Cognitive and cardiovascular aging:
The effects of multivitamin supplementation

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Abstract

Cognitive aging is an issue of increasing concern in our aging population. Finding ways to enhance cognition in older adults may delay the onset of cognitive impairment, improving quality of life and reducing associated costs in society.

Cognitive outcomes are especially dependent on cardiovascular health. Arterial stiffness increases with age and may affect cognition via detrimental effects on the brain. Augmentation index is a measure of arterial stiffness but there is little research on its relationship with cognitive performance. The first study presented in this thesis investigated the association between cognitive performance on a computerised test battery, and augmentation index and augmentation pressure. After controlling for confounding variables, a relationship was observed between augmentation index and Spatial Working Memory, in both younger and older adults. This was the first study to examine these relationships separately in older and younger age groups, and results indicated that the strength of the relationship was the same in younger and older adults.

Research suggest that supplementation with vitamins, minerals and phytonutrients may be beneficial for cognition and cardiovascular function, especially in older adults. Results from two randomised controlled trials of multivitamin, mineral, and herbal supplements are presented in this thesis. In the Women's Study, healthy females aged 55-65 years supplemented daily for 16 weeks with a multivitamin formulated to meet the requirements of older women. Baseline and follow-up testing of cognition, cardiovascular function, and blood biomarkers was conducted. Tests included a computerised cognitive battery; augmentation index, augmentation pressure and brachial blood pressures; and blood markers of vitamins, inflammation, oxidative stress and homocysteine. The Men's Study followed the same protocol as the Women's Study, using a multivitamin formula with ingredients tailored to older men, in healthy males aged 55-65 years.

Multivitamin supplementation had no effect on performance on cognition or cardiovascular function measures in either the Women's study or the Men's study. However, assessment of blood biomarkers revealed some beneficial changes due to supplementation. These included increases in vitamin B6 and vitamin B12 in both the Women's and Men's studies. CRP was reduced in the Women's study, indicating reduced inflammation. In the Men's study there was a marginal effect on protein carbonyls, indicating reduced oxidative stress; and decreased total and LDL cholesterol.

Despite no effect on cognition or cardiovascular function, the results of blood biomarkers suggest a possible role for multivitamins in reducing risk factors for cognitive decline and cardiovascular impairment in older adults.

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In the study reported in Chapter 8, cognitive and cardiovascular data from my own participants is compared to data from a group of younger participants who were participating in another study at the university (Pipingas et al. 2013). I was not involved in that study and would like to thank the researchers who provided me with this baseline data set.

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Declaration

This thesis contains no material which has been accepted for the award of any other degree or diploma, except where due reference is made in the text of this thesis. To the best of my knowledge this thesis contains no material previously published or written by another person except where due reference is made in the text. Where the work is based on joint research or publications, the relative contributions of the respective authors are disclosed in the text.

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List of Abbreviations

ACC	anterior cingulate cortex
ACE	acetylcholinesterase
CRP	C-reactive protein
DP	diastolic pressure
ERP	event related potential
fMRI	functional magnetic resonance imaging
MCI	mild cognitive impairment
MMSE	Mini Mental State Examination
MP	mean pressure
MRI	magnetic resonance imaging
NHANES	National Health and Nutrition Examination Survey
NHMRC	National Health and Medical Research Council
NO	nitric oxide
PET	positron emission tomography
PWV	pulse wave velocity
RDI	reference daily intakes
SAM	s-adenosyl methionine
SP	systolic pressure
TICS	Telephone Interview for Cognitive Status
WAIS	Wechsler Adult Intelligence Scale

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1 Introduction

Cognitive decline is an inevitable part of the aging process, affecting all older adults to a greater or lesser degree. In some it may present as mild forgetfulness and slowing of mental speed; in others mental deterioration results in dementia, a behavioural syndrome characterized by severe loss of memory and at least one other cognitive impairment, reducing the capacity for independent living (American Psychiatric Association, 2013). Deterioration in mental performance is a process that begins as early as the third decade of life but has its greatest impact in much later years, particularly after age 65-70 where there is an accelerated decline (Finkel, Reynolds, McArdle, & Pedersen, 2005; Rabbitt & Lowe, 2000). Memory is affected, along with mental speed and other cognitive domains. Even without dementia, cognitive aging can affect mental performance in a way that impacts daily life (D. C. Park & Gutchess, 2000). This thesis is based on this concept, and endeavours to contribute to the existing scientific knowledge of cognitive aging, and how cognitive aging processes might be addressed to improve cognition in older adults.

The experience of debilitating mental decline in later years is one that occurs with increasing frequency in our aging society. Data from the Australian Bureau of Statistics' 2009 Survey of Disability, Ageing and Carers indicated that around 110,000 Australians had dementia, including Alzheimer's disease, with prevalence increasing with age, reaching 28% of people aged 100 years or more (ABS, 2009). A report commissioned by Alzheimer's Australia forecasts that incidence will rise due to the increasing population and increasing proportion of the population in older age groups, reaching more than 940,000 cases by 2050 (Deloitte Access Economics, 2011). This follows similar trends worldwide and forecasts an enormous social and financial cost (Brookmeyer, Johnson, Ziegler-Graham, & Arrighi, 2007). Anything that can be done to delay cognitive decline may substantially reduce the personal and social burden. For example, if the onset of Alzheimer's disease could be delayed by two years, the relative risk for lifetime development of the disease would be 0.77, a substantial reduction in dementia cases (Brookmeyer et al., 2007). Thus there is a need for cost-effective measures to alleviate this burden.

Supplementing with vitamins, minerals and plant extracts may assist in the prevention or delay of cognitive impairment in later years. Poor nutrition plays a role in the development of cognitive decline and cognitive impairment has been associated with reduced levels of many nutrients, including B vitamins (Bryan & Calvaresi, 2004; Ramos et al., 2005), antioxidant vitamins (Masaki et al., 2000), and some minerals (Marcellini et al., 2006; Rayman, 2012). Other plant components such as flavonoid compounds are not considered dietary essentials but may also provide protection against cognitive decline (J. P. E. Spencer, 2009; Webb, 2011). The elderly, that is, those over 65 years of age, are particularly vulnerable to vitamin and mineral deficiency due to undernutrition, impaired absorption capability or increased demand (Marian & Sacks, 2009). This, in addition to the epidemiological evidence, provides a biological rationale for using nutritional supplements and suggests that supplements might play beneficial roles in the maintenance of cognitive function into old age. Nevertheless, results from randomized controlled trials of supplements have generally been equivocal. Further research is needed to clarify the role of nutritional supplements in preventing or delaying cognitive decline.

A further motivation for the investigation of nutritional supplements for enhanced cognition is the increasing use of supplements within the community. Sales of vitamins, herbal preparations and other complementary and alternative medicines are on the rise (Euromonitor International Ltd, 2013a), with people choosing to supplement for many reasons, including cognitive improvement or protection (Laditka, Laditka, Tait, & Tsulukidze, 2012). However products are often untested and their efficacy is uncertain (MacLennan, Myers, & Taylor, 2006).

This thesis reports on the effects of two multivitamin, mineral and herbal supplements on cognitive function in older adults. In assessing such effects it is additionally useful to examine physiological factors that are associated with cognitive decline. Increased oxidative stress, increased inflammation and hyperhomocysteinemia are all associated with poorer cognitive function in older adults (Akiyama et al., 2000; Barja, 2004; Dufouil, Alpérovitch, Ducros, & Tzourio, 2003). Various nutrients that are associated with cognition have also been demonstrated to impact these markers, such as B vitamins

and antioxidants (Chin et al., 2011; Homocysteine Lowering Trialists' Collaboration, 2005). Thus it can be supposed that functional changes in cognition might be mediated via these factors, that is, through the effects of nutrients on oxidative stress, inflammation or homocysteine. For this reason blood markers for these were observed in the present trials, including C-reactive protein and fibrinogen for inflammation, protein carbonyls for oxidative stress, and homocysteine.

Of the various factors that impact cognitive decline, perhaps the greatest influence is cardiovascular health; the role that the cardiovascular system plays in cognition is fundamental. The cardiovascular system can affect the brain directly, and the biological markers that are common to cardiovascular and cognitive health are numerous (Gorelick et al., 2011). Cardiovascular function not only has a profound impact on cognition but is itself a vital health issue for the elderly, with ischemic heart disease being the largest cause of mortality in Australia (ABS, 2013). Age is the major risk factor for cardiovascular disease, as it is for cognitive decline, so it is fitting to investigate the two themes concomitantly. For these reasons, cardiovascular function was a primary focus of the present research.

This thesis comprises eleven chapters, including reviews of the scientific literature and reports of original research. Cognitive aging is reviewed in **CHAPTER 2**. The aim of the chapter is to provide a general understanding of the nature of cognitive aging and given the broad scope of the topic, it provides an overview of the scientific literature. It covers research of different domains of cognition, giving attention to those that are most susceptible to the course of aging: processing speed, episodic memory, executive function and spatial ability. Cognitive aging does not affect everyone equally and a number of factors that impact cognitive decline are considered, in particular education and gender. The chapter also explores methodological issues in cognitive aging, which is an important consideration for the studies in this thesis.

CHAPTER 3 examines the processes of cardiovascular aging and how this affects cognition. Specifically, it looks at the arterial system and blood flow, how structure and function change with age, and how this impacts cognition via effects on the brain. Brachial blood pressures are standard measures that are currently used in cardiovascular

risk assessment, however variables associated with aortic pressures have been proposed to be additionally useful (McEniery, Wilkinson, & Avolio, 2007). This includes augmentation index, which is a measure of arterial stiffness (O'Rourke & Hashimoto, 2008). Recent research on augmentation index in the context of cardiovascular risk assessment and cognitive decline is evaluated in this chapter. Other cardiovascular risk markers are also described, in particular inflammation and cholesterol.

CHAPTER 4 reviews vitamins, minerals and phytonutrients, and their role in cognitive and cardiovascular health. There are many nutrients which have been demonstrated to impact cognitive and cardiovascular health, and many nutrients which are contained in a multivitamin supplement. Many of these have extensive scientific literature devoted to them, so comprehensive reviews are not possible in this thesis. An overview and update on recent literature is presented, with greater space dedicated to the nutrients most relevant to the present studies.

These chapters each give a general review of the subject matter to bring the reader up to date with the current knowledge in the respective themes. In Chapters 5 and 6 the focus becomes narrower, providing comprehensive reviews of specific relevant subjects. The review in **CHAPTER 5** examines clinical trials of multivitamin supplements for cognition in older adults. Studies evaluating multivitamins are gaining in number as interest in the field grows. However due to aging processes, results in older adults may differ from those observed in younger adults and thus deserve separate attention. **CHAPTER 6** examines the use of augmentation index in clinical studies evaluating the effects of vitamin supplements. Little research exists to demonstrate whether augmentation index is indeed susceptible to nutritional supplementation, and if so, which supplements might be effective. This is an important consideration for the clinical trials reported in Chapters 9 and 10, where the effects of multivitamin supplementation on augmentation index are assessed. These two reviews are particularly relevant to the present studies, and inform their aims and hypotheses.

Three inter-related studies form the original research for this thesis, with the broad aim of assessing the effects of multivitamin supplementation on cognition in older adults. The first is a cross-sectional study examining the relationship between cognition and

cardiovascular function; the following studies investigate the effects of multivitamin supplements in older adults. The studies share several aspects of their methodology, which is described in **CHAPTER 7.** In each of the studies a computerized cognitive test battery was used to assess cognitive function. This battery has been used in previous studies of cognitive aging and supplementation (E. Harris et al., 2012; Pipingas et al., 2010). Augmentation index and augmentation pressure were used as cardiovascular measures, in addition to brachial systolic and diastolic pressure measurements. These variables were examined in all three studies. Two multivitamin formulas were used: "Swisse Women's Ultivite Multi-Vitamin, Mineral & Anti-Oxidant with Herbs 50+ Years" (Women's formula) and "Swisse Men's Ultivite Multi-Vitamin, Mineral & Anti-Oxidant with Herbs 50+ Years" (Men's formula). Both formulas contain a range of vitamins, minerals and herbal ingredients, but differ in the quantities of each nutrient and in the selection of herbal ingredients, with dosages tailored to the requirements of each gender. The two supplements were used in separate studies but are described in detail in the Methods chapter. Details of the blood biomarker assessment in the multivitamin trials are also provided.

Cardiovascular function is affected by age and also relates to cognitive performance. The present studies have assessed cardiovascular health using augmentation index, a validated measure of arterial stiffness. However, there is little data regarding its relationship with cognition. Therefore, the aim of the study in **CHAPTER 8** is to elaborate on the relationship between cognition and cardiovascular function as measured by augmentation index. Specifically, the study asks whether augmentation index can predict cognitive performance as assessed by the battery of computerized cognitive tasks. Although there is scarce data in the scientific literature, based on other measures of arterial stiffness a relationship between augmentation index and cognition is anticipated (Rabkin, 2012). In this study the relationship is examined in older and younger adults to see whether the relationship changes across adulthood. Augmentation pressure is also included because it has been suggested that this variable is more relevant in older adults (Fantin, Mattocks, Bulpitt, Banya, & Rajkumar, 2007). Baseline data from the present multivitamin studies is used, along with baseline data from a separate supplementation study in younger adults (Pipingas et al., 2013). Observation of a relationship between these cardiovascular and cognitive variables is useful for the

interpretation of results of the multivitamin studies, as it may reveal mechanisms of action.

CHAPTER 9 includes the study of the Women's formula; a 16-week intervention in women aged 55-65 years. It reports the effects of the supplement on cognition, cardiovascular function and associated blood biomarkers. Cognition is affected by multiple factors in later years and this study aims to investigate a range of these that are particularly relevant to nutrition and nutritional supplements. Therefore blood biomarkers for oxidative stress, inflammation and lipid concentrations are assessed, along with appropriate vitamin levels. Cardiovascular function is also examined using augmentation index and other blood pressure variables. The computerized test battery covers a range of cognitive domains, chosen for their particular sensitivity to decline with age (Pipingas et al., 2010). By examining these variables a broad picture of the physiological and behavioural effects of the supplement will be gained. **CHAPTER 10** reports on the clinical trial of the Men's formula; a corresponding 16-week study in men aged 55-65 years. This study tests the effects of the Men's formula multivitamin supplementation on the same cognitive, cardiovascular and blood variables. These two chapters describe the considerable practical research that has been undertaken for this thesis, the results of which contribute to our scientific understanding of the role of nutritional supplement for cognitive decline.

Finally, **CHAPTER 11** discusses the broad outcomes of the group of studies in the context of existing research and outlines some future directions for research in nutritional supplementation for cognitive aging.

2 Cognitive Aging

This chapter examines the nature of age-related cognitive decline. It discusses which particular cognitive functions decline with age and which are maintained. The impact of normal and pathological aging on specific cognitive processes is addressed, including processing speed, episodic memory, executive function and spatial abilities. Factors

which influence the course of cognitive aging are considered, in particular, gender and education. Finally, methodological issues pertaining to research in cognitive aging are discussed.

The exact mechanisms behind cognitive aging are still unclear; however two important contributors will be examined in later chapters: cardiovascular function (Chapter 3) and nutritional factors (Chapter 4). Age-related changes in cognitive performance are substantially mediated by observable changes in the structure and function of the brain (Fjell & Walhovd, 2010). These changes are briefly addressed but it is outside the scope of this chapter to include a full review of the considerable literature on this topic.

The scientific literature relating to cognition is extensive and this chapter is necessarily a rather superficial summary, with greater focus on areas that are most relevant to the present investigations. The research papers that are cited are not intended to be an exhaustive account of the literature but are used as illustrative examples. The aim is to provide a general account of cognitive aging and the issues relating to cognitive testing in older adults.

Throughout this thesis reference is made to impairment syndromes of cognitive aging. These include dementia, Alzheimer's disease, and mild cognitive impairment (MCI), which are defined here for clarity.

Dementia is a general term that refers to the behavioural manifestation of neural impairment. The symptoms include reduced memory performance which presents with impairment in at least one other cognitive function, such as aphasia, ataxia, agnosia or executive function (American Psychiatric Association, 2013). The impairment must be sufficiently serious to impact the individual's ability to function in social or occupational settings. There are various forms of dementia which are defined based on etiology, including Alzheimer's disease and vascular dementia, which are the two most common dementias in the elderly.

Alzheimer's disease is the most common cause of dementia in the elderly. It is characterized by gradual onset of dementia, with deterioration in cognitive function,

particularly memory function (Savla and Palmer, 2005). Neurological features of Alzheimer's disease include brain atrophy as well as beta amyloid deposits and neurofibrillary tangles (Braskie and Thompson, 2013).

Mild cognitive impairment (MCI) is a term which refers to an intermediate stage of cognitive impairment, where memory function is reduced but not sufficiently severe to warrant a diagnosis of dementia (Feldman and Jacova, 2005). Individuals with MCI have an increased risk of subsequent dementia (Panza et al., 2005).

2.1 The pattern of cognitive aging

There are two broad categories of cognitive change that occur with normal aging. One is an increase in general knowledge and know-how, for example, vocabulary and other acquired knowledge; this can be referred to as crystalized abilities (Christensen, 2001). The other is a decline in cognitive performance, in particular, deterioration in memory, spatial abilities, reasoning and mental speed (Salthouse, 2010); also referred to as fluid abilities. Bäckman and co-workers (2004) describe a pattern of cognitive aging whereby tasks which are highly automatized, have limited speed demands, or depend on prior experience remain stable. Conversely, tasks that require new learning, speed, and mental flexibility are greatly affected. Importantly, the course of cognitive decline follows a similar pattern whether it occurs as a result of normal aging, or due to pathological processes (Bäckman et al., 2004). It can therefore be difficult to tell if early pathological decline is occurring, on the basis of cognitive testing.

Research supports the notion that knowledge increases with age and experience. For example, data from the Betula project, a large longitudinal study of cognition throughout adulthood, indicated there was an increase in semantic memory (vocabulary and general knowledge) from middle adulthood up until early old-age (55-65 years) although it decreased in older old-age (70-80 years) (Nyberg et al., 2003). Similarly, another study observed that semantic representation (in this case, knowledge about the categories or attributes of items) was preserved in old age, despite slower responses (Eustache, Desgranges, Jacques, & Platel, 1998). In the Canberra Longitudinal Study,

crystalized abilities, as measured by the National Adult Reading Test (NART), remained stable after seven years of follow up in elderly participants (Christensen, 2001).

Interestingly, in normal aging, forgetting rates are stable across the lifespan (Fjell et al., 2005). Although less information is encoded overall, it is retained in older people in the same proportion as it is in younger people (Salthouse, 1992). This suggests that memory loss is due to processes involved with encoding memories, rather than retrieving them.

2.1.1 Cognitive decline

Although some functions do improve, other mental faculties show clear decline with age. Specifically, performance impairment is evident in cognitive speed, memory, executive function and spatial abilities, among others. There is still some debate regarding the nature of the impairments. Some authors argue that there is evidence of a single general factor underlying cognitive decline (Malec, Ivnik, & Hinkeldey, 1991; Salthouse, 2010). This is proposed to be processing speed, that is, a general slowing in cognitive ability which affects performance in all other cognitive domains (Salthouse, 1996). However, other research has shown decrements in specific areas in addition to processing speed. In our own research we have demonstrated that different cognitive functions decline at different rates, with the greatest decline observed in tasks assessing spatial working memory and contextual memory (Pipingas et al., 2010). Changes in specific cognitive domains are discussed in greater detail in the relevant sections below.

Cognitive decline becomes most apparent in the elderly, however a reduction in performance can be observed from early adulthood (Pipingas et al., 2010; Salthouse, 2009). In cross-sectional studies, decline is observed to be linear and mild throughout adulthood, becoming steeper after 70 years of age (Rabbitt, Diggle, Smith, Holland, & Mc Innes, 2001). In longitudinal studies the decline does not appear to be as steep, but this might be due in part to practice effects (Salthouse, 2010) (also see Section 2.3.2 for the implications of cross-sectional and longitudinal research).

Following is a discussion of the scientific literature relating to particular cognitive domains, and how they are affected in old age.

2.1.2 Processing speed

That cognitive speed is reduced with increasing age is a robust finding that has been reported consistently for nearly a century (Salthouse, 1996). A more or less linear decline across adulthood, the speed with which cognitive processes can be carried out begins to deteriorate from the early twenties (Nilsson, 2003; Salthouse, 1996).

The effects of cognitive slowing can be observed in simple tasks that assess response time, such as a computerized reaction time task where participants push a button in response to a target stimulus, or a choice reaction time task in which participants push one of two or more buttons in response to different target stimuli. In computerized batteries, response time and choice response time tasks are consistently reported to show decrements with increasing age. In our own cognitive battery the correlation with age was $r = 0.301$ for Simple Response Time and $r = 0.474$ for Choice Response Time for adults aged from 21 to 86 years (Pipingas et al., 2010). Similarly, other computerized batteries have demonstrated slower response times in healthy elderly, including the CANTAB (Robbins et al., 1998) and CogState (Lim et al., 2012).

Traditional paper-and-pen tasks also demonstrate slowing with age. For example, the Coding and Symbol Search tasks of the Wechsler Adult Intelligence Scales (WAIS) have a significant speed component and are very sensitive to age (Holdnack, Zhou, Larrabee, Millis, & Salthouse, 2011; Wisdom, Mignogna, & Collins, 2012).

2.1.2.1 Effect of slowing on other cognitive domains

A general reduction in mental processing speed may manifest as poorer performance in tasks which are more complex than those described above, affecting performance in accuracy as well as response time. The processing speed theory was comprehensively addressed in an influential paper by Salthouse (Salthouse, 1996). He contended that processing speed does manifest as a simple slowing and that it affects other cognitive

functions as well, such as memory and executive function. This occurs through two mechanisms, the limited time mechanism and the simultaneity mechanism. The limited time mechanism explains that accuracy may be affected when there is a time limit for processing. Later mental operations cannot be completed because earlier operations have taken too long, that is, time to complete the cognition runs out. In the simultaneity mechanism, "the products of early processing may be lost by the time that later processing is completed". Information decays over time thus slower processing would result in less information being available. That is, errors can occur by not being able to concurrently access earlier and later processes. Effects of this would be observed in tasks of working memory.

Other research supports the processing speed theory. For example, in a large study in twins it was determined that processing speed could account for most of the variation in cognitive performance as well as the acceleration of cognitive decline in later years (Finkel et al., 2005). The study also noted that a significant proportion of the genetic influences on cognitive ability were mediated by genetic factors that affected processing speed. In a further study, this group reported that processing speed was the best indicator of age-related changes in spatial function and memory, although not verbal ability (Finkel, Reynolds, McArdle, & Pedersen, 2007).

Contrarily, it has also been argued that the reverse might be true, since many other functions underpin performance on a processing speed task (Eckert, 2011). The trailmaking ("Connections") task was used as an example. Performance of this task requires many cognitive processes, including stimulus perception, working memory, decision making, motor planning and praxis, and performance evaluation. This requires the coordination of several neural systems which may themselves be affected with age, and these can have additive effects on the speed at which the task is performed.

2.1.2.2 Processing speed and the brain

Data from brain imaging studies provide evidence of the nature of age-related processing speed changes. Structural changes in both grey matter and white matter have potential to impact processing speed. Grey matter loss could contribute to cognitive

slowing because it could impair the quality of neural signals and increase noise, which may slow recognition and response processes (Eckert, 2011). Loss of myelination (white matter) could slow rates of signal conduction along neural pathways, thereby slowing processing speeds.

White matter lesions and brain atrophy have been associated with cognitive decline. In an MRI investigation of people with cerebral small vessel disease, the extent of white matter lesions was associated with the level of cognitive decline in processing speed and executive function (Prins et al 2005). Similarly, longitudinal association between baseline periventricular white matter hyperintensities and reduced processing speed has been observed (van den Heuvel et al., 2006). This relationship was maintained longitudinally over three years, with poorer cognitive performance being associated with increasing volume of the lesions.

Cognitive slowing may also be attributed to a decrease in the efficiency of neural networks, or cortical connectivity. Cortical networks were examined in 342 healthy elderly people using diffusion tensor imaging (Wen et al., 2011). Investigators observed that global, as opposed to local, network efficiency was associated with processing speed in the Digit Symbol Coding and Trail Making A tasks. They concluded that because processing speed was global, many types of lesion may produce a reduction in processing speed.

Despite the general cognitive slowing that is a well-established corollary of age, it is apparent that other functions also decline and this cannot be wholly explained by slowing. That is, further decline is evident in particular functions, including memory, executive function and visuospatial function. These are discussed below.

2.1.3 Episodic memory

Episodic memory is one of the most affected processes in cognitive aging. Episodic memory is defined as the memory for personally experienced events (Tulving, 2002); it allows the re-experiencing of past events through conscious recollection. This not only

includes recall and recognition of factual information, but also includes the many surrounding circumstances of an experience, including location, time, or other qualities. Thus tasks that require recollection of contextual information are also relevant.

A decline in episodic memory performance was reported in the Betula study (Nilsson, 2003). Cross sectional data of episodic memory was examined using a composite score of episodic memory tasks including free and cued recall, source recall (context) and recognition. A clear episodic memory decline was observed from age 35 (the youngest group examined), with a steeper decline in older age groups, from age 65.

There is a greater deterioration in free recall compared with recognition and this is well established (Nyberg et al., 2003). Decline in free recall may be due to impairment in either memory encoding or retrieval. Poor performance in both recall and recognition suggests a problem at the encoding level. To illustrate this point, investigators on the Kungsholmen Project discussed their series of studies where a group of 1800 elderly people (75 years plus) were studied longitudinally for up to 13 years (Bäckman et al., 2004). They compared free recall and recognition, using word recall tasks. It was observed that the age-associated decline in performance was stronger in free recall although it was still apparent in the recognition tasks. That older participants were sometimes unable to recognise previously presented material suggests a deficiency in the encoding of memories. The stronger effect observed on free recall indicates an additional difficulty with memory retrieval.

Performance in episodic memory depends in part on the availability of contextual details for correct recollection of information (Nilsson, 2003), however the contextual details themselves may pose a greater difficulty for elderly individuals. The theme of context has been examined in a variety of tasks, with the aim of assessing details surrounding or relating to the content, rather than the content itself. Results from a meta-analysis of studies investigating memory context and content revealed that declines in memory performance for contextual information were greater than memory for content (W. D. Spencer & Raz, 1995). The authors argued that the source of information is more difficult to remember (e.g. male or female voice), as are other details such as the colour that a word was presented in, or spatial location of objects.

They acknowledged however that this may in part be due to the more demanding nature of contextual tasks.

A related hypothesis proposes that older people have an impaired ability to merge unrelated attributes into a cohesive whole memory. In studies using the paired associates task, the participants are shown two items (pictures, words etc) that are either related or unrelated. It was demonstrated that older adults performed poorly on recall of the association, while performing almost as well as young people on the recognition part of the task (Naveh-Benjamin, 2000; Naveh-Benjamin, Hussain, Guez, & Bar-On, 2003). This was particularly true when the items were semantically unrelated, demonstrating that when there were pre-existing associations older adults were able to rely on these in their performance.

A contextual deficit is also observed in other conditions. For example, the Contextual Memory task in our cognitive battery uses spatial location as the context (Pipingas et al., 2010). Participants are required to recall the location on the screen where they first saw an item presented – i.e. top, bottom, left or right. This function showed greater impairment with age than the recognition memory tasks.

2.1.3.1 Episodic memory and the brain

The hippocampus, located in the medial temporal lobe, is an important structure for memory performance. Evidence from imaging studies suggests it plays a role in the organisation of information from different cortical regions, allowing memory encoding and retrieval (Dickerson & Eichenbaum, 2010). Hippocampal volume is related to episodic memory performance, for example it was associated with score on the Wechsler Logical Memory test in a group of elderly people aged 80 years or more (Lye et al., 2006). Atrophy of the hippocampus is associated with poorer verbal memory in older adults (Golomb et al 1993). It is also related to disease severity in Alzheimer's disease and to Mini Mental State Examination (MMSE) score in MCI, with hippocampal volume loss over one year being correlated with worsening cognitive outcome (Morra et al., 2009). There is also evidence of beta-amyloid deposition leading

to hippocampal atrophy and consequent episodic memory impairment, providing a link between Alzheimer's pathology and cognitive performance (Mormino et al., 2009).

The prefrontal cortex is also implicated in episodic memory decline. The prefrontal cortex is active during encoding and retrieval of memories (Vannini et al., 2011) and function is also altered with age (Rajah & D'Esposito, 2005). There is substantial atrophy of the region during aging (Salat et al., 2004). In Alzheimer's disease, increased activity in the prefrontal cortex indicates compensatory behaviour, as this was correlated with performance on episodic memory tasks in Alzheimer's patients (Grady et al., 2003).

While the hippocampus and prefrontal cortex have been the focus of much research in episodic memory decline, other research has demonstrated that white matter also plays an important role. Prefrontal white matter is particularly susceptible to aging and is associated with episodic memory impairment (Gunning-Dixon, Brickman, Cheng, & Alexopoulos, 2009). White matter hyperintensities are associated with episodic memory decline, with both the volume and location of these showing relationships with performance on memory tasks (E. E. Smith et al., 2011). Measures of white matter integrity, which indicate the intactness of individual pathways in the brain, have also been associated with episodic memory loss. In particular, disruptions to white matter integrity have been observed in the medial temporal lobe during a measure of delayed verbal memory (Goldstein et al., 2009). Also, white matter tracts connecting frontal and temporal cortical regions as well as frontal to subcortical structures are disrupted, leading to poorer episodic memory function (Lockhart et al., 2012).

2.1.4 Executive function

Executive function refers to the coordination and regulation of cognitive performance. It encompasses a range of behaviours including intentionality, interference management, inhibition, planning and social regulation (Amieva, Phillips, & Della Sala, 2003). While executive function is generally considered to be a regulator of complex behaviour, there remains a lack of clarity as to what, exactly, executive function tasks measure. The

following excerpt from a review on executive functions provides a neat description of the processes included under this general category:

> *"Executive abilities allow us to shift our mind set quickly and adapt to diverse situations while at the same time inhibiting inappropriate behaviors. They enable us to create a plan, initiate its execution, and persevere on the task at hand until its completion. Executive functions mediate the ability to organize our thoughts in a goal-directed way and are therefore essential for success in school and work situations, as well as everyday living" (Jurado & Rosselli, 2007)*

The authors argue that "executive function" does not appear to be a unitary system, referring to four different modalities: attentional control, planning, set shifting and verbal fluency.

Another group identifies three separable functions that are frequently considered in the scientific literature. These are mental set shifting, information updating and monitoring, and inhibition (Miyake et al., 2000). Set shifting is the process of changing focus between multiple tasks or mental sets. Information monitoring and updating refers to information in working memory and is thus closely related to this construct. It maintains task-relevant information and it actively manipulates it, evaluating incoming information and revising existing contents. Inhibition refers to the ability to inhibit behavioural responses that are dominant or automatic, when the need arises. In a factor analysis, these three functions were clearly distinguished from each other but also shared some commonality, with moderately high correlations among them (Miyake et al., 2000).

Impairment in these functions can be observed in patients with frontal lobe damage, who exhibit "dysexecutive syndrome" and whose skills in planning, organization, reasoning and/or decision making may be disrupted (Hanna-Pladdy, 2007). An age-related decline in performance on executive tasks has also been observed. For example, across an age range of 20-81 years, age-related cognitive decline was observed in updating and inhibition, and non-significantly in shifting (Fisk & Sharp, 2004). The

investigators pointed out that there was a relationship between these variables and processing speed, in addition to the relationship with age. Age-related decline has also been observed in other executive function tests, including the Wisconsin Card Sorting Test, which assesses set shifting or switching; fluency tasks including letter and category fluency; and the Tower of London test, which measures planning (Bryan & Luszcz, 2000).

Executive tasks tend to have low internal reliability and test-retest reliability, probably due to the task complexity and the fact that executive function controls other cognitive processes (that may themselves impact on performance). However, executive functions have been demonstrated to be associated with activities of daily living, and so may reflect an elderly person's capacity for independent living (Jurado & Rosselli, 2007).

2.1.4.1 The Stroop task

The studies in this thesis used a computerized version of the Stroop task as a measure of executive function. The Stroop task aims to cause "interference" by eliciting an automatic response that must be inhibited. There can be up to three control tasks and an "interference" task. These include reading colour words (i.e. red, green, blue, yellow) printed in black ink; naming the colour of a series of coloured bars; reading colour words printed in incongruous colours; and naming the colour of the ink of these words, ignoring what the word reads (interference task) (Cohn, Dustman, & Bradford, 1984). The latter is a more difficult task, as participants must inhibit the impulse to read the word and focus only on the colour it is presented in. The "interference effect" is the difference in time it takes to name the colours in the latter task. Other studies have only used one control task, for example, reading the colour names in black ink or matching colours; in other versions the task has been computerized (Pipingas et al., 2010).

Normative data for the Stroop task was established in the Maastricht Aging Study for 1,856 adults aged 24 to 81 years (van der Elst, van Boxtel, van Breukelen, & Jolles, 2006). The study used a paper version of the task, asking participants to read aloud 100 items in each of three subtasks: reading colour names in black ink, naming colours of solid coloured patches, and the interference task. Age and level of education affected

performance on all tasks. Performance had a curvilinear relationship with age, showing a decline with age that decreased more rapidly with older age. Higher education was associated with better performance at all ages. There was also an interaction between age and education, in that the magnitude of decline was moderated by a person's level of education, that is, those with less education showed a steeper decline in old age than those with more education. The study also observed that women performed better than men.

Other studies have found similar age-related deterioration. In a paper version of the task, all participants performed worse in the interference task compared with the control tasks, however older adults (61-90 years) performed significantly worse than younger participants (21-50 years) (Cohn et al., 1984).

There is debate whether the age-related decline observed in the Stroop task is due to general slowing or to an inhibition effect that is additional to this. For example, some investigators argue that an independent factor is unnecessary to explain the decline in Stroop performance with age, and that it can almost entirely be explained by general slowing (Uttl & Graf, 1997). Furthermore, in a meta-analysis of twenty Stroop studies, it was determined that the decline in interference effect was due to general cognitive slowing (P. Verhaeghen & De Meersman, 1998) . Not all authors agree with this conclusion. Comparing young and old adults, and patients with Alzheimer's disease, another study observed that Stroop interference increased in old age and with Alzheimer's disease, with greater deterioration in Alzheimer's patients. The age effect was not merely due to cognitive slowing. In addition to taking longer, Alzheimer's patients made more errors of naming in the incongruent task (Spieler, Balota, & Faust, 1996). Furthermore, as discussed in greater detail below, brain imaging studies tend to support the "inhibitory deficit hypothesis" (West & Alain, 2000).

There are other points to consider when assessing Stroop task performance: the interference effect can be reduced with practice in young and old adults (Dulaney & Rogers, 1994); age effects on Stroop are associated with age-related changes in colour perception (Ben-David & Schneider, 2010); poorer Stroop performance is found in those with less education (Seo et al., 2008); and finally, the decline in Stroop

performance is greater in men than in women (van der Elst, van Boxtel, van Breukelen, & Jolles, 2008).

2.1.4.2 Executive function and the brain

Brain studies corroborate the evidence from cognitive trials of a deficiency in executive function with aging. Executive behaviours are considered "frontal lobe functions". Neuropsychological evidence indicates that damage to frontal lobes can result in impaired ability to control and regulate behaviour (Lezak, 2004).

The involvement of the prefrontal cortex in executive control is well established, but other parts of the frontal lobes as well as other brain regions are also relevant, including subcortical structures (Jurado & Rosselli, 2007). Specific regions are thought to be associated with different modalities within executive function, for example, the anterior cingulate cortex is associated with error processing and conflict monitoring whereas the dorsolateral prefrontal cortex is involved with set maintenance (Fassbender et al., 2004).

The Stroop task in particular has been associated with the anterior cingulate cortex (ACC) in brain studies using various methods. The ACC was associated with Stroop performance in a positron emission tomography (PET) study looking at regional cerebral blood flow (Pardo, Pardo, Janer, & Raichle, 1990). In an event related potential (ERP) study of Stroop performance, the anterior cingulate cortex was activated with a later activation of the left temporo-parietal cortex (Liotti, Woldorff, Perez Iii, & Mayberg, 2000). During a functional magnetic resonance imaging (fMRI) investigation older people demonstrated increased activity in the ACC in Stroop Incongruent blocks compared with younger participants (Milham et al., 2002). Other brain regions exhibited reduced responsiveness, including the dorsolateral prefrontal cortex and parietal cortex, areas that have been associated with control of attention. The authors suggested that in aging, a loss of inhibitory control means there is a greater intrusion of irrelevant information into working memory, thereby impairing performance.

Other processes that contribute to decline in executive function include decreased neurotransmitter function or a decrease in horizontal dendrites, which may play

inhibitory role in brain (Cohn et al., 1984), or impaired regional efficiency of neural networks in various cortical regions (Wen et al., 2011).

2.1.5 Spatial function

Spatial function refers to the perception and processing of spatial properties such as shape, size, distance, location, relative position, orientation and direction. It is also termed visuospatial function in the scientific literature due to the visual nature of the assessment tasks. Spatial function is necessary for navigation and way finding, and is also relevant to other everyday tasks such as reaching for objects or reading an analogue clock face. It can involve spatial attention, spatial memory and spatial orientation (Drag & Bieliauskas, 2010).

The construct of spatial function can be further divided according to the frame of reference. Egocentric frames of reference consider the position of an object in relation to the self, whereas an allocentric frame of reference considers an object is in terms of reference to other features of the environment, or another object (Iachini, Iavarone, Senese, Ruotolo, & Ruggiero, 2009). This can be exemplified by contrasting the experience of reading a map (allocentric) compared with driving through the streets (egocentric). It has been proposed that allocentric, orientation-free representations are stored in long term memory and interact with the egocentric system which updates relations to objects from the self-orientation (Avraamides & Kelly, 2008). The Spatial Working Memory task that is used in the present studies takes an allocentric perspective, with white squares appearing in a reference grid, which are related to the grid and to each other.

Spatial function is particularly sensitive to age-related decline (Pipingas et al., 2010). However, assessing spatial function in the elderly poses some difficulties because many popular tasks that purport to measure spatial function also require other cognitive abilities. Consequently it is difficult to attribute performance specifically to visuospatial competence (Iachini et al., 2009). For example, the Rey-Osterrieth Figure and the Block Design task from the WAIS require both planning and praxis, cognitive functions which

may also be affected with age or dementia. It is not yet clear whether the particular sensitivity of spatial ability to age is because it is itself more sensitive to normal aging or because the tasks used to measure it are more complex than other tasks (Drag & Bieliauskas, 2010). In general the more complex the task, the more likely it is that multiple cognitive functions may be required, e.g. executive function, language, or long term memory. Nevertheless, well-validated tasks such as the Visual Spatial Learning Test, WAIS Block Design, Clock Drawing and the Rey-Osterrieth Complex Figure, which all have a substantial spatial component, do demonstrate significant decline with age.

The Visual Spatial Learning Test requires participants to recall the positions of various abstract designs within a 6x4 grid. The task shows decline in the elderly, with progressively less information being recognized or recalled with increasing age. Retention in the delayed task remains similar (Malec et al., 1991), indicating a deficit in encoding into memory rather than retrieval of stored information. This encoding deficit is consistent with evidence from episodic memory literature (discussed above). A further study compared performance of a group of patients with dementia, predominantly Alzheimer's disease, to a control group. Dementia patients performed poorly in comparison and made a greater number of intrusion errors (false positives) (Malec et al., 1992).

The Block Design Subtest of the WAIS shows a decline in performance from around midlife, with a considerable impairment exhibited by later life (Wisdom et al., 2012). A variation in performance is also observed, with older people demonstrating greater diversity of test scores than younger people, denoting that some individuals decline more than others on this task.

The Rey-Osterrieth Complex Figure taps into visuospatial perception and memory as well as spatial constructional ability. There is a copy condition where the figure is directly copied, as well as immediate and delayed memory conditions, where the figure is drawn from memory. Performance in all of these conditions declines with age (Peña-Casanova et al., 2009). However, as the task also requires planning and problem solving

the age-related decline cannot be presumed to be entirely a spatial deficit, and executive functions might also play a role.

Clock drawing is very sensitive to cognitive decline, particularly abnormal decline and has been demonstrated to be better at discriminating MCI and Alzheimer's dementia patients from the normal elderly (Royall, Cordes, & Polk, 1998; Tuokko, Hadjistavropoulos, Miller, & Beattie, 1992; Yamamoto et al., 2004). Performance on clock drawing is also predictive of later cognitive deterioration (Ferruci et al., 1996). However, this task is also correlated with executive functions and other cognitive abilities (Shulman, 2000).

Other less commonly used spatial tasks have also demonstrated significant age-related decline in performance. In one study, museum and office settings were used to examine age-related changes in episodic spatial memory (Uttl & Graf, 1993). In the first experiment, participants were required to recall on a map particular items in the museum setting. The office setting included a relocation test, where participants had to physically replace items within the office. In this egocentric spatial task they observed that the older group performed more poorly than the younger group.

In a further example, a visual-spatial memory test was developed which involved remembering the location of numbered circles on the screen and entering them in sequential order (Maki, Yoshida, & Yamaguchi, 2010). They demonstrated impairment in visuospatial function in patients with MCI, with even greater impairment observed in dementia patients. Thus spatial abilities are particularly relevant to pathological cognitive decline.

From the examples cited above it is evident that age-related performance decrements can be observed in a wide variety of spatial tasks. Despite the difficulty in isolating the spatial component of any given test, evidence suggests that visuospatial function may actually be more susceptible to aging than other cognitive functions. In a series of three experiments, two groups of participants (young vs old) were tested on verbal and visuospatial tasks, including processing speed, memory span and learning (Jenkins, Myerson, Joerding, & Hale, 2000). In all three conditions the older group performed

more poorly than the younger group, however, the older group performed even more poorly on visuospatial tasks compared with verbal tasks. Another study demonstrated a more rapid deterioration of visuospatial function using the Visual Reproduction task of the Wechsler Memory Scale III when compared to the Logical Memory task (Haaland, Price, & Larue, 2003).

Due to additional aging effects on working memory, spatial working memory may be more susceptible to aging than other spatial functions. A slowing of response and a decrease in accuracy in mental imagery tasks has been observed in older adults, which the investigators determined was mediated by declines in working memory rather than sensorimotor speed (Briggs, Raz, & Marks, 1999). This suggests that spatial working memory may be particularly useful in research on cognitive aging.

In the above studies it may be argued that the greater decline in the visuospatial tasks is due to task complexity rather than a particular deficit in spatial ability. Simpler tasks might do better at isolating spatial cognition. The spatial task that is used in the present study, Spatial Working Memory, has been demonstrated to be highly sensitive to cognitive decline (Pipingas et al., 2010). In this task, participants are required to memorise patterns of white squares in a 4x4 grid. The task exercises working memory for spatial information, with a low requirement for manual dexterity or other cognitive functions. It is interesting to note that this task showed the steepest gradient for change with age in our study of normal adults (Pipingas et al., 2010). This lends support to the suggestion from that spatial functions are affected early and acutely in the process of cognitive decline (Jenkins et al., 2000).

2.1.5.1 Spatial function and the brain

Brain research supports evidence from cognitive trials which indicates reduced spatial abilities in both normal aging and dementia. Impairment in spatial cognitive function can be explained by the fact that the underlying brain structures are particularly vulnerable to the effects of normal aging and degenerative disease (Iachini et al., 2009). Of relevance are frontal and parietal regions, as well as parts of the temporal lobe, especially the hippocampus.

The hippocampus is frequently implicated in spatial working memory, with reduced activity being associated with poorer performance (Klencklen, Després, & Dufour, 2012). The prefrontal cortex has also been associated with spatial working memory however its role may be related to the memory component rather than the spatial nature of a task (Kessels, Postma, Wijnalda, & De Haan, 2000). Furthermore, memory for spatial contextual information appears to be dependent on the hippocampus, with reduced volumes of the hippocampal head interfering with encoding capacity (Maillet & Rajah, 2011). Prefrontal cortex and parietal activation patterns appear to be reorganized in the elderly during spatial working memory performance, suggesting compensatory mechanisms are in play (Piefke, Onur, & Fink, 2012).

Spatial navigation is also affected with age, with older adults again showing reduced activation in the hippocampus compared with younger adults (Moffat, Elkins, & Resnick, 2006). Differences were also observed in other brain regions including parts of the parietal lobe, again suggesting an alteration in cognitive strategies in older adults, in compensation for hippocampal deficiency. A recent review of navigation studies noted differences between normal aging and Alzheimer's disease (Lithfous, Dufour, & Després, 2013). The prefrontal cortex and hippocampus demonstrate alterations in normal aging, but there are additional changes in Alzheimer's disease, in regions including the parahippocampal gyrus, caudate nucleus and parietal lobe. The authors suggest that impaired navigation ability may be a means of identifying those at higher risk of dementia.

2.2 Factors that impact cognitive aging

All people experience some cognitive decline with age, that is, the decline in mean performance of a group is not solely due to the worsening performance of a subset of individuals (Salthouse, 2010). However there are numerous factors that can influence the degree or extent to which cognitive performance is affected in old age. Factors that exacerbate deterioration tend to do so in a manner that reflects normal aging (Bäckman

et al., 2004). Because of this, we cannot determine the source of cognitive impairment by examining the pattern of performance on various cognitive tasks.

Examination of the factors that impact cognitive decline may provide insight for potential therapeutics. Cardiovascular function (Chapter 3) and nutritional status (Chapter 4) are two important issues that will be considered separately. Here we briefly review several other issues that may influence the progression of cognitive decline.

2.2.1 Education and intelligence

Education has a protective effect on cognition with age, with more highly educated people tending to perform better on cognitive tests in old age, when compared with those with a lesser education (Anstey & Christensen, 2000; Van Hooren et al., 2007). This is also true in longitudinal studies where educated people tend to decline at a slower rate than their uneducated peers (Bäckman et al., 2004).

The protective effect is not uniform across the cognitive spectrum. Backman et al (2004) discussed how education is more protective in tasks which have "high strategic demands" and demonstrates benefits in a pattern consistent with the declines seen in cognitive aging. Describing the data from the Kungsholmen Project, they noted that education was associated with better performance in tasks more susceptible to aging: free recall, block design, digit span, verbal fluency and clock drawing. Tasks such as recognition and trail-making did not benefit from higher education (Bäckman et al., 2004).

In the EClipSE Study, investigators examined 872 participants who had donated their brains at death. Years of education was associated with decreased risk of dementia, however there was no evidence of protection against neurodegenerative or vascular pathology. Thus it appears that education provides a compensatory benefit – those with higher education are able to compensate for their pathology (Brayne et al., 2010).

That education provides a protective effect has been disputed by some researchers. Analysis of data from more than 1000 participants over 12 years revealed that although education was associated with better cognitive performance it did not attenuate the *rate* of decline. That is, the rate of change was similar across groups. The investigators attribute this difference in findings to improved longitudinal statistical methods (Zahodne et al., 2011).

This implies that those with higher education are merely starting from a higher baseline performance, a proposition which corresponds to suggestions that education is a proxy for higher intelligence. This was argued in a series of papers by Steinberg and co-workers, who examined older adults aged 56-99 years on a range of cognitive tests. They reported that WAIS-R full scale intelligence was more closely correlated than level of education with a range of tasks, including Boston Naming Test, Trail Making, Stroop, Weschler Memory Scale-Revised and Auditory Verbal Learning Test (AVLT) and visual spatial learning test (VSLT) (Steinberg, Bieliauskas, Smith, & Ivnik, 2005a, 2005b; Steinberg, Bieliauskas, Smith, Ivnik, & Malec, 2005; Steinberg, Bieliauskas, Smith, Langellotti, & Ivnik, 2005).

A protective effect of education, intelligence, or both, supports the brain reserve hypothesis (Valenzuela & Sachdev, 2006b). This meta-analysis of more than 29,000 cases determined that high brain reserve (education, IQ, occupation and mental activity) reduced dementia risk by half, over a mean follow up of seven years. An accompanying systematic review by the same authors determined that high brain reserve was also associated with reduced cognitive decline (Valenzuela & Sachdev, 2006b).

Education and intelligence are important considerations in that cognitive impairment may be masked by the better performance of educated individuals, or conversely, older people of low education might be misclassified as impaired (Mejia, Gutiérrez, Villa, & Ostrosky-Solís, 2004).

2.2.2 Gender

Women and men may differ in their cognitive abilities and the manner in which aging affects these. It is typically reported that women perform better on tests of verbal functions, while men perform better on tests of visuospatial function, and this has demonstrated in younger and older adults (Herlitz, Nilsson, & Bäckman, 1997; Maitland, Intrieri, Schaie, & Willis, 2000). Also, women have been demonstrated to outperform men on measures of episodic memory, which is particularly relevant to cognitive aging (Herlitz et al., 1997; Nilsson, 2003). On the other hand, women are more likely to develop dementia (Deloitte Access Economics, 2011).

Despite these differences being reported in many studies, it is also true that cognitive differences are quite small (Hyde, 1981) and that cognitive similarities between the sexes far outweigh the differences (Hyde, 2005). Some authors dispute that gender differences exist at all when the data is rigorously analysed (Wallentin, 2009).

Further to this, gender differences in cognition may be a proxy for other variables, particularly in the elderly. For example, education may impact gender differences in cognition. In current cohorts of elderly people women are less educated than men, which would have bearing on their performance on education-sensitive tasks (Gerstorf, Herlitz, & Smith, 2006). One large study found that older women were less educated than older men and there was a mediating effect of education on women and men's performance on a number of cognitive tasks (Jorm, Anstey, Christensen, & Rodgers, 2004).

To complicate the issue further, cross-sectional analysis of performance in the elderly may be confounded by selective effects, in that men die younger so the surviving men may be those who are more cognitively intact. On the other hand, women are more likely to get dementia and prodromal stages of this may also be unwittingly observed in cross-sectional studies.

Even so, there are reasons to expect that patterns of cognitive aging should differ between women and men because factors that impact cognitive aging differ between the

sexes. For example, cardiovascular disease and hormone changes differentially affect women and men (Compton, Van Amelsvoort, & Murphy, 2001; Y. Zhang et al., 2012).

2.2.3 Other factors

It is outside the scope of this chapter to explore all influences in cognitive aging. Additional factors reported by Medical Research Council Cognitive Function and Ageing Study in the UK included perceived poor health, history of stroke, exposure to general anaesthetic and Parkinsons disease (Yip, Brayne, & Matthews, 2006). Others considerations include level of social activity (Glei et al., 2005), exercise (Heyn, Abreu, & Ottenbacher, 2004), genetic factors (S. E. Harris & Deary, 2011), and mental health (Bierman, Comijs, Jonker, & Beekman, 2005). It is also important to acknowledge the many interrelationships between these variables, which increase the complexity of research into cognitive aging.

2.3 Methodological issues in cognitive testing

A range of methodological considerations apply to cognitive aging, including the physical limitations of the elderly, as well as study design issues.

2.3.1 Physical limitations

Cognitive test performance may be impeded by physical limitations that occur in the elderly, for example, visual integrity or motor performance. Van Boxtel et al (2001) demonstrated that on Stroop sub-tasks, various visual debilities reduced the speed of response: low contrast sensitivity was associated with word naming; red/green colour weakness with colour naming; and poorer distant acuity with the interference task (Van Boxtel, Ten Tusscher, Metsemakers, Willems, & Jolles, 2001). Similarly, simulated visual impairment by reduction of contrast sensitivity significantly slowed performance in young people (Wood et al., 2009). Furthermore, a relationship between visual

function and processing speed was observed in a cross-sectional study of older adults (Clay et al., 2009).

Age-related slowing of motor responses can also impact cognitive task performance. Two ERP studies using choice reaction time tasks indicated that motor responses are slowed in older adults and that slower response times were not an indication of slower stimulus processing or response selection, but rather movement generation. Only those ERP components relating to movement were prolonged in the elderly (Falkenstein, Yordanova, & Kolev, 2006; Yordanova, Kolev, Hohnsbein, & Falkenstein, 2004).

2.3.2 Cross-sectional versus longitudinal designs

Cross-sectional and longitudinal study designs each have their advantages and disadvantages. Cross-sectional data is subject to cohort effects. Educational attainment is an obvious example, since older adults are generally less educated than younger adults (Anstey & Christensen, 2000) but other less obvious or unknown cohort effects may also influence the data interpretation. Longitudinal data which assesses the same individuals over time avoids this hazard but may be influenced by practice effects, which can inflate scores over time and attenuate the effects of decline on task performance (Salthouse, 2010). Longitudinal data may also be confounded by survival rates, whereby the poorer performers over time are lost to dementia or death (Bäckman et al., 2004). Similarly, poorer performers are less likely to complete a study due to poor health. This was illustrated in a study using participants from the Rotterdam Study and the Leiden 85-plus study. It was found that cross-sectional variation in cognitive performance was greater when using the whole sample rather than the subgroup that later had follow up data recorded (Euser, Schram, Hofman, Westendorp, & Breteler, 2008).

2.3.3 Computerized testing

While traditional tests such as those found in the WAIS have the advantage of well-established norms, they may not have the precision required to measure small changes in cognition that occur longitudinally or to detect changes in cognitive function due to a drug or nutritional intervention. Computerized testing has the advantage of millisecond timing, which may be particularly useful for detecting small changes. Other advantages include consistency in test presentation and accurate scoring (Schmitt, Benton, & Kallus, 2005).

On the other hand, young adults may be more familiar and comfortable with the use of computers than past and current cohorts of elderly individuals, which may facilitate their performance, potentially exacerbating age-related differences in performance.

2.3.4 Task difficulty

It may be argued that the sensitivity of a task to cognitive aging is due to the level of difficulty of the task, rather than an indication of domain-specific cognitive decline. For example in the Kungsholmen Project it was observed that age-related declines in fluid abilities were attenuated in easier tasks using familiar stimuli (Bäckman et al., 2004). The more complex a task, the more likely it will require multiple cognitive functions, thereby increasing opportunities for cognitive impairment to affect performance (Drag & Bieliauskas, 2010). It has been demonstrated that increasing cognitive load increases the time it takes to complete a task, as well as the number of errors (Rypma, Berger, & D'Esposito, 2002). Thus, response time and accuracy are indicators of task difficulty.

The studies in this thesis have used a computerized cognitive battery that has been previously shown to be sensitive to age-related decline (Pipingas et al., 2010). In this battery the easiest tasks were the Simple and Choice Reaction Time tasks – these had the fastest response times and showed the least change with age compared with the more challenging tasks. While this supports a role for task difficulty in eliciting cognitive impairment, for the more complex tasks this argument did not hold.

Immediate and Delayed Recognition had the slowest mean response times and poorest accuracy, indicating greatest difficulty. These tasks did not demonstrate the greatest change with age however, which occurred in the Spatial Working Memory and Contextual Memory tasks (Pipingas et al., 2010). This suggests that while task difficulty may play a role in detecting cognitive impairment, choosing domain-specific tasks is also important.

2.4 Summary and conclusion

All people suffer from some degree of cognitive deterioration in later years. Although cognitive aging means an increase in some abilities such as general knowledge and vocabulary, of greater concern are the abilities that decline. Cognitive deficiencies become apparent in various domains. Processing speed is particularly susceptible to decline and may underlie impairment in other abilities, although it is unlikely to be the sole cause of deterioration. Episodic memory is also affected, especially in dementia. Executive functions deteriorate and more recently, spatial abilities have gained attention in the aging literature. Given the significant overlap between domains, it is difficult to isolate any specific function, however cognitive testing of the elderly should include tasks that have high dependency on these susceptible abilities.

There are particular brain regions implicated in cognitive aging and these substantiate the findings from the cognitive literature. The hippocampus, important for episodic memory and spatial abilities, and the prefrontal cortex, involved with executive functions, are particularly affected. Evidence of cerebrovascular disease and general brain attrition is also observed in aging and is related to poorer cognitive function.

Despite the considerable knowledge surrounding cognitive aging, further research is required before it is fully understood. Investigation of the factors that influence the course of decline and interventions which may delay or prevent it is imperative if we are to support the increasing number of people suffering decline and dementia in our aging population.

3 The Cardiovascular System & Cognition

This chapter provides an overview of the structure and function of the cardiovascular system and how it relates to cognitive function. The discussion takes place in the context of aging, where cognitive and cardiovascular systems suffer concurrent decrements with increasing age. Ischemic heart disease and cerebrovascular disease are the two leading causes of death in Australia (ABS, 2013), highlighting the value of research in this area. However it is also imperative to understand cardiovascular aging because of its substantial contribution to cognitive impairment in later life, the importance of which is being increasingly understood (Gorelick et al., 2011).

To begin, this chapter presents some background information on the arterial system and blood flow. This information is provided in order to explain the measures used in the present studies and to place in context the recent research on changes that occur with age. Following this is a discussion of blood pressure measurements. This includes peripheral blood pressure measures, which are the standard systolic and diastolic readings that are taken from the brachial artery in the upper arm. Their use in assessing cardiovascular risk in research and clinical settings are discussed. In addition to peripheral blood pressures, the studies in this thesis have assessed central blood pressure variables using the method of pulse wave analysis. These variables include augmentation index and augmentation pressure, both measures of arterial stiffness. They were chosen because they provide important information on the health of the cardiovascular system, above that provided by brachial blood pressure measures. The methodology behind these variables is explained and the suitability of the technique is evaluated.

A description of the age-related changes that occur in the cardiovascular system follows, with particular focus on arteriosclerosis, or arterial stiffening. This is a major age-related change that has important implications for cardiovascular health in the elderly and also plays a role in cognitive decline. The advantages of central pressure measurements in cardiovascular aging research are also discussed.

The direct effects of cardiovascular changes on cognition are examined next, focusing on brain changes with cardiovascular causes including vascular cognitive impairment, dementia and cerebral small vessel disease. A discussion of the important role of cardiovascular impairment in cognitive decline is presented. This role is further emphasized by the evaluation of the many cardiovascular risk factors that are also implicated in cognitive decline. These include hypertension and arterial stiffness; however there has been little use of central blood pressure measures in cognitive aging research. Markers of inflammation and cholesterol are two important blood biomarkers for cardiovascular health and these are also associated with cognition; these are briefly addressed.

The research relating to the cardiovascular system is vast and complex, and there are many topics which are outside the scope of this chapter. For example, glucose, obesity, and metabolic syndrome all play important roles in cardiovascular health (Romeo, Lee, & Shoelson, 2012). They may also affect cognitive function, possibly via improvement in endothelial and cerebrovascular function (Sinn & Howe, 2008). Molecular and genetic factors, which are also important in cardiovascular aging (Lakatta & Sollott, 2002), are also excluded from the discussion here.

The objective of this chapter is to develop a comprehensive understanding of the cardiovascular system and how arterial aging and injury influence cognitive function. In addition, this chapter aims to illustrate the utility of central pressure measurements in assessing cardiovascular health in aging.

3.1 Arterial system and blood flow

This section gives a brief outline of blood flow in the arteries of the systemic circulation, which is necessary in understanding the impact of arterial changes in the aging individual as well as the methods used in the current studies.

3.1.1 Arterial blood flow and the cardiac cycle

Blood flow in the arterial system begins in the left ventricle of the heart which squeezes blood into the aorta, then the arteries, arterioles and lastly to the capillaries in the target organs where exchange of molecules and gases occurs. During ventricular systole, the ventricle contracts and there is an initial rapid ejection of blood into the aorta causing a sharp rise in ventricular and aortic pressures. The aorta stretches in response and the blood flow into it decelerates as pressure increases. At the end of systole the aortic valve closes and ventricular pressure drops abruptly before the mitral valve (between the left atria and left ventricle) opens. As the mitral valve opens, blood from the left atria fills the left ventricle. This period is diastole (M. N. Levy & Pappano, 2007).

As will be discussed later in the chapter, the fact that blood flow is pulsatile is central to cardiovascular aging. It is pulsatile because ventricular ejection is intermittent. However, the structure and function of the arterial tree enables the pulses to be transformed into a steady flow, providing a consistent blood supply to the peripheral organs (Nichols, O'Rourke, & McDonald, 2011).

3.1.2 Structure and function of the arterial tree

Anatomical differences in the various part of the arterial tree ensure each part is suited to the task it performs. The composition of the blood vessel wall varies depending on its location and function (M. N. Levy & Pappano, 2007). In general, the arterial wall is made up of several layers. The innermost layer is the endothelium, a single layer of cells attached to the basal lamina, which together make up the intima. This is surrounded by the internal elastic lamina, a large layer of elastic fibers. The middle layer is the media which comprises connective tissue and smooth muscle cells arranged circumferentially. The adventitia is the outermost layer and is mostly connective tissue, collagen and elastin, but may also have some smooth muscle cells and nerves. The adventitia provides strength and structure to the artery. Most arteries and arterioles are arranged with these layers, however the capillaries, where gas exchange, molecular transport and

diffusion take place, are mostly composed of endothelial cells and basement membrane (M. N. Levy & Pappano, 2007).

There are three distinct regions in the arterial tree: the large arteries; the conduit arteries; and the arterioles. A constant flow of blood is maintained by changes in vascular resistance. The large, proximal arteries (e.g. the aorta and the carotid artery) are elastic – they contain elastin and collagen fibers which allow the vessel wall to stretch to store the increased volume of blood that is received during systole. This also provides a cushioning effect, damping the increase in pressure. During diastole there is passive recoil of the aorta and the arterial walls return to shape, expelling the blood into the conduit arteries. Conduit arteries are the long, muscular arteries that distribute blood to the periphery. They also assist in smoothing the blood flow. Muscle tone in the smooth muscles changes, altering the caliber of the artery and thereby varying the blood flow and arterial pressure. (M. N. Levy & Pappano, 2007).

Distally, the microcirculation is also able to alter resistance by changing caliber according to the requirements of nearby organs and tissues. The microcirculation comprises the resistance vessels –small arteries, arterioles and capillaries – which are so named because they provide the greatest resistance to blood flow. Contraction and relaxation of the smooth muscle fibers here leads to constriction and dilation of the artery. This occurs in response to arterial perfusion so that blood is supplied to match the metabolic requirements of the tissue. The mechanics of these arterial regions ensure that the necessary blood is provided to target organs in a steady flow, and the intermittent nature of the heart-pumped blood is converted to a smooth flow by the time it reaches the capillaries. While smooth flow does occur for most organs and tissues, for those where there is high blood flow increased arterial caliber means that pressure pulsations may reach further along the arterial tree. In particular, the brain and kidneys experience high blood flow so the blood vessels leading there are more dilated and pulsations are transmitted more strongly, and this has potential to cause damage to these organs (O'Rourke & Hashimoto, 2008).

3.1.3 Endothelium and atherosclerosis

The endothelium is of particular interest because it serves as the interface between the blood and the underlying cells. The endothelium plays a part in many important functions including molecular transport, regulation of coagulation and thrombosis, maintenance and modulation of vasomotor tone, vessel wall growth and remodeling, as well as inflammation and immune functions (Thiriet, 2008). The endothelium is in direct contact with the circulating blood and is therefore exposed to pulsatile flow and any circulating molecules (Creager, Loscalzo, & Dzau, 2006).

Certain characteristics of blood pressure and blood flow affect the endothelium, including shear stress and pressures which cause tensions within the arterial wall. These forces on the endothelium contribute to the generation of basal tone in smooth muscle cells, and thus regulate the caliber of the artery through contraction and relaxation of the vascular smooth muscle cells (M. N. Levy & Pappano, 2007). Nitric oxide (NO) is an important mediator of these functions, acting as a vasodilator. Availability of NO can affect arterial compliance; decreased NO can lead to functional arterial stiffness and subsequent increased blood pressure (Feletou, 2011).

Atherosclerosis is a progressive disease which involves the buildup of fatty plaques in the arteries. The process begins when shear forces cause a local endothelial injury, which increases the adhesiveness and permeability of the endothelium. Monocytes and lipids accumulate at the site of the damage, leading to formation of plaques and thickening of the intima and media. This causes narrowing and stiffening of the artery, increasing blood pressure. There is also risk of plaque rupture and subsequent blocking of the coronary arteries due to thrombus formation, which may result in myocardial infarction and sudden death. Stroke may also occur due to blockage of the cerebral arteries (Santos & Nasir, 2009).

Atherosclerosis is not a consequence of the normal aging process but a progressive pathological condition. However, since it develops gradually over many years, age is a risk factor for its associated complications (Lakatta & Levy, 2003).

3.2 Brachial blood pressure measures

Blood pressure customarily refers to the brachial systolic and diastolic blood pressures. Systolic pressure is the maximum arterial pressure that occurs during contraction of the left ventricle, or systole; diastolic pressure is the lower arterial pressure that occurs during diastole, between contractions of the left ventricle. The two are usually measured by a cuff sphygmomanometer over the brachial artery and are therefore measures of pressure in the peripheral arterial tree. It was initially observed within the Framingham Heart Study that increased systolic and diastolic pressures are important risk factors for cardiovascular events. According to the Framingham model, hypertension is a reading of 140mmHg or higher during systole or 90mmHg or higher during diastole (Chobanian et al., 2003). The ease of measurement and significant prognostic value of these variables has led to widespread uptake of the technique in the clinical setting.

According to the Framingham model, **systolic blood pressure**, whether treated or untreated, is the major predictor of general cardiovascular disease in women and in men is second in importance only to age (D'Agostino Sr et al., 2008). It is also a biomarker of arteriosclerosis (O'Rourke & Hashimoto, 2007). Reductions in systolic blood pressure lead to improved clinical outcomes, including for stroke (Probstfield, 1991). Systolic pressure increases linearly in normal aging (Franklin et al., 1997).

Diastolic blood pressure follows a different trajectory, increasing until about age 60 and then declining after (Franklin et al., 1997). Increased diastolic blood pressure is now acknowledged to be less important in coronary heart disease risk prediction, and may only be relevant in young adults where it increases in parallel with systolic blood pressure. In older adults, diastolic blood pressure has an inverse relationship with coronary heart disease, that is, low diastolic pressure is associated with greater risk of disease. For example in individuals with systolic blood pressures over 120mmHg, coronary heart disease risk increased with decreased diastolic blood pressure (Franklin, 1999). A comparable observation was made in another study which observed survival rates in hypertensive individuals over 80 years of age (Oates, Berlowitz, Glickman, Silliman, & Borzecki, 2007). They reported that higher blood pressure in the

"controlled" range (that is, up to 139 mmHg systolic and 89mmHg diastolic) was associated with greater likelihood of survival during five years follow up.

Pulse pressure is another common blood pressure variable that is used more commonly in research. It is the difference between systolic and diastolic pressures, indicating how much the arterial pressure is increased as the heart beats. It varies according to the volume of blood that is ejected during systole and importantly the compliance of the arteries, with pulse pressure increasing as arteries stiffen (Nichols et al., 2011). As such it is sometimes used as a proxy for arterial stiffening (Mourad, Blacher, Blin, & Warzocha, 2001). Pulse pressure was slightly superior to systolic and diastolic pressures when predicting coronary heart disease risk in the Framingham study (Franklin et al, 1999). Pulse pressure also increases with normal aging. For example, in a large sample of French adults aged 18-103, it was noted that there was an increase in isolated systolic hypertension across adulthood. In subjects over 65 years, the age-related increase in systolic pressure was associated with a concomitant decrease in diastolic pressure, leading to an increased pulse pressure. The authors attributed this to accelerating stiffening of arteries in older adults (Mourad et al., 2001).

One further measure of peripheral arterial pressure is the **mean arterial pressure**, which is the average pressure in the artery over time. The more accurate method of calculating mean arterial pressure is by tracing the arterial pressure wave and measuring the area under the curve, divided by the time interval. However it is frequently estimated using only the systolic and diastolic pressures, using the formula of one third of the pulse pressure plus diastolic pressure (MP = DP + 1/3 (SP-DP). The mean pressure depends mainly on arterial blood volume and arterial compliance. In a large trial of male physicians aged 40-84 years mean arterial pressure (along with systolic and diastolic pressure) was predictive of cardiovascular risk (Sesso et al., 2000). Although it has significant predictive capacity, mean arterial pressure is less frequently used in clinic and research (Safar & Smulyan, 2006).

Despite the utility of hypertension as a marker for cardiovascular disease, O'Rourke and Hashimoto (2007) point out that 'hypertension' is a level of blood pressure that has been set arbitrarily. In some older people, hypertension is not due to disease but is

simply a corollary of aging (O'Rourke & Hashimoto, 2007). In fact most people have reached hypertensive blood pressure levels by the age of 90, and it is estimated that there is a lifetime risk of 90% even for individuals who are normotensive at age 55 (Chobanian, 2009). Furthermore it can be difficult to untangle the effects of normal aging from cardiovascular pathology due to similarity of presentation. Other authors argue that the two are interrelated, that age-related changes provide an optimal environment for the progression of vascular disease. They argue that "unsuccessful vascular aging" promotes cardiovascular disease (Lakatta & Levy, 2003). The mechanisms of cardiovascular aging and cardiovascular pathology are discussed later in this chapter.

3.3 Central blood pressure measurement

Arterial stiffness is a key feature of arterial aging and may present early in the progression of cardiovascular disease or be an indicator of premature vascular aging. Non-invasive methods of observing vascular changes may allow for earlier or more targeted interventions and improve outcomes (A. M. Wilson, 2006). Changes due to arterial stiffness can be observed by recording the pulse pressure waveform.

3.3.1 The pulse pressure wave

When blood is ejected from the left ventricle, a pressure wave is propagated down the aorta and along the arterial tree. The speed of this pressure wave varies depending on arterial compliance, that is, the wave will travel faster in a stiffer artery as there is reduced "cushioning" to dampen its travel. The pulse pressure wave is distinct from the flow of blood, which does not move as fast (M. N. Levy & Pappano, 2007). Measuring the speed of the pulse pressure wave gives an indication of the compliance of the arteries. Resistance increases in peripheral vessels, consequently the pulse wave increases in velocity as it moves outward. It also changes shape (Nichols et al., 2011).

The pulse pressure waveform can be measured noninvasively. Using the technique of applanation tonometry, a highly sensitive micromanometer (tonometer) is placed on the skin over the maximal arterial pulsation at the site of a peripheral artery, such as the carotid or radial artery. The artery is flattened against the underlying bone and a high-fidelity sensor records the underlying pressure (Nichols et al., 2011). The waveform has been shown to be highly accurate when compared to invasively-recorded pressure waveforms (Kelly, Hayward, Avolio, & O'Rourke, 1989).

By applying a generalized transfer function to recordings made at peripheral sites, blood pressure levels in the aorta can be calculated. This method was validated in patients who were undergoing cardiac catheterization, where the central pressures could be measured more directly. Pulse pressure waveforms recorded were from the carotid or radial arteries. These were used to estimate the central (aortic) waveforms and a transfer function was applied. The resulting estimate of the central pressure waveform was compared to those measured directly and was found to be a good approximation of the true measurements (Söderström, Nyberg, O'Rourke, Sellgren, & Pontén, 2002).

The central pulse pressure waveform has a distinctive shape comprising an initial systolic peak and a second peak which represents the reflected wave (Figure 3.1). This second peak represents the summation of many centrally-travelling waves that have been reflected at points in the arterial tree where there has been a change in impedance, for example, changes in diameter or stiffness of the artery, or where an artery branches. Reflected waves occur in healthy, young adults but they may be altered by factors related to aging, including vascular stiffening and remodeling, and changes in vasomotor tone due to endothelium changes (Safar, Levy, & Struijker-Boudier, 2003).

The timing of wave reflections is important and optimal timing occurs when the reflected wave returns to the aorta during diastole. In healthy, young adults the wave reflection is timed so that the reflected pressure wave arrives in the aorta as the aortic valve shuts. This maintains pressure and facilitates perfusion of the coronary vessels. When arteries are stiffer, both the outgoing and reflected waves travel faster and the reflected wave may return to the aorta while still in systole. This elevates aortic systolic pressure, increasing the workload of the heart which needs to pump harder to push the

blood out against the returning pressure wave (O'Rourke, 2007). A further complication is that diastolic pressure is subsequently reduced, which may impair perfusion of the coronary arteries. Importantly, this resultant increase in central pulse pressure would not be apparent by observation of peripheral pulse pressure (Mitchell, 2008).

3.3.2 Central pressure measures

Where systolic and diastolic blood pressure measures provide minimum and maximum values of peripheral pressure, pulse wave analysis can provide additional information about the condition of the cardiovascular system which may be helpful. The studies in this thesis employed the technique of applanation tonometry to record the pulse pressure waveform at the radial artery, then applied a generalized transfer function to calculate central pulse pressure waveform (SphygmoCor System, AtCor Medical Pty Ltd, Australia). This technique provides a number of potentially useful variables including central (aortic) systolic pressure, central diastolic pressure, augmentation pressure and the augmentation index. A consensus document by leading researchers in the field asserts that accumulating research demonstrates the utility of these central pressure measures as an additional tool in cardiovascular risk management (Agabiti-Rosei et al., 2007). Another consensus document states that pulse wave analysis has demonstrated "independent predictive value" for cardiovascular events in people with hypertension or coronary disease (Laurent et al., 2006).

Augmentation pressure is the portion of central systolic pressure that is attributable to wave reflection. In healthy young adults, peak aortic pressure occurs with peak flow during systole. When reflected waves return early the systolic pressure is increased; the amount of the increase is referred to as augmentation pressure, and is measured in mmHg. This occurs in older adults and can be seen in Figure 3.2 where the peak flow appears as a shoulder in the upward curve of the pressure wave. The peak of the waveform is the summation of outgoing and incoming pressure waves, so subtracting peak flow pressure from total pressure gives augmentation pressure (Nichols et al., 2011).

Augmentation index is a related measure; it tells the degree to which the peak of the systolic pressure wave in the aorta is augmented due to the early return of the reflected wave by comparing the augmentation pressure to the peak flow pressure. When reflected waves return early (i.e. during systole) the pressure in the aorta is increased due to the additive effect of the outgoing and returning pressure waves. Because reflected waves travel faster in stiffer arteries, the augmentation index is increased where arterial stiffening is present (Nichols et al., 2011). Augmentation index is used as a measure of arterial stiffness although some have refuted this definition (Cameron, 2007). Despite early reservations, the methodology of the augmentation index and other central measures are now recognized by the scientific community. Reproducibility and repeatability of the technique are sound, at a level similar to other blood pressure and heart rate measures, and the transfer function is now well accepted (Laurent et al., 2006; O'Rourke & Hashimoto, 2008).

As a measure of arterial stiffness, augmentation index is an alternative to **pulse wave velocity (PWV)**, which is considered the "gold standard" of arterial stiffness measurement (Laurent et al., 2006). PWV measures the speed at which the pulse waves travel through the arteries (Hirata, Kawakami, & O'Rourke, 2006). It is measured by recording the pulse at two points in the arterial tree, for example, the carotid and femoral arteries, and is defined as the time it takes divided by the distance travelled. Because the speed at which the pulse pressure wave travels increases with increased arterial stiffness, it is a good indicator of this measure (Hirata et al., 2006).

3.3.3 Central pressure measures and cardiovascular health

A number of associations have been observed between cardiovascular health and central pressure measures using pulse wave analysis. It has been demonstrated that older adults with established hypertension not only have higher brachial blood pressure, but also higher central augmentation pressure and central augmentation index, when compared with healthy controls (Izzo Jr & Shykoff, 2001). Studies have found that central measures are associated with cardiovascular risk, including augmentation index, central systolic pressure and pulse pressure (van Trijp et al., 2006; Williams et al., 2006). A

further study demonstrated that central pulse pressure was more strongly related to cardiovascular events than brachial pulse pressure in adults aged 18-88 years (M. J. Roman et al., 2007) indicating that for some groups central pressure measurements may be better indicators for risk assessment.

A meta-analysis of longitudinal studies investigated the capability of central pressure measures to predict cardiovascular events and all-cause mortality (Vlachopoulos et al., 2010). Findings indicated that for cardiovascular events, a 10mmHg increase in central systolic pressure increased the risk by 8.8%; a 10mmHg increase in central pulse pressure increased risk by 13.7%; and for a 10% increase in augmentation index the risk increased by 31.8%. Furthermore, a 10% increase in augmentation index increased the risk of all-cause mortality risk by 38.4%. This analysis combined studies of predominantly patient groups including those with cardiovascular and renal problems so the data cannot be extended to normal populations. However it shows the potential of central blood pressure variables in health assessment and risk prediction.

Although not directly applicable to healthy populations, examination of patient groups is informative. One notable example that was included in the meta-analysis was a study which demonstrated that augmentation index was independently associated with mortality in end stage renal patients (London et al., 2001). In the study, a 10% increase in augmentation index was associated with a risk ratio of 1.51 for all-cause mortality and 1.48 for cardiovascular mortality. This patient group is of interest because of the susceptibility of the kidneys to increased pressure pulsations in hypertension; the brain is similarly susceptible to increased pulsations due to greater blood requirement (Decarli, 2012; O'Rourke & Safar, 2005).

Observation of central pressure measures may be useful in a clinical setting when prescribing antihypertensive medication. Different types of medication have different mechanisms of action which may result in a similar reduction in brachial systolic blood pressure but differential effects on central pressure measurements. In the Anglo-Scandinavian Cardiac Outcomes Trial (ASCOT) trial (Dahlot et al 2005) more than 19,000 participants aged 40-79 years with high cardiovascular risk were randomized to one of two blood-pressure-lowering medication regimens. The two arms of the study

recorded different clinical outcomes despite similar effects on brachial blood pressures. Further investigation was conducted in the (Conduit Artery Function Evaluation (CAFE) study, a substudy of ASCOT (Williams et al., 2006). Researchers observed that calcium channel blockers were associated with lower central systolic pressure compared with β-blockers. The authors of this study suggested that this explains the different clinical outcomes that were observed in ASCOT. This indicates that assessing central blood pressure measures could improve clinical outcomes by a more targeted choice of therapies.

3.4 Age-related changes in the arterial system

A number of structural and functional changes occur within the arterial system with age. Arteries become stiffer in the same manner as in hypertension. This is partly due to structural changes in the arterial wall and partly due to cellular factors (Zieman, Melenovsky, & Kass, 2005). Because of the pulsatile nature of blood flow, repetitive strain occurs in the elastin fibers of the arterial wall, causing them to eventually fatigue and fracture. With the loss of the elastin fibers the artery dilates and the pressure load is transferred to collagen (O'Rourke & Hashimoto, 2007). Whereas elastin can stretch up to 60%, collagen fibers are stiffer and normally provide structural integrity to the arterial wall (Thiriet, 2008). Consequently the artery becomes stiffer and more dilated. Because the proximal arteries are the most distensible they stretch more with each pulse than do the more distal arteries, so the effects of repetitive strain are greatest. This point is illustrated by the observation of a greater age-related increase in pulse wave velocity in the aorta compared with the brachial artery (McEniery et al., 2005). High blood pressure can magnify the effects of age on the arteries. When blood pressure is high, the pulse pressure is increased and greater stretch occurs in the central arteries. This leads to additional fatigue of elastin and earlier arterial stiffening (O'Rourke & Hashimoto, 2008).

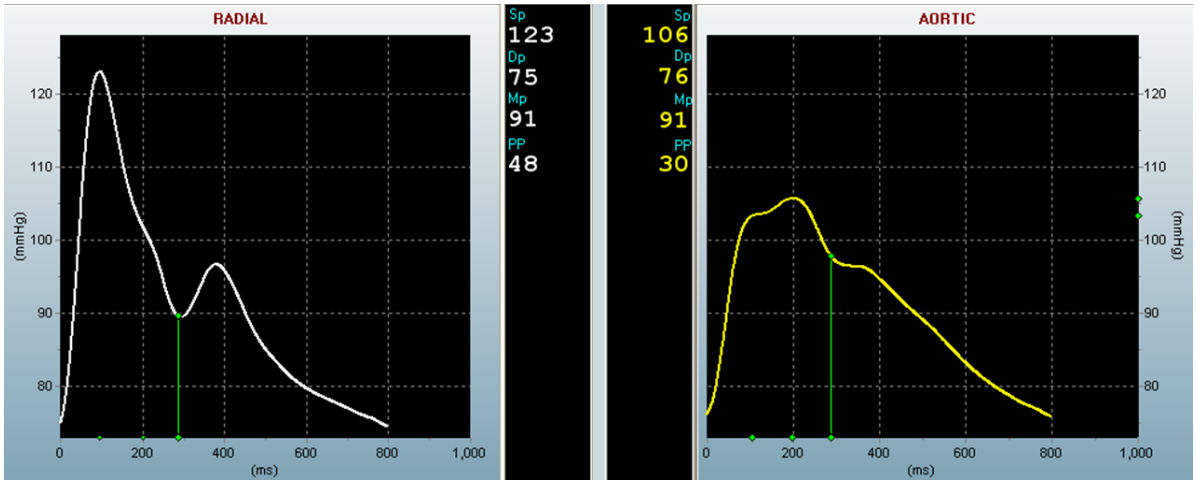

Figure 3.1. Radial and aortic pulse pressure waveforms of a 24 year old female. Augmentation index = 109%, augmentation pressure = 2 mmHg. The radial waveform shows an initial systolic peak and a second peak representing the reflected wave. In the aortic waveform, the systolic peak appears as a shoulder in the upward curve of the pressure wave, and the second peak represents the additive effect of the reflected wave on the systolic peak.

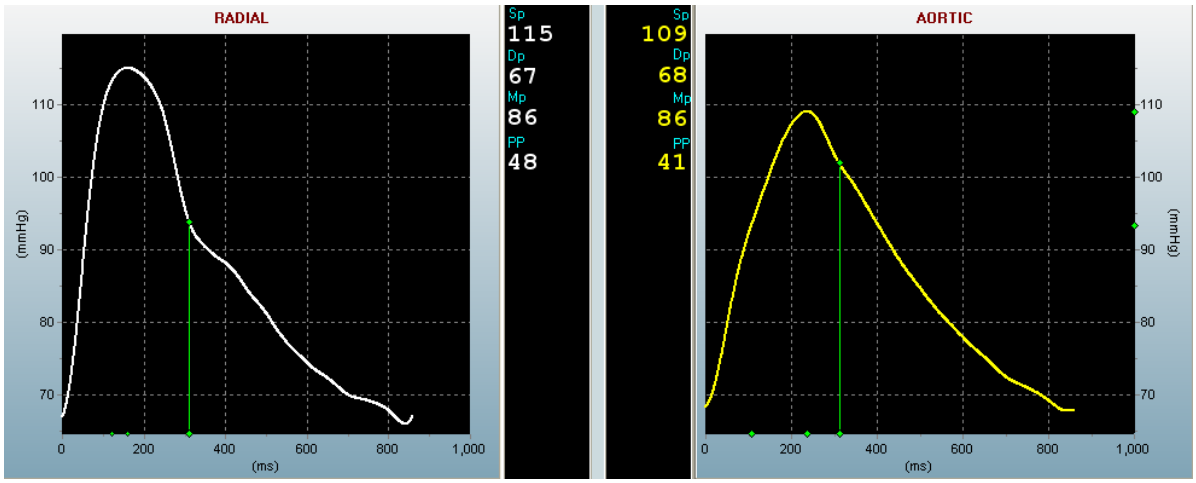

Figure 3.2. Radial and aortic pulse pressure waveforms of a 63 year old female. Augmentation index = 39%, augmentation pressure = 16 mmHg. Despite normal systolic and diastolic blood pressures, this participant has very high augmentation index, indicating increased arterial stiffness. The radial waveform shows little separation of systolic peak and reflected wave. The single peak appearing in the aortic waveform indicates that the reflected wave has arrived in the aorta during systole.

With age, endothelial function gradually deteriorates. This may be due to many factors that occur throughout the lifetime including genetic factors, lifestyle (e.g. diet, smoking and physical activity), disease, and the aging process itself (Gates, Strain, & Shore, 2009). This decline in function is associated with structural changes such as thickening of the endothelial layer, hyperplasia of endothelial cells, and hypertrophy of the basement membrane. Functionally, reduction in NO-mediated vasodilation may occur due to decreased synthesis of NO or faster breakdown of NO by superoxide anions. The decrease in NO production with age is due to a reduction in endothelium-derived nitric oxide synthase (eNOS) activity. This affects NO-dependent (endothelial-dependent) vasodilation that normally occurs in response to physical stress (Maruyama, 2012). With atherosclerosis (which can also increase with age) these problems may be exacerbated further (Nichols et al., 2011).

The consequences of arterial stiffness are potentially dire. As the proximal aorta stiffens there is an increase in blood pressure which becomes further increased as a result of early wave reflection (see section 3.3.1). High systolic blood pressure leads to an increase in left ventricular oxygen requirements, left ventricular hypertrophy, and eventually left ventricular failure (Mitchell et al., 2010). High systolic blood pressure increases stress on the arteries due to higher pulse pressure, and predisposes the individual to atherosclerosis, plaque rupture and arterial occlusion. Weakened vessels may rupture and hemorrhage. Sensitive organs such as the brain and kidney may be damaged because pulsations are not cushioned and the impedance mismatch between the aorta and conduit arteries which normally causes wave reflections is reduced, leading to greater transference of pulsatile energy into the microvasculature and susceptible organs (Mitchell, 2008). Because of this it is argued that the brain should be considered an end-organ in vascular disease, due to the impact that pulsations can have in cognitive impairment (Decarli, 2012).

Early wave reflection also leads to a steeper fall in aortic blood pressure during diastole which can lead to decreased coronary perfusion and myocardial ischemia (O'Rourke & Hashimoto, 2008). In addition to this, there is a slower contraction and relaxation of the heart which increases the duration of systole, further increasing late systolic blood pressure. Because of the increased load, coronary blood flow requirements are increased

and simultaneously less able to be met because of the decreased diastolic blood pressure and duration of diastole. This may result in ischemia (O'Rourke & Hashimoto, 2007).

Given the serious consequences of arterial stiffness, it is of great importance to consider how to counteract such effects. O'Rourke and Hashimoto (2008) discuss whether arterial stiffness is a modifiable risk factor. They conclude that age-related large artery stiffness is not modifiable because the breakdown of elastin is irreversible. However they suggest that smaller muscular arteries may be a target for intervention. Contraction of the smooth muscles narrows the artery making it more stiff, which increases blood pressure. And the converse is true; relaxation of the smooth muscle dilates the artery and reduces blood pressure. Consequently, interventions that affect smooth muscle tone in the arterial wall, which support NO production and promote endothelium-dependent vasodilation, might be relevant here. This is pertinent to the multivitamin studies in this thesis because it is a possible means by which the nutrients may exert an effect. For example, antioxidant vitamins and ginkgo enhance NO production (Farbstein, Kozak-Blickstein, & Levy, 2010; Koltermann et al., 2007).

3.4.1 Central blood pressure measures in aging

Pulse wave analysis is particularly relevant to cardiovascular aging. Arterial stiffness increases with age and this is reflected in marked changes in the pulse pressure wave contour. Age-related changes may be observed in pressure wave variables that are absent or underestimated when relying on brachial cuff measurements (O'Rourke, 2007). These central measures may better reveal the effects of age-related arteriosclerosis.

The most comprehensive study of age-related changes in pulse wave variables included 4001 healthy, normotensive participants and examined the effects of age on a range of cardiovascular variables (McEniery et al., 2005). They observed that peripheral systolic blood pressure increased across the adult lifespan whereas diastolic pressure increased until age 50 and then decreased. This led to an observed increase in the peripheral pulse pressure after 50 years of age. Central systolic blood pressure also increased after age

50 however this occurred to a greater extent than it did in the periphery, indicating that peripheral measures may underestimate the pressure changes that occur with age.

The study also observed that augmentation index and augmentation pressure both increased with age, however augmentation index followed a nonlinear trajectory: the relationship with age decreased after 50 years (McEniery et al., 2005). These findings concur with those of another study that also observed that augmentation index increased in adults up until age 55, with augmentation pressure continuing to increase linearly after age 55 (Fantin et al., 2007). Authors suggested that augmentation pressure would be a better indicator of arterial stiffness in the elderly than augmentation index.

The trajectory can be accounted for by the relative contributions of incident and reflected waves to the central systolic blood pressure. This was examined in normotensive participants across the whole adult lifespan (Namasivayam, McDonnell, McEniery, & O'Rourke, 2009). Using 60 years as an age cut-off point, investigators determined that in younger adults, wave reflection was the greater contributor to elevations in aortic systolic blood pressure than the incident wave. After age 60, *both* incident and reflected waves contributed comparably to elevated systolic pressure in the aorta. This was true for both women and men. They explain that increased aortic impedance (stiffening) increases the magnitude of the incident pressure wave, which in older adults contributes to increased aortic systolic blood pressure. This also explains why the augmentation index increases with age only until about age 60. From around 60 years, the incident and reflected waves both contribute, and because augmentation index is a ratio (of augmented pulse pressure to total pulse pressure) the augmentation index is stabilized because both the numerator and denominator are concurrently increasing.

The principle is illustrated further in the comparison of younger and older adults by McEniery et al. (2005). Here, the relationships between age (across a 10-year age bracket) and peripheral pulse pressure, central pulse pressure, and aortic pulse wave velocity were stronger for older adults. On the other hand the relationship between age and augmentation index was stronger in young adults. Augmentation pressure and brachial pulse wave velocity were similar in both groups (McEniery et al., 2005).

The finding that augmentation index does not continue to increase in the elderly is important when considering the use of central pressure measurements in research, as it might be more appropriate to use augmentation pressure as a measure of arterial stiffness.

3.5 Cardiovascular function and cognition in aging

The health of the cardiovascular system is crucial to the proper functioning of the brain and cognition. Cardiovascular problems can impact cognition in various ways and cardiovascular risk factors and markers have demonstrated relationships with cognition and brain pathology, particularly in the aging brain. This section investigates cognitive impairment that is due to cardiovascular factors, including vascular cognitive impairment and vascular dementia. The contribution of the cardiovascular risk factors to cognitive decline is also discussed, in particular hypertension and arterial stiffness. Finally, shared risk factors for cardiovascular disease and cognitive decline are addressed, including inflammation and cholesterol and their blood biomarkers. Oxidative stress and homocysteine are considered in the following chapter.

3.5.1 Vascular cognitive impairment

Any cognitive impairment that has vascular etiology is covered by the general term of vascular cognitive impairment. This includes vascular dementia and cognitive impairment due to stroke (O'Brien, 2006). Other cognitive disorders may also be exacerbated by vascular pathology, including Alzheimer's dementia (Gorelick et al., 2011). Various vascular conditions can also impact cognition. These include hypertension, atherosclerosis, transient ischemic attacks, infarcts and silent infarcts, and cerebral small vessel disease (Erkinjuntti & Gauthier, 2009).

Stroke due to large vessel disease can lead to cognitive impairment, with a sudden and severe loss of function or even death. Ischemic stroke is due to cerebral infarction, a blockage of the blood supply usually caused by a blood clot. Brain hemorrhage is

another form of stroke, caused by leaking or bursting of blood vessels (Lindley, 2008). The cardiovascular etiology of stroke is clear and reduction of cardiovascular risk factors is also important for reducing the risk of stroke (M. F. Elias et al., 2004).

Cerebral small vessel disease is suggested to be the main cause of brain damage in vascular cognitive impairment (Pantoni, 2010). Presentation of cerebral small vessel disease varies. It may occur as arteriosclerosis with microatheroma (atherosclerosis) or microaneurysms; it may cause hemorrhage in cerebral arteries; or narrowing of the arterial lumen and consequent chronic ischemia may lead to white matter lesions and lacunar infarcts (Pantoni, 2010). White matter lesions, or leukoaraiosis, are associated with cognitive impairment in cross-sectional studies (Breteler et al., 1994) and longitudinally with cognitive decline (Arboix, 2011; Jokinen et al., 2011) and brain atrophy (Nitkunan, Lanfranconi, Charlton, Barrick, & Markus, 2011). Cerebral small vessel disease also confers a higher risk of disability and death (A. J. Zhang, Yu, & Wang, 2010). Importantly, the progressive nature of the disease recommends it as a possible target for therapeutic intervention, and because it is associated with cardiovascular disease, improvement of cardiovascular risk factors should be pursued (Pantoni, 2010; Richardson et al., 2012).

Vascular dementia includes several forms of dementia that are defined by ischemic or hemorrhagic cerebrovascular disease, or brain lesions with cardiovascular etiology. Memory impairment and other cognitive dysfunctions are a defining feature of the disease. With vascular dementia the brain shows evidence of cerebrovascular lesions which may or may not be temporally associated with the cognitive impairment; however the impairment often proceeds in a step-wise manner (G. C. Roman, 2003). Subcortical lesions are often present (Jellinger, 2008b). Heterogeneity of presentation has led to a lack of clear diagnostic criteria for vascular dementia, which are not clearly defined in the scientific literature, making comparisons between studies difficult (Jellinger, 2008a; Wiederkehr, Simard, Fortin, & Van Reekum, 2008).

Cardiovascular risk factors are important in the etiology of vascular dementia. A meta-analysis of longitudinal and prospective studies indicated that hypertension is a significant risk factor (Sharp, Aarsland, Day, Sønnesyn, & Ballard, 2011), however

hypotension may also contribute, via hypoperfusion of the brain leading to white matter lesions (Moretti et al., 2008). In a large study of dementia incidence and metabolic syndrome, the risk of vascular dementia was increased with metabolic syndrome, triglycerides and diabetes (Raffaitin et al., 2009). Similarly, there was an association between metabolic syndrome and vascular dementia in the Italian Longitudinal Study on Ageing, and this was mediated by inflammation (Solfrizzi et al., 2010). Smoking is also a risk factor (Rusanen, Kivipelto, Quesenberry Jr, Zhou, & Whitmer, 2011) whereas physical exercise is protective (Aarsland, Sardahaee, Anderssen, & Ballard, 2010).

3.5.2 Cardiovascular risk factors and cognitive decline

The diseases just discussed are frank examples of the relationship between cardiovascular dysfunction and cognitive impairment. However relationships between cardiovascular and cognitive functions can be observed in apparently healthy older adults and cognitive impairment may be observed in older adults with cardiovascular pathology but no dementia. Some examples from large-scale epidemiological studies illustrate this point.

The Framingham Heart Study has developed a general risk profile for cardiovascular disease (coronary heart disease, cerebrovascular events, peripheral artery disease and heart failure), which estimates ten-year risk based on age, diabetes, smoking, treated and untreated systolic blood pressure, total cholesterol, HDL cholesterol and diabetes status (D'Agostino Sr et al., 2008). This risk profile has been shown to be associated with decline in cognitive performance over 10 years (Kaffashian et al., 2011). Similarly, the Framingham stroke risk profile is associated with poor cognitive performance in cross-sectional studies (M. F. Elias et al., 2004; Llewellyn et al., 2008). The Framingham risk factors for stroke are broadly similar to the general cardiovascular risks, with the addition of atrial fibrillation.

The relationship between cardiovascular risk factors and cognition was investigated in the Tromso study, a large study of 55-74 year old adults in Norway (Arntzen, Schirmer,

Wilsgaard, & Mathiesen, 2011). They measured blood pressure, body mass index (BMI), blood lipids, health and demographic questionnaires including cardiovascular disease, and compared these with cognitive performance on word memory, digit symbol and finger tapping tasks. They found that cardiovascular risk factors including systolic blood pressure, smoking and diabetes were associated with poorer cognitive performance. Similarly, in the Rotterdam study, people with evidence of cardiovascular disease demonstrated poorer cognition (Breteler et al., 1994). Investigators compared the cognitive performance of healthy older adults to those with previous vascular events, plaques in the carotid arteries, and/or evidence of atherosclerotic disease in the peripheral arteries. Participants were 55-94 year old residents of Rotterdam, the Netherlands. They observed that cognitive performance was worse and more variable for those with cardiovascular disease. They estimated a prevalence of one fifth to one third of the population having atherosclerosis, and suggested that interventions to counter this may provide large improvements in cognitive function at a population level.

Relationships between cardiovascular health and cognition have also been observed longitudinally. A large study in the USA observed relationships between cardiovascular variables and cognitive decline. Baseline diabetes, hypertension, APOEe4 and incident stroke were all related to decline over 14 years in cognitive tasks including delayed recall, digit symbol substitution and word fluency. However other cardiovascular risk factors including baseline smoking, alcohol, cholesterol, BMI and carotid intima-media thickness were unrelated (Knopman, Mosley, Catellier, & Coker, 2009).

3.5.3 Hypertension and cognition in the elderly

Hypertension is an important cardiovascular risk factor and is also highly relevant to cognition, particularly in aging. Blood pressure is associated with cognitive performance however the relationship is not straightforward. Some studies find that high blood pressure is related to cognitive impairment, while others observe an inverse relationship. For example, a large, multicenter study observed that elevated systolic blood pressure was associated with impairment in executive function but not memory, in adults 65-94 years (Kuo et al., 2004). Another study observed that elderly adults with

hypertension were approximately ten percent slower in their cognitive responses than normotensive counterparts (Harrington, Saxby, McKeith, Wesnes, & Ford, 2000). With regard to dementia, hypertension may confer greater risk in the elderly. Investigators observed a robust relationship with incident vascular dementia in persons over 65 years when heart disease or diabetes were also present, but found no relationship between hypertension and incident Alzheimer's dementia or cognitive performance in a seven-year follow up (Posner et al., 2002).

On the other hand, *lower* blood pressure was associated with poorer cognitive performance in a study of adults aged 70-85 years (Paran, Anson, & Reuveni, 2003). Similarly, in a longitudinal study of participants aged 80 years or more, the risk of dementia over two years was decreased with higher blood pressure in people using antihypertensive medication (Ruitenberg et al., 2005). In a large cross-sectional study, a pattern was observed whereby cognitive performance on the MMSE was better with lower blood pressure in participants aged 60-74, but that the pattern reversed in those aged 80 plus (Obisesan et al., 2008). This study highlights how conflicting observations might be accounted for by the age of the subjects under investigation.

These seemingly contradictory findings echo the nonlinear relationship observed between diastolic blood pressure and cardiovascular risk described in Section 3.2 above, and suggests that hypertension is dangerous in younger adults, but that lower blood pressure might be a greater risk for the oldest-old, perhaps because it leads to hypoperfusion of the brain (Qiu, Winblad, & Fratiglioni, 2005). In fact, cerebral hypoperfusion is associated with dementia and likely precedes onset of the disease (Ruitenberg et al., 2005).

Pulse pressure is also a risk factor for cognitive decline, with several studies demonstrating that pulse pressure predicts later cognitive impairment. For example, the Betula study observed that dementia patients had higher pulse pressure (and systolic pressure) 10 years before being diagnosed (Nilsson et al., 2004). The Baltimore longitudinal study of aging also observed that performance on various memory tasks deteriorated with higher pulse pressure (Waldstein et al., 2008). Contrarily, the

Honolulu-Asia Aging study found that pulse pressure was unrelated to incident dementia, but that systolic pressure was related (Freitag et al., 2006).

Cognitive impairment that is associated with hypertension implies an effect of hypertension on the brain, and in fact it does have an observable impact on the brain. One study observed that hypertension is related to a reduction of white matter volume (Raz, Rodrigue, & Acker, 2003). Another observed that increased blood pressure exacerbated regional age-related deterioration in white matter; higher pulse pressure was seen to amplify the deterioration in anterior brain regions whereas in participants with hypertension there was further deterioration in posterior regions (K. M. Kennedy & Raz, 2009). Similarly, a relationship was observed between high systolic blood pressure and cerebral white matter lesions, where systolic pressure was measured up to 20 years prior to the MRI examination (Van Dijk et al., 2004).

Further corroborating the detrimental effect of hypertension, antihypertensive treatment is protective of cognition. A review of longitudinal studies of hypertension and aging reported that acetylcholinesterase (ACE) inhibitors had a positive effect on cognition, and Ca^{2+} antagonists improved cognition and reduced the risk of vascular cognitive impairment (Amenta, Mignini, Rabbia, Tomassoni, & Veglio, 2002). Not all classes of antihypertensive medications were advantageous, with diuretics and beta blockers not demonstrating benefits. These findings echo the CAFÉ study described above (Williams et al., 2006), which found improved central pressure variables and clinical outcomes after treatment with calcium channel blockers compared with β-blockers. Together these studies suggest that central blood pressure measures have potential to assist in better choice of treatment for not only cardiovascular risk but cognitive risk too. Consideration of these data also raises the possibility that central pressure measurements might be a more sensitive tool for assessing improvements due to other treatments, including nutritional supplements or dietary changes. Furthermore, the mechanisms of action of the more beneficial drugs could suggest a theoretical basis for prescribing particular nutritional or herbal interventions.

3.5.4 Arterial stiffness and cognition

Arterial stiffness is an important risk factor for cardiovascular disease and this is also robustly associated with cognition. Measures of pulse wave velocity, the 'gold standard' in assessment of arterial stiffness (Laurent et al., 2006), have been frequently associated with cognition. A meta-analysis investigating the relationship between pulse wave velocity and longitudinal decline in MMSE performance found that increased arterial stiffness significantly predicted cognitive decline (Pase, Herbert, Grima, Pipingas, & O'Rourke, 2012). Another meta-analysis which examined cross-sectional data found that arterial stiffness was associated with poorer cognitive function, and was higher in patients with vascular dementia compared with controls or patients with Alzheimer's dementia (Rabkin, 2012). Furthermore, the strength of the association between arterial stiffness (as measured by pulse wave velocity) was demonstrated to increase with increasing age.

The risk of damage to the brain by arterial stiffness can be explained by the transfer of high pressure pulsations into the microvasculature of the brain, causing damage to these small vessels and surrounding brain matter, with a consequential deterioration in cognitive performance (Mitchell et al., 2011).

3.5.5 Central pressure measures and cognition

Fewer studies have examined cognition in relation to central blood pressure variables. Central pressure measures may be particularly relevant to cognitive aging because of the association between arterial stiffness and cognitive impairment which has been established using methods of pulse wave velocity. Furthermore, augmentation index and augmentation pressure reflect increased pressure pulsations which have potential to damage the brain (Mitchell, 2008). Thus it is of interest to study these variables in relation to cognition and the aging brain.

A relationship between cognition and central pressure measures was demonstrated in middle-aged, healthy adults (40-65 years) (Pase et al., 2010). Using a computerized

battery of cognitive tasks, it was demonstrated that increased arterial stiffness, as measured by central pulse pressure and augmentation index, was associated with poorer performance on episodic memory accuracy (central pulse pressure) and speed of memory (central pulse pressure and augmentation index). This means that participants with higher arterial stiffness were slower at recalling than those with lower arterial stiffness. The effect was specific to memory, measures of attention were unrelated to arterial stiffness.

In another example, a study of 198 elderly adults with subjective memory complaints used the SphygmoCor system in conjunction with MRI and a cognitive battery. Although augmentation index was not associated with memory impairment, it was associated with the presence of white matter hyperintensities (Kearney-Schwartz et al., 2009). This was observed after taking into account other cardiovascular risk factors. As discussed previously, white matter hyperintensities are a sign of small vessel disease, which may produce serious cognitive repercussions in the long term.

Given that augmentation index and augmentation pressure have different relationships with age (Fantin et al., 2007), it is of interest to observe whether these variables demonstrate similar or different relationships with cognition in young and old adults. The relationship between cognition and augmentation index is investigated in the study reported in Chapter 8.

3.6 Shared biomarkers in cognitive and cardiovascular aging

There are a number of blood biomarkers which are risk factors that apply to both cognitive and cardiovascular health during aging. These include markers of oxidative stress and inflammation, homocysteine, and blood lipids. Oxidative stress and homocysteine will be addressed in the following chapter because they are closely related to nutritional factors. Inflammation and cholesterol are discussed here.

3.6.1 Inflammation

Inflammation is part of the body's immune response. It occurs acutely in response to injury, such as physical damage or infection, instigating a series of events with the aim of restoring tissue to its normal state (Ward, 2010). It may also occur chronically as a result of health and lifestyle issues, including diet, exercise, and adiposity (Galland, 2010; Hassan, Latif, & Yacoub, 2012; Kasapis & Thompson, 2005). There are serious health implications for chronic inflammation. It has also been proposed that inflammation is the common underlying factor in age-related diseases (Chung et al., 2009).

There are numerous biomarkers of inflammation. Two that are particularly relevant include C-reactive protein (CRP) and fibrinogen. CRP is the gold standard of inflammatory markers and the most widely used in cardiovascular studies (Biasillo, Leo, della Bona, & Biasucci, 2010). High sensitivity CRP is a more recent immunoassay measurement that can obtain values in the normal range of healthy individuals (0.1-3mg L^{-1}), compared with older assays which were less sensitive (2-10mg L^{-1}). CRP is independently related to coronary heart disease (Buckley, Fu, Freeman, Rogers, & Helfand, 2009). Fibrinogen plays a role in the coagulation cascade. It is also strongly associated with cardiovascular risk (Danesh et al., 2005).

The contribution of inflammation to cardiovascular disease is well-recognized. It is central to the atherosclerotic process, including the initiation and progression of atherosclerotic lesion as well as regulating plaque stability (Wong, Meredith, Lin, & McManus, 2012). An early meta-analysis of prospective studies found that risk of coronary heart disease was significantly increased with higher concentrations of various inflammatory markers including fibrinogen and CRP (Danesh, Collins, Appleby, & Peto, 1998). Ongoing research has corroborated these findings (Danesh et al., 2004). Of particular relevance to the present studies, CRP has been associated with arterial stiffness as measured by pulse wave velocity but not augmentation index in one study (Yasmin et al., 2004), and with both of these measures in another (Mahmud & Feely, 2005).

The role of inflammatory markers in cognitive decline has been demonstrated quite consistently in several large-scale longitudinal studies. In the Maastricht Aging Study, higher baseline CRP was associated with impaired performance on memory tasks at baseline and after six years follow-up (Teunissen et al., 2003). Other inflammatory markers were also associated with diminished cognition. Comparable findings were observed in the Health ABC Study and the Helsinki Aging Study, which both observed increased risk ratios of cognitive decline with increased CRP over two and five years, respectively (Tilvis et al., 2004; Yaffe et al., 2003). Fibrinogen was independently associated with declines in reasoning, fluency and processing speed in the Edinburgh Artery Study after a 16-year follow up (Rafnsson et al., 2007). More recently, the Cardiovascular Health Study All Stars cohort indicated that the rate of increase in inflammatory markers was predictive of cardiovascular events and cognitive function. A doubling in CRP over nine years was associated with significant impairment (Jenny et al., 2012). Not all studies have concurred. Data from the Rotterdam and Leiden 85-Plus studies found only moderate associations between inflammatory markers and cognitive performance or decline, with authors concluding that they were not useful markers of risk (Schram et al., 2007).

Increased inflammatory markers have also been observed in dementia and Alzheimer's disease, even before clinical onset of the disorder (M. J. Engelhart et al., 2004; Schmidt et al., 2002). CRP is neurotoxic in vitro and may contribute to brain dysfunction via cerebral atherosclerosis, leading to leukoaraiosis or stroke (Kuo et al., 2005). Inflammation is thought to be both a contributor to and a consequence of Alzheimer's disease pathology (Akiyama et al., 2000).

3.6.2 Cholesterol

Having been first established as a risk factor for cardiovascular disease in the Framingham study in the 1960s, the role of high cholesterol in heart disease is now well-established (Castelli, Garrison, & Wilson, 1986). High cholesterol is a serious risk factor for heart disease and intervention to decrease blood lipids via medication and

lifestyle changes is a major therapeutic aim (Frayn, 2008). On the other hand, the association between cholesterol and cognitive decline and dementia is less clear.

Midlife total cholesterol is related to the risk of Alzheimer's disease and this has been demonstrated in several large longitudinal studies (Kalmijn et al., 2000; Kivipelto & Solomon, 2006; Whitmer, Sidney, Selby, Claiborne Johnston, & Yaffe, 2005). Furthermore, midlife cholesterol was associated with neuritic plaques and neurofibrillary tangles in an autopsy study conducted as part of the Honolulu-Asia Aging study, and this occurred in a dose-response manner (Launer, White, Petrovitch, Ross, & Curb, 2001). However the relationship does not hold in later life, where there is a possible inverse relationship. That is, higher cholesterol is associated with a reduced risk of dementia (Kivipelto & Solomon, 2006).

Contradictory relationships are also observed for cognition in elderly people without dementia. In a large study of older women, high total cholesterol and LDL were unrelated to cognitive impairment as measured by the MMSE and a range of other cognitive tasks (Mielke et al., 2008). On the other hand, in the Framingham study, people with total cholesterol in the "desirable" range performed poorly on cognitive tasks of fluency, executive function and attention, compared to those with higher levels of total cholesterol (P. K. Elias, Elias, D'Agostino, Sullivan, & Wolf, 2005). Also, the Melbourne Women's Midlife Health project found better memory with higher LDL and total cholesterol measures, in a cross-sectional study of women aged 52-63 years (Henderson, Guthrie, & Dennerstein, 2003).

Relationships between cholesterol and cognition or dementia were addressed in a group of meta-analyses by Anstey et al. (2008). Results suggested that there was a link between midlife but not late-life cholesterol and Alzheimer's disease. On the other hand there was no association between cholesterol and vascular dementia, or in studies which investigated "any dementia". This suggests the involvement of cholesterol in Alzheimer's pathology, echoing the results of the autopsy study cited above. For cognitive decline the results were mixed and did not undergo meta-analysis. However it was observed that while some studies showed that high total cholesterol in late life was

associated with a decreased risk of cognitive decline, evidence was insufficient to indicate a relationship.

Since then, a large longitudinal study has reported that high total cholesterol in mid-life is associated with poorer cognitive function in later life, particularly in episodic memory and fluency (A. Solomon et al., 2009). However this study also observed that total cholesterol decreased over time, with greater decreases being associated with poorer cognitive function except in people who used statins. Statins are a cholesterol-lowering medication, and their use has been proposed to be protective of Alzheimer's disease and cognitive decline, although evidence for this is currently lacking (Benito-León, Louis, Vega, & Bermejo-Pareja, 2010; McGuinness, Craig, Bullock, & Passmore, 2009).

In summary, it appears that there may be a risk for cognitive decline with high cholesterol at midlife, but this relationship changes by later life, where there is a risk for cognitive decline with low cholesterol. Kivipelto (2006) discuss that the nature of cholesterol-cognition relationships might change over time since many age-related variables also change. They suggest that age should be taken into account when setting optimal serum lipid profiles. However a better understanding of cholesterol and cognition may be required before this can be realized.

3.7 Summary and conclusion

Cardiovascular health is of major importance as we age. While brachial blood pressures are a useful indicator of cardiovascular health in aging, measures of arterial stiffness including augmentation index can provide additional information. In the elderly, arterial stiffness is a serious condition that can lead to increased mortality.

Cardiovascular health impacts cognition and there is much overlap between cognitive and cardiovascular impairment. Hypertension and arterial stiffness are important risk factors for cognitive decline, although little research has examined augmentation index in the context of cognition. This will be addressed in the study in Chapter 8, where the

relationship between cognitive function and augmentation index and augmentation pressure is examined in younger and older adults.

Inflammation and cholesterol are additional cardiovascular risk factors that are important in cognitive decline. In the following chapter another key risk factor will be addressed: nutrition and the potential benefits of nutritional supplementation to forestall the development of cognitive and cardiovascular deterioration. This includes biomarkers for oxidative stress and homocysteine, which are particularly relevant to nutritional supplementation.

4 Nutritional Supplements in Cognitive and Cardiovascular Aging

The previous chapter examined the relationship between cognitive and cardiovascular health, and some of the important risk factors that are common to both. Poor nutrition is another risk factor for cognitive and cardiovascular decline in old age, which suggests that addressing nutritional insufficiencies may improve outcomes. This applies to vitamins, minerals and phytonutrients, which are the subject of this chapter.

Vitamins and minerals are organic compounds that are required in the diet to maintain health (Bender, 2003). They are involved in innumerable physiological activities throughout the body, including many functions in the brain. The broad range of vitamins including A, B-group, C, D and E variously play important roles in neuroprotection, neurotransmission, homeostatic regulation, antioxidant activities, energy metabolism and DNA synthesis (D. O. Kennedy & Haskell, 2011). Certain minerals are also important for brain function and cardiovascular health. In addition to these nutritional requirements, various plant extracts have purported benefits to cognition and cardiovascular function.

There are minimum daily requirements for vitamins and minerals which have been set by the Australian National Health and Medical Research Council (NHMRC) and other organisations around the world. These "recommended dietary intakes" or "reference daily intakes" (RDI) are determined by expert groups of advisers and may differ between organisations. RDI are determined from mean requirements, plus two standard deviations (Bender, 2003), however individual requirements are likely to be different. Kennedy and Haskell (2011) noted that the scientific literature that provides a basis for determining many nutrients' RDI is incomplete. They also suggest that if a certain level is a minimum requirement, then the optimal intake is likely "some way above" that. While minimum requirements are adequate to prevent overt disease associated with deficiency, determining the level required for sufficient reserves in the body and maintenance of optimal metabolic activity is unknown and perhaps unknowable (Bender, 2003). The possibility exists that providing nutrients at levels above RDI is

beneficial. This is especially true for older adults, who are at increased risk of nutritional deficiency, particularly for vitamins including folate, vitamin B_6, vitamin B_{12}, and vitamin D; and minerals including zinc, calcium, magnesium, as well as phytonutrients (Wahlqvist & Tienboon, 2011).

This chapter is divided into sections, each dedicated to a nutrient group: B vitamins; antioxidant vitamins; vitamin D; phytonutrients; and minerals. Each section first provides a brief description of the nutrient and the role it plays in body, and then reviews the literature regarding its relationship with cognition and cardiovascular function, including the results of trials of supplementation.

4.1 B vitamins and homocysteine

B vitamins are a group of water-soluble vitamins that play important roles in many biological processes including cognition and cardiovascular function. They include:

B_1 – thiamine
B_2 – riboflavin
B_3 – niacin
B_5 – pantothenic acid
B_6 – pyridoxine
B_7 – biotin (or vitamin H)
B_9 – folate
B_{12} – cobalamin

The focus of this review is on folate, vitamin B_6 and vitamin B_{12}, which have substantial literature devoted to them in the context of cardiovascular and cognitive health. While several others of these vitamins have some neuropsychological actions or associations, these are outside the scope of this review.

Folate is a general term which includes folic acid and the various derivatives of tetrahydrofolate, the reduced form of folic acid (NHMRC, 2006). The various forms of folate are most frequently found in food, whereas free folic acid is more commonly used in food fortification and supplementation because it has a higher bioavailablility (Bender, 2003). Folate is found in fresh leafy green vegetables, sprouts, nuts, fortified

cereals and organ meats. Folate in food is unstable; it is easily destroyed by light, heat, cooking or storage, during which as much as 100% can be lost (Braun & Cohen, 2010). Folate deficiency in developed countries is common at around 8-10% of the population (Bender, 2003). The NHMRC recommends an intake of 400µg of folate per day in adults (NHMRC, 2006). Folate acts as a methyl donor in many biological reactions, particularly related to metabolism of amino acids and nucleic acids. It is involved in DNA and RNA synthesis, and is required for the conversion of homocysteine to methionine (Bender, 2003).

Folate fortification of cereal products was introduced in Australia between June 2007 and September 2009 (Food Standards Australia New Zealand, 2009). The level of folic acid fortification provides, for example, around 120µg per 3 slices of bread (Australian Institute of Health and Welfare, 2011). Motivation for the mandatory fortification is the prevention of neural tube defects that can occur in children of mothers who have insufficient intake of folate during pregnancy. It aims to increase the folate intake of women of childbearing age (Food Standards Australia New Zealand, 2009). Fortification of cereal products has proven to be a successful public health strategy in the USA, where the prevalence of folate deficiency declined from 16% to 0.05% of the population after fortification (Pfeiffer, Caudill, Gunter, Osterloh, & Sampson, 2005). On the other hand, high levels of folate can mask deficiency of vitamin B12, leading to anemia and cognitive impairment in older adults (Morris, Jacques, Rosenberg, & Selhub, 2007). This, and other potential complications of high folate intake, suggest caution in the use of widespread folate fortification (Smith, Kim, & Refsum, 2008).

Vitamin B_{12}, or cobalamin, also comes in various forms. Cobalamin refers to a group of cobalt-containing compounds (called corrinoids) which act as vitamins in the body (Bender, 2003). The various vitamers of cobalamin occur in different quantities or proportions throughout the body. Cyanocobalamin and hydroxocobalamin are forms that are commonly used in pharmaceutical preparations. In food, vitamin B_{12} is found in animal protein products such as meat, seafood, eggs and dairy. Because it is synthesized by bacteria it is not found in plants (Bender, 2003). Most cases of deficiency are due to impaired absorption or metabolism, which is quite common, particularly in the elderly (Joosten et al., 1993). For example, an Australian study of elderly people living in

residential care facilities indicated that 14% were deficient in vitamin B_{12} and a further 36% had equivocal levels (Mirkazemi, Peterson, Tenni, & Jackson, 2012). The NHMRC recommends an intake of 2.4µg of B_{12} per day for adults (NHMRC, 2006).

Vitamin B_{12} acts as a cofactor in many biological reactions which are essential for normal cell function. It is required for growth and replication of cells, production of red blood cells and metabolism of carbohydrate, lipids and protein (Braun & Cohen, 2010). As a cofactor it is required for the conversion of homocysteine to methionine; this means that vitamin B_{12} deficiency can also lead to a functional deficiency of folate. Vitamin B_{12} is the essential prosthetic group in methionine synthetase. In the absence of vitamin B_{12}, methyltetrahydrofolate cannot be demethylated to free tetrahydrofolate (Bender, 2003).

There are six vitamers of **vitamin B_6**; the principle active form is pyridoxal 5'-phosphate (PLP), whereas pyridoxine hydrochloride is the usual form used for supplements (NHMRC, 2006). Vitamin B_6 is widely available in many foods and is particularly found in fish, organ meats, legumes, eggs, nuts, potatoes and bananas (Braun & Cohen, 2010). It is also synthesized by the intestinal flora (Bender, 2003). Heat may reduce the bioavailability, with up to 40% lost in cooking (Braun & Cohen, 2010). Although deficiency is rare because it is found so widely in so many foods, marginal inadequacy is more common, with 10-20% of people in developed countries having marginal or inadequate status (Bender, 2003). Deficiency is particularly relevant for the elderly. Intake decreases with age (Kant & Block, 1990) and one study observed that vitamin B_6 was below the normal range in nearly half (48%) of community living elderly and three quarters of elderly living in institutions (Bates, Pentieva, Prentice, Mansoor, & Finch, 1999). The NHMRC (2006) recommends an intake of 1.3mg/day for adults, with increased intakes for those aged over 50 years to 1.5mg for women and 1.7mg for men.

Broadly, vitamin B_6 acts as a cofactor for several enzymes and is important in the metabolism of amino acids. It also modulates steroid hormone action and regulates gene expression (Bender, 2003). Vitamin B_6 is associated with homocysteine, in that it is required in the reaction of homocysteine conversion to cysteine, via cystathionine. The

relationship of homocysteine with vitamin B_6 is not as strong as the relationship with folate however, as most of homocysteine is converted to methionine rather than cysteine (Bender, 2003).

Homocysteine is a sulphur amino acid whose metabolic activity is connected with the B-group vitamins, particularly folate, vitamin B_{12} and vitamin B_6 (Trabetti, 2008). It is implicated in cardiovascular disease and cognitive impairment, and has been proposed as a likely pathway via which B vitamins may exert benefit.

Homocysteine is produced from the demethylation of s-adenosyl methionine (SAM). SAM is used in many reactions as a methyl donor (e.g. in methylation of DNA, RNA, hormones, lipids and neurotransmitters) (Trabetti, 2008). Homocysteine can then be converted back into methionine, which occurs with the greater part of homocysteine, or it can be converted into cystathionine. When homocysteine is converted to cystathionine, vitamin B_6 is required as a cofactor in the reaction. Similarly, vitamin B_{12} and folate are required for the conversion of homocysteine back into methionine (Bender, 2003). Therefore, deficiencies of folate, vitamin B_{12}, and/or vitamin B_6 can lead to elevated homocysteine, or hyperhomocysteinemia.

It is well established that supplementation with folate and/or vitamin B_{12} reduces blood concentrations of homocysteine. The "Homocysteine Lowering Trialists' Collaboration" has conducted meta-analyses to examine this effect. The first, conducted in 1998, determined that folate supplementation in the range of 0.5 to 5mg daily could reduce homocysteine by 25%. The addition of vitamin B_{12} to the supplement reduced this by a further 7%, whereas the addition of vitamin B_6 did not add any further benefit (Clarke, 1998). In 2005 they conducted another meta-analysis and determined dose-response relationships between the amount of vitamin in the supplement and the percentage decrease in blood homocysteine concentrations. Again, vitamin B_{12} had a small additional benefit whereas vitamin B_6 provided no significant benefit (Homocysteine Lowering Trialists' Collaboration, 2005). Both meta-analyses reported that changes were greater in those who had higher homocysteine and lower folate at baseline.

Other studies have observed a significant effect of homocysteine reduction with vitamin B_6 supplementation alone (Mansoor et al., 1999; McKinley et al., 2001).

In addition to dietary deficiency, homocysteine may be increased due to impaired renal function or to genetic abnormalities affecting the enzymes that metabolize it, particularly methylenetetrahydrofolate reductase (Steed & Tyagi, 2011).

4.1.1 Homocysteine, B vitamins and cardiovascular aging

The Hordaland Study has investigated homocysteine in over 18,000 adults. The study observed that homocysteine increased with age and was higher in men than in women (Nygard et al., 1995). It was also determined that high homocysteine was a risk factor for cardiovascular mortality and hospitalization for cardiovascular disease, particularly in older adults and those with increased cardiovascular risk factors (Refsum et al., 2006). In support of these findings, a meta-analysis of studies examining homocysteine and ischemic heart disease and stroke concluded that it was a moderate risk factor for both of these diseases (Clarke et al., 2002).

Homocysteine is thought to contribute to the atherosclerotic process via oxidative stress and consequent endothelial dysfunction. Auto-oxidation of homocysteine produces reactive oxygen species which increases inflammation of the vessel wall (Debreceni & Debreceni, 2012). Other contributions include suppression of nitric oxide synthase and thus reduced NO activity; increased platelet activation and adhesion; increased oxidative damage to the endothelium; thrombosis promotion through alteration of the procoagulant-anticoagulant balance; and impairment of DNA methylation and altered gene expression, which may also possibly lead to vascular smooth muscle cell proliferation (Trabetti, 2008). In various studies, homocysteine has been associated with oxidative stress, impaired flow-mediated vasodilation, decreased bioavailability of NO, and proliferation of smooth muscle cells (Debreceni & Debreceni, 2012).

High homocysteine is also associated with increased arterial stiffness (Bortolotto et al., 1999; D. Levy et al., 2007) and this might occur via functional or structural arterial

changes. Arterial stiffness can be induced by acutely increasing homocysteine via methionine load, indicating a functional effect on arterial stiffness (Nestel, Chronopoulos, & Cehun, 2003). High homocysteine may also mediate vascular remodeling by triggering matrix metalloproteinases, specialized enzymes that are involved in the breakdown and synthesis of the extracellular matrix (Steed & Tyagi, 2011).

Early research indicated that homocysteine was a causative factor in cardiovascular disease (Wald, Law, & Morris, 2002). But despite homocysteine remaining a significant risk factor for cardiovascular end points, clinical trials involving the reduction of homocysteine have not produced the expected benefits to cardiovascular health (Antoniades, Antonopoulos, Tousoulis, Marinou, & Stefanadis, 2009). There is ongoing debate in the scientific literature to try to account for this apparent discrepancy, with some authors suggesting methodological issues are at play and a causative role cannot be ruled out (Debreceni & Debreceni, 2012; Ueland & Clarke, 2007).

4.1.2 Homocysteine and the brain

In addition to the detrimental effects that cardiovascular damage may have in the brain, various lines of research have demonstrated that homocysteine is neurotoxic. An early study used cerebrocortical cultures derived from embryonic rats and demonstrated that homocysteine directly causes neurotoxicity via activation of the *N*-methyl-D-aspartate (NMDA) subtype of glutamate receptor (Lipton et al., 1997). Many further studies since then have demonstrated in vitro the effect of homocysteine on various types of cerebral cells. For example, homocysteine increased measures of oxidative stress in rat hippocampus (Streck et al., 2003). Particularly of note is one study that demonstrated that homocysteine could induce neurodegeneration in cultured rat cerebellar neurons when applied in micromolar concentrations over three days (Zieminska & Lazarewicz, 2006).

Correlational studies in humans have demonstrated a detrimental association between homocysteine and gross brain health. For example, in the Chicago Health and Aging

Study higher homocysteine was associated with increased white matter hyperintensities and cerebral infarcts, and these wholly accounted for the relationship between homocysteine and cognition (Tangney, Tang, Evans, & Morris, 2009). Similarly, another study reported that homocysteine was increased in patients with cerebral small vessel disease compared with normal controls, and that homocysteine concentration was independently associated with age-related white matter changes as measured by MRI. It was also associated with decline in cognitive status, however this association did not remain after adjustment for covariates (Pavlovic et al., 2011). In a recent example which examined older patients with atherosclerosis, high homocysteine was related to the progression of ventricular enlargement over four years, and a concomitant decline in cognition (Jochemsen et al., 2012). In Alzheimer's patients, high homocysteine at baseline was similarly associated with disease progression and greater atrophy of the medial temporal lobe after three years (Clarke et al., 1998).

Research does not consistently find such associations. In a study examining grey matter volume it was observed that participants with high homocysteine had reduced grey matter volume, but that this relationship could be accounted for by age and other cardiovascular risk markers (Ford & Almeida, 2012). Also, homocysteine was unrelated to hippocampal atrophy in another MRI study (Morra et al., 2009).

4.1.3 Homocysteine and cognitive aging

The role of homocysteine in cognition has been researched extensively, with nearly 600 articles returned in a Scopus search using these terms. A comprehensive review is therefore not possible in this chapter. An overview of the recent literature and results from larger studies is provided here.

There are many correlational studies that testify to a relationship between homocysteine and cognition function in older adults. In one example, the Rotterdam Scan Study, homocysteine was associated with cognitive performance in a large group of elderly adults without dementia (Prins et al., 2002). The relationship was not mediated by changes in brain structure (cerebral infarcts, white matter lesions or atrophy) as

measured by MRI. The relationship with cognition was largely driven by those with very high homocysteine, that is, greater than 14 μmol/L. This differs from results observed in a previous study from the Rotterdam cohort, in which they found no relationship between cognition and homocysteine at baseline, or for homocysteine and cognitive decline at follow up after 2.7 years (Kalmijn et al., 1999). This study had a large sample (702 participants) but they used the MMSE to measure cognition, and this may not be a sufficiently sensitive tool in a healthy population with participants as young as 55 years.

The relationship between homocysteine and cognition was also examined in the Framingham Offspring Cohort using a various cognitive tasks such as the Wechsler Memory Scales, abstract reasoning, trailmaking and naming. It was observed that the relationship was significant in the 60-plus age group but not the younger groups (M. F. Elias et al., 2005). The National Health and Nutrition Examination Survey (NHANES) examined more than 2000 adults aged 60 years or more for memory performance using a delayed story recall task. Participants with low folate and those with hyperhomocysteinemia had significantly poorer recall, and those with low folate together with high homocysteine fared worst of all (M.S. Morris, Jacques, Rosenberg, & Selhub, 2001).

A correlation between homocysteine and cognitive decline does not infer causality. Elderly people with memory impairment may self-neglect, with poorer eating habits leading to deteriorating nutritional status, including reduced folate and consequent increased homocysteine (S. M. Smith et al., 2006). However, some prospective studies are now providing evidence in favour of a causative role. Prospective studies improve our understanding by investigating whether nutritional deficiency and elevated homocysteine precedes memory impairment or vice-versa. Several studies have examined this question with findings generally supportive of a causative role of homocysteine in cognitive decline.

The Epidemiology of Vascular Aging study (EVA) examined older adults and found an association between cognitive decline and homocysteine, with hyperhomocysteinemic participants (>15 μmol/L) nearly three times more likely to demonstrate cognitive

decline at the four-year follow up than those with low homocysteine (<10 µmol/L) (Dufouil et al., 2003). Similarly, higher homocysteine was associated with cognitive decline over three years in older men in the Veterans Affairs Normative Aging Study. The effect was specific to spatial copying and recall memory (Tucker, Qiao, Scott, Rosenberg, & Spiro, 2005).

Homocysteine also increases the risk of dementia. For example, elevated homocysteine at baseline doubled the risk of dementia at a four-year follow up in the Conselice Study of Brain Ageing (Ravaglia et al., 2006). Also, in the Framingham study, high homocysteine was associated with incident Alzheimer's disease at eight years, with dementia risk almost doubling in those with concentrations over 14µmol/L (Seshadri et al., 2002). Homocysteine is also associated with the rate of cognitive decline in Alzheimer's patients; those with higher concentrations declined faster (Oulhaj et al., 2010). The relationship may be mediated by vascular disease, which is increasingly acknowledged to be a significant contributor to Alzheimer's disease (Gorelick et al., 2011). In one study it was observed that Alzheimer's disease patients with comorbid vascular disease had a far greater risk of elevated homocysteine than those without vascular disease, when compared with healthy controls (odds ratios of 10.0 and 2.2 respectively) (J. W. Miller, Green, Mungas, Reed, & Jagust, 2002). These patients may be particularly at risk of rapid cognitive decline.

The association between homocysteine and the development of dementia has been demonstrated to extend as far as midlife. The Prospective Population Study of Women looked at midlife homocysteine and found that high levels were significantly associated with development of dementia in later life, with follow up interval of 35 years (Zylberstein et al., 2011).

Despite these significant associations, homocysteine is not the whole story of cognitive decline. Elevated homocysteine is not a prerequisite for neurodegenerative disease, which also occurs in people with normal homocysteine levels (Doherty, 2008). Also some researchers have noted that homocysteine makes only a small contribution to the variance in cognitive impairment (J. W. Miller et al., 2003). If this is the case, any benefit associated with reducing homocysteine might be expected to be mild. On the

other hand, major influences on cognitive performance in the elderly such as age and education are not modifiable and cannot be targeted for intervention.

4.1.4 B vitamins and cognitive aging

Blood levels of B vitamins, particularly folate, have been inversely correlated with cognitive decline, suggesting these vitamins may play a protective role in the brain. Since these vitamins reduce homocysteine, it is plausible that this is how the beneficial effect is produced. However, B vitamins also play roles in neurotransmitter production, signal transduction, protein synthesis in the nervous system, and may act as antioxidants (Braun & Cohen, 2010), actions which have potential to affect cognitive performance. Improved cognition might also occur via the effects of B vitamins on mood and stress; these have been demonstrated to be reduced with B vitamin supplementation (Long & Benton, 2013; Stough et al., 2011).

B vitamins appear to be beneficial to cognition. For example, in the SALSA study there was a relationship between folate and cognitive function scores, as well as with incident dementia (Ramos et al., 2005). This was after controlling for homocysteine, vitamin B_{12} and other variables. Furthermore, the relative risk of cognitive impairment and dementia decreased with increasing folate concentration. Similarly, an Australian study examined dietary intake of folate, vitamin B_{12} and vitamin B_6 and self-reported cognitive function and mood in middle aged adults. Dietary intake of folate and vitamin B_6 was associated with better memory in women, and vitamin B_6 and vitamin B_{12} in men (Bryan & Calvaresi, 2004).

A large study including more than 2000 elderly participants found that in cross-sectional analysis, folate was lower and homocysteine was higher in people with memory impairment, but there was no difference in vitamin B_{12} between the impaired and healthy (Nurk et al., 2005). Furthermore, high baseline homocysteine measured six years earlier was associated with increased risk for memory impairment at follow up. It was also demonstrated that a decline in homocysteine levels over the six years was associated with better memory scores, whereas an increase was associated with poorer

scores. Similarly, increasing folate was associated with a better memory score and declining in folate with a lower score. Although the data seem to suggest that the biochemical status precedes the memory impairment, the participants were not tested for memory at baseline, so changes in memory could not be assessed.

Those findings are in contrast to the Leiden 85+ study, which measured homocysteine, vitamin B_{12} and folate in elderly adults. They reported that although homocysteine and the vitamins were correlated with cognition at baseline and yearly for the four years that participants were tested, baseline data did not predict a faster decline in cognitive performance (Mooijaart et al., 2005). The authors concluded that homocysteine and B vitamins are not a causative factor in cognitive decline but a consequence of it, probably due to poorer diet in those with cognitive impairment. However this conclusion contradicts data from other studies. For example, a seven-year prospective study observed that baseline vitamin B_{12} level was associated with better cognitive performance at follow up on a range of cognitive tasks assessing episodic memory, executive function and speed. Folate level was associated with global cognition and verbal tests (Hooshmand et al., 2012).

Not all prospective studies have confirmed a relationship between B vitamins and cognition. In the Nurse's Study no relationship was observed between cognition and folate or vitamin B_{12}. This study examined a subset of 635 participants whose cognitive performances were followed up over four years, with folate and vitamin B_{12} having been observed 15 years earlier. They used the Telephone Interview for Cognitive Status (TICS, a telephone test similar to the MMSE), immediate and delayed recall, category fluency (animals) and digit span backwards (J. H. Kang, Irizarry, & Grodstein, 2006). Similarly, only baseline low folate and not homocysteine, vitamin B_6 or vitamin B_{12}, predicted cognitive decline after seven years, in high functioning people aged 70-79 years (Kado et al., 2005). This was despite all variables being associated with cognition at baseline.

4.1.5 Brain pathology associated with B vitamins

B vitamins have been implicated in Alzheimer's dementia. For example, lower blood levels of vitamin B_{12} and folate were found in histologically confirmed Alzheimer's disease (Clarke, 1998). Other research suggests the benefits are limited to folate. For example, folate intake protected against development of Alzheimer's disease in a large longitudinal observational study of people aged 65 years or more. In this study, those who had the highest intake of folate had the lowest risk of dementia after six years, whereas intake of vitamin B_6 and vitamin B_{12} conveyed no protection (Luchsinger, Tang, Miller, Green, & Mayeux, 2007). Similarly, MCI patients with low serum folate were more likely to convert to Alzheimer's disease over a 3-year follow up (Ravaglia et al., 2006).

Vitamin B_{12} may protect against cognitive decline in healthy adults but it is uncertain whether it can maintain cognition in people with dementia, with studies generally finding no benefit of higher blood levels of vitamin B_{12} (Oulhaj et al., 2010; Small & Bäckman, 1998). Recently a systematic review of prospective cohort studies concluded that there was insufficient evidence of a relationship between serum vitamin B_{12} concentrations and cognitive decline or dementia. However, they considered that many studies did not have a sufficient follow-up interval, or used cognitive tests that were not sensitive enough (O'Leary, Allman-Farinelli, & Samman, 2012). The authors also noted that the studies which used more sensitive markers of vitamin B status were those that found associations with cognition or dementia.

Evidence from brain studies may contribute to our understanding of the relationship between cognition and B vitamins. For example, vitamin B_{12} was associated with brain volume loss over five years (but folate was not) in older adults with no cognitive impairment (Vogiatzoglou et al., 2008). Vitamin B_{12} was also associated with the severity of white matter lesions in the Rotterdam Scan Study (De Lau, Smith, Refsum, Johnston, & Breteler, 2009). These studies argue for a role of vitamin B_{12} in neuroprotection.

4.1.6 Intervention studies of B vitamins for cognition

The effects of B vitamin supplementation on cognition have been examined in numerous studies. However, controlled trials of B vitamin supplementation have had limited success in improving cognition in normal or impaired elderly. A number of systematic reviews and meta-analyses have been conducted to evaluate the data regarding these effects.

A Cochrane Review investigated studies of folic acid, whether singly or in combination with vitamin B_{12}, and concluded that there was insufficient evidence either for or against folic acid in the treatment or prevention of cognitive decline or dementia, but urged further investigation of the subject (Malouf & Evans, 2008). Similarly, a systematic review of vitamin B_{12} examined six placebo-controlled trials of vitamin B_{12} for cognition and found benefit in only one study (Moore et al., 2012). Recently a comprehensive meta-analysis of placebo-controlled trials looked at B vitamins, homocysteine reduction, and the effects on cognition (Ford & Almeida, 2012). They concluded that vitamin B_6, vitamin B_{12} and folate did not affect cognition, whether or not they succeeded in reducing homocysteine and regardless of whether the vitamins were used singly or in combinations. Their conclusion applied to normal elderly subjects and those with dementia. The authors also determined that studies with a longer duration of supplementation were no more likely to elicit benefits than shorter interventions.

Despite the negative findings of these meta-analyses some studies have found positive cognitive outcomes, suggesting there are conditions which these supplements may be effective. For example, the Folic Acid and Carotid Intimamedia Thickness (FACIT) trial was a large randomised, placebo controlled trial which demonstrated improved information processing speed and sensorimotor speed after three years of supplementation with folic acid. Participants in the study were aged 50-70 years and had high homocysteine, placing them at greater risk of vascular disease (Durga et al., 2007).

Another important example is the Vitacog trial, where participants with MCI were given B vitamin supplements for two years, reducing homocysteine by 30%. Supplementation slowed the rate of cognitive decline in the treatment group, having an effect on the clock drawing task. Furthermore, those who began the study with homocysteine above the median level also demonstrated a significant benefit in MMSE score and verbal learning and fluency tasks (de Jager, Oulhaj, Jacoby, Refsum, & Smith, 2012). An earlier report from the study described the results of MRI examinations of these participants: they observed reduced brain atrophy in the treatment group over the two year intervention, particularly in those with high baseline homocysteine. Also, greater whole brain atrophy was associated with poorer cognitive performance at follow up (A. D. Smith et al., 2010). This tells a compelling story whereby B vitamin supplementation reduced homocysteine with a consequent reduction in brain atrophy and associated cognitive decline. It can be reasoned then that B vitamins may be protective in susceptible individuals in the longer term.

4.2 Antioxidant vitamins and oxidative stress

4.2.1 Oxidative stress

The free radical theory of aging was first proposed by Harman in 1956, who hypothesized that the aging process was caused by free radical damage to DNA, lipids and proteins (Harman, 1956). Further research since then has supported a role of oxidative stress in aging (Ames, Shigenaga, & Hagen, 1993).

Oxidative stress is defined as an increase in free radicals, or reactive oxygen species (ROS), where antioxidant defences are insufficient (Touyz & Schiffrin, 2008). Free radicals in the body increase with exposure to oxidants such as cigarette smoke or pollution, but also increase cumulatively over the lifespan as a result of normal metabolic processes (Barja, 2004). A decreased capacity of cells to respond to oxidative stress can also lead to a detrimental imbalance (K. J. Davies, 2000). Consequences of oxidative stress include modification of gene expression; suspension of cell growth or

apoptosis; lipid peroxidation, which can cause cell membranes to become rigid and reduce permeability; DNA oxidation, which can disrupt the replication process, causing mutation and cell death; and accumulation of oxidized proteins within cells that can disrupt cell function (K. J. Davies, 2000). Oxidative stress is an important contributor throughout many stages of the atherosclerotic process, with oxidation of LDL being a starting point (Singh & Jialal, 2006). Accordingly it plays a major role in cardiovascular disease.

The brain is particularly sensitive to oxidative stress due to its high oxygen uptake (Halliwell, 2006). Reactive oxygen species can cause cell death in neurons and astrocytes (Barja, 2004). Oxidative stress can also disrupt the protective blood brain barrier (Freeman & Keller, 2012).There is substantial evidence for oxidative stress being present in Alzheimer's disease, with markers of protein, lipid and DNA oxidation in patients (Bennett, Grant, & Aldred, 2009). Oxidative stress is thought to be a contributor to Alzheimer's disease pathology, not merely a consequence of the disease process (Sayre, Perry, & Smith, 2007).

Oxidative stress is observed in cognitive decline even without the presence of dementia. For example, markers of oxidative stress were associated with cognitive decline in the EVA study (Berr, Richard, Gourlet, Garrel, & Favier, 2004). Similarly, measures of free radicals in blood were inversely correlated with cognitive performance on the MMSE in elderly participants (Maugeri et al., 2004). F2α-isoprostanes and protein carbonyls were correlated with cognitive performance in healthy elderly and these biomarkers were also lower in those with high fruit and vegetable intake (Polidori et al., 2009). Studies such as these infer a detrimental role of oxidative stress in cognitive aging and have prompted investigation of ways to minimise oxidative damage and improve cognitive function.

4.2.2 Antioxidant vitamins

An antioxidant is any substance that has the capacity to neutralize a free radical. Dietary sources include the vitamins A, C, and E, beta-carotene, and other carotenoids including lycopene. Another important group of antioxidants that are found in food are the flavonoids (see Section 4.4). Although the focus of this section is on antioxidants, these vitamins have other functions in the body in addition to their roles as antioxidants.

Vitamin A is a term that includes any of the substances that provide vitamin A activity, including retinoids (retinol and derivatives) and carotenoids (α- and β-carotene and lycopene). Preformed vitamin A is mainly found in animal foods including red meat, fish and dairy (Braun & Cohen, 2010). Precursors to vitamin A are also found in oils, fruits and vegetables (NHMRC, 2006). Vitamin A requirements are expressed in µg retinol equivalents, because the different forms have differing biological activity. Daily requirements are 700µg for women and 900µg for men. Due to the limited capacity for the body to eliminate excess vitamin A and potential toxicity, upper limits are also important and are set at 3000µg daily for both women and men (NHMRC, 2006).

Vitamin A is important for eye health, growth and bone formation, immune function, and cancer prevention, among other functions (Braun & Cohen, 2010). It also has free-radical scavenging capacity and in this respect may be important for cardiovascular and cognitive health; however vitamins C and E have been the focus of research in this regard.

Vitamin C (ascorbic acid) has many functions in the body but its role as an antioxidant is of major importance (Bender, 2003; Braun & Cohen, 2010). The RDI is 45mg/day for both women and men (NHMRC, 2006). Deficiency causes scurvy, which is rare in developed countries since vitamin C is commonly found in fruit and vegetables, although marginal deficiency can sometimes be found (Braun & Cohen, 2010). While vitamin C is commonly referred to as an antioxidant, it can act as a pro-oxidant in presence of transition metals (Bender, 2003) and it may also act as a prooxidant in the atherosclerotic plaque (Farbstein et al., 2010). Other roles of vitamin C which may be

relevant to cognition include acting as a cofactor in the synthesis of catecholamines, and a possible role in modulation of neurotransmitters (Harrison & May, 2009).

Vitamin E includes eight different molecules, including four tocopherols and four tocotrienols which act as antioxidants (Bender, 2003). There are also synthetic products that have vitamin E activity. The form most utilized in the body is α-tocopherol (Azzi & Stocker, 2000). Vitamin E is found in green vegetables, nuts and grains, dairy and eggs (Braun & Cohen, 2010). An RDI is not provided by NHMRC, instead they provide an 'average intake' level based on populations in Australia and New Zealand, which are assumed to be sufficient because these populations are healthy. For women this is 7mg per day and for men it is 10mg per day (NHMRC, 2006). As an antioxidant, vitamin E scavenges lipid radicals and is considered the most important antioxidant for lipid preservation (Azzi & Stocker, 2000).

4.2.3 Antioxidant vitamins and cardiovascular health

Due to the role of oxidative stress in cardiovascular health, antioxidants have been the focus of considerable research. Fruit and vegetable intake are protective against cardiovascular disease and it has been proposed that the antioxidant content of these foods is the reason for this (Bowman, Bassuk, & Gaziano, 2008). Vitamins C and E in particular may play important roles in atherosclerosis.

A review by Farbstein (2010) evaluates the evidence that vitamins C and E are important in the process of atherosclerosis. In summary, studies have demonstrated that vitamin E enhances NO release, has antithrombotic and vasodilation functions and inhibits inflammatory processes and cellular adhesion. It may also play a role in reducing cell proliferation, suppressing vascular smooth muscle cell proliferation and boosting survival of vascular smooth muscle cells that have been oxidized by LDL. Vitamin C defends against the atherosclerosis in various ways. It promotes endothelial cell proliferation, prevents endothelial cell apoptosis, reduces oxidative stress and inflammation by augmenting the production of NO, and reverses endothelial cell dysfunction (Farbstein et al., 2010).

Despite plausible mechanisms and some epidemiological evidence, intervention trials of vitamins C and E have not demonstrated clear benefits in cardiovascular outcomes, and may even be detrimental. Some examples include a four-and-a-half year study where no differences between vitamin E and placebo for cardiovascular outcomes were observed in vascular disease and diabetes patients aged 55 plus (Yusuf, 2000). An extension to this study also reported no benefit of vitamin E supplementation to cardiovascular events, cancer incidence or death in the same patients after seven years (Lonn et al., 2005). Similarly, a very large trial (n= 29,133) investigated α-tocopherol, β-carotene, or both versus placebo over five to eight years. Participants were male smokers aged 50-69 years. They observed no benefit for 50mg α-tocopherol and an increased risk of myocardial infarction for 20mg β-carotene (Törnwall et al., 2004). In contrast to these studies, a recent meta-analysis has concluded there is a benefit for vitamin C for systolic and diastolic blood pressures (Juraschek, Guallar, Appel, & Miller I, 2012).

Of concern are reports of a detrimental effect of antioxidant supplementation. In a meta-analysis of trials using vitamin E supplementation, it was concluded that all-cause mortality was increased with supplementation (E. R. Miller et al., 2005). A recent Cochrane review similarly warned of the potential for increased mortality with vitamin E, beta carotene and possibly vitamin A (Bjelakovic, Nikolova, Gluud, Simonetti, & Gluud, 2012). Conversely, two recent meta-analyses concluded there was no evidence of danger with antioxidant supplements (Myung et al., 2013; Ye & Song, 2008).

Although intervention trials of single antioxidant vitamins (C, E and beta-carotene) for prevention of cardiovascular events have not demonstrated any benefit, using combination of vitamin C and vitamin E may be more beneficial due to interactions between the vitamins (Bowman et al., 2008). The pattern is similar to that observed with B-group vitamins, where clinical trials have not shown any benefit vitamin supplementation for cardiovascular health or mortality. Farbstein et al. (2010) suggest that intervention studies may have failed to detect improvements because of methodological issues. They noted that many studies have been conducted with patient groups or older participants where atherosclerotic processes had probably already begun. Evidence suggests that benefits may occur in the early stages of atherosclerosis

and in these cases it is too late. They also suggest that the dose given in some trials might be too high, affecting both beneficial and detrimental effects of the vitamins. Also, naturally occurring forms of vitamins, for example vitamin E, differ from synthetic forms and these may have differing effects in vivo.

4.2.4 Antioxidant vitamins and arterial stiffness

The literature examining the effects of antioxidant vitamins on arterial stiffness is comparatively sparse, particularly in the elderly. Studies in non-elderly adults suggest there may be a role. For example, vitamin C (500mg ascorbic acid daily) for four weeks was demonstrated to reduce peripheral systolic and diastolic blood pressure as well as central augmentation index, in diabetic patients (Mullan, Young, Fee, & McCance, 2002). Also, an improvement in endothelial function (measured by flow-mediated dilation) and arterial stiffness (pulse wave velocity and augmentation index) was observed in patients with essential hypertension after eight weeks of supplementation with vitamin C (1mg) and vitamin E (400IU) combined (Plantinga et al., 2007). This is similar to a later study which observed a reduction in blood pressures in hypertensive adults with vitamin C plus E supplementation over eight weeks(Rodrigo, Prat, Passalacqua, Araya, & Bachler, 2008).

However, the SUVIMAX study (Supplementation en Vitamines et Mineraux Antioxydants) observed only a tendency for pulse wave velocity after seven years of combined antioxidants (vitamin C, E, beta carotene, selenium and zinc), which did not reach statistical significance (Zureik et al., 2004). In another example, vitamin C did not reduce arterial compliance or blood pressure in old or young men after 30 days of supplementation with 500mg vitamin C (Eskurza, Monahan, Robinson, & Seals, 2004). A recent review concluded that existing literature does not support a role of micronutrients in reducing arterial stiffness; however the authors acknowledged that the evidence is lacking (Pase, Grima, & Sarris, 2011). This subject will be examined again in closer detail in Chapter 6, where the effects of nutritional supplements on augmentation index are systematically reviewed.

4.2.5 Antioxidant vitamins and cognition

There is abundant but inconclusive research regarding the role of antioxidants in cognitive aging and dementia. Assessment of the benefits of antioxidants is complicated by various factors. Sample characteristics (age, gender, patient vs healthy populations) and study outcomes (different cognitive tests, dementia incidence) may produce different results. The particular antioxidant under investigation – vitamin C, E, beta-carotene, flavonoids, or a combination of these – may alter the outcome. Also, vitamins and other antioxidants have multiple functions in the body, so any effect may be attributable to factors other than its antioxidant activity.

4.2.5.1 Epidemiological studies of antioxidant vitamins and cognition

Studies that directly assess the relationship between antioxidants and cognition or dementia in older adults have produced inconsistent results. These studies have investigated blood levels of antioxidant vitamins, dietary intake, and use of supplements, and how these relate to cognition. Some examples from large epidemiological studies are discussed here.

Various studies have reported that blood levels of the antioxidants β-carotene, vitamins A, C and E are significantly different between dementia sufferers and normal controls. Specifically, individuals suffering from vascular dementia or Alzheimer's dementia were found to have significantly depleted vitamins A, C, and E and those suffering from vascular dementia also show lower beta-carotene (Foy, Passmore, Vahidassr, Young, & Lawson, 1999). Similarly, increased plasma vitamin C (La Rue et al., 1997) and serum vitamin E and dietary vitamin E intake (Ortega et al., 2002) have been associated with better cognitive performance in healthy elderly people.

Antioxidant vitamins are obtained in the diet predominantly via fruit and vegetable intake (Braun & Cohen, 2010), and studies have shown a benefit for diets rich in these foods. For example, Polidori (2009) divided participants into groups of high versus low fruit and vegetable intake. Blood levels of vitamin E and lycopene were greater in the

high intake group than the low intake group. Cognitive scores were also better in the high intake group, and blood vitamin E and carotenoids were correlated with cognitive performance. Similarly, in the Nurses' Health Study, a large longitudinal study, researchers investigated fruit and vegetable consumption and cognitive performance in more than 13,000 aging women. They found that greater intake of some vegetables, particularly green leafy and cruciferous vegetables, was associated with better cognitive performance. However other foods such as legumes or fruit intakes, which are also known to have high antioxidant capacity, were unrelated (J. H. Kang, Ascherio, & Grodstein, 2005). The Hordaland Health study also found fruit and vegetable intake was associated with better cognition in the elderly, and this was observed in a dose-dependent manner (Nurk et al., 2010).

Estimated intakes of particular antioxidant vitamins have also been studied. In one study, an association between past dietary intake of vitamin A and vitamin E with cognitive performance was observed, where past status was recorded six years before cognitive testing (La Rue et al., 1997). Dietary intake and antioxidant supplement use was assessed in the Rotterdam Study. It was reported that high baseline intake of vitamin C and vitamin E from food – but not from supplements – was associated with lower risk of developing Alzheimer's disease during the six-year follow up (M.J. Engelhart et al., 2002). These studies conflict with results reported by the Honolulu-Asia Aging Study, a longitudinal investigation into the health characteristics of more than 8000 Japanese-American men that began in 1965. They reported no protection against dementia for dietary intake of antioxidants (Laurin, Masaki, Foley, White, & Launer, 2004). On the contrary, use of antioxidant supplements was shown to be favourable, particularly for long term users. Use of combined vitamin C and E supplementation was significantly associated with better cognitive function and reduced incidence of vascular dementia, and the effect was stronger for long term use of this combination. There was no protective effect observed for Alzheimer's dementia (Masaki et al., 2000).

Other reports on supplement use have been inconsistent. For example, in the Nurses' Health Study, female nurses aged 70 to 79 who were long term users of combined vitamin C and E supplements demonstrated better performance on several cognitive

tests than those who had never used supplements, with a trend towards better performance for longer duration of use (Grodstein, Chen, & Willett, 2003). Another large study investigated antioxidant use over a 10-year period in participants aged 65 years or more. Although they found that current users of antioxidant supplements were about a third less likely to develop cognitive impairment or decline, they did not find any further benefit for participants who had supplemented the long term, or between high and low dose users (Gray et al., 2003).

4.2.5.2 Supplementing vs. dietary antioxidants

As described above, there are sometimes conflicting reports regarding antioxidant intake, depending on whether the antioxidants were obtained in the diet or as a supplement. A distinction between dietary and supplemental sources of antioxidants may be important for several reasons. Firstly, different sources might differ critically in their biochemical properties. For example, the various forms of natural vitamin E have different levels of activity and synthetic forms (which may be used in supplements) differ from natural forms, having reduced bioavailablity (Burton et al., 1998) and biological activity (Azzi & Stocker, 2000). Importantly, α-tocopherol has functions that are specific to that form and not necessarily related to their antioxidant function (Azzi & Stocker, 2000).

A further reason to distinguish dietary and supplemental vitamins is that supplements might only provide benefit to those who have low dietary intake. For example, in the Chicago Health and Aging Project (CHAP) it was observed that healthy elderly people with higher intake of vitamin E from either diet or supplements had less cognitive decline over three years than those with low vitamin E intake (M. C. Morris, Evans, Bienias, Tangney, & Wilson, 2002). However they also found that supplementing with vitamin E had a protective effect only for people who had low dietary intake of vitamin E; for those with high dietary vitamin E no further protective effect was found. Similar findings were observed in the Nurses' Health Study discussed above, where benefits of supplementation were more apparent in those with low dietary intake of α-tocopherol (Grodstein et al., 2003). Furthermore, in another report on the CHAP, the researchers observed a dose-response protective effect of dietary vitamin E on incidence of

Alzheimer's disease, whereas supplements did not provide any benefit (M. C. Morris et al., 2002). Taken together, these studies suggest that supplementing over satisfactory dietary intake of vitamin E may not provide any additional benefit. In contrast, the use of vitamin C and vitamin E supplements was associated with less cognitive decline in the Honolulu-Asia Aging Study whereas dietary intake was not (Laurin et al., 2004; Masaki et al., 2000), suggesting that supplementation is in itself beneficial.

Another consideration is that antioxidant vitamins may act synergistically, providing greater benefit when taken together. One role of vitamin C is to 'recycle' oxidized vitamin E (Bender, 2003), which implies that vitamin E may be more effective when taken with vitamin C. Interestingly, participants of the Nurses' Health Study who used vitamin C and vitamin E together demonstrated better cognitive performance that those who used either supplement singly (Grodstein et al., 2003). Similarly, current use of both vitamin C and vitamin E combined was associated with a reduced incidence of vascular dementia and mixed dementia, whereas use of either supplement alone was unrelated (Masaki et al., 2000). In another example, researchers examined elderly people aged 65 years or more and found that those who supplemented with vitamin E in combination with vitamin C (or multivitamins containing vitamin C) had a significantly reduced prevalence of dementia at baseline and a reduced incidence at a three-year follow-up (Zandi et al., 2004).

These epidemiological studies all suggest a benefit of supplementing with antioxidant vitamins. However for best evidence, efficacy needs to be tested in randomized controlled trials.

4.2.5.3 Intervention studies of antioxidant vitamins for cognition

Several large randomized controlled trials have been conducted to investigate the effects of antioxidant supplements on cognition or dementia, with conflicting results. A trial of vitamin E supplementation in MCI patients found no benefit after three years of supplementation, with supplement and placebo groups equally likely to convert to dementia (Ronald C Petersen et al., 2005). This is in contrast to an earlier study of vitamin E which was demonstrated to slow the progression of Alzheimer's disease after

two years supplementation, in elderly people with moderately severe dementia (Sano et al., 1997).

Studies investigating cognitive performance in healthy participants are similarly inconsistent. One large study investigated women over 65 years who had cardiovascular disease or associated risk factors, providing antioxidant supplementation with vitamin E, vitamin C or beta carotene for five years. Antioxidants did not improve cognitive function (measured by the TICS) over five years, although a small post-treatment association between vitamin C and cognitive performance was observed (J. H. Kang et al., 2009). Similarly, the Women's Health Study investigated vitamin E in healthy elderly women for five years and did not observe any benefit to cognition (Jae Hee Kang, Cook, Manson, Buring, & Grodstein, 2006). In contrast to these findings, the SUVIMAX study used a combination supplement containing vitamin C, vitamin E, beta-carotene, zinc and selenium (zinc and selenium are mineral antioxidants) over four years, in participants aged 45-60 years. Participants in the supplement group demonstrated better episodic memory, and for non-smokers with low baseline vitamin C, verbal memory was also improved (Kesse-Guyot et al., 2011).

The Physicians' Health Study has also demonstrated cognitive benefits with antioxidant supplements. Researchers investigated beta carotene supplementation in male physicians, and found an association between beta-carotene supplementation and cognition. This was observed in participants who had been supplementing over the long term (mean 18 years), and not in those who had been supplementing for only one year, suggesting that benefits are slow to appear or that they occur at early stages in the disease process (Grodstein, Kang, Glynn, Cook, & Gaziano, 2007).

That positive outcomes were observed in several large studies indicates that a benefit of antioxidants for cognition is likely, at least under certain circumstances. However the heterogeneity of the studies makes it difficult to discern exactly what these circumstances are, particularly when seemingly similar studies have opposing outcomes. To find answers to this it may help to look more closely at mechanisms of action of supplements. For example, an interesting study conducted by Lloret et al (2009) looked at markers of oxidative stress in Alzheimer's disease patients after six

months of vitamin E supplementation. They found that "responders" to vitamin E, that is, those with reduced oxidative stress markers, maintained or improved cognitive performance across six months. On the other hand, "non-responders", those with oxidative stress markers unchanged, deteriorated. They concluded that it is important to assess the efficacy of supplementation in reducing oxidative stress in the individual before prescribing vitamin E supplement. This study has important implications for interpretation of past research findings as well as for the design of future studies; if different people respond differently to a supplement, this could go some way to explaining the inconsistent results found in both cognitive and cardiovascular research. It also highlights advantage of assessing blood biomarkers in trials of vitamin supplements.

4.3 Vitamin D

Vitamin D has been associated with a range of psychiatric conditions and more recently has been investigated for a role in cognitive decline and dementia. An association between vitamin D status and cognitive decline has been supported by recent meta-analyses of cross-sectional studies. One meta-analysis of 10 studies indicated that Vitamin D was significantly lower in Alzheimer's disease patients compared to normal control subjects (Annweiler, Llewellyn, & Beauchet, 2013). Another similarly found a reduced risk for Alzheimer's disease with higher vitamin D levels, and also found an association between vitamin D and cognitive function or cognitive impairment (Balion et al., 2012).

Supporting this, increasing biological evidence corroborates a relationship between cognition and vitamin D, with testimony coming from various disciplines. The biological rationale for vitamin D in cognitive impairment is plausible, with proinflammatory cytokines playing a role (McCann & Ames, 2008). It has been argued that anti-inflammatory activity of vitamin D may protect against Alzheimer's disease pathology; a study comparing aged and young rats demonstrated a significant impairment in learning in the aged rats which was improved with vitamin D supplementation, via reduction of age-related changes in inflammation (Briones &

Darwish, 2012). Further research has indicated that vitamin D receptors are found in the brain, including in the hippocampus, an important structure for memory, and animal studies suggest that vitamin D affects neurotransmitter function, particularly the dopaminergic system (Eyles, Burne, & McGrath, 2013). In elderly adults, vitamin D deficiency was correlated with larger cerebral ventricles, suggesting it may be involved in brain atrophy (Annweiler, Montero-Odasso, et al., 2013).

Despite these associations there has been little investigation of the effects of vitamin D supplementation for cognition. Two trials have found opposing results. The first found no benefit for cognition after four weeks of supplementation (Przybelski et al., 2008). The second did observe a benefit, in particular for executive functions (Annweiler et al., 2012). However this study was not a randomized trial; participants who were prescribed vitamin D for any reason were compared with those who were not. Further intervention studies in humans are needed.

4.4 Phytonutrients

Phytonutrients are various compounds found in food that have potential health benefits, although they are not essential in the diet (Cassidy & Kay, 2011). Classes of compounds which have been studied for their health benefits include the polyphenols, phytoestrogens, sterols and stanols, and glucosinolates (Salter & Andrew, 2012). These have various effects in the body, for example, sterols and stanols have been demonstrated to lower cholesterol (Buttriss, 2012). Also the phytoestrogens, in particular soy isoflavones, have been investigated for their beneficial actions in cognitive and cardiovascular health, and benefits have been observed for cholesterol levels, arterial stiffness and cognitive function (Wiseman, 2012).

Among the more beneficial compounds are the flavonoids, a group of polyphenols that occur abundantly in foods. They are particularly found in fruit and vegetables, often as the pigment providing colour (Bender, 2003). Examples include the flavonols, which are found in tea and grapes; flavanones found in citrus; and anthocyanidins, which are the purple pigments found in berries. Herbs such as Ginkgo biloba and St Mary's thistle

also have high flavonoid content (Braun & Cohen, 2010). Flavonoids have been proposed as the reason that diets high in fruits and vegetables are protective against cardiovascular disease and cognitive decline (Hooper et al., 2008).

Initially the benefits provided by flavonoids were thought to be via antioxidant mechanisms (Halliwell, Rafter, & Jenner, 2005) but more recently additional functions have been discovered. For the prevention of cardiovascular disease these include inhibition of inflammation and antiplatelet activities (García-Lafuente, Guillamón, Villares, Rostagno, & Martínez, 2009). In relation to brain function, flavonoids provide protection against neuroinflammation, enhance neural function, inhibit neurodegeneration, and improve cerebral blood flow, which may enable neurogenesis in the hippocampus (J. P. E. Spencer, 2009).

Many of the aforementioned fruits, vegetables and other plant foods have been studied for their benefits to cognitive and cardiovascular health. One example that has received significant attention is Ginkgo biloba, which is contained in substantial amounts in the supplements used in the present studies.

4.4.1 Ginkgo biloba

Ginkgo biloba is a species of tree whose leaves are used in herbal medicine, particularly Chinese medicine (Mahadevan & Park, 2008). Because the chemical composition of the leaves vary according the plant's origin and the seasons, extractions may be standardized. The extract EGb 761 is a standardized ginkgo extract that is used in many studies. Use of this extract is advantageous because it provides consistent bioactivity and enhances comparability between studies.

Ginkgo leaves contain abundant bioactive substances, including terpenoids and many flavonoids (Mahadevan & Park, 2008). As such it has antioxidant activity, but it also exhibits several other activities that are relevant to cognitive and cardiovascular health. Effects that are relevant to cognition include effects on neurotransmitter function, and neuroprotective actions including defence against beta-amyloid neurotoxicity and cell

damage due to ischemia (Ahlemeyer & Krieglstein, 2003). In vitro studies have demonstrated these beneficial actions of ginkgo extracts. In one example, ginkgo was protective against beta amyloid toxicity, preventing death of cultured hippocampal cells (Bastianetto et al., 2000). In neuroblastoma cells ginkgo similarly prevented cell apoptosis, possibly via antioxidant effects (Shi et al., 2009).

Despite these promising findings in vitro studies, intervention studies using ginkgo extracts have not demonstrated consistent benefits to cognition. In 2009, a Cochrane Review concluded that the evidence for cognitive benefit in healthy elderly or dementia patients was unconvincing (Birks & Evans, 2009). They referred to recent large studies, the majority of which had failed to show any benefits. Contrary to this, another systematic review determined that there was some benefit for Alzheimer's disease. They reviewed studies of at least 16 weeks duration. Although there was substantial heterogeneity in results, they concluded there was a benefit in activities of daily living for studies which used high-dose extracts. There was also a suggested benefit for cognitive function and psychopathological symptoms. Notably, this review was specific to Alzheimer's disease whereas the Cochrane review included all types of dementia. This could possibly dilute the benefits, particularly if the protective action of ginkgo is related to beta amyloid, a hallmark of Alzheimer's disease.

The cardiovascular effects of ginkgo are better established and multiple cardiovascular benefits have been demonstrated with ginkgo extracts. Endothelial effects include enhancement of NO production, improved endothelial function, protection of vascular endothelium against oxidative stress, and enhanced endothelium-dependent vasodilation (Koltermann et al., 2007; X. S. Li, Zheng, Lou, Lu, & Ye, 2009; Y. Wu et al., 2008). Clinical benefits have also been demonstrated for cardiovascular diseases. In healthy elderly and in patients with coronary artery disease, improvement in coronary circulation was observed (Y. Wu et al., 2008; Y. Z. Wu, Li, Zu, Du, & Wang, 2008). Other clinical benefits that have been demonstrated include improved symptoms in peripheral arterial disease and small vessel disease, and decreased platelet aggregation in thrombosis (Zhou et al., 2004).

4.5 Minerals

Minerals in the nutritional context are inorganic elements that are required by the body for normal functioning (E. P. Solomon, Berg, Martin, & Ville, 1996). Essential trace minerals are required in minute microgram amounts and include iron, iodine, zinc, chromium, selenium, manganese, molybdenum and copper. Other minerals are required in larger amounts. These are calcium, magnesium, phosphorous, potassium and sodium(E. P. Solomon et al., 1996). The essential minerals have minimum daily requirements and are often found in multivitamin supplements, including those used in the present research (listed in Table 7.1). It is also important to note that some minerals, particularly the essential trace minerals, are toxic in high amounts (Frausto da Silva & Williams, 2001).

Iron, calcium and phosphate are stored in the body and can be used at times of dietary deficiency, but other minerals need to be regularly supplied in the diet (Brody, 1998). Overt deficiency of minerals in industrialized countries is rare, with the exception of iron deficiency anaemia (Webb, 2011). However, as for vitamins, the elderly are more likely to be undernourished and therefore may benefit from supplementation (Margetts, Thompson, Elia, & Jackson, 2003; Troesch, Eggersdorfer, & Weber, 2012).

The quantity of research investigating supplementation with single minerals in relation to cognitive and cardiovascular health varies from mineral to mineral, although on the whole this area is relatively unexplored. In this section each mineral is briefly addressed except for copper, manganese and molybdenum for which there were no supplementation trials in humans.

Calcium supplements are widely used for bone health, but in relation to cognition there is little research available. One large study that investigated calcium in combination with vitamin D observed no benefit for incident cognitive impairment in women aged 65 years plus (Rossom et al., 2012). Evidence for their use in cardiovascular health is conflicting. Although some trials have indicated a benefit for reduction of hypertension, a Cochrane review concluded the evidence for this is weak (Dickinson, Nicolson, Cook, et al., 2006). Lately the use of calcium supplements has been associated with increased

cardiovascular risk. Studies have observed an increased risk of myocardial infarction and possibly cardiovascular-related death for those who supplement with calcium (Bolland et al., 2010; Xiao et al., 2013).

Chromium has been studied for its beneficial effects on insulin sensitivity but there is relatively little research on the cognitive and cardiovascular effects of this mineral. Age-related decreases in chromium levels have been demonstrated (S. Davies, Howard, Hunnisett, & Howard, 1997). Chromium supplementation for 12 weeks improved inhibitory control and increased activation in several brain regions in the only study of this mineral for cognition (Krikorian, Eliassen, Boespflug, Nash, & Shidler, 2010). Chromium supplementation may also reduce LDL cholesterol (Press, Geller, & Evans, 1990; Rabinovitz et al., 2004).

Iron is the mineral that is most susceptible to deficiency, although elderly people are not especially susceptible (Hercberg, Preziosi, & Galan, 2001). Iron deficiency anaemia can cause cognitive and cardiovascular impairment; however care must be taken with supplementation because high levels of iron storage in the body are associated with endothelial dysfunction and cardiovascular events (Jelani & Katz, 2010). A recent meta-analysis of trials of iron supplementation concluded there was some evidence for a benefit to attention, concentration, and intelligence in children and adults, but there were no studies investigating effects in elderly adults (Falkingham et al., 2010).

Magnesium is involved in synaptic transmission, which theoretically could be impacted by deficiency (Billard, 2006). Studies in humans are scarce, although a study in a mouse model demonstrated improved learning with increased brain magnesium (Slutsky et al., 2010). Magnesium supplementation is used as a treatment for depression, demonstrating it has psychoactive capacity (Eby & Eby, 2010), but whether it has a role in cognitive aging has not been investigated. Magnesium deficiency is implicated in cardiovascular disease and higher magnesium intake has been associated with reduced risk of coronary heart disease (Al-Delaimy, Rimm, Willett, Stampfer, & Hu, 2004; Burford-Mason, 2013). Furthermore a recent meta-analysis concluded that circulating and dietary magnesium is protective against cardiovascular disease (Del Gobbo et al., 2013). In contrast, an earlier meta-analysis of trials of magnesium supplementation in essential

hypertension concluded there was only weak evidence of a benefit (Dickinson, Nicolson, Campbell, et al., 2006).

Phosphorus is necessary for bone health but increased intake is occurring due to the inclusion of phosphorus in processed foods (Takeda, Yamamoto, Yamanaka-Okumura, & Taketani, 2012). High phosphorus in serum is associated with arterial stiffness (Ix et al., 2009) and high dietary phosphorous was demonstrated to decrease flow-mediated dilation, indicating impaired endothelial function (Shuto et al., 2009). Phosphorus has not been examined in relation to cognitive aging.

Potassium has potential benefits to cardiovascular health, and supplementation reduces hypertension, particularly salt-induced hypertension (Whelton & He, 1999). This may occur via antioxidant mechanisms (Ando, Matsui, Fujita, & Fujita, 2010). A recent four-week trial of potassium supplementation in healthy participants found no changes to blood pressure and augmentation index but a small increase in pulse wave velocity, indicating greater arterial stiffness (Matthesen, Larsen, Vase, Lauridsen, & Pedersen, 2012). This suggests that the benefits of potassium supplementation may apply only to those with increased salt intake. Potassium supplementation has not been investigated for cognition.

Selenium acts as an antioxidant and blood level decreases are observed with increasing age (K. Park et al., 2011). Reduced selenium is also associated with cognitive decline (Rayman, 2012) and it has also been widely studied in relation to Alzheimer's disease, where altered levels may play a role in pathogenesis. Proposed mechanisms include formation of amyloid plaques and neurofibrillary tangles, alteration of neurotransmitter metabolism, and antioxidant actions (Loef, Schrauzer, & Walach, 2011). Despite this, a systematic review of trials of selenium supplementation concluded there is no evidence of benefit for cognitive decline or Alzheimer's dementia (Loef et al., 2011). Selenium has also been frequently investigated in relation to protection against cardiovascular disease, since low selenium levels are associated with an increased risk of coronary heart disease (Flores-Mateo, Navas-Acien, Pastor-Barriuso, & Guallar, 2006). Beneficial actions of selenium that may reduce risk include anti-inflammatory activity, inhibition of platelet aggregation and prevention of lipid oxidation (Rayman, 2012).

While a recent meta-analysis of trials of selenium supplementation for cardiovascular health concluded that selenium provides no protection (Rees et al., 2013), others contend that the existing data is inconclusive, with methodological issues and a lack of mechanistic understanding accounting for the inconsistent findings in clinical studies (Joseph, 2012).

Zinc has been studied in regard to cognitive development of children (Gogia & Sachdev, 2012) but there is less research on its role in cognitive aging. Animal studies suggest that zinc may play a role in learning and memory, and particularly age-related decline (Mott & Dingledine, 2011). Furthermore, zinc levels in plasma were related to MMSE score in older Europeans (Marcellini et al., 2006). One trial of zinc supplementation found an improvement in spatial memory in healthy older adults after three months (Maylor et al., 2006). However, the role of supplementation for cognitive aging remains uncertain (Mocchegiani, Bertoni-Freddari, Marcellini, & Malavolta, 2005).

In summary, low levels of several minerals are associated with cognitive and cardiovascular impairment in the elderly. Mineral deficiencies are common in the elderly and supplementation has potential to address this (Ervin & Kennedy-Stephenson, 2002). On the other hand, excessive intake of certain minerals can be detrimental. For cognitive and cardiovascular aging research on minerals is scarce and inconclusive; our understanding could be greatly improved with further randomized controlled trials of mineral supplements.

4.6 Summary and conclusion

This chapter has provided an overview of vitamins, minerals and herbal extracts, as they relate to cardiovascular and cognitive health. For many supplements there is some evidence of benefits; however trials of particular nutrients are inconsistent and the clinical benefits of supplements, if any, are yet to be resolved. Nevertheless, this chapter has demonstrated that there is a good rationale for studying supplements for cognitive and cardiovascular health.

The broad overview provided in this chapter will become more focussed in the following two chapters. While this chapter has focussed on individual nutrients or classes of nutrients, it has not addressed the possible synergistic effects that may occur by providing a range of nutrients together in a multivitamin. In Chapter 5, studies investigating multivitamin supplements for cognition in older adults are systematically reviewed, taking a more detailed approach. Chapter 6 similarly involves an in-depth review, examining the effects of vitamin supplements on augmentation index.

5 Multivitamins and Cognition in the Elderly

The previous three chapters have provided overviews of the scientific literature in three broad subjects: cognitive aging; cardiovascular aging and cognition; and nutritional supplementation. In this chapter the focus becomes more specific, with a short review of randomized controlled trials of multivitamin supplementation for cognition in the elderly. Trials of the elderly were chosen in particular because the cognitive changes that take place in old age make this group qualitatively different from younger adults and children. This provides a context for the intervention studies in this thesis, and lead into the aims and hypotheses for the research.

While Chapter 4 provided a review of individual nutrients, here the review is specific to multivitamins. Multivitamin supplements are worthy of separate consideration because they contain a mix of ingredients which may provide a different outcome when compared to a single vitamin or class of vitamins. Because different nutrients may act at different stages of a metabolic process, multiple nutrients might be required to complete a given process. This can be seen where vitamin B_{12} and folate are both required in the conversion of homocysteine to methionine: a greater effect on homocysteine is observed with combination supplements (Homocysteine Lowering Trialists' Collaboration, 2005). The same argument applies to different classes of vitamins. For example, various B vitamins and antioxidant vitamins have been demonstrated to improve NO production through different processes, suggesting maximum effect would be gained when combining them (G. Wu & Meininger, 2002). Thus, provision of a range of nutrients in one supplement has a theoretical advantage and may result in stronger effects. A further consideration is that participating subjects might be deficient in or have suboptimal levels of different vitamins due to their individual diets, so there is a greater chance of redressing any deficiencies.

The nutritional supplement industry is growing and was worth $23 billion in the US and $1.8 billion in Australia in 2012 (Euromonitor International Ltd, 2013a, 2013b). Multivitamins in particular are a popular choice of supplement (Millen, Dodd, & Subar, 2004; Murphy, White, Park, & Sharma, 2007). A national survey in Australia reported

that around 15% of adults over 50 years used multivitamin supplements (Morgan et al., 2012). Despite this, the majority of research has focussed on single vitamins or combinations of a select few nutrients. Given the possible advantage of wide-range multivitamins described above, including synergistic effects or the capacity to address a range of insufficiencies, it is worthwhile to examine the data on multivitamins as a distinct entity.

The difficulty of this approach is that there are endless ways that a "multivitamin" might be formulated and there is no standard that exists against which to compare any given formulation. For the purpose of this review multivitamins were considered to be any supplement that contained combinations of vitamins from at least two classes: the B-group vitamins, antioxidant vitamins and other vitamins (e.g. vitamin D). Investigations of supplements which additionally contained essential minerals, herbal ingredients and flavonoids were also included. Studies which included supplements with other potentially psychoactive ingredients such as caffeine or omega-3 were excluded. This definition was formed in order to assess what might commonly be thought of as a commercial multivitamin supplement without the confounding effects of other potentially psychoactive substances.

The definition of "older adults" here describes those at least 50 years of age. Studies involving healthy participants and those with cognitive impairment were considered. The focus on older adults is due to their increased susceptibility to cognitive impairment and brain deterioration. Because of these age-related changes the effects of interventions may differ in older adults, making them a qualitatively different subject group than younger adults and deserving of independent consideration. Furthermore, older adults are more likely to suffer nutritional deficiency due to decreased food intake and problems with nutrient absorption (Wahlqvist & Tienboon, 2011). Therefore an older group might be particularly disposed to benefit from supplementation.

The intention of this section is to scrutinise the studies to discover the differences between those that have found benefits with supplements and those that have not. What characteristics of the trial are important: duration, participants, type of cognitive assessment, or specific supplement ingredients? This will help to apprise what might be

the best approach to assessing efficacy of supplements, and also what results might be expected from the intervention trials in this thesis. Accordingly, this review will help to inform the aims and hypotheses of the present research.

5.1 Intervention studies of multivitamin supplements in the elderly

The Scopus database was searched for intervention studies using combination supplements for cognitive impairment. Given the fairly broad definitions described above, it was surprising to find few randomized trials that examined the effects of multivitamins on older adults. A summary of the ten studies identified is provided in Table 5.1.

Two of the ten studies identified investigated multivitamin effects in elderly people with dementia. It is worthwhile to consider these separately because cognitive function in dementia is different to normal aging due to the severity of cognitive impairment. These two studies found no effect on cognition after supplementation. The earlier of these was a small study (n=15) which used a limited range of vitamins over six weeks (Burns, Marsh, & Bender, 1989). Despite the lack of cognitive (MMSE) and behavioural changes, the supplements did appear to benefit the patients; carers reported improved appetite and weight gain in the multivitamin group.

The other study of dementia patients was larger, including 89 participants with mild to moderate dementia (Sun, Lu, Chien, Chen, & Chen, 2007). The lack of effect in this study is a more convincing finding given its larger sample size and more comprehensive cognitive testing. In addition to the MMSE the investigators used the Alzheimer's Disease Assessment Scale – Cognitive subscale (ADAS-Cog), a battery which includes language, comprehension, recall, and naming; as well as the Cognitive Abilities Screening Instrument, a battery incorporating attention, orientation, memory, construction and fluency tasks. The intervention took place through six months, with a broad range of vitamins and minerals. The main drawback of this study is that both supplement and control group participants were additionally prescribed angiotensin-converting enzyme (ACE) inhibitors, which may have obscured potential benefits of

Table 5.1. Studies examining the effect of multivitamin supplementation on older adults

Study (first author, year)	**Supplement**	**Duration**	***n***	**Participants**	**Measures**	**Results**
Burns, 1989	Vitamins B1, B_2, B_3, B_6 + Vitamin C	6 weeks	15	Dementia patients in care, mean age 81	MMSE, behaviour disturbance.	No cognitive effects. No effect on behaviour disturbance measure.
Sun, 2007	ACE + MV + minerals vs. ACE + placebo	26 weeks	89	Mild to moderate dementia, 50+	ADAS-Cog battery, Cognitive Abilities Screening Instrument, MMSE.	No cognitive effects.
Cockle, 2000	Vitamins A + Bs + C	24 weeks	139	Healthy elderly, 60-83	Critical flicker fusion, choice RT, memory scanning, word recognition, MMSE. Blood vitamins.	No cognitive effects. Increased vitamin levels.
Gariballa, 2007	MV + minerals + carb, fat, protein (in drink)	6 weeks	445	Hospitalized elderly, 65+	Abbreviated mental test.	No cognitive effects.
McNeill, 2007	MV + minerals	12 months	910	Healthy elderly, 65+	Digit span, verbal fluency.	No cognitive effects.
Wolters, 2005	MV + minerals	6 months	220	Healthy females, 60-91	WAIS symbol search, pattern recognition. Vitamins, homocysteine, MMA.	No cognitive effects. Vitamins increased, homocysteine decreased.

Continued…

Harris, 2012	MV + minerals + herbs + flavonoids	8 weeks	51	Sedentary men, 50-74	Computerized cognitive battery. Vitamin B_{12}, folate, homocysteine	Improved performance on contextual memory subtest only. Vitamin B_{12} and folate increased, homocysteine decreased.
Macpherson, 2012	MV + minerals + herbs + flavonoids + probiotic	16 weeks	56	Women with subjective memory complaints, 64-82	Computerized cognitive battery. Vitamin B_{12}, folate, homocysteine	Improved performance on spatial working memory subtest only. Vitamin B_{12} and folate increased, homocysteine decreased.
Summers, 2010	MV + minerals + herbs (incl flavonoids, ginkgo, ginseng) + amino acids	4 months	113	Independently living with no dementia, 50-75	MMSE, name learning paired associates, word recall. Homocysteine	Improved performance on name learning and word recall. Homocysteine decreased.
Wouters-Weseling, 2005	MV + minerals + plant extracts /flavonoids + protein, fats (enriched drink)	6 months	67	"frail" elderly in assisted living, no dementia, 65+	Word learning, category fluency, word recognition. Vitamin B_{12}, homocysteine	Improved performance on word learning and category fluency. Vitamin B_{12} increased and homocysteine decreased.

MV – multivitamin; ACE – acetylcholinesterase inhibitor

supplementation. Nevertheless, the outcome of these two studies is not unexpected. As mentioned in the previous chapter, it might be difficult to reverse cognitive impairment with nutritional supplementation. With the exception of vitamin E (Sano et al., 1997) many other studies of vitamins have not been able to produce cognitive improvements in dementia sufferers (Malouf & Evans, 2008; R. C. Petersen et al., 2005), and these two studies of multivitamins in dementia patients concur.

The remaining eight studies were in elderly adults with no diagnosed cognitive impairment. Four of these observed improved cognitive performance after a multivitamin supplement intervention (E. Harris et al., 2012; MacPherson, Ellis, Sali, & Pipingas, 2012; Summers, Martin, Cunningham, Deboynton, & Marsh, 2010; Wouters-Wesseling et al., 2005). It is not immediately clear why these four were able to demonstrate benefits where the others were not. Overall, the study durations ranged from six weeks to 12 months, and in studies with positive results the supplementation period ranged from eight weeks to six months. Thus, it does not appear that duration of supplementation was a crucial factor. The subject groups were somewhat heterogeneous, including healthy elderly or those with risk factors including memory complaints, sedentary lifestyle or general frailty. Two of the studies that found improvement with supplementation included participants as young as 50 years of age (E. Harris et al., 2012; Summers et al., 2010).

Sample sizes ranged from 15 to 910 participants and interestingly it was some of the smaller studies which found benefits. While smaller studies are more likely to produce a type 1 error, or false positive result (Christley, 2010), this is not a likely explanation in for these investigations. The studies found cognitive changes in the anticipated direction (i.e. improvement, not worsening) and in tasks that were more likely to benefit (i.e. memory).

The tasks used in the different studies were varied, but it is notable that benefits were observed on memory tasks in all four positive studies, with additional benefits in category fluency in one study (Wouters-Wesseling et al., 2005). This is pertinent because memory is particularly susceptible to decline in the elderly and may therefore be more responsive to intervention. However, studies with negative findings also tested

memory with no evidence of benefit (Cockle, Haller, Kimber, Dawe, & Hindmarch, 2000; McNeill et al., 2007; Sun et al., 2007). A meta-analysis which examined multivitamins and cognition in adults of all ages determined that after combining data across studies, there was an effect of multivitamins on immediate free recall but not on other measures (Grima, Pase, MacPherson, & Pipingas, 2012). Results from the individual studies in older adults suggest that multivitamin supplementation affects a variety of memory tasks, which could indicate the more widespread impairment in the cognitive functioning of older adults.

More sensitive cognitive tests such as a computerized test battery might be more likely to detect a response to intervention, and these were used in two of the studies that had positive finding (E. Harris et al., 2012; MacPherson et al., 2012). Conversely, insensitive tests such as the MMSE and the AMT (abbreviated mental test) are unlikely to find small changes even in a fairly large sample, due to the substantial improvement in cognition required to improve the score.

One important difference between the positive and negative findings is that the supplements used in the four positive studies included ingredients in addition to vitamins and minerals. Each of them contained plant extracts or flavonoids. In the studies from our group the supplements contained additional herbal ingredients, including flavonoids and various herbal ingredients. The study by Wouters-Weseling (2005) involved an enriched drink which also contained herbal extracts and flavonoids, as well as protein, carbohydrate and fats. The supplement used in Summers (2010) included a wide range of vitamins and minerals, plus herbal ingredients and other plant extracts. By contrast, only one of the negative studies contained extra ingredients, and this study used a fairly insensitive cognitive measure, the Abbreviated Mental Test (Gariballa & Forster, 2007). This raises the possibility that flavonoids in particular are a beneficial addition to multivitamin supplements.

It is also worth noting that changes were observed in relevant blood biomarkers in all four of the positive studies. Only two of the negative studies measured bloods, and these results also indicated changes in beneficial directions. However these findings are in keeping with the hypothesis that positive physiological changes are required in order to

elicit cognitive benefits. Assessment of blood biomarkers is important for corroboration of cognitive findings.

5.2 Summary and conclusion

In summary, the evidence for an effect of multivitamin supplementation on cognition in older adults remains uncertain. In dementia patients cognitive benefits are doubtful. In healthy older adults the results are more promising, particularly for supplements containing plant extracts in addition to broad-spectrum vitamins and minerals. However further research is needed to clarify these effects.

The present studies investigate the effects on older adults of broad-spectrum multivitamin supplements in a way that optimises the chances of observing an effect, if one exists. The studies use the same sensitive computerized cognitive battery that was used in two of the studies with positive findings (E. Harris et al., 2012; MacPherson et al., 2012). Blood biomarkers are also included to assess whether uptake of the supplements is achieved and physiological responses are observed. Assessment of augmentation index is also included to examine potential mediating effects of cardiovascular function.

6 Vitamin Supplements and Augmentation Index

Two studies in this thesis examine the effects of multivitamin supplementation on cognition and a range of risk factors, including cardiovascular function. The variable that is being used to assess cardiovascular function is augmentation index. This is a relatively new measure of arterial stiffness that is gaining in popularity, but as yet there are relatively few trials examining the effects of vitamin supplementation on this measure.

This chapter comprises a review of randomized controlled trials of the effects of various vitamin supplements on augmentation index. It asks specifically whether augmentation index is sensitive to intervention with vitamin supplementation, that is, can supplements affect cardiovascular function in a way that can be measured by augmentation index? If so, which nutrients have demonstrated such an effect? Augmentation index is a primary outcome measure in the studies in this thesis and this review will help to inform us why changes due to multivitamin supplementation might be expected.

The purpose of this chapter is to comprehensively review studies that examine the effect of vitamin supplementation on augmentation index. The aim is to ascertain whether augmentation index is indeed sensitive to different forms of nutritional supplementation, and which nutrients might provide cardiovascular benefits that can be measured by augmentation index.

As discussed in Chapter 4, B-group and antioxidant vitamin supplements have various effects on the cardiovascular system including the arterial tree. A recent review which examined effects on various arterial stiffness measures for nutritional supplements found that omega-3 and soy isoflavones improved arterial stiffness (Pase et al., 2011). This indicates that arterial stiffness can be modified by supplementation. However this review determined that there was insufficient evidence in relation to other nutrients. This review looks specifically at studies which have used augmentation index as an outcome measure, and focus on nutrients that are found in multivitamin type supplements.

Since there is relatively little research available, this review includes all existing studies that have assessed central augmentation index before and after intervention with vitamin supplements, regardless of the age or health status of the subjects, the intervention period, or whether the study was blinded or open label. This is in order to get the broadest picture possible, while acknowledging the data are limited.

A search was conducted in the Scopus database using the terms "augmentation index" and "vitamin" returned 38 results. Further searches were then conducted replacing "vitamin" with specific names, namely: ascorbic, tocopherol, folate, folic, cobalamin, pyridoxine, pyridoxal, carotenoid, or retinol. Studies were screened to identify those that were randomized, controlled trials, and that used central augmentation index as an outcome measure. Studies that contained additional nutrients to those stated, for example, caffeine or omega 3, were not included. Flavonoid studies were not included because that these were generally conducted using food and drink interventions rather than supplements. Food and drinks might have different effects to a supplement, making these studies less relevant to the present multivitamin studies. Therefore studies of flavonoid interventions were not included. After elimination of studies which did not meet these criteria, 19 studies remained (Table 6.1). These include studies of supplements containing folate, vitamin B_{12}, vitamin C, vitamin E or vitamin D, either singly or in combinations. These are discussed in turn. There were no studies of multivitamin supplements.

6.1 Augmentation index and B vitamins

Few studies have investigated the effects of these B vitamins on augmentation index, and these were all conducted in patients with renal disease. Augmentation index is particularly relevant for renal disease patients because they have increased cardiovascular disease and show increased arterial stiffness. In fact arterial stiffness can cause damage to the kidneys, which can be viewed as a susceptible end-organ in the same way as the brain (Mitchell, 2008). In turn, kidney damage is thought to exacerbate arterial stiffness (Kanbay, Afsar, Gusbeth-Tatomir, & Covic, 2010).

Table 6.1. Trials investigating augmentation index and vitamin supplementation

Author & Year	Supplement	Duration	Participants	Result
Tochihara, 2008	Folic acid, 5mg administered either before or during haemodialysis	Acute	10 patients with end stage renal disease	Decreased augmentation index by 4.7% in patients
Koyama, 2010	Folate 15mg/d +/- Methylcobalamin 500µg 3/week after haemodialysis	3 weeks	40 patients receiving long term haemodialysis	Carotid augmentation index decreased in B_{12} + folate group but not in folate alone. This was an open label study
Zoungas, 2006	Folic acid 15mg/d	3.6 years	315 patients with chronic renal failure, age 24-79	No effect
Wilkinson, 1999	Vitamin C, 2g	Acute	8 healthy males, age 20-42	Augmentation index reduced by 9.6%
Kelly, 2008	Vitamin C, 2g	Acute	26 healthy adults, age 21-26	No effect
Wilkinson, 2001	Methionine 100mg/kg +/-Vitamin C, 2g	Acute	8 healthy males, age 20-42	Combination methionine + vitamin C reduced augmentation index by 10.5% where methionine alone had no effect.
Mullan, 2004	Vitamin C, 2g	Acute	12 healthy males age 20-34	Attenuated or prevented the increase in augmentation index caused by hyperglycaemia
Mullan, 2002	Vitamin C, 500mg/d	4 weeks	30 patients with type 2 diabetes, age 45-70	Augmentation index reduced by 16%, systolic and diastolic pressures reduced by 9.9mmHg and 4.4mmHg respectively

Plantinga, 2007	Vitamin C, 1g/d + Vitamin E, 400 IU/d	8 weeks	30 males with essential hypertension age 42-60	A tendency to reduction of augmentation index (p = 0.09), PWV reduced, FMD improved
Rasool, 2006	Vitamin E (tocotrienol rich), 80, 160 or 320mg/d	2 months	36 healthy males	No effect
Rasool, 2008	Vitamin E (tocotrienol rich), 50, 100 or 200mg/d	2 months	36 healthy males	Augmentation index reduced in 50, 100 and 200mg groups by 6.59%, 8.72% and 6.27% respectively. Pulse wave velocity decreased in 100mg and 200mg groups.
Breslavsky, 2013	Vitamin D 1000 IU/d	1 year	47 diabetic patients	Significant reduction in augmentation index in supplement group with no change in placebo group
Larsen, 2012	Vitamin D 75µg (3000 IU)	20 weeks	130 hypertensive adults	Study took place over dark winter in Denmark. No change in augmentation index. Reduction in central systolic BP
Marckmann, 2012	Vitamin D 40,000 IU per week	8 weeks	52 chronic kidney disease patients with low vitamin D	No effect
Gepner, 2012	Vitamin D3 2500 IU	4 months	114 post-menopausal women	No effect on augmentation index or other CV risk factors

Augmentation index has been demonstrated to predict mortality or survival in patients with end stage renal disease (London et al., 2001).

Folic acid or folate was investigated in three studies including one study where folic acid was administered in combination with vitamin B_{12}. The largest of the studies was a randomized, placebo-controlled trial of 315 patients with chronic renal failure, which found no effect on augmentation index after 3.6 years of daily folic acid supplementation at 15mg, despite a 20% decrease in homocysteine (Zoungas et al., 2006). The authors mentioned that despite significant homocysteine decrease, these levels were not normalized with folate supplementation and this might explain why an effect on cardiovascular variables was not achieved.

A two-arm, open label study investigated three weeks supplementation in 40 patients receiving long term haemodialysis (Koyama et al., 2010). They likewise found no improvement with folate supplementation provided alone, however they did find an improvement in carotid augmentation index with a combination of folate and methylcobalamin (vitamin B_{12}). Biochemical analysis also indicated that methionine, homocysteine and ADMA (asymmetric dimethylarginine, a nitric oxide synthase inhibitor) were reduced after supplementation with either supplement, but more so after the combined supplementation. They attributed the change in augmentation index to the greater success in lowering homocysteine with the combined supplement.

In contrast, an open-label, cross-over study compared the effects of pre- versus intra-haemodialysis doses of 5mg of folic acid in patients with end stage renal disease. A 4.7% reduction in augmentation index was observed when folic acid was supplied intra-haemodialysis, whereas a single oral dose before haemodialysis had no effect (Tochihara, Whiting, Barbara, & Mangoni, 2008). Supplementing intra-haemodialysis also prevented the folate loss due to haemodialysis, suggesting that timing of supplementation and/or absorption is important in this patient group.

These studies provide some limited evidence that arterial benefits from B-group vitamins might be observed with augmentation index. It is uncertain whether any effects

on augmentation index can be observed in healthy adults or the elderly as this has not yet been investigated.

6.2 Augmentation index and vitamin C and vitamin E

Antioxidant vitamins have been studied comprehensively in the context of cardiovascular health (see section 4.2), however only eight studies have directly looked at augmentation index with these supplements.

Four articles report investigations of augmentation index after a single 2g dose of vitamin C in healthy adults. In one study, augmentation index decreased by 9.6% after a single 2mg dose of vitamin C (Wilkinson et al., 1999). A second study by the same authors investigated the effects of 2mg of vitamin C combined with 100mg/kg of methionine, and found a similar result to their previous study with a 10.5% reduction in augmentation index (Wilkinson et al., 2001). The change was attributed entirely to vitamin C since the methionine-only arm of this study had no impact on augmentation index. A third study investigated the effects of vitamin C in the context of hyperglycaemia, a condition which increases augmentation index. Healthy participants were injected with 2g of vitamin C or placebo before initiation of hyperglycemia. It was demonstrated that pre-treatment with vitamin C attenuated or completely prevented the change induced by hyperglycaemia (Mullan, Ennis, Fee, Young, & McCance, 2004). Of the single-dose studies, only one found no effect of vitamin C at the same 2g dosage (Kelly et al., 2008). This was a larger study (n=26) with a crossover design. The authors argue that vitamin C at that dose has no effect, and point to the small sample size (n=8) in the Wilkinson et al. (1999) study, suggesting it may have been a chance finding. However this seems unlikely, given that positive results were found in the two additional studies cited above, albeit under different conditions.

Four further studies investigated ongoing supplementation with antioxidant vitamins. A finding from one randomized controlled trial argues for a beneficial effect of vitamin C (Mullan et al., 2002). In this study, patients with type 2 diabetes (a group at high risk of cardiovascular complications) were examined before and after six weeks of daily

supplement of 500mg vitamin C. A 16% decrease in augmentation index was observed, with concurrent reductions in brachial systolic and diastolic blood pressures.

In addition to these studies which investigated vitamin C alone, a further study considered the effects of daily vitamin C (1g) combined with vitamin E (400 IU) over 4 weeks, in a crossover design with a 4-week wash-out period between vitamin and placebo interventions (Plantinga et al., 2007). Participants were aged 42-60 years and had essential hypertension. There was a tendency to reduction in augmentation index that was not significant ($p = 0.09$). However, this study also investigated pulse wave velocity and flow-mediated dilation, which both improved significantly over the intervention period. This implies that the combination of vitamins did provide a benefit to arterial stiffness, to which the other measures were more sensitive. Notably, brachial blood pressure measures were unchanged in this study.

Two vitamin E studies from the same group investigated vitamin E at varying doses over two months in healthy male participants. The first study found no effect on augmentation index after supplementation with a tocotrienol-rich formulation of vitamin E (Rasool, Yuen, Yusoff, Wong, & Rahman, 2006). In contrast, the second study found that augmentation index improved between 6.27% and 8.72% with 50, 100 or 200mg daily of another tocotrienol-rich vitamin E supplement (Rasool, Rahman, Yuen, & Wong, 2008). The second study used a "self-emulsifying" gel capsule designed to improve absorption, which may explain the dissimilar findings.

These two studies highlight an important issue in nutritional supplement research, that supplements must be successfully absorbed to elicit a benefit. In the Rassool et al. (2006) study, a lower absorption may have precluded significant changes in arterial function. In a similar vein, Kelly et al. (2008) in the example cited above failed to achieve a reduction in oxidative stress markers with vitamin C supplementation, and this may better explain why they observed no effect on arterial function. This can be compared with the study by Plantinga et al. (2007), where oxidative stress markers were reduced with combined vitamin C and E, and benefit to arterial stiffness was observed.

In summary, it appears that vitamin C does have the potential to affect augmentation index, but further research is needed to substantiate these findings. There is insufficient evidence for an effect of vitamin E on augmentation index.

6.3 Augmentation index and vitamin D

Interest in vitamin D for cardiovascular health has been increasing in recent years (see Chapter 4 for discussion). Vitamin D deficiency is associated with high augmentation index (Al Mheid et al., 2011). In 2012, three trials of vitamin D supplementation were published in a range of participant groups. None of these showed reductions in augmentation index with Vitamin D.

The largest of these studies examined 130 adults with hypertension, and took place in winter in Denmark where there is reduced daylight hours and increased risk of vitamin D deficiency (Larsen, Mose, Bech, Hansen, & Pedersen, 2012). After twenty weeks supplementation with vitamin D, there was no change in augmentation index. However, a significant decrease in central systolic blood pressure was observed. Similarly, there was a lack of effect on augmentation index in another study of 52 patients with chronic kidney disease and low vitamin D status after supplementing for eight weeks (Marckmann et al., 2012). The third study examined 114 postmenopausal women. Four months of vitamin D supplementation had no effect on augmentation index or any other measure of cardiovascular risk (Gepner et al., 2012).

These studies suggest that vitamin D does not affect augmentation index over a range of health conditions, or that any cardiovascular benefits are not reflected in augmentation index. However, a fourth trial of vitamin D for augmentation index has recently been reported (Breslavsky et al., 2013). In this study there was a significant decrease in augmentation index in type 2 diabetic patients who supplemented with vitamin D for one year, suggesting there may still be a role for vitamin D in reducing augmentation index.

6.4 Summary and conclusions

In summary, the limited studies available provide tentative support that some supplements, in particular folate, vitamin B_{12}, vitamin C and vitamin E, have potential to alter augmentation index in a beneficial manner. Vitamin C especially has demonstrated effects both acutely and in longer term studies up to two months. However, the majority of studies are small with short intervention periods or are single-dose studies. Subject groups under investigation are heterogeneous, making comparisons difficult.

To date there are no studies that investigate the effects of multivitamins on augmentation index. Also, there are no studies assessing supplementation benefits in healthy elderly groups. Given that some studies have provided evidence of a beneficial effect of supplements on augmentation index, this subject is worthy of greater attention. Arterial stiffness increases with age and with cardiovascular conditions that increase with age, so a beneficial effect of supplementation in older adults is especially relevant. The studies in this thesis are the first to examine the effects of multivitamin supplementation on augmentation index in healthy elderly adults.

7 General Methods

7.1 Introduction

The original research in this thesis comprises three studies and these are reported in the subsequent chapters. These studies are:

Chapter 8: Augmentation index and cognition in younger and older adults.
This study investigated whether augmentation index and augmentation pressure predicts performance on cognitive task measures, and whether this relationship is different in younger (20-35 years) and older (55-65 years) adults.

Chapter 9: The effects of multivitamin supplementation in older women.
Hereafter referred to as the "Women's study", this project was a randomized, double-blind, placebo-controlled trial which investigated the effects of 16 weeks of multivitamin supplementation with a formula that was designed for older women. Effects on cognition, augmentation index, and a range of blood biomarkers were assessed.

Chapter 10: The effects of multivitamin supplementation in older men.
This study was also a randomized, double-blind, placebo-controlled trial that followed the same protocol as the Women's study but used a multivitamin supplement that was formulated particularly for older men. It similarly examined effects on cognition, augmentation index, and a range of blood biomarkers. It is hereafter referred to as the "Men's study".

The rationale for each of the studies is provided in their respective chapters. The purpose of this chapter is to describe in detail aspects of the methodology used. There is considerable overlap in the studies because the Women's and Men's studies followed the same protocol. Furthermore, the Augmentation Index and Cognition study used baseline data from Women's and Men's studies, combined with baseline data from

another trial in young people which had a similar protocol (the results of that trial are not reported in this thesis). To avoid repetition, the comprehensive details of participant characteristics, testing instruments and measures, treatments, and testing procedures are reported together in this chapter. Study design and statistical analyses are discussed in the particular study chapters.

7.2 Participants

Participants in the studies were healthy female and male participants in two age groups: "older" and "younger". The older group were healthy females and males aged 55 to 65 years. They participated in the Women's study (N = 68) and Men's study (N = 48) respectively. In addition, baseline data for these participants were included in the Augmentation Index and Cognition study (N = 116).

The younger group were healthy males and females aged 20 to 35 years. They were drawn from a larger sample of participants who were taking part in a separate multivitamin study at the university (Pipingas et al., 2013). The present author was not involved with that trial and the results of the intervention are not reported in this thesis. The larger sample included 216 adults aged 20-45 years however this range was considered too broad and a younger, narrower subgroup was selected to be included in the Augmentation Index and Cognition study (n = 106).

7.2.1 Exclusion criteria

Volunteers were excluded from the studies if they reported any of the following: smoking; current or recent use of herbal supplements, multivitamin supplements, or combinations of vitamins and minerals; use of psychoactive medication; psychiatric or psychological disorder; nutritional intolerances or allergies; heart disease or stroke; serious head injury; diabetes; neurological disorders; sleep apnoea; or any current alcohol or drug dependency or substance use. In addition, older participants who were taking blood-thinning medication were excluded, with the exception of those taking

100mg or less of aspirin per day, provided they had no prior history of heart disease. This was to avoid potential interaction with the treatment ingredients. Also, younger participants were excluded if they were taking any prescription medication (except the contraceptive pill) or nutritional supplement.

7.2.2 Participant recruitment

Participants were recruited via advertising in local newspapers and university noticeboards, via a participant recruitment agency, and through word-of-mouth. Participants in the younger group were also recruited via radio and television, and via social media. Notices asked for volunteers who were aged 20-45 years or 55-65 years, who were generally in good health and were not currently taking multivitamins.

7.2.3 Informed consent

All participants who attended at baseline were provided with an Explanatory Statement and signed Informed Consent (Appendix A) before proceeding with testing.

7.2.4 Participant compensation

Older participants were compensated $40 for their participation in the studies. Participants in the Women's study who underwent EEG testing at baseline were compensated a further $20 (EEG is not reported in this thesis).

Younger participants were paid $40 per session they attended (Pipingas et al., 2013).

7.3 Screening instruments and procedures

Volunteers who responded to the request for participants were initially given an account of the research and advised what their participation would involve. Those who agreed and were interested were then screened for the exclusion criteria above.

Volunteers who passed the telephone screening and agreed to participate were assessed again on attendance at baseline testing, to determine any health issues that may have been missed during the telephone screening.

7.3.1 Mini Mental State Examination (MMSE)

Older participants underwent further screening with the Mini Mental State Examination (Folstein, Folstein, & McHugh, 1975). The MMSE is a short screening instrument that is designed to quickly assess cognitive abilities, including orientation, memory, attention span and construction (Lezak, 2004). A score of 30 is possible, with 24 often used as a cut-off for dementia. For the present studies, a minimum of 27 was chosen as requisite, in order to select cognitively healthy older adults.

7.4 Treatment and placebo

7.4.1 Multivitamin tablets

The supplements used in the Women's and Men's studies were, respectively, Swisse Women's Ultivite Multi-Vitamin, Mineral & Anti-Oxidant with Herbs 50+ Years ™ and Swisse Men's Ultivite Multi-Vitamin, Mineral & Anti-Oxidant with Herbs 50+ Years™ provided by Swisse Vitamins Pty Ltd. These are commercial multivitamin tablets that are widely available in Australia and are registered with the TGA (Appendix B). Both supplements contain a range of vitamins, minerals, herbs, and probiotics. The full ingredients are listed in Table 7.1. The differences between the formulas are due to differing nutritional requirements between women and men, for example, there are differences in recommended daily intakes for women and men (NHMRC, 2006).

Women's Ultivite 50+™ tablets were dark pink in colour, the Men's Ultivite 50+™ tablets were dark green. Tablets were packaged in blister packs of 10 individual tablets. Participants were provided with 13 packets (130 tablets) to allow for 16 weeks of supplementation, with approximately an extra two weeks to allow for delays in their return appointment.

7.4.2 Placebo tablets

Placebo tablets were identical to their respective multivitamins in shape, colour and packaging. They contained a starch product with 2mg of vitamin B_2 (riboflavine) in order to give them a comparable smell and to produce a similar colouration of the urine. This compares to 30mg and 35mg of riboflavine that was included in the Women's and Men's formulas.

7.4.3 Randomisation and blinding

Randomisation was performed by Swisse Vitamins Pty Ltd and was conducted in blocks of four with an allocation ratio of 1:1. Multivitamin and placebo tablets were provided by Swisse Vitamins Pty Ltd and given to the investigator in numbered packets according to the allocation schedule. Swisse Vitamins Pty Ltd held the allocation schedule until data collection was completed, thus all investigators were blind to allocation for the duration of data collection. Numbers were allocated sequentially as participants enrolled in the study.

7.4.4 Compliance

Participants were instructed to take one tablet per day, during or immediately after a meal. They were asked to give back all empty packets and any unused tablets when they returned for post-treatment testing. Remaining tablets were counted to ascertain compliance.

Table 7.1. Ingredients of Swisse Ultivite 50+ Women's & Men's Formulas, including reference daily intakes as recommended by NHMRC, where applicable.

VITAMINS AND MINERALS	Women's	RDI	Men's	RDI
Retinyl Acetate (= 2500 IU of vitamin A)	862.5 µg	700 µg	862.5 µg	900 µg
d-Alpha-Tocopheryl Acid Succinate (= vitamin E 24.2 IU)	20 mg	7 mg	25 mg	10 mg
Thiamine Hydrochloride (vitamin B1)	30 mg	1.1 mg	35 mg	1.2 mg
Riboflavine (vitamin B_2)	30 mg	1.1 mg	35 mg	1.3 mg
Nicotinamide (vitamin B_3)	20 mg	14 mg	25 mg	16 mg
Calcium Pantothenate (vitamin B_5) (= pantothenic acid 64.13mg)	70 mg	4 mg	75 mg	6 mg
Pyrodoxine Hydrochloride (vitamin B_6) (= pyridoxine 24.68mg)	30 mg	1.5 mg	25 mg	1.7 mg
Cyanocobalamin (vitamin B_{12})	115 µg	2.4 µg	120 µg	2.4 µg
Cholecalciferol (vitamin D3) (=200 IU vitamin D)	5 µg	10 µg	5 µg	10 µg
Biotin (vitamin H)	150 µg	25 µg	200 µg	30 µg
Folic Acid	500 µg	400 µg	500 µg	400 µg
Calcium Ascorbate Dihydrate (vitamin C) (= ascorbic acid 165.3mg)	200 mg	45 mg	200 mg	45 mg
Phytomedadione (vitamin K1)	60 µg	60 µg	70 µg	70 µg
Calcium Orotate (= calcium 10mg)	100 mg	1000mg	100 mg	1300mg
Magnesium Aspartate Dihydrate (= magnesium 6.74mg)	100 mg	320mg	100 mg	420mg
Selenomethionine (= selenium 26µg)	65 µg	60µg	65 µg	70µg
Molybdenum Trioxide (= molybdenum 45µg)	67.5 µg	45µg	67.5 µg	45µg
Chromium Picolinate (= chromium 50 µg)*	402 µg	25µg*	402 µg	35µg*
Manganese Amino Acid Chelate (= manganese 3mg)	30 mg	5mg	40 mg	5.5mg
Ferrous Fumerate (= iron 5mg)	16.01 mg	8mg	16.01 mg	8mg
Copper Gluconate (= copper 1.2mg)*	8.57 mg	1.2mg*	12.14 mg	1.7mg*
Potassium Iodide (= iodine 149.83µg) (= potassium 46.18µg)*	196 µg	150µg 2800mg*	196 µg	150 µg 3800mg*
Zinc Amino Acid Chelate (= zinc 15mg)	75 mg	8mg	100 mg	14mg

**No RDI has been determined, adequate intake is listed*

continued...

PROBIOTICS AND PLANT EXTRACTS	Women's	Men's
Lactobacillus rhamnosus	80 million organisms	80 million organisms
Lactobacillus acidophilus	80 million organisms	80 million organisms
Bifidobacterium longum	35 million organisms	35 million organisms
Vaccinium Macrocarpon Fruit Dry (patented cranberry PACRAN)	800 mg	1000 mg
Citrus Bioflavoloids Extract	20 mg	20 mg
Silybum Marianum Dry Fruit (St. Mary's thistle) (= flavanolignans calculated as silybin 17.14mg)	1500 mg	1700 mg
Ginkgo Biloba Leaf Dry (= Ginkgo flavonglycosides 4.8mg and ginkgolides and bilobalide 1.2mg)	1000 mg	1000 mg
Crataegus Monogyna Fruit Dry (Hawthorn)	100 mg	120 mg
Cynara Scolymus Leaf Dry (Globe artichoke)	50 mg	50 mg
Lecithin Powder – Soy Phosphatidylserine Enriched Soy (= phosphatidylserine 2mg)	10 mg	10 mg
Scutellaria Lateriflora Herb Dry (Skullcap)	50 mg	50 mg
Spearmint Oil	2 mg	2 mg
Tagetes Erecta Flower Dry (Marigold) (Lutein esters calculated as lutein (of Tagetes erecta) 1mg)	100 mg	100 mg
Ubidecarenone (Co-enzyme Q10) (from patented Ultrasome CoQ10)	2 mg	3 mg
Urtica Dioica Leaf Dry (Nettle)	100 mg	50 mg
Vaccinium Myrtillus Fruit Dry (Bilberry) (= anthocyanosides 324mcg)	100 mg	100 mg
Vitis Vinifera Dry Seed (Grape seed) (= procyanidins 7.9mg)	1000 mg	1000 mg
Bacopa Monnieri Whole Plant Dry (Bacopa) (= bacosides calculated as bacoside A 1.125mg)	50 mg	-
Cimifuga Racemosa Root & Rhizome Dry (Black cohosh)	200 mg	-
Curcuma Longa Rhizome Dry (Turmeric)	100 mg	-
Silica Colloidal Anhydrous (= silicon 9.35mg)	20 mg	-
Tunera Diffusa Leaf Dry (Damiana)	500 mg	-
Withania Somnifera Root Dry (Ashwagandha)	500 mg	-
Dulacia Inopiflora Root Dry (Muirapuama)	-	200 mg
Serenoa Repens Fruit Dry (Saw palmetto) (= fatty acids 27mg)	-	300 mg
Tribulus Terrestris Fruit & Root Dry (Tribulus)	-	1000 mg

7.5 Measurements

The following demographic information, cognitive tasks, cardiovascular and blood biomarker variables were included in the study.

7.5.1 Demographic information

The following demographic information was obtained for participants in all three studies.

7.5.1.1 Age and date of birth

Although the age ranges were limited in the present studies, measurable change in cognitive and cardiovascular health may still be observed over a period of 10 to 15 years (Salthouse, 2009). Age is therefore included as a covariate in relevant analyses.

7.5.1.2 Education

The total number of years of education completed by each participant was recorded. Education is used as a covariate because the level of education can influence cognitive performance (Valenzuela & Sachdev, 2006a). Furthermore, educational attainment is subject to cohort effects (Paul Verhaeghen, 2003) which are particularly relevant to the Augmentation Index and Cognition study, which compares younger and older adults.

7.5.1.3 Body mass index (BMI)

Height and weight were recorded and BMI was calculated as kilograms per metre squared. BMI increases with age (Guo, Zeller, Chumlea, & Siervogel, 1999) and can influence cognitive performance (Gunstad et al., 2007). Also, people with a high BMI are more susceptible to cardiovascular problems (P. W. Wilson, D'Agostino, Sullivan, Parise, & Kannel, 2002). It was recorded for possible use as a covariate.

7.5.1.4 Medication and supplementation use

Participants were asked to provide information on any medications and nutritional supplements they were taking. Medications provide information on the health status of the individual and inform us of possible health conditions that were overlooked by the participant in the screening procedures. Information regarding supplement use ensured that participants had not been taking nutritional supplements that would interfere with the aims of the study.

7.5.2 Computerized cognitive tasks

The Swinburne University Computerized Cognitive Assessment Battery (SUCCAB) is a set of eight computerized cognitive tasks covering a broad range of cognitive abilities including processing speed, memory and executive functions. The battery is appropriate for the current studies because it has previously been demonstrated to be sensitive to both age-related cognitive decline (Pipingas et al., 2010), and nutritional intervention (Pipingas et al., 2008; Macpherson et al., 2012).

Responses were made on a response box with four coloured buttons arranged in the positions of compass poles as follows: top/yellow, bottom/green, left/blue, right/red. Participants were read standardized instructions and were given a brief practice prior to each task to familiarise the participant with the test. Two equivalent versions of the battery were administered; all participants were given version A at baseline and version B at post-treatment. Response times were recorded in milliseconds. The battery took approximately 45 minutes to complete. On the day of testing participants followed their usual diet but were asked to refrain from caffeine for three hours before the testing session.

Simple Reaction Time

A single white square was presented in the centre of the screen at random intervals. Participants responded as quickly as possible with a right button press.

Choice Reaction Time

Red squares and blue triangles were randomly presented in the centre of the screen, with a randomized delay. Participants responded as quickly as possible with a left/blue button press or right/red button press.

Immediate and Delayed Recognition Memory

Participants were initially shown a series of 40 abstract images that were presented on the screen for three seconds each, with no inter-stimulus interval. In the Immediate condition, a second series of 40 images followed, half of which had just been presented, the other half were new. Participants had to determine which images they had seen and respond with a left or right button press.

The delayed condition used the remaining 20 images from the initial set randomized with another set of new images. These were presented as the last task of the test battery, approximately 40 minutes after the initial presentation. Again participants indicated with a left or right button press whether they recognized each image.

Stroop – Congruent, Incongruent and Interference

The words RED, YELLOW, GREEN, and BLUE were presented on the screen one at a time for 1.7 seconds with 0.5 seconds inter-stimulus interval. In the congruent condition the text was presented in the colour matching the written word and participants responded by pressing the matching coloured button. In the incongruent condition the colour of the word did not match. Participants ignored the written word and pressed the button corresponding to the colour of the text. The Stroop effect was calculated by subtracting the Congruent response time from the Incongruent response time. This is considered to be the "interference effect", which represents inhibitory function (Cohn et al., 1984).

Spatial Working Memory

A 4x4 grid was presented on the screen, with six spaces filled with white squares, making a pattern. This was displayed for three seconds then the pattern disappeared. White squares then appeared one at a time in four of the spaces, for two seconds each.

Participants responded with a yes or no button press, according to whether the square was in one of the spaces that matched the pattern. There were 14 different grid patterns presented, with a total of 56 responses elicited.

Contextual Memory

Photographs of 20 everyday items were presented one at a time at the top, bottom, left or right of the screen for three seconds with no inter-stimulus interval. Subjects were required to memorise where on the screen the objects were presented. On completion of the series, the pictures were presented again in the centre of the screen in randomized order. Participants responded with a top, bottom, left or right button press corresponding to the location of the original presentation.

7.5.3 Augmentation index, augmentation pressure and blood pressure

Brachial blood pressure, augmentation index and augmentation pressure were assessed as measures of cardiovascular health. The Augmentation Index and Cognition study investigated whether they could predict performance on cognitive tasks. The Women's and Men's studies assessed whether measurable changes could be observed after supplementation with multivitamins.

7.5.3.1 Brachial blood pressure

Brachial blood pressures provide maximum (systolic) and minimum (diastolic) blood pressures in the brachial artery, and are thus a measure of pressures in the periphery of the arterial tree. Blood pressure was measured in the left arm using an electronic inflating sphygmomanometer device. Participants were seated in a chair.

7.5.3.2 Augmentation index and augmentation pressure

Augmentation index and augmentation pressure are surrogate measures of arterial stiffness (Laurent et al., 2006). They were obtained using the method of pulse wave analysis, which records the pulse-pressure waveform at the radial artery and applies a generalized transfer function to determine the waveform at the aorta (central pressure

waveform). The variables are calculated based on features of the central waveform and are calibrated to brachial systolic and diastolic pressures. They therefore reflect pressures in the central arterial system.

Augmentation pressure is the amount by which the peak of the systolic pressure wave is augmented due to early wave reflection and is measured in mmHg. Augmentation index is calculated as the ratio of augmentation pressure to pulse pressure. Although augmentation index is the more commonly used variable in the scientific literature, augmentation pressure has also been included because it has been argued that it is a more suitable index in older populations (Fantin et al., 2007). A more detailed explanation of the theory behind these variables has already been provided in section 3.3.

The present studies used the SphygmoCor System (AtCor Medical, NSW). Systolic and diastolic blood pressures were entered into the Sphygmocor program. The pulse pressure waveform was recorded from the radial artery as the participant was seated with their left arm resting on the table. The pressure tonometer was applied to the radial artery of the left arm and the pulse pressure waveform was recorded.

The Operator Index is a calculation to determine the internal consistency of the recording. Scores can be obtained from 1-100, with higher scores representing a more consistent, higher-quality recording. The operating manual provided by the manufacturer recommends an Operator Index of at least 80 to obtain an accurate reading. Therefore if the Operator Index was less than 80 the recording was repeated until an acceptable score was achieved (where possible). Data with an Operator Index less than 80 were excluded from the analysis.

7.5.4 Blood biomarkers

A range of blood biomarkers were assessed in the Women's and Men's studies. These markers were taken to assess different aspects of health that might be impacted by multivitamin supplementation. The aim of these tests was to get a broad overview of the

effects that the multivitamin supplementation might have on physiology, particularly in relation to cardiovascular and cognitive health.

Participants attended a commercial blood collection centre (Gribbles Pathology) on the morning following their testing session at Swinburne University of Technology. Participants underwent venepuncture and provided seven vials of blood. Six of these underwent analysis by Gribbles Pathology according to their standard protocol and were reported along with the reference ranges. One vial was forwarded to Southern Health for protein carbonyl analysis.

7.5.4.1 Vitamin B_6 and vitamin B_{12}

A considerable body of research has indicated relationships between vitamin B_6 and/or B_{12} and cognitive performance, particularly in older adults (see section 4.1 for review). Furthermore, a previous study from our group has demonstrated a substantial increase in these vitamins after supplementation with a multivitamin (E. Harris et al., 2012).

Vitamin B_6 was measured in whole blood as pyridoxal-5'-phosphate. It was collected in a tube containing heparin and wrapped in foil to prevent degradation of the sample by light. It was analysed using high-performance liquid chromatography (HPLC). The reference range was 35-110 nmol/L (nanomoles per litre). Vitamin B_{12} was measured in serum. Blood was collected in a serum separator tube and analysed with a competitive immunoassay using direct, chemiluminescent technology. Concentrations above 180 pmol/L (picomoles per litre) were considered normal; less than 150 pmol/L was deemed deficient.

7.5.4.2 Folate

Red cell folate results appeared suspect upon analysis of the data. Although they were predominantly within the stated reference range, the pattern of results was inconsistent, with no correlation between baseline and post-treatment folate concentrations. Also folate changes in some individuals appeared too large to be credible, particularly in

comparison to previous folate assessments conducted by our group. Folate was therefore excluded from the analysis.

7.5.4.3 Homocysteine

Homocysteine is a risk factor for cardiovascular disease and is associated with poorer cognitive performance and dementia in older people (see section 4.1 for review). Homocysteine can be reduced by supplementation with vitamin B_{12} and folate, and to a lesser extent, vitamin B_6. The B vitamins in the present formulations are at a level considered to be sufficient to achieve homocysteine reduction (Clarke, 1998). Serum homocysteine was measured in micromoles per litre (µmol/L), using the same methods as for vitamin B_{12}. The stated reference range was 5 – 15 µmol/L.

7.5.4.4 Antioxidant vitamins

For vitamin C and vitamin E a large number of tests were reported as substantially below the reference range, indicating errors in the collection and transport of the blood samples. The tests were discontinued during the study.

7.5.4.5 Oxidative stress

Oxidative stress biomarkers are products of oxidating reactions, with different biomarkers indicating damage to protein, DNA or lipids. As markers of oxidative stress they may provide information about in vivo effects of pro-oxidant or antioxidant interventions (Griffiths et al., 2002).

Protein carbonyls are particularly suited as markers of oxidative stress because they appear early in disease processes, are relatively stable, and can be measured easily in blood samples (Dalle-Donne, Rossi, Giustarini, Milzani, & Colombo, 2003). They are a general marker of protein oxidation. Their occurrence is the major change that is observed with the oxidation of proteins (Griffiths et al., 2002).

Protein oxidation levels also increase with age, and this may be due to an increase in oxidative stress, a decrease in antioxidant function, or changes in the rate of degradation

of these products (Stadtman, 2006). However, protein carbonyl levels are markedly increased in patients with Alzheimer's disease or MCI, when compared with age-matched controls (Bermejo et al., 2008; Greilberger et al., 2008). Because of this they are considered a useful marker of the disease process (Dalle-Donne, Giustarini, Colombo, Rossi, & Milzani, 2003).

In this study protein carbonyl concentration in plasma was measured using a Cayman analysis kit. Samples were collected in EDTA and frozen at -80ºC until they were analysed in batches. The results were provided in nmol/ml, which differs from the typical reporting of nmol/mg, meaning that direct comparisons with other studies or with typical values are not able to be made. However the test is valid for comparisons within this study.

7.5.4.6 Inflammatory markers

High-sensitivity C-reactive protein (CRP)

CRP is a marker of inflammation and an indicator of cardiovascular risk (Biasillo et al., 2010). It has also been associated with cognitive impairment and dementia (Hedges, Farrer, & Brown, 2012). The reference range provided by the pathology company indicated that less than 11.1 mg/L was normal. While levels this high may indicate acute disease states, much lower levels are associated with risk of cardiovascular disease, since mildly elevated CRP occurs with atherosclerosis. The American Heart Association has suggested that people with levels over 3mg/L be considered at risk (Pearson et al., 2003). For this reason, the high-sensitivity assay is required, to differentiate between those with low inflammation and those with very mildly elevated CRP (Bassuk, Rifai, & Ridker, 2004).

High-sensitivity CRP was measured in serum. Blood was collected in a serum-separator tube (SST) and analysed with immunoturbidimetric test.

Fibrinogen

Fibrinogen is a nonspecific marker of inflammation. It has been demonstrated to contribute to the risk profile of cardiovascular disease (Danesh et al., 2005) and

cognitive decline (Rafnsson et al., 2007). The reference range provided for fibrinogen was 2-4 g/L. Blood was collected in a sodium citrate tube.

7.5.4.7 Lipids

A report by the Australian Institute of Health and Welfare states that high cholesterol is one of the most important contributors to cardiovascular disease, noting that the effect is focussed in the elderly (Begg et al., 2007). High cholesterol is a risk factor for cognitive decline and dementia (Anstey et al., 2008). High total cholesterol is detrimental to health due to the negative effects of low-density lipoprotein (LDL), whereas high density lipoprotein (HDL) is considered to be a beneficial form of cholesterol. Therefore the ratios among these variables are also important (Fernandez & Webb, 2008).

Cholesterol levels were measured in µmol/L. Reference ranges were given as follows: LDL less than 2.5 µmol/L; HDL greater than 1.0 µmol/L; and triglycerides less than 1.5 µmol/L. Samples were collected in a serum-separator tube and analysed using the following methods: Cholesterol oxidase for total cholesterol; lipase/glycerol kinase for triglycerides; and elimination for HDL.

7.5.4.8 Blood safety assessment

A number of blood markers were undertaken to ensure the safety of the supplements, as changes in these may indicate toxicity or a detrimental effect in the body.
Kidney and liver function tests were included. Blood samples were collected in SST tubes and various analytical methods were used as follows: Ion-selective electrode (ISE) analysis was used for sodium, potassium and chloride; urease-kinetic method for urea; enzymatic method for bicarbonate; biuret endpoint for total protein; BMP for albumin; chemical oxidation for bilirubin; g-glut-3-carboxynitro for gamma glutamyl transpeptidase (GGT); AMP buffer rate (IFCC) for alkaline phosphatase (ALP); the International Federation of Clinical Chemistry (IFCC) modified method was used for aspartate transaminase (AST) and alanine transaminase (ALT).

7.6 Procedures

All three studies followed the same procedures for baseline testing. This comprised a testing session of one and a half hours. Participants were asked to refrain from drinking caffeinated beverages for three hours before the testing sessions.

Baseline testing sessions were conducted in the following order:

Explanatory statement and informed consent
Review of eligibility screening data received during the telephone interview
Personal and demographic information
MMSE screening
Health questionnaires completed by the participant (data not included in this thesis)
Blood pressure recording
Pulse wave analysis assessment
Height and weight
Computerized cognitive tasks

At the conclusion of each testing session, older participants in the Women's and Men's studies were given a referral to Gribbles Pathology blood collection centre to have a blood sample taken. They were instructed to fast from 10pm the night before the test and to go first thing in the morning. Participants attended a Gribbles blood collection centre convenient to them. They were asked to attend the following day or as soon as possible after the baseline testing session, and were also instructed not to begin supplementation until after they had attended for blood testing.

In the Women's and Men's studies, participants returned after 16 weeks of supplementation with the multivitamin for follow up testing. Participants who attended this session followed the same protocol as at baseline, with the exception of informed consent and eligibility screening. They returned all unused supplements and were asked to guess whether they had been taking multivitamin or placebo tablets. Follow up testing sessions were conducted at the same time of day as the baseline session. Participants were requested not to take their supplement on the day of follow-up testing, in order to control for potential acute effects of the supplement.

7.7 Summary and conclusion

This chapter has provided details of the methodology used in the studies that are reported in the following chapters.

8 Augmentation index and cognition: Relationships in younger and older adults

8.1 Introduction

The aim of this thesis is to assess the benefits of nutritional supplementation on cardiovascular and cognitive function. As discussed in Chapter 3, cardiovascular function is a key determinant of cognitive health and there are many ways in which cardiovascular and cognitive variables interrelate. In particular, arterial stiffness can impact the brain via increased arterial pulse pressure entering cerebral blood vessels, causing damage to the brain matter (Mitchell, 2008). This in turn is related to impaired cognitive function.

There are various measures and markers of arterial stiffness, including pulse wave velocity and pulse pressure, and these have been demonstrated to predict cognitive performance in older adults. This includes the cognitive domains of memory, speed and executive function (Poels et al., 2007; Waldstein et al., 2008; Watson et al., 2011), functions that are particularly affected in aging. However, little research has investigated the relationship between cognition and arterial stiffness using augmentation index. This more recently developed measure of arterial stiffness is relatively easy to obtain from an individual and may provide valuable information on the health of the cardiovascular system, above that afforded by traditional brachial measures (Izzo Jr, 2005).

To date, only two studies have investigated the relationship between augmentation index and cognition. One study examined the relationship using a computerized cognitive battery (CDR) in a group of adults aged 40-65 years. Investigators observed a relationship between augmentation index and speed of memory, but not in other cognitive domains (Pase et al., 2010). However, the study did not look at how the relationship might change across adulthood, an important consideration because both augmentation index and cognitive performance are altered dramatically with increasing

age (McEniery et al., 2005; Rabbitt & Lowe, 2000). The other investigated elderly adults with subjective memory complaints. They did not observe a relationship between augmentation index and memory impairment, but augmentation index was associated with the presence of white matter hyperintensities (Kearney-Schwartz et al., 2009). On the other hand, pulse wave velocity, another measure of arterial stiffness, has been observed to have increasing correlation with cognition with increasing age (Rabkin, 2012).

Further to this, it is important to note that research has indicated that augmentation index is a more appropriate index of arterial stiffness in younger adults, whereas augmentation pressure may be more useful in the elderly (Fantin et al., 2007). This is because augmentation index increases up to around age 50-55 and then begins to level off, whereas augmentation pressure continues to rise. On the other hand, cognitive ability slowly decreases across adulthood, with a steeper decline occurring from around 70 years, with great individual differences (Rabbitt & Lowe, 2000). Given these changing trajectories, it might be supposed that that the relationship between cognition and augmentation index or augmentation pressure also changes across the adult lifespan. The present study examines these relationships in a group of younger adults versus a group of older adults.

It was hypothesized that the central pressure measures, augmentation index and augmentation pressure, would be related to cognition in both younger and older adults, and that these relationships would be different in the two age groups. Specifically, this study tested the hypothesis that there is a relationship between cognitive performance on a computerized battery and cardiovascular function as measured by augmentation index and augmentation pressure, in younger and older adult groups. It was also hypothesized that these cognitive-cardiovascular relationships would be stronger in the older adult group. In addition, it was hypothesized that cognition would be more strongly correlated with central pressure variables than with brachial blood pressure measurements.

This investigation is useful for the overall aims of the present thesis. In addition to being important endpoints in themselves, if relationships between augmentation

index/augmentation pressure and cognition are established they could help to explain how a benefit in cognition is mediated (supposing such a benefit is observed). That is, changes in these variables due to vitamin supplementation might provide an indication as to whether cardiovascular benefits underlie cognitive changes.

8.2 Methods

The current study examined the baseline data from the Women's and Men's studies that are reported in Chapter 9 and Chapter 10 of this thesis, and compared it with the baseline data from another study which examined the effects of nutritional supplementation in younger adults (Pipingas et al. 2013). (Please note that the author was not involved with the study design or data collection for that trial and the results of the intervention are not reported in this thesis). A detailed description of the participants, measurements and procedures was provided in the previous chapter. A brief account is presented here.

The trials were registered at the Australian New Zealand Clinical Trials Registry, trial ID ACTRN12608000117314. Ethics approvals for the studies were obtained from the Swinburne University Human Research Ethics Committee (Appendix A).

8.2.1 Participants

The study comprised a total of 215 adults, including 106 younger participants (20-35 years of age) and 109 older participants (55-65 years). All participants were healthy non-smokers who had no history of psychiatric, psychological or neurological disorder, head injury, stroke, diabetes, drug use or alcohol dependency. They were not currently supplementing with herbal supplements, multivitamins, or combinations of vitamins and minerals.

Recruitment of participants involved advertisements in newspapers and noticeboards and word of mouth. A participant recruitment agency was also used. Volunteers initially

contacted the research team by telephone, where they were advised what their participation would encompass and were then screened according to the inclusion and exclusion criteria. All participants provided written informed consent.

8.2.2 Measures and procedure

Participants attended Swinburne University of Technology for the testing session, which was undertaken as part of their participation in one of three multivitamin supplement trials (the Women's study and Men's study reported in this thesis, and a third multivitamin study that is not reported here). They were asked to refrain from caffeinated drinks for three hours before their testing session. At the beginning of the session, details of their date of birth, educational attainment, medication and supplementation details were recorded. Height and weight were also measured to determine body mass index (BMI). The MMSE was administered to the older group, where a cut-off of 27/30 determined eligibility into the study.

Following these measures, brachial systolic and diastolic pressures were recorded using an electronic sphygmomanometer device. Central augmentation index and central augmentation pressure were then acquired via applanation tonometry of the radial artery, using the SphygmoCor System (AtCor Medical, NSW).

Cognitive testing involved a battery of computerized cognitive tasks covering a range of cognitive abilities. The tasks included were Simple Reaction Time, Choice Reaction Time, Immediate Recognition Memory, Stroop Congruent, Stroop Incongruent, Spatial Working Memory, Contextual Memory, and Delayed Recognition Memory.

For further details of the measures and procedures used in the study, please refer to Chapter 7.

8.2.3 Statistical analysis

Data was analysed using SPSS version 20. Except where stated otherwise, data met the assumptions required for each analysis, including random sampling, independence of observations, normal distribution, linearity and homogeneity of variance. Significance level was set at $p < 0.05$ and due to the number of comparisons marginally significant relationships are not reported for the primary outcome measures.

T-tests were conducted to examine differences between younger and older groups on demographic and cardiovascular measures. For differences between groups on the cognitive measures, univariate analyses of covariance (ANCOVA) were conducted with cognitive test score (response time or accuracy) as the dependent variable, age group as the independent variable and education as the covariate. This was to eliminate possible cohort effects of education on cognitive performance (Paul Verhaeghen, 2003). Levene's tests of equality of variance were used to assess the differences in spread of scores between younger and older groups where relevant.

Bivariate correlations were employed to examine the relationships between the cognitive measures and educational attainment, and to examine relationships between cognitive and cardiovascular measures for younger and older groups separately.

For significant correlations, further analyses were undertaken to assess whether the cardiovascular measures predicted cognitive performance after controlling for age and education. Hierarchical regression models were conducted for each cognitive measure, with the cognitive test score as the dependent variable, age entered at stage one, education entered at stage two, and the cardiovascular measure at stage three.

8.3 Results

8.3.1 Demographic information

Descriptive data for the younger and older groups regarding age, education and BMI are presented in Table 8.1. The younger participants were significantly more educated than the older participants ($t(213) = 2.46$, $p = 0.015$, equal variances not assumed). In addition to a lower mean number of years of formal education, the older group's distribution was more widely spread, and Levene's test for equality of variances indicated that this was significant ($F(1,213) = 29.39$, $p < 0.001$). This shows that the younger group were more alike in their high level of education whereas the older group had a mix of people with high and low educational attainment. As expected, education was related to performance on some tasks. In the younger group it was correlated with the Delayed Recognition task ($r = 0.247$, $p = 0.012$). In the older group it was correlated with Simple Reaction Time ($r = 0.207$, $p = 0.035$) and Spatial Working Memory ($r = 0.237$, $p = 0.014$).

The younger group had a significantly lower mean BMI than older ($t(196) = -5.079$, $p = < 0.001$), however BMI was not significantly related to cognitive performance.

Table 8.1. Means and standard deviations for age, education and BMI

	M	*SD*	Min	Max	*n*
Younger					
Age (years)	27.1	3.9	20	35	106
Education (years)	16.7	2.4	10	23	106
BMI (kg/m^2)	23.7	4.4	15.4	37.1	90
Older					
Age (years)	60.0	3.0	55	65	109
Education (years)	15.6	4.1	6	27	109
BMI (kg/m^2)	27.0	4.7	18.5	49.2	108

8.3.2 Difference between younger and older adults on cognitive tasks

Results of cognitive task assessments for older and younger groups are presented in Table 8.2. As anticipated, the younger group performed significantly better on the cognitive task battery compared with the older group, for both response time and accuracy. This was true after co-varying for educational attainment. Results of ANCOVA are presented in Table 8.3 for each cognitive measure. The difference in performance between age groups was significant for all measures except for accuracy on the Delayed Recognition task (however response time for this task was significantly different between the groups).

Education was a significant covariate for Spatial Working Memory accuracy ($F(1,207) = 6.601$, $p = 0.011$) and was marginally significant for Stroop Interference ($F(1,201) = 3.34$, $p = 0.069$). That is, the age differences in cognitive task performance were partly due to differences in education for those tasks only. However, the relationship between age group and these cognitive variables remained significant after education was controlled for (Table 8.3). This indicates that the observed differences in cognitive performance between young and old participants were generally not due to cohort differences in education level.

8.3.3 Cognitive variability

There was a greater diversity in performance between participants in the older group than in the younger group. Levene's tests of equality of variances indicated that significantly more variance was observed in the older group for accuracy and response times on most tasks, with the exception of Immediate Recognition accuracy and the Simple Response Time task (Table 8.3).

Table 8.2. Cognitive task results for younger and older groups

Task		*M*	*SD*	Min	Max	*n*
Younger Group						
Accuracy	Simple Recog.	78.3	11.25	43.3	100.0	102
(%)	Spatial	89.4	9.26	62.5	100.0	103
	Contextual	86.6	10.56	55.0	100.0	100
	Delayed Recog.	71.0	12.08	43.3	100.0	104
Response	Simple Response	252.9	30.13	197.9	344.7	104
Time (ms)	Choice Response	374.1	45.87	279.8	511.9	94
	Simple Recog.	874.4	100.80	667.3	1106.8	104
	Stroop Interfer.	109.1	87.42	-113.99	369.08	105
	Spatial	754.4	134.47	483.4	1131.1	103
	Contextual	786.0	112.13	585.4	1118.5	103
	Delayed Recog.	901.2	109.14	596.3	1203.4	104
Older Group						
Accuracy	Simple Recog.	69.0	12.34	36.7	96.7	105
(%)	Spatial	70.9	14.21	35.7	98.2	108
	Contextual	75.0	15.22	40.0	100.0	106
	Delayed Recog.	68.8	10.24	40.0	90.0	104
Response	Simple Response	269.4	27.70	208.8	348.9	105
Time (ms)	Choice Response	480.0	59.46	364.2	653.5	105
	Simple Recog.	1126.6	146.62	856.7	1533.9	105
	Stroop Interfer.	159.6	139.41	-41..9	752.2	100
	Spatial	1112.7	186.14	749.1	1653.1	108
	Contextual	1094.3	135.16	770.1	1499.7	106
	Delayed Recog.	1072.0	132.97	787.7	1489.2	105

ms: milliseconds

Table 8.3. ANCOVA results for differences between young and old groups on cognitive performance, after controlling for education

Task	*F*	*DF* error	*p*	Partial Eta squared	Levene's Test *F*	Levene's Test *p*
Accuracy(%)						
Simple Recog.	31.6	203	<0.001	0.135	1.18	0.279
Spatial	112.3	207	<0.001	0.352	15.98	<0.001
Contextual	34.4	202	<0.001	0.146	14.07	<0.001
Delayed Recog.	1.543	204	0.216	0.008	4.72	0.031
Response Time (ms)						
Simple RT	18.5	205	<0.001	0.083	0.07	0.788
Choice RT	186.4	195	<0.001	0.489	3.70	0.056
Simple Recog.	200.3	205	<0.001	0.494	11.02	0.001
Stroop Interfer.	20.0	201	<0.001	0.091	7.844	0.006
Spatial	238.7	207	<0.001	0.536	4.893	0.028
Contextual	303.9	205	<0.001	0.597	4.589	0.033
Delayed Recog.	97.0	205	<0.001	0.321	5.611	0.019

ms: milliseconds

8.3.4 Difference between younger and older adults on cardiovascular measures

Observed values for central cardiovascular and brachial blood pressure measures are presented in Table 8.4. The older group had substantially higher values for augmentation index, augmentation pressure and brachial blood pressures than the younger group, and t-tests indicated that the differences were significant (Table 8.5). The difference was greatest for augmentation index, revealing how severely this variable is affected by age.

Table 8.4. Cardiovascular measures for younger and older groups

	M	*SD*	Min	Max	*n*
Younger group					
Central Augmentation Index	8.6	11.01	-21.7	30.3	96
Central Augmentation Pressure	2.4	3.06	-5	10	96
Systolic Pressure	117.5	11.10	90	146	97
Diastolic Pressure	74.3	7.34	57	90	97
Older group					
Central Augmentation Index	26.4	10.20	-6	55	107
Central Augmentation Pressure	10.3	4.57	-2	22	98
Systolic Pressure	130.1	12.52	99	159	103
Diastolic Pressure	81.3	8.02	63	97	104

Table 8.5. T-tests for difference between young and old groups on blood pressure measures

					Levene's Test	
	t	*df*	*p*		*F*	*p*
Central Augmentation Index	11.957	201	<0.001		0.853	0.357
Central Augmentation Pressure	-14.138	169.921	<0.001	*	14.29	<0.001
Systolic Pressure	-7.488	198	<0.001		0.768	0.982
Diastolic Pressure	-6.364	199	<0.001		1.113	0.293

*equal variances not assumed

8.3.5 Central pressure measures and age

Only the younger group showed significant correlations between age and the central cardiovascular measures, augmentation index and augmentation pressure. Figure 8.1 shows the slopes of the regression equations for these relationships. For augmentation index, the relationship with age was stronger in younger adults ($r^2 = 0.112$, $p = 0.001$) than in older adults ($r^2 = 0.024$, not significant); this is indicated by a steeper slope. A similar pattern was observed for augmentation pressure: the relationship was greater in younger adults where the relationship was identical to that of augmentation index ($r^2 = 0.112$, $p = 0.001$); in the older adults it was not significant ($r^2 = 0.018$). This might be partly accounted for by the wider age range in the younger group.

8.3.6 Cardiovascular variability

There was significantly greater variance in the older group for augmentation pressure, as indicated by Levene's test (Table 8.5). Interestingly this was not observed for augmentation index or for brachial systolic and diastolic pressures, suggesting that augmentation pressure is more sensitive to individual differences in age-related cardiovascular change.

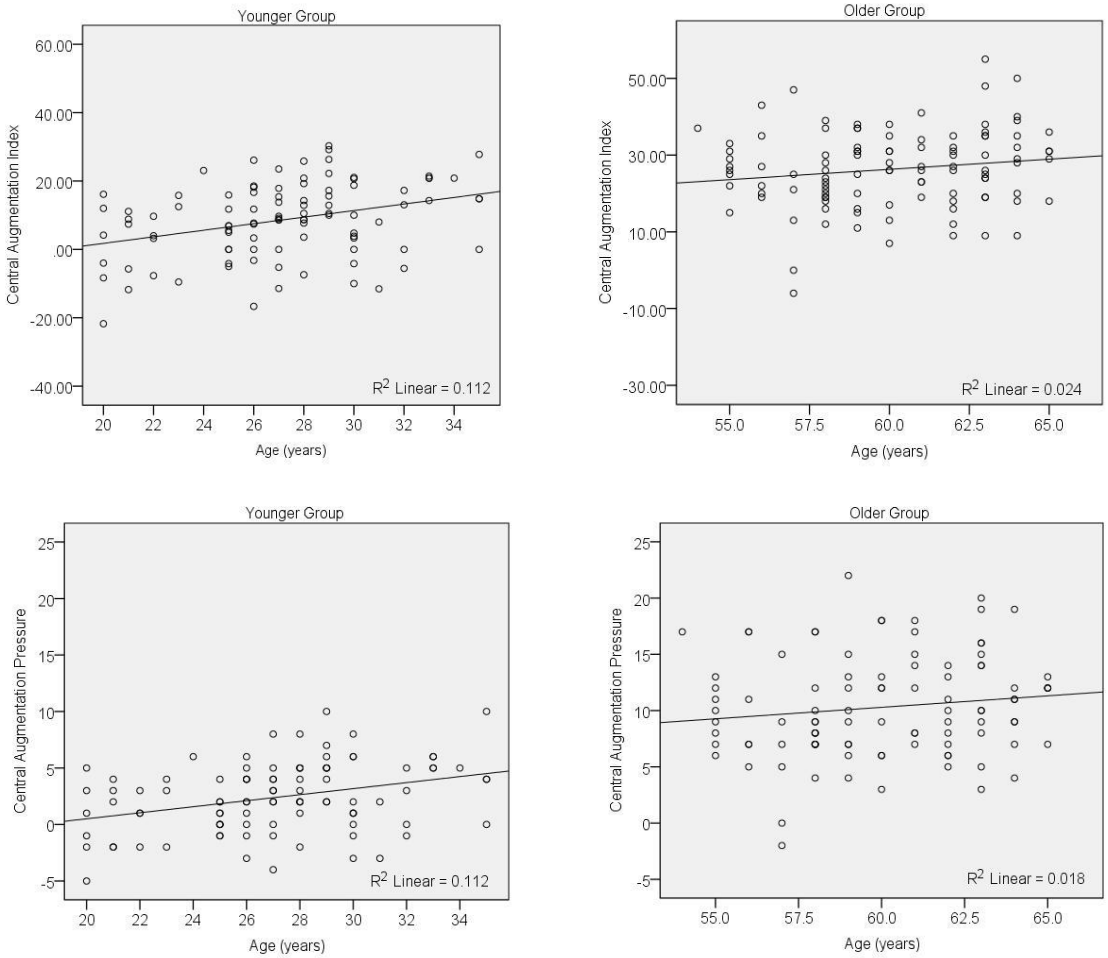

Figure 8.1. Regression slopes of augmentation index and augmentation pressure for younger and older adults.

8.3.7 Relationship between cognitive tasks and cardiovascular measures in younger and older groups

8.3.7.1 Cognition, augmentation index and augmentation pressure

A number of significant correlations were observed between the cognitive tasks and augmentation index or augmentation pressure (Table 8.6). The Spatial Working Memory task was particularly associated with the central cardiovascular measures. Higher accuracy and faster response time were associated with lower augmentation pressure and augmentation index in both older and younger participants. The Simple Recognition task also demonstrated some relationships with central cardiovascular measures. In both the younger and older groups, higher accuracy on this task was correlated with lower augmentation pressure. These relationships were in the anticipated direction, that is, better cognitive performance was associated with lower scores on the central cardiovascular measures (i.e. better cardiovascular health).

Hierarchical multiple regressions were used to assess how well the cardiovascular variables predict performance on cognitive tasks, after controlling for the effects of education and age (Table 8.7). For augmentation index, Spatial Working Memory accuracy remained significant in the younger and older groups and Spatial Working Memory response time remained significant in the older group only. For augmentation pressure, Spatial Working Memory accuracy also remained significant in the younger group. The relationship between Simple Recognition accuracy and augmentation pressure was not significant after controlling for age and education.

Relationships between augmentation index and Spatial Working Memory were significant in younger and older adults; moderation analysis was used to determine whether the strength of the relationship was the same in younger and older adults. Because the relationships were differentially significant for accuracy and response time in the two age groups, these variables were first combined to create an overall performance measure (accuracy divided by response time). This would account for any differences in speed-accuracy trade-off that may have occurred between the groups.

Table 8.6. Two-tailed Pearson's correlations between cardiovascular measures and cognitive task performance

	Aug Index		Aug Pressure		Brachial Systolic		Brachial Diastolic	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*
Younger								
Simple Recogniton %	-0.151	0.075	***-0.181**	**0.042**	-0.025	0.405	*** 0-.207**	**0.023**
Spatial Working Memory %	***-0.277**	**0.003**	***-0.300**	**0.002**	0.109	0.145	-0.109	0.146
Contextual Memory %	-0.029	0.393	-0.022	0.419	-0.012	0.453	-0.126	0.115
Delayed Recognition %	-0.028	0.395	-0.071	0.249	0.072	0.245	-0.031	0.381
Response Time *ms*	-0.108	0.149	-0.059	0.285	0.149	0.075	0.105	0.155
Choice Response *ms*	-0.047	0.334	-0.016	0.441	-0.035	0.375	0.069	0.263
Simple Recognition *ms*	0.107	0.153	0.079	0.225	-0.13	0.276	-0.043	0.059
Stroop Interference *ms*	-0.116	0.132	-0.106	0.153	-0.062	0.276	-0.161	0.059
Spatial Working Mem *ms*	***0.239**	**0.010**	***0.232**	**0.012**	**† -0.202**	**0.024**	-0.077	0.228
Contextual Memory *ms*	-0.044	0.339	-0.031	0.384	-0.094	0.184	0.024	0.409
Delayed Recognition *ms*	-0.065	0.266	-0.101	0.168	-0.108	0.149	-0.087	0.201

ms: milliseconds

continued…

Table 8.6 continued…

	Aug Index		Aug Pressure		Brachial Systolic		Brachial Diastolic	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*
Older								
Simple Recognition %	-0.100	0.158	***-0.190**	**0.034**	***-0.169**	**0.048**	-0.099	0.164
Spatial Working Memory %	***-0.272**	**0.003**	**-0.152**	**0.069**	0.026	0.397	0.149	0.067
Contextual Memory %	-0.071	0.238	-0.044	0.336	-0.023	0.411	-0.045	0.330
Delayed Recognition %	-0.042	0.337	-0.027	0.398	-0.023	0.412	0.012	0.455
Response Time *ms*	0.106	0.145	0.032	0.381	**† -0.181**	**0.037**	**†-0.253**	**0.006**
Choice Response *ms*	0.076	0.224	0.058	0.288	0.012	0.455	-0.057	0.289
Simple Recognition *ms*	0.002	0.493	-0.033	0.376	-0.001	0.497	-0.015	0.443
Stroop Interference *ms*	0.075	0.232	-0.011	0.460	-0.052	0.311	-0.096	0.179
Spatial Working Memory *ms*	***0.236**	**0.008**	***0.189**	**0.033**	0.009	0.463	-0.065	0.257
Contextual Memory *ms*	0.134	0.090	0.126	0.133	0.049	0.314	-0.025	0.401
Delayed Recognition *ms*	-0.045	0.325	0.035	0.369	0.057	0.287	-0.042	0.341

ms: milliseconds

Table 8.7. Selected hierarchical regressions of cardiovascular variables on cognitive performance, after controlling for education and age

Cardiovascular Predictor	Dependent Variable	Whole model†				Change statistics		
		B	Beta	*R*	Adj. R^2	R^2 Change	*F*	*p*
Younger group								
Simple Recognition %	Aug Index	-0.119	-0.117	0.241	0.026	0.012	1.098	0.297
	Aug Pressure	-0.527	-0.144	0.254	0.032	0.018	1.698	0.196
Spatial Working Memory %	Aug Index	-0.200	-0.238	0.507	0.233	0.049	5.982	***0.016**
	Aug Pressure	-0.742	-0.245	0.511	0.237	0.053	6.492	***0.013**
Spatial Working Memory *ms*	Aug Index	1.913	0.157	0.356	0.098	0.021	2.207	0.141
	Aug Pressure	6.385	0.145	0.353	0.096	0.019	1.924	0.169
Spatial Working Memory Performance (% / ms)	Aug Index	-0.063	-0.225	0.456	0.182	0.044	5.003	***0.028**

ms: milliseconds

continued…

Table 8.7 continued…

Cardiovascular Predictor	Dependent Variable	Whole model†				Change statistics		
		B	Beta	*R*	Adj. R^2	R^2 Change	*F*	*p*
Older group								
Simple Recognition %	Aug Pressure	-0.535	-0.198	0.195	0.006	0.038	3.500	0.065
Spatial Working Memory %	Aug Index	-0.124	0.095	0.291	0.053	0.018	1.735	0.191
	Aug Pressure	-0.334	-0.107	0.279	0.048	0.011	1.111	0.295
Spatial Working Memory *ms*	Aug Index	2.693	0.228	0.337	0.083	0.050	4.884	***0.030**
	Aug Pressure	6.014	0.148	0.291	0.055	0.021	2.110	0.150
Spatial Working Memory performance (% / ms)	Aug Index	-0.050	-0.222	0.388	0.125	0.048	5.660	***0.019**
Simple RT	Diastolic BP	-0.748	-0.216	0.292	0.056	0.043	4.371	***0.039**

†Whole model includes age and education as predictor variables, *significant at $p < 0.05$, *ms: milliseconds*

After controlling for age and education, augmentation index predicted Spatial Working Memory performance in the young group and in the older group. Moderator regression analyses were conducted with age group, augmentation index and their interaction term (age group multiplied by augmentation index) entered as predictor variables and Spatial Working Memory as the dependent variable. The interaction term was not significant (p = 0.570), indicating there was no difference in the relationship between groups.

8.3.7.2 Cognition and brachial blood pressures

Several significant bivariate correlations were observed between cognitive tasks and brachial blood pressures (Table 8.6). In the Simple Recognition task, better accuracy was associated with lower diastolic pressure in the younger group and lower systolic pressure in the older group.

Contrary to expectations, faster performance on the Spatial Working Memory task was significantly correlated with higher systolic pressure in the younger group. In the older group, faster performance on the Response Time task was associated with higher systolic and diastolic pressures. These results appear to contradict the notion that better cardiovascular health equals better cognitive performance.

For significant correlations, hierarchical multiple regressions were used to determine whether these variables predict performance on cognitive tasks after controlling for the effects of age and education. The only relationship that remained significant was a very small predictive relationship for diastolic pressure and Simple Reaction Time in the older group (Table 8.7). All other brachial blood pressures were unrelated to cognitive performance after controlling for age and education.

Given the conflicting results for brachial blood pressure variables, chance findings cannot be ruled out. Only five of the 44 correlations between brachial pressure and cognition were significant, and three of these were in the reverse direction to the anticipated. Furthermore, only one remained significant after controlling for confounding variables. These data do not provide a compelling argument for a relationship between brachial blood pressure and cognition in this participant group.

8.4 Discussion

This study provides further evidence for the hypothesis that augmentation index and augmentation pressure are associated with cognition in younger and older adults. In particular, Spatial Working Memory was correlated with augmentation index in both age groups. Relationships were not observed on other cognitive tasks, however the Spatial Working Memory task has been demonstrated in previous research to be the most sensitive of the cognitive battery. It is especially sensitive to aging and to changes due to nutritional supplementation (Pipingas et al., 2010; Pipingas et al., 2008). It is not surprising that this task in particular has demonstrated sensitivity to arterial stiffness, as measured by augmentation index and augmentation pressure.

Spatial function is particularly susceptible to age-related decline and dementia (Malec et al., 1992; Pipingas et al., 2010; Royall et al., 1998). Decline in spatial function is associated with changes in the prefrontal cortex and hippocampus (Klencklen et al., 2012) and these regions have been demonstrated to be sensitive to vascular variables. For example, arterial stiffness as measured by pulse pressure was associated with white matter deterioration in frontal regions (K. M. Kennedy & Raz, 2009), and another study observed that participants with higher arterial stiffness exhibited reduced blood flow in frontal white matter and in the hippocampus (Tarumi, Shah, Tanaka, & Haley, 2011). Adding to this evidence, augmentation index was associated with white matter hyperintensities in another study which examined periventricular and subcortical structures, demonstrating it is associated with brain changes (Nakano, Munakata, Shimaura, Asano, & Ohkuma, 2012). Thus a role for increased augmentation index in spatial decline in particular is plausible.

In a previous study which showed a relationship between augmentation index and cognition, the pertinent cognitive domain was speed of memory and there was no specific correlation with spatial working memory (Pase et al., 2010). That study also used a computerized cognitive battery suggesting that the tasks are somewhat comparable. However, the tests differ in their format and it might be that our battery has a more sensitive spatial assessment due to task difficulty. Further research using a range of validated cognitive tasks would help to elucidate this issue.

The hypothesis that augmentation index would demonstrate stronger relationships with cognition than brachial blood pressure measures was also supported in this study. There was no clear pattern of correlation for systolic or diastolic pressure and some analyses found that higher blood pressure was associated with better cognitive performance, which is contrary to expectations. It is true that some studies have found that lower blood pressure in older adults is associated with poorer health outcomes, particularly for low diastolic pressure (Paran et al., 2003). In the case of cognition, lower blood pressure could lead to reduced cerebral blood flow, affecting brain structure and cognitive performance (Qiu et al., 2005). However, this would be expected in participants who were more elderly than those in the present sample and does not generally apply to younger adults. Thus it is likely that the significant correlations observed for brachial blood pressures are chance findings, resulting from multiple tests. Augmentation index and augmentation pressure appeared to have a more consistent pattern of relationships with the cognitive tasks.

Augmentation index was a better predictor of cognitive performance than augmentation pressure, which did not remain a significant predictor of cognitive performance in the older group after controlling for age and education. Changes in arterial properties that occur with age result in an increase in augmentation index until around 50-55 years when it levels out, whereas augmentation pressure continues to rise (Fantin et al., 2007). This suggests that augmentation pressure might be a better candidate for observing a relationship with deteriorating cognition in an older group of adults. This was not observed in this study, in fact a relationship between cognition and augmentation pressure was significant in the younger group but not the older group. This unexpected finding may be due to the fact that the anticipated age-related changes described by Fantin et al. (2007) were not observed in the present participants. Augmentation pressure was not significantly associated with age in the older group but followed a similar pattern to augmentation index and appeared to level out.

While the findings in this study with regard to Spatial Working Memory were significant, they were not particularly strong and were limited to this task. Other measures of arterial stiffness have demonstrated relationships with cognition, including cognitive domains such as executive function and episodic memory (Rabkin, 2012), so

it was unexpected that a more robust role for augmentation index in cognition was not observed. It is possible that the relationships were weakened by controlling for age. Augmentation index and cognition are both markedly affected with age. Arterial stiffness increases in old age due to breakdown of elastin in the arterial wall (Zieman et al., 2005). This occurs inevitably but is hastened by cardiovascular risk factors such as hypertension (O'Rourke & Hashimoto, 2008). However for cognition, age-related changes may be partly mediated by arterial stiffness (see Chapter 3 for discussion). Nevertheless, it is necessary to control for the effects of age because of the many other age-related factors that affect cognitive and cardiovascular impairment.

A number of other interesting observations can be made on this data. The older group had a greater diversity of cognitive test scores than the younger group, reflecting greater cognitive variability. Although the older group were in a smaller age bracket (11 years compared with 15 years for the younger group) the cognitive task results of the older group were spread more widely. This reflects the individual differences in cognitive performance of older adults, where some are operating at the optimum for their age group while others have deteriorated more substantially. This agrees with other research which shows increasing individual differences in cognitive aging (Hultsch, MacDonald, & Dixon, 2002).

Patterns of educational achievement among the participants also correspond with other research showing cohort differences, in that people today receive more years of formal education than in the past (Anstey & Christensen, 2000). In this sample, the younger participants were more highly educated and more consistently so. In the older group educational attainment was as low as six years and as high as 27 years. Despite these differences, educational attainment had little impact on cognitive test scores for the participants in this study.

A limitation of the present study is the inclusion of participants who were taking preventative cardiovascular medication, including for cholesterol and blood pressure. These medications might distort the true effects of age on the cardiovascular system, particularly in the older group where medication was more common. However, the

majority of participants were not taking any medication so this is unlikely to have had an overwhelming effect on the data.

8.5 Summary and conclusion

This study explored the relationships between cognitive tasks and cardiovascular measures. It was demonstrated that augmentation index was a significant predictor of cognitive performance, in particular for Spatial Working Memory, after controlling for age and education. This is the first study to demonstrate this relationship in younger and older adult groups separately. In addition, this study observed that augmentation pressure was also a significant predictor of performance on Spatial Working Memory for the younger group only.

This study has also provided a clearer understanding of the differences between younger and older adults in cognition and cardiovascular function, using a computerized cognitive battery and the central pressure measures, augmentation index and augmentation pressure. These variables were used in the clinical trials reported in the following chapters to measure the effects of multivitamin, mineral and herbal supplementation in older adults. Importantly, this study has also demonstrated the sensitivity of the variables to aging.

9 The effects of multivitamin supplementation in older women: cognition, cardiovascular function, and associated blood markers

9.1 Introduction

The prevalence of cognitive impairment is increasing, with women suffering a greater share of cognitive decline including dementia (Deloitte Access Economics, 2011). Cognitive impairment places a growing burden on the health care system and has enormous personal implications for quality of life for the individual as well as being distressing for the families who care for those with impairment.

Cardiovascular health is also of increasing importance to the health and mortality of women, with ischemic heart disease and cerebrovascular disease now the two leading causes of death for women in Australia (ABS, 2013). This has implications for cognitive health too, due to the negative effects of poor cardiovascular health on cognition (Erkinjuntti & Gauthier, 2009).

Cognitive decline and cardiovascular disease share a number of known risk factors, including poor nutritional status (Gillette-Guyonnet et al., 2007; Guardamagna, Abello, Cagliero, & Lughetti, 2012). Also, cognitive and cardiovascular aging are associated with a range of physiological markers which might be causal to the process. High homocysteine is associated with poorer cognition and dementia and is a risk factor for cardiovascular disease (Prins et al., 2002; Refsum et al., 2006). The inflammatory response is integral to the atherosclerotic process and is also associated with poorer cognition (Teunissen et al., 2003; Wong et al., 2012). Increased inflammatory markers are also present in dementia (M. J. Engelhart et al., 2004). Oxidative stress has been extensively studied in relation to both cognitive and cardiovascular health. Markers of oxidative stress are increased in people with cardiovascular disease, cognitive decline and dementia (Barja, 2004; Holtzman, 2008).

These markers have been demonstrated to be sensitive to supplementation with various nutrients. Homocysteine is reduced by supplementation with B-group vitamins, particularly folate and B_{12}. Many nutrients are purported to have anti-inflammatory actions, including quercetin (Davis, Murphy, & Carmichael, 2009), vitamin D (Shab-Bidar et al., 2012), and vitamin B_6 (M. S. Morris, Sakakeeny, Jacques, Picciano, & Selhub, 2010; Shen, Lai, Mattei, Ordovas, & Tucker, 2010). Oxidative stress markers can be reduced with antioxidant vitamins (Chin et al., 2011) and plant extracts including flavonoids (Young, Shand, McGregor, Scott, & Frampton, 2006).

The use of vitamin supplementation is widespread in Australia and other western countries, with a larger proportion of women choosing to supplement. For example, one study reported that 62% of women aged 50-59 years supplemented compared with 49.7% of men in the same age group (E. T. Kennedy, Luo, & Houser, 2013). Multivitamins are one of the more popular choices of supplementation (Sebastian, Cleveland, Goldman, & Moshfegh, 2007; Timbo, Ross, McCarthy, & Lin, 2006). People may choose to supplement for various reasons, with memory improvement cited as a common reason among older adults (Marinac et al., 2007). However the benefits of multivitamins for this purpose are not clear.

Although epidemiological data has provided associations between nutritional variables and cognitive decline, dementia and cardiovascular disease, intervention studies aiming to improve cognitive and cardiovascular outcomes have had limited success. These studies were reviewed in Chapter 4, where it was observed that the effects of supplementation on cognition have been inconsistent, with a few studies finding cognitive benefits but many others not. Similarly, for cardiovascular outcomes, a number of large trials have failed to find a benefit of supplements including B vitamins and antioxidants, and it has even been suggested that vitamin E may be detrimental to cardiovascular health.

There are methodological issues that may help to account for this apparent contradiction. Firstly, nutritional supplements may act in a preventative capacity, not as a treatment for disease. In this case, studies might benefit from observing changes in individuals who do not have existing disorders, that is, without dementia or

cardiovascular disease. While not be able to reverse damage, in healthier populations nutritional supplements may alter risk factors in a beneficial way. For cognitive studies this means examining markers such as homocysteine, oxidative stress biomarkers and inflammatory markers. For cardiovascular studies, it would be useful to assess modifiable measures relating to arterial function or other risk markers such as lipids.

Secondly, it might be advantageous to use a broad range of nutrients such as in a multivitamin. This would address the different nutritional requirements that might occur between different individuals. It would also provide a range of nutrients so that the benefits of a given nutrient are not limited by shortage of another; for example, both folate and B_{12} are involved in homocysteine metabolism and vitamin C can 'recycle' or 'refresh' vitamin E (Bender, 2003). Both of these examples raise the possibility that combinations of nutrients may produce stronger effects.

Thirdly, for cognitive function, it is imperative that appropriate tests are selected. Tests such as the MMSE or TICS are sometimes used in aging research, but might not be sensitive enough to pick up subtle changes in supplement studies (J. H. Kang et al., 2009; Summers et al., 2010). Computerized tests which assess faculties known to decline with age would be a better choice due to millisecond resolution. For example, our computerized battery has been demonstrated to be sensitive to age and nutritional supplementation (E. Harris et al., 2012; Pipingas et al., 2010).

9.1.1 Aims and hypotheses

This study was a randomized, controlled trial of a multivitamin, mineral and herbal supplement, which investigated the effects of 16 weeks supplementation on older women. Cognitive function, cardiovascular function and a range of blood biomarkers were assessed.

The primary outcome measures for cognition were performance on computerized cognitive tasks, in particular the Spatial Working Memory, Contextual Memory and Stroop Interference tasks of the computerized battery. These tasks assess abilities that

are most susceptible to cognitive decline, including spatial functions, episodic memory and inhibition (executive function). Of the tasks in the cognitive battery, these were previously demonstrated to be most sensitive to age-related decline (Pipingas et al., 2010). Furthermore, the Contextual Memory task was shown to improve after eight weeks supplementation with multivitamins in older men at risk of cardiovascular disease (E. Harris et al., 2012). Also, a composite score derived from the memory tasks in the battery was improved after 16 weeks in older women with memory complaints (MacPherson et al., 2012). It was hypothesized that performance on these tasks would improve after supplementation. The remaining cognitive tasks were included as secondary outcome measures.

For cardiovascular function the primary outcome measures were augmentation index and augmentation pressure. As demonstrated in the previous chapter, these are measures of arterial stiffness which increase substantially with age and are associated with cognition. Augmentation index is more commonly used in research however it has been proposed that augmentation pressure might be a better index of arterial stiffness in older adults (Fantin et al., 2007). Augmentation index has been shown to decrease after supplementation with antioxidant vitamins (Mullan et al., 2002; Rasool et al., 2008). As measures of arterial stiffness they are sensitive to endothelium-dependent vasodilation, which is mediated by NO (McEniery 2006). Homocysteine and oxidative stress can modify NO availability, potentially affecting arterial stiffness (Trabetti, 2008). It was therefore hypothesized that multivitamin supplementation would decrease augmentation index and augmentation pressure. Brachial blood pressures were assessed as secondary outcome measures. This is the first study to examine multivitamin effects on augmentation index and augmentation pressure.

As discussed above, blood biomarkers are associated with poorer cognition and cardiovascular dysfunction. Biomarkers were assessed to provide information on the physiological effects of the supplement and to provide information regarding possible mechanisms of action, if cognitive and cardiovascular benefits were observed. These included B-group and antioxidant vitamins; the inflammatory markers CRP and fibrinogen; protein carbonyls as a measure of oxidative stress; the lipid profile; and

blood safety measures. Blood biomarkers were considered to be secondary outcome measures.

9.2 Method

A detailed explanation of the trial design, participants, measurements, procedures and statistical analyses was provided in Chapter 5. This section gives a short outline of the methods applicable to this study.

The study was a double-blind, placebo-controlled trial, investigating the effects of 16-weeks supplementation with a multivitamin formulation on older women. The study was registered with the Australian New Zealand Clinical Trials Registry, trial ID ACTRN12608000117314. Ethics approval for the study was obtained from the Swinburne University Human Research Ethics Committee (Appendix A).

The study was funded by Swisse Vitamins Pty Ltd, who also supplied the multivitamin and placebo tablets. Staff at Swisse Vitamins Pty Ltd conducted the randomisation of the supplements and held the allocation schedule until after the data collection was complete. The company had no further input into the design of the study, analysis of the data or interpretation of results.

9.2.1 Participants

Participants were healthy females aged 55 to 65 years who responded to advertisements in local newspapers and seniors publications, on noticeboards, or via word-of-mouth. Exclusion criteria included: smoking; use of multivitamins or combinations of vitamins or herbal supplements; psychological or neurological disorders; history of heart disease or stroke; and those taking blood-thinning medication (except if less than 100mg aspirin).

Volunteers were initially screened for the exclusion criteria via telephone and again on attendance at the baseline testing session. In addition, the MMSE was conducted at baseline, with a cut-off set at 27 from a possible score of 30 to determine eligibility into the study. All participants gave written informed consent.

9.2.2 Power calculation

Two previous studies conducted by our group, which tested nutraceutical products and used the same cognitive test battery, obtained moderate eta-squared (η^2) values of 0.484 (Harris et al., 2012) and 0.314 (MacPherson, Ellis, Sali, & Pipingas, 2012). Using an effect size of 0.4, alpha of 0.05 and power of 0.80, G*Power software 3.1.7 was used to calculate the required sample size (Faul, Erdfelder, Lang, & Buchner, 2007). It was determined that 52 participants were required for the study to have sufficient power to detect a moderate effect size.

9.2.3 Treatment and placebo

The multivitamin supplement was Swisse Women's Ultivite Multi-Vitamin, Mineral & Anti-Oxidant with Herbs 50+ Years ™ by Swisse Vitamins Pty Ltd. It contains vitamins, minerals, plant extracts and probiotics. For a full list see Table 7.1. The placebo tablets contained starch with a small amount of vitamin B_2 (riboflavin) to produce a similar smell and some colouration of the urine.

Both tablets were dark pink in colour and were the same size and shape. They were packaged in identical blister packs with instructions to “take one tablet per day, during or immediately after a meal”.

Swisse Pty Ltd provided the tablets and conducted the randomisation. Tablets were provided to the investigator in numbered packets according to the allocation schedule. Swisse Pty Ltd held the allocation schedule until data collection was complete, ensuring investigators were blind to the allocation. Unused tablets were returned at follow up and counted to determine compliance.

9.2.4 Measures

Details of the measures used in the study are provided in Chapter 7. A short summary is provided here.

Demographic measures included date of birth, number of years of education, body mass index and details of medication or supplement use.

Cognitive testing comprised a battery of eight computerized cognitive tests, which took approximately 45 minutes to complete. Tasks included Simple Reaction Time, Choice Reaction Time, Immediate and Delayed Recognition Memory, Stroop Congruent and Incongruent, Spatial Working Memory and Contextual Memory.

Cardiovascular measures included central augmentation index, central augmentation pressure, brachial systolic pressure and brachial diastolic pressure. Augmentation index and augmentation pressure were obtained using the SphygmoCor System (AtCor Medical, NSW). Brachial pressures were recorded using an electronic sphygmomanometer device.

Blood biomarkers were assessed using a commercial pathology company. Tests included vitamin B_6, vitamin B_{12}, folate, homocysteine, high-sensitivity C-reactive protein, fibrinogen, protein carbonyls, full lipid profile, kidney and liver function tests. Vitamin C and vitamin E were assessed for several participants at baseline but were discontinued due to errors in collection and transport of the samples.

9.2.5 Procedure

Participants attended two testing sessions (baseline and post-treatment) at Swinburne University of Technology. The duration of the sessions was 1.5 hours. Participants were asked to refrain from drinking caffeine for three hours before the sessions.

After participants gave informed consent, they provided demographic information and were screened using the MMSE. Questionnaires were then completed (data not included here). Next the participant's blood pressure was taken and the pulse pressure waveform was recorded. Height and weight were then measured. Finally the computerized cognitive tasks were completed.

Upon finishing testing the participants were provided with a referral for blood testing and instructions to fast overnight and attend the blood collection centre early in the morning.

9.2.6 Statistical analysis

Data analysis was performed using IBM SPSS 20. Repeated measures analysis was used to determine the effects of multivitamin supplementation on cognitive tasks, cardiovascular measures and blood markers.

Data was screened to ensure it met the assumptions for repeated measures ANOVA. The assumptions were met except where stated otherwise in the results section. To avoid undue distortion of the data, values more than three standard deviations from the mean were not included in the analysis (Hair, 1998).

Significance levels were set at $p < 0.05$, however results with significance levels $p < 0.10$ are also noted, to acknowledge any non-significant trends in the data. For the primary outcome measures, adjustments were made to correct for multiple comparisons. For the three cognitive tasks, significance was calculated as $0.05 / 3 = 0.016$. For the central pressure measures it was $0.05 / 2 = 0.025$.

9.3 Results

One hundred and sixty-two women responded to the advertisements and underwent telephone screening. Of these, 68 passed the screening procedures and enrolled in the study; 33 were randomly allocated to the multivitamin group and 35 to the placebo group. Six participants did not return for post-treatment testing; three participants became unavailable because they had gone interstate or abroad, two elected to withdraw from the study, and one began medication during the intervention period that was an exclusion criteria. Therefore 62 women completed the study and were used in the analyses, with 31 in each group.

9.3.1 Baseline demographics

The multivitamin and placebo groups were well matched on demographic variables. Independent t-tests indicated that there were no significant differences between the group means for any of the screening variables at baseline ($p > 0.10$).

Table 9.1. Participant demographics at baseline

	Placebo (n = 31)				Multivitamin (n = 31)			
	M	*SD*	Min	Max	*M*	*SD*	Min	Max
Age - *years*	60.1	3.4	54	65	60.2	3.2	55	65
Education - *years*	14.7	3.8	9	24	16.0	3.9	11	23
BMI - kg/m^2	26.3	4.4	18.5	36.5	26.8	6.2	19.0	49.1

9.3.2 Concurrent supplement and medication use

Supplementation

Two-thirds of participants (66.7%) were not taking any concurrent supplements. Of the supplements reported, fish oil, glucosamine and occasional vitamin C were the most common (Table 9.2). In addition, there was one instance each of lysine, garlic &

horseradish, blueberry, acidophilus, magnesium, evening primrose oil, kelp, and calcium, and two instances of coenzyme Q-10 and vitamin D3. Participants continued to take their regular supplements during the study.

Table 9.2. Reported supplement use in placebo and multivitamin groups

	Placebo Group (*n*=31)	Multivitamin Group (*n*=31)	Total
Glucosamine	7 (22%)	6 (19%)	13 (21%)
Fish Oil	3 (10%)	5 (16%)	8 (13%)
Vitamin C	2 (6%)	3 (10%)	5 (8%)

Medication

More than half of the participants were taking no concurrent medication. The most commonly used medications were preventative cardiovascular medications, such as ACE inhibitors or calcium channel blockers for control of blood pressure, or statins for cholesterol reduction. Five participants were taking hormone replacement therapy.

Table 9.3. Reported medication use in placebo and multivitamin groups

	Placebo Group	Multivitamin Group	Total
No medications	21 (67%)	14 (45%)	35 (56%)
Blood pressure	6 (19%)	6 (19%)	12 (19%)
Cholesterol	4 (13%)	6 (19%)	10 (16%)
Aspirin	3 (10%)	3 (10%)	6 (10%)

9.3.3 Compliance

Participants returned all unused supplements when they returned for post-treatment testing and remaining tablets were counted to ascertain compliance. Mean compliance was 95.8% (minimum = 82%, maximum = 100%).

9.3.4 Success of placebo

Participants were asked to guess whether they were taking the multivitamin supplement or the placebo (Table 9.4). Fisher's Exact Test revealed a marginally significant difference between the beliefs of participants in the multivitamin and placebo groups ($p = 0.075$) suggesting they were marginally more likely to correctly guess what the supplements were.

Table 9.4. Number of participants who believed they were taking the multivitamin or placebo.

		Believed Placebo	Believed Multivitamin	Didn't Know	Total
Placebo Group	*n*	15	4	11	30
	%	50%	13%	37%	100%
Multivitamin Group	*n*	7	10	10	27
	%	26%	37%	37%	100%

9.3.5 Adverse events

There were no adverse events reported during the study. After the study had concluded, all participants were given two containers of Women's Ultivite 50+ in appreciation of their involvement. One participant who had been in the placebo group reported a possible reaction to the supplement, in the form of a skin rash. She was referred to her general practitioner and the event was reported to the Swinburne University Human Research Ethics Committee.

9.3.6 Cognitive tasks

Cognitive test results are provided in Table 9.5. Independent samples t-tests were conducted to examine baseline differences between multivitamin and placebo groups in cognitive task performance: no significant differences between groups in either accuracy or response time were observed ($p > 0.05$). Due to anticipated ceiling effects,

accuracy data was not analysed for Reaction Time, Choice Reaction Time and the Stroop Congruent task.

Repeated measures ANOVAS revealed no effect of supplementation on cognition, including for the primary outcome measures, Spatial Working Memory, Contextual Memory and Stroop Interference. Therefore the hypothesis that these tasks would improve with multivitamin supplementation was not supported. Significant or marginally significant main effects were observed for several tasks, indicating improvement from baseline to post-supplementation (i.e. practice effects). Specific details of the cognitive task results are as follows:

Spatial Working Memory

No effect of supplementation was observed on the Spatial Working Memory task. Significant practice effects were evident for accuracy ($F(1,54) = 5.179$, $p = 0.027$, partial $\eta^2 = 0.088$) and response time ($F(1,54) = 12.986$, $p = 0.001$, partial $\eta^2 = 0.194$).

Contextual Memory

Accuracy on the Contextual Memory Task declined significantly across both groups from baseline to follow up ($F(1,53) = 4.406$, $p = 0.041$, partial $\eta^2 = 0.077$). No significant changes were observed in response time.

Stroop Task (Interference effect)

There was no significant effect of supplementation on the Stroop task.

Simple Reaction Time

There was no effect of multivitamin supplementation on the Simple Reaction Time task.

Choice Reaction Time

No effect of supplementation was observed for Choice Reaction Time.

Immediate Recognition Memory

There was no effect of multivitamin supplementation on Simple Recognition Memory. Significant practice effects were evident for accuracy ($F(1,50) = 18.224$, $p < 0.001$,

partial $\eta^2 = 0.267$) and response time ($F(1,50) = 9.354$, $p = 0.004$, partial $\eta^2 = 0.158$) indicating improved performance from baseline to follow-up.

Delayed Recognition Memory

There was no significant effect of supplementation on the Delayed Recognition Memory task, however significant practice effects were evident for accuracy ($F(1,52) = 7.795$, $p = 0.007$, partial $\eta^2 = 0.130$) and response time ($F(1,52) = 10.320$, $p = 0.002$, partial $\eta^2 = 0.166$).

9.3.7 Cardiovascular function

Means and standard deviations of cardiovascular measures are presented in Table 9.6.

9.3.7.1 Augmentation index and augmentation pressure

No effect of multivitamin supplementation was observed for the primary outcome measures, augmentation index and augmentation pressure. The hypothesis that these central pressure measures would be reduced with multivitamin supplementation was not supported.

9.3.7.2 Brachial blood pressures

There was no effect of multivitamin supplementation on systolic or diastolic blood pressure. There were main effects of a reduction in systolic pressure ($F(1,55) = 11.3$, $p = 0.001$, partial $\eta2 = 0.013$) and in diastolic pressure ($F(1,55) = 9.83$, $p = 0.003$, partial $\eta2 = 0.171$).

Table 9.5. Cognitive tests: Means and standard deviations for accuracy and response time

		Baseline		Post-treatment		
		M	*SD*	*M*	*SD*	*n*
Spatial Work. Mem. *%*	Placebo	67.1	13.8	70.5	10.6	30
	Multi	68.7	13.2	71.4	13.3	26
Spatial Work. Mem. *ms*	Placebo	1200.0	190.2	1132.2	126.8	30
	Multi	1145.7	166.0	1099.2	161.3	26
Contextual *%*	Placebo	74.3	16.7	73.2	11.7	28
	Multi	78.5	12.7	71.1	13.0	27
Contextual *ms*	Placebo	1129.7	157.3	1081.0	134.6	29
	Multi	1070.0	123.0	1073.3	139.4	27
Stroop Interference *ms*	Placebo	205.4	133.7	216.7	141.5	27
	Multi	186.5	134.9	188.1	138.9	23
Stroop Congruent *ms*	Placebo	775.3	91.6	784.3	87.6	29
	Multi	776.4	109.2	744.8	93.2	27
Stroop Incongruent *%*	Placebo	94.1	6.2	93.7	9.7	28
	Multi	94.0	11.4	94.9	6.0	23
Stroop Incongruent *ms*	Placebo	978.8	147.7	1000.4	140.1	28
	Multi	940.5	184.7	918.5	176.5	23
Simple RT *ms*	Placebo	278.0	27.1	274.3	33.4	27
	Multi	275.7	26.8	272.4	29.3	25
Choice RT *ms*	Placebo	490.8	60.7	484.5	57.5	27
	Multi	498.5	66.4	501.3	65.4	27
Immediate Recog *%*	Placebo	67.4	11.7	76.4	9.0	28
	Multi	70.3	13.0	78.2	8.0	24
Immediate Recog *ms*	Placebo	1151.1	160.9	1086.7	132.8	28
	Multi	1083.9	101.9	1054.2	127.2	24
Delayed Recognition *%*	Placebo	68.0	8.7	75.2	10.4	28
	Multi	71.2	9.4	73.6	12.3	26
Delayed Recognition *ms*	Placebo	1063.8	141.2	1019.9	125.6	28
	Multi	1076.8	128.3	1022.9	147.9	26

Table 9.6. Means and standard deviations of cardiovascular measures

		Baseline		Post-treatment		
		M	*SD*	*M*	*SD*	*n*
Augmentation Index *%*	Placebo	32.0	10.3	29.2	7.3	29
	Multi	27.1	9.9	25.1	11.0	28
Augmentation Pressure *mmHg*	Placebo	12.6	4.8	11.6	3.7	28
	Multi	10.2	4.2	8.9	4.5	28
Systolic Pressure *mmHg*	Placebo	130.4	16.7	125.7	15.8	29
	Multi	126.2	11.4	121.8	10.4	28
Diastolic Pressure *mmHg*	Placebo	81.0	9.8	76.6	10.3	29
	Multi	79.0	8.2	77.0	8.4	28

9.3.8 Blood biomarkers

Independent samples t-tests indicated that participants in the multivitamin and placebo groups did not differ in their baseline blood assays ($p > 0.05$). One exception was for folate, which is discussed in greater detail below.

9.3.8.1 B vitamins and homocysteine

Means and standard deviations for B vitamins and homocysteine are presented in Table 9.7.

Vitamin B_6

There was a significant treatment effect for vitamin B6 ($F(1,49) = 75.7$, $p < 0.001$ partial $\eta 2 = 0.526$) (Figure 9.1). This was due to substantial increases in vitamin B6 for participants supplementing with the multivitamin, compared with only a very small change in those taking placebo.

Table 9.7. Means and standard deviations for homocysteine and B vitamins

		Baseline		Post-treatment		
		M	*SD*	*M*	*SD*	*n*
Homocysteine *µmol/L*	Placebo	13.0	2.9	14.4	3.9	31
	Multivitamin	12.9	3.0	12.6	2.5	28
Vitamin B_{12} *pmol/L*	Placebo	300	78	298	91	31
	Multivitamin	300	103	435	143	28
Vitamin B_6	Placebo	88	28	118	92	28
	Multivitamin	104	53	467	215	23
Folate *nmol/L*	Placebo	744	290	800	228	30
	Multivitamin	922	180	933	309	28

Vitamin B_6 was also a good indicator of compliance. Figure 9.2 shows individual changes in Vitamin B_6 for women in the multivitamin and placebo groups. There were clear individual increases in the multivitamin group, whereas the changes in the placebo group clustered around zero.

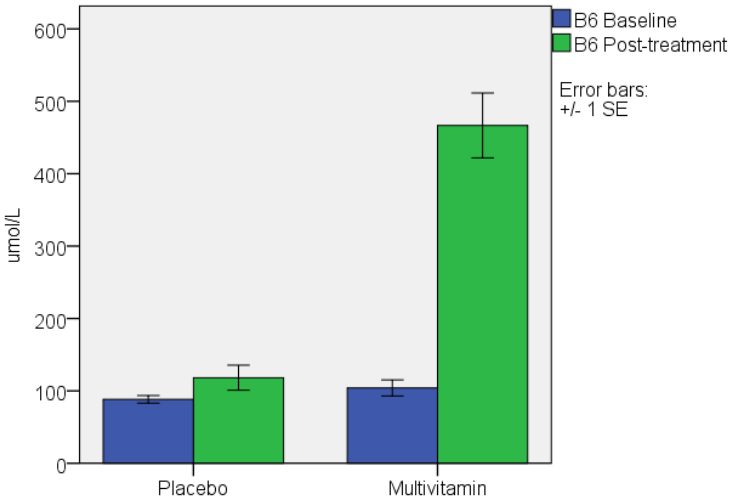

Figure 9.1. Mean vitamin B_6 concentrations for multivitamin and placebo groups. Error bars show ±1 standard error.

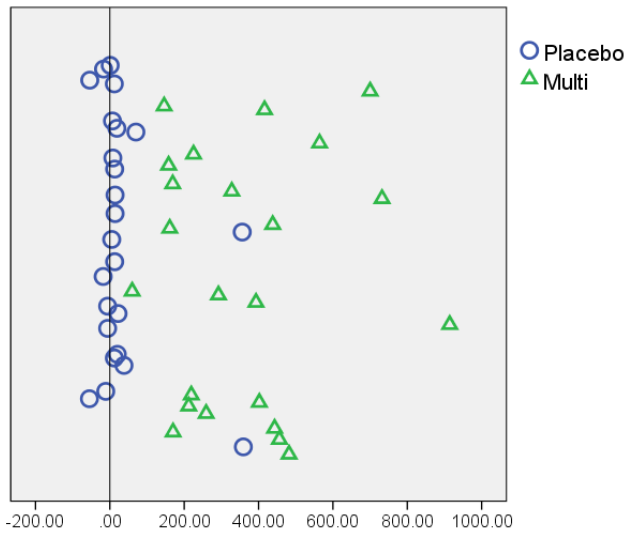

Figure 9.2. Change in vitamin B6 for individual women in the multivitamin and placebo groups. The Y-axis represents a de-identified participant code.

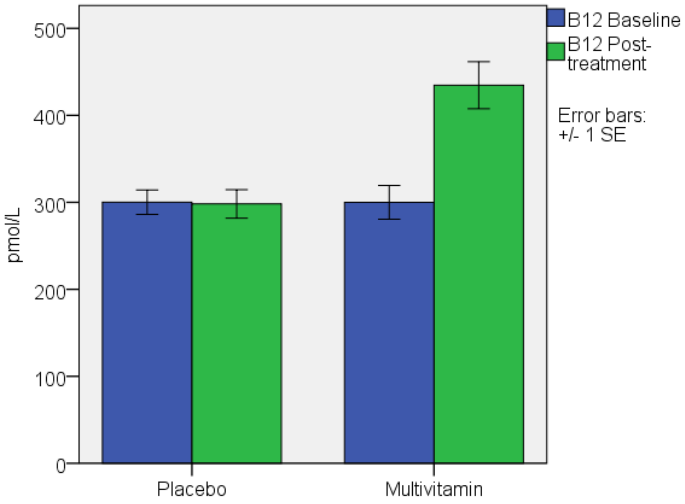

Figure 9.3. Mean vitamin B_{12} concentrations for multivitamin and placebo groups. Error bars show ±1 standard error.

Vitamin B_{12}

There was a significant treatment effect for vitamin B_{12} ($F(1,57) = 29.9$, $p < 0.001$ partial $\eta^2 = 0.344$). This was due to an increase in B_{12} in the multivitamin group compared with no change in the placebo group (Figure 9.3).

Folate

No significant changes in folate were observed after multivitamin supplementation. This was an unexpected result and appears suspect for several reasons. Firstly, there was considerable variation in the individual participant data and folate levels pre- and post-supplementation were uncorrelated, even in the placebo group. This is unusual because red cell folate represents tissue stores of folate which are reasonably stable and less sensitive to fluctuations in the diet (Galloway & Rushworth, 2003). Related to this, some unlikely findings were observed in that some individual participants in the multivitamin group appeared to greatly decrease their folate levels over the course of the study and some in the placebo group greatly increased their levels. Secondly, the supplements each contained 500mcg of folic acid which is more than sufficient to increase folate levels (Homocysteine Lowering Trialists' Collaboration, 2005). The participants in the study were not deficient in folate at baseline but levels were not so high that supplementation would not have any effect. Finally, vitamin B_6 and vitamin B_{12} increased in the supplement groups, so it is unlikely that participant non-compliance produced the negative findings. This pattern suggests that there was an error in the sampling, transport, analysis or reporting of the folate samples. Folate has been shown to be stable for 72 hours when kept at 4ºC but for only 24 hours at room temperature (Zemlin, Essack, Rensburg, Keller, & Brinkmann, 2010), indicating that incorrect handling of the samples could affect results. Errors that occurred with the vitamin C and vitamin E testing corroborate the conclusion that folate results were unreliable.

Homocysteine

There was a significant effect of supplementation on homocysteine ($F(1,57) = 6.02$, $p = 0.017$, partial $\eta^2 = 0.095$), however this was due to an increase in homocysteine in the placebo group ($t(30) = -2.65$, $p = 0.013$) rather than a decrease in the multivitamin group, which was not significant (Figure 9.4).

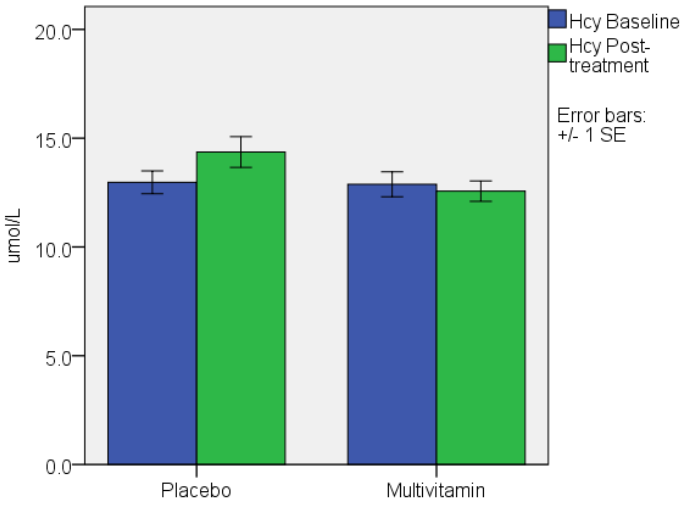

Figure 9.4. Mean homocysteine concentrations for multivitamin and placebo groups. Error bars show ±1 standard error.

9.3.8.2 *Inflammatory markers*

Fibrinogen

There were no significant treatment effects observed for fibrinogen.

High Sensitivity C-Reactive Protein (CRP)

The distribution of CRP was highly positively skewed. A logarithmic transformation was applied (log10(x)), which improved the distribution. Analysis of the transformed data revealed a significant effect of multivitamin supplementation on CRP ($F(1,51) = 4.47$, $p = 0.039$, partial $\eta^2 = 0.081$). This was due to a reduction in CRP in the multivitamin group which was marginally significant ($t(23) = 2.02$, $p = 0.055$) compared to a non-significant change in the placebo group (Figure 9.5).

Table 9.8. Means and standard deviations of inflammatory biomarkers and protein carbonyls

		Baseline		Post-treatment		
		M	*SD*	*M*	*SD*	*n*
CRP *mg/L*	Placebo	1.9	2.2	1.8	2.1	27
	Multi	1.9	2.5	1.1	1.2	24
Fibrinogen *g/L*	Placebo	3.1	0.3	3.0	0.4	30
	Multi	3.0	0.5	3.0	0.5	26
Prot Carb *mol/mL*	Placebo	19.6	7.1	17.6	3.5	24
	Multi	18.2	7.05	19.0	5.1	24

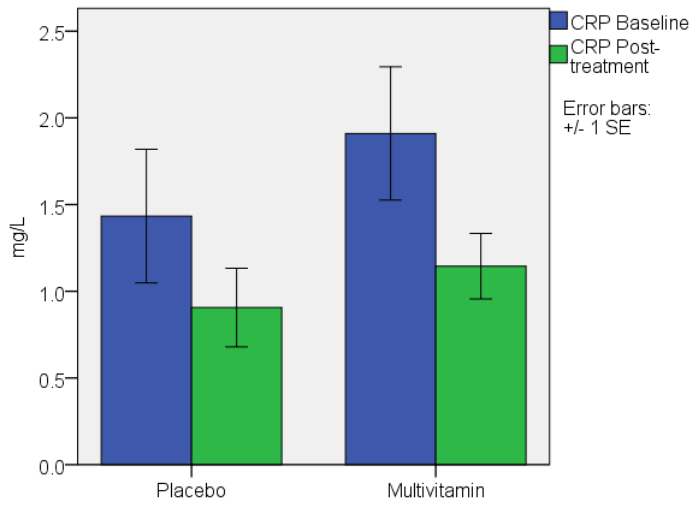

Figure 9.5. Mean C-reactive protein concentrations for multivitamin and placebo groups in the Women's study. Error bars show ±1 standard error.

9.3.8.3 Protein carbonyls

Means and standard deviations for protein carbonyls are presented in Table 9.8. There was no effect of multivitamin supplementation on protein carbonyls.

9.3.8.4 Lipid profile

Results of lipid measures are presented in Table 9.9. There were no treatment effects for any of the lipid measures.

Significant main effects were observed including an increase in HDL ($F(1,58) = 3.81$, $p = 0.056$, partial $\eta^2 = 0.062$) and a decrease in LDL ($F(1,58) = 9.10$, $p = 0.004$, partial $\eta^2 = 0.136$).

Table 9.9. Means and standard deviations of lipid variables

		Baseline		Post-treatment		
		M	*SD*	*M*	*SD*	*n*
Total Chol *mmol/L*	Placebo	6.0	1.1	5.8	0.9	31
	Multi	5.7	0.8	5.8	0.8	29
HDL *mmol/L*	Placebo	1.80	0.40	1.88	0.48	31
	Multi	1.90	0.38	2.07	0.56	29
LDL *mmol/L*	Placebo	3.70	0.97	3.40	1.05	31
	Multi	3.37	0.66	3.11	0.96	29
Triglyceride *mmol/L*	Placebo	1.2	0.5	1.2	0.6	31
	Multi	1.0	0.4	1.1	0.4	28
LDL/HDL ratio	Placebo	2.2	0.7	2.1	0.7	31
	Multi	1.8	0.5	1.8	0.5	29
Chol/HDL ratio	Placebo	3.5	0.9	3.4	0.8	31
	Multi	3.1	0.6	3.1	0.6	29

9.3.8.5 General biochemistry

There were no treatment effects for the general biochemistry measures, which included sodium, potassium, chlorine, bicarbonate, urea, creatinine or estimated glomerular filtration rate, eGFR (data not shown). All values were within reference range at baseline and remained so after the supplementation period.

9.3.8.6 Liver function tests

There were no treatment effects for ALP, albumin, bilirubin, or GGT (data not shown). Significant treatment effects were observed for AST and ALT. Means and standard deviations for AST and ALT are provided in Table 9.10.

Table 9.10. Means and standard deviations for AST and ALT.

		Baseline		Post-treatment		
		M	*SD*	*M*	*SD*	*n*
AST *U/L*	Placebo	23.0	5.7	21.3	5.3	29
	Multivitamin	21.4	45.0	26.2	9.7	29
ALT *U/L*	Placebo	22.9	10.1	20.9	9.1	29
	Multivitamin	18.1	4.0	22.9	5.4	27

AST (aspartate transaminase)

There was a significant main effect indicating an increase in AST across all participants ($F(1,56) = 4.89$, $p = 0.031$, partial $\eta^2 = 0.080$). The interaction was also significant, with a greater increase in the multivitamin group than in the placebo group ($F(1,56) = 22.84$, $p < 0.001$, partial $\eta^2 = 0.290$). Although the increases were significant, they were small and remained well below the reference value of <41 U/L (Figure 9.6).

ALT (alanine transaminase)

Increases were also observed in ALT. There was a marginally significant main effect for ALT ($F(1,53) = 3.514$, $p = 0.066$, partial $\eta^2 = 0.062$). The interaction was significant ($F(1,54) = 24.316$, $p < 0.001$, partial $\eta^2 = 0.310$), indicating a greater increase in the multivitamin group. However, means still remained well below the reference value of <51 U/L (Figure 9.7).

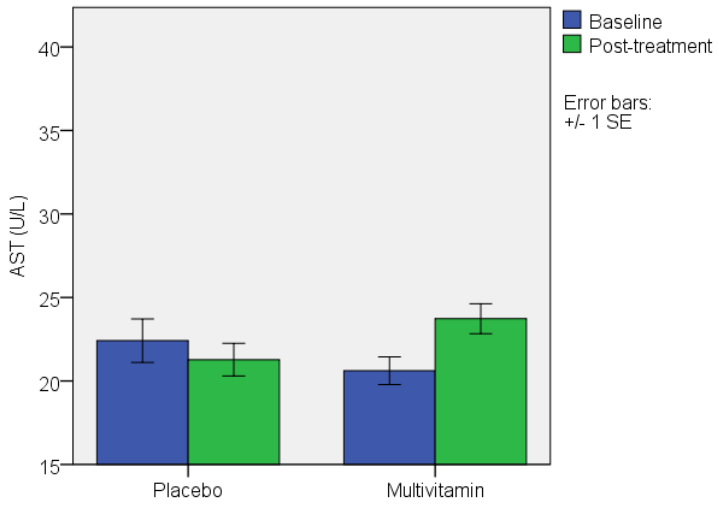

Figure 9.6. Mean AST concentrations for multivitamin and placebo groups in the Women's study. Error bars show ±1 standard error. Y-axis is scaled to the reference range.

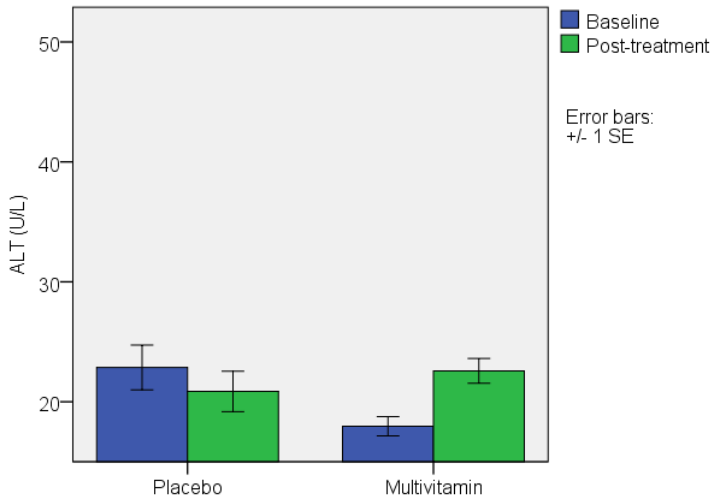

Figure 9.7. Mean ALT concentrations for multivitamin and placebo groups in the Women's study. Error bars show ±1 standard error. Y-axis is scaled to the reference range (<51 U/L).

9.4 Discussion

The present investigation found no effect of 16-weeks multivitamin, mineral and herbal supplementation on cognitive task performance or the central pressure measures augmentation index and augmentation pressure, in women aged 55-65 years. Therefore, the hypothesis that these primary outcome measures would be improved with supplementation was not supported. However, some beneficial changes to blood biomarkers were observed, including an increase in vitamin B_6 and vitamin B_{12}, and a reduction in the inflammatory marker CRP. In addition there were small changes in two liver function tests, AST and ALT. Other blood markers remain unchanged or exhibited changes that were likely due to random variation.

9.4.1 Cognitive measures

Cognition was a primary outcome measure for this study. Cognitive effects were not observed after supplementation, despite the advantages of the present methodology. Computerized tests were used that have previously been demonstrated to be age-sensitive and responsive to nutritional supplementation (E. Harris et al., 2012; Pipingas et al., 2010). The supplement contained a broad range of vitamins, thus addressing potential inadequacies of a range of nutrients (D. O. Kennedy & Haskell, 2011). Furthermore, the study population was old enough to be experiencing measurable cognitive decline but were not suffering from any serious cognitive impairment such as dementia. Compliance in the study was also good, as indicated by the tablet counts and corroborated by the considerable increases in vitamin B_6 and B_{12} in the supplement group.

The lack of effect of multivitamin supplementation on cognition in this study is therefore a convincing finding, with no apparent trends observable in the data. Existing research has demonstrated the difficulty in producing measurable cognitive benefits with vitamin supplementation (Debreceni & Debreceni, 2012). This seems to contradict epidemiological research which indicates better cognitive outcomes with higher vitamin

status or intake, including for B-group vitamins (Nurk et al., 2005; Ramos et al., 2005) or antioxidant vitamins (M.J. Engelhart et al., 2002; Grodstein et al., 2003).

The present results are also inconsistent with previous multivitamin research by our group using the same cognitive battery, which has demonstrated improvements to cognition in older women with self-reported memory complaints (MacPherson et al., 2012), and older men at high risk of cardiovascular disease (E. Harris et al., 2012). Both of those studies observed beneficial changes to blood biomarkers. In contrast, in the present study only the inflammatory marker CRP was reduced whereas fibrinogen, homocysteine and protein carbonyls remained unchanged. If benefits are to be mediated by these pathways then this may explain why the treatment was unsuccessful; cognitive benefits might only occur where nutrients alter the physiology in a beneficial way. It might also be that the present participants, being healthier and/or younger than previous samples, required a longer intervention period to elicit cognitive changes.

9.4.2 Augmentation index and augmentation pressure

Cardiovascular variables were also unchanged in this study. Augmentation index has previously been reduced with chronic supplementation with 50-200mg vitamin E in healthy younger men (Rasool et al., 2008) and 1000mg vitamin C in patients with type 2 diabetes (Mullan et al., 2002). Another study which investigated a combination of vitamin C and E found a marginally significant effect on augmentation index in hypertensive males, with significant effects in other measures of arterial stiffness (Plantinga et al., 2007). In the present study, the doses of vitamin C (200mg) and vitamin E (20mg) were much lower amounts than those given in the studies cited above, and might not have been sufficient to elicit a change.

The effect of B vitamins on augmentation index is less clear. Existing research is in the context of kidney disease which might not be applicable to the present investigation of healthy older women (see Chapter 6). However, some improvement in augmentation index has been observed in renal patients with, for example, combination folate and vitamin B_{12} for three weeks (Koyama et al., 2010). On the contrary, a large study

investigating folic acid supplementation for 3.6 years observed no benefit to augmentation index (Zoungas et al., 2006). Despite the weak support for an effect of B vitamin supplementation on augmentation index in the scientific literature, there is a theoretical rationale to suggest that reduction of homocysteine may lead to reduced arterial stiffness via endothelium-dependent vasodilation. This may occur for example through improved NO availability or reduced auto-oxidation of homocysteine leading to reduced oxidative stress (Debreceni & Debreceni, 2012).

There was a main effect of reduction in systolic and diastolic blood pressures during the course of the study, with both groups significantly improved at post-treatment testing. This could be due to improved lifestyle behaviours in the intervention period as a result of the focus on physical health variables during the baseline testing session (blood pressure, weight, etc.). Taking part in the study may have been part of their motivation to improve their health; a daily tablet may have served as a reminder of their health status.

9.4.3 Blood biomarkers

It was unexpected that homocysteine was not reduced in the multivitamin group in this study. A reduction in homocysteine is a generally dependable finding with B vitamin supplementation, including multivitamin supplementation (E. Harris et al., 2012; Haskell et al., 2010). The amount of folate and vitamin B_{12} provided in the vitamin was enough to reduce homocysteine according to previous research (Homocysteine Lowering Trialists' Collaboration, 2005). Also, the mean homocysteine level in the participants was high enough at baseline to allow for improvement. There was an increase in homocysteine in the placebo group and it could be argued that the supplement prevented a similar increase from occurring in the multivitamin group. However there is no obvious reason for an increase in homocysteine to have occurred in the participants at that time.

Similarly, protein carbonyls remained unchanged indicating that the antioxidant vitamins, vitamin C and vitamin E, and the phytonutrient antioxidants did not alter the

level of oxidative stress. Protein carbonyls have previously been shown to decrease with antioxidant supplementation (Chin et al., 2011).

The lack of effect on homocysteine and the oxidative stress marker, protein carbonyls, indicate that two major mechanisms through which benefits might be mediated were not altered. One explanation might be due to lack of absorption. Vitamin B_6 and B_{12} did increase substantially during the study however due to errors in testing of vitamin C, vitamin E and folate we cannot observe whether blood levels of these vitamins were altered. Folate in particular is crucial for homocysteine reduction (Clarke, 1998). Importantly, Rasool et al. (2008) found a benefit to augmentation index only after supplementation with a vitamin E tablet that was designed for better absorption, a previous study with the same design but using a different supplement did not improve augmentation index (Rasool et al., 2006).

For the cholesterol measures, both the multivitamin and placebo groups showed a significant increase in HDL and decrease in LDL. This improvement is consistent with the main effect of improvement in systolic and diastolic blood pressure, and may similarly be due to improved lifestyle behaviours in both groups during the course of the study.

For blood safety markers, there was a significant effect of supplementation on AST and ALT, with both of these markers increasing after supplementation with the multivitamin but not after placebo. Although these results are suggestive of a detrimental effect of the supplementation on liver function, the actual change was very small and remained well within the reference ranges.

9.4.4 Practice effects

Improvement across both groups were observed in several cognitive tasks, including accuracy in the Simple Recognition, Spatial Working Memory, Contextual Memory and Delayed Recognition tasks, and for response time in the Simple Reaction Time, Spatial Working Memory and Delayed Recognition tasks. This indicates that all participants'

performance was better when they returned for second round regardless of whether they were in the multivitamin or placebo group. This might have been due to familiarity with the task, that is, practice effects. However, the effects were quite strong and practice effects of this magnitude were not observed previously in the test battery (Pipingas et al., 2010), suggesting an alternative explanation.

As mentioned above, systolic and diastolic pressures were reduced for both multivitamin and placebo groups. Furthermore, HDL was increased and LDL was decreased across both groups, both beneficial changes for cardiovascular health (Frayn, 2008). Participation in the study may have led to an improvement in lifestyle behaviours in some participants, as a result of observing their own baseline health variables such as excess weight or high blood pressure. Improvements in cardiovascular measures might have led to the improvement in cognition; alternatively, underlying health behaviours might have contributed to both cognitive and cardiovascular changes.

9.4.5 Limitations and future directions

The present study possibly had too small a sample size. Power calculation determined that 52 participants were sufficient, however the effect size that was used for the calculation was based on our previous studies in which the participants had key differences. Women in this study were healthy older adults aged 55-65 years. In the Macpherson et al. (2012) study, the women were older and suffered subjective memory complaints. In the E. Harris et al. (2012) study, participants were men aged 50-70 who were at risk due to sedentary lifestyles. It might be that a much larger sample is required to observe changes in healthy older adults. Future research may benefit by targeting at-risk older adults, or using a larger participant group.

A further limitation is the duration of supplementation. If vitamins act in a preventative manner, it might be necessary to supplement for much longer, i.e. years, to observe the benefits. This would help to explain why epidemiological research shows relationships between cognition and cardiovascular function whereas trials of supplements

infrequently observe significant changes: epidemiological research observes data that reflects a lifetime of health behaviours.

Finally, due to problems with blood testing we could not observe whether important the nutrients in the multivitamin were absorbed. Changes in blood levels of folate, vitamin C and vitamin E could provide important information as to why the supplement produced limited benefits. If there was a problem with absorption then benefits to the outcome measures would be unlikely.

Despite these limitations, it can be stated that multivitamin supplementation did not affect cognition or cardiovascular function in this group of healthy older women.

9.5 Summary and conclusion

This study investigated the effects of a multivitamin, mineral and herbal supplement in women aged 55 to 65 years. There were no effects of supplementation on the primary outcome measures, including cognitive performance on a computerized battery, and cardiovascular function as measured by augmentation index and augmentation pressure.

Beneficial changes due to supplementation were observed in some blood variables, which were secondary outcome measures. This included an increase in vitamin B_{12} and vitamin B_6, and a reduction in CRP. Improvements in these markers might have beneficial effects on cognition and cardiovascular function in the long term. However, a lack of effect on other blood biomarkers (e.g. homocysteine and protein carbonyls) may also explain why changes in cognitive and cardiovascular variables did not occur. That is, the pathways that mediate these variables were unchanged.

Significant main effects were observed in some cognitive tasks, including Immediate Recognition, Spatial Working Memory, Contextual Memory, and Delayed Recognition. This may have been due to difference in difficulty between baseline and follow up versions of the test or due to health improvements in the participants. There were main effects for systolic and diastolic blood pressures which decreased in both multivitamin

and placebo groups. Also, mean LDL cholesterol was reduced and HDL cholesterol increased across both groups.

The following chapter reports on findings from the Men's study, which investigated the effects of a Men's formula multivitamin, mineral and herbal supplementation on cognition and cardiovascular function, in men aged 55 to 65 years.

10 The effects of multivitamin supplementation in older men: cognition, cardiovascular function, and associated blood markers

10.1 Introduction

The previous chapter examined the effects of a multivitamin supplement in women aged 55-65 years. In this chapter we similarly examine multivitamin effects, however in men of the same age. There are several reasons for studying men and women in separate trials.

There are gender differences in the aging process and thus benefits of a given supplement may affect men and women differently. For example, women have a higher prevalence of dementia (Deloitte Access Economics, 2011) and poorer cognitive function during the illness (Irvine, Laws, Gale, & Kondel, 2012). On the other hand, in the healthy elderly, women tend to have better memory than men (Gerstorf et al., 2006). Hormonal differences can also impact cognition, with studies showing that testosterone in elderly men may be protective (Holland, Bandelow, & Hogervorst, 2011) whereas estrogen differences between men and women may contribute to disparities in cognitive outcome (Janicki & Schupf, 2010). Such differences argue for separate studies for men and women.

In addition to this, males and females have different daily requirements for vitamins, with men requiring higher doses of some vitamins due to larger body size (NHMRC, 2006). Therefore tailoring a supplement to provide optimal benefit requires different formulas for men and women. The supplements used in the present studies reflect these differences by including different dosages of many of the vitamins and minerals (see Table 7.1 to compare ingredients). Furthermore, the present supplements also contain differences in herbal ingredients which reflect other gender differences in older adults. Examples include saw palmetto in the Men's formula, which is used to treat benign prostatic hyperplasia, and black cohosh in the Women's formula, used for menopausal

symptoms (Braun & Cohen, 2010). Another important difference in the current supplements is that only the Women's formula contains Bacopa Monniera, a herb with demonstrated cognitive effects (Pase, Kean, et al., 2012). Because of these gender-specific ingredients it would be inappropriate to give the same supplement to both male and female groups. Also the two products cannot be assumed to have identical effects so combining data would be inappropriate.

Although there are important differences between the genders, men are likewise susceptible to cognitive decline and cardiovascular disease. In Australia, dementia prevalence in men aged 80 to 85 years is 12.1%, and increases to 37.2% in those over 95 years of age (Deloitte Access Economics, 2011). Cardiovascular diseases are a major cause of death in Australian men, with ischemic heart disease and cerebrovascular disease listed as the number one and three leading causes of death in Australia in 2011; dementia and Alzheimer's disease are placed sixth (ABS, 2013).

Men also use nutritional supplements and although with a lesser frequency than women, in older age groups prevalence of supplementation reaches nearly 50% (E. T. Kennedy et al., 2013). Despite their widespread use, the efficacy of multivitamin supplementation has not been well-established in the scientific literature. Intervention trials are inconsistent and only one study has examined cognitive effects specifically in older men; this was a study of men at risk of cognitive decline due to cardiovascular risk factors (E. Harris et al., 2012).

In our previous study using the same cognitive battery, a multivitamin supplement elicited cognitive benefits after eight weeks of supplementation in men aged 50-70 years (E. Harris et al., 2012). The formula was a multivitamin, mineral and herbal supplement that was designed for men, and is therefore similar to the formula for this study. However the present supplement includes ingredients targeted to older adults. One notable difference in the present supplements is the addition of Ginkgo biloba which might provide additional benefit to cognition (Mahadevan & Park, 2008). Furthermore, in the previous study, the participants were at risk of cognitive decline due to cardiovascular risk factors. Here we examine healthy older males, in a protocol consistent with the female cohort that was reported in the previous chapter.

10.1.1 Aims and hypotheses

The aim of this investigation was to examine the effects of 16 weeks supplementation with a multivitamin, mineral and herbal formulation in healthy older men. This randomized, placebo-controlled trial examined effects of supplementation on cognition, cardiovascular function and related blood biomarkers were evaluated.

Primary outcome measures were proposed for cognitive and cardiovascular measures. For the cognitive tasks, the Spatial Working Memory, Contextual Memory and Stroop Interference tasks from the computerized cognitive battery were specifically chosen as the primary outcome measures. The Contextual Memory task was improved in our previous study of the effects multivitamin supplementation in older men, and the Spatial Working Memory and Stroop tasks have previously been shown to be sensitive to age-related cognitive decline and nutritional supplementation (E. Harris et al., 2012; Pipingas et al., 2010). It was hypothesized that performance on these tasks would improve after supplementation.

Augmentation index and augmentation pressure were the primary outcome measures for cardiovascular function. These are measures of arterial stiffness that increase with age and are related to cognition (see Chapter 3 for a review). Augmentation index has decreased in response to vitamin and flavonoid supplementation (Mullan et al., 2002; Young et al., 2006). Thus it was hypothesized that these measures would improve after 16 weeks of supplementation with the multivitamin formula.

Secondary outcome measures included the remaining tasks in the cognitive battery, as well as brachial systolic and diastolic pressures. Blood biomarkers were also considered to be secondary outcome measures. Homocysteine, inflammatory markers, and protein carbonyls were hypothesized to improve after 16 weeks of supplementation with the nutritional formula. Blood vitamin levels were hypothesized to increase due to supplementation.

10.2 Method

Chapter 7 provided a complete account of the methods used in this study, including trial design, participants, measurements, procedures and statistical analyses. This section gives a brief summary of these. The study was a randomized, double-blind, placebo-controlled trial. It investigated the effects of 16-weeks multivitamin supplementation in older men. The study was registered with the Australian New Zealand Clinical Trials Registry, trial ID ACTRN12608000117314. Ethics approval for the study was obtained from the Swinburne University Human Research Ethics Committee (Appendix A).

Funding for the study and supply of the multivitamin and placebo tablets were provided by Swisse Vitamins Pty Ltd. Staff at Swisse Vitamins Pty Ltd conducted the randomisation of the supplements but the company had no further input into the design of the study, analysis of the data or interpretation of results.

10.2.1 Participants

Healthy men aged 55 to 65 years were sought via advertisements in local newspapers, seniors publications, on noticeboards, or through word-of-mouth. Initial telephone screening excluded volunteers who were smokers; used multivitamins, vitamin combinations or herbal supplements; had a history of psychological or neurological disorder, heart disease or stroke; and anyone taking blood thinning medication (with the exception of those taking less than 100mg aspirin). On attendance at baseline testing, participants were screened using the MMSE, with a score of 27/30 being the minimum for participation in the study. All participants gave written informed consent.

10.2.2 Power calculation

The power calculation for this study is the same as for the Women's study. It was based on two previous studies conducted by our group, which obtained moderate eta-squared (η^2) values of 0.484 (Harris et al., 2012) and 0.314 (MacPherson et al., 2012). G*Power

software 3.1.7 (Faul et al., 2007) was used to calculate the required sample size based on an effect size of 0.4, alpha of 0.05 and power of 0.80; 52 participants were required to have adequate power to detect a moderate effect size.

10.2.3 Treatment and placebo

The supplement used in the study was Swisse Men's Ultivite Multi-Vitamin, Mineral and Antioxidant with herbs 50+ years™ by Swisse Vitamins Pty Ltd. It contains vitamins, minerals, plant extracts and probiotics. A full list of ingredients is provided in Table 7.1. The placebo tablets were the same colour, size and shape as the multivitamins. They consisted of starch and a small amount of vitamin B_2 (riboflavin) which was included to give the tablets a similar smell and produce colouration of the urine. Both tablets were packaged in blister packs with instructions to "take one tablet per day, during or immediately after a meal". Participants were instructed to return any unused tablets, which were counted to verify compliance.

Randomisation was conducted by Swisse Pty Ltd and the tablets were supplied in packets that were numbered according to the allocation schedule. To ensure that investigators were blind to treatment, Swisse Pty Ltd held the allocation schedule until the conclusion of data collection.

10.2.4 Measures

An extended account of the measures used in the study is given in Chapter 7. The following is a brief description of the variables that were assessed.

Demographic measures included date of birth, number of years of education, body mass index and details of medication or supplement use.

Cognitive testing comprised a battery of eight computerized cognitive tests. Tasks included Simple Reaction Time, Choice Reaction Time, Immediate and Delayed

Recognition Memory, Stroop Congruent and Incongruent, Spatial Working Memory and Contextual Memory. The battery took approximately 45 minutes to complete.

Cardiovascular function measures included central augmentation index, central augmentation pressure, brachial systolic pressure and brachial diastolic pressure. Augmentation index and augmentation pressure were obtained using the SphygmoCor System (AtCor Medical, NSW). Brachial pressures were recorded using an electronic sphygmomanometer.

Blood samples were collected via venepuncture at a blood collection centre convenient to the participant. Samples were analysed for vitamin B_6, vitamin B_{12}, folate, homocysteine, high-sensitivity C-reactive protein, fibrinogen, protein carbonyls, full lipid profile, kidney and liver function tests. Vitamin C and vitamin E were assessed for several participants at baseline but were discontinued due to errors in collection and transport of the samples. Blood measures were conducted by a commercial pathology company.

10.2.5 Procedure

Baseline and post-treatment testing sessions were conducted at Swinburne University of Technology, taking approximately 1.5 hours each. Participants were asked not to drink caffeinated beverages for three hours before the sessions.

Testing sessions proceeded in the following order: informed consent, demographic information, MMSE screening, questionnaires (data not included), blood pressure, central pressure measures, height and weight, computerized cognitive tasks. Upon completion the participants were given a referral for blood testing. They were advised to fast overnight and attend the blood collection centre early the following morning.

10.2.6 Statistical analysis

Individual variables were inspected to determine whether they met the assumptions for repeated measures ANOVA. Details of any divergence from the assumptions are given in the relevant results section. Values greater than three standard deviations from the mean were considered outliers and were not included in the analyses (Hair, 1998).

IBM SPSS 20 was used to conduct the data analyses. Repeated measures ANOVAs were performed to ascertain the effects of multivitamin supplementation on cognitive tasks, cardiovascular measures and blood markers. Significance levels were set at $p < 0.05$. Results with significance levels $p < 0.10$ are also noted, to investigate any trends in the data. For the primary outcome measures, adjustments were made to correct for multiple comparisons. For the three cognitive tasks, significance was calculated as 0.05 / 3 = 0.016. For the central pressure measures it was 0.05 / 2 = 0.025.

10.3 Results

Eighty-four men responded to the advertisements and underwent telephone screening. Of these, 48 passed the screening procedures and attended the first testing session. Further assessment disqualified four volunteers, three due to multiple health issues and one who did not achieve the minimum MMSE score of 27/30. Therefore 42 men were enrolled in the study, with 21 randomly allocated to the multivitamin group and 21 to the placebo group. One participant from the placebo group chose to withdraw from the study, therefore 41 men completed the study and their data were used in the analyses.

10.3.1 Baseline demographics

The multivitamin and placebo groups were well matched on demographic variables (Table 10.1). Independent t-tests indicated that there were no significant differences between the group means for any of the screening variables at baseline ($p > 0.05$).

Table 10.1. Means and standard deviations of participant characteristics at baseline

	Placebo (*n* = 20)				Multivitamin (*n* = 21)			
	M	*SD*	Min	Max	*M*	*SD*	Min	Max
Age - *years*	59.1	2.3	55	65	60.2	3.2	55	65
Education - *years*	16.5	3.8	11	27	16.0	3.9	11	23
BMI - kg/m^2	26.8	2.9	21.4	32.3	26.8	6.2	19.0	49.2

10.3.2 Concurrent supplement and medication use

Nutritional supplements

The majority of participants (80%) were taking no concurrent supplements. Three participants were taking fish oil supplements, three were taking glucosamine, two were taking vitamin C, and one was taking saw palmetto. Participants were asked to continue their usual regime while participating in the study.

Medication

Over half of the participants (56%) were taking no concurrent medication. The most common medications used by participants were preventative cardiovascular medications, for control of blood pressure or cholesterol (Table 10.2).

Table 10.2. Number of participants taking concurrent medications

	Placebo Group (*n* = 20)	Multivitamin Group (*n* = 21)	Total
No medications	10 (50%)	13 (62%)	23 (56%)
Blood pressure	6 (30%)	4 (20%)	10 (24%)
Cholesterol	3 (15%)	2 (10%)	5 (12%)
Aspirin	2 (10%)	2 (10%)	4 (10%)

10.3.3 Compliance

Participants returned all unused tablets when they attended post-treatment testing. Remaining pills were counted. Compliance was good, with participants taking an average of 95.8% of their allocated tablets (minimum = 80.4%, maximum = 100%).

10.3.4 Success of placebo

Participants were asked if they knew whether they were taking the multivitamin supplement or the placebo (Table 10.3). Fisher's Exact Test found that there was no significant difference between the beliefs of participants in the multivitamin and placebo groups ($p = 0.332$), indicating that participants were not able to guess which tablets they were taking.

Table 10.3. Number of participants who believed they were taking the multivitamin or placebo.

		Believed Placebo	Believed Multivitamin	Didn't Know	Total
Placebo	*n*	11	0	4	15
	%	73%	0%	27%	100%
Multivitamin	*n*	12	3	6	21
	%	57%	14%	29%	100%

10.3.5 Adverse events

There were no adverse events reported during the study.

10.3.6 Cognitive tasks

Results of cognitive task performance are reported in Table 10.4. Independent samples t-tests indicated that there were no significant differences at baseline between multivitamin and placebo groups in cognitive task accuracy or response time ($p > 0.05$).

Analysis of the cognitive tasks did not reveal any effect of supplementation for the multivitamin formula for the primary outcome measures: Contextual Memory, Spatial Working Memory and Stroop Interference. Similarly, there was no effect of supplementation on the secondary cognitive outcome measures.

Two specious findings were observed. Significant interactions were found in the Stroop Congruent task and marginally in the Choice Reaction Time task, as described below. These were due to changes in the placebo group rather than being an effect of supplementation. Furthermore, significant or marginally significant main effects were observed for several tasks. This indicates that all participants' performance improved when they returned for follow-up testing, regardless of whether they were in the multivitamin or placebo group.

Following are details for the findings of each cognitive task:

Contextual Memory

There was a significant main effect for Contextual Memory. Performance accuracy on the task was poorer in both groups from baseline to follow-up ($F(1,33) = 9.781$, $p = 0.004$, partial $\eta^2 = 0.229$).

Spatial Working Memory

Significant practice effects were evident on the Spatial Working Memory task for accuracy ($F(1,35) = 8.407$, $p = 0.006$, partial $\eta^2 = 0.194$) and marginally for response time $F(1,35) = 3.631$, $p = 0.065$, partial $\eta^2 = 0.094$).

Stroop – Interference Effect

There was a marginally significant effect observed for the Stroop - Interference Effect ($F(1,30) = 3.401$, $p = 0.075$, partial $\eta^2 = 0.102$). However this reflected an improvement in the placebo group after the intervention period, with little change observed in the multivitamin group (Figure 10.1). This is considered a specious finding.

Simple Reaction Time

There were no significant changes for Simple Reaction Time.

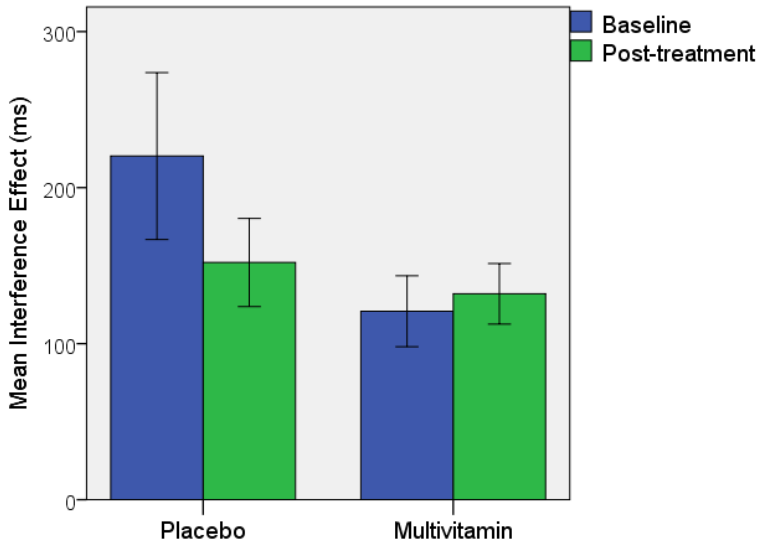

Figure 10.1. Stroop Interference Effect before and after supplementation with multivitamin and placebo. Error bars are +/- 1 standard error.

Choice Reaction Time

A marginally significant effect was observed for response time on the Choice Reaction Time task ($F(1,35) = 3.915$, $p = 0.056$, partial $\eta^2 = 0.101$). This was due to poorer performance at post-treatment testing in the placebo group rather than an improvement in the multivitamin group. This is considered a specious finding as cognition is unlikely to have deteriorated significantly during the study period.

Simple Recognition Memory

Significant practice effects were evident in the Simple Recognition Memory task for both accuracy ($F(1,33) = 23.211$, $p < 0.001$, partial $\eta^2 = 0.413$) and response time ($F(1,33) = 8.697$, $p = 0.006$, partial $\eta^2 = 0.209$). This indicates improved performance from baseline to follow up across all participants.

Delayed Recognition Memory

Significant practice effects were evident on the Delayed Recognition Memory task for response time ($F(1, 38) = 6.789$, $p = 0.014$, partial $\eta^2 = 0.180$).

10.3.7 Cardiovascular function

Means and standard deviations of cardiovascular function variables are presented in Table 10.5.

Augmentation index and augmentation pressure

There were no significant changes to augmentation index or augmentation pressure.

Brachial blood pressures

There was no effect of supplementation on systolic or diastolic blood pressure. There was a trend towards a main effect of reduction in systolic pressure across both groups ($F(1,38) = 3.48$, $p = 0.070$, partial $\eta^2 = 0.084$).

10.3.8 Blood biomarkers

Independent samples t-tests indicated that participants in the multivitamin and placebo groups did not differ in their baseline blood assays ($p > 0.05$). One exception was for folate, which is discussed below.

Table 10.4. Cognitive tasks: Means and standard deviations for accuracy and response time

		Baseline		Post-treatment		
		M	*SD*	*M*	*SD*	*n*
Spatial Working Memory *%*	Placebo	78.1	9.9	81.5	9.2	16
	Multi	74.9	16.1	77.0	15.7	21
Spatial Working Mem. *ms*	Placebo	1023.3	136.3	996.2	114.8	16
	Multi	1018.2	181.4	974.7	182.8	21
Contextual Memory *%*	Placebo	69.7	13.0	63.8	12.5	16
	Multi	73.4	17.7	64.2	16.9	19
Contextual Memory *ms*	Placebo	1068.7	131.4	1081.5	116.0	16
	Multi	1076.9	135.2	1060.6	168.1	19
Stroop Interference *ms*	Placebo	220.2	207.1	152.0	109.3	15
	Multi	120.8	93.8	131.9	79.9	17
Stroop Congruent *ms*	Placebo	745.6	80.5	779.7	94.7	16
	Multi	743.7	99.1	725.6	101.7	21
Stroop Incongruent *%*	Placebo	91.5	15.3	93.2	7.6	15
	Multi	97.8	3.4	97.5	4.2	17
Stroop Incongruent *ms*	Placebo	969.9	164.9	938.0	105.9	15
	Multi	851.3	127.2	853.9	99.4	17
Simple RT *ms*	Placebo	261.7	32.1	257.1	27.2	17
	Multi	260.2	24.3	258.3	16.5	20
Choice RT *ms*	Placebo	461.6	42.1	487.7	63.3	17
	Multi	466.7	56.1	457.4	50.3	20
Immediate Recog *%*	Placebo	70.7	12.0	79.8	7.6	15
	Multi	67.0	12.2	76.7	8.7	20
Immediate Recog *ms*	Placebo	1128.6	173.8	1094.9	160.0	15
	Multi	1143.5	152.8	1059.6	179.0	20
Delayed Recog *%*	Placebo	66.0	14.3	70.5	7.6	14
	Multi	69.0	10.0	70.7	14.0	20
Delayed Recog *ms*	Placebo	1122.5	162.4	1081.2	154.9	14
	Multi	1073.1	95.2	1014.4	146.8	19

Table 10.5. Means and standard deviations of cardiovascular measures

		Baseline		Post-treatment		
		M	*SD*	*M*	*SD*	*n*
Systolic Pressure *mmHg*	Placebo	131.9	11.5	128.0	9.8	18
	Multi	136.1	13.9	134.2	13.0	22
Diastolic Pressure *mmHg*	Placebo	82.2	6.2	80.7	9.4	18
	Multi	84.4	9.7	82.7	7.1	22
Augmentation Index	Placebo	18.7	8.0	18.8	8.9	14
	Multi	23.1	8.3	22.5	12.1	19
Augmentation Pressure *mmHg*	Placebo	6.8	3.1	6.6	3.4	18
	Multi	9.3	4.8	8.4	5.0	21

10.3.8.1 B vitamins and homocysteine

Means and standard deviations for B vitamins and homocysteine are in Table 10.6.

Table 10.6. Means and standard deviations for homocysteine and B vitamins

		Baseline		Post-treatment		
		M	*SD*	*M*	*SD*	*n*
Homocysteine µmol/L	Placebo	15.5	3.9	16.2	3.2	17
	Multi	15.1	3.4	13.7	2.8	21
Vitamin B_{12} *pmol/L*	Placebo	284	100	249	84	15
	Multi	265	63	342	120	19
Vitamin B_6 *nmol/L*	Placebo	151	120	160	155	13
	Multi	121	89	335	134	15
Folate *nmol/L*	Placebo	601	187	614	156	17
	Multi	683	298	793	303	19

Vitamin B_6

Multivitamin supplementation significantly increased vitamin B_6 levels compared with placebo $F(1,26) = 25.5$, $p < 0.001$ partial $\eta^2 = 0.495$) (Figure 10.2). Post-hoc t-tests indicated that the change was significant in the treatment group ($t(14) = -7.17$, $p < 0.001$) but not in the placebo group.

Vitamin B_6 was also a good indicator of compliance. Figure 10.3 shows individual participants' changes in Vitamin B_6. There were clear individual increases in the multivitamin group but not in the placebo group.

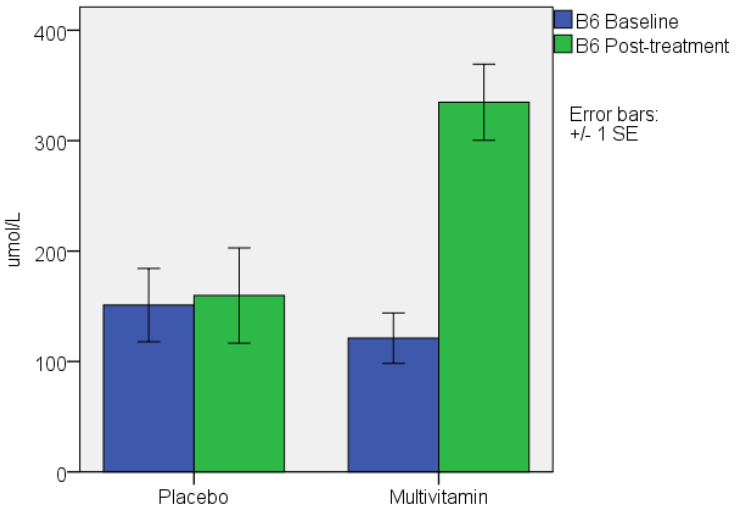

Figure 10.2. Mean vitamin B_6 concentrations for multivitamin and placebo groups. Error bars show ±1 standard error.

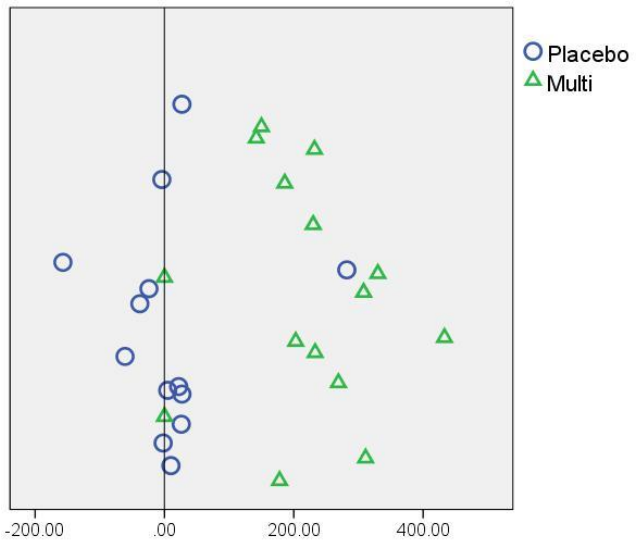

Figure 10.3. Individual changes in vitamin B6 for men in the multivitamin and placebo groups. Y-axis represents a de-identified participant code.

Vitamin B_{12}

Multivitamin supplementation significantly increased vitamin B_{12} levels compared with placebo ($F(1,32) = 14.1$, $p = 0.001$ partial $\eta^2 = 0.306$) (Figure 10.4). Post-hoc t-tests indicated a significant increase in the multivitamin group ($t(18) = -3.22$, $p = 0.005$) and the decrease in the placebo group was also significant ($t(14) = 2.45$, $p = 0.028$).

Folate

There was no significant effect of multivitamin supplementation on folate. This was an unexpected result. As described in Section 9.3.8.1 of the Women's study, patterns of folate results indicate that there were errors in the blood testing and analysis procedures.

Homocysteine

There was a significant treatment effect on homocysteine ($F(1,36) = 4.40$, $p = 0.043$, partial $\eta^2 = 0.109$) (Figure 10.5). This effect appeared to be due to a decrease in homocysteine in the multivitamin group and an increase in the placebo group. Paired samples t-tests indicated that the decrease in the multivitamin group was marginally significant ($t(20) = 1.949$, $p = 0.066$) whereas the increase in the placebo group was not significant ($p = 0.308$).

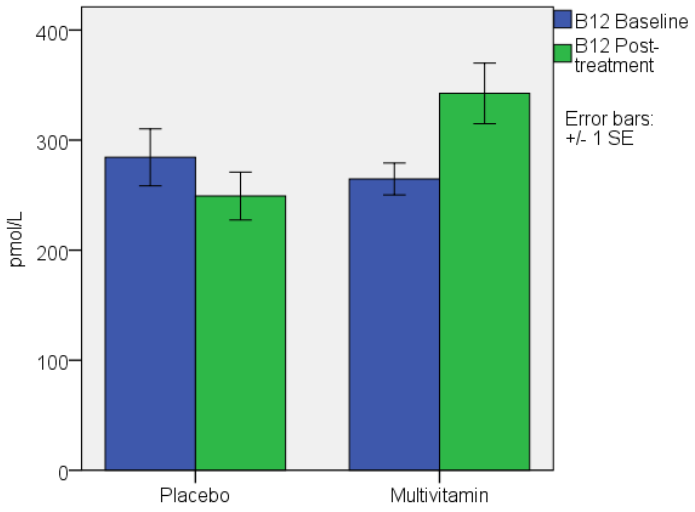

Figure 10.4. Mean vitamin B_{12} concentrations for multivitamin and placebo groups. Error bars show ±1 standard error.

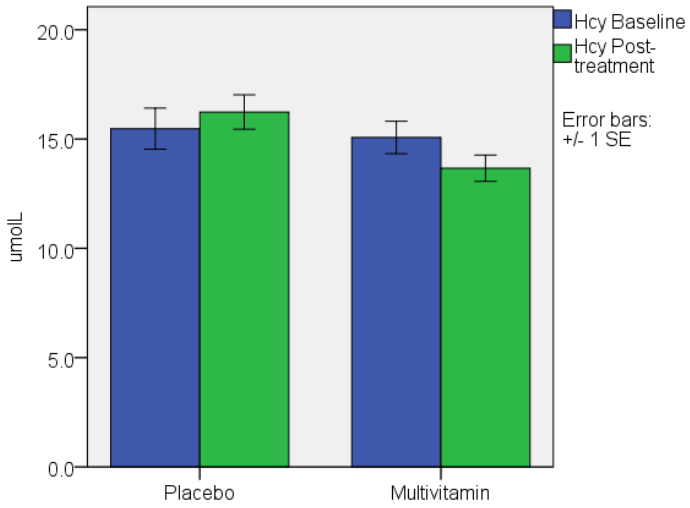

Figure 10.5. Mean homocysteine concentrations for multivitamin and placebo groups. Error bars show ±1 standard error.

10.3.8.2 Inflammatory markers

Means and standard deviations of the inflammatory markers are presented in Table 10.7.

Table 10.7. Means and standard deviations of inflammatory biomarkers

		Baseline		Post-treatment		
		M	*SD*	*M*	*SD*	*n*
CRP *mg/L*	Placebo	1.4	1.5	0.9	0.9	15
	Multi	1.9	1.7	1.1	0.8	20
Fibrinogen *g/L*	Placebo	3.0	0.6	3.1	0.3	14
	Multi	2.9	0.4	2.9	0.5	20

Fibrinogen

There was a marginally significant treatment effect for fibrinogen ($F(1,32) = 2.10$, $p = 0.069$, partial $\eta^2 = 0.062$), however this was due to a reduction in fibrinogen in the placebo group and therefore not an effect of supplementation. Post-hoc t-tests indicated that the changes were not significant in either the multivitamin or placebo group, thus it is likely to be a chance finding.

High Sensitivity C-Reactive Protein (CRP)

CRP data were highly positively skewed so a Log transformation was applied, resulting in a more normal distribution appropriate for ANOVA.

There was a significant overall reduction in CRP in both groups ($F(1,33) = 8.05$, $p = 0.008$, partial $\eta^2 = 0.196$). This was possibly due to a seasonal effect because CRP tends to be higher in the winter months and more of the participants were enrolled during this time.

10.3.8.3 Protein carbonyls

Results of protein carbonyls are presented in Table 10.8.

Table 10.8. Means and standard deviations of protein carbonyls.

		Baseline		Post-treatment		
		M	*SD*	*M*	*SD*	*n*
Protein Carbonyl *mol/mL*	Placebo	19.1	5.2	19.4	4.5	15
	Multi	20.5	5.1	16.4	4.6	15

There was a marginally significant reduction in protein carbonyls after supplementation with the multivitamin ($F(1,28 = 3.491$, $p = 0.072$, partial $\eta^2 = 0.111$). There was a 20% reduction in protein carbonyls in the multivitamin group compared with only a very small change in the placebo group (Figure 10.6). Post-hoc t-tests indicated that the change in the multivitamin group was significant ($t(14) = 2.59$, $p = 0.021$) whereas the change in the placebo group was not significant.

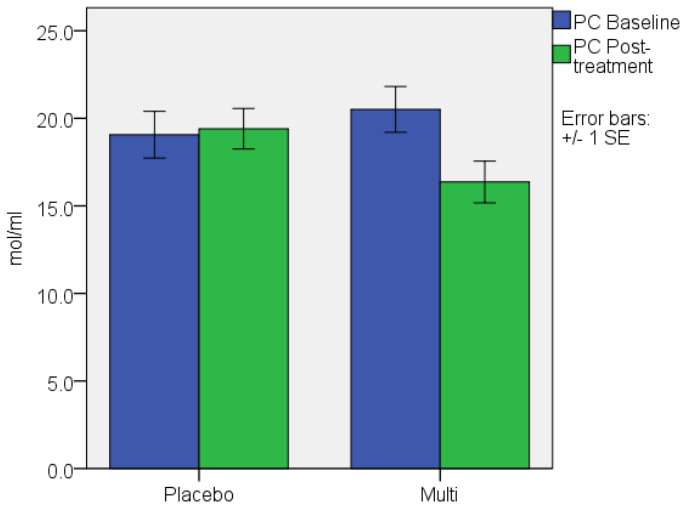

Figure 10.6. Mean protein carbonyl concentrations for multivitamin and placebo groups. Error bars show ±1 standard error.

10.3.8.4 Lipid profile

Results of blood lipid testing are presented in Table 10.9. Multivitamin supplementation had significant effects on several lipid measures. There was a significant treatment effect for LDL ($F(1,35) = 5.25$, $p = 0.028$, partial $\eta^2 = 0.130$), which decreased in the multivitamin group ($t(19) = 2.64$, $p = 0.016$) but not in the placebo group ($t(16) = 0.229$, $p = 0.822$) (Figure 10.7). There was also a marginally significant treatment effect for total cholesterol ($F(1,36) = 3.34$, $p = 0.076$, partial $\eta^2 = 0.085$) with total cholesterol similarly decreasing in the multivitamin group only (Figure 10.8). However post-hoc t-tests were not significant for total cholesterol.

These changes were reflected in cholesterol ratios. The LDL/HDL ratio decreased in the multivitamin group after supplementation ($F(1,36) = 5.60$, $p = 0.023$, partial $\eta^2 = 0.135$) (Figure 10.9), as did the cholesterol/HDL ratio ($F(1,36) = 5.26$, $p = 0.028$, partial $\eta^2 = 0.127$) (Figure 10.10). These were confirmed by post-hoc t-tests. For LDL/HDL the t-test was significant in the multivitamin group only ($t(20) = 2.83$, $p = 0.010$). Similarly, for cholesterol/HDL the t-test was marginally significant in the multivitamin group ($t(20) = 2.06$, $p = 0.053$) but non-significant in the placebo group.

There was an overall increase in triglycerides ($F(1,35) = 5.35$, $p = 0.027$, partial $\eta^2 = 0.133$), with both multivitamin and placebo groups presenting with increased triglyceride concentrations at post-treatment.

Table 10.9. Means and standard deviations of lipid variables

		Baseline		Post-treatment		
		M	*SD*	*M*	*SD*	*n*
Total Chol *mmol/L*	Placebo	5.6	0.9	5.7	1.1	17
	Multi	5.6	0.7	5.3	0.9	21
HDL *mmol/L*	Placebo	1.54	0.30	1.52	0.36	17
	Multi	1.51	0.42	1.46	0.39	20
LDL *mmol/L*	Placebo	3.42	0.67	3.44	0.79	17
	Multi	3.45	0.59	3.06	0.89	20
Triglyceride *mmol/L*	Placebo	1.2	0.5	1.4	0.6	16
	Multi	1.3	0.5	1.4	0.6	21
LDL/HDL ratio	Placebo	2.3	0.6	2.2	0.7	17
	Multi	2.5	0.7	2.3	0.5	21
Chol/HDL ratio	Placebo	3.7	0.7	3.8	0.8	17
	Multi	4.0	0.9	3.8	0.7	21

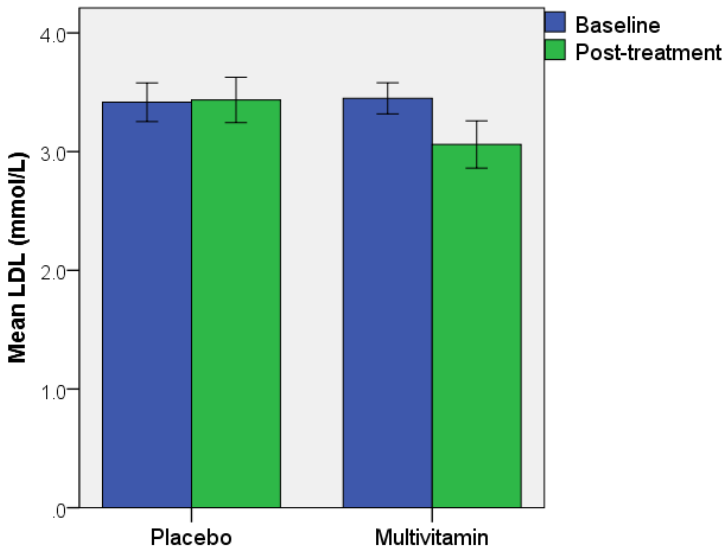

Figure 10.7. Mean LDL concentrations for multivitamin and placebo groups. Error bars show ±1 standard error.

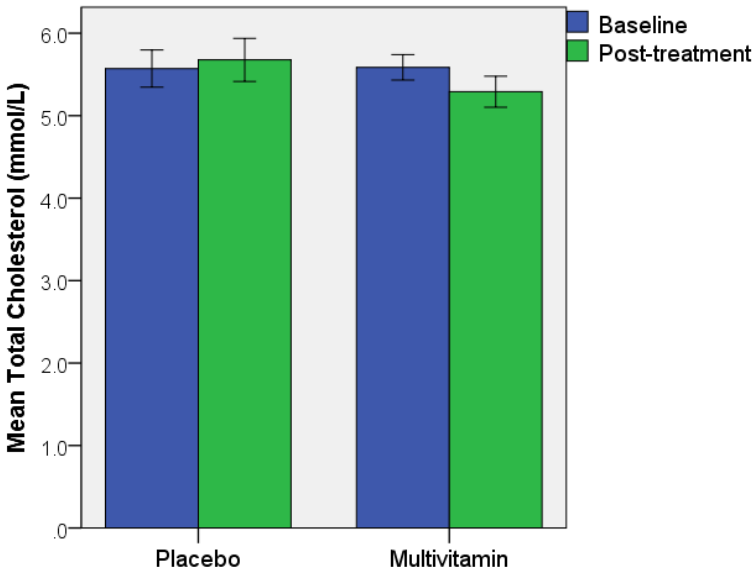

Figure 10.8. Mean total cholesterol concentrations for multivitamin and placebo groups. Error bars show ±1 standard error.

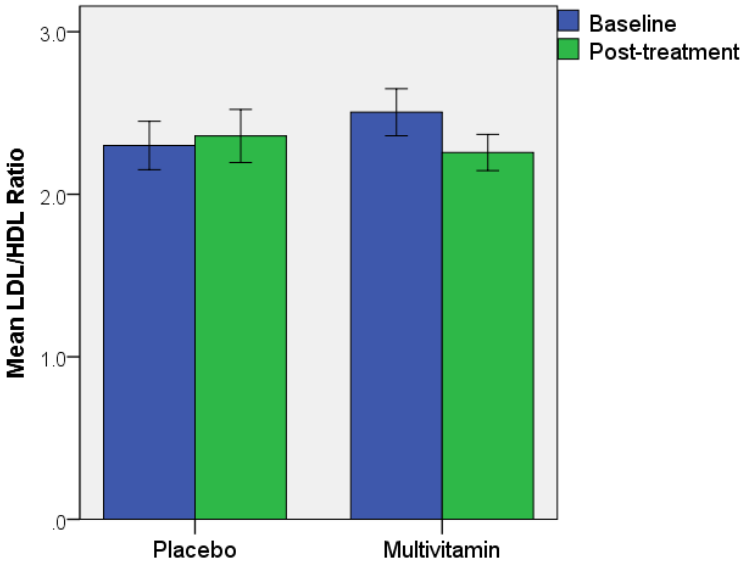

Figure 10.9. Mean LDL/HDL ratio for multivitamin and placebo groups. Error bars show ±1 standard error.

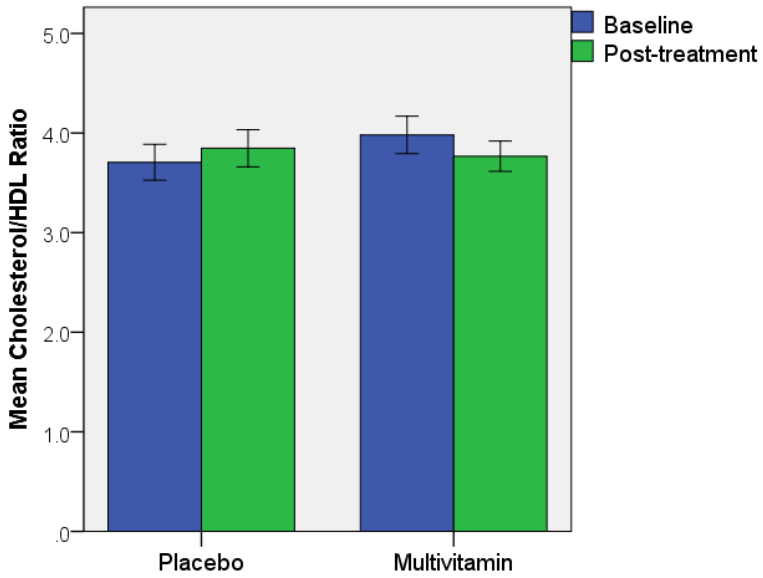

Figure 10.10. Mean cholesterol/HDL ratio for multivitamin and placebo groups. Error bars show ±1 standard error.

10.3.8.5 General biochemistry

There were no treatment effects for the general biochemistry measures, which included sodium, potassium, chlorine, bicarbonate, urea, creatinine and eGFR (data not shown). All values were within reference range at baseline and remained so after the supplementation period.

10.3.8.6 Liver function tests

There were no treatment effects for total protein, ALP, albumin, bilirubin or GGT (data not shown).

In the previous study (Chapter 9), there were significant increases in AST and ALT after supplementation with Women's formula, compared with placebo. Changes were small and remained within the reference ranges. It is worth noting that there were similar increases in this study, which did not reach significance (possibly due to a

smaller sample size in this study). AST was increased in the multivitamin group compared with placebo but this was not significant ($F(1,35) = 1.981$, $p = 0.168$). ALT increased across both multivitamin and placebo groups, and this was marginally significant ($F(1,35) = 3.590$, $p = 0.066$, partial $\eta^2 = 0.093$). The change was larger in the multivitamin group and this was also marginally significant ($F(1,35) = 3.411$, $p = 0.066$, partial $\eta^2 = 0.093$). These data suggest that multivitamin supplementation may increase AST and ALT, however, mean changes remained well below the reference values.

Table 10.10. Means and standard deviations for selected liver function tests.

		Baseline		Post-treatment		
		M	*SD*	*M*	*SD*	*n*
AST *U/L*	Placebo	24.2	6.0	24.0	7.1	16
	Multi	23.1	6.2	25.0	5.9	21
ALT *U/L*	Placebo	27.5	12.2	27.6	9.4	16
	Multi	25.6	7.9	30.5	9.9	21

10.3.9 Discussion

This study investigated the effects of 16 weeks multivitamin, mineral and herbal supplementation in men aged 55-65 years. There was no effect observed on cognitive or cardiovascular outcome measures, although there were some interesting observations made in secondary outcome measures for blood biomarkers.

10.3.9.1 Cognitive tasks

No effects of supplementation were observed in the cognitive task measures. This contradicts our previous study which found an improvement in Contextual Memory over a shorter time period of only eight weeks with another multivitamin, mineral and herbal supplementation (E. Harris et al., 2012). Participants in that study were aged 50

to 74 years, compared with 55 to 65 years in the present study, thus it might be that the older participants were more able to benefit from supplementation.

The present results agree with those from the Women's study reported in the previous chapter, which similarly observed null findings for cognition. Furthermore, of the eight studies identified in Chapter 5 that examined multivitamin effects in older adults without dementia, only half demonstrated a benefit for cognition. The studies which demonstrated benefits used sensitive cognitive tasks, gave supplements which contained herbal ingredients in addition to vitamins and minerals, and examined older adults without dementia (see Table 5.1 for a summary of these studies). The present study was comparable in these key factors, ensuring optimal methodology for observing effects. Although this study had a smaller sample size, there were no trends in the data to suggest that the null findings were due to insufficient power. The outcomes are therefore convincing that the supplement did not improve cognition in this group of healthy older men. Consideration of the blood biomarkers may assist in the interpretation of these findings (see section 10.3.9.3 below).

10.3.9.2 Cardiovascular function

There were no changes to the primary outcome measures, augmentation index or augmentation pressure. While augmentation index has been shown in some studies to be sensitive to supplementation with vitamin C and folate (Koyama et al., 2010; Mullan et al., 2002), other studies have found no benefit for vitamin C, or for several years of folate supplementation (Kelly et al., 2008; Zoungas et al., 2006). The Women's study in this thesis similarly observed no effect.

The strongest evidence for beneficial effect of vitamins on augmentation index comes from studies of vitamin C. Some studies which demonstrated an effect augmentation index used 2g acutely or 500mg for one study over four weeks (Mullan et al., 2002; Wilkinson et al., 1999). The present supplement contained only 200mg of vitamin C, which may have been insufficient to elicit change. Also we did not measure acute effects of the supplement so it is possible that the effect on augmentation index is transient one. In future research it would be beneficial to assess augmentation index

both acutely and after a period of ongoing supplementation. It would also be useful to assess blood levels of vitamin C; unfortunately the problems that occurred with blood testing in this study meant we were unable to assess changes in vitamin C concentrations due to the multivitamin.

Previous studies assessed patient groups rather than healthy adults and this may also have accounted for the changes they observed in augmentation index with vitamin supplements. In the four-week vitamin C trial the group under investigation had type 2 diabetes (Mullan et al., 2002). Folate studies which have also demonstrated an effect on augmentation index examined chronic renal disease patients during haemodialysis (Koyama et al., 2010). These groups are likely to have functional arterial stiffness (Potenza, Gagliardi, Nacci, Carratu, & Montagnani, 2009; Santoro et al., 2010). By comparison, the arterial stiffness that is found in healthy older adults is at least partly due to age-related structural changes that are not amenable to improvement (O'Rourke & Hashimoto, 2007). In these healthy older adults the age-related arterial stiffness that was observed in the Augmentation Index and Cognition study (Chapter 8) may have been primarily due to structural factors rather than functional disturbance, which would preclude any effects of interventions.

10.3.9.3 Blood biomarkers

Several changes in blood biomarkers were observed after supplementation with the multivitamin, indicating improvements to these physiological measures.

Vitamins B_{12} and B_6 were increased with supplementation however these increases did not have a substantial effect on homocysteine. Although the statistical interaction for homocysteine was significant, inspection of the data indicated that this might be partly due to the increase in homocysteine observed in the placebo group. Post-hoc t-tests indicated that the decrease in the multivitamin group was only marginally significant. This is important for the interpretation of the cognitive and cardiovascular results. Homocysteine is a risk factor for cognitive and cardiovascular impairment (M.S. Morris et al., 2001; Refsum et al., 2006) and is a possible pathway through which vitamins exert a benefit to cognition and cardiovascular function. In the Vitacog study it was

observed that B vitamin supplementation improved cognition in MCI patients via successful reduction of homocysteine (de Jager et al., 2012). Similarly, Koyama et al. (2010) suggested that the reduction in augmentation index observed in their study was due to a more successful reduction of homocysteine that was achieved with combined folate and vitamin B_{12}. Homocysteine reduction was marginal in the present study, thus cognitive and cardiovascular benefits were less likely.

The inconclusive results regarding homocysteine were unexpected because reduction with B vitamins has been consistently observed in previous research and the vitamin levels contained in the supplement were sufficient to achieve a reduction (Clarke, 1998). It is unfortunate that the blood folate results were unreliable, given that folate plays such a significant role in homocysteine reduction. Observing effects on folate levels may have provided further explanation.

Several changes in blood lipid concentrations due to multivitamin supplementation were also observed, in beneficial directions. These include a reduction in total and LDL cholesterol, and improvements in LDL/HDL and total cholesterol/HDL ratios in the multivitamin group. This is an improved health outcome because maintenance of cholesterol levels within range is important for cardiovascular health and increased levels of total and LDL cholesterol are risk factors for cardiovascular disease (Frayn, 2008). On the other hand, low cholesterol is associated with poorer cognitive function in older adults, although this was shown in groups older than the participants in this study (Kivipelto & Solomon, 2006). Of the ingredients found in the current supplement, cholesterol reduction has been associated with probiotics, St Mary's thistle and vitamin B_5 (Braun & Cohen, 2010). A study of older adults with cardiovascular disease observed reduced cholesterol, triglycerides and LDL as well as an increase in HDL after two months of vitamin C plus vitamin E supplementation (Karajibani, Montazerifar, Hashemi, Bolour, & Dikshit, 2011). With regard to multivitamins there is little research available. One study of obese Chinese women observed an improvement to the lipid profile after six months multivitamin supplementation, with reductions in total cholesterol and LDL, as well as an increase in HDL (Y. Li, Wang, Zhu, Feng, & Sun, 2010). Another study found beneficial changes to the lipid profile of diabetic participants with four months of multivitamin supplementation (Gunasekara,

Hettiarachchi, Liyanage, & Lekamwasam, 2011). However other research contradicts these findings. The Women's study reported in this thesis found no changes to the lipid profile after multivitamin supplementation. Also a larger study of low dose antioxidant supplementation over 7.5 years accomplished no reduction in cholesterol levels, with even a possible increase observed (Hercberg et al., 2005). A recent review of dietary and supplement interventions for improving the lipid profile concluded that vitamin C, tocotrienols and several other nutrients did not have evidence of efficacy (Huang, Frohlich, & Ignaszewski, 2011). On the whole, research on nutritional supplements for cholesterol reduction is limited and further research is required to elucidate a role for multivitamins, and supplements in general, in this capacity.

There was a reduction in protein carbonyl concentrations by 20% in the supplement group. This substantial reduction did not reach significance, however, the low sample size may account for this. Protein carbonyls are produced by protein oxidation, and are thus impacted by antioxidant activity. The Men's formula contains the antioxidant vitamins C and E as well as flavonoids, which have antioxidant activity. Numerous reports have demonstrated a reduction in protein carbonyls with these ingredients. This includes studies of vitamin E supplements (Chin et al., 2011), a flavonoid plus vitamin C formula (Young et al., 2006), vitamin C plus vitamin E, as well as with a fruit and vegetable supplement (Bloomer, Goldfarb, & McKenzie, 2006). A reduction of protein carbonyls is therefore an indication of the antioxidant function of the multivitamin. Although the marker was considerably reduced in the multivitamin group, this did not translate to benefits in cognition or cardiovascular function. However, these effects may occur over the long term, as indicated by longitudinal studies of antioxidant intake (Grodstein et al., 2003; Masaki et al., 2000).

Inflammatory markers were not significantly changed with supplementation. Inflammation is important in atherosclerosis, and high-sensitivity CRP and fibrinogen predict long term cardiovascular risk (Danesh et al., 2004). Flavonoids and some vitamins are thought to have anti-inflammatory properties from in vitro studies (Brighenti et al., 2005; de Pascual-Teresa, Moreno, & García-Viguera, 2010) suggesting that they may be effective for in vivo reduction of inflammation. Furthermore, higher flavonoid intake is associated with lower CRP (Ock, Chung, Claycombe, & Song,

2008). However reduction of inflammatory biomarkers with nutritional supplements is not consistently achieved. For example, in one study, vitamin C plus E reduced high-sensitivity CRP in cardiac surgery patients (Castillo et al., 2011). On the other hand, no effect on inflammation was observed with antioxidants in rheumatoid arthritis patients (Bae, Jung, Lee, Yu, & Sung, 2009) and no association of reduced inflammation with ginkgo or other flavonoid-containing supplementation was observed in the NHANES survey (Kantor et al., 2012). Thus, anti-inflammatory effects of nutrients that have been observed in vitro are not necessarily translated into clinical benefits, and this has been observed in this study.

10.3.9.4 Practice effects

Practice effects were observed for a number of cognitive tasks, including Spatial Working Memory, Simple Recognition and Delayed Recognition. These tasks also showed practice effects in the Women's study. These tasks are some of the most sensitive to cognitive aging and practice effects could potentially mask any effects of supplementation. For future studies it would be worthwhile to examine these tasks for level of difficulty, or to give a practice session before baseline testing to reduce the effects of practice. However, as stated in the Women's study, improved health behaviours during the study may also have affected results. Main effects were observed for CRP and marginally for systolic blood pressure, and these may have impacted performance on the cognitive tasks.

10.3.9.5 Limitations and future directions

The small sample size (N = 42) is a limitation for this study, with 52 being the number of participants deemed to be adequate in the power analysis. The group was smaller than planned owing to a poor response to advertising as well as health issues which disqualified some volunteers. Although the data did not suggest any trends for the main outcome measures, a larger sample would have given a clearer indication of the effects of the supplement on blood measures, particularly for protein carbonyls and homocysteine. Furthermore, a larger sample would enable analysis of subgroups. In particular it would be interesting to observe effects of supplementation on those

participants with high homocysteine, who may be more amenable to improvement with supplementation, as was observed in the Vitacog study (de Jager et al., 2012).

10.3.10 Summary and conclusion

This study found no effect of 16-weeks multivitamin supplementation on older males for cognition or cardiovascular function. Improvements were seen in some measures of cholesterol and marginally for homocysteine and protein carbonyls. Importantly, the changes in blood measures were observed in beneficial directions, suggesting that over the long term multivitamin supplementation could play a protective role for cognition and cardiovascular function.

11 General Discussion

The present thesis is set in the context of our aging population, where there is increasing incidence of cognitive decline and dementia in older adults. Nutritional factors and cardiovascular health each play important roles in cognitive outcomes in the elderly. The aim of the thesis was to contribute to the understanding of cognitive and cardiovascular aging, and to examine whether the negative effects of these might be ameliorated using multivitamin, mineral and herbal supplementations.

Three studies were conducted to address these issues. In the first study, the relationships between cognition and augmentation index and augmentation pressure were examined in younger and older adults. Augmentation index is a measure of arterial stiffness, but little research has investigated its relationship with cognition, and whether this relationship increases with age as it does for other measures of arterial stiffness. Augmentation pressure has been proposed to be a better measure of arterial stiffness in older adults. Two further studies examined the effects of multivitamin supplementation on cognition, cardiovascular function and associated blood biomarkers. Several lines of research have indicated that nutritional supplements are beneficial for cognition and cardiovascular health in the elderly, however trials of supplements have been inconsistent. This chapter provides a general discussion of the outcomes of these investigations, and examine what the present findings contribute to our understanding, in the context of existing research.

This chapter firstly provides a summary of the key findings from each of the three studies. Following is a discussion of the general outcomes from the Women's and Men's multivitamin studies. This considers where the findings from the two multivitamin studies were similar or different, and why; and how the overall outcomes contribute to the research on vitamin supplements for older adults.

Methodological issues are also addressed. These include a consideration of dosage and duration of supplementation in vitamin research, the observation of practice effects in the present studies, and an evaluation of the utility of the central pressure measures that

were used in the studies. Also, the unexpected pattern of age-related changes in augmentation index and augmentation pressure that was observed in the study is considered. Finally, a reflection of the limitations of the present research will provide some indicators for future research in the area of nutritional supplementation in cognitive and cardiovascular aging.

11.1 Summary of key findings

11.1.1 Augmentation index and cognition in younger and older adults

This study examined cognitive performance, augmentation index and augmentation pressure in younger (20-35 years) and older (55-65 years) adults. The primary outcome measure was the relationship between cognitive performance on a computerized battery and these arterial stiffness measures, after controlling for age and education. It was hypothesized that augmentation index and augmentation pressure would predict performance on the cognitive tasks in both younger and older adults.

Augmentation index predicted performance on the Spatial Working Memory task in both younger and older adults. Furthermore, it was observed that the relationship between these cognitive and cardiovascular variables was the same in the older group as it was in the younger group. That is, the strength of the relationship did not differ significantly between younger and older adults. Augmentation pressure predicted performance on Spatial Working Memory in the younger group only. Other cognitive measures were unrelated to augmentation index and augmentation pressure.

In addition to the primary outcomes, the study observed that the younger and older groups differed in demographic variables, cardiovascular measures and cognitive performance, in a manner that was expected according to the age difference. Older adults were less educated, performed more slowly and less accurately on cognitive tasks, and had poorer physiological health measures including higher BMI, blood pressures, augmentation index, and augmentation pressure. However, older adults did not conform to expected trajectories of age-related change in augmentation index and

augmentation pressure. Both augmentation index and augmentation pressure were more strongly associated with age in the younger group; it was unexpected that augmentation pressure did not continue to have a strong correlation with age in the older group.

11.1.2 Effects of multivitamin supplementation on older women

This study investigated the effects of 16-weeks supplementation with a multivitamin, mineral and herbal supplement on healthy women aged 55 to 65 years. While previous research has demonstrated inconsistent effects of supplementation, it was hypothesized that a broad-spectrum supplement might elicit benefits in susceptible populations, if sensitive cognitive, cardiovascular and blood biomarker tests were used. The primary outcome measures were cognitive performance on a battery of computerized tests, and cardiovascular function as measured by augmentation index and augmentation pressure. There was no effect of supplementation on these primary outcome measures.

Secondary outcome measures included systolic and diastolic blood pressures and a range of blood biomarkers including vitamin B_6, vitamin B_{12}, CRP, fibrinogen, protein carbonyls, lipids, and safety measures. Several of the blood markers were altered after supplementation with the Women's formula. Vitamin B_6 and vitamin B_{12} significantly increased after supplementation whereas the inflammatory marker CRP was reduced. Furthermore, two markers of liver function were increased: ALT and AST.

Main effects were also observed in the study. As a whole, the participant group had increased HDL cholesterol and decreased LDL, along with decreased systolic and diastolic blood pressure. Practice effects were observed on several cognitive tasks.

11.1.3 Effects of multivitamin supplementation in older men

Following the protocol of the Women's study described above, the Men's study investigated the effects of 16-weeks supplementation with a multivitamin, mineral and herbal supplement in healthy men aged 55 to 65 years. Separate studies for men and women are warranted because of differences in nutritional requirements which

necessitate different supplements, as well as gender differences in the aging process. It was anticipated that beneficial changes in cognition, cardiovascular function and blood biomarkers would be observed with supplementation.

The primary outcome measures were performance on the computerized cognitive tests, and cardiovascular function as measured by augmentation index and augmentation pressure. No effect of supplementation was observed for these variables.

Secondary outcome measures comprised systolic and diastolic blood pressures; as well as blood biomarkers including vitamin B_6, vitamin B_{12}, CRP, fibrinogen, protein carbonyls, lipids, and blood safety measures. Changes due to supplementation were observed in several of these variables. Blood levels of vitamin B_6 and vitamin B_{12} were significantly increased. Total cholesterol and LDL were significantly reduced and there was a substantial but only marginally significant reduction in protein carbonyl concentration, a measure of oxidative stress. Additionally, two markers of liver function increased: ALT and AST.

Main effects were also observed for the Men's study. There was an overall reduction in CRP, and an overall increase in triglycerides. There was also a trend to overall reduction in systolic blood pressure. Practice effects were observed on several cognitive tasks and these were the same tasks that observed practice effects in the Women's study.

11.2 Multivitamin studies: A discussion of general outcomes

This section considers the findings from the Women's and Men's studies together, to compare and contrast the findings, and provide and overall picture of the effects of the supplements. The studies were conducted in women and men of the same age, who were all in good health and had no cognitive complaints. The formulas were similar in that they provided a full range of vitamins and minerals, but differed in the dosages of some constituents as well as in several pertinent herbal ingredients. The studies followed the same testing protocol, enabling comparison between results.

11.2.1 Primary outcome measures: Cognitive performance and cardiovascular function

Evidence from in vitro and epidemiological studies, and known associations between various nutrients and cognitive and cardiovascular outcomes, point to a beneficial effect of vitamins, minerals and phytonutrients on cognitive and cardiovascular health (see Chapter 4 for review). On the other hand, intervention trials of supplements do not regularly demonstrate benefits of these types of supplements, and this may be partly due to methodological issues. The present studies aimed to optimise the methodology by targeting an older population, using supplements with wide ranging nutrients, and choosing tests that are sensitive to aging and to nutritional supplementation.

The primary outcome measures of cognitive performance and cardiovascular function were unchanged in both the Women's and Men's studies. The lack of effect observed for cognition is in contrast to two previous studies by our group which found memory improvements in response to multivitamin supplementation, using the same computerized cognitive battery. These included one study examining the effects of a multivitamin, mineral and herbal combination on older men who were at increased risk of cognitive decline due to lifestyle factors including sedentary occupation (E. Harris et al., 2012), and another which examined effects in women aged 65 plus years with self-reported memory complaints (MacPherson et al., 2012). This study in elderly women used the same multivitamin supplement as was used in the present Women's study. A key difference in the present studies is that participants were healthy and were not selected for characteristics that increase their risk of cognitive or cardiovascular impairment. Furthermore, the previous studies included participants who were older than those in the Women's and Men's studies reported here. Thus the inclusion of participants with poorer health or older age may be required to observe cognitive benefits due to supplementation.

This suggestion is consistent with results from previous studies of multivitamins in older adults that were reported in the review in Chapter 5. In the review it was observed that four studies observed cognitive improvements in older adults after multivitamin

supplementation, including the two studies by our group and two other studies (Summers et al., 2010; Wouters-Wesseling et al., 2005). Each of these studies included participants who were older than in the present studies, and only one of those examined healthy elderly subjects (Summers et al., 2010). In contrast, four studies reported no change after multivitamin supplementation (Cockle et al., 2000; Gariballa & Forster, 2007; McNeill et al., 2007; Wolters, Hickstein, Flintermann, Tewes, & Hahn, 2005). These trials were similarly in participants older than those in the Women's and Men's studies, but interestingly, three of the four were in healthy elderly, the exception being a six-week trial of elderly in a hospital setting (Gariballa & Forster, 2007). Thus it appears that it is more difficult to elicit changes in older adults who are healthy.

The studies in this thesis deliberately chose a younger group of adults than past studies. The reasoning was that vitamin supplements were unlikely to reverse existing damage in those with dementia pathology, which has rarely demonstrated cognitive improvement with nutritional supplementation (Burns et al., 1989; Sun et al., 2007). However, supplementation might be able to improve cognition via functional improvement to associated risk processes, including the inflammatory response, oxidative stress and cardiovascular function, in adults who have not yet suffered irreversible injury. This was not observed in this research, and in light of results from the present studies, it is proposed that a certain level of poor health may be required in order for cognitive benefits to be readily observed over a 16 week period. A reversal of negative physiological conditions may have a larger effect on those in poorer health and result in greater changes to associated risk markers; changes which might then observed in improved cognitive task performance and cardiovascular function.

Other authors have argued that multivitamin studies should be conducted in non-elderly adults who are healthy and cognitively intact (D. O. Kennedy & Haskell, 2011). The outcomes of the present studies argue the opposite, suggesting that at-risk adults would be a better target group for interventions. This would be particularly true for smaller studies which have reduced statistical power to detect small changes. However, larger studies that included younger adults may also be informative. There is less cognitive variation in younger adults, meaning smaller changes in response to supplementation might be more discernible.

The suggestion that it would be constructive to target less healthy populations in research on supplementation and cognition could also apply to cardiovascular function. Neither the Women's study nor the Men's study observed an improvement to augmentation index or augmentation pressure in healthy older adults after multivitamin supplementation. Previous studies of the effects of nutritional supplements on augmentation index have predominantly been done in patient groups, and many have examined acute effects (see Table 6.1). Thirteen trials have been conducted chronically, that is, with ongoing supplementation over days, weeks or years. Of these, only three examined healthy adults; the rest were in patients or other at-risk groups. Of the three studies, only one demonstrated a benefit of supplementation to augmentation index. This was for two months of vitamin E supplementation in younger men (Rasool et al., 2008). The other studies demonstrated no effect on augmentation index after two months of supplementation with another vitamin E formula in younger men (Rasool et al., 2006) or after four months of vitamin D3 in healthy post-menopausal women (Gepner et al., 2012). Therefore, with the inclusion of the present studies, only one of five studies has demonstrated a chronic supplementation effect on augmentation index in healthy adults.

On the other hand, in patient groups, four out of seven studies investigating chronic effects of various vitamin supplements have reported effects on augmentation index. These include studies in diabetic patients, who responded to four weeks vitamin C supplementation (Mullan et al., 2002) or one year of vitamin D (Breslavsky et al., 2013). Similarly, patients with kidney disease had reduced augmentation index after three weeks of folate supplementation (Koyama et al., 2010) and there was a marginal reduction in augmentation index in hypertensive middle aged men after eight weeks of combined vitamin C plus vitamin E supplementation (Plantinga et al., 2007). The three studies that reported no change included a study of folate supplementation in renal failure patients (Zoungas et al., 2006) and two studies of vitamin D, one in hypertensive adults (Larsen et al., 2012) and another in kidney disease patients (Marckmann et al., 2012). While several differences among the augmentation index studies may account for these observations, the data is not inconsistent with the hypothesis proposed above: that

the benefits of supplementation might be more readily seen in subjects with poorer health or some level of impairment.

If changes due to supplementation are more easily observed in an impaired group, this does not mean that some benefits are not occurring in younger or healthier adults. Similarly, a lack of effect on broad outcome measures such as cognitive performance or cardiovascular function does not necessarily indicate that a supplement is entirely ineffective. In the case of healthy adults it may be necessary to examine physiological markers that are known to impact cognition and cardiovascular function in older people, in order to determine whether supplementation is providing any benefit. A reduction of risk markers has the potential to protect against impairment over the long term.

11.2.2 Blood biomarkers: A comparison of the Women's and Men's studies

Despite a lack of effect on cognitive performance and cardiovascular function, the Women's and Men's studies did observe some beneficial changes in biochemical measures. The blood biomarkers were considered to be secondary outcome measures and there were several changes observed in them after supplementation. These differed between studies, with the exception of vitamin B_6 and vitamin B_{12}, which both increased significantly after supplementation.

The increases in vitamin B_6 and vitamin B_{12} observed in both studies were expected because the Women's and Men's formulas contained substantial amounts of each vitamin; in fact they were at levels many times greater than the recommended daily intakes. Previous research has similarly demonstrated increases in blood levels of these vitamins after supplementation, including studies examining specific B vitamin supplementation as well as other multivitamin studies in older adults (Cockle et al., 2000; E. Harris et al., 2012; MacPherson et al., 2012). It is important to note that these changes can be observed after multivitamin supplementation, given the popularity of these supplements in the general population (Millen et al., 2004; Sebastian et al., 2007).

Changes in other biomarkers also occurred in the multivitamin studies, but these differed between the Women's and Men's studies. In particular, inflammatory markers, protein carbonyls and homocysteine are of interest. This is because cognitive decline and poor cardiovascular health are associated with increased chronic inflammation, oxidative stress and higher levels of homocysteine, and it has been argued that these risk markers are a cause of impairment and not just an indicator of it (K. J. Davies, 2000; Trabetti, 2008; A. M. Wilson, Ryan, & Boyle, 2006). For example, inflammation is involved in atherogenesis (Wong et al., 2012) and contributes to Alzheimer's disease processes (Akiyama et al., 2000). Oxidative stress also contributes to age-related diseases including cardiovascular and cognitive disorders (Ames et al., 1993). Reactive oxygen species cause brain cell death and contribute to Alzheimer's pathology and vascular dementia (Barja, 2004; Bennett et al., 2009). Homocysteine effects on the cardiovascular system include increased oxidative stress and suppression of nitric oxide synthase (Trabetti, 2008). It is also neurotoxic (Obeid & Herrmann, 2006). Therefore, interventions that alleviate these symptoms may have cognitive and cardiovascular benefits in the longer term.

Changes to these biomarkers were observed differentially in the Women's and Men's studies, and to account for this we must consider factors which differ between the studies. These include gender differences in general; factors that are specific to the current participant groups; and effects which are related to the different supplements used.

CRP is a marker of inflammation which has significant prognostic value in cardiovascular risk evaluation (Biasillo et al., 2010) and also predicts cognitive decline and dementia (Kuo et al., 2005). In the present trials the Women's study observed an effect of supplementation on CRP but the Men's study did not. This echoes findings from a cross-sectional study, which found that use of multivitamins was associated with lower CRP in women, whereas there was no such association in men (Scheurig, Thorand, Fischer, Heier, & Koenig, 2008). The authors suggested that supplements may interact with gender-specific factors to produce a reduction in CRP. There are also gender differences in population CRP levels. A recent report from the NHANES study indicated that more women had elevated CRP than men (32% vs. 20%), however in

women it was not associated with as high a risk of mortality (Doran, Zhu, & Muennig, 2013). In the present research, men and women had similar mean levels of CRP at baseline, although the women had a greater variation.

In addition to gender factors, the divergent outcomes for CRP that were observed in the two studies may be due to ingredients that were only included in the Women's formula. Several of these have anti-inflammatory actions, including Ashwagandha and black cohosh (Verma & Kumar, 2011; Yang, Chik, Li, Cheung, & Lau, 2009). In particular, turmeric and Bacopa Monnieri may have played a role in reduction of CRP. Turmeric has multiple anti-inflammatory actions, which have been ascribed to the component curcumin (Aggarwal & Harikumar, 2009). Bacopa monnieri also contains a number of different compounds which have anti-inflammatory properties, including flavonoids and bacosides, and it has demonstrated in vitro anti-inflammatory action (Viji & Helen, 2011; Volluri, Bammidi, Chippada, & Vangalapati, 2011). These ingredients, which were not included in the Men's formula, may have been instrumental in the change in CRP that was observed in the Women's study.

The Men's study observed a reduction in homocysteine due to multivitamin supplementation but the Women's study did not. Homocysteine is particularly sensitive to folate levels because folate plays a major role in homocysteine reduction, being necessary for the conversion of homocysteine to methionine (Bender, 2003). The Women's and Men's supplements each contained 500µg of folic acid, which is the required dose to achieve maximum reduction of homocysteine, which was previously reported to be a 25% reduction (Clarke, 1998). Although a significant finding, the level of homocysteine reduction achieved in the Men's study was only 9.3%, substantially less than that. Also, according to their data, the inclusion of vitamin B_{12} should reduce homocysteine a further seven per cent. Since vitamin B_{12} substantially increased in men and women in the studies, the poor response is likely to be related to folate, and may be a result of folate malabsorption.

There are a number of factors which can impair folate uptake. For example, atrophic gastritis is present in around 30% of older adults, and this can lead to poorer absorption of several nutrients including folate (Wahlqvist & Tienboon, 2011). Other dietary

components may also interfere with absorption, including consumption of alcohol or green tea (Alemdaroglua, Dietz, Wolffram, Spahn-Langguth, & Langguth, 2008; Halsted, Villanueva, Devlin, & Chandler, 2002). In addition, medications, including anti-inflammatories, antacids and diuretics, may also impact folate metabolism (Wahlqvist & Tienboon, 2011). The possibility of a problem with folate absorption in the present participants is corroborated by their high baseline levels of homocysteine; those with existing absorption impairment would be likely to have higher homocysteine at baseline and also be less likely to respond to supplementation.

Comparing the Men's and Women's studies, the men did have higher baseline homocysteine which could arguably result in a stronger reduction. In fact their levels were quite high at baseline; the average in the multivitamin group was 13.7µmol/L and in the placebo group was 15.5µmol/L, the latter being above the maximum reference range of 15µmol/L. Although these baseline levels were higher than in the Women's study (multivitamin and placebo groups had 12.9µmol/L and 13.0µmol/L respectively at baseline), other research has observed a multivitamin effect on homocysteine in participants with baseline levels much lower than this. In our previous study in older men, baseline homocysteine was only 11.2µmol/L, and a reduction in homocysteine occurred after only eight weeks of multivitamin supplementation (E. Harris et al., 2012). Furthermore, the Homocysteine Lowering Trialists' Collaboration meta-analysis (2005) observed that women are generally more responsive than men to homocysteine reduction with vitamin supplementation. Thus the present results are unexpected and require further scrutiny.

An additional explanation is that the supplement did not address other factors that are causing elevated homocysteine in the present participants. Lifestyle factors, such as low fruit and vegetable intake, insufficient exercise, and high alcohol and coffee consumption are all associated with elevated homocysteine levels (Panagiotakos, Pitsavos, Zeimbekis, Chrysohoou, & Stefanadis, 2005). These potential influences were not measured in the present studies, but they may have contributed to the high baseline levels of homocysteine that were observed in the present participants, as well as the poor response to supplementation, since multivitamin supplementation would not affect these lifestyle practices.

There was a 20% reduction in protein carbonyls in the Men's study which did not occur in the Women's study. Although this was only marginally significant, due to the size of the reduction it is worth consideration. Protein carbonyls have previously been demonstrated to be sensitive to supplementation with combinations of vitamins including multivitamins (Dragsted et al., 2004; Peng, Jones, & Watson, 2000). A further study demonstrated protein carbonyl reduction in older adults after six months of vitamin E supplementation (Chin et al., 2011). Interestingly, this occurred concomitantly with an increase in blood vitamin E. The same study also investigated a group of younger adults and observed no change in blood vitamin E or protein carbonyls. In the present study it could not be observed whether vitamin C and vitamin E changes differed between the groups because of discontinuation of these tests during the study. This might have assisted in corroborating the change in the Men's study, if antioxidant vitamin levels had been demonstrated to increase.

The change in protein carbonyls suggests that the antioxidant vitamins (or other herbal ingredients with antioxidant properties) may have had some physiological effect on oxidative stress in the Men's study only. There were no ingredients specific to the Men's formula that would account for divergent results; however baseline protein carbonyl levels were higher in the Men's study than in the Women's study, perhaps allowing for a stronger effect of supplementation. Nevertheless, the change in the Men's study did not reach significance. It is also possible that vitamin absorption was low across all participants, or alternatively, that the dosage or duration of supplementation were insufficient to elicit significant effects in healthy older adults. Dosage and duration of supplementation are discussed in section ## below.

The Men's study also observed improvements in the lipid profile that were not observed in the Women's study. Baseline lipids were at healthier levels in the Men's group than the Women's group, suggesting that effect differences were not related to poorer baseline health in the men. As discussed in the report of the Men's study (Chapter 10), improvements to the lipid profile have been observed after supplementation with antioxidant vitamins (Karajibani et al., 2011) and multivitamins (Gunasekara et al., 2011). Also, of the ingredients found in the Men's formula, probiotics, St Mary's thistle

and vitamin B_5 are associated with cholesterol reduction (Braun & Cohen, 2010). Each of these ingredients was found in similar dosages in the Women's formula, where there was no supplement effect observed. A recent review examined the effects dietary interventions and supplements on the lipid profile (Huang et al., 2011). The authors concluded there was no evidence for an effect of vitamin C, chromium or tocotrienols, however they determined that there was evidence for other dietary interventions. In addition to the usual cardiovascular risk reduction strategies such as a Mediterranean diet and a low fat diet, food components such as soy protein and phytosterols were effective in cholesterol reduction. Also green tea, red wine, and red yeast rice extract were considered to be potentially effective. Although these phytonutrients were not included in the present supplements, the Women's and Men's formulas contained many plant extracts which also contain flavonoid compounds. It is possible that improvements to the lipid profile may occur with the present supplements, and with the positive effects observed in the Men's study, further research is recommended.

Despite these significant changes in biomarkers, it must be noted that the effects were limited to a few measures. Chance findings are a possibility, considering the number of tests undertaken in the projects. However, the biomarker findings are in anticipated directions and are plausible when compared with previous research. Nevertheless, a lack of effect on several important biomarkers indicates that mechanisms which have the potential to influence cognitive and cardiovascular outcomes were not affected by the supplements. This may have been a crucial factor in the lack of change in cognitive and cardiovascular outcomes.

11.3 Methodological considerations

Several issues relating to methodology need to be considered to enable proper interpretation of the findings from the studies. These include the dosage and duration of supplementation; the meaning of practice effects, or main effects, that were observed in the data; the utility of the central pressure measures as indicators of cardiovascular function; and the observation of unexpected relationships between age and central pressure measures. These are discussed in turn in this section.

11.3.1 Dosage and duration of supplementation

In light of null findings for cognition and cardiovascular function, the issues of nutrient dosage and duration of supplementation should be considered. This is because a lack of effect could be due to insufficient quantities or inadequate uptake of the required nutrient into the body, as opposed to a lack of efficacy. Although vitamin B_6 and vitamin B_{12} were substantially increased, the effect of supplementation on other blood vitamin levels could not be determined due to problems with blood testing that affected vitamin C, vitamin E collection and folate test results. However, dosage and duration of supplementation can be discussed with reference to past research.

Dosage and duration requirements are particularly relevant for antioxidant vitamins. Some studies have demonstrated a cognitive benefit for antioxidant vitamins in healthy adults over the longer term. For example, in the SUVIMAX study, participants supplemented with 120mg vitamin C, 6mg β-carotene, 30mg vitamin E, 100µg selenium and 20mg zinc. These dosages were comparable or lower than in the formulas used in the present studies, but the duration of the study was four years (Kesse-Guyot et al., 2011). Another large study, the Physician's Health Study, investigated supplementation with β-carotene. No cognitive improvements were observed after one year of supplementation but benefits were evident after 18 years (Grodstein et al., 2007). A further study of Alzheimer's disease patients demonstrated cognitive benefits over two years with a large dose of vitamin E (Sano et al., 1997). This suggests that antioxidant benefits for cognition may take years to appear, which is understandable if the effects are mainly preventative.

In contrast, cardiovascular studies with antioxidant vitamins have not demonstrated a benefit even in large studies over long periods (Törnwall et al., 2004; Yusuf, 2000). This is despite several lines of evidence which suggest a role of antioxidants in cardioprotection, including antithrombotic and vasodilation activities. In addition, cardiovascular benefits have been demonstrated with antioxidant vitamins, in relation to arterial stiffness. Several studies have observed an acute effect of vitamin C on augmentation index and other measures of arterial stiffness (see Table 7.1 for a summary of these studies). Furthermore, effects have been seen after vitamin C and

vitamin E supplementation in studies ranging from four weeks to two months (Mullan et al., 2002; Plantinga et al., 2007; Rasool et al., 2008). The supplements used in those studies contained much higher doses of vitamin C and vitamin E than Women's and Men's formulas, so it is possible that the present supplements did not contain sufficient dosages of antioxidant vitamins to produce effects.

Dose and duration of supplementation is also relevant for B vitamins. Although they generally demonstrate an effect of homocysteine reduction, cognitive and cardiovascular benefits are rarely observed, and results from a meta-analysis of B vitamin studies concluded that dose and duration did not improve likelihood of observing effects (Ford & Almeida, 2012). An important exception to this is the Vitacog trial, where elderly participants with MCI were supplemented with B vitamins for two years, at doses much higher than the present sample. A reduction in homocysteine was observed and this was related to reduced brain atrophy and maintenance of cognitive function. Effects were stronger in participants with high baseline homocysteine (de Jager et al., 2012; A. D. Smith et al., 2010). This indicates that a longer duration of supplementation is able to produce cognitive benefits in susceptible participants.

11.3.1.1 Acute vs. chronic effects

Changes to central pressure measures were not observed in the studies, despite previous research which shows that these variables are sensitive to the effects of supplements (Chapter 6). It is possible that the maximal effects on arterial stiffness are acute effects; that is, they affect arterial stiffness in a temporary manner, with no long-term effect on cardiovascular health. It is known that vitamin C and vitamin E have immediate effects on endothelial function. For vitamin C, this occurs via multiple mechanisms associated with NO, including the reduction of oxidative stress as well as mechanisms associated with increased NO synthesis (Holowatz, 2011). For vitamin E, mechanisms are attributed to reduction of lipid peroxidation and maintenance of nitric oxide homeostasis (Mah et al., 2013). Acute vitamin E has also been demonstrated to reduce methionine-induced impairment of endothelial function (Raghuveer, Sinkey, Chenard, Stumbo, & Haynes, 2001). Therefore, it is possible that maximum effect of these supplements occurs in the short term, with little lasting benefit, and it is those effects that have been

reported in the earlier studies. It is also possible that short term changes in cardiovascular function affect cognition, for example via improved blood flow.

In the present study the participants were asked not to take the supplement on the day of follow-up testing. This would reduce the chance of observing acute effects of ingredients, which may have dissipated in the time since taking the last supplement. Consideration of acute versus chronic effects has wider implications. Participants in our previous multivitamin study in older men (E. Harris et al., 2012) were not asked to refrain from taking the supplement on the day of follow-up testing, which allows the possibility that the cognitive effects observed in that study were also a result of acute effects of the supplement.

Acute effects on cognition may act via an effect on mood. Our previous study reported effects on mood, with alertness increasing and general negative mood symptoms declining after the eight weeks of supplementation (E. Harris et al., 2011). Research on adult males aged 30 to 55 years similarly reported increased alertness, concentration and mental stamina while taking multivitamin/mineral supplements (D. O. Kennedy & Haskell, 2011). These subjective mood states, which may be transient effects of supplementation, could also affect performance on cognitive tasks. Nevertheless, attributing efficacy solely to acute effects would contradict other research which has demonstrated benefits with longer term interventions, as well as epidemiological evidence that shows long term benefits of supplementation (Chapter 4).

11.3.2 Practice effects

Practice effects were observed on cognitive tasks in both the Women's study and the Men's study, and these followed a similar pattern in each. Notably, the tasks that improved were the more difficult tasks and thus likely to be more amenable to improvement. For Contextual Memory performance was worse at follow up in both studies, suggesting that the version of the battery given then was more difficult. Conversely, performance improved in both studies for three other cognitive tasks: Simple and Delayed Recognition and Spatial Working Memory. There are several

possibilities that could account for the fact that both studies observed a similar pattern of main effects in the cognitive tasks.

Firstly, the tasks might have been easier (and the Contextual Memory task more difficult) in the follow up version of the cognitive battery than they were in the baseline version. It is also possible that true practice effects occurred; that is, the participants improved at follow up due to familiarity and experience with the task (Rabbitt et al., 2001). Practice effects were not reported in the published studies which used the same cognitive battery, however our own analysis of the data set from Pipingas et al. (2010) indicates that only Spatial Working Memory demonstrated any significant practice effects.

Another possibility is that the observed improvements were due to a placebo effect, perhaps due to expectancy. Placebos have been demonstrated to have significant impact on health outcomes in a variety of settings, including cardiovascular and cognitive studies (Finniss, Kaptchuk, Miller, & Benedetti, 2010). Analysis of participants' beliefs about the tablets indicated that there was no difference between groups in what they believed they were taking. However, relatively few participants believed they were taking a multivitamin compared to those who believed they were taking a placebo, regardless of what they were actually taking. An expectation of improvement in the participants is therefore unlikely to have occurred in this group.

An alternative explanation is that improved performance in the group as a whole reflects improvements in health over the course of the study. In particular, main effects in the form of beneficial changes were observed in cholesterol measures in the Women's study, and in CRP in the Men's study. Similarly, systolic and diastolic blood pressures were reduced overall in the Women's study, with a trend to overall reduction in systolic pressure in the Men's study. These variables have all been associated with cognition so it is plausible that they influenced cognitive performance in the present studies. Group changes in health may have occurred due to participation in the studies, making subjects more aware of their health (for example, observations of their weight and blood pressure at baseline testing). As a result they may have modified their health behaviours during

the study, leading to group improvements in health variables and cognition. An assessment of participants' health behaviours might be useful for future research.

11.3.3 Is augmentation index a useful tool for supplementation and aging studies?

The lack of effect of supplementation on augmentation index and augmentation pressure raises the question of whether these measures are useful techniques in the research setting. Augmentation index is associated with increased cardiovascular risk, including cardiovascular events and mortality (Vlachopoulos et al., 2010) but its utility in intervention studies is still unclear. Also, there is little research regarding its relationship to cognition. The present studies found no change in augmentation index or augmentation pressure after supplementation, and the relationship with cognition was only significant for the most sensitive task in the computerized cognitive battery, Spatial Working Memory.

Augmentation index may be less sensitive to supplementation than other indicators of cardiovascular function. With regard to the nutritional intervention studies reviewed in Chapter 6, there were some studies that demonstrated stronger effects using alternative measures of arterial stiffness. For example, Plantinga et al. (2007) demonstrated a significant effect of vitamin C and vitamin E on pulse wave velocity and flow-mediated dilation whereas the effect on augmentation index was marginal. Similarly, Curtis et al. (2013) observed no effect on augmentation index although pulse wave velocity improved after isoflavone supplementation. Other studies found within-group changes to augmentation index that did not remain significant in interaction analyses (Naissides, Pal, Mamo, James, & Dhaliwal, 2006; Ruel et al., 2013).

Generally, null findings may indicate either a lack of effect of an intervention or a lack of sensitivity of the instruments that measure outcomes. In the present studies, the failure of augmentation index to change with multivitamin supplementation is likely to be due to a lack of change in cardiovascular health rather than a problem with the methodology. This is because a lack of effect corresponds to results in other variables

including cognition, the other primary outcome. Also there was not a consistent pattern of reduction in blood biomarkers that would be likely to influence augmentation index, including inflammatory markers, protein carbonyls and homocysteine.

With regard to cognitive aging, the present research revealed that augmentation index was a better predictor of cognitive performance than brachial systolic or diastolic pressures. However, the relationship between augmentation index and cognition was limited to the Spatial Working Memory task. This cognitive battery is very sensitive to aging, and other tasks in the battery have similarly demonstrated a strong decline with age (Pipingas et al., 2010). However these age-sensitive tasks were unrelated to augmentation index and augmentation pressure. Nevertheless, it might be that Spatial Working Memory is particularly related to augmentation index due to specific effects of arterial stiffness on brain structures related to spatial function. This question has not been addressed in the present studies, however other hemodynamic variables including pulse wave velocity and carotid pulsatility index have been associated with extent of white matter lesions and cognitive function on memory, speed and executive function tests (Mitchell et al., 2011). In one study augmentation index was associated with total volume of white matter hyperintensities, but not with cognition (Kearney-Schwartz et al., 2009). Further research assessing the relationship between augmentation index, brain pathology and various susceptible cognitive faculties would be advantageous.

Other methods of assessing arterial stiffness including pulse wave velocity have demonstrated relationships with a range of cognitive functions including spatial function (M. F. Elias et al., 2009; Rabkin, 2012). Research comparing the relationships between cognition and various measures of arterial stiffness may be beneficial to determine the optimal tool for future research.

11.3.4 Age-related change in augmentation index and augmentation pressure

Augmentation index and augmentation pressure did not follow the expected pattern of age-related change. Previous research has described a trajectory where augmentation index increases until around age 55 and then levels off, whereas augmentation pressure

continues to rise (Fantin et al., 2007; McEniery et al., 2005). In the present study, which examined discrete younger and older groups rather than a continuous age range, this should result in a weaker correlation between augmentation index and cognition in older adults compared with younger adults. This was observed, the relationship in older adults was not statistically significant. It would also be expected that in the older group, age would be more closely correlated with augmentation pressure than augmentation index, but the relationships were similar in the present participants.

Differences in participant demographics may account for the conflicting results. For example, in the older group, including participants who were taking preventative cardiovascular medication might have ameliorated the strength of age-related changes. Close to half of the women and men in the studies were taking some form of medication, including for blood pressure, blood thinning (aspirin) or cholesterol reduction. Due to the number of participants taking these medications, it was not feasible to run sub-group analyses comparing participants who were taking medication with those taking no medication.

11.4 Limitations and future directions

The sample size of the multivitamin studies was a limitation for this research, particularly for healthy older adults in the multivitamin studies, who may have smaller responses to supplementation. A larger sample would have allowed subgroup analyses to determine whether participants with poorer baseline health had greater responses. In the present studies, it did not appear that there were any trends towards changes in the primary outcome measures, indicating that for healthy adults, multivitamin supplementation does not affect these variables over a period of four months. Future research may benefit by including larger participant numbers. Also, as discussed previously, it might be prudent to target older adults who are in poorer health or at greater risk of impairment. This would allow for greater changes to the processes underlying cognitive impairment and increase the likelihood of observing improvements.

The inclusion of participants who were taking preventative cardiovascular medications might be considered a limitation of the study. However the use of medication in general, and preventative cardiovascular medications in particular, is pervasive in the older adult population. An Australian survey indicated that of adults aged over 50 years, 87.1% had used at least one conventional or complementary medicine within the previous 24 hours, with the vast majority taking at least one conventional medicine (Morgan et al., 2012). For participants aged 50-64 years, 29.9% were taking antihypertensive medication, 21.5% were taking lipid-lowering medication, and 12.7% were taking anticoagulants. In our healthy participant group aged 55 to 65 years, the prevalence was slightly lower than this but still reached nearly half for any medication. Therefore it is relevant to investigate whether multivitamin supplementation is effective in a group that is representative of the general population, and also whether there is an association between central pressure measures and cognition in this group. Nevertheless, these medications might have obscured the effects of multivitamin supplementation in those participants, and also affected the age-related changes that were observed in the Augmentation Index and Cognition study.

Related to this is the inclusion of participants who were taking various supplements. These were fewer in number than those taking medication, and were predominantly supplements that were not included in the multivitamins, such as fish oil and glucosamine. However it is possible that some of these supplements may have effects which confounded the effects of the multivitamin in participants who were taking them.

Another limitation is that we did not examine the acute effects of the multivitamin supplement. As discussed above, acute effects may result from supplementation due to alterations in cardiovascular function or mood, including alertness, and these may have contributed to positive findings in previous research. Future studies would benefit by assessing cognition and cardiovascular function on the first day of supplementation, as well as after the intervention period.

The present investigations included a high number of tests, in cognitive, cardiovascular and biochemical domains. A high number of tests increases the likelihood of a Type I error, or false positive (Tabachnick & Fidell, 2001). The lack of effect observed in the

cognitive and cardiovascular measures makes this point redundant for the primary outcome measures however it should be taken into account for the blood biomarkers. Although the effects on homocysteine, CRP, protein carbonyls and the lipid profile were plausible and consistent with previous research on supplements, these were not corrected for multiple measures, and should be considered with this caveat in mind.

11.5 Summary and conclusion

The aim of the thesis was to improve our understanding of cognitive and cardiovascular aging, and to investigate whether cognitive performance or cardiovascular function could be improved in older adults after supplementation with multivitamin, mineral and herbal formulations.

Cardiovascular health is important for maintenance of cognitive function in older age and there are many risk factors that are associated with both outcomes. In this thesis the association between cognition and cardiovascular function was assessed using a computerized cognitive battery and central pressure measurements, augmentation index and augmentation pressure. A relationship was observed in both younger and older adult groups on the Spatial Working Memory task. This was the first study to examine these relationships in distinct older and younger adult groups, and the results indicated the relationship between augmentation index and Spatial Working Memory was the same in younger and older adults.

The use of vitamins, minerals and phytonutrients may be beneficial for cognition and cardiovascular health, especially in older adults. Multivitamins contain a wide range of nutrients including vitamins, minerals and phytonutrients that can address the different insufficiencies that may occur across individuals. Additionally, they may enhance effects through possible synergies among nutrients. Multivitamin, mineral, and herbal supplements were assessed in two studies in this thesis: the Women's study and the Men's study. The supplements were formulated specifically for women and men, and were supplied to older adults for a period of 16 weeks. Participants were assessed for cognition, cardiovascular function, and associated biomarkers.

Supplementation had no effect on cognitive performance on the computerized battery, or on augmentation index or augmentation pressure in either the Women's study or the Men's study. However, assessment of blood biomarkers that are related to cognitive decline and cardiovascular impairment in older adults indicated that there were some beneficial changes resulting from the supplements. This is important because an effect on risk markers suggests there multivitamins may play a role in prevention of cognitive and cardiovascular deterioration in the long term.

Therefore, despite the limited effects in the present study, further research is warranted to elucidate the role of nutritional supplements in cognitive and cardiovascular aging. The importance of reducing the impact of aging and delaying the onset of disease states is increasingly important as the population ages. The changes observed in these studies are small; however the long term benefits may have a considerable impact at a national level and, if confirmed by further research, may amount to a considerably reduced social and financial burden in relation to cognitive and cardiovascular aging. Furthermore, the finding of an association between augmentation index and Spatial Working Memory indicates that it could be a useful midlife biomarker of risk for cognitive impairment, indicating the need for lifestyle intervention for susceptible individuals.

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Appendix A. Explanatory Statement and Informed Consent

Explanatory Statement

SWINBURNE UNIVERSITY OF TECHNOLOGY

Project Title
The Swinburne Multivitamin Study: Effects of vitamin and herbal supplementation on cognition, brain electrical activity and cardiovascular measures in older adults.

Principal investigators
Elizabeth Harris, Dr Andrew Pipingas, Dr Richard Silberstein

Background and study aims
Various dietary supplements have been reported to have positive effects on mental processes such as memory and attention, and to improve well-being. Supplements may also affect brain electrical activity. This study will investigate the effects of vitamin supplements on mental processes (e.g. memory and attention), brain electrical activity, cardiovascular health and general well-being.

If you agree to participate in the study you will be given a 16-week course of a multivitamin supplement or a placebo. The products that will be used in this study are Swisse Men's Ultivite 50+ and Swisse Women's Ultivite 50+. These multivitamins are widely available over-the-counter. They contain vitamins, minerals, antioxidants and herbs. The study will be placebo controlled. This means that during the study you will not know if you have been given Swisse Ultivite or the placebo, however you will be advised on completion of the study. Participants who are assigned the placebo during the study will receive a 16-week supply on completion of the study.

Are you eligible to participate in the study?

To participate in the study you will need to be:

- Aged 55 to 65 years
- Generally in good health and a non-smoker
- No history of psychiatric disorder, neurological disease, diabetes or food intolerance/allergy
- Free from epilepsy and have no history of epilepsy (only applicable if participating in brain electrical activity testing)
- Not currently taking any form of dietary supplementation
- You will need to be able to attend two weekday, daytime testing sessions at Swinburne University of Technology and have two blood tests over a 16 week period.

What does the study require of you?

- The study will take place over 16-weeks. You will complete two rounds of testing, one in the first week and one after 16 weeks. Testing involves computer tasks, questionnaires, brain electrical activity recording and blood testing. During the 16 weeks you will take the multivitamin or placebo once per day.

- On the first day of the study you will attend the Brain Sciences Institute. After you have provided some personal details you will complete a number of pen and paper tasks to assess your cognitive functioning (e.g. memory) and general wellbeing.

- Next we will record your height and weight, blood pressure and pulse pressure. Following this you will complete some computer tasks for memory and attention. These will be explained to you and you will be given time to practice each task. Questionnaires and computer tasks will take approximately 1½ hours to complete.

- *Due to funding limitations, only female participants will complete the following brain electrical activity recording:* After a short break we will record your brain electrical activity (electroencephalogram, or EEG). A 64-electrode cap will be placed on your head and a water-based gel will be used to provide contact between the electrodes and your scalp. Goggles will be fitted to produce a dim white flicker whilst you perform a number of computer tasks. Once you have completed the tasks the cap will be removed and your hair will be washed. The total duration of the EEG session is approximately 1½ hours. Thus the total time for the first testing session will be approximately 3 hours.

- Before leaving the Brain Sciences Institute you will be given an information sheet with details of the study. This will include dates, times, contact names, addresses and telephone numbers. You will also be given a 16-week supply of the multivitamin or placebo. You will need to take one tablet each morning with breakfast for the 16-week duration of the study.

- On the morning after testing you will be required to go to a pathology clinic to have a blood sample taken. You will need to fast from the evening before. You will be given information regarding the location of these clinics. Please note that it is important that you do not begin taking the supplements until after you have your blood test.

- After 16 weeks you will return to the Brain Sciences Institute for the second testing session. The testing procedure will be repeated.

- Other than outlined above, you will not be expected to change your daily routine in any way during the study. We encourage you to keep your normal eating and exercise habits throughout the study, so that any changes do not interfere with results.

Remuneration

A payment of $20 (male participants) or $30 (female participants who complete EEG testing) will be made to you for each session completed at the Brain Sciences Institute. That is, you will receive $40/$60 in total for completing both sessions. Female participants who complete EEG testing will be paid extra as compensation for the extra time taken for this testing. Female participants who elect not to complete EEG testing will receive $20 for each session.

While we would like you to undertake both sessions you are free to withdraw from the study at any time and you will be paid for the sessions that you have attended. Payment will be made by a cheque, mailed to you in the weeks following completion of the study.

Participants who were assigned to the placebo group will also be given a free 16-week supply of multivitamin on completion of the study.

Other points to note

Your personal details will remain strictly confidential. All data will be associated with a numerical code rather than a name. Scores may be published in a scientific journal as group data, or may be provided to other researchers, but individuals will not be able to

be identified. As a participant in this research you will not be entitled to restrict in any way how the results of this study are used.

Disclosure of funding source

The costs associated with this project are funded in part by Swisse Vitamins Pty Ltd. In addition Swisse have provided a PhD research scholarship for Elizabeth Harris to assist with her studies in the area of "nutraceutical interventions and cognitive decline." Swisse will not restrict in any way the publication of research findings that emerge from this study

Any questions can be directed to Liz Harris on 9214 5656.

If you have a query that the investigator has not been able to satisfy you are advised to contact: Dr. Andrew Pipingas on 9214 5215, or email apipingas@swin.edu.au

Complaints

If you have any concerns or complaints about the conduct of this project, please contact:
Research Ethics Officer
Office of Research & Graduate Studies (H68)
Swinburne University of Technology
P O Box 218, HAWTHORN VIC 3122
Tel (03) 9214 5218 or resethics@swin.edu.au

SWINBURNE UNIVERSITY OF TECHNOLOGY

Informed consent

Project title
The Swinburne Multivitamin Study: Effects of vitamin and herbal supplementation on cognition, brain electrical activity and cardiovascular measures in older adults.

Principal investigators
Elizabeth Harris
Dr Andrew Pipingas
Dr Richard Silberstein

I ……
(Name of Participant)

- Have read, or had read to me, and have understood the information provided in the Explanatory Statement and agree to participate in the study. Any questions I have asked have also been answered to my satisfaction.

My agreement is based on the understanding that:

- Any personal information that I provide will remain confidential
- My involvement entails undertaking a series of blood tests, computerized cognitive tests, brain electrical activity recording and psychometric tests, on two occasions over a 16-week period
- My involvement entails taking a nutritional supplement or placebo on a daily basis for 16 weeks
- I do not have any history of psychiatric disorder, neurological disease, diabetes or food intolerance/allergy
- I do not have epilepsy or have no history of epilepsy *(this only applies if you are taking part in the brain electrical activity testing)*
- I am free to withdraw from the study at any time
- Information and test results collected from me as part of the study may be published as group results, or provided to other researchers, on the condition that I cannot be identified
- I am not entitled to restrict in any way how the results of this study may be used.

Name of participant: ……

Signature: …….. Date: ……

Name of witness: ……

Signature: …….. Date: ……

Appendix B Therapeutic Goods Administration Product Information

Therapeutic Goods Administration
Product Information

ARTG Number:	**140129**
ARTG Label Name:	**Swisse Men's Ultivite Multi-Vitamin, Mineral & Anti-Oxidant with Herbs 50+ Years F**
Sponsor:	**Swisse Vitamins Pty Ltd**
Type of Therapeutic Good:	**Medicine**
Product Id:	**227252**

ARTG Number:	**140130**
ARTG Label Name:	**Swisse Women's Ultivite Multi-Vitamin, Mineral & Anti-Oxidant with Herbs 50+ Years F**
Sponsor:	**Swisse Vitamins Pty Ltd**
Type of Therapeutic Good:	**Medicine**
Product Id:	**227253**

Appendix C: Ethics Documentation

The author declares that all conditions pertaining to this ethic clearance were properly met, and annual and final reports have been submitted.

>>> Keith Wilkins 20/11/2006 9:57 am >>>
To: Dr Andrew Pipingas/Ms Elizabeth Harris, BSI, FLSS

Dear Andrew and Elizabeth

SUHREC Project 0607/073 The Swinburne Multivitamin Study: Effects of vitamin and herbal supplementation on cognition, brain electrical activity and cardiovascular measures in older adults Dr A Pipingas, Ms E Harris Approved Duration To 31/05/2007

I am pleased to advise that, on the basis of consideration of your resubmitted revised application (email of 9 November 2006) and further revision/clarification (email of 17 November 2006), the Chair of Swinburne's Human Research Ethics Committee (SUHREC) has endorsed the project. Ethics Clearance has therefore been given for the project to proceed as per standard conditions here outlined.

- All human research activity undertaken under Swinburne auspices must conform to Swinburne and external regulatory standards, including the current National Statement on Ethical Conduct in Research Involving Humans and with respect to secure data use, retention and disposal.

- The named Swinburne Chief Investigator/Supervisor remains responsible for any personnel appointed to or associated with the project being made aware of ethics clearance conditions, including research and consent procedures or instruments approved. Any change in chief investigator/supervisor requires timely notification and SUHREC endorsement.

- The above project has been approved as submitted for ethical review by or on behalf of SUHREC. Amendments to approved procedures or instruments ordinarily require prior ethical appraisal/ clearance. SUHREC must be notified immediately or as soon as possible thereafter of (a) any serious or unexpected adverse effects on participants and any redress measures; (b) proposed changes in protocols; and (c) unforeseen events which might affect continued ethical acceptability of the project.

- At a minimum, an annual report on the progress of the project is required as well as at the conclusion (or abandonment) of the project.

- A duly authorised external or internal audit of the project may be undertaken at any time.

Please contact me if you have any concerns or queries about on-going ethics clearance and if you need a signed ethics clearance certificate. The SUHREC project number should be cited in communication.

Best wishes for the project.

Yours sincerely

Keith Wilkins
Secretary, SUHREC

****

Keith Wilkins
Research Ethics Officer
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Tel: 9214 5218