Evaluation of the Effects of Acute Caffeine Supplementation on Selected Cognitive Domains in Older Women

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2022

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A thesis presented in partial fulfillment of the requirements for the degree of

Master of Science
in
Nutrition and Dietetics

at Massey University, Albany
New Zealand

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2022

Abstract

Caffeine is the most frequently consumed psychoactive substance globally and has been shown to enhance various aspects of cognition, particularly in younger adults. Previous research has demonstrated that acute intake of low to moderate doses of caffeine (e.g., 100 mg to 300 mg) significantly improves lower-order cognitive functions such as processing speed and attention in younger adults. The impact of caffeine on higher-order cognitive domains such as memory and executive function remains unclear. Since older women experience age- and hormonal-related changes that may negatively affect cognition, this age group may be more likely to experience greater functional improvements in cognitive performance following caffeine supplementation compared to younger adults. This pilot study aims to evaluate the effect of acute caffeine supplementation on selected cognitive domains including processing speed, sustained attention, memory, and executive function, in post-menopausal women, aged 55 to 79 years. Twelve female participants (mean age ± SD = 63.75 ± 6.81 years) took part in a randomised placebo-controlled, double-blind, crossover study. Participants were asked to abstain from caffeine for 24 hours before each of three repeated cognitive testing sessions (separated by two-week intervals): baseline, 45 minutes post-ingestion of 100 mg caffeine, and 45 minutes post-ingestion placebo. Repeated measures ANOVA (treatment × time) indicated that 100 mg of caffeine supplementation significantly improved movement time ($p = 0.04$) in a five-choice reaction time (RTI) task, compared to placebo. There was a significant improvement over time for Rapid Visual Information Processing (RVP) sensitivity score ($p = 0.04$) and correct responses ($p = 0.02$), and Spatial Working Memory strategy score ($p = 0.03$). However, post-hoc analysis indicated no significant differences between caffeine and placebo supplementation. The key finding of the current study is 100 mg of caffeine supplementation significantly enhanced processing speed in older post-menopausal women but did not improve other cognitive processes including attention, memory, and executive function. In line with other research, caffeine supplementation may only affect performance on simpler tests requiring lower-order cognitive functions. This pilot study contributes to the growing body of research on caffeine and cognition, through a unique examination of older healthy women across a range of cognitive functions. However, further studies are necessary on a larger population scale and perhaps, utilising different doses of caffeine, to corroborate these findings.

Key words: caffeine, older women, ageing, cognition, cognitive performance

Acknowledgements

Firstly, I would like to say a big – thank you and express my sincere gratitude to my wonderful supervisors; Dr Judy Thomas, Associate Professor Kay Rutherfurd-Markwick, and Dr Cheryl Gammon, for having the confidence in me, and providing consistent support and guidance throughout the entire thesis project. Without my supervisors, I would not have been able to complete this thesis and made it through my master's degree.

Secondly, I would like to say a big thank-you to Dr Hajar Mahazery for her time and help with SPSS data analysis and statistics for this research project.

Thirdly, many thanks to all participants that took part in this study, without whom this project would not have been possible.

To conclude, a big thank you to my family, partner, friends and classmates for their consistent motivation, unconditional support and putting up with me over the past two intense years, without whom I would not have been able to complete my master's degree in a COVID pandemic.

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List of Abbreviations

AD	Alzheimer's Disease
ADORA2A	Adenosine 2a receptor gene
ADP	Adenosine diphosphate
AMP	Adenosine monophosphate
ATP	Adenosine triphosphate
CANTAB	Cambridge Neuropsychological Test Automated Batteries
CNS	Central nervous system
COMPASS	Computerised Mental Performance Assessment System
COWAT	Controlled Oral Word Association
CRT	Choice Reaction Time
CYP450	Cytochrome P-450 enzymes
DAG	Diacylglycerol
IP3	Inositol triphosphate
JEF	The Jansari Assessment of Executive Function
MtSVS	Match-to-Sample Visual Search
NREM	Non-rapid eye movement
NZ	New Zealand
PAL	Paired Associated Learning
RTI	Reaction Time
RVP	Rapid Visual Information Processing
SNP	Single Nucleotide Polymorphism
SRT	Simple Reaction Time
SWM	Spatial Working Memory
UK	United Kingdom
USA	United States of America
VRT	Visual Recognition Time

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Chapter 1: Introduction

1.1 Background

Caffeine is the most widely consumed psychostimulant globally, with an estimated 80% of the world's population consuming various caffeinated foods or beverages daily (reviewed in Heckman et al., 2010 and Willson, 2018). Caffeine is a pure alkaloid and is chemically known as 1,3,7-trimethylxanthine (Fredholm, 2011). It is a bitter, plant-derived substance, naturally found in over 60 plant species including coffee beans, tea leaves, kola nuts, guarana berries, cocoa beans, and is also able to be produced synthetically (Fredholm, 2011).

Caffeine is widely known for its stimulatory effects including enhancing the activity of the central nervous system (CNS) to promote alertness and alleviate sleepiness, as well as improving mood and physical performance (reviewed in Cappelletti et al., 2015 and McLellan et al., 2016). However, an individual may experience adverse effects from caffeine ingestion such as insomnia, restlessness, anxiety, jitteriness, gastrointestinal distress, and tachycardia (Cappelletti et al., 2015; Heckman et al., 2010). Multiple reviews have concluded that consumption of low to moderate doses of caffeine (e.g., 50 mg to 300 mg) significantly improves lower-order cognitive functions such as processing speed, perceptual-motor function, and complex attention (e.g., using reaction time, response accuracy and vigilance tests) (Cappelletti et al., 2015; Heckman et al., 2010; McLellan et al., 2016; Snel & Lorist, 2011). However, results have been more equivocal for the effects of caffeine supplementation on higher-order cognitive functions such as learning, memory (e.g., episodic/working memory, recall tests) and executive function (e.g., strategic planning, decision making tasks) (Cappelletti et al., 2015; McLellan et al., 2016; Snel & Lorist, 2011).

In 2020, it was estimated that there were 727 million individuals aged 65 years and over in the world, with a prediction that this population group will increase twofold by 2050 (United Nations, 2020). In New Zealand alone, census statistics indicated there were 715,200 older adults (aged 65 years and above) in 2018, with a forecast that this would increase to 1.36 – 1.51 million older adults by 2048 (Statistics New Zealand, 2020). Therefore, it is important to identify strategies that can improve the quality of life of older individuals and reduce the social and economic burden on the health care system. Adults above 65 years of age can experience gradual physical, psychological, and cognitive age-related decline, hence consuming an ergogenic aid such as caffeine may be beneficial to overall health. Caffeine is widely consumed by middle-aged adults for various reasons including alleviating sleepiness, promoting alertness, and improving mood (Lieberman et al., 2019; Mitchell et al., 2014; Robillard et al., 2015).

In their review, which examined the effects of caffeine on cognitive, physical, and occupational performance, McLellan et al. (2016) reported that the majority of caffeine and cognition research has primarily focused on studying the effect of caffeine supplementation in the younger population, between the ages of 20 to 40 years, demonstrating improvements in lower-order cognitive functions such as processing speed and complex attention. Caffeine studies that were conducted in older adults typically investigated the effects of chronic caffeine consumption on attenuating cognitive decline or decreasing the risk of dementia and Alzheimer's Disease (AD) (McLellan et al., 2016). Limited research has shown that cognitive performance in older adults is also enhanced by acute caffeine supplementation. Since older, post-menopausal women experience age- and hormonal-related changes that may negatively affect cognition (Weber et al., 2014), caffeine supplementation may show a greater functional response in this cohort compared to younger adults. However, there is a gap in the current literature on caffeine's acute functional effects on overall cognitive function among this population group.

1.2 Study Justification

The present research aims to evaluate the effects of acute caffeine consumption versus placebo on different cognitive domains in healthy post–menopausal women, aged 55 – 79 years.

A review by McLellan et al. (2016) reported that a number of caffeine studies have demonstrated that low to moderate caffeine consumption (e.g., 50 mg to 300 mg) significantly enhances processing speed and complex attention in young adults, with results more equivocal for memory and executive function. Assessing cognitive performance in younger populations may result in ceiling effects, which are inherent in some standardised cognitive assessments. The occurrence of ceiling effects may mask the functional effects of caffeine on some cognitive functions. For the current study, the selected cohort are healthy older, post-menopausal women aged 55 to 79 years. Ceiling effects may be less likely to occur in this population particularly since this study utilises Cambridge Neuropsychological Test Automated Batteries (CANTAB) cognitive assessments that have extended levels of difficulty. Adults aged 80 years and above were not included in this study as they tend to experience more precipitous cognitive decline and are at higher risk of cognitive impairments in comparison to adults less than 80 years of age, who are relatively active and have more stable cognitive function (reviewed in Deary et al., 2009).

Limited research has studied the effect of caffeine consumption on cognitive performance in older women, who experience age- and hormonal-related changes that may impact negatively on cognition (Weber et al., 2014). As such, older women may show a greater functional response in cognitive testing after caffeine ingestion compared to younger adults. Waer et al. (2021) reported that 100 mg caffeine improved simple reaction time in a middle-aged cohort. Haskell-Ramsey et al. (2018) reported that older women demonstrated improved digit vigilance accuracy and reaction time in a selective attention task after supplementation with 100 mg caffeine. Neither of these studies found any significant effects for higher-order cognitive functions using an older cohort. Thus, further studies are required in older women to further elucidate the effect of caffeine supplementation on both lower-order processes (e.g., processing speed and attention) and higher-order functions (e.g., memory and executive function).

Cognitive assessments utilised in previous caffeine studies commonly have a time constraint, that is participants are instructed to complete a task as quickly as possible. As such, cognitive performance can be confounded by processing speed, particularly in older cohorts where speed may be slower. Having a range of cognitive tests, both timed and untimed, can further demonstrate whether caffeine only improves cognitive functions requiring speeded responses.

Despite a large body of work in the area of caffeine and cognition, there remains a gap in the research for investigating the functional effects of caffeine consumption on a wide range of tests, with and without time constraints, in older women. The present thesis addresses the gaps in the research by examining the effects of 100 mg caffeine supplementation versus placebo on cognitive function in post-menopausal women - using timed and untimed, extended difficulty tasks from across a range of cognitive domains including processing speed, sustained attention, memory, and executive function.

1.3 Purpose of the Research Study

1.3.1 Aim

This pilot study aimed to investigate the effects of acute caffeine consumption on selected cognitive domains in older, post-menopausal women (aged 55 to 79 years).

1.3.2 Objectives

- To examine the effects of caffeine supplementation (100 mg) on cognitive performance compared to placebo.
- To investigate the effects of 100 mg of caffeine supplementation on selected domains of cognition including processing speed, sustained attention, memory, and executive function.

1.4 Structure of the Thesis

The present thesis is comprised of four chapters. Chapter 1 provides background for the current study, including a study justification, the aim, and objectives. Chapter 2 is a narrative review of the current relevant literature on caffeine and cognition. Chapter 3 is a research manuscript prepared for the journal *Nutrients*, which outlines the materials and methods used for data collection and analysis, following with the study's results, discussion, and a conclusion. Finally, Chapter 4 summarizes the main findings of the thesis project, further elaborating on the strengths and limitations of the study, with recommendations for future research.

1.5 Researcher’s Contributions

Table 1: Researchers' contributions to the thesis study

Authors	Contributions
Neelam Khindria	Research study proposal, composed review of current literature, thesis manuscript, recruitment of participants, data collection, data entry and analysis, formulation of results, discussion, and conclusion.
Dr Judy Thomas	Study design, ethics application, provided supervision for the conduct of research, feedback on all chapters, assisted with data collection and the approval of this thesis.
Associate Professor Kay Rutherfurd-Markwick	Study design, provided supervision for conduct of research, feedback on all chapters and the approval of this thesis.
Dr Cheryl Gammon	Study design, provided supervision for conduct of research, feedback on all chapters, assisted with the materials used for intervention, and the approval of this thesis.
Dr Hajar Mazahery	Provided supervision and assistance with data analysis and results interpretation.

Chapter 2: Literature Review

2.1 Introduction

Caffeine is perceived as the world's oldest and most widely consumed psychostimulant, with 80% of the world's population consuming a range of caffeinated foods and beverages daily (reviewed in Heckman et al., 2010 and Willson, 2018).

Caffeine is found naturally in over 60 plant species, including coffee beans *(Coffea Arabica and Coffea Robusta)*, tea leaves *(Camellia Sinensis)*, kola nuts *(Cola Acuminate),* guarana berries *(Paullinia Cupana)*, and cocoa beans *(Theobroma Cacao*) (Heckman et al., 2010; Willson, 2018). Caffeine can also be extracted from various natural sources or synthetically produced to be added to popular processed foods and beverages such as energy drinks, sodas, sports supplements and chewing gum (reviewed in Reyes & Cornelis, 2018). Not surprisingly, with the broad range of caffeine–containing products that are available, an individual's dietary sources of caffeine vary depending on multiple factors, including country, age, ethnicity, and gender (reviewed in Reyes & Cornelis, 2018 and Verster & Koenig, 2018). For example, coffee and tea are commonly consumed by adults in many countries, while kola (cola) drinks are preferred among children and teenagers (Reyes & Cornelis, 2018).

Caffeine consumption has been shown to be associated with various stimulatory effects, including enhanced alertness, mood, and improved exercise performance whilst combating fatigue or tiredness (reviewed in McLellan et al., 2016). McLellan et al. (2016) reported low to moderate caffeine doses (e.g., 50 mg to 300 mg) consistently show improvements in specific cognitive functions such as reaction time, vigilance, and attention, whereas caffeine's effect on higher-order cognitive functions such as memory and executive function is more often debated in the literature. Interestingly, long term (e.g., 10 – 20 years) habitual moderate consumption of 200 mg to 400 mg caffeine daily is linked to a decreased risk of developing cognitive disorders, including dementia and AD (Eskelinen et al., 2009; Gardener et al., 2021; Zhang et al., 2021).

Adverse effects that an individual may experience due to caffeine consumption include insomnia, restlessness, anxiety, jitteriness/tremor, gastrointestinal upset, and cardiovascular-related issues such as tachycardia (reviewed in Cappelletti et al., 2015 and Heckman et al., 2010). It is possible that these acute adverse effects may negate any potential cognition-enhancing benefits of caffeine in affected individuals.

This chapter explores trends in caffeine consumption, the pharmacokinetics and pharmacodynamics of caffeine, and genetic influences on caffeine metabolism. The primary functions and respective testing measures of the main cognitive domains, cognitive ageing, and the acute effects of caffeine consumption on cognitive function, including an analysis of previous studies investigating the effects of caffeine consumption on cognition, are included.

Several comprehensive reviews have concluded that caffeine has positive functional effects on physical performance, cognitive performance, cognitive ageing, mood, and behaviour (Cappelletti et al., 2015; Lorist & Tops, 2003; McLellan et al., 2016; Snel & Lorist, 2011; Willson, 2018). However, studies investigating the impact of acute caffeine intake on cognition across a wide range of domains (from perceptual-motor activity to higher-order executive functioning) are limited, particularly in healthy older women. Although, both older men and women experience age–related changes in overall cognitive function, older women undergo hormonal transition from peri-menopause to post-menopause that may be associated with declines in cognition (Weber et al., 2014). Therefore, this cohort may have greater functional response to caffeine consumption to show improvements in cognitive assessments compared to younger adults.

2.2 Trends in Caffeine Consumption

In the US, the average amount of caffeine consumed by adults is estimated to be 150 mg – 260 mg/day (e.g., 1.8 mg – 3.0 mg·kg bw^{-1}) (Fulgoni et al., 2015), while in the United Kingdom (UK) it is 80 mg – 250 mg/day, in Ireland (Fitt, 2013) and Australia ranges between 110 – 190 mg/day (4.5 mg·kg bw^{-1}) (Watson et al., 2016), and in Europe and Asia it ranges between 20 mg to 300 mg/day (e.g., 0.4 mg – 5 mg·kg bw^{-1}) (European Food Safety Authority, 2015; Verster & Koenig, 2018).

In New Zealand, more than 73 percent of the population consume caffeine daily (Thomson & Schiess, 2010), with an average intake of approximately 200 mg to 300 mg/day (e.g., 3.5 mg – 4.0 mg·kg bw^{-1}). However, a recent study (Stachyshyn et al., 2021) of 314 New Zealanders, ranging in age from 16 to 50 years estimated the daily caffeine consumption of this population to be lower at 146.73 mg/day.

There is currently no recognised reference standard or "safe limit" (e.g., Acceptable Daily Intake) for daily caffeine consumption (Food Standards Australia & New Zealand, 2019). Numerous caffeine reviews and food standard agencies have concluded that for the general population, caffeine consumption of up to 400 mg/day is 'safe' for healthy adults (e.g., dosage of 5.7 mg·kg bw^{-1} for a 70 kg adult) (reviewed in Heckman et al., 2010; Nawrot et al., 2003 and Reyes & Cornelis, 2018). The recommended daily dosage of caffeine for children and teenagers aged 12 to 18 years is a maximum of 100 mg (2.5 mg·kg bw^{-1} daily for a 40 kg person), and zero mg of caffeine for children aged under 12 years (Food Standards Australia & New Zealand, 2019; Thomson & Schiess, 2010). Pregnant, lactating and breastfeeding women are recommended to restrict their daily intake of caffeine to 200 mg/day (3 mg·kg bw^{-1}) (Food Standards Australia & New Zealand, 2019; Thomson & Schiess, 2010)

2.3 Pharmacokinetics of Caffeine

Caffeine (Figure 1) is chemically defined as 1, 3, 7-trimethylxanthine with an alkaloid backbone conferring its bitter taste (Fredholm, 2011).

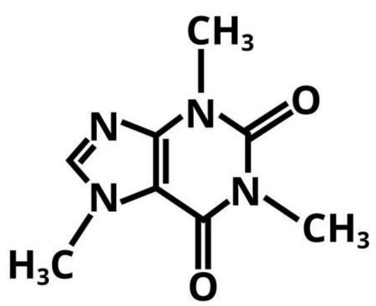

Figure 1. Structure of caffeine (Fredholm, 2011)

2.3.1 Caffeine Absorption, Metabolism and Distribution in the Human Body

Following consumption, caffeine is rapidly and completely absorbed in the small intestine of the gastrointestinal tract (Blanchard & Sawers, 1983; Marks & Kelly, 1973).

In healthy adults, caffeine usually peaks in blood plasma (Nehlig, 2004) approximately 30 to 90 minutes after consumption, with almost 100 percent bioavailability and a half-life of 3½ - 10 hours (Blanchard & Sawers, 1983; Marks & Kelly, 1973). Caffeine metabolism occurs predominantly in the liver and is catalysed by hepatic microsomal cytochrome P-450 (CYP) enzymes, which demethylate caffeine into three major pharmacologically active metabolites: paraxanthine (1,7 dimethylxanthine; 84%), theobromine (3,7 dimethylxanthine; 11%), and theophylline (1,3, dimethylxanthine; 5%) (Gu et al., 1992; Lelo et al., 1986; Miners & Birkett, 1996). Caffeine and the three metabolites distribute throughout the body, easily crossing all cell membranes including the blood brain barrier due to their hydrophilic and lipophilic nature to exert their effects on cardiovascular function (vasodilatation), renal function (diuresis), and the CNS (psychostimulant) (Gu et al., 1992; Lelo et al., 1986; Miners & Birkett, 1996).

2.3.2 Caffeine Elimination

The clearance rate of caffeine can vary 40-fold between individuals, primarily due to genetic factors (72.5%) (e.g., CYP1A2, ethnicity) and external/environmental factors (27.5%) (e.g., alcohol consumption, pregnancy, smoking status, medication use) (reviewed in Yang et al., 2010). Cytochrome P-450 (CYP) enzymes, specifically the P-450 1A2 enzyme, encoded by the *CYP1A2* gene is primarily responsible for caffeine metabolism (Berthou, 1991; Miners & Birkett, 1996). The vast majority of caffeine metabolites are excreted in the urine (~85–88 percent), with theobromine and theophylline being quickly excreted in the urine (~3-6 hours) compared to paraxanthine (~8-10 hours) (Cornish & Christman, 1957; Goldstein et al., 2010; Magkos & Kavouras, 2005). A small percentage (less than 5 percent) of caffeine is excreted unchanged (Cornish & Christman, 1957).

2.4 Pharmacodynamics of Caffeine

Caffeine and its dominant metabolite, paraxanthine are known to stimulate the CNS, resulting in enhancement of alertness and vigilance (Willson, 2018). The other two minor metabolites, theobromine and theophylline are typically used in medications, albeit at higher doses, to facilitate diuretic, vasodilatory and respiratory effects (Gu et al., 1992; Lelo et al., 1986; Miners & Birkett, 1996).

Caffeine's psychostimulatory effect is achieved due to its similarity in molecular structure to adenosine (Figure 2) (Fredholm, 2011). Caffeine binds with adenosine receptors in the brain, resulting in wakefulness and sleep inhibition (reviewed in Farah & Paula, 2019; Holst & Landolt, 2015; Willson, 2018).

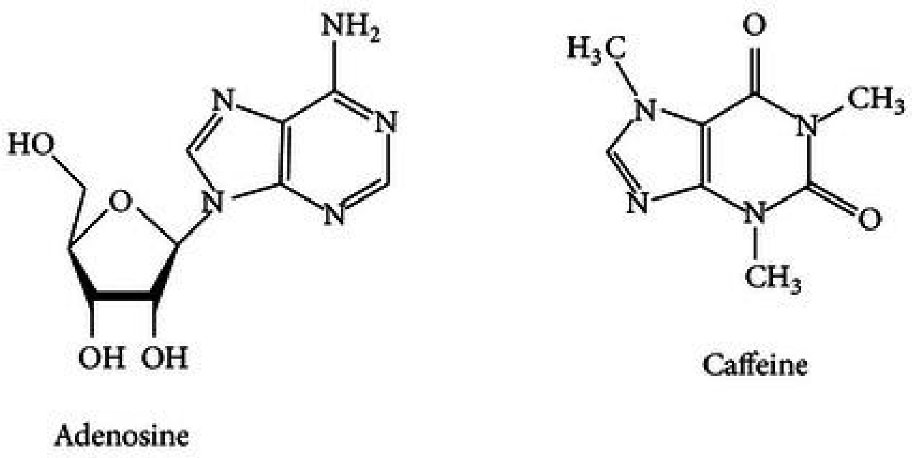

Figure 2. The molecular structures of adenosine and caffeine (Fredholm, 2011)

Adenosine is an ubiquitous endogenous nucleoside present in all cells of the body (Stockwell et al., 2017). It is a neuromodulator and a somnogenic substance, with a key inhibitory effect resulting in suppression of neuronal and brain activity (Holst & Landolt, 2015). Crucial functions of adenosine includes assisting in maintaining body homeostasis via sleep-wake regulation, modulation of blood glucose, lipid levels and metabolism (Holst & Landolt, 2015; Stockwell et al., 2017)

Adenosine is formed from the breakdown of the energy–rich molecule adenosine-tri-phosphate (ATP), dephosphorylating into adenosine-di-phosphate (ADP), adenosine-mono-phosphate (AMP) and adenosine (Holst & Landolt, 2015). Utilisation of ATP leads to an accumulation of adenosine, leaking from the intracellular neuronal network into the extracellular network via a nucleoside transporter (Holst & Landolt, 2015; Stockwell et al., 2017). The extracellular adenosine binds and activates four G-protein-coupled receptor subtypes: A1 (neuroprotective), $A2_A$ (neurodegenerative), $A2_B$ and A3 (Table 2) (reviewed in Dunwiddie & Masino, 2001; Jacobson & Gao, 2009; Stockwell et al., 2017).

Table 2: Adenosine receptors and their respective locations and functions

Adenosine Receptors	Location	Functions
A1[1,2]	Extensively on excitatory neurons in the brain, high abundance in pre and post synaptic neurons	Promotes sleep (facilitates delta activity in non-rapid eye movement (NREM), inhibits lipolysis, lowers heart rate, reduces renal blood flow
$A2_A$[1,2]	Widely on post-synaptic membranes in the dorsal striatum	Promotes sleep, regulates metabolism (associated to the reward feeding system), facilitates vasodilation and hypotension
$A2_B$[1,3]	Mainly in the brain and the periphery with low abundance	Regulates metabolism, modulates blood glucose homeostasis and lipid levels
A3[4]	Scarce, low presence in neurons, highly expressed in the lung and liver	Vasodilatory effects (e.g., afferent arterioles)

[1] (Holst & Landolt, 2015)
[2] (Arslan et al., 2000)
[3] (Polosa & Zeng, 2006)
[4] (Lu et al., 2015)

The activation of these four receptors located in the heart, brain, lung and liver have multiple effects due to their interaction with various signaling molecules, such as choline, inositol triphosphate (IP3), and diacylglycerol (DAG) (reviewed in Arslan et al., 2000; Holst & Landolt, 2015; Stockwell et al., 2017). Stimulating the release of these signaling molecules generates second messengers (e.g., cAMP) that regulate a variety of cellular responses such as suppressing or arousing neuronal activity, vascular functionality, immune system modulation, platelet aggregation, and blood cell regulation (Arslan et al., 2000; Holst & Landolt, 2015; Willson, 2018).

Caffeine primarily binds to the A1 and $A2_A$ adenosine receptors (reviewed in Ribeiro & Sebastião, 2010), consequently blocking adenosine from binding to these receptors, resulting in a reduction in adenosine's inhibitory effects on neural activity, promoting alertness (Ribeiro & Sebastião, 2010). Via this mechanism caffeine is able to exert its excitatory effects and alleviate tiredness and sleepiness (Ribeiro & Sebastião, 2010; Willson, 2018).

2.5 The Role of Genetics

Genetic variation influences an individual's response to caffeine consumption by affecting their rate of caffeine metabolism and hence clearance rate from the body, (Farah & Paula, 2019; Yang et al., 2010). This section will focus on two extensively researched genes that influence caffeine's mechanisms of action. These are *CYP1A2*, rs762551 (cytochrome P450 1A2), which alters the rate of caffeine metabolism, and *ADORA2A*, rs5751876 (adenosine $A2_A$ receptor), which impacts caffeine's psychostimulatory effects on adenosine receptors (reviewed in Tennent et al., 2020; Yang et al., 2010).

2.5.1 CYP1A2

The cytochrome P450 enzyme CYP1A2, coded by the *CYP1A2* gene is responsible for the rate at which caffeine is metabolised. There is one single nucleotide polymorphism (SNP) present at rs762551 of the *CYP1A2* gene (Cornelis et al., 2006; Sachse et al., 1999). The SNP at rs762551 has three genotype variations which are homozygote wildtype A/A, heterozygote A/C and homozygote C/C. These genotype variations alter CYP1A2 enzymatic activity and the rate of caffeine metabolism (Cornelis et al., 2006; Sachse et al., 1999). The wild–type A/A allele is referred to as "fast caffeine metaboliser" and increases the activity of the enzyme in individuals carrying this genotype, promoting a more rapid caffeine breakdown (up to four-fold higher) by the liver compared to individuals who carry alleles C/C and A/C, which are typically considered as "slow caffeine metabolisers" (Tennent et al., 2020; Yang et al., 2010). In "slow" caffeine metabolisers, the breakdown of caffeine is prolonged, resulting in caffeine remaining in the body for longer periods (Tennent et al., 2020; Yang et al., 2010). Extended exposure to high amounts of caffeine in the body may pose a risk, causing adverse effects such as increased heart rate (Cornelis et al., 2006), vasoconstriction and increased risk of hypertension (Tennent et al., 2020; Yang et al., 2010).

2.5.2 ADORA2A

A SNP (rs5751876) in the adenosine *A2A* gene (*ADORA2A*) influences an individual's sensitivity to caffeine's effects on sleep quality and anxiety by increasing A1 and $A2_A$

adenosine receptor (AR) expression (Domschke et al., 2012; Rétey et al., 2007). There are three genetic variants: homozygote C/C and T/T and heterozygote C/T (typically known as 1976 C→T) (Domschke et al., 2012). The 1976 C/C genotype is linked to poor sleep quality and increased likelihood of experiencing caffeine-induced insomnia or greater sleep disruptions (Domschke et al., 2012; Rétey et al., 2007). Non-habitual caffeine consumers who carry the heterozygote C/T polymorphism may experience caffeine's anxiogenic effects and may be at risk of panic disorder (recurring episodes of high anxiety and panic attacks) (Cornelis et al., 2007; Rogers et al., 2010). Similarly, the homozygote 1976 T/T polymorphism allele is linked to caffeine–induced anxiety post moderate to high caffeine intake (~150 mg and more), along with elevated systolic blood pressure and risk of panic disorders (Cornelis et al., 2007; Rogers et al., 2010). Other *ADORA2A* SNPs, such as rs2298383 and rs4822492 have also been associated with caffeine-induced anxiety (Domschke et al., 2012), however, due to the scope of this study, these two SNPs will not be discussed further.

2.6 Cognition, Cognitive Function and Testing

Cognition is a multifaceted construct encompassing various mental processes that are typically categorised into broad domains of cognitive functioning such as executive functioning; learning and memory; perceptual motor function; processing speed; complex attention and language/verbal skills/social cognition (Harvey, 2019). These functions are thought to be inter-dependent and hierarchical in nature, from basic sensory and perceptual processes to higher-level executive functioning such as decision-making, planning and problem-solving (Harvey, 2019).

Neuropsychological testing is commonly employed to diagnose cognitive impairment, or to test particular functions or domains influenced by the effects of a specific intervention, treatment or supplement such as caffeine, in both clinical and healthy populations (Miller, 1992). Table 3 outlines standardised cognitive assessments that are widely utilised to assess performance in specific cognitive domains. Computerised neurocognitive assessments such as the Cambridge Neuropsychological Test Automated Batteries (CANTAB) are comprised of extended levels of difficulty to reduce potential ceiling effects which occur when the majority of subjects achieve maximum scores in any cognitive assessment (Fray et al., 1996). Reduced ceiling effects and randomisation of stimuli are favourable characteristics to reduce practice effects that may occur with repeated testing in intervention-based studies (Fray et al., 1996). The CANTAB has been extensively validated and previously shown to have sensitivity for acute changes in cognitive performance post various treatments (Durlach, 1998; Falconer et al., 2009), including post consumption of a nutritional supplement such as caffeine (Attwood et al., 2007).

Table 3: Cognitive domain functions and their respective neurocognitive assessments

Cognitive Domain	Functions	Examples of Cognitive Tests
Executive Function[1,3,5,6]	Planning, problem solving, decision-making/judgement, abstract reasoning, impulse control, behaviour control, working memory, inhibition, responding to feedback, fluency, and mental flexibility.	Wisconsin Card Sorting Test, Trail Making Test Part B, the Stroop Interference subtest, Delis-Kaplan Executive Function System, Jansari Assessment of Executive Function (JEF) and specific measures within word fluency tasks, **Spatial Working Memory (SWM)**
Learning & Memory[2,4,5,6]	Registering, storing, and retrieving information, episodic/semantic memory, short/long term memory, recall, recognition memory, associative/non-associative learning, procedural memory.	Auditory verbal recall, visual recognition test, Rey complex figure test, digit span, memory comparison test, **Paired Associates Learning (PAL)**
Perceptual – Motor Function[1,2,5,6]	Visual perception, perceptual-motor coordination/motor imagery, visuo-constructional reasoning, motor speed and praxis skills	Object recognition, organizational strategies, drawing tests (e.g., Mini-Mental State Examination and Montreal Cognitive Assessment), reaction time tasks, construction, or perceptual tests (e.g., similarly to the Rey Complex Figure)

Cognitive Domain	Functions	Examples of Cognitive Tests
Processing Speed[1,2,5,6]	Information processing speed, response latency, motor response time/reaction time and accuracy	Trail Making Test, Detection tasks, Simple reaction time, **Choice reaction time (CRT)**
Complex Attention[1,2,5,6]	Sustained attention, selective/divided attention, processing speed, attention span, focussed/complex attention	Digit Span test, Stroop test, sequential operation series, Corsi Blocks, selective or sustained attention/vigilance tasks, **Rapid Visual Information Processing (RVP)**
Language/Verbal Skills/Social Cognition[1,2,4,5,6]	Recognising emotions, object naming, word finding and fluency, insight, grammar and syntax, receptive language	Boston naming test, Controlled Oral Word Association Test (COWAT), fluency tests, reading and comprehension assessments

Note: The four cognitive assessments highlighted in bold font were employed for this research study.

[1] (Committee on Psychological Testing et al., 2015)
[2] (Kipps & Hodges, 2005)
[3] (Rabinovici et al., 2015)
[4] (Brem et al., 2013)
[5] (Harvey, 2019)
[6] (Daffner et al., 2015)

2.7 Cognitive Senescence

Globally, in 2020, there were estimated to be 727 million individuals aged 65 years and above, with a projection that this age group will double by 2050 (United Nations, 2020). In 2018 in New Zealand, 15.2 % of the New Zealand population were estimated to be over 65 years of age (Statistics New Zealand, 2020). The ageing process relates to the various physical, psychological, physiological, and social changes experienced by older adults that may impact quality of life (reviewed in Urtamo et al., 2019). Cognitive health is a key component of upholding independence, healthy ageing, and quality of life (Evans et al., 2018; Mumme et al., 2019). Although cognitive senescence occurs naturally and progressively with age, there is significant inter-individual variability in the rate and degree of this change. Normal age-related cognitive changes may result in slower processing speed, greater difficulty in sustaining attention and recalling information (reviewed in Harada et al., 2013). The incidence of certain cognitive disorders increases with age, including mild cognitive impairment, dementia (e.g., Lewy body dementia or vascular dementia), and AD (Deary et al., 2009).

Reviews of previous longitudinal and cross-sectional studies have concluded that cognitive functions such as processing speed, memory, complex attention, spatial orientation, and executive functioning peak at 20–30 years of age, and gradually decline with age (Deary et al., 2009; Hartshorne & Germine, 2015). In contrast, vocabulary/verbal functions remain relatively stable with advancing age (Deary et al., 2009; Harada et al., 2013; Hartshorne & Germine, 2015). In addition, ageing is associated with a linear decrease in fluid cognition (comprised of executive function, learning and memory, and processing speed) among individuals aged 40 to 70 years, in comparison to crystallized cognition (language/verbal skills/social cognition), which remains steady throughout the life span of an individual (Deary et al., 2009; Harada et al., 2013; Murman, 2015). However, older adults over the age of 80 tend to experience a more sudden and rapid decline in overall cognitive function (Deary et al., 2009; Harada et al., 2013; Horning & Davis, 2012).

The transition from pre-menopause to post-menopause results in major hormonal alterations which have been shown to negatively impact specific cognitive functions such as memory and fluency tasks (Weber et al., 2014). Given the age- and hormonal-related cognitive changes that can be experienced by post-menopausal women, this population may demonstrate greater functional improvements in cognitive testing post caffeine consumption, compared to the younger populations that are most often studied. Limited studies have specifically examined the effects of acute caffeine consumption on cognitive function in older, post-menopausal women. Therefore, further research is required to examine the effect of caffeine consumption on various aspects of cognition in this population.

2.8 The Effects of Caffeine Consumption on Cognitive Function

Numerous acute, placebo-controlled, caffeine studies have demonstrated that caffeine consumption of 50 mg – 400 mg in healthy adults (aged 18 to 80 years) significantly (p = <0.05) enhances specific cognitive functions (Table 4). Participants recruited for these caffeine trials included naïve, or habitual (e.g., low, moderate, and high) caffeine consumers. An overview of these prior caffeine studies is summarised in Table 4. Chronic and long-term caffeine studies were not included in Table 4 as the present research focuses on the effects of acute caffeine consumption.

Acute caffeine interventions conducted in healthy adults show that caffeine consumption at low to moderate doses (e.g., approximately 75 mg to 300 mg) significantly improves lower-level cognitive functions such as increasing information processing speed, increasing accuracy and decreasing response times on tests of attention, vigilance, and alertness (Hasenfratz & Bättig, 1992; Haskell-Ramsay et al., 2018; Rosenthal et al., 1991; Waer et al., 2021). The majority of acute caffeine research indicates that caffeine has less impact on higher-levels of cognitive functioning, with performance mostly unchanged on tests of memory, executive functioning, decision making, problem solving, and strategic planning (Attwood et al., 2007; Childs & Wit, 2006; Haskell et al., 2005; Hindmarch et al., 1998; Valladares et al., 2009).

Evidence suggests that moderate to long–term habitual caffeine consumption (400 mg and more daily) may be advantageous for short- and long-term memory, as well as decreasing the risk of cognitive decline and dementia in older adults (> 65 years of age) (Eskelinen et al., 2009; Gardener et al., 2021; Zhang et al., 2021). Constant stimulation of adenosine receptors is thought to lead to Aβ-amyloid accumulation and neurodegeneration, resulting in cognitive decline (Gardener et al., 2021). Due to its action as an adenosine receptor antagonist, chronic caffeine intake may reduce Aβ-amyloid accumulation and hence may decrease the risk of cognitive decline and dementia (reviewed in Ribeiro & Sebastião, 2010; Santos et al., 2010).

Table 4: Summary of findings of acute studies investigating the effects of acute caffeine consumption on cognition in healthy adults

Study	Study Design	Participants	Caffeine Dose	Performance Metric	Cognitive Domains	Caffeine Treatment vs Placebo
Ali et al., 2016	Randomised, double-blind, placebo-controlled crossover-study Caffeine abstinence: 48 hours Trials were conducted during days 5 – 8 and 18 – 22 days of oral contraceptive pill cycle (10 washout period)	10 females (24 ± 4 years with low to moderate consumers of caffeine, > 0 – 300 mg · day^{-1})	Approximately 350 mg (6 mg·kg^{-1} body mass, average bodyweight: 59.7 ± 3.5 kg)	Stroop test, CRT Computer software: COMPASS	Processing speed & attention, and executive function	NS effect of caffeine in Stroop Test NS improvements on reaction time and correct responses in CRT

Study	Study Design	Participants	Caffeine Dose	Performance Metric	Cognitive Domains	Caffeine Treatment vs Placebo
Attwood et al., 2007	Placebo-controlled, double-blind, crossover study Caffeine abstinence: roughly 12 hours (from 2300 hours the previous night) Intersession intervals were not less than 24 hours or exceed 14 days	45 (18 male, 27 females, range 18 to 41 years), separated into 2 groups: moderate and high caffeine users (<200 mg/day and >200 mg/day)	400 mg	SRT, CRT, MtSVS & RVP Computer software: CANTAB	Memory, processing speed & sustained attention	Significant caffeine-by-group interactions for SRT reaction, where high consumers were significantly faster than moderate consumers on SRT. Moderate consumers were slower after caffeine relative to placebo for movement time Significant main effect of caffeine for CRT, but no effect of group. High consumers were significantly faster after caffeine intake compared to placebo. NS effects on MtSVS and RVP cognitive tasks
Brunyé et al., 2010	Within-participants study with four levels of a double-blind independent variable Caffeine abstinence: 12 hours Five visit trials (one normal consumption session, four test sessions), all visits were separated by at least 3 days	36 young adults (10 male, 26 females, mean age of 20.11 years), all high caffeine consumers (mean consumption: 592.3 mg/day)	0 mg 100 mg 200 mg 400 mg	Attention Network Test	Attention, alerting, orienting, and executive control	Caffeine enhanced vigilance and the executive control of visual attention (response inhibition) only for the highest dose of 400 mg

Study	Study Design	Participants	Caffeine Dose	Performance Metric	Cognitive Domains	Caffeine Treatment vs Placebo
Childs & Wit, 2006	Double blind, placebo-controlled, within-subject study Caffeine abstinence: Refrained from eating and drinking from midnight prior of trial visit (roughly 8 – 12 hours) Four trial visits, with each trial visit 72 hours apart	102 (51 males, 51 females, age range of 18 to 35 years), all participants were light, nondependent caffeine consumers (<300 mg per week)	0 mg 50 mg 150 mg 450 mg	Digit symbol substitution test, visual vigilance task, digit span task and stop task	Working, verbal and episodic memory, sustained attention, psychomotor speed	450 mg ↓ reaction time in visual vigilance task 150 mg and 450 mg ↑ correct responses for visual vigilance task NS main effects seen for memory
Hasenfratz & Battig, 1992	Placebo-controlled, crossover study Caffeine abstinence from 8:00 PM prior trial visit	20 females (mean age: 28.3 years, age range of 21 – 39 years) Moderate to high caffeine consumers (average of 5.4 cups of coffee/day)	250 mg	Numerical "Stroop" task	Complex attention	250 mg ↓ reaction time in Stroop Task

Study	Study Design	Participants	Caffeine Dose	Performance Metric	Cognitive Domains	Caffeine Treatment vs Placebo
Haskell et al, 2005	Placebo-controlled, double-blind, balanced crossover study Caffeine abstinence: 12 hours Four trial visits were conducted 48 hours apart	48 (29 males, 19 females, age range of 18 – 46 years, mean age of 23.4 years), separated into two distinctive groups: 24 habitual caffeine consumers and 24 non-habitual caffeine consumers	0 mg 75 mg 150 mg	SRT, CRT, digit vigilance, immediate and delayed word recall, numeric working memory, spatial memory, RVP, tracking task, logical reasoning, sentence verification task, serial subtraction task, and serial threes Computer software: Cognitive Drug Research battery	Memory, processing speed & accuracy, sustained attention, and executive function (e.g., sentence task, subtraction task and serial threes)	75 mg of caffeine significantly improved SRT 75 mg and 150 mg significantly speeded up digit vigilance reaction time 150 mg ↓ numeric working memory reaction time NS effect for spatial memory tasks Significant group differences in the number of false alarms generated RVP task were, non-consumers producing more false alarms than consumers 75 mg and 150 mg significantly improved sentence verification accuracy NS effects for subtraction tasks

Study	Study Design	Participants	Caffeine Dose	Performance Metric	Cognitive Domains	Caffeine Treatment vs Placebo
Haskell-Ramsey et al, 2018	Randomised, placebo-controlled, double-blind, counterbalanced-crossover study Caffeine abstinence from 12 PM the day prior trial visits but not exceeding 24 hours Three trial visits, with seven days apart from each visit	59 (30 males, 29 females, age range: 20 – 80 years) who were regular caffeine consumers (roughly ≥150 mg caffeine/day)	0 mg 5 mg (decaffeinated) 100 mg	Word presentation, immediate word recall, picture presentation, SRT, digit vigilance, numeric working memory, verbal fluency, delayed word recall, RVP, delayed word recognition, delayed picture recognition Computer software program employed: COMPASS	Episodic memory, sustained attention, working memory & language, psychomotor speed, and accuracy	100 mg caffeine ↑ in digit vigilance accuracy and ↓ in digit vigilance reaction time 100 mg ↓ RVP reaction time compared to placebo for both younger and older subjects NS for learning & memory tasks
Hindmarch et al, 1998	Within subject, complete five-way crossover Caffeine abstinence: Evening prior trial visit	19 (9 males, 10 females, mean age: 29.2 years ± 6.07 years)	400 ml (containing 100 mg of caffeine) of black tea, coffee, caffeinated water, decaffeinated tea, or plain water at three separate times: 0900,1400 and 1900 hours	CRT and short-term memory task	Processing speed & memory	NS effects on CRT reaction time NS effects on memory test

Study	Study Design	Participants	Caffeine Dose	Performance Metric	Cognitive Domains	Caffeine Treatment vs Placebo
Karayigit et al., 2020	Randomised, placebo-controlled, double-blinded, counter-balanced, crossover study Caffeine abstinence: 24 hours prior testing session Five trial visits, separated by 48 to 72 hours	17 females (M ± SD: 23 ± 2 years, weight: 64 ± 4 kg), naïve caffeine consumers (<25 mg/day)	Low: 3 mg/kg/bm (~190mg/kg) High: 6 mg/kg/bm (~380mg/kg) Decaffeinated coffee (placebo)	Modified arrow version of Eriksen Flanker Test Computer software: Inquisit Lab 5.0	Psychomotor/processing speed & accuracy	Faster reaction time for 6 mg/kg/bm (high) compared to placebo, but NS differences between low and high dosage NS effects for low caffeine
Konishi et al., 2008	Randomised, placebo-controlled, double-blinded study Caffeine abstinence: Three days	100 (50 males, 50 females, age range of 22 to 59 years)	200 mg	Symbol digit coding test (SDC), Stroop test, Shifting attention test (SAT) & Four-Part Continuous Performance Test (FPCPT) Computer software: Cognitrax® and CNS Vital Signs	Psychomotor speed & accuracy, attention, cognitive flexibility, executive function (decision making in SAT task) & working memory (FPCPT)	200 mg ↑ correct responses and ↓ errors made on the SAT task NS effects on SDC, Stroop test and FPCPT

Study	Study Design	Participants	Caffeine Dose	Performance Metric	Cognitive Domains	Caffeine Treatment vs Placebo
Rosenthal et al., 1991	Placebo-controlled study design Received caffeine twice daily after 8 and 5 hrs in bed the night prior	36 males (age range of 19 – 35 years)	0 mg 75 mg 150 mg	Multiple Sleep Latency Test, auditory vigilance task	Sleep latency, psychomotor speed & attention	75 mg and 150 mg ↑ average daily sleep latency 75 mg and 150 mg ↓ vigilance reaction time
Soar et al., 2016	Double-blind, repeated measure study Caffeine abstinence: two hours prior trial visit Two trial sessions, one week apart from each other	43 (17 males & 26 females, mean age: 28 years) Regular caffeine users	50 mg (1.8 g of Nescafe coffee granules + 200 ml of water) Decaffeinated coffee (1.8 g of Nescafe® decaffeinated coffee granules + 200 ml water)	JEF & Stroop Colour-Word task Computer software: JEF	Executive function (e.g., planning, adaptive thinking, creative thinking, decision making), prospective memory	50 mg significantly improved JEF tasks in planning, creative thinking, and prospective memory 50 mg ↓ reaction time on Stroop task NS for correct responses in Stroop-Colour Word
Valladares et al., 2009	Double blind, counter-balanced, placebo-controlled, crossover, repeated measure study Caffeine abstinence from 11 AM on testing day (four hours prior to testing) Two trial visit, seven days apart	24 (age range of 19 – 38 years, mean age: 26.5 years), light to moderate caffeine consumers (consuming 2-4 cups of coffee daily)	250 mg	N-back task	Working memory	NS for working memory tasks 250 mg ↓ mean reaction time in N- back task compared to placebo

Study	Study Design	Participants	Caffeine Dose	Performance Metric	Cognitive Domains	Caffeine Treatment vs Placebo
Waer et al., 2021	Randomised, placebo-controlled, double-blind, crossover study Caffeine abstinence from 6:00 PM the night before testing session Four trial visits separated by at least four days apart	19 older, post-menopausal females (mean age = 52 ± 3 years), who were low habitual caffeine consumers (<200 mg/day of caffeine)	100 mg 400 mg	SRT Computer software: Superlab 4.5 program	Processing speed	100 mg ↓ reaction time for SRT NS outcomes for 400 mg

Abbreviations: NS: Not Significant, [↑]: significant increase (*p* = <0.05); [↓]: significant decrease (*p* = <0.05), Computerised Mental Performance Assessment System (COMPAS), Choice Reaction Time (CRT), Simple Reaction Time (SRT), Match-to-Sample Visual Search (MtSVS), Rapid Visual Information Processing (RVP), Symbol digit coding test (SDC), Shifting attention test (SAT), Four-Part Continuous Performance Test (FPCPT), and The Jansari assessment of Executive Functions (JEF).

2.8.1 Processing Speed and Attention

Cognitive tasks that assess processing speed and attention typically measure simple responses to stimuli under timed conditions (i.e., participants are generally instructed to respond as quickly as possible) (Harvey, 2019). Assessments of processing speed measure the time required for cognitive and motor functioning to rapidly process information and respond to simple or complex stimuli, including the simple reaction time (SRT), visual recognition time (VRT), and choice reaction time (CRT) tests (Harvey, 2019). Attention is divided into two classifications: selective attention, which measures concentration focused on relevant and critical information, and sustained attention or vigilance, where attentiveness is measured over a period of time (Harvey, 2019). Tests of attention include rapid visual information processing (RVP) and vigilance tasks (Harvey, 2019). The ability to attend to stimuli with good cognitive processing speed underlies the ability to complete higher-level more complex tasks and is thought to be the strongest predictor of overall cognitive performance (Harvey, 2019).

Several acute studies (Table 4) demonstrate that in participants ranging from 18 to 80 years of age caffeine supplementation (75 mg to 400 mg) results in improved reaction times in tests of processing speed (e.g., SRT, CRT) compared to placebo (Attwood et al., 2007; Haskell-Ramsay et al., 2018; Karayigit et al., 2020; Waer et al., 2021). Performance on tests of attention and vigilance (e.g., digit vigilance task, RVP, and Stroop Test) are also shown to be affected by caffeine supplementation of 75 mg to 450 mg with improved reaction times and response accuracy compared to placebo (Brunyé et al., 2010; Childs & Wit, 2006; Hasenfratz & Bättig, 1992; Haskell-Ramsay et al., 2018; Haskell et al., 2005). Rosenthal et al. (1991) showed that 45-minutes post caffeine supplementation with low to moderate caffeine doses (75 mg and 150 mg) leads to significantly improved reaction time in an auditory vigilance task, and even increased sleep latency in 36 young males. The majority of acute caffeine studies measuring processing speed and attention were conducted in a younger population (with an age range of 18 to 41 years) (Ali et al., 2016; Attwood et al., 2007; Brunyé et al., 2010; Childs & Wit, 2006; Hasenfratz & Bättig, 1992; Hindmarch et al., 1998; Karayigit et al., 2020; Rosenthal et al., 1991; Soar et al., 2016; Valladares et al., 2009). Only two studies in Table 4 examined the impact of caffeine supplementation in older women.

These are Waer et al. (2021) who recruited nineteen healthy middle-aged women (mean age = 52 ± 3 years) and Haskell-Ramsay et al. (2018) who recruited sixteen healthy older females aged 61 to 80 years. These studies showed improvements in processing speed using a simple reaction time task ($p = 0.01$) (Waer et al., 2021), and attention/vigilance in a digit vigilance task ($p = 0.01$), and RVP reaction time ($p = 0.02$) (Haskell-Ramsay et al., 2018) 30-minutes post consumption of 100 mg caffeine supplementation. A higher dose of caffeine, 400 mg did not show any reduction for reaction time in SRT task (Waer et al., 2021).

2.8.2 Memory

Memory is defined as the process of storing and recalling information (Harvey, 2019). Memory is comprised of multiple subcategories (Table 3), however, working memory (short-term) and episodic (long-term) memory are popularly assessed to study the effects of caffeine (Table 4). Working memory pertains to information retained for a short period of time, in contrast to episodic memory, that engages with working memory to encode, store and retrieve information for a prolonged period of time (Harvey, 2019).

Acute interventions with 100 mg to 400 mg of caffeine supplementation have shown that supplementation has no significant impact on either working or episodic memory (Attwood et al., 2007; Haskell-Ramsay et al., 2018; Haskell et al., 2005; Hindmarch et al., 1998; Valladares et al., 2009). However, Valladares et al. (2009) showed 24 young, light to moderate caffeine consumers exhibited an improvement in reaction time (but not accuracy) in a working memory task, post 250 mg caffeine supplementation compared to placebo, following only four hours of caffeine abstention. The majority of acute caffeine trials that have included memory tasks were conducted in younger rather than older adults, with younger healthy adults likely to show high memory scores pre-caffeine ingestion, thus leading to ceiling effects. Interestingly, one study found that 450 mg of caffeine supplementation impaired performance on a working memory task (participants significantly decreased the number of digits remembered in the digit span task) in 102 young adults, aged 18 to 35 years (Childs & Wit, 2006). Therefore, caffeine's functional effects on various aspects of memory remains unclear and requires further research.

2.8.3 Executive Function

Executive function is comprised of complex higher-level cognitive processes including reasoning, problem solving, monitoring performance, responding to feedback, implementation of strategies, inhibition and flexibility, and decision-making (Harvey, 2019; McLellan et al., 2016).

Limited research has examined the effects of caffeine consumption on executive function, and results are unclear and equivocal. Due to the many cognitive processes comprising "executive function", there is a large diversity in tests utilised in previous studies. As well, the difficulty in separating executive function from other underlying abilities needed to complete the task, such as attention or speed of processing (Soar et al., 2016) also complicates such studies. One study in which 36 young, high caffeine consumers, consumed 100 mg or 200 mg of caffeine, showed no significant improvements in executive function compared to placebo (Brunyé et al., 2010). However, the highest dose (400mg) improved executive control of visual attention (response inhibition) in an attention network task (Brunyé et al., 2010). Another study found a significant effect post 200 mg of caffeine supplementation only on Shifting Attention Task (cognitive flexibility task), but no effect observed for the Stroop Test (interference) (Konishi et al., 2018). Ali et al. (2016) also did not find any significant effect using 350 mg of caffeine supplementation on Stroop Test in ten young females. Soar at al. (2016) who employed a non-immersive computerised assessment assessing executive functioning called The Jansari Assessment of Executive Functions (JEF) in 43 young adults showed an improvement in executive functioning (planning, creative thinking, prospective memory) post 50 mg caffeine supplementation. Surprisingly this study exhibited effectiveness on executive function following use of a low dose of 50 mg but after only two hours of caffeine abstention prior to the testing session. This is in contrast to most studies that have used a longer abstinence period due to caffeine's half-life of 3 ½ hours to 10 hours.

2.9 Summary of Literature and the Importance of the Current Study

Caffeine supplementation has been shown to improve selected cognitive processes including alertness, attention/vigilance, and processing speed. The psychostimulant effect of caffeine as an adenosine receptor antagonist likely underlies its effect on these lower-order cognitive functions. Whereas it is not clear whether caffeine consistently facilitates memory and executive functioning. The majority of these caffeine studies in Table 4 were conducted largely among younger adults. There are limited studies that have investigated the functional effects of caffeine on cognition in older adults. Since older, post-menopausal women experience normal age- and hormonal-related changes that negatively affect cognitive function, this cohort may be more likely to experience greater functional improvements in cognitive testing following caffeine supplementation compared to younger adults. Thus, caffeine's functional improvements on cognitive function may enhance quality of life in the growing NZ ageing population.

Chapter 3: Research Study Manuscript

The following chapter is composed of a manuscript prepared for the journal *Nutrients*. Abstracts for this journal have a 200-word maximum, with the original article no longer than 5000 to 7500 words. Additional methods and results referred to in the manuscript can be found in the Appendices (A - I).

3.1 Title

A pilot study evaluating the effects of caffeine consumption on selected cognitive domains in older, post-menopausal women aged 55 to 79 years.

3.2 Abstract

The effect of low to moderate doses of caffeine consumption on cognitive performance has been well documented, particularly in younger adults. Results show caffeine improves attention and processing speed tasks but its effects on higher-level cognitive domains such as memory and executive function remain unclear. Since older, post-menopausal women experience age- and hormonal-related changes that may affect cognition, caffeine consumption may show a greater functional response in this cohort compared to younger adults. This study investigates the effect of acute caffeine consumption in older women, aged 55–79 years, on processing speed, attention, memory, and executive function. Twelve participants completed a placebo-controlled, double-blind, crossover study. Participants completed three repeated 30-minute cognitive assessment batteries (two weeks apart): baseline, 45 minutes post 100 mg of caffeine supplementation and 45-minutes post placebo. Compared to placebo, caffeine supplementation significantly improved movement time (p = 0.04) in a choice reaction time test. There were no significant differences between caffeine supplementation and placebo on performance in attention, memory, or executive function tests. Overall, results showed 100 mg of caffeine consumption only improved processing speed in older women. Further studies should be conducted on a larger population to corroborate the key finding.

Keywords: caffeine, older women, ageing, cognition, cognitive performance

3.3 Introduction

Caffeine is the world's most consumed psychoactive substance (reviewed in Reyes & Cornelis, 2018). It is perceived as a 'stimulant' and is widely known for its arousal effects by enhancing the activity of the CNS to promote alertness and alleviate sleepiness (reviewed in Cappelletti et al., 2015; McLellan et al., 2016).

McLellan et al. (2016) concluded that caffeine consumption (100 mg to 300 mg) significantly improves lower-order cognitive functions such as processing speed, perceptual-motor function, and complex attention (e.g., reaction time, response accuracy). However, results remain equivocal among studies investigating the effect of caffeine supplementation on higher-order cognitive functions such as learning and memory (e.g., episodic/working memory, recall) (Valladares et al., 2009), and executive function (e.g., strategic planning, decision making) (Ali et al., 2016; Konishi et al., 2018; Soar et al., 2016).

The number of older individuals (aged 65 years and above) in New Zealand is forecast to increase over the coming years, from 715,200 in 2018 to 1.36–1.51 million by 2048 (Statistics New Zealand, 2020). Therefore, it is crucial to identify strategies that might improve the quality of life of older individuals and reduce the social and economic burden on the health care system. Generally, over the age of 65, adults will start to experience physical, psychological, and cognitive age-related decline. A limited number of studies have reported that older adults may have a greater functional response to ergogenic aids such as caffeine to improve cognitive performance (Haskell-Ramsay et al., 2018; Waer et al., 2021). Caffeine is widely consumed among adults (Lieberman et al., 2019; Mitchell et al., 2014) for alleviating sleepiness and improving alertness (Robillard et al., 2015) or commonly, in a social setting. However, there is limited research on caffeine's acute functional effects on overall cognitive function among women in an older age cohort.

To date, the majority of research that studied the effects of caffeine consumption on cognitive performance has primarily focused on the younger population, between the ages of 18 to 41 years. Multiple caffeine studies that were conducted on the younger cohort have demonstrated that caffeine supplementation improves lower-order cognitive functions such as processing speed and complex attention (Attwood et al., 2007; Brunyé et al., 2010; Childs & Wit, 2006; Hasenfratz & Bättig, 1992; Haskell et al., 2005; Karayigit et al., 2020). However, many studies have failed to show any significant improvement post caffeine supplementation on memory and executive function tasks (Ali et al., 2016; Haskell-Ramsay et al., 2018; Haskell et al., 2005; Hindmarch et al., 1998; Valladares et al., 2009). One study by Konishi et al. (2008) had equivocal results finding significant effects of caffeine supplementation (200 mg) on only one of the two executive function tests used, the Shifting Attention Task (cognitive flexibility), but no effects observed for the Stroop Test (interference).

Caffeine studies conducted in older adults have typically examined the effects of chronic caffeine intake on attenuating cognitive decline or decreasing the risk of dementia and AD (McLellan et al., 2016). Only two studies with similar study design and population cohort to the current study examined the functional effects of acute caffeine supplementation on various aspects of cognition in older women (Haskell-Ramsay et al., 2018; Waer et al., 2021). Waer et al. (2021) studied 19 older women with low habitual caffeine intake and showed that 100 mg of caffeine supplementation improved reaction time in a simple reaction time (SRT) task, whereas no reduction was seen at the higher dose of 400mg. Haskell-Ramsey et al. (2018) recruited 59 healthy participants (where 16 of the 59 participants were older women), and showed post caffeine supplementation of 100 mg significantly improved digit vigilance accuracy and decreased reaction time in a rapid visual information processing (RVP) task. Although scores for learning and episodic memory tasks were lower in the older group than the younger cohort post caffeine ingestion, the study failed to show any significant interaction of treatment and age group on learning and memory tasks.

In summary, studies of the functional effects of caffeine on cognitive performance, largely conducted in younger adults, demonstrate low to moderate doses of caffeine supplementation improve specific cognitive processes such as processing speed and attention. However, there is limited research investigating the impact of acute caffeine ingestion on both lower- and higher-order cognitive functions in older adults, particularly post-menopausal women. Thus, this pilot study aimed to investigate the effect of caffeine consumption on various domains of cognitive functions such as processing speed, attention, memory, and executive function in post-menopausal women, aged 55 to 79 years.

3.4 Materials and Methods

Ethical approval for the study was obtained from Massey University Human Ethics Committee: Human Ethics Southern A Committee (SOA 21/05; Appendix A).

3.4.1 Participants

Healthy, post-menopausal women aged between 55 to 79 years, living in Auckland who regularly consume caffeine were recruited through poster advertisements (Appendix B). Interested participants were invited to complete a health screening questionnaire (Appendix C) to assess study eligibility. Participants were excluded if they had a menstrual cycle in the past two years or were on hormone replacement therapy. Additional exclusion criteria included previous adverse reactions to caffeine, or having health conditions such as anxiety, dementia or other psychiatric disorders, head trauma, sleep disorders, arthritis, dementia, visual/hearing impairment, or cardiovascular disease. Fourteen older women who met the inclusion criteria were recruited to participate in this pilot study. All participants were provided with an information sheet outlining the study procedure (Appendix D) and informed of the benefits and potential risks of the study, before providing written consent (Appendix E).

3.4.2 Intervention

Participants ingested a capsule (Vegie methylcellulose capsules, NZ Nutritionals, New Zealand) containing either 100 mg of pure caffeine powder or 100 mg of maltodextrin (placebo). The capsules were prepared by a pharmacist in the School of Sport, Exercise, and Nutrition Laboratory, Massey University (Auckland, NZ). The caffeine and placebo capsule bottles were uniquely labelled to ensure that the primary researcher and participants were blinded to the intervention received.

3.4.3 Study Design and Procedure

This study was an acute, double-blind, placebo-controlled, randomised within–subject crossover pilot study, where each participant served as their own control. Randomisation of participants to treatment order was completed using the Microsoft Randomiser program, by an independent party. Participants attended Massey University, Auckland (New Zealand) for three visits at two-week intervals. Participants were asked to abstain from consuming any caffeinated food or beverages (e.g., coffee, tea, chocolate) for at least 24 hours prior to each visit, which took place at the same time of day.

During the first visit baseline cognitive testing was conducted. Participants were provided with a glass of water (no intervention), required to complete a demographic questionnaire (Appendix F), and asked to sit quietly for a 45-minute waiting period, and then complete a 30-minute cognitive assessment, which included a range/selection of tests to assess different domains on an iPad. The second and third visits were the intervention trials where participants consumed one capsule (either caffeine or placebo) with water, followed by a 45-minute waiting period, before completing the same 30-minute cognitive assessment (Appendix G illustrates the detailed study protocol for all three visits and testing sessions).

3.5 Cognitive Measurements

The Cambridge Neuropsychological Test Automated Battery (CANTAB) is a selection of computerised neuropsychological tests validated for use for both cognitively normal and impaired adults (Fray et al., 1996), with demonstrated test-retest reliability (Karlsen et al., 2020). CANTAB tests are conducted on an iPad touchscreen but are based on traditional neuropsychological tests. The CANTAB tasks are graded in nature allowing for extended levels of ability while avoiding ceiling effects in normal healthy subjects (Fray et al., 1996). Cognitive assessments were conducted in a standardised, quiet room, using an iPad mounted on a desk at eye level. For each task, standardised audio instructions were given, and several practice trials were provided to familiarise participants with each cognitive assessment. The 30-minute cognitive assessment was comprised of four, extended difficulty level cognitive tasks with randomised stimuli: five-choice reaction time (RTI), rapid visual information processing (RVP), paired-associate learning (PAL), and spatial working memory (SWM). These tests have been used to assess processing speed, attention, memory, and executive function, respectively. In addition to the test descriptions below, Appendix H provides a summary of the outcome variables measured in each test.

3.5.1 Reaction Time (RTI)

The RTI task is a test of processing speed and requires participants to react to a yellow spot appearing briefly inside one of five circles at the top of the screen, by letting go of the central button and quickly selecting the circle where the yellow spot appeared. The RTI task provides measures of reaction time and movement time.

3.5.2 Rapid Visual Information Processing (RVP)

The RVP task is a test of attention and requires participants to follow a chain of single numbers from 2 to 9, which appear briefly in the centre of the screen in a pseudo-random order at the rate of 100 digits per minute. Participants are required to correctly identify the target sequences (e.g., 2–4–6, 4–6–8, or 3–5–7) as quickly as possible by touching the central button of the screen after the last digit of a target sequence. The RVP task provides measures of sensitivity to target sequences, total correct responses, and probability of false alarms.

3.5.3 Paired Associate Learning (PAL)

The PAL task is a test of memory/learning and requires participants to focus on each of several boxes displayed on the screen as each reveals a different pattern, which emerge randomly one at a time. The aim of this task is for participants to remember the location of each pattern when an identical pattern is shown at the centre of the screen. If a wrong box is chosen, all boxes will open again to remind the participant of each pattern location. The PAL task provides measures of first attempt correct memory score, errors to success, total attempts, and total errors.

3.5.4 Spatial Working Memory (SWM)

The SWM task is a test of executive function (planning/strategy) and requires participants to search for one token at a time in each of several boxes. All tokens found are required to be collected to fill up the empty side bar to complete the task. The key task instruction is that the system will never hide another token in the same box. Hence, once a token is found in a box, the subject should not revisit the same box to look for another token. The SWM measures number of errors and calculates a strategy score.

3.6 Statistical Analysis

All statistical analyses were completed using IBM SPSS (Version 26.0, BM, New York, USA). The variables were tested for normality using the Kolmogorov–Smirnov, Shapiro–Wilk tests and normality plots. Normally distributed data was expressed as mean ± SD. Repeated measure analysis of variance was conducted with treatment and time as within-subject factors, and treatment order as the between-subject factor, and inclusion of the interaction between these two was performed to assess the effect of treatment order on treatment effect. The assumptions for repeated measure analysis (including normality and sphericity) were tested. Significance level was set at $p<0.05$. Sphericity was tested using the Mauchly's test, and in the case of violation, the Greenhouse-Geisser test was used to adjust for lack of sphericity (none). Where significant effect of treatment was found, post-hoc analyses with Holm-Bonferroni correction were undertaken to adjust for multiple comparisons. A partial eta-squared (ηp^2) of 0.01, 0.06, and 0.14 was considered small, medium, and large effect size, respectively.

3.7 Results

3.7.1 Participants

Forty participants expressed interest in the study. Of these 24 were screened and 16 met the eligibility criteria (Section 3.4.1) and were invited to participate in the study. Two participants withdrew after familiarisation visit (first testing session) and two more withdrew before completing their final visit due to a COVID-19 lockdown (refer to Appendix I for a detailed participant flowchart). Twelve participants completed all three visits (mean age ± SD = 63.75 ± 6.81 years, age range = 56 – 75 years). One participant failed to complete one RTI task, thus all three RTI outcomes from this participant were excluded from the results. Socio-demographic characteristics of the participants are summarized in Table 5.

Table 5: Socio–demographic characteristics of recruited participants (N=12)

Socio-Demographic Parameters	Frequency (N)	Percentage (%)
Age (years)		
55 - 59	4	33
69 - 69	4	33
≥ 70	4	33
Education Level		
Secondary Level	2	17
Diploma/Trade Level	3	25
University Level	7	58
Occupation Status		
Employed (e.g., professional services/manager/community worker/technician/labourer)	12	100
Unemployed/retired	0	0
Ethnicity		
European	8	66
Māori/New Zealand European	2	17
Asian	2	17

3.7.2 Reaction Time (RTI)

The outcome for RTI tasks showed a significant effect of treatment*time for mean movement time (p = 0.04, η^2_p = 0.559, Table 6). Pairwise comparisons showed that caffeine consumption significantly improved movement time from baseline to post-supplementation compared to placebo. There was no interaction with treatment order (p = 0.228, $\eta 2_p$ = 0.309).

There was no significant effect of treatment*time for reaction time (p = 0.20, η^2_p = 0.309).

Table 6: Results for RTI cognitive tasks for each trial visit

Cognitive outcomes	Visits			*p*-value	
	Baseline	Post caffeine	Post placebo	Treatment effect	Treatment order
Five-Choice Reaction Time					
Reaction time	398.1 ± 52.1	387.5 ± 53.8	381.6 ± 50.8	0.20	0.13
Movement time	315.8 ± 117.3	272.6 ± 82.2[a]	275.9 ± 83.0	0.04*	0.23

All data are reported as mean ± SD. Reaction and movement time were measured in ms. *Significant effect repeated measures analysis ($p < 0.05$). [a] Post caffeine significant difference from baseline post hoc analysis ($p < 0.05$).

3.7.3 Rapid Visual Information Processing (RVP)

The RVP A sensitivity score showed a treatment*time effect ($p = 0.04$, $\eta^2_p = 0.518$, Table 7), but pairwise comparisons showed that the sensitivity score improved from baseline to post-supplementation for both caffeine and placebo.

Similarly, there was a treatment*time effect for RVP correct responses ($p = 0.02$, $\eta^2_p = 0.600$), but post–hoc analysis showed improved correct responses from baseline to post supplementation for both caffeine and placebo treatments. There was no effect of treatment*time for probability of false alarms ($p = 0.24$) and no interactions with treatment order for any of the RVP cognitive tasks.

Table 7: Results for RVP cognitive tasks for each trial visit

Cognitive outcomes	Visits			*p*-value	
	Baseline	Post caffeine	Post placebo	Treatment effect	Treatment order
Rapid Visual Information Processing					
RVP A[1]	0.90 ± 0.07	0.94 ± 0.04[a]	0.93 ± 0.06[b]	0.04*	0.96
Probability false alarms	0.02 ± 0.04	0.01 ± 0.02	0.03 ± 0.08	0.24	0.50
Correct Responses[2]	34.8 ± 10.7	42.1 ± 7.76[a]	41.5 ± 9.58[b]	0.02*	0.69

All data reported as mean ± SD. *Significant effect repeated measures analysis ($p < 0.05$). [1] Sensitivity score (0-1). [2] Correct responses score (0 – 54). [a] Post caffeine significant difference from baseline post hoc analysis ($p < 0.05$). [b] Post placebo significant difference from baseline post hoc analysis ($p < 0.05$).

3.7.4 Paired Associate Learning (PAL)

Table 8 displays the outcomes of PAL tasks at baseline, post caffeine and post placebo consumption. There was no effect of treatment*time for errors to success ($p = 0.46$, $\eta^2_p = 0.158$), and total attempts ($p = 0.17$, $\eta^2_p = 0.324$).

There was a trend of improved first attempt memory score over time (p = 0.08, η^2_p = 0.222) and reduced total errors over time (p = 0.08, η^2_p = 0.532), for both caffeine and placebo supplementation.

There was no significant interaction with treatment order on any PAL measure ($p > 0.05$).

Table 8: Results for PAL cognitive tasks for each trial visit

Cognitive outcomes	Visits			*p*-value	
	Baseline	Post caffeine	Post placebo	Treatment effect	Treatment order
Paired Associates Learning					
First Attempt Memory[1]	12.0 ± 3.93	13.9 ± 3.89	13.1 ± 2.84	0.08	0.22
Errors to Success	2.42 ± 1.56	1.58 ± 1.78	2.08 ± 1.08	0.46	0.06
Total Attempts	7.92 ± 1.68	7.08 ± 1.38	7.08 ± 1.44	0.17	0.24
Total Errors	14.0 ± 7.19	11.3 ± 8.55	9.67 ± 4.25	0.08	0.53

All data reported as mean ± SD. [1] First attempt to memory score (0-24).

3.7.5 Spatial Working Memory (SWM)

There was a significant treatment*time effect for strategy score (p = 0.031, η^2_p = 0.536) (Table 9), but post-hoc analysis indicated that there was significant improvement over time for both caffeine and placebo interventions. There was a trend for decreased errors over time for both caffeine and placebo treatments (p = 0.08, η^2_p = 0.424). There were no interaction effects with treatment order on the two SWM measures (p = > 0.05).

Table 9: Results for SWM cognitive tasks for each trial visit

Cognitive outcomes	Visits			*p*-value	
	Baseline	Post caffeine	Post placebo	Treatment effect	Treatment order
Spatial Working Memory					
Errors	15.4 ± 9.5	7.58 ± 9.19	8.83 ± 9.23	0.08	0.91
Strategy score	9.17 ± 1.99	6.42 ± 3.09[a]	6.67 ± 3.8[b]	0.03*	0.94

All data reported as mean ± SD. *Significant effect repeated measures analysis ($p < 0.05$). [a] Post caffeine significant difference from baseline post hoc analysis ($p < 0.05$). [b] Post placebo significant difference from baseline post hoc analysis ($p < 0.05$).

3.8 Discussion

3.8.1 Purpose of the Study

The present research sought to examine the effects of acute caffeine consumption on various cognitive domains, specifically processing speed, attention, memory, and executive function in older, post-menopausal women.

3.8.2 The Effect of Caffeine on Processing Speed

In the current study, we found 100 mg of caffeine supplementation significantly improved processing speed, resulting in reduced movement time in a choice reaction time task (p = 0.04). This outcome was predicted as caffeine promotes alertness through its effects as an adenosine antagonist (Penetar, 1994). The result is consistent and largely in line with other studies of younger adults (Attwood et al., 2007; Haskell et al., 2005; Karayigit et al., 2020), that demonstrate approximately 75 mg to 400 mg of caffeine supplementation significantly improves processing speed compared to placebo, using simple and/or choice reaction time tasks. These previous studies applied 12 to 24 hours of caffeine abstention duration, which is similar with the current study in which participants refrained from any caffeine consumption for 24 hours prior to cognitive testing. In addition, our finding was aligned with one study that had a similar older population group. Waer et al. (2021) showed 100 mg of caffeine supplementation improved reaction time in a SRT task (p = 0.01), but no significant effect was observed for 400 mg of caffeine supplementation. Perhaps in older adults a higher caffeine dose may not further enhance processing speed.

Using CANTAB computerised testing, we were able to differentiate reaction time (time taken to release the response button after presentation of stimulus) and movement time (time taken to release the response button and select the target stimulus) in RTI measures of processing speed. Most of the previous caffeine studies discussed only refer to 'reaction time' without clarification of whether time is measured after presentation of the stimulus or after release of the start button. Only one study that used CANTAB and measured movement time was Attwood et al. (2007). This study indicated that 400 mg of caffeine supplementation did improve SRT and CRT reaction time but not movement time in 45 younger adults aged 18 to 41 years. Therefore, further studies utilising computerised cognitive testing which measure movement time in addition to reaction time are required and may allow corroboration with our findings.

3.8.3 The Effect of Caffeine on Sustained Attention

In the current study, acute caffeine consumption of 100 mg did not significantly improve sustained attention compared to placebo. Both caffeine and placebo interventions resulted in improvements over time for the attention sensitivity score and correct responses in the RVP task. Our findings differed from other studies using acute moderate caffeine doses (e.g., 75 mg to 250 mg) that showed significant improvements in attention related tasks for younger adults (Childs & Wit, 2006; Hasenfratz & Bättig, 1992; Haskell et al., 2005; Rosenthal et al., 1991). One study by Haskell-Ramsay et al. (2018) that is similar to our study in its older population cohort and 100mg caffeine dose reported improvements in digit vigilance accuracy and reaction time, and RVP reaction time for caffeine supplementation compared to placebo. Additionally, studies that utilised a higher dose of caffeine supplementation (e.g., 400 mg) in younger adults observed improved performance on vigilance and attention related tasks (Brunyé et al., 2010; Childs & Wit, 2006). However, it is important to note that previous studies utilise a wide range of cognitive tests (e.g., RVP, digit vigilance/span, sequential operation series, Stroop tasks) to assess different aspects of attention such as complex attention, sustained attention, selective/divided attention, processing speed, attention span, focussed attention and/or vigilance. Variability in results among studies is likely given the use of different tests (and measures) which vary in task instructions and cognitive demands which may be differentially affected by caffeine.

Based on our findings, it is suggested that practice effects may also have occurred, which is common for repeated cognitive testing (Bartels et al., 2010). The interval between repeated visits of 14 days in our study was longer than previous caffeine and cognition crossover studies reviewed, in which the repeated testing period varied from 24 hours to 14 days. One limitation of short, repeated testing periods is that high frequency cognitive testing increases the risk of practice effects in healthy participants, regardless of age (Bartels et al., 2010), which may adversely impact study outcomes. An extended period of three months or more between each cognitive testing session has been shown to reduce the risk of practice effects (Bartels et al., 2010) and perhaps, is required to remove these confounding effects and capture the impact of caffeine's functional effect on attention related cognitive tasks in older women.

3.8.4 The Effect of Caffeine on Memory and Executive Function

The PAL memory tasks showed no significant effects post caffeine consumption compared to placebo. Although there were trends observed for both caffeine and placebo treatments such as improved first attempt memory score and reduced errors over time, these improvements were likely due to practice effects. The reported findings for PAL measures are in accordance with several studies (Attwood et al., 2007; Childs & Wit, 2006; Haskell-Ramsay et al., 2018; Hindmarch et al., 1998; Valladares et al., 2009), that demonstrated low to high doses of caffeine (75 mg to 400 mg) had no significant improvements on memory tasks compared to placebo. One study by Haskell et al. (2005) showed that 150 mg of caffeine consumption in younger adults decreased reaction time in a numeric working memory test, but no significant improvements were seen for the spatial memory task (Haskell et al., 2005). Many of these studies typically measure learning, episodic and working memory functions by using a broad range of spatial and verbal memory tests. Therefore, results of caffeine effects may differ depending on the tests utilised, and whether the task is timed or non-timed.

Similarly, no significant effect was observed for executive function post caffeine consumption compared to placebo. We found an improvement in strategic scoring for the SWM task over time for both caffeine and placebo treatments. Relatively limited studies have investigated the functional effects of caffeine on higher level executive function and our findings were consistent with these studies showing non-significant or equivocal results (Ali et al., 2016; Haskell et al., 2005), suggesting executive functioning may not be as susceptible to caffeine as lower-order processes (Konishi et al., 2018). Since there are multiple functions considered "executive" in nature, prior research has utilised a range of cognitive software or tests such as Attention Network Test, Jansari Assessment of Executive Function, Stroop Task, and Shifting Attention to analyse changes. Some of these tests have a time constraint, which may be affected by processing speed, particularly in older cohorts where processing speed may be slower.

The current study utilised a non-timed, extended level task of executive function to measure strategy for locating tokens in hidden boxes, which was thought to be a suitable test for normal healthy adults not confounded by speed. However, if participants actively try to recall the strategy used in baseline testing and apply that knowledge to repeated testing, practice effects may have occurred.

Interestingly, one study observed a significant impact from 50 mg of caffeine supplementation on different aspects of executive function such as planning, creative thinking, and prospective memory in 43 young adults, using a non-timed constraint JEF cognitive software test (Soar et al., 2016). The study only required a two-hour caffeine abstention prior to testing, which differed from most caffeine studies, which commonly utilise a longer abstinence period due to caffeine's half-life of three and half hours to ten hours.

Based on all the outcomes discussed, the effects of caffeine on various aspects of executive functioning remains equivocal. Further research is required to compare the effect of different doses of caffeine on executive function using a variety of tests that measure different aspects of executive function.

3.9 Conclusion

The main finding from the present pilot study indicated that acute consumption of 100 mg caffeine (equivalent to one cup of a plunger/drip coffee, close to one shot of espresso) significantly improved processing speed (movement time) in older women but failed to improve other cognitive functions including sustained attention, memory, and executive function. Further research is required in a larger older adult population to give greater statistical power and using an extended period between repeated cognitive testing sessions to reduce practice effects. This may allow the functional effects of caffeine on overall cognition to be shown in an older cohort.

Chapter 4: Conclusion

4.1 Summary of Main Findings

The current pilot study aimed to investigate the effects of caffeine consumption on various cognitive functions in older, post-menopausal women aged 55 to 79 years, and determined that acute caffeine supplementation improved processing speed (movement time) compared to placebo. This thesis was able to replicate outcomes found in similar caffeine studies in older participants (Haskell-Ramsay et al., 2018; Waer et al., 2021), where acute consumption with 100 mg of caffeine significantly improved processing speed. Although some memory and executive-function related tasks showed improvement over time, there were no statistically significant differences between caffeine supplementation and placebo. In conclusion, acute ingestion of 100 mg caffeine in twelve older women improved processing speed but did not lead to improvement in other cognitive functions, including sustained attention, memory, and executive function, as measured using selected CANTAB tests.

4.2 Strengths

To our knowledge, this pilot study is unique in its examination of the effects of acute ingestion of 100 mg caffeine on a range of lower- to high-order cognitive functions, in normal healthy post-menopausal women. Previous caffeine research has typically studied the impact of caffeine consumption on cognitive performance in younger populations. However, older women experience age- (reviewed by Murman, 2015) and hormonal-related (Weber et al., 2014) changes that may negatively affect cognitive function. Hence, this population group may be more likely to experience greater functional improvements in cognitive testing following caffeine supplementation in comparison to a younger population who may demonstrate maximal baseline cognitive performance. In addition, this research solely focuses on healthy older, post-menopausal women (not taking hormone replacement therapy), and not peri-menopausal women or older men, thereby reducing any variation due to hormones (e.g., oestrogen) and sex that may contribute to inter-individual differences in cognitive performance.

This research studied the effect of acute caffeine consumption on multiple cognitive functions including lower- and higher-order cognitive processes such as processing speed, attention, memory, and executive function. Few previous studies have analysed this wide range of cognitive functions, measuring performance on both speeded and non-time constrained tasks, to seek to identify which specific functions may be enhanced by caffeine supplementation.

Strengths of this study also included the study design and cognitive testing software utilised. This is a randomised, double-blind, crossover pilot study in which participants served as their own control. In addition, CANTAB is a computerised cognitive assessment tool with previously demonstrated reliability and validity (Fray et al., 1996). A wide range of cognitive assessments were utilised, including timed and untimed tasks, to reduce confounding effects of slowed information processing speed in older adults. Participants were presented with randomised stimuli in each test, to reduce practice effects that tend to occur with repeated testing. The tests chosen included extended levels of difficulty to reduce ceiling effects that are sometimes demonstrated in healthy cognitively normal participants. The stimuli were based on numbers and symbols, to reduce cultural bias. All cognitive assessments were conducted on an iPad which participants were familiar with using.

4.3 Limitations

This study has several limitations that may have affected the study's findings. These include the small sample size (n= 12), limited ethnic and demographic diversity, and the short wash-out period between repeat testing.

The small sample likely meant the study was underpowered to detect significant differences between caffeine and placebo. Therefore, future studies should utilise greater participant numbers.

The cohort was not as ethnically diverse as New Zealand's population. In terms of demographics, the participants were active, independent, employed and the majority held a tertiary qualification. These factors may have influenced their cognitive function in comparison to the wider population of older women who include retirees. Therefore, future studies are encouraged to explore various recruitment strategies to employ a broader demographic study sample.

The short 14–day washout period between repeat testing sessions led to practice effects which are common in cognitive testing studies. Therefore, future trials should increase the interval of the washout period to reduce the risk of practice effects.

4.4 Use of the Findings

The present study may guide the design of future larger studies investigating the effects of caffeine on cognitive function in an older cohort.

4.5 Future Directions

- Future studies should be conducted using a larger sample size which will provide greater statistical power to detect any significant differences between caffeine supplementation and placebo on cognition.
- The effects of different doses of caffeine on multiple cognitive processes in post-menopausal women should be determined using a randomised controlled, double-blind, crossover study. This will enable researchers to investigate the dose-response effects of caffeine on cognitive function.
- The older New Zealand population is ethnically and socio-demographically diverse. A wider range of recruitment procedures applied to recruit a larger and broader sample (e.g., wider age groups with different employment status, occupation, ethnic subgroups, and education level) will provide a better representation of the population of older women in New Zealand.
- Future trials investigating caffeine supplementation could utilise tests incorporating language and verbal responses, in addition to visuo-spatial tasks to test a wider range of functions in post-menopausal women.
- Future studies could compare the effects of acute caffeine supplementation on various cognitive functions in naïve, low, and high habitual caffeine consumers.

- Similar studies to those outlined above could also be carried out in healthy older men to determine if similar effects are observed between older men and women.

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Appendices

Appendix A: Massey University Human Ethics Committee Approval Letter SOA 21/05

HoU Review Group

Reviewer Group

A/Pro Kay Rutherfurd

Researcher: Dr Judy Thomas

Title: Evaluating the effects of caffeine consumption on different cognitive domains in older women – a pilot study

Dear Judith

Thank you for the above application that was considered by the Massey University Human Ethics Committee: Human Ethics Southern A Committee at their meeting held on 05/05/2021.

On behalf of the Committee, I am pleased to advise you that the ethics of your application are approved.

Approval is for three years. If this project has not been completed within three years from the date of this letter, reapproval must be requested.

If the nature, content, location, procedures, or personnel of your approved application change, please advise the Secretary of the Committee.

If you wish to print an official copy of this letter, please logon to RIMS (http://rims.massey.ac.nz), and under the Reporting section, View Reports you will find a link to run the Ethics Committee Report.

Yours sincerely

Professor Craig Johnson

Chair, Human Ethics Chairs' Committee and Director (Research Ethics)

Appendix B: Poster Advertisement

Appendix C: Participant Health Screening Questionnaire

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

COLLEGE OF HEALTH
TE KURA HAUORA TANGATA

Participant Screening Questionnaire

Project Title: Evaluating the effects of caffeine consumption on different cognitive domains in older women – a pilot study

Name:	
Gender:	**☐M ☐F ☐Non-binary ☐Prefer not to answer**
Date of Birth:	
Address:	
Phone:	
Email:	

Please read the following questions carefully. If you have any difficulty, please advise the researcher who is conducting your tests.

Please answer all the following questions by ticking the correct box.

The information provided by you on this form will be treated with the strictest confidentiality.

Q1. Have you had a menstrual period in the last 2 years?

☐YES

☐NO

Q2. Are you currently taking hormone replacement therapy (HRT)?

☐YES, I am currently on HRT

☐YES, I have taken HRT but do not currently

☐NO, I do not and have never taken HRT

Q3. Have you ever been diagnosed with or are taking medications for any of the following conditions? Answer yes by ticking the box and then, please provide more detail on your condition and what medications you are taking.

☐Heart disease: Click or tap here to enter text.

☐High blood pressure: Click or tap here to enter text.

☐Arrythmias/heart palpitations/high heart rate: Click or tap here to enter text.

☐High blood cholesterol: Click or tap here to enter text.

☐Anxiety: Click or tap here to enter text.

☐Previous head trauma: Click or tap here to enter text.

☐Dementia/Alzheimer's disease: Click or tap here to enter text.

☐Other brain/psychiatric conditions: Click or tap here to enter text.

☐Arthritis: Click or tap here to enter text.

☐Sleep disorders: Click or tap here to enter text.

☐Visual/hearing impairment: Click or tap here to enter text.

Q4. How often do you drink tea? Click or tap here to enter text. **(# times per day) OR**

Click or tap here to enter text. **(# times per week)**

Q5. How often do you drink coffee? Click or tap here to enter text.**(# times per day) OR**

Click or tap here to enter text. **(# times per week)**

Q6. What type of tea or coffee do you most often consume? (Espresso, decaf, earl grey, etc) Click or tap here to enter text.

Q7. How often do you consume energy drinks (e.g. Red Bull, Monster)?

Click or tap here to enter text. **(# times per day) OR**

Click or tap here to enter text. **(# times per week)**

Q8. How often do you consume soft drinks? (e.g. Coca-cola, pepsi)

Click or tap here to enter text. **(# times per day) OR**

Click or tap here to enter text. **(# times per week)**

Q9. Describe how caffeinated products normally affect you.

Click or tap here to enter text.

Q10. Have you ever had any adverse reactions from consuming caffeine?

☐Yes If Yes, what were the reactions? Click or tap here to enter text.

☐No

Q11. Which electronic or mobile devices do you regularly use? Click or tap here to enter text.

Thank you for completing the screening questionnaire.

☐Tick the box to indicate you have read, understood, and completed this questionnaire yourself.

Appendix D: Participant Information Sheet

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

COLLEGE OF HEALTH
TE KURA HAUORA TANGATA

INFORMATION SHEET

Evaluating the effects of caffeine consumption on different cognitive domains in older women – a pilot study

Invitation to Participate in the Research Study

My name is Neelam Khindria, and I am a postgraduate student undertaking a thesis project to complete a Master of Science degree in Nutrition and Dietetics at Massey University. I am under the supervision of Dr Judy Thomas, Associate Professor Kay Rutherfurd-Markwick and Dr Cheryl Gammon from the School of Health Sciences at Massey University.

Project Description and Invitation

Caffeine is the most consumed psychoactive substance in the world. It can be consumed via coffee, teas, energy drinks and some foods such as chocolate. Caffeine is typically known for its effects on the brain to increase alertness and reduce fatigue. Recent studies have demonstrated that caffeine may also lead to enhanced performance on other cognitive/mental tasks, but the results are unclear regarding which cognitive functions are most sensitive to caffeine's effects. Much of this previous research has been conducted with young adult populations. New Zealand's older-aged population is forecast to double over the next 20 years and finding ways to improve the physical and brain/cognitive health of this population will have important social implications. As well, older individuals who may be experiencing normal age-related cognitive changes, may show greater sensitivity to the cognitive enhancing effects of caffeine. By the inclusion of only women (aged 55-79) who are post-menopausal, variability due to sex hormone fluctuations on cognition will be limited. A number of cognitive functions will be tested in response to acute ingestion of a caffeine supplement versus placebo including: reaction time, attention, memory and higher executive functions such as strategy and planning. This is a pilot study and therefore the knowledge gained from this study may be used to inform other similar studies with more participants.

Participant Identification and Recruitment

Approximately 15-20 participants will be recruited for this study.

To participate in this study, you must be:

- Female
- Between the ages of 55-79 years old
- Have not had a menstrual period in the last 2 years
- Able to consume caffeine (if you don't consume any caffeine or have had any adverse reactions to caffeine in the past, you cannot take part in this study)

Risks/discomforts as a result of this study may include:

- Feeling a bit tired, having difficulty concentrating and possibly a headache due to not consuming caffeine for 24 hours prior to each visit
- Feeling a bit nervous about completing cognitive tests
- Feeling a bit jittery following caffeine consumption (caffeine dose is 100mg – equivalent to one cup of brewed/plunger coffee)

Before commencing this study, you will be asked to complete a health screening questionnaire and a participant demographic questionnaire. If you have a medical condition or are taking a medication that may affect cognitive testing, you may be excluded from the study. All information collected on these questionnaires is strictly confidential and will only be used for the purpose of this study.

As acknowledgement of your time and possible expenses if travelling to Massey University, you will be provided with a $30 petrol (MTA) or Westfield voucher upon completion of the study.

Te Kunenga ki Pūrehuroa
Massey University – School of Health Sciences, College of Health
Private Bag 102904, North Shore, Auckland 0745, New Zealand **T** +64 9 213 6653 www.massey.ac.nz

Project Procedures

First Visit - Familiarisation

Before the main trials for this pilot study begin you will be asked to come into the research unit at Massey University (Albany campus) to undergo familiarisation and baseline cognitive testing. You will be required to not consume any caffeine-containing beverages or foods for 24 hours prior to this visit. You will also be asked to consume a light meal two hours prior to your visit. When you arrive, you will be asked some basic demographic questions and shown how to complete the cognitive tests on an iPad so you know what to expect.

Forty-five minutes after arrival, you will complete the cognitive tests in a separate quiet testing room without distractions. The cognitive tests take approximately thirty minutes. The cognitive tests are not pass/fail and are not being used for diagnostic purposes. Your performance on these tests will only be used to compare against your own performance following caffeine or placebo capsules to determine if there is a differential effect on the cognitive functions tested. The cognitive functions tested include: reaction time, attention, memory and higher executive functions such as strategy and planning.

The familiarisation visit will take approximately 90 minutes in total.

Second and Third Visits - Main Trials

The main trials will consist of 2 further visits to the Massey University research unit, each requiring approximately 80 minutes of your time. These trials will occur at a similar time of day to your baseline visit. You will be required to not consume any caffeine-containing beverages or foods for 24 hours prior to each visit. You will also be asked to consume a similar light meal to the first visit, two hours prior to your visit. When you arrive at the research unit, in one trial you will be given a caffeine capsule (100mg – similar dose to a cup of coffee) and in the other trial you will be given a placebo capsule (maltodextrin) to take with a glass of water. You will not be told which capsule you are receiving. Following capsule ingestion, you will be required to sit for 45 minutes, during which time you may read or do another quiet activity, before you are taken into the testing suite to complete the 30 minute cognitive testing protocol.

Visits will be scheduled a minimum of 2 weeks apart.

If you feel a bit jittery after taking the capsule which may contain caffeine and are worried about this, please discuss your concerns with the researcher present.

Some people feel a bit nervous about cognitive testing, this is normal. You will be given the opportunity to ask any questions about the study at any time. You may bring a whānau member or support person to each visit (but not into the cognitive testing room to ensure a standardised testing environment). These tests will **not** be used to diagnose any neurocognitive disorders. Some of the tasks will be easy, whilst others are designed to be challenging. However, if you have any concerns about the cognitive tasks conducted you should discuss this in the first instance with your primary health care practitioner. Other support and information may be found through the following organisations:

Brain Research NZ - http://www.brnz.ac.nz/community/community-groups
Neurological Foundation - https://neurological.org.nz/what-we-do/
Mental Health Foundation of New Zealand - https://mentalhealth.org.nz/our-work/resource-and-information-service
Samaritans Aotearoa New Zealand - https://www.samaritans.org.nz/

Data Management

All data collected will be used only for this study.

No names will be used when reporting data. Participants will be identified only by a unique study identification code and all data forms will use this code.

All hard copies of data forms will be stored in a locked filing cabinet only accessible to the researchers at Albany Campus, Massey University in a restricted access building. The electronic data from cognitive testing will be identified by unique identification code only (not your name) and will be stored on computers, which are protected by passwords and accessible only to the researchers. The project data will be stored as outlined above for 5 years.

Individual cognitive test results will not be provided, but a summary of the general outcomes of the study will be sent to you. Outcomes from this project may be published in scientific journals or presented at relevant conferences. These outcomes will be summary data only and not identify individual participant data. As this is a pilot study the knowledge obtained may also contribute to the design of future studies utilizing larger sample sizes.

Participant's Rights

You are under no obligation to accept this invitation. If you decide to participate, you have the right to:

- decline to answer any particular question;
- withdraw from the study up until two weeks following data collection;
- ask any questions about the study at any time during participation;
- provide information on the understanding that your name will not be used unless you give permission to the researcher;
- be given access to a summary of the project findings when it is concluded.

Project Contacts

Ms Neelam Khindria (School of Sport, Exercise and Nutrition, Massey University); email n.khindria@massey.ac.nz

Dr Judy Thomas (School of Health Sciences, Massey University); phone (09) 2136665; email j.thomas1@massey.ac.nz

Associate Professor Kay Rutherfurd-Markwick (School of Health Sciences, Massey University), email k.j.rutherfurd@massey.ac.nz

Dr Cheryl Gammon (School of Health Sciences, Massey University), email c.gammon@massey.ac.nz

- If you have any questions about the study, please contact any one of the above researchers

Committee Approval Statements

This project has been reviewed and approved by the Massey University Human Ethics Committee: Southern A, Application 21/05. If you have any concerns about the conduct of this research, please contact Dr Negar Partow, Chair, Massey University Human Ethics Committee: Southern A, telephone 04 801 5799 x 63363, email humanethicsoutha@massey.ac.nz.

Compensation for Injury

If physical injury results from your participation in this study, you should visit a treatment provider to make a claim to ACC as soon as possible. ACC cover and entitlements are not automatic and your claim will be assessed by ACC in accordance with the Accident Compensation Act 2001. If your claim is accepted, ACC must inform you of your entitlements, and must help you access those entitlements. Entitlements may include, but not be limited to, treatment costs, travel costs for rehabilitation, loss of earnings, and/or lump sum for permanent impairment. Compensation for mental trauma may also be included, but only if this is incurred as a result of physical injury.

If your ACC claim is not accepted you should immediately contact the researcher. The researcher will initiate processes to ensure you receive compensation equivalent to that to which you would have been entitled had ACC accepted your claim.

Thank you for considering participating in this study.

Appendix E: Participant Consent Form

MASSEY UNIVERSITY
COLLEGE OF HEALTH
TE KURA HAUORA TANGATA

Evaluating the effects of caffeine consumption on different cognitive domains in older women – a pilot study

CONSENT FORM FOR STUDY VOLUNTEERS

This consent form will be held for a minimum period of five (5) years

I have read the Information Sheet and have had the details of the study explained to me. My questions have been answered to my satisfaction, and I understand that I may ask further questions at any time.

I have been given sufficient time to consider whether to participate in this study and I understand participation is voluntary.

I understand that I have the right to withdraw from the study at any time and to decline to answer any particular questions.

I agree to provide information to the researcher on the understanding that my name will not be used without my permission. (The information will be used only for this research and publications arising from this research project will contain group summary data only.)

I agree to participate in this study under the conditions set out in the Information Sheet.

Signature: ____ **Date**__________________

Full Name (printed) ____

Phone Number_____________________ **Age** ______________ **Date of Birth** ________

Participant code ☐

Appendix F: Participant Demographic Questionnaire

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

COLLEGE OF HEALTH
TE KURA HAUORA TANGATA

Study Identification Number

Evaluating the effects of caffeine consumption on different cognitive domains in older women – a pilot study

Participant questionnaire

Please complete the following form. All the information you give us is in confidence and will be used only for the purposes of this study. If you need any help to complete the form, please ask one of the research team.

Which ethnic group(s) do you belong to? Tick whichever applies to you (you may tick more than one box)

☐ European
☐ Māori
☐ Pacific Peoples
☐ Asian
☐ Indian
☐ Middle Eastern/Latin American/African
☐ Other (*Please state which other ethnicity or ethnicities you belong to)*

____

What is your first language? ________________________________

What is your highest educational level (choose one)?

☐ No qualifications

☐ Primary school

☐ Secondary school

☐ Post-secondary certificate, diploma, or trade diploma

☐ University degree

What is your current living arrangement?

☐ Living alone

☐ Living with others

If living with others, how many others do you live with and what is their relationship to you (e.g. Husband, wife, partner, son, daughter, grandson, granddaughter, flatmate, boarder, etc)

____

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COLLEGE OF HEALTH
TE KURA HAUORA TANGATA

Study Identification Number

Do you live in a retirement village or community?

☐ Yes

☐ No

Which of the following best describes your current work situation? (please tick as many as apply)

☐ Paid employment

Occupation and number of hours of paid employment per week?

____

☐ Volunteer work

Position and number of volunteer hours per week?

____

☐ Fully retired

☐ Semi-retired

☐ Other (e.g. caregiver, studying, homemaker), please describe

____

During your working life, what is/was your main occupation?

☐ Labourer (e.g. Cleaner, food packer, farm worker)

☐ Machinery operator/driver (e.g. Machine operator, store person)

☐ Sales worker (e.g. Insurance agent, sales assistant, cashier)

☐ Community or personal service worker (e.g. Teacher aide, armed forces, hospitality worker, care)

☐ Technician/trades worker (e.g. Engineer, carpenter, hairdresser)

☐ Professional (e.g. Accountant, doctor, nurse, teacher)

☐ Manager (e.g. General manager, farm manager)

☐ Other *(Please Specify)* ______________________________

This is the end of the questionnaire, thank you for participating.

Appendix G: A Detailed Study Procedure Flowchart

Research study advertised in newsletters (e.g., Massey University Research), social platforms (e.g., Neighbourly), and local cafés and supermarkets. Participants that met eligibility criteria were recruited and booked for their first visit.

↓

Two days prior to each visit, a reminder email was sent out to participants which included the following directions:

I. Requirement to abstain from any caffeinated food and beverages (e.g., coffee, tea, chocolate) 24 hours before visit.
II. Consume a light meal 2 hours before visit (e.g., toast with juice/milk).
III. Bring along a book/kindle/personal device/activity for self – entertainment during the 45 – minute waiting period.

↓

Familiarisation Visit/1st Visit

I. Upon arrival, participants were asked to confirm their caffeine abstention for 24 hours prior to visit and the time of their last meal. Then, the 45-minute wait period commenced.
II. Participants were required to complete two forms: Demographic Questionnaire and Consent Form.
III. 30-minute CANTAB cognitive assessment was conducted once the waiting

↓

2nd Visit

I. Confirmation of caffeine abstention 24 hours prior to their visit and the time of last meal.
II. Participants were asked to consume either a caffeine or placebo capsule (researcher and participant blinded), followed by 45-minute waiting period.
III. 30-minute CANTAB cognitive assessment was conducted once waiting period ended.

↓

3rd Visit (same as Visit 2)

I. Participants were provided with the alternative capsule not received at visit 2 (placebo or caffeine), followed by 45-minute waiting period.
II. The identical 30-minute CANTAB assessment was completed as per prior visit.
III. $30 Westfield/MTA vouchers (participant's choice) were given at the end of the visit as reimbursement for travel costs for their participation.

Appendix H: CANTAB Cognitive Task Measures and Descriptions

Cognitive Task Measure	Description	Sense	Units	Min	Max
RTI					
Reaction Time	The mean duration it took for subject to release the response button after the presentation of the target stimulus.	-ve	ms	100	5100
Movement Time	The mean duration it took for subject to release the response button and select the target stimulus after flashing yellow.	-ve	ms	100	5100
RVP					
RVP A	The signal detection measure of a subject's sensitivity to the target sequence, regardless of response tendency; measures how good the subject is at detecting target sequences.	+ve	-	0	1
Probability of False Alarm	The number of sequence presentations that were false alarms divided by the number of sequence presentations that were false alarms plus the number of sequence presentations that were correct rejections: [False Alarms ÷ (False Alarms + Correct Rejections)].	-ve	-	0	1
Total correct responses/hits	The total number of correct target sequences selected by subject.	+ve	-	0	54
PAL					
First Attempt Memory	The number of times a subject successfully chosen the accurate box on their first attempt when recalling the pattern locations.	+ve	-	0	20

Cognitive Task Measure	Description	Sense	Units	Min	Max
Errors to Success	The mean number of attempts made by a subject in order to successfully complete the stage.	-ve	-	0	15
Total Attempts	The number of attempts made by each subject.	cx	-	4	16
Total Errors	The number of times subject mistakenly selected an incorrect box.	-ve	-	0	68
SWM					
Errors	The number of times the subject incorrectly revisits a box in which token has previously been found.	-ve	-	0	153
Strategy	The number of times a subject begins a new search pattern from the same box they started with previously. If subject always begin their search for the token from the same starting point, we infer that the subject is employing a planned strategy for finding the tokens. A low score indicates high strategy use (e.g., 1 = subject always begin search from the same box) and a high score indicates that subject begin their searches from many different boxes.	-ve	-	2	14

Sense: An indication whether a higher score indicates better (+ve) or worse (-ve) performance, or if measure sense is complex (cx)

Units: The units in which the measure is reported (e.g., percentage or milliseconds)

Min: The minimum possible score for the measure

Max: The maximum possible score for the measure

Appendix I: Participants Recruitment Flowchart