$_{12}$<148pmol/L) and 24.3% were marginally deficient (148-222pmol/L). Females had significantly lower mean plasma homocysteine concentrations ($M=10.0$, $SD=3.10$) and significantly higher serum vitamin B$_{12}$ concentrations ($M=322.3$, $SD=216.52$) compared to males ($M=11.2$, $SD=3.05$; $M=268.6$, $SD=136.43$, respectively); no gender differences were observed for serum folate. Average LS scores, reported in Table 13 are only slightly above the neutral point of 20 on the scale. This is considerably lower than previously reported in older adults (Pavot et al., 1991) and Australian adults (Gannon & Ranzijn, 2005). Correlations between folate, vitamin B$_{12}$, and homocysteine were as expected. Homocysteine was negatively correlated with folate and vitamin B$_{12}$, and folate and vitamin B$_{12}$ were positively correlated. PA, NA, and LS were highly correlated with each other from baseline to 18 months. PA and homocysteine were negatively correlated both at baseline and 18 months; vitamin B$_{12}$ at baseline was negatively correlated with LS at 18 months.
Table 13

Means, standard deviations, and Pearson’s correlations between folate, vitamin B12 and homocysteine with PA, NA and LS baseline and 18 months.

<table>
<thead>
<tr>
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<th>1</th>
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<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>M</th>
<th>SD</th>
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<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>1 B_{12}^a</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>297.32</td>
<td>185.42</td>
</tr>
<tr>
<td>2 Folate^b</td>
<td>0.21</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>25.58</td>
<td>9.42</td>
</tr>
<tr>
<td>3 Homocysteine^c</td>
<td>-0.26</td>
<td>-0.44</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>10.56</td>
<td>3.14</td>
</tr>
<tr>
<td>4 PA</td>
<td>0.03</td>
<td>-0.09</td>
<td>-0.13</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>22.17</td>
<td>4.89</td>
</tr>
<tr>
<td>5 NA</td>
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<td>-0.04</td>
<td>0.01</td>
<td>-0.17</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>10.90</td>
<td>3.98</td>
</tr>
<tr>
<td>6 LS</td>
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<td>-0.03</td>
<td>-0.08</td>
<td>0.34</td>
<td>-0.32</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>20.64</td>
<td>5.05</td>
</tr>
<tr>
<td>18 month</td>
<td></td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>7 B_{12}^a</td>
<td>0.76</td>
<td>0.14</td>
<td>-0.15</td>
<td>0.02</td>
<td>0.03</td>
<td>-0.05</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>305.17</td>
<td>189.39</td>
</tr>
<tr>
<td>8 Folate^b</td>
<td>0.18</td>
<td>0.61</td>
<td>-0.27</td>
<td>-0.01</td>
<td>-0.03</td>
<td>0.02</td>
<td>0.16</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>26.21</td>
<td>9.88</td>
</tr>
<tr>
<td>9 Homocysteine^c</td>
<td>-0.25</td>
<td>-0.29</td>
<td>0.70</td>
<td>-0.10</td>
<td>0.00</td>
<td>-0.06</td>
<td>-0.22</td>
<td>-0.40</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>11.82</td>
<td>3.65</td>
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<tr>
<td>10 PA</td>
<td>0.00</td>
<td>-0.04</td>
<td>-0.10</td>
<td>0.65</td>
<td>-0.20</td>
<td>0.31</td>
<td>0.05</td>
<td>0.07</td>
<td>-0.15</td>
<td>-</td>
<td>-</td>
<td>22.53</td>
<td>4.28</td>
</tr>
<tr>
<td>11 NA</td>
<td>0.01</td>
<td>-0.05</td>
<td>0.04</td>
<td>-0.19</td>
<td>0.58</td>
<td>-0.24</td>
<td>0.02</td>
<td>-0.03</td>
<td>0.04</td>
<td>-0.27</td>
<td>-</td>
<td>10.44</td>
<td>3.49</td>
</tr>
<tr>
<td>12 LS</td>
<td>-0.14</td>
<td>-0.02</td>
<td>-0.05</td>
<td>0.35</td>
<td>-0.31</td>
<td>0.70</td>
<td>-0.08</td>
<td>0.02</td>
<td>-0.06</td>
<td>0.39</td>
<td>-0.34</td>
<td>20.89</td>
<td>4.85</td>
</tr>
</tbody>
</table>

^a pmol/L; ^b nmol/L; ^c µmol/L; p<0.05
Covariates include gender, age, use of cardiovascular medications, and physical health; indicators of PA, NA and LS, and co-varying residuals for these indicators between baseline and 18 months are not included for simplicity.

Figure 17. Indirect effects cross-lagged regression model including covariates.
Main analyses

Mediation was assessed using a cross-lagged path analysis (see Figure 17) to allow causal inferences to be drawn from the non-experimental, longitudinal data. The autoregressive component accounts for the dependence between the repeated measures of constructs, inter-correlations between all variables at baseline account for time point dependence, and the residuals associated with each of the latent construct indicators were allowed to co-vary across time. With only two-waves of data, mediation is inferred from the product of the paths from baseline independent variable (folate, vitamin B_{12}, or their interaction) to the mediator (homocysteine) measured at 18 months, and from baseline mediator (homocysteine) to the dependent variable (PA, NA, or LS) measured at 18 months. The combination of three independent and three dependent variables resulted in a total of nine potential mediation relationships being assessed. Nutrient variables were scaled\(^6\) to reduce residual variances and aid model convergence, and centered to aid parameter interpretation. Homocysteine and vitamin B_{12} were both positively skewed, however natural log transformations did not alter the pattern of results, and thus untransformed variables are reported here.

Confirmatory factor analysis model estimation

Models were estimated in Mplus Version 5.21 (Muthén & Muthén, 1998-2007) using the Weighted Least Squares Mean- and Variance-Adjusted (WLSMV) estimator due to the use of categorical (item-level) data. Models were assessed based on absolute, comparative, and residual based fit statistics; models indicate acceptable fit when there is little discrepancy between the estimated and the actual variance-covariance matrix. A non-significant chi-square (\(\chi^2\)) distribution and a Root Mean Square Error of Approximation

\(^6\) According to (Gelman, 2008): value divided by two times its standard deviation
(RMSEA) less than 0.06 indicate acceptable absolute fit (Hu & Bentler, 1999), a 
Comparative Fit Index (CFI) and Tucker Lewis Index (TLI) greater than 0.95 indicate 
good comparative fit (Hu & Bentler, 1999), and a Weighted Root Mean Square Residual 
(WRMR) less than 0.90 indicates good residual based fit.

Covariates

Bivariate correlations were used to determine which of twelve potential 
demographic and health covariates were significantly ($p<0.05$) related to both the 
nutrients and SWB outcomes. Gender, age, use of cardiovascular medications, and 
physical health satisfied these criteria at baseline, and so were included in the final model. 
These variables were specified as covariates by regressing all baseline nutrition and SWB 
variables onto these four variables.

Direct effects from vitamin B$_{12}$, folate and their interaction on subjective wellbeing

The direct effects model assesses whether vitamin B$_{12}$, folate, or the interaction 
between the two are causally related to PA, NA, or LS at 18 months, controlling for both 
the prediction of these by PA, NA, and LS at baseline, and for covariates. Note that 
homocysteine is also included in this model but only related to itself, via auto-regression. 
The direct effects model demonstrated good fit to the data ($\chi^2(173)=337.120$, $p<0.05$; 
CFI=0.96; TLI=0.98; RMSEA$^7=0.05$; WRMR=1.03), and all stability coefficients were 
large and significant (PA $\beta=0.78$; NA $\beta=0.76$; LS $\beta=0.81$; vitamin B$_{12}$ $\beta=0.76$; folate 
$\beta=0.67$; interaction $\beta=0.56$; homocysteine $\beta=0.71$; all $p<0.001$), suggesting that baseline 
levels of SWB components and nutrients are strong predictors of their subsequent 18 
month measurement. vitamin B$_{12}$, folate, and their interaction weakly but significantly 

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$^7$ 90% Confidence intervals are not available with the WLSMV estimator
predicted PA 18 months later (vitamin $B_{12}$ $\beta=0.15$, $p=0.011$; folate $\beta=0.14$, $p=0.037$; interaction $\beta=0.14$, $p=0.020$), beyond the prediction afforded by baseline PA. Vitamin $B_{12}$, folate, and the interaction between the two did not contribute to the prediction of NA or LS beyond baseline measures of these constructs.

**Indirect effects from vitamin $B_{12}$, folate and their interaction on subjective wellbeing via homocysteine**

The indirect effects model demonstrated good fit to the data ($\chi^2(170) = 327.500$, $p<0.05$; CFI=0.97; TLI=0.98; RMSEA=0.05; WRMR=1.04) and again, all stability coefficients were all large and significant (PA $\beta=0.62$; NA $\beta=0.73$; LS $\beta=0.83$; vitamin $B_{12}$ $\beta=0.77$; folate $\beta=0.62$; interaction $\beta=0.73$; homocysteine $\beta=1.23$; all $p<0.001$). Parameter estimates for baseline homocysteine to PA, NA, and LS at 18 months were all weak and non-significant; folate and the interaction with vitamin $B_{12}$ were not significant predictors of homocysteine at 18 months, but $B_{12}$ at baseline was a significant predictor of homocysteine at 18 months ($\beta=-0.12$, $p<0.001$). Mediation is assessed within two-wave, cross-lagged analyses as the product of the path from the independent variable (baseline) to the mediator (18 month), and the path from the mediator (baseline) to the dependent variable (18 month); if the product of these two paths is significantly different from zero then we can infer partial mediation. With three separate, but related predictors – vitamin $B_{12}$, folate, and their interaction – and three separate, but related outcomes – PA, NA, and LS –, and one mediator – a total of nine potential mediations were assessed here. Indirect effects for each of the nine models ranged from 0.0001 to 0.010, and Sobel’s Z test (Sobel, 1982) confirmed that none were significantly different from zero.
Table 14

*Standardised parameter estimates for cross-lagged regression models; direct and indirect effects from respective models.*

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>18month</th>
<th>β</th>
<th>S.E.</th>
<th>Est/S.E.</th>
<th>p-value</th>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>$B_{12}$ --$&gt;$ PA</td>
<td></td>
<td></td>
<td>-0.18</td>
<td>0.04</td>
<td>-2.55</td>
<td>.011</td>
</tr>
<tr>
<td>$B_{12}$ --$&gt;$ NA</td>
<td></td>
<td></td>
<td>-0.11</td>
<td>0.06</td>
<td>-1.44</td>
<td>.150</td>
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<tr>
<td>$B_{12}$ --$&gt;$ LS</td>
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<td></td>
<td>-0.02</td>
<td>0.06</td>
<td>-0.34</td>
<td>.734</td>
</tr>
<tr>
<td>Folate --$&gt;$ PA</td>
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<td></td>
<td>0.14</td>
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<td>2.09</td>
<td>.037</td>
</tr>
<tr>
<td>Folate --$&gt;$ NA</td>
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<td>0.02</td>
<td>0.05</td>
<td>0.27</td>
<td>.787</td>
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<tr>
<td>Folate --$&gt;$ LS</td>
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<td>-1.42</td>
<td>.155</td>
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<tr>
<td>$B_{12}$ x Folate</td>
<td></td>
<td></td>
<td>0.14</td>
<td>0.03</td>
<td>2.33</td>
<td>.020</td>
</tr>
<tr>
<td>$B_{12}$ x Folate</td>
<td></td>
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<td>0.11</td>
<td>0.05</td>
<td>1.59</td>
<td>.113</td>
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<tr>
<td>$B_{12}$ x Folate</td>
<td></td>
<td></td>
<td>0.04</td>
<td>0.06</td>
<td>0.58</td>
<td>.562</td>
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<tr>
<td><strong>Indirect Effects</strong></td>
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<tr>
<td>$B_{12}$ --$&gt;$ Hcy</td>
<td></td>
<td></td>
<td>-0.12</td>
<td>0.03</td>
<td>-4.05</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Folate --$&gt;$ Hcy</td>
<td></td>
<td></td>
<td>-0.02</td>
<td>0.05</td>
<td>-0.41</td>
<td>.681</td>
</tr>
<tr>
<td>$B_{12}$ x Folate</td>
<td></td>
<td></td>
<td>0.01</td>
<td>0.03</td>
<td>0.45</td>
<td>.654</td>
</tr>
<tr>
<td>Hcy --$&gt;$ PA</td>
<td></td>
<td></td>
<td>0.02</td>
<td>0.06</td>
<td>0.30</td>
<td>.766</td>
</tr>
<tr>
<td>Hcy --$&gt;$ NA</td>
<td></td>
<td></td>
<td>0.06</td>
<td>0.09</td>
<td>1.14</td>
<td>.256</td>
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<tr>
<td>Hcy --$&gt;$ LS</td>
<td></td>
<td></td>
<td>0.08</td>
<td>0.10</td>
<td>1.70</td>
<td>.091</td>
</tr>
</tbody>
</table>

Both models fit using WLSMV estimator; direct effects model fit statistics,

\[ \chi^2(173)=337.120, \ p<0.05, \ CFI=0.963, \ TLI=0.977, \ RMSEA=0.049, \ WRMR=1.025; \]

indirect effects model fit statistics, \[ \chi^2(170)=327.500, \ p<0.05, \ CFI=0.965, \ TLI=0.977, \]

RMSEA=0.049, WRMR=1.037

*Hcy, homocysteine; LS, Life Satisfaction; NA, Negative Affect; PA, Positive Affect.*
Discussion

Results suggest a direct effect of vitamin B_{12}, folate, and their interaction on Positive Affect (PA), but not on Negative Affect (NA) or Life Satisfaction (LS). Only vitamin B_{12} significantly predicted homocysteine 18 months later and homocysteine did not predict PA, NA, or LS, thus providing no support for any indirect effects of vitamin B_{12}, folate, or their interaction on PA, NA or LS through homocysteine as the mediator.

Negative associations between folate and vitamin B_{12} at baseline, and homocysteine 18 months later reflect the causal relationship of folate and vitamin B_{12} to levels of homocysteine (Bottiglieri, 1996). Folate and vitamin B_{12} are necessary for the methylation of homocysteine to methionine such that deficiencies in either result in impaired methionine production and therefore in increased levels of homocysteine (Bottiglieri, 1996). Cross-sectional analyses reported here suggest that folate contributes more to homocysteine than vitamin B_{12}, consistent with previous reports (Bjelland et al., 2003). The relationship between baseline vitamin B_{12} and 18 month homocysteine was of a similar magnitude; however, the relationship was considerably attenuated between folate at baseline and 18 month homocysteine. Accounting for autoregressive effects of homocysteine further attenuated both relationships, reducing that between folate and homocysteine to non-significance. This suggests that vitamin B_{12} influences homocysteine in this sample, but that folate does not. There are two potential explanations: First, previous reports have been made based on either cross-sectional (Bjelland et al., 2003), or longitudinal relationships without accounting for prior levels of homocysteine, which provided a considerably attenuated estimate in our own sample. Second, this could be explained due to the low rates of folate deficiency present in this sample (6.8%), whereas previous studies have recorded much higher rates of deficiencies (Morris et al., 2003),
particularly in clinical populations (Bottiglieri et al., 2000; Carney, 1967). Lower rates of folate deficiency could be due to mandatory folate fortification introduced in Australia in 2009.

A similar pattern of results emerged when assessing the effect of homocysteine on the three components of SWB. Despite the presence of cross-sectional relationships between homocysteine and PA, longitudinal analyses suggested that homocysteine does not cause levels of PA 18 months later, suggesting that homocysteine may be a marker for levels of PA rather than a cause. There were no observed relationships between homocysteine and either NA or LS. If homocysteine is only a marker for low levels of PA, then what is the cause? Homocysteine is known to be influenced by a variety of genetic and nutritional factors (Abraham & Cho, 2010) but high homocysteine is most commonly due to B-vitamin deficiencies, including folate and vitamin B$_{12}$. One possibility is that folate and/or vitamin B$_{12}$ levels influence PA, and that therefore, elevated homocysteine is associated with PA due to its dependence on these B-vitamins. There were no cross-sectional relationships between folate or vitamin B$_{12}$ with PA; however, cross-lagged analyses accounting for both autoregressive effects and the inter-correlation between PA, NA, and LS demonstrated a causal relationship from folate ($\beta=0.14$), vitamin B$_{12}$ ($\beta=0.18$), and their interaction ($\beta=0.14$) on subsequent levels of PA. There were no direct effects from folate, vitamin B$_{12}$, or their interaction on subsequent levels of NA or LS. One previous study with older people found a relationship between elevated homocysteine and lower life satisfaction (Jensen et al., 1998), though another found no relationship between folate and vitamin B$_{12}$ with mood in a similar sample (Cassidy et al., 2004). Both these studies are consistent with our own cross-sectional results; we found an association between homocysteine and PA, and no relationship between folate and vitamin B$_{12}$ with
PA cross-sectionally. This highlights the importance of longitudinal data to accurately reflect the proposed biological pathways between these nutrients and homocysteine.

Although SWB and mental ill health are largely independent constructs, there is clear construct overlap between depression and NA and they are typically reported to correlate around 0.4 to 0.6 (e.g., Crawford & Henry, 2004). Despite this, there were no cross-sectional or longitudinal relationships between folate, vitamin B$_{12}$, and homocysteine with NA, either before (Table 13), or after (Table 14) controlling for statistically relevant confounding variables. One other study found no overall effect of folate supplementation on NA using the same measure as that used here (Williams et al., 2005) despite a decrease in plasma homocysteine; however, there was an inverse association between increased folate and decreased variability in NA. This study was also conducted with healthy participants, without diagnosis of depression and with normal levels of folate and vitamin B$_{12}$. This suggests that there may be no relationship between NA with folate, vitamin B$_{12}$, or homocysteine in those without diagnosed depression, and with relatively normal levels of folate, vitamin B$_{12}$, and homocysteine. This conclusion is consistent with previous research that has found an effect of folate supplementation on mood when based on either nutrient- or mood-deficient populations (Carney, 1967; Guaraldi et al., 1993; Reynolds et al., 1970).

The explanation that homocysteine is cross-sectionally related to PA due to its dependence on folate and vitamin B$_{12}$, rather than exerting a causal influence on PA is further supported by results reported in CVD research. Homocysteine has been independently associated with cardiovascular risk (e.g., Kumar, Jayaraman & Muralidharan, 2012). This relationship is considerably attenuated in prospective studies
(Christen et al., 2000; Hankey & Eikelboom, 1999; Malinow, Bostom & Krauss, 1999), though others have reported a causal effect of homocysteine on CVD (Wald, Law & Morris, 2002). Preventative approaches have therefore targeted homocysteine, with recent intervention trials assessing whether lowering homocysteine through folate and vitamin B<sub>12</sub> supplementation reduces the risk of CVD. Supplementation with some combination of folic acid, Vitamin B<sub>6</sub>, and/or vitamin B<sub>12</sub> have consistently reported no effect on cardiovascular events (Albert et al., 2008; Armitage, 2010; Ebbing et al., 2008; Lonn et al., 2006) up to 7.3 years follow-up (Albert et al., 2008), despite lowering homocysteine levels in all of these trials. Three explanations have been proposed; homocysteine is a risk marker for CVD, a consequence of CVD (Brattström & Wilcken, 2000), or is simply a proxy for low SES and poor nutrition.

Others have reported evidence that suggests folate deficiency alone may account for the risk of cardiovascular events, with homocysteine just a marker for low folate (Verhaar, Stroes & Rabelink, 2002). This conclusion is supported by Verhaar and colleagues (1998) who reported that folate was beneficial for hypercholesterolemia, independent of its effect on lowering homocysteine, and by Lewis and colleagues (2006) who reported an association between genotype MTHFR C677T and depression. This genotype influences functioning of the folate metabolic pathway, therefore further supporting a causal relationship from folate to depression.

It has been well established that CVD and depression are bi-directionally associated (CVD -> depression: Freedland et al., 2003; Schleifer et al., 1989; depression -> CVD: Barefoot & Schroll, 1996; Everson et al., 1996; Frasure-Smith, Lespérance & Talajic, 1995; Van der Kooy et al., 2007). Prevalence of depression in patients with CVD
ranges from 16-23% (Musselman et al., 1998), while depression predicts future cardiac events (Aromaa et al., 1994), and increased mortality (Ahern et al., 1990; Frasure-Smith, Lеспérance & Talajic, 1993; Frasure-Smith et al., 1995) in patients with coronary heart disease. Others have also reported depression as an independent risk factor in the progression of CVD (Aromaa et al., 1994; Brozek et al., 1966; Everson et al., 1996; Lichtman et al., 2008; Murphy et al., 1987; Ostfeld et al., 1964; Simonsick et al., 1995; Wassertheil-Smoller et al., 1996). This suggests that folate and/or vitamin B₁₂ deficiencies, rather than elevated homocysteine could be the common pathogenesis underlying the link between CVD and mental health.

The conclusion that homocysteine is cross-sectionally related to PA due to it being a marker of folate and vitamin B₁₂ deficiencies is supported by the finding that homocysteine does not mediate the relationship between folate, vitamin B₁₂, or their interaction, on PA, NA, or LS. Previous reports have not explicitly tested a mediation model; Kim and colleagues (2008) reported a similar pattern of results; they found that while the relationships from folate and vitamin B₁₂ to depressive symptoms were dependent on vitamin B₁₂ and/or homocysteine, and on folate and/or homocysteine respectively, and the relationship between homocysteine and depressive symptoms was attenuated once folate and vitamin B12 were controlled for (Kim et al., 2008). However, others have found that the relationship between homocysteine and depressive symptoms was independent on folate and/or vitamin B₁₂ (Sachdev et al., 2005) and that the relationship between vitamin B₁₂ and depressive symptoms was dependent on folate and/or homocysteine concentrations (Robinson et al., 2011). Though these authors did not specifically address the temporal order of mediation and baseline levels of the outcome variable.
When only two waves of data are available it is not possible to satisfy temporal criteria necessary to demonstrate causality. Two approaches are commonly used – the half longitudinal design and cross-lagged designs. The first approach uses cross-sectional data to estimate one path, thus assuming the causal structure is in equilibrium and failing to account for autoregressive effects (Maxwell & Cole, 2007). The second approach assumes stationarity between the mediator and the outcome variable (Maxwell & Cole, 2007); that is, the degree of change in PA, NA, and LS caused by homocysteine does not change (across time) from wave to wave, this approach also accounts for autoregressive effects when estimating all causal paths. Path analysis was used to estimate these models based on three clear advantages: (1) parameters are estimated simultaneously, (2) residuals are allowed to co-vary across time, and (3) PA, NA, and LS are able to be modelled as latent constructs, thereby removing measurement error from these constructs. For indirect effects – all cross-sectional correlations (bar one from homocysteine to NA which remained the same and non-significant) that therefore did not control for autoregressive effects, over-estimated relationships compared to the longitudinal associations found when controlling for the influence of prior levels of the outcome variable.

**Limitations**

Several limitations should be considered when interpreting findings from this study. Generalisability is limited to older adults because nutrient absorption and therefore dietary requirements are known to differ across the lifespan (NHMRC, 2005). This sample also had low levels of vitamin B$_{12}$ deficiency, folate deficiency, hyperhomocysteinemia and depression; as such, the results cannot be applied to patient groups or populations with significantly impaired diets. Serum measurements of vitamin B$_{12}$ and folate have been criticised because they reflect recent dietary intake whereas Red Blood Cell (RBC)
measurements provide a more accurate reflection of body stores and are not affected by recent diet. However, serum vitamin B\textsubscript{12} and folate demonstrated good stability across 18 months here; based on Table 13, serum vitamin B\textsubscript{12} at baseline explained 58.4\% of the variance in serum vitamin B\textsubscript{12} 18 months later, and serum folate at baseline explained 36.8\% of the variance in serum folate 18 months later. Furthermore, only 16.6\% changed categories for vitamin B\textsubscript{12} (i.e., from deficient to non-deficient), and only 8.4\% for folate, thus providing further evidence for the broad stability of serum vitamin B\textsubscript{12} and serum folate over 18 months.

**Future directions**

Future research in this area should include measures of positive mental functioning, such as SWB used here, and extend methods beyond the use of single, isolated nutrients to more accurately reflect nutritional intake. For instance, future research in this area could test additional potential nutritional pathways by considering the use of dietary patterns. However, omnibus delivery of nutrients, such as via Berocca\textsuperscript{®} (Carroll et al., 2000; Kennedy et al., 2010; Schlebusch et al., 2000) do not offer the best solution because specific nutrient responses cannot be identified. The current study assessed a commonly hypothesised pathway from folate and vitamin B\textsubscript{12} to mental health through homocysteine. Oxidative stress is another potential mechanism that could be explored to explain the link between folate and vitamin B\textsubscript{12} with mental and cardiovascular health, given its implication in the pathogenesis of CVD (Griendling & Alexander, 1997), and the fact that folate possesses antioxidant potential (Verhaar et al., 1998). Analyses of nutritional pathways and dietary patterns will also be enhanced with the use of longitudinal data to correctly reflect the temporal nature of these hypotheses. As
we have shown here, cross-sectional correlations can conceal the true nature of data patterns. Where limited resources impose constraints on longitudinal data collection the use of retrospective data-collection methods can be useful, such as the Lifetime Diet Questionnaire (Hosking, Nettelbeck & Wilson, 2010), a recently developed tool designed to access dietary intake across the lifespan.

**Conclusion**

This is the first study to assess the role of folate, vitamin B$_{12}$, and homocysteine for SWB in a representative sample of older community-dwelling adults with low levels of B-vitamin deficiencies and without diagnosed depression. Results can therefore not be applied within clinical settings for patients presenting with mood disorders. These results suggest that higher levels of folate and vitamin B$_{12}$ are beneficial for positive mental health in the community, thus having the potential to benefit all non-clinical, aged populations. Recommendations for optimal levels of folate and vitamin B$_{12}$ in non-clinical, aged populations for positive mental health are based on the observation of a direct effect of these B-vitamins on PA, rather than on their ability to lower levels of homocysteine.
**Context**

Folate, vitamin $B_{12}$ and homocysteine have long been implicated in mental illness, and growing evidence suggests they may play a role in mental health. Elucidation of these relationships is confounded due to the dependence of homocysteine on available levels of folate and vitamin $B_{12}$.

**Design**

Serum folate, serum vitamin $B_{12}$, plasma homocysteine and SWB were measured at baseline and 18 months later in older, community-dwelling healthy ($N=391$) adults. Cross-lagged mediation models allowed us to assess whether folate and/or vitamin $B_{12}$ had a direct influence on SWB, or whether the relationship was mediated by homocysteine.

**Contribution**

Folate and vitamin $B_{12}$ predicted subsequent levels of PA independent of baseline SWB, gender, use of cardiovascular medications and physical health. This was a direct relationship that was not mediated by homocysteine.

**Implications**

The connection from folate and vitamin $B_{12}$ to PA in healthy adults offers an easily modifiable preventative measure to ill health with potential benefits at the population-level.
Chapter 5. Paper 3

*n*-3 Polyunsaturated Fatty Acids and Subjective Wellbeing in Healthy Older Adults:
A Randomised, Double-Blind Intervention Trial

The aim of the third Paper was to assess whether a nutritional intervention was able to affect the normative trajectory of SWB over 18 months. Recent research in this area has found promising results for a potential therapeutic role of *n*-3 long-chain polyunsaturated fatty acids (*n*-3 LC PUFAs) for depression (see Appleton et al., 2006, 2010; Lin & Su, 2007). A number of rigorous RCTs have been recently published with mixed results (e.g., Antypa et al., 2012; Gracious et al., 2010); however, there have been fewer RCTs conducted on healthy participants (e.g., Fontani et al., 2005; van de Rest et al., 2008). Whether *n*-3 LC PUFAs have a benefit for mood levels in the general population without clinically diagnosed depression remains unanswered; establishing a benefit here may have population-level implications.

The research question addressed was whether supplementation of *n*-3 LC PUFAs could affect the normative development of SWB in older, community living adults over 18 months when compared to placebo. To answer this question we conducted a double-blind, randomised, placebo-controlled trial (RCT). The aim was to incorporate the model of SWB obtained from the analyses in Paper 1; however, this presented a problem. Our initial conception of analyses for the RCT was to perform latent growth curve modelling (LGCM). This was based on several grounds. Firstly, longitudinal nutritional interventions have traditionally been assessed using either independent samples *t*-tests at each measurement point or Analysis of Covariance (ANCOVA) models to incorporate
repeated measures and potential covariates. Arguments against the theory behind this approach are long standing (Matthews et al., 1990); the basic premise of which is that there is often no theoretical reason to hypothesise a difference at the particular time points specified. That is, we hope to alter the trajectory of one group with an intervention, but our measurement of this trajectory is often arbitrary and performed at convenient, equally spaced intervals. As part of our study protocol outcome variables were measured for all participants at baseline, 6, 12 and 18 months. How is this interpreted, for example, if we see a significant difference at 6 months but not at 12 or 18 months? These measurement points were not based on a priori hypothesis about the specific effects of n-3 LC PUFAs versus placebo at 6, 12 and 18 months, thus specific interpretations of this potential pattern of results are difficult to substantively interpret. Statistical techniques have long been available to assess growth as a more continuous construct; however, it has only been relatively recently that these techniques have been easily accessible through their application in software such as AMOS (Arbuckle, 2006) and Mplus (Muthén & Muthén, 1998-2007). We therefore thought it imperative that such techniques were applied here.

Because these methods are based on the entire trajectory of the outcome variable across the study length they provide more power to find an effect, and they enable a greater scope of research questions to be addressed. Whereas traditionally the questions that could be answered were simply the difference between groups on an outcome measure (e.g., t-tests & ANOVA), whilst potentially controlling for covariates (e.g., ANCOVA), LGCM allows us to assess whether any statistically significant heterogeneity in the individual growth trajectories of SWB, including starting points and potential growth, can be accounted for by the intervention and/or the level of SWB. Specifically, there are three main questions that we can investigate (Byrne, 2012):
Question 1 Are there inter-individual differences in the starting point and growth trajectory of SWB in the population (variances of the intercept and slope)?

Question 2 Do self-perceptions of SWB differ between treatment and placebo group?

Question 3 Does the rate at which self-perceived SWB change over time differ depending on group status (i.e., treatment versus placebo) and/or level of SWB?

These questions are of greater clinical significance and allow us to explore the question of whether the treatment is only successful for particular groups within our sample, for example, those with low baseline levels of the treatment, or those with low SWB at baseline.

There was one major drawback to using LGCM; we were unable to completely replicate the model of SWB as recommended in Paper 1. LGCM is based on Structural Equation Modelling (SEM) and path analysis where variables are estimated as observed or latent, and paths between these are the parameters estimated. Such models are unidentified when the number of parameters to be estimated exceeds the degrees of freedom. Because we were estimating the effects of several covariates, the treatment grouping, and an intercept and slope term (i.e., the baseline values and potential growth), there were not enough degrees of freedom remaining to additionally model SWB as three latent, inter-correlated components of PA, NA and LS. However, several strategies were adopted in an attempt to replicate this model as closely as possible. Firstly, we decided to free up available degrees of freedom by using the latent factor scores to create observed variables
for PA, NA and LS rather than estimating these as latent factors based on item responses. This provides a slightly different theoretical perspective – latent variables are reflective, that is, item responses are thought to tap into some aspect of the latent variable. Creating a composite, however, suggests that the items are *everything we need to know* about the construct. However, we were still able to capture the relative individual item contributions by using each items factor loading on the latent construct from Paper 1 to weight its relative contribution to the construct – that is, an item with a loading of .7 *contributes more* to the latent construct than an item with a loading of .5. By freeing up these parameters we were able to retain the inter-correlations between the three components of SWB in line with the recommendations from Paper 1. We felt this was the best option to allow us to use LGC modelling and to approximate the recommendations from Paper 1 as closely as possible.
n-3 Polyunsaturated Fatty Acids and Subjective Wellbeing in Healthy Older Adults: A Randomised, Double-Blind Intervention Trial

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To be submitted for publication

Statement of Contributions

This chapter is a co-authored manuscript. Please refer to Appendix 1 for a statement of author contributions.
Abstract

There is increasing recognition of the role that omega-3 polyunsaturated fatty acids (n-3 PUFAs) play in a variety of health outcomes. Evidence regarding their therapeutic potential for mental health is equivocal, partly due to differences in measuring n-3 PUFAs, different populations, and how mental health is assessed. We tested the effect of n-3 LC PUFA supplementation on Subjective Wellbeing using a randomised, double-blind, placebo-controlled design. \( N = 391 \) well-functioning, community-dwelling, older (65-91yrs) adults received either 1720mg DHA plus 600mg EPA, or placebo, daily for 18 months. Subjective Wellbeing was defined by the inter-correlations of its three components – Positive Affect (PA), Negative Affect (NA), and Life Satisfaction (LS). Latent growth curve analyses were employed with PA, NA, and LS modelled as three related growth processes for the entire sample. Treatment group, initial n-3 LC PUFAs (EPA+DHA) and initial n-3 PUFAs (EPA+DHA+DPA+ALA) from erythrocyte membranes were included as potential predictors of initial status and rate of change across 18 months in PA, NA, and LS. Results indicated that treatment group did not predict change in PA, NA, or LS. Initial n-3 LC PUFAs (EPA+DHA) significantly predicted baseline PA, but not baseline NA or LS, or change in PA, NA or LS. Total n-3 PUFAs (EPA+DHA+DPA+ALA) did not predict baseline PA, NA, or LS; it did however, predict rate of change in both NA and LS over 18 months. Thus, there is some evidence for the potential role of n-3 PUFAs in decreasing the growth of NA and increasing the growth of LS over 18 months in otherwise healthy, older individuals.
**n-3 Polyunsaturated Fatty Acids and Subjective Wellbeing in Healthy Older Adults: A Randomised, Double-Blind Intervention Trial**

Omega-3 polyunsaturated fatty acids (n-3 PUFAs) are essential fatty acids that must be obtained from dietary sources such as oily fish and walnuts. Oily fish provide a direct dietary source of n-3 long-chain (LC) PUFAs, whereas dietary sources such as walnuts and flaxseed provide a source of n-3 short-chain (SC) PUFAs and vegetable oils provide a source of the parent n-3 PUFA, α-linolenic acid (ALA). n-3 LC PUFAs have been associated with many physical health benefits from coronary health (Rissanen et al., 2000; Yokoyama, 2007) to eye health (Christen et al., 2011); however, evidence regarding their therapeutic role in the treatment of mental health remains equivocal.

**Biological mechanisms**

Several biological mechanisms have been proposed to account for how n-3 PUFAs may influence mood. n-3 PUFAs may have an indirect effect on mood via the mediating role of chronic inflammatory responses. Chronic inflammation involves the continual stimulation of pro-inflammatory immune cells, which can result in damage to healthy cells. n-3 PUFAs have been shown to have anti-inflammatory effects (Farooqui, Ong & Horrocks, 2006; Su, 2009) and it has been suggested that chronic overactive inflammatory responses play a role in depression (Das, 2004) by inducing oxidative stress (Su, 2009). Furthermore, arachidonic acid (AA; an n-6 PUFA) can be pro-inflammatory (Le-Niculescu et al., 2011; Su, 2009), which could account for results that suggest the ratio of n-3 LC PUFAs to n-6 FAs is associated with depression (Adams et al., 1996).
Another potential mechanism concerns the influence of $n$-$3$ PUFAs on dopaminergic and serotonergic functions (Vaddadi, 2006). Change in $n$-$3$ LC PUFA concentration in the brain could lead to a decrease in dopamine and an increase in serotonin receptor density in the frontal cortex (Su, 2009), with both neurotransmitters thought to play a role in the aetiology of depression (Su, 2009). Additionally, $n$-$3$ LC PUFAs are involved in the regulation of signal transduction (Su, 2009) via their influence on cell membrane fluidity (Morgan & Jorm, 2008), providing another mechanistic account for their role in the pathophysiology of depression.

**Omega-3 polyunsaturated fatty acids and mental health**

Low prevalence rates of mental disorders observed in countries with high fish consumption (Noaghiul & Hibbeln, 2003) suggest that $n$-$3$ LC PUFAs may be important for mental health. Western dietary changes, including increased $n$-$6$ PUFA and decreased $n$-$3$ PUFA intake during the 20\textsuperscript{th} century (Blasbalg et al., 2011) may account for higher incidence of mental illness reported during this same period (Klerman & Weissman, 1989). Deficits in $n$-$3$ LC PUFAs have been shown in individuals with both mild (Mamalakis et al., 2002) and major depression (Maes et al., 1999; Peet et al., 1998), and older participants with depressive symptoms have been found to have a lower percentage of $n$-$3$ LC PUFAs and a higher ratio of $n$-$3$ LC PUFAs to $n$-$6$ PUFAs compared to non-depressed controls (Tiemeier et al., 2003).

Cross-sectional studies have reported significant beneficial associations between fish consumption and both depressive symptoms (Tanskanen et al., 2001) and self-reported mental health, after adjusting for confounders (Silvers & Scott, 2002). Similar results have been found based on $n$-$3$ PUFA intake calculated from food frequency
questionnaires (FFQ); n-3 LC PUFA intake was found to be inversely associated with risk of chronic depressive symptoms in women (Colangelo et al., 2009), in women at 32 weeks gestation (Golding et al., 2009), and in university students (Yary & Aazami, 2011), after controlling for confounders. A large-scale (N=2982) UK study reported an association between depressed mood and n-3 LC PUFA intake calculated solely from fish, though this relationship was attenuated once age and an index of multiple deprivation were controlled (Appleton et al., 2007). Objective measures of fatty acid status support these relationships; inverse associations have been found between self-rated depression in healthy adults and DHA levels in adipose tissue (Mamalakis et al., 2002), and associations have been reported between depression and erythrocyte EPA levels, the ratio of AA to EPA, and of n-6 LC to n-3 LC PUFA in a small sample of moderate-to-severely depressed participants (Adams et al., 1996). It should also be noted that the reverse direction of causality has not been eliminated as a potential source of the relationship between n-3 LC PUFAs and mood; as suggested by Kamphuis and colleagues (2006), depression often leads to appetite loss and decreased food consumption.

Longitudinal observational research has not confirmed the nature of any relationship between n-3 LC PUFAs and mental health. In a large population-based study of older Finnish men (N=29,133) Hakkarainen and colleagues (2004) found no association between fish or n-3 LC PUFA intake calculated from dietary history questionnaires and either self-reported depressed mood, major depressive episode, or suicide in the following nine years. Another population-based study of Swedish adult women found that those with a high intake of fish, n-3 LC PUFA, or n-6 PUFA, calculated from a FFQ, had a lower prevalence of psychotic-like symptoms approximately 11 years later (Hedelin et al., 2010). The reported relationship was non-linear with the strongest reduced risk for
psychotic-like symptoms with an intermediate intake of fish or PUFA (Hedelin et al., 2010). In a large French cohort \((N=13,017)\) it was found that an intake of fatty fish higher than 0.1\% of total energy intake was significantly associated with a decreased risk of a depressive episode and of recurrent depressive episodes, but not of a single depressive episode, approximately six years later (Astorg et al., 2008). When the same cohort was analysed again as a nested case-control study (Astorg et al., 2009) using serum \(n\)-3 LC PUFA concentrations, no association was found between \(n\)-3 LC PUFA and risk of depression eight years later.

Intervention trials have sought to establish causal associations. Some have reported a beneficial effect of \(n\)-3 PUFAs for adults with moderate-to-major depression (da Silva et al., 2008; Frangou, Lewis, & McCrone, 2006; Jazayeri et al., 2008; Nemets et al., 2002; Peet & Horrobin, 2002; Rondanelli et al., 2010; Stoll et al., 1999; Su et al., 2003; Su et al., 2008), with interventions ranging from four (Nemets et al., 2002) to 16 weeks (Stoll et al., 1999), and with EPA alone (Frangou et al., 2006; Nemets et al., 2002; Peet & Horrobin, 2002), or with EPA and DHA (da Silva et al., 2008; Rondanelli et al., 2010; Stoll et al., 1999; Su et al., 2003; Su et al., 2008). Similar trials have reported no benefit for adults with moderate-to-major depression (Bot et al., 2010; Carney et al., 2009; Freeman et al., 2008; Greynier et al., 2007; Keck et al., 2006; Marangell et al., 2003; Rees et al., 2008; Rogers et al., 2008; Silvers et al., 2005), with comparable trial lengths from six weeks (Marangell et al., 2003; Rees et al., 2008) to 16 weeks (Greynier et al., 2007; Keck et al., 2006) of supplementations, and with DHA alone (Marangell et al., 2003), EPA alone (Bot et al., 2010; Keck et al., 2006), or a combination of the two (Carney et al., 2009; Freeman et al., 2008; Greynier et al., 2007; Rees et al., 2008; Rogers et al., 2008; Silvers et al., 2005). Recent randomised control trials of postpartum depressive symptoms
all reported no benefit of supplementation on depressive symptoms with 200mg/d DHA (Llorente et al., 2003), 800mg/d DHA (Makrides et al., 2010), and both 220mg/d DHA and a combination of DHA and AA (220mg/d each; Doornbos et al., 2009) compared to placebo, up to 6 months postpartum.

Eight randomised control trials have been conducted with healthy adults, where four have reported a significant treatment effect (Antypa et al., 2012; Fontani et al., 2005; Hamazaki et al., 1996; Kiecolt-Glaser et al., 2011), and four have reported no treatment effect (Antypa et al., 2009; Bradbury et al., 2004; Hamazaki et al., 1998; van de Rest et al., 2008). Hamazaki and colleagues (1996) found that during stress, extraggression remained stable across 12 weeks with a DHA-rich supplementation, compared to an increase in the placebo group, in healthy university students. They sought to replicate these results with healthy university students under non-stressful conditions with DHA treatment alone for 12 weeks; however, whilst extraggression remained stable in the DHA group, it decreased in the placebo group. The trials were methodologically very similar, the disparate results may reflect the utility of DHA only under stressful conditions, or could be due to differences in palmitic acid, vaccenic acid and linoleic acid between groups. Palmitic acid and vaccenic acid were both significantly lower in the DHA group (Hamazaki et al., 1996) and both have been associated with depression (Astorg et al., 2009; McNamara et al., 2010), thus potentially accounting for the observed decrease in extraggression. Furthermore, in their 1998 trial (Hamazaki et al. 1998) linoleic acid, which has been associated with a decreased risk of depression (Astorg et al., 2009), was significantly higher in the control group, which may account for the observed decrease in extraggression observed in the control group.
More recently, three RCTs (Antypa et al., 2012; Fontani et al., 2005; Kiecolt-Glaser et al., 2011) have all reported positive effects of n-3 LC PUFA supplementation. Fontani and colleagues (2005) reported significant improvements in the depression, anxiety, fatigue, anger and vigour subscales from the Profile of Mood States (POMS) following five week EPA-rich supplementation in 49 healthy adults. Similarly, Antypa and colleagues (2012) reported significant improvements in the depression and anxiety subscales of the POMS following a 16 week EPA-rich supplementation on young adults who had recovered from depression, though no improvements were reported for depressive symptoms as measured by the Beck Depression Inventory (BDI). Kiecolt-Glaser and colleagues (2011) also reported a significant treatment effect on anxiety measured with the Beck Anxiety Inventory (BAI) following 12 week supplementation with EPA and DHA in 68 healthy medical students, but not on depression measured with the Centre for Epidemiological Studies – Depression scale, similar to that reported by Antypa and colleagues (2012).

Another three similar RCTs, however, reported no beneficial effect of n-3 LC PUFA supplementation on mood in healthy adults. Bradbury and colleagues reported no reduction in stress across six weeks with a low-dose DHA-rich supplement in 30 stressed university staff. Neither low- or high-dose EPA+DHA treatment improved mood or depressive symptoms over 26 weeks in 302 community-dwelling, non-depressed, older (>65 years) adults, and no treatment effect was observed with a four week EPA-rich supplementation on self-reported mood states in 54 healthy university students.
Three meta-analyses suggested that positive results may be due to heterogeneity and publication bias (Appleton et al., 2006, 2010; Lin & Su, 2007); all three found some evidence for a therapeutic role of \( n\)-3 LC PUFAs on those diagnosed with depressive mood (Appleton et al., 2006, 2010; Lin & Su, 2007). The two meta-analyses conducted by Appleton and colleagues (2006, 2010) further concluded that there was little evidence for a beneficial role of \( n\)-3 LC PUFAs in those without a diagnosis of depression.

We investigated whether 18-month supplementation with DHA-rich \( n\)-3 PUFA benefits Subjective Well-being (SWB) measured via its three components – Positive Affect (PA), Negative Affect (NA), and Life Satisfaction (LS) in a sample of 391 healthy older adults. Group differences were assessed using Latent Growth Curve modelling following Byrne’s (2012) multiple domain approach. This allows us to assess the impact of the treatment group and total \( n\)-3 LC PUFA concentration from erythrocyte membranes measured at baseline – on initial levels of PA, NA, and LS, and on their rate of change over 18 months.

The three questions to be addressed are:

1. Does being in the treatment group predict rate of change in self-reported PA, NA, or LS over 18 months?
2. Does baseline \( n\)-3 LC PUFA (EPA+DHA) status predict baseline levels of PA, NA, or LS, and/or their rate of change across 18 months?
3. Does baseline \( n\)-3 PUFA (EPA+DHA+DPA+ALA) status predict baseline levels of PA, NA or LS, and/or their rate of change across 18 months?
Method

Participants

Participants (N=391; female=54.1%) were community dwelling older adults aged between 64 and 90 years (M=72.3, SD=5.55yrs) who provided written informed consent prior to participation. All had normal cognitive function at baseline (MMSE>23) and were not taking n-3 fish oil (or algal) supplements, and agreed to not commence supplementation for the trial duration. Exclusion criteria included diagnosis of an intellectual disability, current major clinical depression, diabetes or dementia and any conditions which may result in cognitive impairment, such as a head injury, transient ischemic attacks, strokes, coronary artery bypass surgery, open heart surgery, or a history of drug or alcohol abuse. Participants were also required to be fluent in English.

Materials

Positive and Negative Affect: Positive and Negative Affect Schedule (PANAS; Watson, Clark & Tellegen, 1988)

The PANAS contains 20 mood descriptors (10 positive, 10 negative) and requires respondents to rate ‘to what extent have you felt this way during the past week?’ from 1 (very slightly, or not at all) to 5 (extremely); higher scores indicate greater PA or NA. The PANAS has demonstrated acceptable internal consistency reliability for both PA (range \( \alpha=0.86-0.90 \)) and NA (range \( \alpha=0.84-0.87 \)) in undergraduate students (Watson, Clark & Tellegen, 1988), and has been validated in samples of older adults (PA range: \( \alpha=0.84-0.96 \) and NA range: \( \alpha=0.64-0.91 \)).
Life Satisfaction: Satisfaction with Life Scale (SWLS; Diener, Emmons, Larsen, & Griffin, 1985)

The SWLS is a five-item questionnaire designed to measure global LS, the cognitive component of SWB. Respondents indicate their level of agreement with each item (e.g., item 1, “In most ways, my life is close to my ideal”) on a seven-point Likert scale (1=Strongly Disagree, to 7= Strongly Agree), with higher scores indicating greater satisfaction. The SWLS has demonstrated adequate internal consistency reliability (average $\alpha=0.78$ across 62 articles; Vassar, 2008) and has been validated for use in aged populations ($\alpha=0.83$; Pavot et al., 1991).

Self-Reported Health Status: Short-Form-36 Health Survey, version 2 (SF-36v2; Ware, Kosinski & Dewey, 2000)

Perceived health status was measured with the SF-36v2, a 36 item questionnaire designed to measure physical and mental health using Likert scales to record responses. For the present sample, the physical and mental summary scales were calculated using factor score weights (Hawthorne, et al., 2007) derived from the 2004 South Australian Health Omnibus Survey dataset (SAHOS; Harrison Health Research, 2004).

Socio-Economic-Status (SES)

Socio-Economic Indexes for Areas (SEIFA; ABS, 2008)

SEIFA percentiles of relative disadvantage and advantage were used as a proxy measure of SES. The index measures qualifications, income, education and household
type in 21 questions from the Australian 2006 census. Distribution of all SEIFA scores were divided into 100; the lowest scoring 1% (score of 1) represent relative disadvantage and the top 1% (score of 100) represent relative advantage. Our results are presented as proportion of our sample that had a SEIFA score that fell in the top or bottom quartile of the population.

**Income & Education**

Income was measured on a 15 point scale (following the Australian Census, 2006). Education was measured on a seven point scale in common educational categories from 1= finished primary school, to 7=postgraduate studies.

**Omega-3 polyunsaturated fatty acid status**

Erythrocyte membrane PUFAs were analysed according to Ridges and colleagues (2001) from fasted blood samples and expressed as a percentage of total fatty acids. Two measures of PUFA status were included as predictors of SWB here; *n*-3 LC PUFAs calculated as the sum of EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) expressed as percentage of total fatty acids, and *n*-3 PUFAs calculated as the sum of EPA, DHA, ALA (α-linolenic acid) and DPA (docosapentaenoic acid), again expressed as a percentage of total fatty acids.
Body Mass Index (BMI)

Body Mass Index was calculated as weight (kg)/height (m)²; weight was measured using a calibrated precision scale (UC-321PBT; A&D Medical, Sydney, Australia), and height using a wall-mounted stadiometer (SECA; Hamburg, Germany).

Procedure

Data presented here form part of a larger clinical study on nutrition, wellbeing and cognition in older people; a more detailed methodology of the study protocol can be found in Danthiir et al. (2011). Participants were recruited into this study via local newspaper advertisements and South Australian agencies and organisations for older citizens. Participants who met initial inclusion criteria were screened for dementia using the MMSE (>23). Participants were randomly allocated to treatment groups using a computer generated list administered by a third party with two equal size groups and age-stratification (5-year bands) being the only restrictions. All participants received visually identical capsule bottles and capsules, and all were required to consume four capsules per day for the 18-month trial. The intervention capsules each contained 430mg DHA and 150mg EPA, totalling 1720mg DHA and 600mg of EPA daily. Placebo capsules each contained 990mg low-polyphenol oil, 1.8mg EPA and 1.2mg DHA, to mask capsule flavour differences, totalling 3960mg low-polyphenol oil, 7.2mg EPA and 4.8mg DHA daily. Four assessment sessions (baseline, 6mo, 12mo, & 18mo) were conducted, where fasted blood samples were collected. Paper versions of the questionnaires were mailed to participants approximately 4 weeks prior to each assessment and were returned via reply-paid post prior to their assessment visit.

Results

Statistical analyses

Positive Affect (PA), Negative Affect (NA) and Life Satisfaction (LS) were modelled as reflective congeneric Confirmatory Factor Analytic (CFA) measurement models within each group (treatment vs. placebo) at each time point (baseline, 6mo, 12mo, 18mo) using Mplus Version 5.21 (Muthén & Muthén, 1998-2007). Measurement invariance (i.e., of parameters from each item to the measured construct) for PA, NA, and LS was established between groups at each time point (between treatment and placebo at baseline, 6, 12 and 18 months) and within groups across time (treatment group at baseline, 6, 12 and 18 months, and placebo group at baseline, 6, 12 and 18 months). Thus we can conclude that any observed changes in level of PA, NA or LS across time reflect real changes on the construct rather than differences, for example, in interpretation of the questionnaire items. Establishing invariance allowed us to use the parameter estimates from baseline for each construct, modelled separately, to calculate factor scores for PA, NA, and LS at each time point. This allowed us to model PA, NA and LS at each time point as if they were observed variables, to simplify subsequent analyses.

Group differences were assessed using Latent Growth Curve Modelling (LGCM) with a two-step approach (e.g., see Willett and Sayer, 1994) to evaluate intra-individual and inter-individual change. Following Byrne’s (2012) example of a dual domain approach, we employed a triple domain approach for PA, NA and LS; the three growth curves for PA, NA, and LS were modelled simultaneously to account for the inter-correlation between PA, NA, and LS across time. Within this approach, the entire sample is modelled simultaneously, that is, the growth curve contains individuals from both the
treatment and the intervention group. Then treatment group and total omega-3PUFAs from erythrocyte membranes measured at baseline can then be entered as covariates, to assess their impact on the initial status and rate of change in PA, NA, and LS. Both groups were modelled simultaneously with effect of the intervention assessed as a covariate because this offers a straightforward approach to assess, and interpret, the impact of an intervention when considering multiple inter-related outcomes.

**Preliminary analyses**

The final sample consisted of 355 participants who completed all four time points (n=173 intervention; n=182 placebo). Attrition was minimal, 5.9% from baseline to 6mo, 2.7% from 6mo to 12mo and 0.8% from 12mo to study completion (18mo). Reasons for dropout after baseline assessment included unrelated health issue (44.4%), reaction to the capsules (22.2%), desire to start own supplements (8.3%), family illness (8.3%), deceased (5.6%), lost to follow up (5.6%) and time constraints (5.6%). Although nearly a quarter of drop-outs were due to a reaction to the capsule, this constitutes only 2.3% of the total number of participants who completed all four assessments. Reaction to the capsules included complaints of gastric symptoms (n=6), weight gain (n=1) and reflux (n=1), all apart from one were in the treatment group.

Independent samples *t*-tests confirmed there were no baseline differences on SWB (PA, NA, and LS factor scores) or depression between those who completed and those who did not in either the intervention or placebo group.
Compliance according to count of returned capsules was excellent in both the intervention ($M\%$ compliance $= 97.3$, $SD=4.7$), and placebo ($M\%$ compliance $= 97.7$, $SD=4.5$) groups. Erythrocyte membrane concentrations increased on average by 246.1% ($SD=105.8$) for EPA, 89.4% ($SD=36.4$%) for DHA, with an increase of 74.3% ($SD=27.7$%) in $n$-3 PUFA concentrations in the intervention group. Little change occurred from baseline to 18 months in the placebo group: there was a 6.2% increase in EPA, 6.5% decrease in DHA, and a 3.5% decrease in total $n$-3 PUFAs from baseline to 18 months as measured in erythrocyte membranes. There were no significant differences in erythrocyte membrane EPA, DHA and total $n$-3 PUFAs between the intervention and placebo group at baseline; these were significantly different between the two groups at 6, 12, and 18 months (see Figure 18).
Figure 18. Total n-3 LC PUFA (EPA+DHA) and total n-3 PUFA (EPA+DHA+DPA+ALA), both expressed as a percentage of total fatty acids from erythrocyte membranes in the treatment and placebo group at baseline, 6, 12 and 18 months.

Note: trt, treatment group

Descriptive statistics

Demographic information obtained at baseline is displayed in Table 15. The physical health summary scale from the SF36v2 was almost identical to age matched (65+) South Australian norms (physical: $M=42.7 \ SD=11.8$) indicating average overall physical health. The majority (34.4%) of participants fell in the top quartile of all possible SEIFA scores indicating high relative advantage based on household qualifications,
income and occupations; only 12.8% were in the lowest quartile representing relative disadvantage. Participants had an average of 13.5 years of education ($SD=6.07$) with 41.7% of participants achieving some level of education beyond high school. There were no significant differences in any of these variables between the intervention and placebo group.
Table 15

Participant demographics by total sample, placebo, and treatment group obtained at baseline assessment.

<table>
<thead>
<tr>
<th></th>
<th>Total (N=355)</th>
<th>Placebo (n=182)</th>
<th>Treatment (n=173)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>Age</td>
<td>72.35</td>
<td>5.55</td>
<td>72.26</td>
</tr>
<tr>
<td>BMI</td>
<td>27.26</td>
<td>4.23</td>
<td>27.16</td>
</tr>
<tr>
<td>Years of Education</td>
<td>13.47</td>
<td>6.06</td>
<td>13.80</td>
</tr>
<tr>
<td>SEIFA</td>
<td>58.49</td>
<td>26.19</td>
<td>59.56</td>
</tr>
<tr>
<td>MMSE</td>
<td>28.67</td>
<td>1.33</td>
<td>28.72</td>
</tr>
<tr>
<td>Physical Health</td>
<td>43.63</td>
<td>7.00</td>
<td>43.75</td>
</tr>
<tr>
<td>n-3 LC PUFAs</td>
<td></td>
<td></td>
<td>5.89</td>
</tr>
<tr>
<td>n-3 PUFAs</td>
<td>8.18</td>
<td>1.29</td>
<td>8.19</td>
</tr>
</tbody>
</table>

Note: all differences between placebo and treatment groups p>0.05; n-3 LC PUFAs (EPA+DHA); n-3 PUFAs (EPA+DHA+DPA+ALA) both from erythrocyte membranes and expressed as a percentage of total fatty acids; physical health assessed with the Short-form Health Survey (SF-36v2); BMI, Body Mass Index; MMSE, Mini-mental state examination; SEIFA, Socio-Economic Indexes for Areas.
Individual measurement models

Individual growth curves for PA, NA, and LS modelled as observed variables (calculated from baseline factor scores) for all 355 participants were first assessed to establish the fit, initial status and change in PA, NA, and LS across the 18 months. Results are shown in Table 16 and discussed below.

Change in Positive Affect: The latent growth curve model for PA alone over 18 months provided excellent fit to the data ($\chi^2(5)=3.70, p=0.59; CFI=1.00; TLI=1.00; \text{RMSEA}=0.00 \text{CI}_90=[0.00,0.06]; \text{SRMR}=0.06$). Minimal fluctuation in average values at each of the four time points, shown in Table 16, indicated little average change in PA over 18 months, confirmed by a non-significant slope factor (mean=0.095, $p=0.144$). Variance scores were stable from baseline to 12 months, but were reduced by 18 months suggesting perceptions of PA varied less at the end of the study period; however, the variance of the slope indicated there was no significant deviation from the average rate of change (variance=0.184, $p=0.402$).

Change in Negative Affect: The latent growth curve model for NA alone over 18 months provided excellent fit to the data ($\chi^2(5)=2.27, p=0.81; CFI=1.00; TLI=1.01; \text{RMSEA}=0.00 \text{CI}_90=[0.00,0.04]; \text{SRMR}=0.04$). Again, Table 16 shows minimal average change across the four time points, confirmed by a weak, though significant slope factor (mean=0.006, $p=0.005$). Variance scores were also stable across the 18 months indicating no change in inter-individual variability, confirmed by a non-significant variance estimate of the slope (Variance=0.000, $p=0.375$).
Change in Life Satisfaction: The latent growth curve model for LS alone over 18 months provided excellent fit to the data ($\chi^2(5)=2.21, p=0.82; \text{CFI}=1.00; \text{TLI}=1.01; \text{RMSEA}=0.00 \text{ CI}_{90}=[0.00, 0.04]; \text{SRMR}=0.02$). Table 16 shows minimal average change over 18 months which was confirmed by a small, non-significant slope factor (mean=0.024, $p=0.707$). Variance scores varied slightly across the 18 months, and the variance for the slope indicated there was significant deviation from the average rate of change (variance=0.554, $p=0.003$). Although the average rate of change was non-significant for PA and only small for NA and LS, the presence of inter-individual variability indicated by the significant deviations from initial status values for PA, NA, and LS, and from average change in LS provide justification for investigating whether covariates can account for this variability.
Table 16

Means and variances for combined treatment and placebo groups estimated from individual latent growth curve models for Positive Affect, Negative Affect, and Life Satisfaction.

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>6 months</th>
<th>12 months</th>
<th>18 months</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean</strong></td>
<td></td>
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<tr>
<td>Positive Affect</td>
<td>22.19</td>
<td>22.23</td>
<td>22.26</td>
<td>22.48</td>
</tr>
<tr>
<td>Negative Affect</td>
<td>1.02</td>
<td>1.02</td>
<td>1.00</td>
<td>1.00</td>
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<tr>
<td>Life satisfaction</td>
<td>20.68</td>
<td>20.76</td>
<td>20.71</td>
<td>20.77</td>
</tr>
<tr>
<td><strong>Variance</strong></td>
<td>23.85</td>
<td>23.63</td>
<td>22.92</td>
<td>18.61</td>
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<tr>
<td></td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.04</td>
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<tr>
<td></td>
<td>25.27</td>
<td>24.56</td>
<td>23.98</td>
<td>24.26</td>
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</table>

Three process latent growth curve model

Based on the three well-fitting individual latent growth curves, the next step involved modelling these three slopes simultaneously in a single model to allow for their inter-correlation. This model provided a good fit to the data ($\chi^2(40)=42.315, p=0.371$; CFI=0.999; TLI=0.998; RMSEA=0.012 CI$_{90}=[0.00,0.04]$; SRMR=0.037). This is modelled prior to including the covariates of interest, to confirm there are no issues with model specification and to ensure these results confirm to expectation. Results were as expected, there were significant negative within-domain covariances between the latent initial status and slope factors of PA and LS, suggesting that individuals with higher
baseline levels of PA and LS had less reduction in these constructs over 18 months; the relationship between baseline NA and rate of change in NA over 18 months was not significant. Modelling three growth processes simultaneously resulted in a total of nine potential between-domain factor covariances (i.e., initial status x slope factor within each construct [3]; initial status x initial status between each construct [3]; slope factor x slope factor between each construct [3]); of these, three were significant. There was a significant positive covariance between initial LS level with initial PA level, and a significant negative covariance between initial LS level and initial NA level. The third significant covariance was negative between the average rate of growth for NA and the average rate of growth for LS, indicating, as expected, that increased LS over the 18 months was associated with decreased NA over this same period. Within-time covariances of residuals (a total of 12) were significant only for four covariances between PA, NA, and LS at 6 months and between PA and LS at 12 months.

**Covariates**

Predictors of interest were treatment group, n-3 LC PUFAs (EPA+DHA) and total n-3 PUFA (EPA+DHA+DPA+ALA) concentrations from erythrocyte membranes measured at baseline. These were included as covariates within the final latent growth model which included all three constructs modelled across time, and inter-correlated via the inter-correlated intercept and slope factors (see Figure 19), to assess whether they predict either average initial status or average change in the outcome variables: PA, NA, and LS. This model provided excellent fit to the data ($\chi^2(57)=67.337$, $p=0.164$; CFI=0.995; TLI=0.991; RMSEA=0.022 CI$_{90}$=[0.000,0.040]; SRMR=0.034).
Note: controlling for gender; the intercept and slope factors are inter-correlated between and within each construct, but not presented here for clarity.

Figure 19. Latent growth curve model with PA, NA, and LS modelled as observed variables across time, their intercept and slope factors, and the predictors’ treatment group, n-3 LC PUFAs and n-3 PUFAs.
Correlations obtained from this model are shown in Table 17. The two covariates as predictors were not highly correlated with the outcome variables; treatment group was not correlated with PA, NA or LS across any of the time points. \( n-3 \) PUFA status was only correlated weakly with NA at 18 months, and \( n-3 \) LC PUFA status was weakly correlated with baseline and 6 month measurement of PA.
Table 17

*Pearson correlation coefficients obtained from the final latent growth model between baseline, 6, 12, and 18 month factor scores for PA, NA, LS and their potential predictors.*

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<tbody>
<tr>
<td><strong>Positive Affect</strong></td>
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<td>1. Baseline</td>
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<td>2. 6 month</td>
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<td>3. 12 month</td>
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<td><strong>Negative Affect</strong></td>
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<tr>
<td>5. Baseline</td>
<td>-0.18</td>
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<td>-0.20</td>
<td>-0.19</td>
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<td>6. 6 month</td>
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<td>-0.12</td>
<td>-0.15</td>
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<tr>
<td>7. 12 month</td>
<td>-0.07</td>
<td>-0.04</td>
<td>-0.17</td>
<td>-0.15</td>
<td>0.59</td>
<td>0.57</td>
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<td>13. Trt grp</td>
<td>0.04</td>
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<td>15. n-3 LC PUFA</td>
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<td>0.05</td>
<td>-0.01</td>
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*Bold indicates p-value significant at 0.05 value*

*Note: n-3 PUFA, EPA+DHA+DPA+ALA; n-3 LC PUFA, EPA+DHA; Trt grp, treatment group*
The latent growth curve model results show that treatment group was not a significant predictor of initial status of PA (0.26, \(p=0.646\)), NA (-0.04, \(p=0.477\)), and LS (0.90, \(p=0.100\)); thus supporting the assumptions of random allocation to treatment.

Treatment group was not a significant predictor of average rate of change over 18 months for PA (0.02, \(p=0.909\)), NA (-0.20, \(p=0.286\)), or LS (0.03, \(p=0.789\)).

Total \(n\)-3 LC PUFAs (EPA+DHA) at baseline did not predict average baseline levels of NA (-0.07, \(p=0.145\)) or LS (0.06, \(p=0.186\)), but did predict average baseline levels of PA (0.13, \(p=0.017\)). Total \(n\)-3 LC PUFAs at baseline did not predict average rate of change over 18 months for PA (0.50, \(p=0.302\)), NA (-0.153, \(p=0.315\)) or LS (0.05, \(p=0.442\)).

Total \(n\)-3 PUFAs (EPA+DHA+DPA+ALA) at baseline did not predict any average baseline levels of PA (0.05, \(p=0.430\)), NA (0.121, \(p=0.052\)) or LS (0.11, \(p=0.863\)). It did not predict rate of change in PA (0.05, \(p=0.834\)), but it did significantly predict average rate of change over 18 months in NA (-0.59, \(p<0.001\)) and LS (0.22, \(p=0.015\)).
Discussion

Evidence regarding the role of omega-3 polyunsaturated fatty acids (n-3 PUFAs) for mental health is mixed; we sought to establish the nature of any relationship between n-3 PUFAs and a measure of positive mental health – Subjective Wellbeing (SWB), measured as Positive Affect (PA), Negative Affect (NA), and Life Satisfaction (LS). Specifically, we examined whether initial levels of n-3 LC PUFAs (EPA+DHA), initial n-3 PUFAs (EPA+DHA+DPA+ALA), or whether receiving 2320mg of n-3 LC PUFAs (1720mg DHA, 600mg EPA) per day for 18 months had any impact on rate of change in PA, NA, and LS.

Results suggest that total n-3 LC PUFA status at baseline significantly predicted initial levels of PA. Change in PA across the 18 months was not explained by any of the three predictor variables – treatment group, n-3 LC PUFAs, or n-3 PUFAs; however, change in NA and LS was explained by total n-3 PUFAs.

The only cross-sectional relationship reported was between total n-3 LC PUFAs with PA at baseline. Previous reports have been mixed with some reporting positive associations based various measures of n-3 LC PUFA status and aspects of mental health (Adams et al., 1996; Colangelo et al., 2009; Golding et al., 2009; Mamalakis et al., 2002; Silvers & Scott, 2002; Tanskanen et al., 2001; Yary & Aazami, 2011). However, others (Appleton et al., 2007; Crow et al., 2007; Schiepers et al., 2010) have reported no cross-sectional relationships between intake from fish and depressed mood in otherwise healthy adults after controlling for age and an index of multiple deprivation (Appleton et al.,
2007); between fish consumption and \( n \)-3 LC PUFA from plasma phospholipids in a population-based sample with the mental health component of the SF-36 (Schiepers et al., 2010); and between \( n \)-3 LC PUFAs from serum phospholipids and the mental health component of the SF-36 in healthy adults (Crowe et al., 2007).

These divergent results may be due to the dominance of depressive symptoms as the outcome of interest in the aforementioned studies (Adams et al., 1996; Colangelo et al., 2009; Golding et al., 2009; Mamalakis et al., 2002; Tanskanen et al., 2001; Yary & Aazami, 2011), rather than measures of positive mental health as were employed in our study. Only one of these studies employed a similar outcome; Silvers and Scott (2002) reported a positive relationship between people who consumed some kind of fish and higher scores on the mental health component of the SF-36. Our contrasting results may be due to Silvers and Scott’s (2002) use of a dichotomous variable as a proxy for \( n \)-3 LC PUFA intake (no consumption versus some consumption), additionally the relationship observed by Silvers and Scott (2002) may be due to something other than \( n \)-3 PUFAs in fish.

When these associations were assessed longitudinally, we found that total \( n \)-3 PUFA status at baseline significantly predicted change in NA and LS over 18 months, but not change in PA. Treatment group and \( n \)-3 LC PUFAs did not predict change in PA, NA or LS over 18 months. Of the eight RCTs in healthy adults, four reported no benefit of \( n \)-3 LC PUFAs on mood or depression (Antypa et al., 2009; Bradbury et al., 2004; Hamazaki et al., 1998; van de Rest et al., 2008). Four (Antypa et al., 2012; Fontani et al., 2005; Hamazaki et al., 1996; Kiecolt-Glaser et al., 2011) reported some benefit of
supplementation on measures of mood in healthy adults. Extraggession remained stable (Hamazaki et al., 1998), and improvements were seen in self-reported depressed mood (Antypa et al., 2012; Fontani et al., 2005), anxiety (Antypa et al., 2012; Fontani et al., 2005; Kiecolt-Glaser et al., 2011), anger and vigour (Fontani et al., 2005). These results suggest that n-3 PUFAs may have an effect on specific components of mental health, which are potentially attenuated when considering mental health as a whole. We found no relationship between any of the predictors with PA.

No previous research has assessed the relationship between n-3 LC PUFAs and either PA or NA; though the differential pattern of results observed was anticipated as PA and NA are related, but largely independent constructs, being consistently found to negatively correlate ($r=-0.44$, Diener, Smith, & Fujita, 1995). NA is also more highly correlated with depression ($r=0.77$, Brown, Chorpita & Barlow, 1998; $r_m=0.57$, Watson, Clark & Tellegen, 1988) than PA which is only moderately correlated ($r=-0.53$, Brown, Chorpita & Barlow, 1998; $r=-0.35$, Watson, Clark & Tellegen, 1988), potentially offering one explanation of these results. LS has been found to correlate at -0.72 with depression (Pavot & Diener, 1993), and NA correlates at 0.77, 0.57 with depression, it has also been reported that the relationship between LS and NA is weak at around -0.31 (Pavot & Diener, 1993). This suggests that LS and NA may be related to different aspects of depression, but that both may be influenced by n-3 PUFAs because of this correspondence with depression. This finding is consistent with previous longitudinal studies that have reported a relationship between depression with n-3 LC PUFAs (Astorg et al., 2008; Hedelin et al., 2010), though others have not found this association (Astorg et al., 2009; Hakkarainen et al., 2004). Furthermore, this relationship was found between n-3 PUFAs as the combination of EPA+DHA+DPA+ALA, and not with n-3 LC PUFAs, the
combination of just EPA+DHA, suggesting that these lesser studied components of n-3 PUFAs play a potential role. One study that has assessed ALA as an intervention compared to placebo found that whilst there were no significant differences between the two groups, there was a moderate baseline correlation ($r=-0.45, p<0.007$) between ALA and the Clinical Global Impression Scale (Gracious et al., 2010). This contrasts with our results, we found no baseline relationship between n-3 PUFAs, which included ALA, with PA, NA or LS. One potential reason for these different results could be our use of n-3 PUFAs which also included EPA, DHA and DPA; furthermore, we cannot discount that a relationship exists between baseline ALA levels and change in measures of mental health reported by Gracious and colleagues (2010) as they did not assess this longitudinal relationship via latent growth curve modelling techniques.

**Statistical considerations**

Several key factors required consideration to determine the most appropriate analyses. In relation to the study design, a method appropriate for both two group and repeated measures assessment was necessary. Analysis of the mean difference between two groups (e.g., ANOVA, ANCOVA) is widely criticised because it fails to account for within-group change, and because there is often little theoretical rationale for why a difference should be observed from one time point to the next (i.e., measurement spacing is usually arbitrary) conducted at equal space intervals depending on study length limitations, as was the case here. Latent growth curve modelling provides a method whereby group differences can be assessed at baseline, and change can be assessed across the study period. Several methods are available within this framework. However, we also needed to consider measurement of the outcome, SWB, which is most appropriately
modelled as three reflective, congeneric, inter-correlated factors – PA, NA, and LS. We were therefore unable to use some latent growth curve modelling approaches, such as Muthén and Curran’s (1997) approach for analysing RCTs, due to issues with model convergence and interpretation associated with estimating a large number of parameters. Therefore, we followed the approach recently outlined by Byrne (2012), whereby the growth for the entire sample is modelled in a single growth curve and the treatment is entered into the model as a covariate to assess the effect of the intervention. The main limitation to this approach is that we cannot examine whether there is an interaction between initial levels of our outcome variables and the treatment. That is, whether treatment is differentially effective dependent on initial status of SWB; for example, those with lower PA at baseline may show a greater improvement than those with initially higher PA levels. Significant deviations from mean initial levels were reported here for PA, NA, and LS; however, we were only able to assess whether the treatment, total n-3 LC PUFAs (EPA+DHA) or total n-3 PUFAs (EPA+DHA+DPA+ALA) concentrations were able to account for this variability. Additionally, although it is plausible that differential levels of PA, NA, or LS at baseline may affect response to treatment, there was no theoretical evidence to propose this as a particular research question here.

### Limitations and suggestions for future research

Several limitations should be considered here; firstly, generalisability is restricted to healthy, older adults, without a diagnosis of depression. The use of olive oil as our placebo also needs discussing: selection of an appropriate placebo requires that it does not affect the outcome, and that it adequately matches the treatment in appearance and taste. Low polyphenol oil was selected as some have reported a benefit of polyphenols for
cognition (Solfrizzi et al., 2006). The placebo arm received 990mg olive oil masked with 1.8mg EPA and 1.2mg DHA to ensure taste similarity between the treatment and placebo capsules. Masking of the treatment group was successful with 32% correctly guessing their group allocation with guesses no different from that expected by chance ($\chi^2(2)=0.151, p=0.927$); however, masking of the placebo group was less successful with 52.7% correctly guessing their group, and these guesses were different from what we would expect by chance alone ($\chi^2(2)=42.769, p<0.001$), suggesting that masking of the placebo condition was less successful than the masking of the treatment condition. As this was a double-blind trial, with identical capsule bottles, capsules and taste, we can only hypothesise that more correct guesses occurred in the placebo group due to participants not feeling any change in their SWB.

Nutritional research focused on assessing the effect of single isolated nutrients is subject to criticisms concerning low ecological validity because it fails to consider nutrient interactions (National Research Council, 1989). However, we sought to assess the effect of an $n$-3 LC PUFA supplement on SWB in older people. We justified this choice because $n$-3 PUFAs are increasingly consumed as a supplement by the general population; therefore our study adequately represents the inclusion of $n$-3 PUFAs in diet. Furthermore participants were instructed to maintain their usual diet. Feasibility of an individual to consume the same quantity of $n$-3 LC PUFAs was confirmed; our daily treatment included four capsules with a total of 1720mg DHA and 600mg EPA. Commercially available capsules (Blackmores®) in Adelaide, South Australia contain between 120mg DHA to 1100mg DHA and 100mg EPA to 1700mg EPA. Conversely, you could consume approximately 150g of salmon. Thus, our study dosage is considered achievable for individual diet.
This is the first randomised controlled trial we are aware of that has assessed the impact of \( n \)-3 PUFAs on Subject Wellbeing in normally functioning healthy adults for a period of 18 months. \( n \)-3 PUFAs have shown some positive influence for change in NA and LS here, whilst \( n \)-3 LC PUFAs were associated with PA at baseline, though not change in PA. Whilst the amount of change was small here, we are applying the effect to individuals that do not all need prevention of low SWB, in comparison to treatment interventions that apply the effect to all that need treatment and which would therefore result in larger effects. \( n \)-3 PUFAs had a significant, positive impact on change in NA and LS over 18 months; this was supported by the effect that \( n \)-3 PUFA concentrations had on reducing growth in NA and increasing growth in LS over the study period. This research suggests that \( n \)-3 PUFAs may be viable as a preventative measure to preventing poor mental health in older adults. This recommendation is also supported given the previously well-documented additional benefits (e.g., for heart health) of \( n \)-3 PUFAs. We highlight the importance of using methods that are sensitive to change across time such as latent growth curve modelling, as these provide a richer understanding of the potential relationships present.
**Context**

Omega-3 Polyunsaturated Fatty Acids have been implicated in mental illness. Less evidence exists regarding their relationship with mental health in non-clinical populations.

**Design**

An 18 month randomised clinical trial of EPA+DHA for SWB compared to placebo in healthy older adults \( (n=355) \). The impact of treatment group, initial \( n-3 \) LC PUFA (EPA+DHA) levels, and initial \( n-3 \) PUFA (EPA+DHA+DPA+ALA) levels on rate of change in SWB was assessed via latent growth curve modelling.

**Contribution**

Initial \( n-3 \) PUFAs (EPA+DHA+DPA+ALA) predicted rate of change in NA and LS over 18 months whilst controlling for gender.

**Implications**

The connection from \( n-3 \) PUFAs to reductions in NA and improvements in LS in healthy adults offers an easily modifiable preventative measure to ill health with potential benefits at the population-level.
Chapter 6: General Discussion and Research Conclusions

The research presented in the current thesis sought to examine whether nutrition plays a role in positive mental health. In this way we sought to depart from both traditional views of psychological treatment and disability. Prior to assessing this relationship we first needed to establish how to operationalise the construct *positive mental health*. Subjective Wellbeing (SWB) was chosen due to the availability of valid scales and the frequency and simplicity of its use. One issue that became apparent concerned the lack of consensus for how to empirically operationalise SWB; that is, the underlying empirical structure of SWB was unclear. Four empirical models were assessed here based on models that are implied by the way the components are assessed in the literature (c.f. Busseri & Sadava, 2011). Firstly we demonstrated that these four models produced different results when correlated with personality, and secondly, we were able to provide empirical support for one as the superior model. This model was then employed to assessing whether nutrition plays a role in SWB.

Folate and vitamin B$_{12}$ have long been implicated in mental illness, and more recently, interest has concentrated on omega-3 polyunsaturated fatty acids (*n*-3 PUFAs) and their relationship to mental illness. However, few have assessed these relationships within healthy populations, and fewer still have assessed their role in positive mental health outcomes, such as SWB, rather than depressive symptoms. We sought to assess whether folate and vitamin B$_{12}$ levels influenced subsequent measurement of SWB: more specifically, whether these B-vitamins had a direct effect on SWB, or an indirect effect mediated by the amino acid, homocysteine. Secondly, the role of *n*-3 PUFAs, both long-
and short-chain $n$-3 PUFAs, were assessed as potential predictors of change in SWB over 18 months.

An 18-month randomised controlled trial with 391 older participants was employed to address these aims which were assessed via structural equation modelling. Baseline data allowed us to evaluate measurement of SWB, and comparison between alternative measurement models allowed us to provide support for one model conceptualisation over three others (Paper 1). Where possible, this model of SWB was subsequently employed as the outcome variable in Papers 2 and 3. The role of folate, vitamin B$_{12}$ and homocysteine in SWB was assessed via baseline and end point data using simultaneously estimated cross-lagged mediation models, controlling for baseline SWB, gender, use of cardiovascular medications and physical health as covariates (Paper 2). Finally, using all four assessment points taken across the 18 months, latent growth curve modelling was employed to assess the role of $n$-3 PUFAs on the trajectory of SWB whilst controlling for baseline SWB and gender (Paper 3).

This final chapter presents a discussion of the results of the research presented within this thesis. Firstly, we present an overview, including the aims, results and key findings of each of the three papers. Following this, the significance and implications of this body of research will be presented, including both the larger policy implications and potential individual-level benefits. The limitations of the research that apply to all three papers, including the sampling framework and measurement techniques employed, will then be presented. Four key recommendations for future work are made including recommendations that guidelines be created for calculating SWB and for the measurement expression of $n$-3 PUFAs and that future research include measurement of satisfaction.
within life domains and the simultaneous assessment of measures of wellbeing and ill-being. We conclude this final chapter with a summary of our key findings including the overall significance.

**Research Conclusions – Paper 1:**

**Operationalising Subjective Wellbeing**

The first paper in this thesis sought to highlight that different structural models of SWB can influence subsequently drawn conclusions, and sought to provide empirical support for one structural model that best represented the construct. Each of the four models specified:

- **Model 1:** SWB as three inter-correlated factors (PA, NA, LS),
- **Model 2:** SWB as a higher-order construct,
- **Model 3:** SWB as a composite, and
- **Model 4:** SWB as a causal process

had a slightly different relationship to the Big Five Personality Factors of Openness to Experience (O), Conscientiousness (C), Extraversion (E), Agreeableness (A), and Neuroticism (N), demonstrating that measurement conceptualisation of SWB can influence subsequently drawn conclusions. Furthermore, we were able to provide support for SWB modelled as the inter-correlations between PA, NA and LS specified as reflective, congeneric, first-order factors.

Prior to assessing these four alternative models of SWB, we first established individual measurement models for PA, NA and LS. Results suggested that all three
constructs were best specified as congeneric, whereby the relative importance of each questionnaire item is accounted for. Each construct had acceptable convergent validity, and high internal consistency indicating that the observed variables, that is, the questionnaire items, were adequate indicators of each construct. Two different methods – the paired constructs test (Jöreskog, 1971), and the Average Variance Extracted (AVE; Fornell & Larcker, 1981), both confirmed the discriminant validity between PA, NA and LS.

We next modelled all four representations of SWB, and sought to determine which provided the most accurate representation. Models 1 and 2 were equivalent so we were unable to employ traditional Confirmatory Factor Analytic (CFA) methods to identify the superior model. Instead we relied upon four main pieces of evidence to discriminate between the two by addressing whether a higher-order factor (Model 2) could better explain the relationships between the first-order factors. These four key pieces of evidence are summarised below:

- **Factor Inter-correlations:** correlations between PA, NA and LS in Model 1 were weak-to-moderate, indicating that a higher-order factor is not suitable. **Support for Model 1.**

- **Factor Loadings:** correlations from PA, NA and LS to SWB in Model 2 were substantial and significant. **Support for Model 2.**

- **Average Variance Extracted:** SWB accounted for less than 40 per cent of the variance in PA, NA and LS. Therefore a large amount of variance in PA (73%), NA (78%) and LS (36%) was unexplained by SWB. **Support for Model 1.**
Schmid-Leiman Transformation: PA and NA explained approximately three times the amount of variances in their indicators than SWB did, though the amount of variance explained in LS indicators was approximately half due to LS and half due to SWB. Support for Model 1.

These analyses indicated that Model 1 – SWB as inter-correlated PA, NA and LS – demonstrated a better representation of the data than Model 2, where SWB was modelled as a higher-order factor.

Model 3 was unable to be empirically tested within the CFA framework as composite models are unidentified (i.e., \(d.f. < 0\)). However, we were able to suggest that this model does not provide a superior fit over Model 1 based on theoretical considerations. This model specifies that the sum, of PA, NA and LS cause SWB. Thus, elimination of any one construct should alter the meaning of SWB. This is consistent with prior research, and our own analyses that PA, NA and LS are independent constructs. However, it does not account for the unique variance in PA, NA and LS, and therefore does not accurately represent what we know about SWB. Therefore we retain Model 1 as the most accurate representation.

The final model considered (Model 4) assessed SWB as a causal process whereby PA and NA influence LS. As this model was nested within Model 1 we were able to statistically compare the two; results indicated that the restrictions imposed in Model 4 significantly worsened model fit. Therefore, Model 1 is retained as the best representation of SWB.
Although these four models appear empirically similar they are theoretically quite distinct as demonstrated by Busseri and Sadava (2011). Correlations between SWB, PA, NA and LS with O, C, E, A and N highlighted how these differences can have an impact on analyses. The pattern of correlations found were consistent with prior research, we found the strongest relationships from E to both PA and SWB and from N to both NA and SWB (DeNeve & Cooper, 1998; Schimmack, Schupp & Wagner, 2008). Comparison across models indicated that while all displayed similar patterns of significance and direction, the magnitude of the relationships differed; for example, the relationship between N and SWB differed by 0.45 between Models 2 and 3.

SWB modelled as the inter-correlations between PA, NA and LS (Model 1) was identified as the best representation. Establishing the best representation of SWB was an important first step for our research program. Insofar as possible, this model was to be subsequently employed as the outcome variable in Papers 2 and 3.

Research Conclusions – Paper 2:

The effect of Folate, Vitamin B\textsubscript{12} and Homocysteine on Subjective Wellbeing

Previous reports have implicated folate, vitamin B12 and homocysteine with mental illness and mental health. The aim of Paper 2 was to assess the relationship between folate, vitamin B\textsubscript{12}, homocysteine and SWB measured as the inter-correlations between PA, NA and LS (Model 1, Paper1). We investigated whether homocysteine mediates any relationship from folate, vitamin B\textsubscript{12}, or their interaction to SWB because homocysteine is, in part, influenced by folate and vitamin B\textsubscript{12} concentrations.
Firstly we were able to confirm that correlations between folate, vitamin B\textsubscript{12} and homocysteine were as expected. Folate and vitamin B\textsubscript{12} were positively associated at baseline, and both were negatively correlated with homocysteine at baseline. Consistent with previous literature (e.g., Bjelland et al., 2003), folate demonstrated a stronger relationship with homocysteine ($r=-0.44$, $p<0.05$) than vitamin B\textsubscript{12} did ($r=-0.26$, $p<0.05$). This pattern of results was replicated when assessing the relationships from folate and vitamin B\textsubscript{12} at baseline to homocysteine 18 months later ($r=-0.27$, $p<0.05$ & $r=-0.21$, $p<0.05$, respectively).

We found no evidence of a mediational relationship from folate, vitamin B\textsubscript{12}, or their interaction to PA, NA or LS via homocysteine. However, despite no presence of a relationship at baseline, folate, vitamin B\textsubscript{12} and their interaction were significant, though weak, predictors of PA 18 months later, but not of NA or LS. Furthermore, this relationship controlled for baseline PA, the inter-correlation between PA, NA and NA and was independent of gender, age, use of cardiovascular medications and physical health. A previous study that also assessed folate and vitamin B\textsubscript{12} with positive mood (Cassidy et al., 2004) reported no association between folate and vitamin B\textsubscript{12} with mood, though this was a cross-sectional study. We also found no cross-sectional relationship, thus we highlight the need for longitudinal investigations that additionally control for auto-regressive effects.

Baseline homocysteine did not predict PA, NA or LS at 18 months, despite the presence of a relationship to PA at baseline. This suggests that homocysteine may be associated with measures of mental health solely due to its dependence on folate and vitamin B\textsubscript{12}. This explanation is consistent with the above reported findings that folate and
vitamin B$_{12}$ predict PA 18 months later. Homocysteine is influenced by folate and vitamin B$_{12}$, therefore the presence of a relationship between homocysteine and PA at baseline may reflect the dependence of homocysteine on prior folate and vitamin B$_{12}$ concentrations, which subsequently influenced PA.

**Research Conclusions – Paper 3:**

**Impact of omega-3 Polyunsaturated Fatty Acids on the trajectory of Subjective Wellbeing**

The final Paper presented within this thesis sought to assess the role of omega-3 Polyunsaturated Fatty Acids (n-3 PUFAs) in SWB, via a randomised control trial of 391 older, community-dwelling adults comparing n-3 long chain (LC) PUFAs to placebo over 18 months.

Cross-sectional relationships at baseline were reported between initial n-3 LC PUFA (EPA+DHA) status and PA at baseline when modelled simultaneously within a latent growth curve model. Most beneficial evidence for n-3 PUFAs has been found with EPA and DHA – here, their combination was associated with PA. Interestingly we anticipated a correlation with NA as this demonstrates larger correlations with depression, and several previous studies have reported cross-sectional relationships between depressive symptoms with fish consumption (Tanskanen et al., 2001), n-3 LC PUFA intake calculated (Colangelo et al., 2009; Golding et al., 2009; Yary & Aazami, 2011), adipose tissue DHA levels (Mamalakis et al., 2002), erythrocyte EPA levels, the ratio of AA to EPA, and the ratio of n-6 LC to n-3 LC PUFAs (Adams et al., 1996). We also assessed n-3 PUFAs more broadly, with the combination of EPA, DHA, DPA and ALA expressed as a percentage of the total fatty acids. DHA is another LC n-3 PUFA and ALA
is the parent short chain \(n\)-3 PUFA. These were included to create this broader measure of \(n\)-3 PUFAs as, though limited, there is some evidence for a beneficial role of DPA and the short-chain \(n\)-3 PUFA, ALA, was included because it is the main precursor of these \(n\)-3 LC PUFAs and, although important for health, is poorly converted in the body. We found no evidence to support a cross-sectional relationship between initial \(n\)-3 PUFAs with baseline SWB.

Initial \(n\)-3 LC PUFAs (EPA+DHA) and whether or not an individual was in the treatment or placebo group did not predict rate of change in PA, NA or LS. Initial \(n\)-3 PUFA (EPA+DHA+DPA+ALA) status significantly predicted rate of change in both NA and LS over 18 months. Furthermore, this pattern of significance remained after controlling for gender. Other longitudinal studies in healthy adults have reported mixed findings, with four of eight trials identified reporting a significant treatment effect with a DHA-rich (Hamazaki et al., 1996) or an EPA-rich treatment (Antypa et al., 2012; Fontani et al., 2005; Kiecolt-Glaser et al., 2011) and four reporting no treatment effect with either a DHA-rich treatment (Bradbury et al., 2004; Hamazaki et al., 1998) or an EPA-rich treatment (Antypa et al., 2009; van de Rest et al., 2008). One study that has assessed ALA as an intervention across four months in children with bipolar depression reported a correlation between \(n\)-3 PUFAs with the Clinical Global Impression Scale (CGI), but no significant differences between the placebo and intervention group (Gracious et al., 2010). Conversely, we found no cross-sectional relationship between our measure of \(n\)-3 PUFAs which included ALA, perhaps due to our additional inclusion of EPA, DHA and DPA. Furthermore, it is possible that Gracious and colleagues (2010) may have found a relationship between ALA and change across time if they had applied latent growth curve modelling techniques to their data.
It is interesting to note that the results from Paper 2 suggested that folate, vitamin B₁₂ and their interaction had a significant impact on PA, but not NA or LS, measured 18 months later, whereas our results from Paper 3 suggested that \( n-3 \) PUFAs had a significant effect on NA and LS, but not PA, measured 18 months later.

**General Discussion – Significance and Implications**

This thesis represents a shift in the focus of research in this area away from treatment of disorder toward promotion of positive mental health. Nutrition was investigated as a potential factor to promote SWB as it has been previously dominated by assessment of disorder, and because it offers a relatively easy to modify lifestyle factor that is available to the majority of people. That we were able to suggest causative links between folate and vitamin B₁₂ levels to Positive Affect, and from total \( n-3 \) PUFA status to Negative Affect and Life Satisfaction has significant implications for policy guidelines and individual mental health.

Firstly, because we have assessed promotion of SWB rather than treatment of disorder, these results can potentially be of benefit at the population level, rather than for minority populations with a mental disorder. Advice and recommendations can therefore be disseminated and implemented at a population level and potentially modified at the individual level. Furthermore, we have assessed an outcome that is meaningful and tangible to the majority of the population; we therefore hypothesise that public interest will be greater than interventions centered around more specific and less holistic outcomes, such as cognition.
Significance and Implications – Policy Implications:

Nutritional Recommendations

At this stage we view our results as preliminary; however, replication would enable nutritional recommendations to be made. In line with Benton (2013), we firstly suggest that optimal nutritional recommendations be based on a combination of the psychological, as well as the physiological health implications and benefits associated with vitamins, minerals and essential fatty acids. We believe this represents the first stage in potentially implementing policy changes. If replicated, our results suggest that folate, vitamin B$_{12}$ and $n$-3 PUFAs are important for maintaining and improving SWB and therefore potentially important for preventing mental illness. Recommendations could be made to the public regarding these associations and targeted interventions could be developed for ‘at risk’ populations, as determined by SWB levels. These recommendations are feasible for uptake at the individual level, and public adoption could have a significant impact on reduced costs associated with mental illness. Additionally, folate is already fortified in cereals and grains in Australia due to known links to neural tube birth defects; our research suggests that this may also be beneficial for mental health.

Our results from Paper 3 also have potential significance for commercial responsibility of marketing $n$-3 PUFAs for their health benefits. Whilst folate and vitamin B$_{12}$ supplements are largely unambiguous; there are potential concerns regarding misleading information in commercially available omega-3 products (Turchini, Nichols, Barrow & Sinclair, 2012). In particular, current food labelling legislations allow ALA to be sold as an “omega-3 source” (Turchini, Nichols, Barrow and Sinclair, 2012). Our results suggested a potential benefit of the less well studied ALA and DPA $n$-3 PUFAs, in addition to EPA and DHA. Based on this we suggested that future research consider all
aspects of $n$-3 PUFAs; we extend this here to recommend that commercially available “omega-3” capsules be clearly and specifically labelled.

**Significance and Implications – Policy Implications:**

**Subjective Wellbeing**

Results from Paper 1 have built upon prior attempts at uniformity in how SWB is operationalised. Consistent operationalisations of SWB will aid coherence across the numerous disciplines in which it is now employed as an important outcome variable. Improved research coherence may potentially lead to the development of guidelines for how to calculate SWB, which could increase the clinical relevance of the construct, and aid in clinical diagnoses made within the CSM of mental health. We therefore suggest that this uniformity in calculating SWB has policy implications for clinical guidelines, and secondly for monitoring the success of public policy. If nutritional recommendations for mental health can be made at the population level, then measures of SWB will also be important to monitor the success of this communication via population level changes in SWB, and for determining individual risk of mental illness. Although somewhat outside the scope of this thesis, measures of SWB are increasingly being employed alongside more traditional measures of GDP and GNP in attempt to measure government policy, as introduced in Chapter 1.1. Uniform measurement is therefore necessary for meaningful interpretation of differences to be made both across time, and between groups.
Significance and Implications:

Individual-Level Benefits

Preventative research often results in small effect sizes as the population to which the intervention is applied do not all necessarily require treatment, in comparison to treatment interventions where the population accessed are all in need intervention. This means we are applying an effect to some individuals who may not need prevention, compared to treatment interventions that apply the effect to all that need treatment. Therefore, we hypothesise that the effect sizes obtained here would be larger in populations at risk of low SWB. This is particularly true with our sample as they were highly functioning older adults with relatively high levels of SWB. We view the results here as potentially having a greater impact on populations with low SWB, and hypothesise that the effects would be more substantial in such populations, thereby increasing the overall significance of the findings reported herein.

General Discussion – Limitations

Limitations of the study design and analyses have been previously outlined within each of the three papers and so are not repeated in their entirety here. Instead, we discuss two key limitations that have emerged as common themes across the papers presented. These represent limitations of the sampling framework and of the measurement techniques employed. It is important to consider how these may limit generalisability and therefore interpretation.
General Discussion – Limitations:

Potential limitations due to the sampling framework

Generalisability is limited in two key respects based on the sampling framework employed. Firstly, we cannot assume the generalisability of results to other age groups. Secondly, we cannot necessarily generalise to others of the same age due to the specific characteristics of this older population.

Changes across the lifespan are known to occur in both SWB and nutrition. However, although evidence suggests age-related changes in mean-levels of SWB (e.g., Baird, Lucas & Donnellan, 2010), there is no evidence to suggest that the underlying structure of SWB should alter. Therefore, whilst caution is taken when generalising the results from Paper 1, we suggest replication in a younger sample quite plausible.

Similarly, evidence suggests that nutrient requirements alter in older age (Glick, 1990), and are additionally adversely effected by physical and social factors often associated with aging (Morley, 1997). Therefore, it is possible that nutrition may have a more significant role in the SWB of older individuals due to increased requirements and potentially increased variability of nutrient intakes and re-uptake, limiting generalisability to younger individuals.

Generalisability may also be limited within older adults based on potential selection bias. All research must consider how well a sample constitutes a random sample from the population of interest; what unique characteristics may separate individuals who are interested in your study compared to those who are not? Attempts to minimise these issues were incorporated into the design, for example ensuring recruitment advertisement accessed a variety of groups; however, our sample can still be considered unique. The
main characteristic that potentially separates this sample from other older adults is that they were a sample of well-functioning, community-living adults. Participation required several visits to our clinic therefore potentially eliminating those with mobility issues, with limited access to transport, or who were in full-time employment or were full-time caregivers. Of potentially greater concern was that this sample could be seen as belonging to the worried well. That is, some could have been motivated by the belief that changes in nutrition and wellbeing occur in older age and so actively sought out our trial in attempt to mitigate this. In this regard we can consider that relationships presented in this thesis may in fact, have a larger effect size when including the broader population from which our sample belongs.

**General Discussion – Limitations:**

**Potential limitations due to the measurement techniques employed**

Positive mental health was operationalised via SWB here based on responses to two questionnaires which encompassed the three components of SWB – PA, NA and LS. Given the reliance on SWB of all three papers presented herein, it is worth addressing the potential limitation that may arise from over-reliance on self-report data. SWB is over-reliant on self-reported data due to the subjective nature of the construct. Issues associated with over-reliance on any one method include artificial inflation of relationships due to common method variance. Within our own analyses we were able to account for this as CFA techniques partial out the measurement error associated with use of a common method. However, we do advocate the replication of our preferred model using multi-modal assessment of SWB. This would further eliminate issues associated with self-report data such as experience versus perception, social desirability and motivational biases.
Finally, we must make comment to the simplistic account afforded to what is necessarily a complex interplay between biological, psychological, social and environmental factors with mental health. Controlling for covariates was an important step towards acknowledging the complexity of these relationships, and statistical modelling techniques allowed for us to adequately represent complex bi-directional relationships.

**General Discussion – Future Research Directions**

Five key recommendations for future research are presented here. We suggest that future research attempt to provide guidelines for the uniform calculation of SWB, that all components of n-3 PUFAs be measured, and that guidelines are additionally provided for consistency in expressing n-3 PUFA concentrations. Notable admissions from our own research presented herein include the absence of domain satisfaction within our models of SWB, and the absence of measuring ill-being alongside SWB, we recommend incorporating these aspects into future research. These directions for future research are now presented in detail.

**General Discussion – Future Research Directions:**

**Guidelines for Calculating Subjective Wellbeing**

Inconsistent operationalisations of SWB via PA, NA and LS have been previously highlighted based on theoretical differences (e.g., Busseri & Sadava, 2011); here we have further highlighted the practical differences these can have on subsequently drawn conclusions. We therefore propose the development of clear guidelines for calculating SWB to improve coherence of comparisons and conclusions drawn. Guidelines could include information for how to employ CFA techniques based on the most commonly used measures of PA, NA and LS, generally the PANAS (Watson, Clark & Tellegen,
1988), and the SWLS (Diener, Emmons, Larsen, & Griffin, 1985). Provision of factor scores or normative weights should also be made available for when such methods are unavailable, this would involve normative scores based on different populations; however, we would suggest that this would only be meaningful within individualistic cultures due to the well documented differences in interpretation of SWB questionnaires in collectivist cultures due to differences in evaluation of SWB (e.g., Suh, Diener, Oishi & Triandis, 1998). Replication across diverse populations is important for normative scores to be compiled; however, it is important to note that this need not occur for every population where differential levels of SWB are presumed. Factors scores to provide normative values are based on the extent to which each item is reflective of the construct it is designed to measure. Therefore, whilst different levels of SWB may exist, normative values may well be the same. Testing measurement invariance between populations should be employed to provide an empirical test of the similarity of factor loadings.

**General Discussion – Future Research Directions:**

**Measurement of omega-3 Polyunsaturated Fatty Acids**

Two key recommendations regarding the measurement of $n$-3 PUFAs are made here: (1) to assess aspects of $n$-3 PUFAs beyond EPA and DHA, namely we recommend the additional incorporation of DPA and ALA; and (2) to move toward operationalisation consistency of $n$-3 PUFAs, here we recommend concentrations to be assessed in erythrocyte membranes and operationalised as per cent of total fatty acids.

Current evidence suggests that mental health benefits of omega-3 polyunsaturated fatty acids ($n$-3 PUFAs) are predominantly associated with the long chain (LC) $n$-3 PUFAs – specifically eicosapentanoic acid (EPA) and docosapentaenoic acid (DHA).
These n-3 LC PUFAs have been studied extensively and have been associated with reduced inflammation (e.g., Browning, 2003), coronary health (e.g., Rissanen et al., 2000; Yokoyama, 2007), cognition (e.g., Chiu et al., 2008) and mental health (e.g., Antypa et al., 2012). Docosapentaenoic acid (DPA) is another n-3 LC PUFA predominantly found in red meat rather than oily fish. Though considerably less well studied, there is evidence to suggest it has similar benefits for CVD as EPA and DHA (e.g., Rissanen et al., 2000). The n-3 short chain (SC) PUFA, alpha-linolenic acid (ALA), which is converted, though poorly, in the body into n-3 LC PUFAs has similarly not been studied extensively, though some have reported its association to severity of mental illness (Gracious et al., 2010).

Here we assessed the benefit of EPA+DHA on SWB in addition to assessing both short chain and LC n-3 PUFAs; that is, EPA+DHA+ALA+DPA. Interestingly, we found most evidence for a beneficial effect from the combination of EPA+DHA+DPA+ALA. This suggests that future research should consider all aspects of n-3 PUFAs, rather than the traditional sole focus on EPA and DHA.

We also advocate for standardised assessment of n-3 PUFA status. Research has employed varying measures from fish intake, n-3 PUFA intake from FFQ responses, and concentrations from plasma, serum and erythrocyte membranes, confounding between study comparisons. Establishing uniform guidelines for expressing n-3 PUFA status will enable comparison of research findings, particularly as outcome measures are often also discrepant. Concentrations of n-3 PUFAs in erythrocyte membranes, as employed here, are recommended as they reflect longer term dietary intake and incorporation into tissue (Rise, Eligini, Ghezzi, Colli & Galli, 2007), rather than total fatty acid levels which reflect more recent dietary intake. Furthermore it is recommended that these concentrations are expressed as a per cent of total fatty acids (Milte, Sinn & Howe, 2009), in line with the
development within CVD research of the *omega-3 index* (Harris & von Schacky, 2004). The extension of measurement beyond EPA and DHA, in addition to consistent measurement of n-3 PUFAs will facilitate comparison across studies with variant operationalisations of mental health leading to improved coherence within the field.

**General Discussion – Future Research Directions:**

**Inclusion of Domain Satisfaction**

A notable admission in our conceptualisation of SWB is domain satisfaction, where satisfaction is assessed within certain areas of life such as health, relationships and security. Two potential mechanisms are theorised: The bottom-up approach whereby individuals assess their overall Life Satisfaction (LS) with reference to external factors such as particular domains of their life, and the top-down approach whereby individuals assess their domain satisfaction with reference to internal traits that influence their overall LS (Diener, 1984). Domain satisfaction influences overall LS in the bottom-up approach, and overall LS influences domain satisfaction in the top-down approach, though it appears that both processes are at play in judgements (e.g., Diener, 1984). Future research should attempt to locate domain satisfaction within the models of SWB presented herein. For example, domain satisfaction may form an independent, though correlated first-order factor, it may be an indicator solely of LS, therefore supporting the bottom-up approach, or it may be caused by LS, therefore supporting the top-down approach. It seems likely that both processes are at play in overall and domain approaches. Methods of discriminating between models as presented herein will allow for conclusions regarding the best representation to be made. Furthermore, investigations of the predictors of SWB, such as nutrition, are recommended to further elucidate the relationship between domain satisfaction, LS and SWB, whether via longitudinal associational research or intervention
designs. A bottom-up approach is not entirely inconsistent with our results here, for example, it is possible that the relationship between n-3 PUFAs and LS is mediated by domain satisfaction in health; however, it seems more likely that our results support a top-down approach due to this relationship.

**General Discussion – Future Research Directions:**

**Dual measurement of wellbeing and ill-being**

Much of the previous literature reviewed here has focused on measures of ill-being such as levels of depressive symptoms, even when research has employed healthy populations. Many of the questionnaires employed to measure depressive symptoms – such as the CES-D, are designed to capture levels of depressive symptomatology in the general population, as we have previously discussed, this provides an incomplete view of mental health. We recommend that future work within non-clinical populations employ measures of mental ill-being such as the CES-D alongside measures of SWB for broader representation of mental functioning.

Such a conceptualisation aligns with the Complete State Model (CSM) of mental health proposed by Keyes and Lopez (2001), which suggests that an individual’s mental health can be classified into one of four categories: flourishing (complete mental health), languishing (incomplete mental health), struggling (incomplete mental illness) and floundering (complete mental illness). These are classified along two dimensions, subjective wellbeing to mental illness, and complete to incomplete mental health (see Figure 20). Physical, psychological and psychosocial functioning are highest in those classified as flourishing in life (Keyes, 2002, 2004, 2005a, 2005b), and gains in mental health have been associated with decreased odds of subsequent incidence of mental illness
(Keyes et al., 2010). This suggests that strategies to improve mental health must focus on promotion of mental health in addition to alleviating mental illness, therefore assessment should encompass both dimensions (e.g., Keyes, 2005b).

Figure 20. Mental health and mental illness: the Complete State Model (CSM; Keyes & Lopez, 2002).
We recommend that future research attempt to incorporate this model into investigations of nutrition and mental health in order to assess whether:

- There are significant differences in nutrient values between the four categories of the CSM of mental health;
- Nutritional interventions can promote movement to an improved category within the CSM of mental health;
- Promotion of mental health is uniform across categories within the CSM of mental health; for example, are rates of improvement similar from languishing to flourishing, as they are from floundering to struggling?

This could provide important insight into the proposed benefit of nutrition as evidence in this area is currently dominated by assessment of mental illness within patient populations, with less evidence in non-clinical populations. Few studies have assessed positive mental health within non-clinical populations. This provided a challenge within the current thesis in extrapolating evidence that is based on mental illness across to proposed hypotheses of positive mental health. Addressing the above questions would help to bridge the gap between the two areas of research whilst also providing further empirical assessment of the CSM of mental health.
Final Comments

Each year it is estimated that treatment costs for mental health in Australia exceed AUD$600 million, with one in five Australian adults affected, thus constituting a significant burden for both society and individuals. Traditionally, efforts have been consistent with the biomedical approach by targeting treatment, though increasingly efforts are focused on prevention. Due to increasing evidence that mental health protects against mental illness (e.g., Keyes et al., 2010), it is recommended that promotion of mental health should be employed alongside current risk reduction strategies for prevention of mental illness. It is therefore important to assess the predictors of mental health, here we evaluated nutrition. Nutrition was of interest as a viable predictor of mental health due to its well established role in mental illness. Furthermore, it was seen as providing a holistic approach to prevention, as being of practical use, easy to communicate, modifiable at the individual level, available to all, and consequently, having the potential for far reaching benefits. After establishing the best empirical representation of SWB, our measure of mental health in Paper 1, we were able to investigate whether nutritional components were predictive of subsequent mental health. Folate and vitamin B₁₂ had a significant impact on subsequent levels of PA, whilst controlling for baseline SWB, gender, use of cardiovascular medications and physical health, and n-3 PUFAs had a significant impact on subsequently measured NA and LS whilst controlling for baseline SWB and gender. We therefore conclude that all three nutritional components have some role to play in the prophylaxis of mood for the general population or at-risk groups, and potentially as a replacement therapy when psychotropic medications are undesired, such as during pregnancy.
Establishing the modifiable environmental determinants of mental health is important for promotion of mental health and prevention of mental illness. Elucidating the relationships between SWB and nutrition here has provided evidence for one of these environmental factors. Public knowledge is accumulating regarding the mind-body connection and should be met with empirically supported scientific evidence.


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Appendix 1

Paper 1 – Comparing Structural Models of Subjective Wellbeing:
Choice of Model Affects Relationships with Personality

Manuscript to be submitted for publication

Statement of Contributions

V. Danthiir and N. Burns initiated and obtained funding for the project, and V. Danthiir conceptualised the study design. The data was collected by L.C. Edney and V. Danthiir. L.C. Edney devised the idea for the manuscript, conducted the data analysis and prepared the manuscript. V. Danthiir and N. Burns both provided conceptual input on the analyses, commented on drafts, made suggestions regarding the presentation of material in the paper, and provided editorial input.

I certify that the statement of contribution is accurate.

L. C. Edney (Candidate) Date: 18/12/2013

I certify that the statement of contribution is accurate and I give my permission for the inclusion of the manuscript to be incorporated in L.C. Edney’s submission for the degree of Doctor of Philopsophy from the University of Adelaide.

N. Burns (Co-Supervisor) Date: 18/12/2013

V. Danthiir (Co-Supervisor) Date: 18/12/2013
Paper 2 – Folate, Vitamin B\textsubscript{12}, Homocysteine and Subjective Wellbeing: Relationships in Older, Community-living Adults

Manuscript to be submitted for publication

Statement of Contributions

V. Danthiir and N. Burns initiated and obtained funding for the project, and V. Danthiir conceptualised the study design. The data was collected by L.C. Edney and V. Danthiir. L.C. Edney devised the idea for the manuscript, conducted the data analysis and prepared the manuscript. V. Danthiir and N. Burns both provided conceptual input on the analyses, commented on drafts, made suggestions regarding the presentation of material in the paper, and provided editorial input.

I certify that the statement of contribution is accurate.

L. C. Edney (Candidate) Date: 18/12/2013

I certify that the statement of contribution is accurate and I give my permission for the inclusion of the manuscript to be incorporated in L.C. Edney’s submission for the degree of Doctor of Philosophy from the University of Adelaide.

N. Burns (Co-Supervisor) Date: 18/12/2013

V. Danthiir (Co-Supervisor) Date: 18/12/2013
Paper 3 – $n$-3 Polyunsaturated Fatty Acids and Subjective Wellbeing in Healthy Older Adults: A Randomised, Double-Blind Intervention Trial

Manuscript to be submitted for publication

Statement of Contributions

V. Danthiir and N. Burns initiated and obtained funding for the project, and V. Danthiir conceptualised the study design. The data was collected by L.C. Edney and V. Danthiir. L.C. Edney devised the idea for the manuscript, conducted the data analysis and prepared the manuscript. V. Danthiir and N. Burns both provided conceptual input on the analyses, commented on drafts, made suggestions regarding the presentation of material in the paper, and provided editorial input.

I certify that the statement of contribution is accurate.

L. C. Edney (Candidate) Date: 18/12/2013

I certify that the statement of contribution is accurate and I give my permission for the inclusion of the manuscript to be incorporated in L.C. Edney’s submission for the degree of Doctor of Philopsophy from the University of Adelaide.

N. Burns (Co-Supervisor) Date: 18/12/2013

V. Danthiir (Co-Supervisor) Date: 18/12/2013