Health benefits of peanuts: Effects of peanut consumption on cognition, vascular and cardio-metabolic risk factors and satiety

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Summary

Health concerns are growing worldwide as populations' age and become increasingly overweight. Obesity is associated with increased cardio-metabolic risk and cognitive decline. Peanuts are a snack food rich in unsaturated fatty acids, protein and fibre which have demonstrated satiety benefits and may reduce total energy intake, despite their high energy density. Hi-oleic peanuts have been bred in Australia for their high monounsaturated fat content and are also a source of arginine, resveratrol (in the skins), folate, vitamin E and magnesium which have demonstrated benefits for vascular function and cardio-metabolic health. Epidemiological evidence indicates an inverse association between nut consumption and obesity, inflammation, hyperlipidaemia and glucose intolerance, which all influence vascular health. Studies have suggested that improved vasodilator function in the cerebral arteries with increased blood flow to the brain may be a likely mechanism for improvements in cognitive performance. Hence, this thesis examined the effect of nut consumption on satiety, cardio-metabolic, vascular and cognitive measures. These relationships were examined by conducting two intervention studies and a scoping review of the literature.

A scoping review examining associations between nut consumption, vascular health and cognitive function identified epidemiological and intervention studies which evaluated effects of chronic nut consumption on blood pressure, glucose regulation, endothelial vasodilator function, arterial compliance, inflammatory biomarkers and cognitive performance. Weighted mean changes were estimated where data were available; they indicated that nut consumption can reduce blood pressure and improve glucose regulation, endothelial vasodilator function and inflammation, whilst a limited number of studies suggest nut consumption may also improve cognitive performance.

An intervention study was performed which examined the effects of consuming unsalted Hi-oleic (oleic acid ~75 % of total fatty acids) peanuts and regular peanuts (oleic acid ~ 50 % and higher in polyunsaturated fatty acids) compared with an iso-energetic high carbohydrate snack (potato crisps) on satiety and subsequent energy intake in healthy overweight or obese adults. Total energy intake was 21 % and 17 % lower, during a buffet meal 3 hours after consumption of Hi-oleic and regular peanuts respectively compared with consumption of potato crisps. Over a subsequent four day

period energy intake was ~10 % lower with both peanut types compared with crisps. The reduction in energy intake may be due to the fibre and protein, and possibly the unsaturated fat content, of the peanuts. Despite the reductions in energy intake, no differences in satiety were observed. These findings suggest that peanuts may be a preferred snack food to include in the diet for maintaining a healthy weight.

A second, larger intervention study investigated whether regular daily consumption of Hi-oleic peanuts could influence dietary intake, body composition, inflammation, lipids, glucose regulation, and vasodilator function in the cerebral arteries and hence improve cognitive performance. Sixty one healthy overweight or obese adults aged 50-75 years consumed unsalted Hi-oleic peanuts (56g-84 g/d), with skins intact or an ad libitum nut free diet for 12 weeks, in a randomised cross-over design. Energy intake was increased by 12 % (primarily from monounsaturated fat) compared with the nut free diet with no changes in physical activity. Despite the additional energy intake, no significant changes in body weight, waist circumference or body composition between the two phases was observed. The reason why body composition measures did not change despite an increased energy intake is not clear but one possible explanation may be incomplete nutrient absorption resulting in reduced energy utilisation from the peanuts. Consumption of Hi-oleic peanuts also led to increased vitamin E, folate and magnesium intake. Surprisingly, Hi-oleic peanuts demonstrated no changes in lipids, high sensitive C-reactive protein (an inflammatory marker) (hs-CRP) or glucose regulation compared with the control diet. As the participants commenced the study with healthy lipids, glucose, insulin and hs-CRP levels, this may explain why additional benefits were not observed in this population. No significant changes in blood pressure or arterial elasticity were observed. However, blood pressure improvements in other studies have been observed primarily with habitual long term nut consumption indicating that interventions longer than 12 weeks may be required.

The most prominent finding was a significant improvement in the primary outcome; cerebral vasodilator responsiveness (CVR) increased by 8 % and 9 % in the left and right middle cerebral arteries respectively. These results are comparable to other studies with polyphenols. In addition, improvements in some cognitive tasks were demonstrated compared with the control diet. Measures of processing speed reached significance; other cognitive tasks demonstrated small but non-significant

improvements. No intervention studies have previously investigated the effect of nuts on CVR and only one other study has investigated cognitive measures.

In summary, this research demonstrates unsalted Hi-oleic peanut supplementation (with skins intact) significantly improved vasodilator function in the middle cerebral arteries, increasing blood flow to the brain which is a likely mechanism for improvements in cognitive performance. The mechanism for improved cerebral vascular function is not clear as no benefits in body composition, lipids, glucose regulation or inflammation were observed. In conclusion, this thesis adds to the body of research supporting the inclusion of nuts as part of a healthy diet with benefits for older overweight adults in vascular health and cognitive function compared with control diets.

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Signed declaration

I declare that this thesis presents work carried out by myself and does not incorporate without acknowledgement any material submitted for a degree or diploma in any other university. To the best of my knowledge it does not contain any materials previously published or written by another person except where due reference is made in the text: and all substantive contributions by others to the work presented, including jointly authored publications, is clearly acknowledged.



Jayne Barbour
31/10/14

Chapter 1: Introduction

1.1 Nutrition for cardiovascular and cognitive health

Cardiovascular disease and cognitive impairment are growing worldwide health concerns, particularly as populations' age (Drag & Bieliauskas 2010). Increasing evidence suggests that cardiovascular disease, metabolic syndrome, hypertension, obesity and type 2 diabetes are associated with diminished cognitive functioning and an increase in all types of dementia (Monsuez, Gesquière-Dando & Rivera 2011). Understanding the mechanisms for regulating cognitive functions is important for reducing the impact of declining cognition in older adults. Interventions that slow or prevent such decline are valuable and have become a health priority (Deary et al. 2009).

There are several proposed mechanisms by which cognitive performance may be improved and cognitive decline delayed with nutritional interventions. These include antioxidant, glucose utilization, neuronal membrane function, neurotransmitter and cerebrovascular effects (Ghosh & Scheepens 2009; Riedel, Jorissen & Hogervorst 2003). One of these potential mechanisms may be through maintenance of vascular health and improvement in cerebral blood flow. Impaired dilatation of cerebral arteries may contribute to reduced cognitive performance, due to poor cerebral perfusion (Silvestrini et al. 2006). Endothelial cell integrity is essential for cerebral vascular function and to ensure normal regulation of blood flow for the delivery of nutrients. With the onset of chronic disease over time, endothelial dysfunction may become too severe to respond to intervention, so primary prevention is the key to maintaining blood vessel function especially through the latter years of life (Endemann & Schiffrin 2004).

Certain nutrients and dietary patterns have been shown to improve endothelial function, blood pressure control and cognitive function (Dinges 2006; Esposito, Ciotola & Giugliano 2006; Feart et al. 2009; Ghosh & Scheepens 2009; Youdim, Shukitt-Hale & Joseph 2004). In addition, studies have identified benefits for cerebral blood flow with acute or short term supplementation of resveratrol, fish oil and diet high in nitrate (Jackson et al. 2012; Kennedy et al. 2010; Presley et al. 2011). However, bioactive nutrients need to be consumed on an ongoing basis to achieve optimal results. These have been achieved with 2 week intake of

cocoa flavonols and 12 week intake of wild green oats (Sorond et al. 2008; Wong et al. 2012).

Body weight also plays an important role in cardiovascular and cognitive health. Obesity is associated with cognitive decline as well as increased cardiovascular risk with accompanying reduction in endothelial dysfunction. One of the strategies to maintain a healthy body weight is through the consumption of foods which promote satiety, reducing subsequent energy intake. Hence, dietary interventions to manage body weight are valuable for reducing both cardiovascular risk and cognitive decline (Abbasi et al. 2002; Smith et al. 2011).

Nuts are a nutritious food with wide ranging cardiovascular and metabolic benefits (Coates & Howe 2007). They also have potential satiating properties (Brennan et al. 2010; Kirkmeyer & Mattes 2000) and hence may assist with weight control (Mattes, Foster & Kris-Etherton 2008). Several studies have demonstrated that nuts do not adversely affect body weight despite their high energy intake (Flores-Mateo et al. 2013). Other potential benefits of nut intake include improvements in lipid profile, glucose regulation, inflammation, blood pressure and endothelial function (Casas-Agustench, Bullo & Salas-Salvado 2010; Coates & Howe 2007; Kendall et al. 2011). Few studies however, have investigated vascular outcomes such as arterial compliance and cerebrovascular reactivity (a measure of endothelial function in the cerebral blood vessels) or cognitive outcomes. Furthermore, no studies have investigated whether nuts can increase blood flow to the brain through improvements in cerebral blood vessel function and whether this may be a mechanism for potential improvement in cognitive performance.

This thesis investigates the impact of nut consumption, in particular Hi-oleic peanuts on body weight, cardio-metabolic health, vascular function and cognitive performance compared with a nut free diet and satiety compared with potato crisps. The section below outlines the nutritional benefits of tree nuts and peanuts and how they may influence these outcomes.

1.2 Nutritional and health benefits of tree nuts and peanuts

The Dietary Guidelines for Australian Adults (National Health & Medical Research Council 2013) recognise the high nutritional value of tree nuts and peanuts. They are an excellent source of protein (up to 20 % of protein requirements/serve) and of dietary fibre providing 5–10 % of daily fibre requirements in one serving (30 g). Nuts are also an important source of folate, magnesium, potassium and phytosterols (Coates & Howe 2007) with peanuts having the richest highest content of phytosterols and folate compared with other nuts. They are rich in monounsaturated and polyunsaturated fatty acids which are likely responsible for their established lipid lowering effects (Kris-Etherton & Griel 2006). Large epidemiological studies have consistently revealed an association between frequent nut consumption and reduced incidence of coronary heart disease (Kelly & Sabaté 2006) and reduced risk of type 2 diabetes (Jiang et al. 2002; Nettleton et al. 2008). There is also evidence of improvements in glycaemic control and insulin sensitivity after 4-12 weeks of nut consumption (Adamsson et al. 2011; Casas-Agustench et al. 2011a; Sari et al. 2010). Clinical and epidemiological evidence indicates that nut consumption can enable improvements in inflammatory markers and reduce oxidative stress by being rich sources of antioxidant vitamins and flavonoids. Despite the high fat content of nuts, their consumption has been shown to not increase body weight (Bes-Rastrollo et al. 2007; Casas-Agustench, Bullo & Salas-Salvado 2010; Rana et al. 2007) which may be due to suppression of appetite and fat absorption (Martinez-Gonzalez et al. 2009; Natoli & McCoy 2007; Sabate 2003).

Peanuts are classified as a legume, but as they share many nutritional similarities with tree nuts they are typically all grouped together under the broad classification of nuts. Peanuts, in particular, are a good source of fibre and L-arginine (3 mg/100 g)(United States Department of Agriculture) which has been shown to improve endothelial function (Brock et al. 2006; King 2005; Lekakis et al. 2002; Sánchez-Muniz 2012). They are also a rich in alpha-tocopherol (Jonnala, Dunford & Dashiell 2006), the most active form of vitamin E with potential anti-atherogenic effects (Meydani 2004). Like other nuts, peanuts contain polyphenols particularly in the outer pellicle (skin) (Tsujita, Shintani &

Sato 2014). One such compound found in peanuts is resveratrol which is a key mediator of blood vessel function (Das & Das 2010). However, peanuts are commonly consumed without their skins so this potential benefit is lost. In addition, despite being naturally low in sodium peanuts are commonly consumed as a highly salted product which can also attenuate potential benefits. The Peanut Company of Australia produces a Hi-oleic variety of peanuts with a high level of the monounsaturated fat (MUFA) oleic acid as shown in **Table 1.1** (Peanut Company of Australia). This fatty acid profile makes them less susceptible to oxidation than regular peanuts (high in polyunsaturated fatty acids) thus, improving their shelf life. **Table 1.1** presents the nutrient profile of Hi-oleic peanuts with the percentage of daily nutrient requirements met in a 30g serve. The nutrient profile of regular peanuts compared with Hi-oleic peanuts is presented in **Table 4.1** of the published study "Lower Energy Intake Following Consumption of Hi-oleic and Regular Peanuts Compared with Iso-energetic Consumption of Potato Crisps" in **Chapter 4**.

Table 1.1 Nutrient content of Hi-oleic peanuts and percentage of nutrient reference value (NRV) met by consumption of 1 serve (30g) of the peanuts.

Nutrient	Hi-Oleic peanut*‡ per 100g	% of NRV 1 serve peanuts (30g)†
Energy (kJ)	2376	-
MUFA (oleic) (g)	38	-
PUFA (linoleic) (g)	2	5-8%
SFA (palmitic) (g)	3	-
Protein (g)	26	12-20%
Arginine (g)	3	-
Resveratrol (mg)¶	0.2	-
Fibre (g)	8.5g	8-10%
Vitamin E (mg)	8.3	25-35%
Folate (µg)	240	20%
Magnesium (mg)	168	15%
Potassium (mg)	705	6-11%
Iron (mg)	4.6	17%
Zinc (mg)	3.3	7-13%
Phystosterols (mg)	220	-

Abbreviations: PUFA, polyunsaturated fatty acids, SFA, saturated fatty acids MUFA, monounsaturated fatty acids *(Peanut Company of Australia) ‡(United States Department of Agriculture), †(National Health & Medical Research Council 2006), ¶(Tokusoglu, Unal & Yemis 2005).

1.3 Hypothesis, research and aim of the thesis

The aim of this thesis is to investigate the effects of Hi-oleic peanuts on acute satiety and subsequent effect on short term energy intake (primary outcome, study 1) over 3 days. A second study investigated the effect of Hi-oleic peanuts on cerebral vascular reactivity (primary outcome), body composition, nutrient intake, lipids, glucose regulation, inflammation, arterial compliance, blood pressure and cognitive performance (secondary outcomes) in older overweight/obese adults, compared with a nut free diet. This body of research is comprised of a scoping review and two clinical trials as outlined below.

The potential health benefits of Hi-oleic peanuts are summarised in **Figure 3.1, Chapter 3** as part of the scoping search. The nutrient content of Hi-oleic peanuts with skins, may have benefits for promoting satiety and reducing energy intake with subsequent benefits on body weight. Body weight reduction as a consequence of Hi-oleic peanut consumption may have cardiovascular benefits. In addition, bioactive nutrients in unsalted Hi-oleic peanuts (with skins intact) may reduce cardio-metabolic risk factors with subsequent benefits on endothelial function. Hence, these effects may lead to improvement in vascular health. It was hypothesized that improvement in endothelial function in the brain would allow greater blood flow and hence an increase in the delivery of nutrients and oxygen to improve cognitive performance. More details of these processes are outlined in **Chapter 2**.

1.4 Thesis structure

Chapter 1 - Introduction.

Chapter 2 provides background information on the satiety process, the effect of nut consumption on satiety and body weight as well as the influence of dietary intake on cardio-metabolic, vascular and cognitive function.

Chapter 3 is a scoping review of the literature describing the effects of nut consumption on vascular and cognitive function. This review was published in Nutrition Research Reviews, June 2014, vol.27, pp.131-158.

Chapter 4 presents findings of **Study 1**, investigating acute and short term (over 4 days) effects of Hi-oleic peanuts on satiety, appetite and food intake. This study was published in Appetite, August 2014, vol.82, pp.124-130.

Chapter 5 presents the hypothesis, aims, method and findings of **Study 2**, investigating the effects of 12 week consumption of Hi-oleic peanuts on vascular and cognitive function.

Chapter 6 presents findings of **Study 2**, investigating the effects of 12 week consumption of Hi-oleic peanuts on nutrient intake, energy balance, body composition and cardio-metabolic outcomes

Chapter 7 summarises the main findings and discusses them in the context of wider implications and potential to provide a foundation for future research.

Chapter 2: Background

This Chapter provides background information on the satiety process, the effect of nut consumption on satiety and body weight as well as the influence of dietary intake on cardio-metabolic, vascular and cognitive function.

2.1 The satiety process and underlying mechanisms

Obesity is a major health concern with serious co-morbidities; appetite regulation is one approach to moderate energy intake and weight gain (Kozimor, Chang & Cooper 2013). This section discusses satiety processes, mechanisms and potential benefits of nuts to regulate appetite.

Satiation refers to the feeling of fullness which leads to the termination of a meal and satiety is the feeling of fullness between meals and determines the time until the next meal. There is increasing evidence that several different mechanisms control appetite regulation, with food choice and intake being influenced by both sensory and metabolic factors (Benelam 2009). Metabolic satiety involves neuronal and hormonal signalling from the gastrointestinal tract to the brain. Appetite regulation in humans is complex and is thought to occur through the action of short, medium and long term feedback mechanisms (Chaudhri et al. 2008). These link energy intake, utilization and storage of nutrients to feedback inhibition of food intake (French & Yeomans 2001). Short term appetite is controlled via the central feeding drive and the peripheral satiety system. These include receptors responding to the presence of food in the gastrointestinal tract and gastric distension which promote satiety signals to reduce food intake (Chaudhri et al. 2008). The hypothalamus acts as the control center for hunger and satiety, allowing entry of peripheral peptides through the blood-brain barrier that directly interact with neurons to stimulate or reduce food intake (Austin & Marks 2009). In addition, the vagus nerve is a major site of gut hormone signaling linking the gastointestinal tract to the brain (Chaudhri et al. 2008). A gastric load stimulates the vagal nerve to convey information to areas of the brain responsible for the suppression of food intake. However, gastric distension alone is insufficient to account for all aspects of postprandial satiety. Receptors for a number of gut hormones, including cholecystokinin (CCK), peptide YY

(PYY) and glucagon-like peptide-1 (GLP-1) are present on the vagus nerve. These gut hormones and their actions are sumarised in **Figure 2.1.**

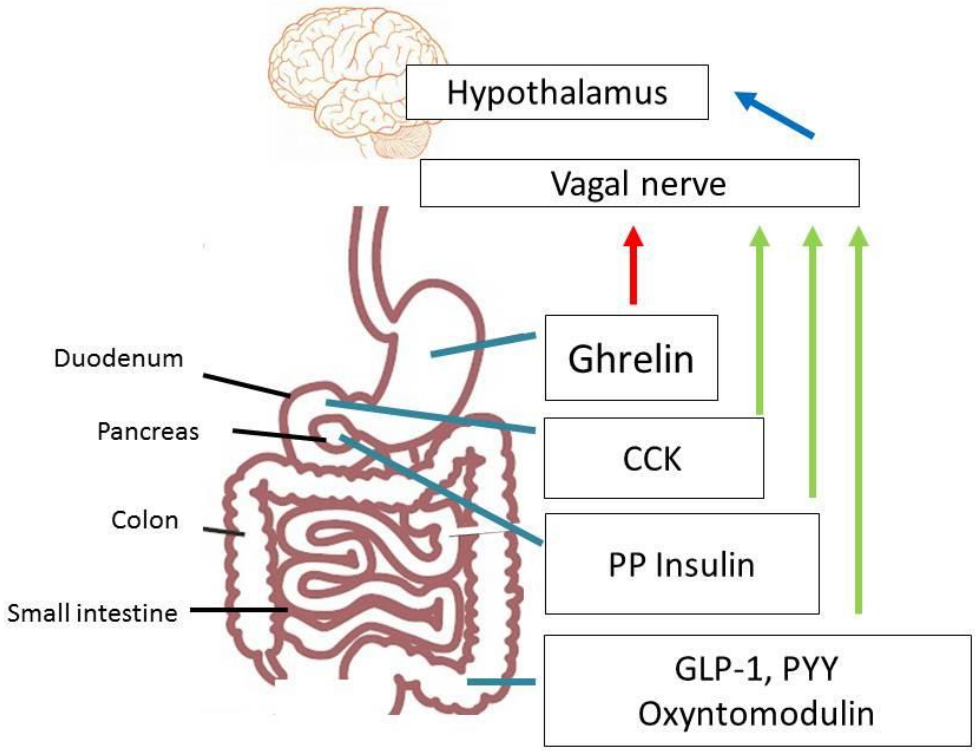


Figure 2.1 Schematic diagram of the intestinal tract indicating where gut hormones insulin, pancreatic polypeptide (PP), glucagon like peptide-1 (GLP-1), cholecystokinin (CKK), peptide YY (PYY), oxyntomodulin and ghrelin are concentrated and their stimulation of satiety ( ) and hunger ( ) through the vagus nerve and brain (Murphy & Bloom 2006).

Increases in circulatory satiety peptides are often small, indicating that satiety may be a result of the cumulative effects of a number of gut hormone responses (Chaudhri et al. 2008). Of these ghrelin is the only known appetite stimulant. In contrast there are many peripheral peptides associated with satiety, produced by the pancreas, gastrointestinal tract and adipose tissue. Cholecystokinin (CCK) plays a role in acute appetite regulation with the greatest response to fat and protein ingestion over 10–30 minutes post-prandially, remaining elevated for up to 5 hours. Peptide YY (PYY) and pancreatic polypeptide (PP) are released into the circulation and peak 1-2 hours post-prandially in proportion to the meal

energy content and are therefore higher after fat intake compared with carbohydrate or protein intake (Austin & Marks 2009; Chaudhri et al. 2008). Oxyntomodulin is released in response to ingestion of nutrients. Glucagon like peptide-1 (GLP-1) is an important peptide which delays gastric emptying. Insulin, another hormonal regulator of appetite increases rapidly after a meal with insulin receptors widely distributed in the brain (Austin & Marks 2009). The following section outlines the influence of diet and more specifically nuts on appetite and possible mechanisms.

2.2 Nutritional modulation of appetite

2.2.1 Protein

There is compelling evidence that protein is the most satiating macronutrient and that consuming an increased amount of protein can reduce energy intake (Slavin & Green 2007). This suggestion is based primarily on studies that increased protein intake with a protein preload and found a reduction in energy intake at subsequent meals (Halton & Hu 2004). The greater satiating effects of a higher-protein diet have been shown over prolonged periods of 12 to 16 weeks with regular consumption (Moran et al. 2005; Weigle et al. 2005). In a 16-week study, subjects consuming a high-protein (34 %), lower-fat diet reported greater post-meal satiety for up to 120 minutes post consumption than subjects consuming a standard protein (18 %) higher-fat diet (Moran et al. 2005). This suggests that a modest increase in protein at the expense of the other macronutrients may promote satiety and facilitate weight loss through reduced energy consumption. Protein has been shown to increase satiety by suppressing ghrelin release, elevating cholecystokinin (CCK) and slowing gastric emptying (Bowen, Noakes & Clifton 2006). In addition, higher-protein diets are associated with increased thermogenesis, which also influences satiety and augments energy expenditure. A study with women consuming a high protein diet demonstrated increased satiety and thermogenesis compared with a high fat diet. These satiety differences were significantly correlated with differences in 24 hour dietary induced thermogenesis (Westerterp-Plantenga et al. 1999). Overall, there is a general consensus in the literature that protein promotes satiety to a greater extent than other macronutrients (Paddon-Jones et al. 2008).

2.2.2 Fibre

Dietary fibre in food has long been regarded as influencing satiety (Howarth, Saltzman & Roberts 2001) and there are several mechanisms by which dietary fibre may influence food intake. These include adding bulk to the diet to promote gastric distension and slowing gastric emptying (Burton-Freeman 2000; Howarth, Saltzman & Roberts 2001). This slower rate prolongs small intestine transit time and slows the absorption of nutrients signalling the central nervous system hence, prolonging satiety (Burton-Freeman 2000). Foods containing dietary fibre also require greater mastication, which slows ingestion adding further to the satiety effect. The lower glycaemic index of fibre containing foods and meals may enhance the release of satiety gut peptides and also slow nutrient absorption (Burton-Freeman 2000). In addition, dietary fibre is not absorbed in the intestine so lowers the energy density (kJ/g) of intake resulting in less energy intake required to feel satiated.

2.2.3 Fatty acids

Fat intake has limited effect on satiety compared with other macronutrients in the diet (Lawton et al. 1993). However, there is growing interest in the effects of different fatty acids on appetite control (Alfenas & Mattes 2003; Casas-Agustench et al. 2009). It has been hypothesised that fat exerts differential effects on satiety depending on degree of saturation and chain length (Maljaars et al. 2009; Poppitt et al. 2010). Absorption via the gut and rate of oxidation have both been suggested as potential mechanisms affecting the satiety response to differing fat sources (Alfenas & Mattes 2003). Monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) are oxidised more rapidly than those with saturated fatty acids (SFA) and short (SCFA) and medium chain fatty acids (MCFA) are more readily oxidised than long chain fatty acids (LCFA) (Poppitt et al. 2010). It has been hypothesised therefore that the ranking order of fatty acids with respect to satiety is PUFA >MUFA >SFA and SCFA>MCFA>LCFA (Alfenas & Mattes 2003; Iyer et al. 2006; Strik et al. 2010).

This hypothesis has been partly supported by a study investigating fat oxidation rates following intakes of olive oil (rich in MUFA), walnuts (rich in PUFA) and dairy products (rich in SFA) (Casas-Agustench et al. 2009). Increased fat oxidation rates were found in the PUFA (34 % greater than baseline levels) and MUFA groups, whilst the SFA group showed decreased fat oxidation compared with baseline. However, the PUFA group demonstrated no difference in perceived satiety and energy intake at a subsequent meal (Casas-Agustench et al. 2009). Infusions of safflower oil (PUFA) and canola oil (MUFA) into the ileum demonstrated significantly increased fullness and reduced hunger ratings than shea oil (SFA) in healthy participants (Maljaars et al. 2009). In contrast, four studies (Kozimor, Chang & Cooper 2013; Lawton et al. 2000; Orosco, Rouch & Dauge 2002; Strik et al. 2010) found the SFA groups to be the most satiating and one study found no difference between MUFA and SFA (Alfenas & Mattes 2003). It was suggested by these authors that SFA is digested and absorbed more slowly than PUFA and MUFA with more time in contact with the endocrine L cells to produce higher levels of PYY hence the increased satiety observed. SCFA and MCFA are thought to be more satiating than LCFA because they are easily digested and absorbed with their relatively seamless uptake into the portal circulation and transportation to the liver for oxidation (Denbow et al. 1992 ; Poppitt et al. 2010). LCFAs have a delayed rate of absorption and enter the lymphatic ducts rather than via the portal vein (Poppitt et al. 2010). Only three studies have investigated the effect of chain length on satiety (Denbow et al. 1992 ; Furuse et al. 1992; Poppitt et al. 2010). Comparison of effects of milk fat (high SCFA), coconut oil (high MCFA), and tallow (high LCFA) on hunger, fullness and energy intake found no differences in these satiety measures (Poppitt et al. 2010). Other studies used animal models (cockerels and mice) and found reductions in energy intake in high SCFA groups; however conclusions are difficult to draw because of differences in absorption and transport of fats in animals compared with humans (Denbow et al. 1992 ; Furuse et al. 1992). Overall, at this current time there is no clear indication of which fatty acids exert greatest influence on satiety.

2.3 Influence of nuts on satiety

Evidence suggests that consumption of nuts induces satiety and may lead to reduction of food intake at subsequent meals and snacks (Alper & Mattes 2001; Hollis & Mattes 2007) due to their protein and fibre content. Similarly, lupin enriched bread (also high in protein and fibre) resulted in higher satiety and a lower energy intake than the control bread with significant reduction in postprandial ghrelin, glucose, and insulin responses in healthy participants (Lee et al. 2006). In addition, whole nuts require more mastication than many other foods particularly those which are highly processed. This activates mechanical, nutrient, and sensory signalling systems that may modify appetitive sensations (Zhu, Hsu & Hollis 2013). Nut studies have primarily compared satiety of whole nuts with nut butter, nut flour, nut oil or other oils, demonstrating that whole nuts have greater satiety value than refined nut products. A recent study (Alves et al. 2014) found High-oleic peanuts contributed to greater sensation of fullness than conventional peanuts, authors proposed this was due to higher diet induced thermogenesis observed with Hi-oleic peanuts. Diet-induced thermogenesis is the acute effect of food ingestion on resting metabolic rate (de Graaf et al. 2004). It has been suggested that the metabolic rate is positively related to the perception of satiety over 24 hours. The increased energy expenditure at rest likely increases oxygen consumption and body temperature (with accompanying increase in temperature in the hypothalamus), both mechanisms are thought to stimulate a decrease in feeding (Westerterp-Plantenga et al. 2002; Westerterp-Plantenga et al. 1999). Few studies have investigated the satiety effects of nuts compared with other snack foods. At this time, no single attribute can account for the high satiety value of nuts; however their protein and fibre content appear to have most evidence for their effect (Tan, Dhillon & Mattes 2014). The ability of nuts to contribute to potential long term satiety, dietary changes (particularly reduction of energy intake) and weight management has not yet been determined. This would be valuable in light of health risks associated with obesity.

2.4 Effect of nut consumption on body weight and body composition

Due to their high fat content, nuts have been considered by the general population to be responsible for body weight increase (Brennan et al. 2010; Flores-Mateo et al. 2013). However, evidence to the contrary suggests that nut consumption is associated with a lower body weight (Bes-Rastrollo et al. 2007; Rana et al. 2007). Two large, prospective cohort studies (the Adventist Health Study and Nurse's Health Study II) showed significant inverse associations between the frequency of nut consumption and body mass index (BMI) (Bes-Rastrollo et al. 2009; Fraser et al. 1992). Frequent nut consumption was also associated with reduced risk of weight gain in the SUN (Seguimiento University of Navarra) cohort after 6 years (Bes-Rastrollo et al. 2007). However, the Physicians' Health Study (Albert et al. 2002) and the Iowa Women's Health Study (Ellsworth, Kushi & Folsom 2001) did not demonstrate this relationship between nut consumption and body mass, possibly due to study design differences.

Evidence from intervention studies indicates that incorporating nuts into the diet has little impact on body mass and body fat (Flores-Mateo et al. 2013). A recent systematic search of 23 clinical trials investigating the effect of chronic nut consumption on body mass demonstrated a small non-significant weighted mean decrease in body weight of 0.47 kg, BMI of 0.40 kg/m², and waist circumference of 1.25 cm (Flores-Mateo et al. 2013). However, in most of the studies, nuts were used in iso-energetic diets so weight changes were not expected. Eight week consumption of 89 g/day (2113 kJ/day) of peanuts in 15 healthy weight adults with no dietary compensation demonstrated a weight gain of only 1kg which was 28 % of the predicted 3.6 kg (Alper & Mattes 2002). Similarly, two studies with ~55-75 g of almonds providing around 1400kJ/day, predicted that weight gains would be 2.5 kg over 10 weeks and 6.4 kg over 6 months. However, there were no weight gains over 10 weeks and 2-10 % of predicted weight gain over 6 months respectively (Fraser et al. 2002; Hollis & Mattes 2007).

Several intervention studies have investigated the effects of incorporating nuts into a weight management regime. A weight reduction program comparing a formula based diet enriched with 84 g/day of almonds with an energy and protein matched diet found more favourable reductions in body weight and BMI, waist circumference and fat mass in the almond group (Wien et al. 2003). However, it is possible that these outcomes could be attributed to the formula based diet as well as the almonds. Mixed nuts and peanut butter providing 35 % of energy from fat compared with a low fat diet over 18 months consumed by overweight adults resulted in greater adherence and hence greater weight loss (4.1 vs. 2.9 kg) with the nut group twice as likely to remain in the study and their diets showing an improved nutrient profile (McManus, Antinoro & Sacks 2001). In contrast, an iso-energetic weight loss diet comparing peanuts and peanut butter (33 % of energy from fat) with a low fat designed to elicit 1kg weight loss per week, with overweight and obese adults demonstrated similar weight loss with both diets (7.2 kg for the peanut group and 6.5 kg for the low-fat group) after 4 weeks (Pelkman et al. 2004).

These studies demonstrated little or no change of body weight with tree nut or peanut consumption, with benefits for improved nutrient profiles. Adherence to the nut diets in most studies was high making them applicable to the wider community. Several mechanisms outlined below have been proposed for the lack of weight gain observed with nut consumption, despite their high energy and fat content.

2.4.1 Dietary compensation

The satiety value of nuts may lead to dietary compensation with a reduction in the total amount or type of other foods consumed. As described above, reduction of energy intake subsequent to nut consumption has been observed (Flores-Mateo et al. 2013), with up to 75% of the energy provided by nuts offset by lower energy intake at later eating events. No single component of nuts appears responsible for this effect. Energy compensation occurs for intake of peanut oil

and other oils but is weaker than whole nuts by about 50%, indicating that several components of the whole nut are responsible (Iyer et al. 2006). An investigation of food attributes on hunger and food intake compared peanuts (500 kcal) with control foods of different characteristics; texture (500 kcal peanut butter), other nuts (500 kcal almonds), macronutrient profile (500 kcal chestnuts), sensory properties (500 kcal chocolate), volume (200 kcal rice cakes) and weight (200 kcal pickles). Hunger ratings did not differ between the foods of the same energy (with the exception of peanut butter which elicited a smaller decline in hunger compared with peanuts) but were significantly different between the high energy foods and low energy foods (rice cakes and pickles) indicating that the energy content was the primary determinant of hunger (Kirkmeyer & Mattes 2000). In addition, volume plays a role in the satiety response as demonstrated with iso-energetic consumption of popcorn and potato crisps. Consumption of popcorn compared with the crisps demonstrated less hunger, more satisfaction, and lower estimates of prospective food intake (Nguyen et al. 2012), with the volume of popcorn consumed 6 times greater than the volume of crisps.

2.4.2 Efficiency of energy absorption

Studies exploring the efficiency of absorption of energy from nuts have consistently demonstrated elevated faecal fat loss with nut consumption. In one study (Levine & Silvis 1980), peanuts provided 95 % of the energy from fat as part of a vegetarian diet, 18 % of the lipid load was lost in the stool with whole peanuts whereas 7.0 % and 4.5 % were lost respectively for peanut butter and peanut oil. A trial with 63 healthy adults compared faecal fat excretion with consumption of 70 g/d of whole peanuts, peanut butter, peanut flour, or peanut oil (Traoret et al. 2008). Faecal fat content was significantly higher when whole peanuts were consumed (5 ± 0.6 %) relative to the other forms (oil, 3.1 ± 0.4 %; butter, 3.1 ± 0.3 %; and flour, 3.8 ± 0.4 %). This corresponded to an energy loss of around 155 kJ/d or 12 % of the peanut lipid. In a trial where the fat content increased from 30 % to 43 % of energy by the addition of peanuts, there was a corresponding faecal fat increase from 3 to 8 % (Haddad & Sabate 2000).

Several trials have demonstrated a dose-response relationship between fat intake and excretion. One trial provided 50–100 g/d of almonds, 25-50 g/d of almonds or no almonds for one month in a cross over trial and reported an increase in faecal energy excretion of 247 ± 63 kJ/d and 113 ± 46 kJ/d for the full and half doses of almonds respectively, compared with no almonds (Kendall et al. 2001).This was supported by another trial observing a dose response from almonds providing 0, 10, and 20 % of total energy (Zemaitis & Sabate 2001) with an estimated energy loss of 10–20 % from the nuts.

This inefficiency in energy extraction from nuts is due to resistance of the cell walls to degradation in the gastrointestinal tract leading to reduced bio-accessibility of nutrients, including fat (Ellis et al. 2003). This is supported by data demonstrating greater energy loss from whole nuts compared with nut butter and nut oil (Traoret et al. 2008) and analyses of faecal samples (Ellis et al. 2003). As a consequence, it is likely that the reported available energy content of nuts is overestimated. This is supported by relatively recent measures of Atwater factors (metabolisable energy) from almonds (Cassady et al. 2009) and pistachios (Baer, Gebauer & Novotny 2012) indicating past calculations have overestimated their energy content. This may also be true for other tree nuts and peanuts.

2.4.3 Increased energy expenditure

Consumption of nuts may augment energy expenditure, such that chronic consumption of peanuts is associated with elevated resting energy expenditure (REE). Consumption of 89 g/day (~2000 kJ/d) of peanuts for 19 weeks demonstrated REE increases of 11 % (~800 kJ) (Alper & Mattes 2001). This amounted to ~ 40 % of the energy from the peanuts. The reason for increased REE with nut consumption is not clear but may reflect their unsaturated fat and protein content. Unsaturated fats are oxidized more readily than saturated fats (Jones, Pencharz & Clandinin 1985) and protein has a high thermogenic effect (Hermsdorff, Volp & Bressan 2007). This is supported by a recent publication (Alves et al. 2014) demonstrating that high-oleic peanuts (82 % of fatty acids as

oleic acid) resulted in higher diet induced thermogenesis than conventional peanuts (52 % of fatty acids as oleic acid). The peanuts contained similar amounts of protein, carbohydrate and total fat so the difference was attributed to the fatty acid composition. Other studies have demonstrated that monounsaturated fatty acid stimulates the sympathetic nervous system more than other fatty acids to increase thermogenesis (Matsuo et al. 1995; Moussavi, Gavino & Receveur 2008).

Although there are insufficient data to support each of these mechanisms alone, together they provide an explanation for the limited body weight increase with nut consumption observed in epidemiological and clinical studies. Estimates are 55–75 % of energy contributed by nuts is offset by dietary compensation, another 10–15 % by faecal loss, and an additional, less well-established estimate of 10 % via increased energy expenditure (Mattes, Foster & Kris-Etherton 2008).

2.5 Effect of nut consumption on cardio-metabolic outcomes

Nuts contain several nutrients which may benefit cardio-metabolic health. These include unsaturated fatty acids, fibre, vitamin E, phytosterols and polyphenols with potential to regulate blood lipids, glucose, insulin sensitivity and inflammation (Coates & Howe 2007).

2.5.1 Effects of nut consumption on blood lipids

Clinical trials have clearly shown that intakes of several varieties of nuts can lower total and low density lipoprotein (LDL) cholesterol by 9-16 %, even in the context of healthy diets (Good, Lavie & Ventura 2009; Griel & Kris-Etherton 2006; Mukuddem-Petersen, Oosthuizen & Jerling 2005). A pooled-analysis of influences of nut consumption on blood lipids revealed the cholesterol lowering effects are greatest in individuals with higher baseline LDL levels or lower body mass index (BMI). This pooled-analysis estimated that a mean daily consumption of 67 g of nuts reduced LDL by 7 %, triglycerides by 5 % and raised HDL: LDL by 8 % (Sabate, Oda & Ros 2010). Nut consumption was also

found to lower triglyceride levels primarily in individuals with hypertriglyceridemia. Similarly, a walnut meta-analysis (Banel & Hu 2009) found that walnut-enriched diets significantly decreased total and LDL cholesterol by 5 % and 7 % respectively with a 5 % reduction in triglycerides and no effect on HDL. These lipid lowering effects of nuts appear to be greatest when their intake is substituted for saturated fat in the diet rather than being added to the diet (Sabate, Oda & Ros 2010).

Another finding from the nut pooled-analysis was a dose response with increasing amounts of nuts manifesting a greater cholesterol lowering capacity (Sabate, Oda & Ros 2010). A trial with hyperlipidaemic healthy adults found that replacing 10 % of energy with almonds (34 g/day) for 4 weeks resulted in a 3 % reduction of LDL and replacing 20 % of energy with almonds (68 g/day) for the same time period gave a 9 % reduction (Sabaté et al. 2003). Similarly 73 g of almonds consumed for 4 weeks by hyperlipidaemic adults produced a 9 % decrease in LDL in compared with 37 g which decreased LDL by 4 % (Jenkins et al. 2002b).

However, not all nut studies have demonstrated reduction in blood lipids. Responses of blood lipid levels appear to be dependent on body mass index (BMI) with obese individuals having an attenuated cholesterol-lowering response to dietary manipulation of fatty acids compared with lean individuals. Obesity is characterized by elevated endogenous production of cholesterol which may explain this (Lefevre et al. 2005). In a study with individuals with metabolic syndrome or obesity, neither walnut nor cashew intake influenced blood lipid levels (Mukuddem-Petersen et al. 2007). Obesity and metabolic syndrome are also associated with reduced intestinal cholesterol absorption, hence additional benefits of plant sterols found in nuts to further reduce cholesterol absorption may not be apparent (Knopp et al. 2003; Simonen et al. 2000).

Studies have found that favourable lipid levels resulting from nut consumption are sustainable over extended periods of time. The PREDIMED trial found that one year consumption of 30g of mixed nuts per day reduced

hypertriglyceridemia in participants with high cardiovascular risk (Salas-Salvado et al. 2008). Similarly 30 g/d of walnuts consumed for 6 months as part of a low-fat diet significantly reduced LDL and increased HDL in patients with type 2 diabetes mellitus (Tapsell et al. 2004).

The cholesterol lowering effects of nuts have been attributed to their high content of unsaturated fat and bioactive compounds, including plant sterols, dietary fibre and antioxidants (Griel & Kris-Etherton 2006). Although several studies have tested the effects on lipids with tree nut and peanut consumption limited research has been conducted with high-oleic peanuts which have a unique fat profile with a much higher content of monounsaturated fatty acids (MUFA) compared with other types of peanuts.

Although nut consumption can elicit a modest reduction in LDL, epidemiological studies estimate regular nut consumption is associated with a 37% reduction in the risk of developing coronary heart disease (Sabate, Oda & Ros 2010). This indicates that other effects of nuts on other cardiovascular risk factors including glucose regulation, endothelial dysfunction and inflammation are also important, which are discussed below.

2.5.2 Effect of nut consumption on glucose regulation and inflammation

Nut consumption has been associated with a reduced risk of type 2 diabetes; evidence to support this comes from large epidemiological studies (Jiang et al. 2002; Nettleton et al. 2008). There is considerable evidence of further benefits of nut consumption for glycaemic control and insulin sensitivity observed after 4-6 weeks of consumption (Adamsson et al. 2011; Kalgaonkar et al. 2011; Sari et al. 2010). However, not all studies have demonstrated benefits with nut intake on glucose regulation and insulin sensitivity (Lovejoy et al. 2002; Mercanligil et al. 2007), making it difficult to reach precise conclusions. Epidemiological and clinical evidence also indicates that nut consumption can enable improvements in inflammatory markers with daily doses of 30g or more able to confer benefits

(Mena et al. 2009; Solà et al. 2012). **Chapter 3** includes a scoping review which provides a more detailed analysis of the literature on the influence of nut consumption on glucose regulation (the term 'glucose regulation' in this thesis refers to both glucose and insulin responses) and inflammation.

2.6 Dietary intake for vascular and cognitive health

2.6.1 Endothelial function and dietary intake for vascular health

As described previously there is emerging evidence that frequent nut consumption beneficially affects cardiovascular risk beyond cholesterol lowering. Mechanisms include antioxidant and anti-inflammatory effects which influence endothelial function (de Lorgeril et al. 2001; Landmesser, Hornig & Drexler 2004). Endothelial dysfunction is associated with increased adhesion molecule expression, inflammatory markers and reactive oxygen species, all of which play important roles in the development of atherosclerosis (Vallance & Chan 2001).The endothelium is the inner lining of blood vessel walls comprising a single layer of endothelial cells; it secretes vasodilator substances including nitric oxide (NO), which are crucial for the maintenance of vascular tone and vascular structure. This vasodilator is produced from L-arginine, by endothelial derived NO-synthase (eNOS) (Lundberg et al. 2008). Endothelial cells join with astrocytes in the brain to form a structure known as the blood brain barrier which acts to limit access to and remove noxious substances from neuronal tissue (Abbott, Rönnbäck & Hansson 2006). Endothelial function of the cerebral blood vessels is essential to allow adequate blood flow for glucose and other nutrients to be transported nutrients to the brain.

Several foods or diets with bioactive nutrients have shown improvement in endothelial function; these include walnuts and pistachios (Sari et al. 2010; West et al. 2010) which are described more fully in the scoping review in **Chapter 3**. Other foods or food patterns shown to improve endothelial function include cocoa (Davison et al. 2008; Scholey et al. 2010; Sorond et al. 2008), blueberries

(Youdim et al. 2000), soy (Cuevas et al. 2001) and Mediterranean diets (Esposito, Ciotola & Giugliano 2006; Fuentes et al. 2001).

Vasoactive nutrients found in peanuts have also demonstrated beneficial effects on endothelial function; these include L-arginine (Heffernan et al. 2010), high oleic acid diets (Fuentes et al. 2001; Pérez-Jiménez et al. 1999), vitamin E (Skyrme-Jones et al. 2000) and resveratrol (Wong et al. 2010).

The mechanisms suggested for the effect of nuts on endothelial function are, indirectly via lipid lowering, reduction in body fat and improved inflammation and glucose metabolism and or directly via the action of vasoactive nutrients (Ganz et al. 2000). Whilst endothelial function declines with age, biological factors can also influence vascular function. These have been observed with inflammation, high blood pressure, high blood glucose, hypertension, hypercholesterolemia, diabetes mellitus, obesity and metabolic syndrome (Endemann & Schiffrin 2004). Possible detrimental factors for endothelial function include oxidative stress associated with obesity and insulin resistance (Roberts, C & Sindhu 2009) and a decrease in insulin-dependent activation of endothelial nitric oxide synthase (eNOS) (Fornoni & Raij 2005). In healthy individuals eNOS derived NO regulates cerebral vascular tone and mediates cerebral blood flow in the brain. During cognitive tasks increased blood perfusion reflects the increased demand for oxygen, glucose and removal of waste products. However as endothelial function declines with age, cerebral blood flow also reduces age with accompanying cognitive decline (Meyer et al. 1988).

It has been hypothesised that cognitive performance may be improved by increasing blood flow regulation in the brain (Krestin et al. 2008; Sinn & Howe 2008). Cerebral vascular reactivity (CVR) (a measure of endothelial function in the brain), has been improved with dietary intervention. These include the administration of resveratrol (Kennedy et al. 2010), cocoa (Fisher, Sorond & Hollenberg 2006; Scholey et al. 2010) and wild green oats (Wong et al. 2012). Several dietary intervention studies have identified benefits for cognitive

performance (Berry et al. 2011) but the measure of CVR with dietary interventions is a relatively new technique and may provide a mechanism for these findings. The following section summarises the effect of dietary supplementation, including nuts on cognitive function.

2.6.2 Dietary intake for cognitive function

There is growing evidence that some components of the diet or specific foods may have a positive effect on cognition. A positive relationship between cognitive performance and bioactive nutrients has been demonstrated in animal and human studies in several flavonoid rich foods including cocoa, blueberries, grape juice, soy, red wine, wild green oats and Ginkgo biloba (Gómez-Pinilla 2008; Macready et al. 2009; Van Dyk & Sano 2007; Youdim et al. 2000).The nutrients vitamin E, monounsaturated fat and resveratrol found in peanuts have also been shown to have a positive relationship with cognitive performance (Morris et al. 2002; Roberts, R et al. 2010; Wong et al. 2010).

In vitro studies with nuts have shown that walnut extract desensitizes microglial cells (associated with neuronal dysfunction) to inflammation (Willis et al. 2010). In animal studies, almond paste has been shown to reduce cholesterol levels and significantly reversed scopolamine induced amnesia (Kulkarni, Kasture & Mengi 2010). Dietary walnut supplementation has been shown to improve cognitive and motor performance in aged rats (Willis et al. 2009). Authors conclude that these effects could lead to nutritional interventions for the prevention and treatment of neurodegeneration and have potential for the management of Alzheimer's disease.

To date, few studies have investigated the effect of nut consumption on cognitive function in humans. These limited data provide promising findings suggesting there may be cognitive benefits with nut intake. The following chapter outlines a scoping review of the effects of nut intake on vascular and cognitive function in humans and the proposed mechanisms for the findings.

Chapter 3: Scoping search of evidence for vascular and cognitive benefits of nut consumption

This chapter comprises of a publication which examines associations between nut consumption, vascular health and cognitive function. It reports on 71 epidemiological or intervention studies in which effects of chronic nut consumption on cardio-metabolic, vascular biomarkers and cognitive performance were evaluated.

This is a co-authored publication. Please refer to **Appendix A** for a statement of authors contributions.

Jayne A. Barbour, Peter R. C. Howe, Jonathan D. Buckley, Janet Bryan and Alison M. Coates. Nut consumption for vascular health and cognitive function. *Nutrition Research Reviews*, June 2014, vol 27, pp. 131–158.

3.1 Nut consumption for vascular health and cognitive function

This chapter is adapted from the published manuscript

3.2 Abstract

Nuts are rich in many nutrients that can benefit multiple cardio-metabolic functions, including arterial compliance, blood pressure, inflammation, glucose regulation and endothelial vasodilatation. Impaired vasodilatation may contribute to impaired cognitive performance due to poor cerebral perfusion. The present narrative review examines associations between nut consumption, vascular health and cognitive function. It includes a systematic search which identified seventy one observational or intervention studies in which effects of chronic nut consumption on blood pressure, glucose regulation, endothelial vasodilator function, arterial compliance, inflammatory biomarkers and cognitive performance were evaluated. Weighted mean changes were estimated where data were available; they indicate that nut consumption reduces blood pressure and improves glucose regulation, endothelial vasodilator function and inflammation, whilst a limited number of studies suggest that nut consumption may also improve cognitive performance. Further clinical trials are warranted to explore relationships between nut consumption, endothelial function and cognitive function.

Key words: Nuts: Endothelial function: Inflammation: Vascular function: Cognition

3.3 Introduction

Cardiovascular disease and cognitive impairment are growing worldwide health concerns particularly as populations' age (Drag & Bieliauskas 2010; Yazdanyar & Newman 2009). In 2006, the worldwide prevalence of Alzheimer's disease was estimated at 26.6 million and by 2050 this is predicted to quadruple (Brookmeyer et al. 2007). Increasing evidence suggests that cardiovascular disease, metabolic syndrome, hypertension, obesity and type 2 diabetes are

associated with diminished cognitive functioning and an increase in all types of dementia (Monsuez, Gesquière-Dando & Rivera 2011). These cognitive changes may be mediated through compromises in the structural and functional integrity of cerebral blood vessels. Cognitive performance refers collectively to mental processes including attention, memory, language, problem solving and decision making (Deary et al. 2009). Understanding the mechanisms for regulating cognitive functions is important to reduce the impact of declining cognition in older adults. Interventions that slow or prevent this condition are valuable and have become a health priority (Deary et al. 2009). One of the mechanisms by which cognitive performance can be improved and cognitive decline delayed may be through maintenance of blood vessel health and improvement in blood flow to the brain (Krestin et al. 2008; Sinn & Howe 2008). Impaired vasodilatation contributes to reduced cognitive performance, due to poor peripheral and cerebral perfusion (Silvestrini et al. 2006). Endothelial cells line blood vessels (including those in the brain), thus maintaining cerebral vascular function to ensure normal regulation of cerebral blood flow for the delivery of nutrients is essential to maintain endothelial cell integrity (Iadecola & Davisson 2008).

It has been hypothesized that inflammation may contribute to cognitive decline (Arfanakis et al. 2013) and to the cardiovascular disease processes (Meigs et al. 2004). This may be a result of endothelial dysfunction (Singhal 2005) associated with reduced nitric oxide (NO) bioavailability. NO is an important vasodilator, produced from L-arginine by endothelial NO-synthase (Lundberg, Gladwin & Weitzberg 2008). Early phases of atherosclerosis involve the adhesion of circulating monocytes to the endothelium (inner lining of blood vessel walls) and their migration to the intima layer. This is a complex disease process mediated by inflammatory responses that involve cytokine production and up-regulation of adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1(VCAM-1) and E-selectin (Rana et al. 2007). An increase in inflammatory cytokines (e.g. C-reactive protein (CRP) and interleukin-6), have been found to be independent predictors of cardiovascular disease and type 2 diabetes (Rana et al. 2007). The endothelium

is crucial for the maintenance of vascular tone and vascular structure; endothelial dysfunction predisposes individuals to complications of atherosclerosis by increasing blood pressure and arterial stiffness, characterized by increased pulse wave velocity and an increase in augmentation index (Vita 2005). Endothelial function declines with age but is also adversely affected by hypertension, hyperglycaemia, dyslipidaemia and obesity individually or collectively known as the metabolic syndrome (Vita 2011).

The principal energy source for the brain is glucose, which must be supplied continuously due to a limited storage capacity (Benton 2001). In addition, a range of nutrients and substrates including oxygen are needed to be delivered via the blood (Bourre 2006), hence cerebral blood flow and substrate transport across the blood brain barrier are primary determinants of brain function (Benarroch 2012). There is a growing interest in the role of nutrition in the causation and prevention of age-related cognitive decline and dementia, more research is needed to understand mechanisms for cognitive decline and possible delay.

As shown in **Table 3.1**, nuts contain a range of nutrients with potential health benefits including improved glucose control and insulin sensitivity (Casas-Agustench, Bullo & Salas-Salvado 2010; Coates & Howe 2007). Despite the high fat content of nuts, nut consumption has not been shown to increase body weight; instead it is associated with improved weight control (Bes-Rastrollo et al. 2007; Rana et al. 2007). There is a substantial body of evidence demonstrating lipid lowering effects of nut consumption (Kris-Etherton & Griel 2006) and large observational studies have consistently revealed an association between frequent nut consumption and reduced incidence of coronary heart disease (Kelly & Sabaté 2006). A meta-analysis of 13 intervention studies using walnuts (Banel & Hu 2009) and a pooled analysis of 25 intervention studies with a range of nuts indicated a consistent cholesterol-lowering effect (Banel & Hu 2009). The analysis in the latter review revealed a 7.4 % reduction of LDL-cholesterol with a mean nut consumption of 67 g per day. Reductions in LDL-cholesterol were dose-dependent, but not dependent on the type of nut consumed

(Sabate, Oda & Ros 2010). The lipid lowering effects may be attributed to the high content of unsaturated fat and fibre in nuts (Kendall et al. 2001). Other bioactive nutrients in nuts may benefit gluco-regulation, endothelial function, blood pressure control (Casas-Agustench et al. 2011b) and inflammation (Casas-Agustench, Bullo & Salas-Salvado 2010). Studies have demonstrated that higher nut consumers are at a significantly lower risk of non-cardiovascular inflammatory disease mortality (Gopinath et al. 2011) and risk of developing type 2 diabetes (Salas-Salvadó, Jordi et al. 2011) than low nut consumers. These benefits may be attributed to their nutrient profile; plant derived omega-3 fatty acids (alpha-linolenic acid (ALA)) found in walnuts have been shown in clinical and epidemiological studies to improve inflammation, arterial compliance, insulin resistance, endothelial function and blood pressure (Mozaffarian 2005; Muramatsu et al. 2010; Paschos et al. 2007; Sala-Vila & Ros 2011). Nuts, especially consumed with their skin intact, have a significant amount of polyphenols (Bolling et al. 2011). The results of many epidemiological studies suggest that the intake of polyphenol-rich foods have a beneficial effect on a large number of cardiovascular risk factors including high blood pressure and poor vascular function (Lecour & Lamont 2011). Polyphenols and vitamin E may have a role in modifying some of the inflammatory mediators (Biesalski 2007; Singh, Devaraj & Jialal 2005) and be beneficial for cognitive performance (Collie & Morley 2007; Joshi & Praticò 2012). Unsalted nuts contain high levels of potassium and magnesium making them a potential food for blood pressure control. However nuts are commonly sold as a highly salted product and in this form can substantially increase the intake of sodium hence reducing their potential benefit. In addition, nuts contain fibre and L-arginine which has been shown to improve endothelial function (Brock et al. 2006; King 2005; Lekakis et al. 2002; Sánchez-Muniz 2012). Studies have investigated the impact of nuts on endothelial function (Casas-Agustench et al. 2011b), however no study has taken the next step and considered whether nuts may have beneficial effects on cerebral vascular function and little research has been conducted on the impact of nut consumption on cognitive performance.

Thus, unsalted nuts contain the precursor, key ingredients for cardio-metabolic benefits needed to enhance blood vessel health which may in turn improve cognitive function and limit cognitive decline as proposed in **Figure 3.1**. Using a systematic search protocol, we reviewed the evidence for the effects of both tree nuts and ground nuts on gluco-regulation, blood pressure, arterial compliance, inflammation, endothelial vasodilator function and cognitive performance. As noted previously there is a large body of consistent evidence demonstrating improvements in lipid regulation with nut consumption (Banel & Hu 2009; Sabate, Oda & Ros 2010), hence this component has not been included in the current review.

Table 3.1 Nutritional composition of nuts (per 100g)

Nutrient	Almonds	Brazils	Cashews	Hazelnuts	Macadamias	Pecans	Peanuts	Pistachios	Walnuts
Energy (kJ)*	2432	2755	2323	2639	3015	2902	2381	2360	2747
SFA (g)*	4	15	8	4	12	7	7	6	6
MUFA (g) *	32	25	24	46	59	40	24	24	9
PUFA (g)*	12	21	8	8	2	21	16	14	47
α-linolenic (g)*	0.0	0.2	0.2	0.1	0.1	0.6	0.0	0.0	6.0
Protein (g)*	21	14	18	17	8	9	25	20	15
Arginine(g)*	2.5	2.2	2.0	2.2	1.2	3.0	1.2	2.2	2.3
Fibre (g)*	13	9	3	10	9	10	9	10	7
Total vitamin E (mg)†	27	4	1	33	1	4	8	7	6
Folate (µg)*	49	22	25	113	11	22	240	51	98
Sodium (mg)*	1	3	12	0	5	0	18	1	2
Potassium (mg)*	733	659	660	680	368	410	705	1025	441
Magnesium (mg)*	270	376	292	163	130	121	168	121	158
Anthocyanins (mg) ‡	184	0	9	501	0	494	16	237	67
Flavonoids (mg)¶	40	29	42	14	9	639	146	87	535
Resveratrol ()§	NA	NA	NA	NA	NA	NA	102	117	NA
Total antioxidant content (with pellicle)‖	0.41	0.25	0.39	0.71	0.42	8.3	2.0	1.3	23.1
Phytosterols (mg)*	114	51	158	85	86	117	22	47	18

Data from: *US Department of Agriculture, Agricultural Research Service,
†Kornsteiner et al 2006,
‡Bolling et al. 2011,
¶Yang et al. 2009,
§Tokusoglu et al 2005,
‖Blomhoff et al. 2006.
Abbreviations: SFA, saturated fatty acid, MUFA, monounsaturated fatty acid, PUFA, polyunsaturated fatty acid

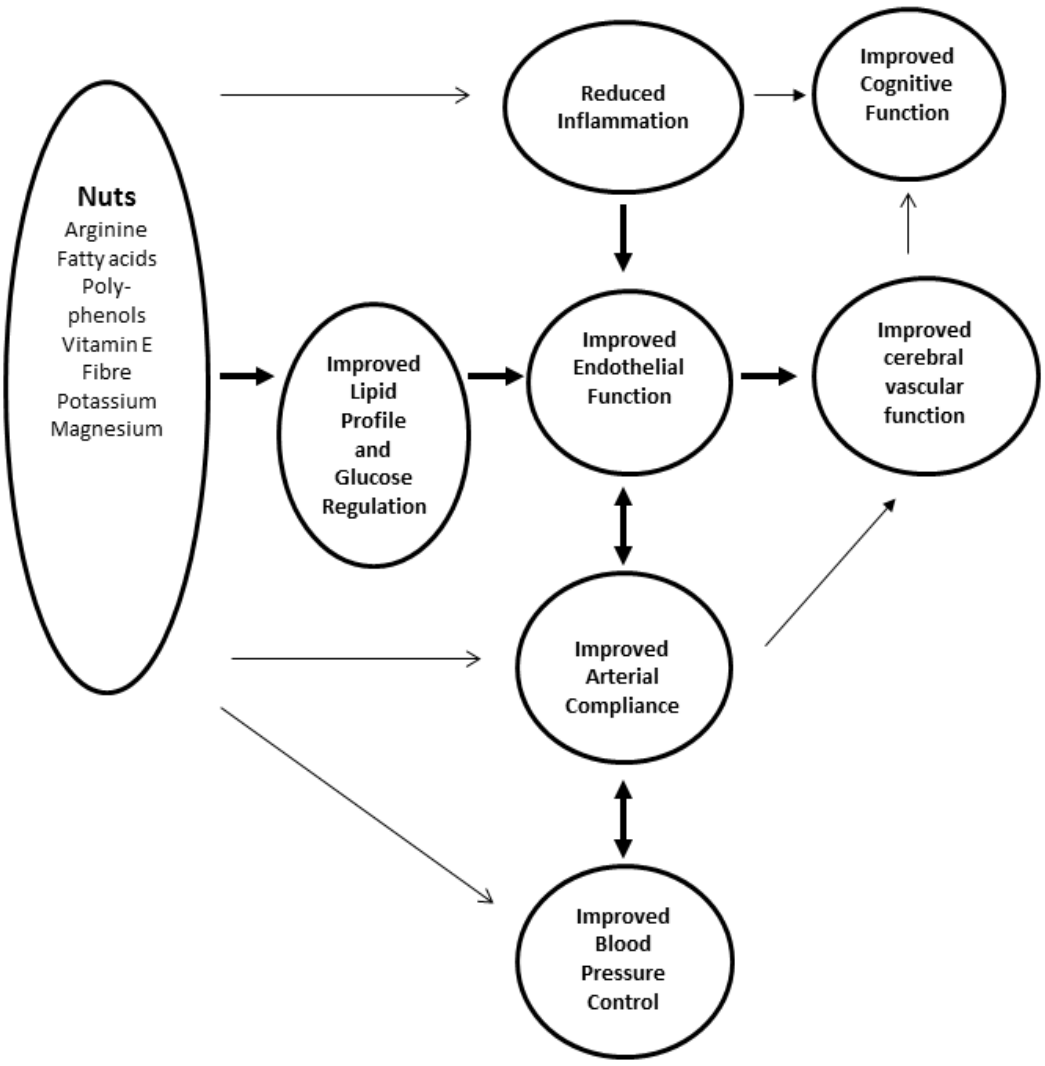


Figure 3.1.Summary of potential effects of nutrients in nuts to improve cardiovascular risk factors (lipid profile, arterial compliance, blood pressure and inflammation) and consequent improvement in endothelial function and potential improvement in cerebral vascular function and hence cognitive performance.

→ = weak evidence ➡ = strong evidence ↔ = bi-directional effect

3.4 Methods

3.4.1 Selection of studies

Medline (via Ovid) and CINAHL (via Ebsco host), Embase databases and the Cochrane Library were searched on 21 November 2012. Search terms used included MeSH (Medical Subject Headings) terms: 'nuts' OR 'almond*' OR 'Brazil nut*' OR 'cashew*' OR 'hazelnut*' OR 'macadamia*' OR 'peanut*' OR 'pecan*' OR 'pistachio*' OR 'walnut*' AND 'endothel*' OR 'FMD' OR 'vascular*' OR 'blood pressure' OR 'arterial compliance' OR 'vasodilatation' OR 'glucose' OR 'insulin' OR 'inflam*' OR 'cognit*'. Limits included 'human only' and 'English language'. In addition, reference lists from the publications identified by the database searches were also manually searched to identify other relevant articles that were not detected by the searches. Studies were included if they met the following criteria: intervention or epidemiological studies in human subjects. Intervention diets included at least one of the following nuts: almonds, cashews, hazelnuts, macadamias, groundnuts, pistachios, walnuts, pecans or Brazil nuts. Intervention studies included assessment of chronic nut consumption for a minimum period of 3 weeks, thereby assessing chronic changes. Published studies were required to be original research and evaluate the effects of nuts on at least one of the following in human subjects: glucose regulation, endothelial vasodilator function, arterial compliance, resting blood pressure, inflammation or cognitive performance.

Studies were excluded if they were non-English-language papers, narrative reviews, systematic reviews, expert opinions, editorials, abstracts, letters to the editor, theses, or animal or in vitro studies. Data were reported for participants who completed studies. Weighted mean changes in glucose regulation, systolic and diastolic blood pressure, CRP, ICAM-1, VCAM-1 and endothelial vasodilator function were calculated for studies that reported available data for calculating a percentage change (changes are between nut and control treatments, in instances where there is no control this is indicated in brackets next to the value "vs. baseline"). Effect size was calculated from change in nut intervention minus change in control divided by standard deviation of the

change. Where no control was used the effect size was calculated as the change from baseline to end of intervention divided by standard deviation. Study sample size was used to weight the calculation of the overall mean percentage change across studies using STATA software (StataIC 11; StataCorp LP). For observational studies relative risk (RR) and odds ratio (OR) are presented as adjusted values.

3.5 Results

The search revealed articles published between March 1993 and October 2013. Of the 4198 articles identified by all databases and nine articles identified from hand searching, 3019 were excluded as duplicates, 114 were excluded because of document type (review, note, letter, proceedings paper, or meeting abstract) and 837 were excluded because they did not assess endothelial function, blood pressure, inflammation, glucose regulation or cognitive performance in conjunction with nut consumption. Of the 237 articles screened (titles and abstracts), 166 were excluded because they did not meet the inclusion criteria. Therefore, seventy-one studies were included in the present review as shown in the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) flowchart (**Figure 3.2**). A total of forty-four studies evaluated blood pressure, thirty-two evaluated glucose regulation, thirty-one evaluated inflammatory markers, nine evaluated endothelial vasodilator function, two evaluated arterial compliance and four evaluated cognitive performance. A total of nine types of nut were used in these studies: almonds, Brazil nuts, cashews, hazelnuts, macadamias, groundnuts, pistachios, pecans and walnuts. The majority of studies examined walnuts, almonds and mixed/any nuts (**Table 3.2**).

Most research measuring the effect of nut consumption on glucose regulation, blood pressure, inflammation, arterial compliance, endothelial vasodilator function and cognition has been performed with walnuts, mixed or non-specified nuts, almonds and pistachios, with only seven studies using groundnuts, hazelnuts, cashews, Brazil nuts or macadamias and no studies with pecans (**Table 3.2**).

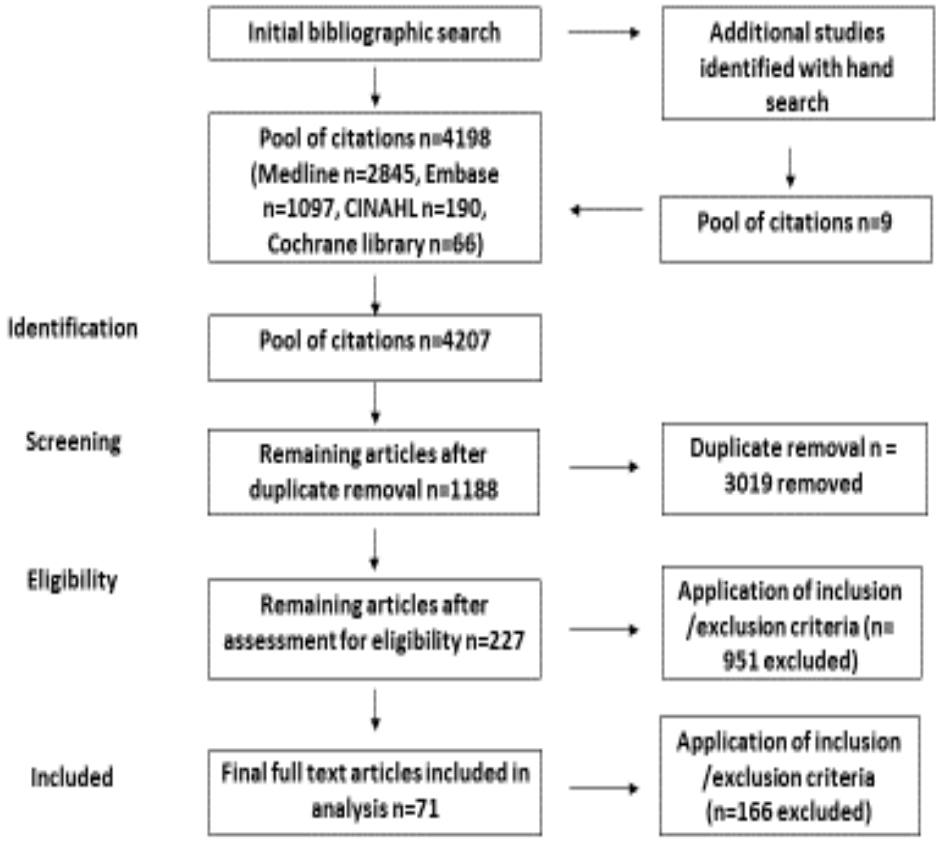


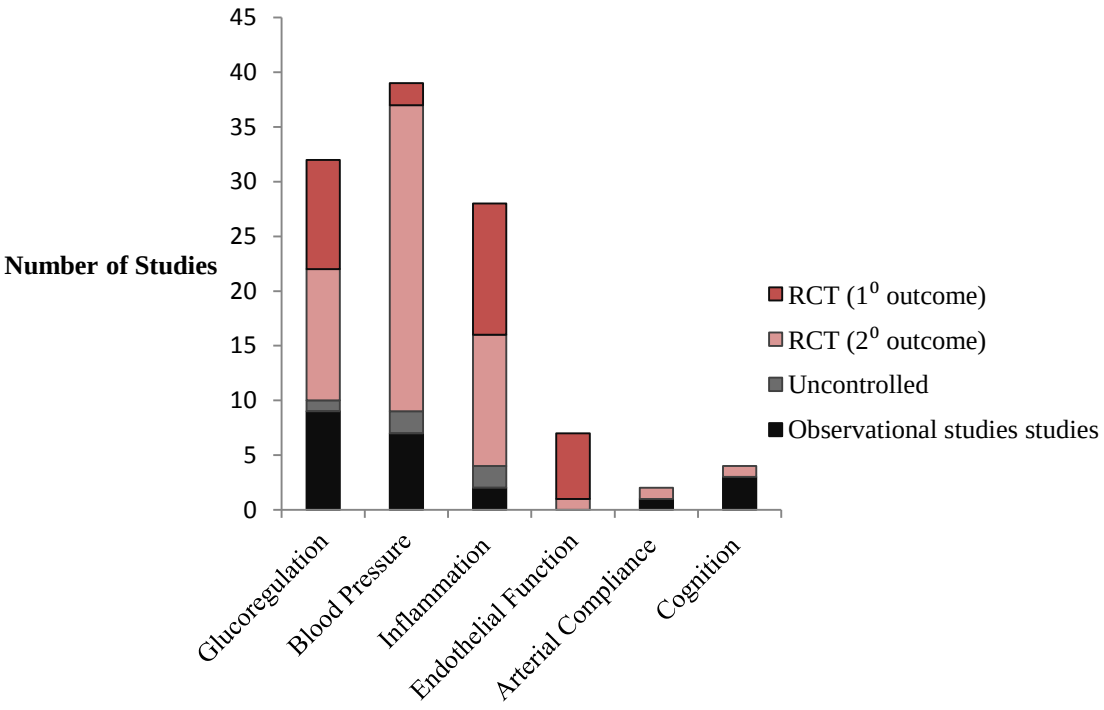
Figure 3.2 PRISMA flow chart showing procedures used to identify studies included in scoping search

Table 3.2 Number of measures of nut consumption on the effect on blood pressure, glucose regulation, inflammation, arterial compliance, endothelial function and cognition (some studies tested more than one type of nut)

Nut type	Blood pressure	Glucose regulation	Inflammation	Arterial compliance	Endothelial function*	Cognition
Walnut	11	9	9	1	4	2
Mixed/any nut	14	12	10	1	2	2
Almond	10	8	8	-	-	-
Pistachio	3	2	1	-	2	-
Hazelnut	1	1	2	-	1	-
Cashew	1	1	1	-	-	-
Brazil	-	-	1	-	-	-
Macadamia	1	-	-	-	-	-
Peanut	1	1	-	-	-	-
Pecan	-	-	-	-	-	-
Total	**42**	**34**	**31**	**2**	**9**	**4**

* endothelial vasodilator function assessed by either flow mediated dilatation or Endopat

Studies are summarised in **Tables 3.3–3.8** and are grouped according to outcomes, presented in order of efficacy (using mean percentage or blood pressure (mmHg) change where available). The following information was also extracted: author and year of publication, number, age and sex of the participants, type of individuals studied (i.e. healthy, hyperlipidaemic, high CHD risk, type 2 diabetes, overweight/obese or metabolic syndrome), study design, length of intervention, type and dose of nut, controls used and effect-size calculations where possible. **Figure 3.3** presents the number of outcome measures and the type of studies reflecting the level of evidence for these studies according to National Health and Medical Research Council guidelines (National Health & Medical Research Council 2000). Most studies were randomised and controlled, providing greater evidence than uncontrolled or non-randomised trials.



RCT = randomised control trial, 1^0 = primary 2^0 = secondary

Figure 3.3 Number of studies measuring blood pressure, endothelial function, inflammation, arterial compliance and cognition as epidemiological, primary or secondary outcome

3.5.1 Effects of nut consumption on glucose regulation

Details of studies measuring effect of nut consumption on gluco-regulation are reported in Table 3.3. Eight observational and 24 intervention trials evaluated the effects of chronic consumption of nuts on gluco-regulation. Nuts consumed included walnuts, pistachios, peanuts, almonds, cashews and mixed nuts, with amounts consumed ranging from 10g to 108g per day (⅓ oz-4 oz) (approximately 2-20 % of energy intake). The duration of consumption ranged from 4 weeks to 16 years. Intervention studies made comparisons with a healthy diet (14 studies), habitual diet (3 studies), high fat diet (1 study) or other food products (5 studies): muffins, pretzels, cereal bar, cheese or another type of nut. One study used no control. Four studies compared habitual or healthy diets with intervention diets including nuts (Nordiet (Adamsson et al. 2011) or a Mediterranean diet (Esposito et al. 2004; Llorente-Cortés et al. 2010; Thomazella et al. 2011).

Tree nuts were associated with a lower prevalence of fasting hyperglycaemia compared with non-nut consumers in the NHANES cohort study (O'Neil et al. 2011). However, a healthy dietary pattern including nuts found no association with fasting glucose or insulin (León, Henríquez & Serra-Majem 2006). It is possible that the amount of nuts consumed was insufficient to show benefits. Nut consumption has also been associated with a reduced risk of type 2 diabetes; evidence to support this comes from large epidemiological studies (Jiang et al. 2002; Martinez-Gonzalez, MA et al. 2008; Nettleton et al. 2008; Pan et al. 2013). The Nurses' Health study demonstrated consumption of nuts (≥ 5 times per week), peanut butter (≥ 5 times per week) or walnuts (≥ twice per week) was associated with a 24 %, 21 % and 15 % lower risk respectively of developing type 2 diabetes (Jiang et al. 2002; Pan et al. 2013) compared with those who never or rarely ate nuts, the effect was greatest in those of healthy body weight (Jiang et al. 2002). In addition the Shanghai Womens' Health study demonstrated peanut consumption was associated with a 22% lower risk of type 2 diabetes (Villegas et al. 2008). The SUN (Seguimiento University of Navarra) study demonstrated a 35 % lower risk of type 2 diabetes with a Mediterranean diet including an unspecified quantity of nuts. However, other components of the

Mediterranean diet including olive oil and a high fibre intake may have also contributed to this outcome (Biesalski 2004). In contrast the Iowa Women's Health Study did not find any association of consumption of foods high in vegetable fat (including nuts) and incidence of type 2 diabetes, (Meyer et al. 2001) which may in part be due to the low mean intake of nuts in this cohort.

Clinical trials examining nut consumption and diabetes risk, glycaemic control or insulin resistance have suggested some beneficial effects. Some short-term intervention studies have shown benefits of nut consumption on glucose homeostasis (Estruch et al. 2006; Sari et al. 2010) and insulin secretion. (Adamsson et al. 2011; Casas-Agustench et al. 2011a; Estruch et al. 2006; Wien et al. 2010). The effects of nuts on insulin sensitivity are influenced strongly by changes in body weight which may have accounted for the changes observed in one of these studies where participants reduced body weight with nut consumption. Longer intervention trials with Mediterranean diets supplemented daily with 20-50 g of walnuts or 30 g of mixed nuts (a mixture of walnuts, almonds and hazelnuts was used in the PREvencion con DIeta MEDiterranea (PREDIMED) trial as reported by Casas Agustench et al. 2011 resulted in a reduction in fasting glucose, insulin and improvement in insulin sensitivity (HOMA) (Esposito et al. 2004) and the incidence of type 2 diabetes by 52% over 4 years (Salas-Salvadó, Jordi et al. 2011). Benefits shown in studies with nuts included as part of the intervention diet (Nordiet (Adamsson et al. 2011) or Mediterranean diet (Esposito et al. 2004)) may have been partly due to other components of these diets (Kalgaonkar et al. 2011). Other studies have not shown benefits; consumption of pistachios, almonds, walnuts and a Mediterranean diet (supplemented with 10 g/day of nuts) revealed no effect on fasting glucose or insulin (Cohen & Johnston 2011; Hongyu et al. 2010; Jenkins et al. 2011; Kasim-Karakas et al. 2004; Li, Z et al. 2010; Llorente-Cortés et al. 2010; Lovejoy et al. 2002; Ma et al. 2010; Mercanligil et al. 2007; Mukuddem-Petersen et al. 2007; Tapsell et al. 2004; Tapsell et al. 2009; Thomazella et al. 2011; Wien et al. 2003; Zaveri & Drummond, S 2009). One study which failed to achieve an improvement in insulin sensitivity supplemented participants' diets with 100 g of almonds per day for 4 weeks. In this study there was a significant

weight gain which may have masked any benefit on insulin control (Lovejoy et al. 2002). Unexpected increases in plasma glucose (but not insulin) were observed with walnut and cashew consumption in women with polycystic ovary syndrome and adults with metabolic syndrome respectively (Kasim-Karakas et al. 2004; Mukuddem-Petersen et al. 2007). Other studies investigated HbA1c in individuals with type 2 diabetes and found 28 g/day of walnuts and 36g/day of almonds reduced HbA1c by 4 % (Cohen & Johnston 2011; Kalgaonkar et al. 2011). However, in other individuals, HbA1c did not change with 37-75 g of mixed nuts, (Jenkins et al. 2011) 30-50 g of walnuts, (Ma et al. 2010; Tapsell et al. 2004; Tapsell et al. 2009) or 57-112 g of almonds /day (for 4 weeks) (Lovejoy et al. 2002). The lack of effect in the latter study may have been due to the short intervention time. Whilst epidemiological studies suggest an association of nut consumption with improvement in gluco-regulation and diabetes risk, not all evidence from randomised controlled trials is supportive. Some inconsistencies in findings may be attributed to variations in the number or health status of the study participants, length of trial, or the dose of nuts used. In addition, it has been shown that when fat intake exceeds 37 % of energy intake this can impact on insulin sensitivity (Rasmussen et al. 2006; Vessby et al. 2001), which could impact on those studies with large doses of nuts.

Weighted mean changes in gluco-regulation indicate significant reductions in; fasting insulin, and HOMA scores of 14% (95 % CI -24, -4.5) and 34 % (95% CI -49, -19) respectively with small non-significant reductions of 2.8 % (95 % CI -6.9, 1.3) and 1% (95 % CI -3, 0.9) for fasting glucose and HbA1c respectively (see **Table 3.9**). This indicates positive effects of nut consumption on the most widely accepted markers of gluco-regulation. Overall, there is considerable evidence of benefits of nut consumption for glycaemic control and insulin sensitivity and observed after 4-6 weeks of consumption. However, inconsistencies make it difficult to reach precise conclusions on the role of nuts. The target population, dose and length of consumption (particularly to observe changes in HbA1c) need to be further considered so that targeted advice can be provided to consumers.

Table 3.3 **Studies measuring effect of nut consumption on glucose regulation**

Observational studies measuring nut consumption, diabetes risk and elevated glucose *(studies presented in order of efficacy)*							
Author	**Time**	**Participants (age = mean (sd) unless indicated**	**Study Design**	**Amount/type of nuts**	**Outcomes**		**Effect**
Martinez-Gonzalez et al. 2008	4yr	N=13380, healthy, M/F, 38(12)yr	Prospective cohort (SUN study) FFQ and incidence of Dm	Tertiles Med diet (unspecified amount of nuts)	**RR** 1.0 0.41 (0.2 to 0.9) 0.17 (0.04 to 0.8)	Med diet ↓ 35% RR incidence of Dm	+
Jiang et al. 2002	16yr	N=137856, healthy women, 46yr (range 34-59yr)	Prospective cohort (Nurses' Health Study) FFQ and incidence of Dm	Quantiles of nuts/seeds Never/rare < 1 time/wk 1-4 times/wk >5times/wk	**RR** 1.0 0.98 (0.9-1.1) 0.91 (0.8-1.0) 0.79 (0.7-0.8)	Nuts ↓ 24% RR, peanut butter ↓21% RR incidence Dm	+
Villegas et al. 2008	5yr	N=64227, healthy women 49yr (range 43-63yr)	Prospective cohort (Shanghai Womens' Health study) FFQ and incidence of Dm	Quintiles of peanut consumption	**RR** 1.0 0.8 (0.7- 0.9) 0.95 (0.82- 1.1) 0.79 (0.7- 0.9) 0.8 (0.7- 0.9)	Peanuts ↓ 20% RR incidence Dm	+
Pan et al. 2013	4yr	N=137856, healthy women, 52(10)yr	Prospective cohort (Nurses' Health study cohorts 1&2) FFQ and incidence Dm	Quintiles of walnut consumption Never/rare <1 serve/wk 1serve/wk ≥2 serves/wk	**RR** 1.0 1.01 (0.95-1.08) 1.01 (0.90-1.13) 1.04 (0.92-1.18)	Walnuts ↓ 15% RR incidence Dm	+
Meyer et al. 2001	11yr	N=7210 , high risk CVD, M/F, 68(6)yr	X- sectional, FFQ, prevalence elevated glucose (PREDIMED)	Quintiles of vegetable fat (incl. nuts)	N/A	No assoc. incidence Dm with nut consumption	0

Table 3.3 (continued)

Cross sectional studies measuring nut consumption, diabetes risk and elevated glucose *(studies presented in order of efficacy)*						
Author	**Time**	**Participants (age = mean (sd) unless otherwise stated)**	**Study Design**	**Amount/type of nuts**	**Outcomes**	**Effect**
Nettleton et al. 2008a	X-sect	N=5011, healthy, M/F, 45-84yr	X-sect, Multi Ethnic Study of Atherosclerosis (MESA), FFQ and prevalence of Dm	Quintiles healthy dietary pattern (incl. any nuts)	↓ 15% RR incidence Dm with nut consumption	+
O Neil et al. 2011	X-sect	N=13 292, general pop. M/F, 19-50+ yr	X-sect, 1999- 2004 National Health and Nutrition Survey (NHANES), 24 hour recall and prevalence elevated glucose	"All" nut group ≥ 7g/d (A)	No assoc. prevalence of elevated glucose with "all" nut consumption.	0
				Tree nut group ≥7g/d (B)	4% ↓ prevalence elevated glucose with tree nut consumption	+
Ibarrola-Jurado et al. 2013	X-sect	N=7210, high risk CVD, M/F, 68(6)yr	X-sect FFQ, prevalence elevated glucose (PREDIMED)	Tertiles (any nuts) <28g/wk 1.0 28-84g/wk 0.95 (0.71-1.29) >84g/wk 0.85 (8.81-1.53)	No assoc. elevated glucose with nut consumption	0

Intervention studies measuring effect of nut consumption on glucose regulation *(studies presented in order of efficacy)*						
Author	**Time**	**Participants** (age = mean (sd) unless otherwise stated)	**Study Design**	**Amount /type nuts**	**Outcome (active vs control)**	**Effect (size)**
Salas-Salvado et al. 2011	4 yr	N=418 (control =134, nuts=145, OO=139) high risk CVD, M/F, 68yr (range 55-80yr)	RCT parallel, Med diet + OO vs. Med diet + nuts vs LF diet (control) (PREDIMED)	30g/d mixed nuts†	↓ 52% RR incidence Dm	+
Esposito et al. 2004	2 yr	N=180 (control=90, Med diet =90), Met-S, M/F, 44(6)yr	RCT parallel, Med diet (incl. nuts) vs prudent diet (control)	20-50g/d walnuts	Glucose ↓5%* Insulin ↓33%* HOMA ↓45%*	+ (0.8) + (0.8) + (0.8)
Wien et al. 2010	16 wk	N=65 (control=32, nut=33) pre-diabetes, M/F, 53(9)yr	RCT parallel, American Diabetes Association diet (control) with/without almonds	56g/d almonds	Glucose ns Insulin ↓32%* HOMA ↓40%* HbA1c ns	0 (0.2) + (1.3) + (0.2) 0 (0.4)
Casas-Agustench et al. 2011	12 wk	N=50 (control=25, nut=25), Met-S, M/F, 52(8)yr	RCT parallel, iso-energetic healthy diet (control) with/without nuts	30g/d mixed nuts†	Glucose ns Insulin ↓33%* HOMA ↓33%*	0 (0.0) + (0.4) + (0.4)

Table 3.3 **(**continued) **Intervention studies measuring effect of nut consumption on glucose regulation** *(studies presented in order of efficacy)*

Author	**Time**	**Participants** (age = mean (sd) unless otherwise stated)	**Study Design**	**Amount /type nuts**	**Outcome (active vs control)**	**Effect (size)**
Adamsson et al. 2011	4 wk	N=86, M/F, (control=42, Nordiet=44), 53(8)yr hypercholesterolaemic	RCT parallel, Nordiet (high fiber, fish, LF dairy, nuts) vs habitual diet (control)	ad libitum almonds	Glucose ns Insulin ↓24%* HOMA ↓25%*	0 (0.0) + (0.5) + (0.6)
Sari et al. 2010(59)	4 wk /arm	N=32, healthy men, 22yr (range 21-24 yr)	Prospective cohort, iso-energetic Med diet (control) with/without pistachios, no washout	80-100g /d pistachios	Glucose ↓9%	+ (0.9)
Kalgaonkar et al. 2011	6 wk	N=31, (almond=14, walnut=17), women with PCOS, age range 20-45yr	Pre/post measures walnuts vs almonds	36g/d walnuts (W) 26g/d almonds (A)	Glucose ns (W vs A) Insulin ns (W vs A) HOMA ns (W vs A) HbA1c ↓4% * (W vs A)	0 0 (0.8) 0 (2.3) + (0.1)
Estruch et al. 2006	12 wk	N=772(control=257, OO=257, nuts=258), high risk CVD, M/F, 69(6)yr	RCT parallel, Med diet + OO vs Med diet + nuts vs LF diet (control) (PREDIMED)	30g/d mixed nuts†	Glucose ↓* data NA Insulin ↓* data NA HOMA ↓* data NA	+ (0.1) + (0.2) + (0.3)
Cohen et al. 2011	12wk	N=13(control=6, nut=6), Dm, 66(3)yr	Pilot study, RCT parallel almonds or cheese (control)	28g/d almonds	Glucose ns Insulin ns HbA1c ↓ 4% *	0 (0.3) 0 (0.7) + (1.5)
Jenkins et al. 2011	3 mth	N=117, (control=37, 37g/d nut=40, 75g/d nut=40), Dm, M/F, 62(10)yr	RCT parallel, 75g/d nut vs.37g/d nut + ½ dose muffin vs muffin (control)	75g or 37g/d mixed nuts‡	Glucose (75g) ns Glucose (37g) ns HbA1c (75g) * HbA1c (37g) ns	0 (0.2) 0 (0.2) + 0
Lovejoy et al. 2002	4 wk/ arm	**Study 1,** N=20, healthy, 25(1)yr	Prospective cohort (no control) habitual diet + almonds	100g/d almonds	Glucose ns Insulin ns	0 (0.2) 0 (0.1)
		Study 2, N=30, Dm, 54(2)yr	RCT X over, high fat (HF) (control) with/without almonds and low fat (LF) (control) with/without almonds	57-113g/d (10%E) almonds	HF glucose ns LF glucose ns HF insulin ns LF insulin ns HF HbA1c ns LF HbA1c ns	0 (0.3) 0 (0.2) 0 0 0 0
Thomazella et al. 2011	12 wk	N=40 (control=19, Med diet=21),males with CVD, 55(5) yr	Prospective controlled study Med diet (incl. nuts) vs. LF diet (control)	10g/d any nuts	Glucose ns	0 (0.4)

Table 3.3 **(**continued) **Intervention studies measuring effect of nut consumption on glucose regulation** *(studies presented in order of efficacy)*

Author	**Time**	**Participants** (age = mean (sd) unless otherwise stated)	**Study Design**	**Amount /type nuts**	**Outcome (active vs control)**	**Effect (size)**
Mercangil et al. 2007	4 wk/ arm	N=15, males, 48(8)yr hypercholesterolaemic,	2 period study, LF diet (control) vs LF + hazelnuts, non iso-energetic	40g/d hazelnuts (12% E)	Glucose ns	0 (0.3)
Llorrente Cortes et al. 2011	12 wk	N= 49(OO=16, nut=15, control=15), high risk CVD, M/F, 66(7)yr	RCT parallel Med diet + OO vs Med diet + nuts vs LF diet (control) (PREDIMED)	30g/d mixed nuts†	Glucose ns	0 (0.4)
Wien et al. 2003	24 wk	N=52 (control=28, nut=24), M/F, overweight/obese, 55(2)yr	RCT parallel, iso-energetic, LC diet + almond vs. CHO (control)	84g/d almonds	Glucose ns Insulin ns HOMA/ HbA1c ns	0 (0.0) 0 (0.3) 0 (0.0)
Zaveri et al. 2009	12wk	N=36 (control=13, cereal bar=14, almond=18), healthy M/F, 40(7)yr	RCT parallel healthy diet with/without nuts or cereal bar (control)	56g/d almonds	Glucose ns Insulin ns	0 0
Li et al. 2013	12 wk	N=31, obese, M/F, (control= 28 pistachio=31), 45(7)yr	RCT parallel iso-energetic prescribed diet + pretzels (control) or pistachios	53g/d pistachios	Glucose ns Insulin ns	0 (-0.6) 0 (1.8)
Tapsell et al. 2004	6 mth	N=41 (control=21, walnut=20), Dm, M/F, 60(8)yr	RCT parallel iso-energetic low fat diet (control) with/without walnuts	30g/d walnuts	HbA1c ns	0 (0.0)
Tapsel et al. 2009	12 mth	N=34 (control=17, walnut=17), overweight, Dm, M/F, 55(9)yr	RCT parallel iso-energetic low fat diet (control) with/without walnuts	30g/d walnuts	Glucose ns Insulin ↑6%* HbA1c ns	0 (0.4) 0 (-0.3) 0 (0.1)
Mukuddem-Peterson et al. 2007	8 wk	N=64 (control=22, walnut=21, cashew=21), Met-S, M/F, 45(8)yr	RCT parallel iso-energetic LF diet (control) with/without walnuts or cashews	63-108g/d walnuts/ cashews	Cashew glucose ↑13%* Walnut glucose n.s	0 (-0.8) 0 (-0.6)
Ma et. al. 2010	8 wk/ arm	N=21, Dm, M/F, 58(8)yr	RCT X over, ad libitum diet (control) with/without nuts not iso-energetic, 8wk washout	56g/d walnuts	Glucose ns Insulin ns HOMA ns HbA1c ns	0 (-0.3) 0 (0.7) 0 (-0.4) 0 (0.0)
Kasim-Karakas et al.2004	3mth	N=17, 34(5)yr, women, PCOS	Prospective cohort iso-energetic habitual diet (control) vs walnuts	106g/d walnuts	Glucose ↑ 19%* Insulin ns HOMA ns	- (-4.5) 0 (0.2) 0 (1.0)

***Abbreviations*:** M/F, males and females, FFQ, food frequency questionnaire, d, days, wk, weeks, mth, months, yr, years, RCT, randomised controlled trial, Met-S, metabolic syndrome, Dm, type 2 diabetes, CVD, cardiovascular disease, PCOS, polycystic ovary syndrome, LC, low calorie diet, CHO, carbohydrate, LF, low fat, OO, olive oil, Med diet, Mediterranean diet, glucose ,fasting glucose, insulin, fasting insulin, HOMA, insulin resistance, N/A, data not available, RR, relative risk, sd, standard deviation, ns, no significant difference, †,mixed nuts = walnuts, almonds and hazelnuts, ‡, mixed nuts = almonds, pistachios, walnuts, peanuts, hazelnuts, pecans, cashews and macadamias, *, $p \leq 0.05$, **+,** significant reduction , 0, no significant change, -, significant increase.

3.5.2 Effects of Nut Consumption on Blood Pressure

Studies measuring the effect of nut consumption on blood pressure are found in **Table 3.4**. Nuts consumed included walnuts, pistachios, peanuts, almonds, cashews, hazelnuts, macadamias and mixed nuts in different forms including oil, whole nuts and nut flour added to baked goods. As with many studies using whole food products, participant blinding was not possible. Amounts consumed ranged from 10 g to 108 g per day (⅓ oz-4oz) (approximately 2-20 % of energy intake). The length of consumption ranged from 3 weeks to 2 years. Whilst there were 36 intervention trials which reported on the effect of chronic consumption of nuts on blood pressure, most measured blood pressure as a secondary outcome. Comparisons were made with a healthy diet (16 studies), habitual diet (7 studies) or other food products including; butter, muffins, processed meat, olive oil, and cocoa. Four studies compared habitual or healthy diets with intervention diets including nuts (Nordiet (Adamsson et al. 2011)) or a Mediterranean diet (Esposito et al. 2004; Llorente-Cortés et al. 2010; Thomazella et al. 2011); only one of the studies reported controlling for salt intake (Adamsson et al. 2011). The remaining studies used control diets with unsalted nuts added as the intervention but overall dietary salt intake was not specified. Four prospective cohort studies measured blood pressure or incidence of hypertension in participants consuming nuts. The Physicians' Study demonstrated a significant reduction in self-reported hypertension after 12 months in those consuming nuts ≥ twice per week, hazard ratio 0.87 (95 % CI 0.79, 0.96) and greatest reduction with consumption ≥ 7 times per week, hazard ratio 0.77 (95 % CI 0.64, 0.93) (Djoussé et al. 2010). However salt intake and changes in weight were not accounted for which could have affected outcomes observed. The CARDIA study demonstrated an inverse relationship between nut consumption and prevalence of hypertension despite those classified as the highest consumers only consuming nuts ≥ 2 times per week, hazard ratio 0.85 (95 % CI 0.64, 0.93) (Steffen et al. 2005). In support of this, the ARIC study also reported nut consumption was inversely related to a reduced risk of hypertension, those who consumed approximately 2 serves of nuts per week were at a lower risk of hypertension than those who rarely or never consumed

nuts, hazard ratio 0.87 (95 % CI 0.77,0.97) (Weng et al. 2013). In contrast, the SUN study demonstrated no association between hypertension and nut consumption after a four year follow up (Martínez-Lapiscina et al. 2010). However the young educated adult sample in this study is less likely to demonstrate improvements in blood pressure with a dietary pattern than older persons who are more likely to have higher blood pressure.

Four cross sectional studies were identified comparing blood pressure or prevalence of hypertension in nut consumers with low/non-nut consumers. The National Health and Nutrition Survey (NHANES) observed a general population and found a 3 % lower risk of hypertension and 1mmHg reduction in systolic and diastolic blood pressure in nut consumers (O'Neil et al. 2011). The Canary Nutrition Survey demonstrated a trend for reduced prevalence of hypertension with higher nut consumption but this did not reach significance (León, Henríquez & Serra-Majem 2006). The Multi Ethnic Study of Atherosclerosis (MESA) in Spain did not find an association with a healthy dietary pattern (incorporating an undetermined quantity of nuts) and blood pressure (Nettleton et al. 2008). The authors suggest that routinely assessed blood pressure may have increased risk factor awareness thereby attenuating associations with dietary intake. No association was found with hypertension and nut consumption in participants with a high risk of cardiovascular disease (Ibarrola-Jurado et al. 2013). However, 90 % of the participants were hypertensive which may have made it difficult to demonstrate a relationship in this population. It is more difficult to account for health benefits from an individual food with observational studies; hence intervention studies are important to isolate effects.

Significant reductions in blood pressure were observed in nine intervention studies (Adamsson et al. 2011; Esposito et al. 2004; Estruch et al. 2006; Jenkins et al. 2008b; Llorente-Cortés et al. 2010; Mena et al. 2009; Toledo et al. 2013; Wien et al. 2003), effect sizes could be calculated in seven of these and were small to large ranging between 0.2 and 1.1. A substantial reduction in systolic blood pressure (14mmHg) was reported in participants who were overweight or obese and mildly hypertensive consuming a diet containing 84 g/day of almonds

for 24 weeks, compared with an iso-energetic high carbohydrate diet (Wien et al. 2003), with some participants reducing or eliminating the use of antihypertensive medications during the duration of the study. A weight reduction of 7 kg (BMI reduction of 2.5 kg/m² (2 kg/m²) was also observed in the participants consuming nuts compared with the control, despite the two groups being prescribed iso-energetic diets which would have accounted for at least some of the reduction in blood pressure observed (Wien et al. 2003). The PREDIMED study tested the consumption of a Mediterranean diet which included 30g /day of mixed nuts compared with a Mediterranean diet devoid of nuts (Estruch et al. 2006). The study found a significant reduction in systolic and diastolic blood pressure of 7mmHg and 3mmHg respectively. This study used a large cohort of 772 participants, sub groups of this study with 49-106 participants also reported similar reductions in blood pressure (Fito et al. 2007; Llorente-Cortés et al. 2010; Mena et al. 2009). A larger cohort of the PREDIMED trial found only a significant reduction in diastolic blood pressure (Toledo et al. 2013). The Nordiet included nuts as part of the intervention diet, (Adamsson et al. 2011) demonstrated reductions in systolic and diastolic blood pressure of 6 mmHg (effect size 0.6) and 2 mmHg (effect size 0.3) respectively. Almonds (23 g/day) consumed of as part of a portfolio diet with plant sterols and soy for one year demonstrated a reduction in systolic and diastolic blood pressure in a single phase prospective study(Jenkins et al. 2008b). However as no control group was used, it is possible that the regular clinic visits in this study increased participant awareness of hypertension as a cardiovascular disease risk factor and other behaviour change may have contributed to reduction in blood pressure in addition to the almond intervention (Jenkins et al. 2008b), without a control group this could not be determined. Consumption of a Mediterranean diet including 20-50g/day of walnuts compared with a prudent diet demonstrated reductions in systolic blood pressure of 3 mmHg (effect size 0.7) in diastolic blood pressure of 2 mmHg (effect size 0.7) (Esposito et al. 2004).

The majority of remaining studies demonstrated either small blood pressure reductions which did not reach significance or no change. A reduction in systolic and diastolic blood pressure was observed with consumption of 40 g/day of

hazelnuts for four weeks from baseline; however this was not significantly different from the reduction observed with cocoa used as the control (Solà et al. 2012). Inclusion of a control food which is not likely to change inflammation or endothelial function may have been a better choice to determine the effects attributable to hazelnuts (Davison et al. 2010). An ad libitum diet with 56 g/day of walnuts consumed by participants with type 2 diabetes, for 8 weeks showed an increase in systolic (effect size -0.8) and diastolic (effect size -0.7) blood pressure. This unexpected result was from the only study which demonstrated a significant increase in blood pressure (Ma et al. 2010). Authors were not able to determine a reason for this increase in blood pressure. However, other factors in the diet such as sodium consumption may have contributed to the blood pressure elevation (despite being prescribed unsalted nuts); sodium intake was not reported or controlled for in this study. Interventions using a portfolio diet (Jenkins et al. 2002a; Jenkins et al. 2003), Nordiet (Adamsson et al. 2011) or Mediterranean diet (Esposito et al. 2004; Thomazella et al. 2011) contained foods other than nuts which may also have been beneficial for improvements in blood pressure, making it difficult to tease out the effects of nuts alone. In contrast, Mediterranean diets in which mixed nuts (Estruch et al. 2006; Llorente-Cortés et al. 2010) replaced olive oil demonstrated improvements in blood pressure, indicating there may be some beneficial effect of nuts above that of other components of the Mediterranean diet. The largest effects of nuts on blood pressure were seen in participants with metabolic syndrome or other risk factor for cardiovascular disease, consuming 30-84g per day of almonds, walnuts or mixed nuts for 4 weeks to 2 years. Significant reductions of 3-14mmHg and 2-3mmHg were observed in systolic and diastolic blood pressure respectively (Adamsson et al. 2011; Esposito et al. 2004; Estruch et al. 2006; Fito et al. 2007; Llorente-Cortés et al. 2010; Mena et al. 2009; Wien et al. 2003). Only 2 of 36 studies measured resting blood pressure as a primary outcome, so the remaining studies may not have been powered to detect small changes. In eight of the nine studies demonstrating blood pressure reductions, nuts were consumed for extended periods of between 12 weeks to two years. Most studies demonstrated no beneficial effect on blood pressure when nuts were consumed for shorter periods (3-12 weeks). This suggests a benefit of nut consumption only after an

extended period of time as indicated with observational studies where habitual nut consumption was associated with reduced blood pressure or reduced prevalence of hypertension.

Weighted mean changes in blood pressure were calculated for 24 of the 36 intervention studies; systolic and diastolic pressure were significantly reduced by 0.73% (95% CI -1.3, -0.2) and 0.75% (95% CI -1.1, 0.4) respectively (see **Table 3.9**). Improvements in blood pressure control were observed particularly when nuts were consumed regularly for extended periods of time. Although the effect of nut consumption on blood pressure is small this may still be clinically meaningful especially when used with other lifestyle measures.

Table 3.4 **Studies measuring effect of nut consumption on blood pressure**

Prospective cohort studies measuring nut consumption and hypertension *(studies are presented in order of efficacy)*						
Author	**Time**	**Subjects**	**Study Design**	**Amount/type of nuts**	**Outcomes** (95%CI)	**Effect**
Steffen et al. 2005	15 yr	N=4304, M/F, healthy, 18-30yr	Prospective cohort (CARDIA) diet history and prevalence of HT	Any nuts <0.1 serve/d 0.1-0.3/day > 0.3/day	**HR** 1.0 0.84 (0.73, 0.98) 0.85 (0.72, 0.99)	Inverse relationship between nut consumption and HT p for trend = 0.04	+
Djousse et al. 2009	12 mth	N=15966, free of HT, M/F, 52yr (range 45-64yr)	Prospective cohort, FFQ and self-reported risk HT (Physicians' Health study)	Any nuts None 1–3serves/mth 1/wk 2–6/wk ≥7/wk	**HR** 1.0 0.93 (0.86, 1.01) 0.94 (0.86, 1.03) 0.87 (0.79, 0.96) 0.77 (0.64, 0.93)	Nut consumption assoc. with ↓ risk hypertension p = 0.01	+
Weng et al. 2013	9 yr	N=9913, healthy M/F, 53(6)yr	Prospective cohort, Atherosclerosis Risk of Communities (ARIC), FFQ and BP	Quintiles nut consumption	**HR** 1.2 (0.98, 1.27) 0.96 (0.87, 1.07) 0.91 (0.81, 1.03) 0.87 (0.77, 0.97)	Nut intake inversely related to incidence HT p = 0.02	+
Martinez-Lapiscina et al. 2009	4yr	N=17177, healthy, M/F, 36yr (25-51yr)	Prospective cohort (SUN) study FFQ and self- reported HT	Any nuts, never/rarely to 2+/wk	**HR** highest vs. lowest intake	No assoc. with HT and nut consumption	0
Cross sectional studies measuring effect of nut consumption on blood pressure/hypertension *(studies are presented in order of efficacy)*						
Author	**Time**	**Subjects**	**Study Design**	**Amount/type of nuts**	**Outcomes**	**Effect**
O Neil et al. 2011	X - sect	N=13 292, general pop. M/F, 19-50+ yr (20% nut consumers)	1999- 2004 National Health and Nutrition Survey (NHANES) 24 hour recall and BP	"All" nut group ≥ 7g/d (A) Tree nut group ≥7g/d (B)	**HT** vs. non consumers ↓ 3% * ↓ 3%*	**BP** vs. non consumers (A)SBP ↓ 1mm Hg (p<0.01) (A)DBP ns (B)SBP ↓ 1mm Hg (p<0.01) (B)DBP ns	+ 0 + 0
Nettleton et al. 2008	X - sect	N=5089, healthy, M/F, 45-84yr	Multi Ethnic Study of Atherosclerosis (MESA) FFQ and BP	Quintiles healthy dietary pattern (incl. any nuts)	no data provided	No assoc. SBP, DBP with healthy dietary pattern	0
Leon et al. 2006	X - sect	N=578, healthy or Met-S, M/F, 18-75yr	Canary Nutrition Survey (ENCA) FFQ and BP	Tertiles (any nuts) Nuts T1 Nuts T2 Nuts T3	**OR HT (**95%CI) 1.0 0.73 (0.41-1.28) 0.83 (0.47-1.46)	No assoc. BP with nut consumption	0

Table 3.4 (continued) **Cross sectional studies measuring nut consumption and blood pressure/hypertension**

Time	Subject	Study Design	Amount/type of nuts	Outcomes	Effect		
Ibarrola-Jurado et al. 2013	X -sect	N=7210 , high risk CVD, M/F, 68(6)yr	X-sect, FFQ and prevalence of HT (PREDIMED)	Tertiles (any nuts) T1 <28g/wk T2 28-84g/wk T3 >84g/wk	**OR HT** 1.0 0.96(0.71–1.29) 1.12 (0.81–1.53)	No assoc. HT with nut consumption	0

Chronic effects of nuts on resting blood pressure *(studies are presented in order of efficacy)*

Author	Time	Subjects (age = mean (sd) unless otherwise stated)	Study Design	Amount /type nuts	Outcome (active vs control)	Effect (size)
Wien et al. 2003	24 wk	N=52 (control=28 nut=24), M/F overweight/obese, 55(2)yr	RCT parallel, iso-energetic, LC diet + almond vs CHO (control)	84g/d almonds	SBP ↓14mm Hg* DBP ns	+ (0.7) 0 (0.0)
Estruch et al. 2006	12 wk	N=772 (control=257, OO=257, nut= 258), high risk CVD, M/F, 69(6) yr	RCT parallel, Med diet + OO vs Med diet + nuts vs. LF diet (control) (PREDIMED)	30g/d mixed nuts†	SBP ↓7 mm Hg* DBP ↓3 mm Hg*	+ (0.3) + (0.2)
Esposito et al. 2004	2 yr	N=180 (control=90, Med diet =90), Met-S, M/F, 44(6)yr	RCT parallel, Med diet (incl. nuts) vs. prudent diet (control)	20-50g/d walnuts	SBP ↓3 mm Hg* DBP ↓2 mm Hg*	+ (0.7) + (0.7)
Adamsson et al. 2011	4 wk	N=86 (control=42, Nordiet=44), M/F, hypercholesterolaemic, 53(8)yr	RCT parallel, Nordiet (high fiber, fish, LF dairy, nuts) vs habitual diet (control)	ad libitum almonds	SBP ↓6mm Hg* DBP ↓3 mm Hg*	+ (0.6) + (0.4)
Llorrente Cortes et al. 2011	12 wk	N= 49(OO=16, nut=15, control=15), high risk CVD, M/F, 66(7)yr	RCT parallel Med diet + OO vs. Med diet + nuts vs. LF diet (control) (PREDIMED)	30g/d mixed nuts†	SBP ↓7mm Hg* DBP ns	+ (0.4) 0 (0.2)
Fito et al. 2007	12 wk	N=372 (OO=123, nut=128, control =127), high risk CVD, M/F, 66(9)yr	RCT parallel Med diet + OO vs Med diet + nuts vs. LF diet (control) (PREDIMED)	30g/d mixed nuts†	SBP ↓*(data N/A) DBP ↓*(data N/A)	+ +
Jenkins et al. 2008	1 yr	N=50, hyperlipidaemic, M/F, 59(1)yr	Single phase prospective study, pre-post measures, sterol+soy+almonds (no control)	23g/4.2MJ/d almonds	(vs. baseline) SBP↓4 mm Hg* DBP↓ 2mm Hg*	 + (0.4) + (0.5)
Mena et al. 2009	12 wk	N=106 (control=36, nut=35, OO=35) Dm, risk CVD, M/F, 66(7)yr	RCT parallel, Med diet + OO vs. Med diet + nuts vs. LF diet (control) (PREDIMED)	30g/d mixed nuts†	(vs baseline) SBP↓ 3mm Hg* DBP↓ 2mm Hg*	 + +
Toledo et al. 2013	4yr	N=7158(control=2064, OO=2345, nuts= 2065), M/F, risk CVD,67(6)yr	RCT parallel, Med diet + OO vs. Med diet + nuts vs. LF diet (control) (PREDIMED)	30g/d mixed nuts†	SBP ns DBP↓ 2mm Hg*	0 (0.2) + (1.1)
Wien et al. 2010	16 wk	N=65 (control=32, nut=33) prediabetes, M/F, 53(9)yr	RCT parallel, American Diabetes Association diet (control) with/without almonds	56g/d almonds	SBP ns DBP ns	0 (0.2) 0 (0.2)
Jenkins et al. 2011	3 mth	N=117, (control=37, 37g/d nut=40, 75g/d nuts=40), Dm, M/F, 62(10)yr	RCT parallel, 75g/d nut vs. 37g/d nut + ½ dose muffin vs muffin (control)	75g or 37g/d mixed nuts‡	SBP ns DBP ns	0 (0.2) 0 (0.2)

Table 3.4 (continued) **Chronic effects of nuts on resting blood pressure** *(studies are presented in order of efficacy)*

Author	**Time**	**Subjects** (age = mean (sd) unless otherwise stated)	**Study Design**	**Amount /type nuts**	**Outcome (active vs control)**	**Effect (size)**
Spaccarotella et al. 2008	8wk/ arm	N=21, healthy, M/F, 66yr (range 45-75yr)	RCT X over, habitual diet (control) with/without walnut (2 wk washout)	75g/d walnuts	SBP ns DBP ns	0 (0.1) 0 (0.2)
Wu et al. 2010	12 wk	N=277 (control=95, flax=94, nut=94), Met-S, M/F, 49(8)yr	RCT parallel iso-energetic LF diet (control) with/without flaxseed or walnuts	30g/d walnut flour	SBP ns DBP ns	0 (0.1) 0 (0.0)
Sari et al. 2010	4 wk /arm	N=32, healthy men, 22yr (range 21-24 yr)	Prospective cohort, iso-energetic Med diet (control) with/without pistachio, no washout	80-100g /d pistachios	SBP ns DBP ns	0 (0.3) 0 (0.1)
Din et al. 2011	4wk /arm	N=30, healthy, M/F, 23(3)yr	RCT X over, non iso-energetic habitual diet (control) with/without walnuts, no washout	15g/d walnuts	SBP ns DBP ns	0 (0.3) 0(-0.1)
Thomazella et al. 2011	12 wk	N=40 (control=19, Med diet=21), males with CVD, 55(5)yr	Prospective controlled study Med diet (incl. nuts) vs. LF diet (control)	10g/d any nuts	SBP ns DBP ns	0 (0.2) 0 (0.1)
Mukuddem-Peterson et al. 2007	8 wk	N=64 (control=22, nut=21, cashew=21), Met-S, M/F, 45yr(8)yr	RCT parallel iso-energetic LF diet (control) with/without walnuts (W) or cashews (C)	63-108g/d walnuts/ cashews	SBP ns W&C DBP ns W&C	0 (0.1) 0 (0.1)
Iwamoto et. al. 2002	4 wk /arm	N=80 healthy, M/F, 24(9)yr	RCT X over, Japanese diet with/without walnuts (no washout)	44-58g/d walnuts	SBP ns DBP ns	0 0
West et al. 2012	4 wk/ arm	N=25, hypercholesterolaemic, M/F, 48(2)yr	RCT X over, LF diet (control) vs. low dose pistachios vs. high dose pistachios, no washout	32-63g/d (A) or 63-126g/d (B) pistachios	A)SBP ns A)DBP ns B)SBP ns B)DBP ns	0 (0.1) 0 (0.1) 0 (0.0) 0 (0.0)
Jenkins et al. 2003	4 wk	N=25 (control=12, Nut=13) hyperlipidaemic, M/F, 59(1)yr	RCT parallel, plant sterol + soy + almond vs statin vs LF diet (control)	14 g/ 4.2MJ almonds	SBP ns DBP ns	0 (0.1) 0 (0.1)
Jenkins et al. 2002b	1 mth /arm	N=27, hyperlipidaemic, M/F, 64(9)yr	RCT X over iso-energetic almonds vs.½ dose almond + ½ dose muffin vs. muffin (control)	36g/73g d almonds	SBP ns DBP ns	0 (0.0) 0 (0.0)
Hiraoka-Yamamoto et al. 2004	3 wk	N=71 (control=24, coconut=23, nut=24), female students, 19(3)yr	RCT parallel, coconut vs. macadamia vs. butter (control)	10g/d macadamias	SBP ns	0 (0.0)
Damesceno et al. 2011	4 wk/ arm	N=18 hypercholesterolaemic, M/F, 56(13)yr	RCT X over iso-energetic Med diet + OO (control) vs. Med diet + walnut vs. Med diet + almond, no washout	40-65g/d walnuts 50-75g/d almonds	SBP ns DBP ns SBP ns DBP ns	0 (0.2) 0 (0..2) 0 (0.1) 0 (0.2)
Olmedilla-Alonso et al. 2008	5 wk /arm	N=25, high risk CVD, M/F, 54yr(8)yr	RCT X over, meat product with/without 20% walnut flour,1 mth washout	19g/d walnuts	SBP ns DBP ns	0(-0.2) 0 (0.0)

Table 3.4 (continued) **Chronic effects of nuts on resting blood pressure** *(studies are presented in order of efficacy)*

Author	**Time**	**Subjects** (age = mean (sd) unless otherwise stated)	**Study Design**	**Amount /type nuts**	**Outcome (active vs control)**	**Effect (size)**
Casas-Agustench et al. 2011	12 wk	N=50 (control=25 nut=25), Met-S, M/F, 52(8)yr	RCT parallel, iso-energetic healthy diet with/without nuts	30g/d mixed nuts†	SBP ns DBP ns	0(-0.2) 0(- 0.1)
Ghadimi et al. 2009	4 wk	N=108 (control=54, peanut=54) hypercholesterolaemic, M/F, 43(10)yr	RCT parallel, habitual diet (control) with/without peanuts	77g/d peanuts	SBP ns DBP ns	0(0.0) 0(0.0)
Ros et al. 2004	4 wk/ arm	N=20, hypercholesterolaemic, M/F, mean 55yr (26-75yr)	RCT X over, iso-energetic Med diet (control) with/without walnuts, no washout	40-65g/d walnuts	SBP ns DBP ns	0 (0.0) 0 (0.1)
Lopez-Uriate et al. 2010	12 wk	N=50 (control=25, nut=25), Met-S, M/F, 52(8)yr	RCT parallel American Heart Assoc. diet (control) with/without nuts, not iso-energetic	30g/d mixed nuts†	SBP ns DBP ns	0 0
Sola et al. 2011	4 wk /arm	N=113(control=28, nut=28, nut +sterol=30, nut+sterol+fibre =27), risk CVD, M/F, 54(9)yr	RCT parallel, iso-energetic cocoa+ nut vs. cocoa+nut+sterol vs. cocoa+ nut+sterol+fibre vs. cocoa (control)	30g/d hazelnuts	SBP ns DBP ns	0 0
Sabate et al. 1993	4 wk /arm	N=18 healthy men, 30yr (range 21-43 yr)	RCT X over , iso-energetic LF diet (control) with/without walnuts, no washout	20% E walnuts (84g /4.4MJ/d)	SBP ns DBP ns	0 0
Edwards et al. 1999	3 wk /arm	N=10 hypercholesterolaemic M/F, 46yr (range 41-64yr)	RCT X-over iso-energetic habitual diet (control) with/without pistachios (20% E)	(20% E) pistachios	SBP ns DBP ns	0 0
Spiller et al. 2003	4 wk	N= 38 (raw=14, roasted=14 butter=10), M/F, 32-74yr hypercholesterolaemic	RCT parallel LF diet + nuts (roasted vs. roasted butter vs. raw (control))	100g/d almonds/almond butter	SBP ns DBP ns	0 0
Jenkins et al. 2002a	4 wk /arm	N=13, hyperlipidaemic, M/F, 65(3)yr,	Prospective cohort LF diet followed by portfolio diet (soy/plant sterol/fibre/nuts)	28g/d almonds	SBP ns DBP ns	0 0
Schutte et al. 2006	8 wk	N=62 (control=21, walnut=20, cashew=21) Met-S, M/F, 45yr	RCT parallel, iso-energetic LF diet (control) with/without walnuts or cashews	63-103g/d walnuts or cashews	SBP ns DBP ns	0 0
Sheridan et. al. 2007	4 wk/ arm	N=15 hypercholesterolaemic, M/F, 60(12)yr	RCT X over, iso-energetic habitual diet (control) with/without pistachios, no washout	56-84g/d pistachio (15% E)	SBP ns DBP ns	0 (0.1) 0 (0.1)
Ma et. al. 2010	8 wk/ arm	N=21, Dm, M/F, 58(8)yr	RCT X over, ad libitum diet (control) with/without nuts not iso-energetic	56g/d walnuts	SBP ↑9 mmHg* DBP ↑4mmHg*	- (-0.8) -(-0.7)

Abbreviations **:** M/F, males and females, HT, hypertension, BP, blood pressure, SBP, systolic BP, DBP, diastolic BP, FFQ, food frequency questionnaire, T1, T2, T3 = tertiles, CI, confidence interval, se std error, d, days, wk, weeks , mth, months, yr, years, OR, odds ratio, HR, hazard ratio, RCT, randomised controlled trial, Met-S, metabolic syndrome, Dm, type 2 diabetes, CVD, cardiovascular disease, LC, low calorie diet, CHO, carbohydrate, LF, low fat, OO, olive oil, Med diet, Mediterranean diet, ALA, alpha linolenic acid, LA, linoleic acid, E, energy, N/A, data not available, sd, standard deviation, ES effect size, ns, no significant difference, †, mixed nuts = walnuts, almonds and hazelnuts, ‡, mixed nuts = almonds, pistachios, walnuts, peanuts, hazelnuts, pecans, cashews and macadamias, *, $p \leq 0.05$, **+,** significant ↓ BP/ HT, 0, no significant change in BP/HT, -, significant ↑ BP

3.5.3 Effects of nut consumption on inflammatory markers

Studies measuring the effect of nut consumption on inflammatory markers are found in **Table 3.5.** The most commonly measured inflammatory marker was C-reactive protein (CRP), reported in 27 of the 31 studies. Other inflammatory markers measured included tumour necrosis factor alpha (TNFα), interleukins (IL) 1, 1β, 6, 7, 18 and cellular adhesion molecules (ICAM-1, VCAM-1 and E-selectin). Four cross sectional studies and 27 intervention trials measuring inflammatory markers with nut consumption were identified. Eleven intervention studies compared nuts with a healthy diet (low fat or Mediterranean diet), five with a Western, American or habitual diet, six studies compared nut consumption with another food product (meat, cocoa, lactose or olive oil), one study compared two types of nut and one study was a single intervention using pre and post measures with no control or comparator food. The range of nuts used included almonds, walnuts, mixed nuts, Brazil nuts, cashews, pistachios and hazelnuts. The amounts ranged from 10-103 g (⅓ oz-4 oz) of nuts per day (approximately 5-25 % of energy intake) for 4 weeks to two years. To date only tree nuts have been tested for effects on inflammatory markers with chronic nut consumption.

In three of four cross sectional studies, nut consumption was associated with lower concentrations of the inflammatory markers CRP, IL-6 or ICAM. The Multi-Ethnic Study of Atherosclerosis (MESA) demonstrated an inverse association between frequency of consumption of nuts and seeds and serum CRP and IL-6 levels (Jiang et al. 2006). This association was moderately attenuated by additional adjustment for body mass index. Two other studies demonstrated a Mediterranean diet pattern (PREDIMED study) (Salas-Salvadó, J. et al. 2008) or an American diet including nuts (O'Neil et al. 2011) was inversely associated with anti-inflammatory markers Surprisingly, a large study (6 309 women with diabetes), which categorised the largest nut consumption of ≥ 5 serves per week, (1 serve = 28 g nuts or 18 g peanut butter) showed no association with inflammatory markers (Li et al. 2009).

Twelve intervention studies demonstrated significant reductions (5-75 %) in inflammatory markers with nut consumption with a variety of nuts. Consumption of 21g-100 g of walnuts, almonds, hazelnuts, pistachios or mixed nuts for four weeks to 2 years in healthy or hypercholesterolaemic participants or those at high risk of cardiovascular disease resulted in significant reductions of CRP (5-75%) (Esposito et al. 2004; Jenkins et al. 2002a; Jenkins et al. 2003; Jenkins et al. 2005; Mena et al. 2009; Rajaram, Connell & Sabaté 2010; Solà et al. 2012; Zhao et al. 2004) or other inflammatory markers (ICAM, VCAM, E-selectin and interleukins) (7-28%) (Canales et al. 2011; Chiang et al. 2012; Esposito et al. 2004; Estruch et al. 2006; Mena et al. 2009; Rajaram, Connell & Sabaté 2010; Ros et al. 2004; Sari et al. 2010; Zhao et al. 2004). A study incorporating walnuts (37 g/day) plus walnut oil (15 g/day) or walnuts and walnut oil plus flax seed as an additional source of ALA demonstrated anti-inflammatory effects compared with an American diet in hypercholesterolaemic individuals (Zhao et al. 2004). The vascular adhesion molecules ICAM-1, VCAM -1 as well as CRP were all reduced significantly with a dose effect found of ALA in the diet with a 75 % reduction in CRP. Participants who consumed 20-50 g of walnuts for two years as part of a Mediterranean diet demonstrated a reduction of CRP (36 %) and interleukins IL-6, IL-7 and IL-18 (9-28 %) when compared with a prudent diet (Esposito et al. 2004). Mediterranean diets in which walnuts (~ 65 g/day) or mixed nuts (30 g/day) replaced olive oil demonstrated improvements in one or more of the inflammatory markers; CRP, ICAM-1, VCAM -1 and IL-6 (Estruch et al. 2006; Mena et al. 2009; Ros et al. 2004). A reduction in CRP was demonstrated in four studies with a diet portfolio containing either almonds (Jenkins et al. 2002b; Jenkins et al. 2003; Jenkins et al. 2005) (14-30g/day) or hazelnuts (30 g/day) (Solà et al. 2012) consumed for 4 weeks, one of these studies found an equivalent reduction to that observed with statin intake in the same individuals (Jenkins et al. 2003). Beneficial improvements in inflammation observed in interventions which contained foods in addition to nuts (portfolio diet (Jenkins et al. 2002a; Jenkins et al. 2003)) or Mediterranean diet (Esposito et al. 2004; Thomazella et al. 2011) may have been attributable to these other components. In contrast, Mediterranean diets in which

walnuts (Ros et al. 2004), mixed nuts (Estruch et al. 2006; Llorente-Cortés et al. 2010) or pistachios (Sari et al. 2010) replaced olive oil demonstrated improvements in one or more of the inflammatory markers; CRP, ICAM-1,VCAM -1and IL-6, indicating there may be some beneficial effect of nuts above that of other components of the Mediterranean diet. One study observed a reduction in CRP with a portfolio diet containing almonds only when participants with baseline CRP of ≥ 3.5 mg/l were excluded from analysis (Jenkins et al. 2005). (CRP levels ≥ 3.5mg/l reflect acute inflammation associated with infection or acute illness which would mask any potential effects of nuts on chronic inflammation) (Gotto 2007). A 25 % reduction in IL-6 was observed with a relatively large dose (80-100 g) of pistachios consumed for 4 weeks (Sari et al. 2010). Consumption of a high-almond diet (68 g/d per 2000 kcal or 8.4 MJ) and a low-almond diet (34 g/d per 2000 kcal or 8.4 MJ) for 4 weeks significantly decreased CRP compared with an iso-energetic control diet in healthy men and women (Rajaram, Connell & Sabaté 2010), E-selectin (a marker of endothelial inflammation) was significantly lower in the higher almond group than control. No dose response relationship was observed with either inflammatory marker in this study. In participants at risk of CVD, statistically significant reduction of cellular adhesion molecules, ICAM -1 (effect size 0.3) and VCAM-1 (effect size 0.4) were demonstrated with relatively low doses (21 g/day) of walnuts added to a meat product compared with the meat product without walnuts. Despite little evidence for the magnitude of nut dose influencing inflammation it is possible that there is a minimum dose required since no studies using <30 g per day demonstrated benefits.

Fifteen studies demonstrated no significant changes in inflammatory markers, although most of these demonstrated small reductions. A recent three arm study compared fatty fish vs. walnuts vs. a fish/nut free diet (control). No significant changes were found between the walnut and the control diets but E- selectin was reduced with the walnut intervention compared with the fish intervention (Chiang et al. 2012). Consumption of 26 g/day almonds or 36 g/day walnuts for 6 weeks and showed a 19 % reduction in IL-6 with the almonds and 20 % reduction in TNFα with the walnuts compared with baseline but this did not

reach significance. In addition, two studies with obese individuals demonstrated small but non-significant improvements in CRP (Maranhao et al. 2011) and Il-6 (Casas-Agustench et al. 2011a) with Brazil nut and mixed nut consumption respectively. Suggested reasons for small but non-significant reductions in inflammatory markers were recruitment of healthy individuals who may only demonstrate limited improvements and diurnal effects of IL-6 which are more difficult to detect than other markers. One study with obese individuals demonstrated small but non-significant improvements in CRP (Maranhao et al. 2011). Increased central adiposity and body weight are associated with increased CRP levels and adipose pro-inflammatory cytokines including IL-6 (Mathieu, Lemieux & Després 2010). It is possible that these individuals may not demonstrate improvements in inflammatory markers with a dietary intervention without weight loss.

The calculated weighted mean changes for all studies revealed reductions in ICAM-1, VCAM -1 and CRP were 8.6 % (95 % CI -20.5, 3.3), 5.8 % (95 % CI -14.1, 2.5) and 12 % (95 % CI -23.6, -0.3) respectively (see table 3.9). In summary, nut consumption has the potential to improve inflammatory markers, particularly with doses of 30 g or greater. This is in line with a qualified health claim for nuts first established by the U.S. Food and Drug Administration (FDA) in 2003; scientific evidence suggests that eating 42 grams (1.5 ounces) per day of most nuts (as part of an overall healthy diet) may be able to reduce risk of heart disease (United States Department of Agriculture).

Table 3.5 **Effects of nut consumption on inflammatory markers** *(studies are presented in order of efficacy)*

Cross sectional studies measuring nut consumption and inflammatory markers

Author	Time	Subjects	Study Design	Amount/type nuts	Measure	Outcome	Effect
Jiang et al. 2006	X-sect	N=6080, healthy M/F, 62yr (range 45-84yr)	X-sect, FFQ and inflammatory markers, (MESA)Multiethnic Study of Atherosclerosis	Rare to > 4 serves/wk any nuts and seeds	CRP	– ve correlation ** r = 0.06	+
					IL-6	– ve correlation ** r = 0.05	+
Salas-Salvado et al. 2008	X-sect	N=772 , high risk CVD, M/F, 68yr (range 55-80ys)	X- sect, FFQ and inflammatory markers (PREDIMED trial)	**Tertiles (any nuts)**	ICAM	↓17%*	+
				T1<3.92g	VCAM	Ns	0
				T2 3.92-10.84g	IL-6	ns	0
				T3>10.84g	CRP	ns	0
O'Neil et al. 2011	X-sect	N=13,292, general pop. M/F, mean 57yr r	1999- 2004 National Health and Nutrition Survey (NHANES), 24 hr recall and CRP	All nus≤ 7g/d	CRP	ns	0
				Tree nuts 7g/d	CRP	↓12% *	+
Li et al. 2009	X-sect	N=6309, women, Dm,	FFQ and inflammatory markers	0 - >5 serves/ wk 1serve = 28g any nuts	ICAM	ns	0
					E-selectin	ns	0

Chronic effects of nuts on inflammatory markers *(studies are presented in order of efficacy)*

Author	Time	Subjects (age = mean (sd)	Study Design	Amount /type nuts	Measures	Outcome (active vs control)	Effect (size)
Zhao et al. 2004	6wk /arm	N=23, hypercholesterolaemic, M/F, 50(2)yr	RCT X over, LA diet (walnut + walnut oil) vs. ALA diet (flax + walnut + walnut oil) vs American diet (control) no washout, iso-energetic	37g/d walnuts +15g/d walnut oil	CRP (ALA)	↓75% *	+
					VCAM (ALA)	↓12% *	+
					E-selectin(ALA)	↓12% *	+
					E-selectin (LA)	↓7%	+
					CRP (LA)	↓35%	+
					VCAM (LA)	↓7%	+
Mena et al. 2009	3mth	N=106 (control=36, OO=45, nuts=35), high risk CVD, M/F, 68(8)yr	RCT parallel, Med diet + OO vs. Med diet + nuts vs LF diet (control) (PREDIMED)	30g/d mixed nuts†	IL-6	↓40%*	+ (0.3)
					ICAM	↓52%*	+ (1.1)
					VCAM	↓33%*	+ (1.5)
					CRP	↓78%*	+ (0.4)
					E-selectin	ns	0 (-0.2)
					P-selectin	ns	0 (0.2)
Esposito et al. 2004	2 yr	N=180 (control=90, Med diet=90), Met-S, M/F, 44(6) yr	RCT parallel, Med diet (incl. walnuts) vs prudent diet (control)	20-50g/d walnuts	CRP	↓36%*	+
					IL-6	↓28% *	+
					IL-7	↓21%*	+
					IL-18	↓ 9%*	+

Table 3.5 (continued) **Chronic effects of nuts on inflammatory markers** *(studies are presented in order of efficacy)*

Author	**Time**	**Subjects** (age = mean (sd)	**Study Design**	**Amount /type nuts**	**Measures**	**Outcome (active vs control)**	**Effect (size)**
Sola et al.2012(86)	4 wk /arm	N=113 (control=28, nut=28, nut+sterol =30, nut+sterol+fibre=27), risk CVD, M/F, 54(9yr	RCT parallel isoenergetic cocoa + hazelnut (A) vs cocoa + hazelnut + phytosterol (B) vs cocoa+hazelnut+ phytosterol+sol fibre (C) vs cocoa (control), no washout	30g/d hazelnuts	VCAM (A)(B) VCAM (C) ICAM (A)(B)(C) IL-6 (A)(B)(C) CRP (A)(B) CRP (C)	ns ns ns ns ns ↓33%*	0 (0.1) 0 (0.0) 0 (0.0) 0 0 0
Sari et al. 2010(59)	4 wk /arm	N=32, healthy men, 22yr (range 21-24 yr)	Prospective cohort, iso-energetic Med diet (control) with/without pistachio, no washout	80-100g /d pistachios	IL-6 CRP TNFα	↓25%* ns ns	+ (0.4) 0 0 (0.0)
Jenkins 2002a(99)	4 wk /arm	N=13, M/F, 65(3)yr, hyperlipidaemic	Prospective cohort LF diet (control) & portfolio diet (soy/sterol/fibre/nuts)	28g/d almonds	CRP	↓ 26%	+
Jenkins et.al. 2003(89)	4 wk	N=46, (control=16, statin=14, nut=16), hyperlipidemic, M/F, 59yr(1)yr	RCT parallel, iso-energetic statin vs. sterol + soy + almonds vs LF diet (control)	14 g/4.2MJ almonds/d	CRP	↓18%*	+ (0.1)
Canales et al. 2011	5 wk/ arm	N=22, risk CVD, M/F, 55(2)yr	RCT X over, meat product (control) with/without walnut, 5 wk washout	21g/d walnuts	VCAM ICAM	↓12%* ↓17%*	+ (0.4) + (0.3)
Jenkins et al. 2005	1 mth / arm	N=34 hyperlipidaemic M/F 55yr(7)yr	RCT X over iso-energetic statin vs phytosterol + soy + almonds vs. LF diet (control), 4 wk washout	14g/4.2MJ almonds	CRP (all subjects) CRP (subjects ≤3.5mg/l)	ns ↓9%*	0 (0.0) + (0.4)
Rajaram et al. 2010	4 wk/ arm	N=25, healthy, M/F, 41(13)yr	RCT X over, low almond diet (L) vs. high almond diet (H) vs healthy heart diet (control), no washout	low almond (L) (34g/8.4MJ)/d high almond (H) (68g/8.4MJ)/d	E-selectin (H) E selectin (L) CRP (H) CRP (L) IL-6 (H) (L)	↓8%* Ns ↓5%* ↓9%* ns	+ (0.5) 0 (0.0) + (0.1) + (0.1) 0 (0.1)
Ros et al. 2004	4wk/ arm	N=20, M/F, hypercholesterolaemic 55yr (range 26-75yr)	RCT X over, iso-energetic Med diet (control) with/without nuts, no washout	~ 65g/d walnuts (32% E)	VCAM ICAM CRP	↓ 12%* ns ns	+ (0.5) 0 (0.3) 0 (0.0)
Estruch et al. 2006	3mth	N=772, (control=257, OO=257, nut=258), high risk CVD, M/F, 69(6) yr	RCT parallel, Med diet + OO vs. Med diet + nuts vs LF diet (control) (PREDIMED)	30g/d mixed nuts†	CRP ICAM VCAM IL-6	ns data N/A* data N/A * data N/A*	0 + + +

Table 3.5 (continued) **Chronic effects of nuts on inflammatory markers** *(studies are presented in order of efficacy)*

Author	**Time**	**Subjects** (age = mean (sd)	**Study Design**	**Amount /type nuts**	**Measures**	**Outcome (active vs control)**	**Effect (size)**
Chiang et al. 2012	4 wk /arm	N=25 hyperlipidaemic M/F 33yr (range 23-65yr)	RCT X-over, iso-energetic fatty fish vs. walnuts vs no nut/fish (control)	43g/10MJ walnuts/d	CRP ICAM TNFα IL-1ß and IL-6 E-selectin	ns ns ns ns ns vs control (↓ vs. fish*)	0 0 0 0 0 +
Kasim-Karakas et al.2004	3mth	N=17, 34(5)yr, women, PCOS	Prospective cohort iso-energetic habitual diet (control) vs walnuts	106g/d walnuts	TNFα	ns	0 (1.0)
Jenkins et al.2011	3 mth	N=117(control=37,37g/d =40, 75g/d=40),Dm, M/F, 62(10)yr	RCT parallel, 75g/d nut vs. 37g/d nut + ½muffin vs muffin (control)	75g/d or 37g/d mixed nuts‡	CRP (full dose) CRP (½ dose)	ns ns	0 (0.1) 0 (0.2)
Jenkins et al. 2002b	1 mth /arm	N=27, hyperlipidaemic M/F 64(9)yr	RCT X over almonds vs.½almond +½muffin vs muffin (control)	73g/d almonds or 36g/d almonds	CRP	ns	0 (0.0)
Adamsson et al. 2011	4 wk	N = 86 (control=42 Nordiet = 44) hypercholesteroleamic M/F, 53(8)yr	RCT parallel, Nordiet (fibre, fish, LF dairy, nuts) vs habitual diet (control)	almonds ad libitum	CRP	ns	0 (0.1)
Thomazella et al. 2011	3 mth	N=40 (control=19, Med diet=21), males with CVD, 55(5)yr	Prospective controlled study, Med diet (incl. nuts) vs LF diet (control)	10g/d any nuts	CRP ICAM VCAM	ns ns ns	0 (0.3) 0 (0.3) 0 (0.0)
Lopez-Uriate et al. 2010	12 wk	N=50 (control=25, nut=25), Met-S, M/F, 5(8)yr	RCT parallel American Heart Assoc. diet (control) with/without nuts, not iso-energetic	30g/d mixed nuts†	ICAM VCAM	ns ns	0 (0.3) 0 (0.6)
Damasceno et al. 2011	4 wk/ arm	N=18, M/F, 56(13)yr, hypercholesterolaemic,	RCT X over, iso-energetic Med diet + walnuts (W) vs almonds (A) vs. OO (control), no washout	40-65g/d walnuts (W) 50-75g/d almond (A)	VCAM (W&A) ICAM (W&A) CRP (W&A)	ns ns ns	0 (-0.2) 0 (0.2) 0 (0.2)
Kalgaonkar et al. 2011	6 wk	N=31(almond=14, walnut=17), women with PCOS, age range 20-45yr	Pre/post measures walnuts vs. almonds	36g/d walnuts (W) 26g/d almonds (A)	IL-6 (W vs A) TNFα (W vs A) IL-1β(W vs A) CRP (W vs A)	ns ns ns ns	0 (1.3) 0 (1.2) 0 (0.9) 0 (0.4)

Table 3.5 (continued) **Chronic effects of nuts on inflammatory markers** *(studies are presented in order of efficacy)*

Author	**Time**	**Subjects** (age = mean (sd)	**Study Design**	**Amount /type nuts**	**Measures**	**Outcome (active vs control)**	**Effect (size)**
Kurdlansky et al. 2006	6 wk	N=41 (control=10, choc=10, nut =10, nut + choc=11), healthy females, 47(9)yr	RCT parallel LF diet (control) vs. choc vs. almond (A) vs choc + almonds (CA)	60g/d almonds	CRP (CA)	ns	0 (0.0)
					ICAM (CA)	ns	0 (0.2)
					VCAM (CA)	ns	0 (0.2)
					CRP (A)	ns	0 (0.2)
					VCAM (A)	ns	0 (0.1)
					ICAM (A)	ns	0 (0.2)
Casas-Agustench et al. 2011	12 wk	N=50 (control=23,nut=27), Met-S, M/F, 52(8)yr	RCT parallel, prudent diet (control) with/without ad libitum nuts	~30g/d mixed nuts†	CRP	ns	0 (0.0)
					lL-6	ns	0 (0.2)
					IL-18	ns	0 (0.0)
Maranhao et al.2011	16wk	N=17 (control=9, nut=8), obese females, 15(3)yr	Pilot RCT parallel Brazil nuts vs. placebo (lactose) not iso-energetic	15-25g/d Brazil nuts	CRP	ns	0 (0.1)
Mukuddem-Peterson et al. 2007	8 wk	N=64 (control=22, nut=21, cashew=21), Met-S, M/F, 45(8)yr	RCT parallel iso-energetic LF diet (control) with/without walnuts or cashews	63-108g/d walnuts/ cashews	CRP	ns (both nuts)	0 (0.1)
Schutte et al. 2006	8 wk	N=62 (control=21, walnut=20, cashew=21) Met-S, M/F, 45yr	RCT parallel, iso-energetic LF diet (control) with/without walnuts or cashews	63-103g/d walnuts or cashews (20% E)	CRP	ns	0
					VCAM	ns	0
					CRP walnut	ns	-(-0.3)
					CRP cashew	ns	-(-0.2)

Abbreviations: M/F, males and females, Met-S, metabolic syndrome, PCOS, polycystic ovary syndrome, Dm, type 2 diabetes, CVD, cardiovascular disease, RCT, randomised controlled trial, LF, low fat, OO, olive oil, Med diet , Mediterranean diet, FFQ, food frequency questionnaire ,T1, T2, T3 = tertiles, d, days, wk= weeks, mth , months, yr, years, CRP, high sensitivity C-reactive protein , ICAM, intercellular adhesion molecule - 1, VCAM , vascular cell adhesion molecule-1, IL-6/7/18, interleukins 6/7/18, TNFα, tumor necrosis factor, ES, effect size, N/A, data not available, †, mixed nuts = walnuts, almonds and hazelnuts, ‡, mixed nuts = almonds, pistachios, walnuts, peanuts, hazelnuts, pecans, cashews and macadamias, *, p <0.05, **, p <0.01, ns, no significant difference, sd, standard deviation, **+,** significant ↓ inflammatory marker, 0, no significant change inflammatory marker, -, significant ↑ inflammatory marker

3.5.4. Effects of nut consumption on endothelial vasodilator function

Studies measuring the effect of nut consumption on endothelial vasodilator function are found in **Table 3.6.** Nine intervention studies measured the effect of nut consumption on endothelial vasodilator function (using either flow mediated dilatation or Endopat) with the dose of nuts ranging between 10 g to 100 g per day for periods ranging from four to twelve weeks. Five of nine studies demonstrated a significant effect.

Endothelial function was significantly improved (24-64 %) in healthy and hypercholesterolaemic participants who consumed 37-100 g of walnuts or pistachios for 4-8weeks.(Ma et al. 2010; Ros et al. 2004; Sari et al. 2010; West et al. 2010) A Mediterranean diet supplemented with 65 g/day of walnuts substituted for olive oil, significantly improved vasodilation by 64 % in hypercholesterolaemic adults (Ros et al. 2004). This study also demonstrated an inverse association between vasodilation and cholesterol-to-HDL ratio suggesting that the effect of walnuts may have been mediated in part through an improved lipid profile (Ros et al. 2004). It is well established that hypercholesterolaemia impairs endothelial function which can be reversed by aggressive cholesterol lowering (Adams et al. 2000). However this study only demonstrated moderate cholesterol lowering indicating that other mechanisms may also play a role. Investigators suggested that phenolic compounds in walnuts may have counteracted the pro-oxidant effects of polyunsaturated fatty acids on LDL. Mediterranean diets supplemented with 20-50 g/day of walnuts (Esposito et al. 2004) and 80-100 g/day of pistachios (Sari et al. 2010) improved endothelial function by 21% and 24 % respectively. Also observed with pistachio consumption was an improvement in glucose levels, lipid parameters, oxidative status and some indices of inflammation which may underlie the improved endothelial function (Sari et al. 2010). A diet with ad libitum consumption (56 g/day) of walnuts improved endothelial function by 45 % (effect size 0.6) in participants with type 2 diabetes (Ma et al. 2010). Consumption of walnuts and walnut oil supplemented with flax seed (to boost

the ALA content of the diet) increased endothelial function by 34 % but no change was observed with the walnut diet alone (West et al. 2010). Four of the five studies using higher doses (56 g-100 g per day) of nuts demonstrated benefits on endothelial function indicating that higher doses may be required to elicit benefits (Ma et al. 2010; Ros et al. 2004; Sari et al. 2010; West et al. 2010).

Four studies did not show significant effects on flow mediated dilatation. A hazelnut-enriched diet consumed by healthy men improved lipid parameters. In spite of this, endothelial functional improvement did not reach statistical significance (Mercanligil et al. 2007). 10-30 g of nuts consumed by participants with either cardiovascular disease risk or metabolic syndrome also demonstrated no benefits on endothelial function (López-Uriarte et al. 2010; Thomazella et al. 2011). Two doses (10 % and 20 % of energy) of pistachios did not demonstrate a reduction in endothelial function (West et al. 2012) despite relatively high doses of up to 126 g/day consumed for 4 weeks. Authors suggested that because the pistachios were roasted this may have reduced polyphenol activity unlike walnuts used in other studies, which were not roasted before consumption.

Calculations of the weighted mean changes from 9 of the 10 studies indicated a 19.7 % (95 % CI 4.3, 35.0) relative increase in vasodilatation with nut consumption (**Table 3.9**). The effects of nuts on endothelial function demonstrate potential benefits, particularly walnuts. However, limited studies have been conducted with other types of nuts which may also demonstrate benefits. Endothelial dysfunction is often detected before increased blood pressure is observed and may be a more sensitive indicator than arterial compliance of early decline in vascular health, hence it may be a better target than blood pressure control or arterial compliance (Ghiadoni, Taddei & Virdis 2012).

Table 3.6 **Chronic effect of nut consumption on endothelial vasodilator function** *(studies are presented in order of efficacy)*

Author	**Time**	**Subjects** (age = mean (sd) unless otherwise indicated)	**Study Design**	**Amount /type nuts**	**Outcome** (active vs control)	**Effect** (Size)
Ros et al. 2004	4wk/ arm	N=20, hypercholesterolaemic M/F, 55yr (range 26-75yr)	RCT X over, iso-energetic Med diet (control)with/without walnuts, no washout	~ 65g/d walnuts (32% E)	↑64%*	+ (0.3)
Ma et al. 2010	8 wk/ arm	N=21, Dm, M/F, 58(8)yr	RCT X over ad libitum diet (control) with/without walnuts, 8 wk washout	56g/d walnuts	↑45%*	+ (0.6)
West et al. 2010	6 wk/ arm	N=20, hypercholesterolaemic, M/F, 49(6)yr	RCT X-over, American diet (control) vs LA diet (flax + walnut + walnut oil) vs ALA diet (walnut + walnut oil) no washout	37g/d walnuts +15g/d walnut oil	ALA ↑34% *	+ (0.4)
					LA ns	0 (0.1)
Sari et al. 2010	4 wk/ arm	N=32, healthy men, 22yr (range 21-24 yr)	Prospective cohort, iso-energetic Med diet (control) with/without pistachios, no washout	80-100g/d pistachios (20% E)	↑24% *	+ (1.0)
Esposito et al.2010	2 yr	N=180 (control=90, Med diet=90), Met-S, M/F, 44(6) yr	RCT parallel, Med diet (incl. walnuts) vs prudent diet (control)	20-50g/d walnuts	↑21%*	+ (0.9)
Mercangil et al 2007	4 wk/ period	N=15, males, 48(8yr hypercholesterolaemic,	2 period study, LF diet (control) vs LF + hazelnuts, non iso-energetic	40g/d hazelnuts (12% E)	ns	0
Lopez-Uriate et al. 2010‡	12 wk /arm	N=50 (control=25, nut=25), Met-S, M/F, 52(8yr	RCT parallel American Heart Assoc. diet (control) (mixed nuts, not isoenergetic	30g/d mixed nuts†	ns	0 (0.0)
Thomazella et al. 2011	3 mth	N=40 (control=19, Med diet =21) males CVD, 55(5)yr	Prospective controlled study Med diet (incl. nuts) vs. LF diet (control)	10g/d any nuts	ns	0 (0.0)
West et al. 2012	4 wk/ arm	N=25, hypercholesterolaemic, M/F, 48(2) yr	RCT X over, LF diet (control) vs. low dose pistachios vs. high dose pistachios, (no washout)	32-63g/d pistachios	ns	0 (-0.10)
				63-126g/d pistachios	ns	0 (0.10)

Abbreviations: M/F, males and females, Met–S, metabolic syndrome, Dm, type 2 diabetes, E, energy, RCT, randomised controlled trial, Med diet, Mediterranean diet, OO, olive oil, LF, low fat, ALA, α linolenic acid, LA, linoleic acid, d, days, wk, weeks, yr, years, E, energy, ns, not significant, sd, standard deviation, +, significant ↑ endothelial function , 0, no significant change endothelial function, *, $p<0.05$ †, mixed nuts = walnuts, almonds and hazelnuts. Vasodilator function measured by flow mediated dilatation (except ‡, measured by Endopat)

3.5.5 Effects of nut consumption on arterial compliance

Studies measuring the effect of nut consumption on arterial compliance are found in **Table 3.7**. These include one cross sectional study and one intervention study. A dose of 15 g/day of walnuts consumed for four weeks demonstrated no effect on arterial stiffness (Din et al. 2011). Whilst this dose is small, investigators chose a realistic amount likely to be consumed in free living individuals for an extended period of time rather than higher doses used in other nut intervention studies. The cross sectional study measured arterial compliance and compared quintiles of a healthy dietary pattern including nuts (Nettleton et al. 2008). No association was found with a healthier diet pattern and measures of arterial compliance with an undetermined quantity of nuts. Few studies have investigated the effects of nuts on arterial compliance therefore more studies in this area are warranted.

3.5.6 Effects of Nut Consumption on Cognitive Performance.

There is little known of the impact of nut consumption on cognitive function. Studies measuring the effect of nut consumption on cognitive performance are found in **Table 3.8**.A five year prospective cohort study demonstrated a positive association between nut consumption and cognitive performance, equivalent to a substantial age reduction effect of 5-8 years in highest nut consumers (amount of nuts not specified) (Nooyens et al. 2011). In addition cognitive performance did not decline over the five year period in highest nut consumers. In a cross sectional study (PREDIMED) an association was found between walnut consumption (but not other nuts) and improvements in performance on tests of working memory (Valls-Pedret et al. 2012). In older adults nut consumption was associated with improved but non-significant scores for executive function in a cross sectional study (Nurk et al. 2010), with a low mean intake of nuts of 5 g per day. Only one intervention study in humans has been performed, this was conducted with students consuming 60 g of ground walnuts per day for eight weeks (Pribis et al. 2012). The study demonstrated a medium effect size (0.4) improvement in inferential reasoning; however other cognitive tests demonstrated no change. Despite the lack of intervention trials, observational

studies indicate that long term consumption of even small amounts of nuts may elicit benefits for cognitive function and reduction in cognitive decline. More evidence is needed from controlled intervention studies before a conclusive benefit can be determined.

Table 3.7 **Effect of nut consumption on arterial compliance**

Cross sectional study measuring nut consumption ansd arterial compliance							
Author	**Time**	**Subjects**	**Study Design**	**Amount /type nuts**	**Measures**	**Outcome**	
Nettleton et al. 2008	X-sect	N=5089, healthy, M/F, range 45-84yr	X sectional, Multi Ethnic Study of Atherosclerosis (MESA) FFQ and AC	Quintiles Healthy Dietary Pattern (incl. any nuts)	LAE, SAE	AC not assoc. with healthy dietary pattern	
Chronic effects of nut consumption on arterial compliance							
Author	**Time**	**Subjects** (age = mean (sd)	**Study Design**	**Amount /type nuts**	**Measure**	**Outcome** (active vs. control)	**Effect (size)**
Din et al. 2011	4 wk/ arm	N=30, healthy males, 23 (3) yr	RCT X-cross over habitual diet (control) (walnuts, not isoenergetic, no washout	15g/d walnuts	PAIx	ns	0 (0.3)
					AP	ns	0 (0.2)

Abbreviations: RCT, randomised controlled trial, sd, standard deviation, d, day, wk, weeks, yr, years, AC, arterial compliance, LAE, large artery elasticity, SAE, small artery elasticity, AP, augmentation pressure, PAIx, peripheral augmentation index, ns, no significant difference, N/A, data not available, 0, no significant change arterial compliance

Table 3.8 **Effects of nut consumption on cognitive function**

Observational studies measuring nut consumption and cognitive function						
Author	**Time**	**Subjects**	**Study Design**	**Amount /type nuts**	**Outcomes**	**Effect**
Valls-Pedret et al. 2012	X-sect	N= 447, risk CVD, M/F, mean 69 yr (55-80yr)	PREDIMED study FFQ + cognitive battery	5g/d (0-60g) all nuts, 1g/d walnuts (0-30g)	Walnuts (not other nuts) assoc. with ↑ working memory, regression coefficient = 1.2 (95% CI 0.06, 2.32), β = 0.15, p = 0.04	+
Nooyens et al. 2011	5 yr	N= 2613 general population 43-70yr	The Doetinchem Prospective Cohort Study, FFQ and cognitive battery	Quintiles any nut consumption (amount not specified)	1) ↑ nut intake assoc. with ↑cognitive function (memory, speed, flexibility and global) p-trend <0·01 2) ↑ 5-8 yr cognitive function in high vs. low nut 3)No ↓ cognitive decline nut consumers over 5 yr	+
Nurk et al. 2010	X-sect	N=2031, M/F, elderly, 70-74yr	X- sect FFQ + cognitive battery	Mean intake nut consumers = 5g/d	Nut intake assoc. with ↑ executive function, semantic memory, ns	0
Chronic effects of nut consumption on cognitive function						
Author	**Time**	**Subjects age (sd**	**Study design**	**Amount /type nuts**	**Outcomes**	**Effect (size)**
Pribis et al. 2012	8 wk	N= 64, M/F students, 21(2)yr	RCT X-over, banana bread (control) with/without walnuts, 6 wk washout	60g/d walnuts	Inferential verbal reasoning ↑ 11% (d = 0·6, p = 0·009) Mood, non-verbal reasoning and memory, ns	+ (0.4) 0

Abbreviation: M/F, males and females**,** CVD, cardiovascular disease, wk, week, d, day, yr, years, FFQ, food frequency questionnaire, sd, standard deviation, n.s., no significant change, +, significant ↑ cognitive performance, 0, no significant change cognitive performance

Table 3.9 **Weighted mean % changes in blood pressure, inflammatory markers and endothelial function with nut consumption**

	Systolic BP		Diastolic BP		C-reactive protein		ICAM-1		VCAM-1		Endothelial vasodilator function	
Weighted mean	-1.3*		-0.7		-12.0*		-8.6		-5.8		19.7*	
SE	0.5		0.3		5.70		5.4		3.8		6.7	
95% CI	-2.3	-0.3	-1.4	0.04	-23.6	-0.3	-20.5	3.3	-14.1	2.5	4.3	35.0
Number of. participants	867		843		745		265		180		189	
No. studies	17		16		20		8		8		8	

Abbreviations: BP, blood pressure, ICAM-1, intercellular adhesion molecule - 1, VCAM-1, vascular cell adhesion molecule-1, SE, standard error, CI, confidence interval, *, statistically significant ($p<0.05$)

3.6 Proposed mechanisms

Several nutrients in nuts may be responsible for observed improvements in cardio-metabolic and cognitive measures. Tree nuts and peanuts have similar nutrient profiles with some variations in micro and macronutrients. From the studies reviewed (with the exception of walnuts which have been more extensively researched than other nuts) it is not possible to determine differences in efficacy between different types of tree nuts and peanuts. Walnuts differ from other nuts in their greater antioxidant capacity, polyphenol and ALA content (see **Table 3.1**). ALA found in walnuts is associated with improved endothelial function (Mozaffarian 2005), inflammation (Winnik et al. 2010) and neuro-protection in animal models (Stark, Crawford & Reifen 2008) and is hypothesised to maintain cognitive function in older adults (Freemantle et al. 2006). Other unsaturated fatty acids in nuts may be beneficial for insulin sensitivity (Risérus 2008) and evidence suggests that higher intakes are associated with a lower risk of type 2 diabetes (Salmerón et al. 2001) whereas higher intakes of saturated fatty acid adversely affect glucose metabolism and insulin resistance (Dangardt et al. 2012; Ebbesson et al. 2010; Vessby et al. 2001). There is also recent evidence to indicate that MUFAs may contribute to improvements in arterial stiffness as well as endothelial function and inflammation (Bellido et al. 2006; Fuentes et al. 2001; Newens et al. 2011; Solfrizzi et al. 2006). Consumption of a Mediterranean diet which is also high in MUFA has been shown to reduce VCAM -1 and E-selectin gene expression by almost half. Animal and human studies have demonstrated that inflammation can be modified by intake of L-arginine (Heffernan et al. 2010). Individuals with hypercholesterolaemia have impaired synthesis of NO; supplementation of 7 g/day L-arginine in this population group has demonstrated benefits, (Clarkson et al. 1996) increasing endothelial dependent dilatation by almost three-and-a-half fold. Nuts contain approximately 2-3 g of arginine per 100 g, hence doses of 30g/day or more used in most studies could partly account for the improvement in endothelial function observed. Nuts also contain fibre and when consumed with their skin intact, contain a significant amount of polyphenols (Bolling et al. 2011; Sanders, McMichael & Hendrix 2000) which have previously been shown to target endothelial cells resulting in improved vascular function (Brock et al.

2006; Ghosh 2009; Wong et al. 2010). Fibre intake can also increase insulin sensitivity (Bodinham, Smith & Wright 2012; Weickert et al. 2006). Vitamin E found in nuts may have a role in modifying some of the inflammatory mediators and may be beneficial for cognitive performance (Joshi & Praticò 2012; Singh, Devaraj & Jialal 2005). Gamma-tocopherol is a powerful antioxidant abundant in walnuts, Brazil nuts and pistachios; however its effect on markers of cardiovascular risk including endothelial function and inflammation has not yet been determined. Nuts are naturally rich in potassium and magnesium which may facilitate blood pressure reductions unless consumed in the salted form (Sacks et al. 2001). In addition magnesium, which has been inversely related to serum CRP levels, has the potential to improve inflammation in individuals with low magnesium status (Nielsen 2010) and magnesium intake is inversely associated with a reduced risk of type 2 diabetes (Dong et al. 2011)

There is emerging evidence that frequent nut consumption beneficially affects cardiovascular risk beyond cholesterol lowering. Key mechanisms include anti-inflammatory, antioxidant, endothelial function, reduction in body fat and improvement in glucose metabolism which play a central role in the development of atherosclerosis (de Lorgeril et al. 2001; Landmesser, Hornig & Drexler 2004). Endothelial function is essential for cerebral vascular function to provide adequate cerebral blood flow to deliver nutrients (primarily glucose and oxygen) to the brain. It has been hypothesised that by improving blood flow regulation in the brain, cognitive performance is also improved (Krestin et al. 2008; Sinn & Howe 2008). Nutritional interventions which have demonstrated improvements in cerebral blood flow include omega-3 fatty acids in fish oil, (Jackson et al. 2012) polyphenols in cocoa (Fisher, Sorond & Hollenberg 2006) and wild green oats (Wong et al. 2012). Anti-inflammatory medications offer some protection from Alzheimer's disease which is consistent with the hypothesis that damage to brain cells is part of an overall inflammatory reaction. If inflammation is the key, then nuts which contain anti-inflammatory nutrients, such as polyphenols, vitamin E and omega-3 fatty acids may prove to be important to reduce damage to the brain.

3.7 Conclusions

The results summarised in the present study provide evidence that regular nut consumption may have a protective effect on both vascular health and cognition. These benefits were evident in trials with doses of higher intakes (≥ 30 g/d) for extended periods (several weeks or longer). These findings further support the use of nuts to reduce cardio-metabolic dysfunction and highlight their potential to maintain or restore endothelial function. This in turn could improve cerebral blood flow and hence cognitive performance as illustrated in **Figure 3.1**. No published studies to date have measured the effect of nut consumption on cerebral blood flow and few studies have measured the impact of nuts on arterial compliance and cognitive performance. Whilst intervention studies have investigated the impact of nuts on endothelial function, only one study has taken the next step and considered whether nuts may have beneficial effects on cognitive performance. Further clinical studies are warranted to determine the type and dose of nut and duration of consumption and which populations may benefit.

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3.10 Research directions informed by background and systematic review

The analysis of the literature in **Chapters 2 and 3** has outlined potential benefits of peanut consumption for several health outcomes. These include improvement in satiety and hence potential for weight management, improvement in cardio-metabolic risk factors, including lipid lowering, glucose regulation and inflammation and potential influences on vascular health and cognitive performance. This provides the background literature for two intervention studies which are described in the following chapters:

Study 1) The effect of peanuts on acute satiety and subsequent energy intake over 4 days.

Study 2) The effect of daily Hi-oleic peanut consumption on vascular and cognitive measures Investigation of the effects of daily peanut consumption (evaluated over 12 weeks) on cardio-metabolic outcomes, body weight, body composition, vascular function and cognitive performance.

The findings from these studies have the potential to further determine the health benefits of regular peanut consumption nuts and underlying mechanisms involved.

Chapter 4: The effect of peanut consumption on satiety

This chapter provides the details of a published study (**Study 1)** entitled "Lower Energy Intake Following Consumption of Hi-oleic and Regular Peanuts Compared with Iso-energetic Consumption of Potato Crisps"

This is a co-authored publication. Please refer to **Appendix A** for a statement of authors contributions.

Jayne A. Barbour, Peter R.C. Howe, Jonathan D. Buckley, Graeme C. Wright, Janet Bryan, Alison M. Coates. (2014). Lower Energy Intake Following Consumption of Hi-oleic and Regular Peanuts Compared with Iso-energetic Consumption of Potato Crisps*, Appetite*, vol 82, pp. 124–130).

Lower energy intake following consumption of Hi-oleic and regular peanuts compared with iso-energetic consumption of potato crisps

Adapted from the published manuscript

4.1 Abstract

Snack foods can contribute a high proportion of energy intake to the diet. Peanuts are a snack food rich in unsaturated fatty acids, protein and fibre which have demonstrated satiety effects and may reduce total energy intake, despite their high energy density. This study examined the effects of consuming Hi-oleic (oleic acid ~75 % of total fatty acids) peanuts and regular peanuts (oleic acid ~50% and higher in poly- unsaturated fatty acids) compared with a high carbohydrate snack (potato crisps) on satiety and subsequent energy intake. Using a triple crossover study design, 24 participants (61 ± 1 years) consumed iso-energetic amounts (56-84 g) of Hi-oleic or regular peanuts or (60-90 g) potato crisps after an overnight fast. Hunger and satiety were assessed at baseline, 30, 60, 120 and 180 minutes following snack consumption using visual analogue scales, after which a cold buffet meal was freely consumed and energy intake measured. The same snack was consumed on 3 subsequent days with energy intake assessed from dietary records. This protocol was repeated weekly with each snack food. Total energy intake was lower following consumption of Hi-oleic and regular peanuts compared with crisps, both acutely during the buffet meal (−21 %; $p < .001$ and −17 %; $p < .01$) and over the 4 days (−11 %; $p < .001$ and −9 %; $p < .01$). Despite these reductions in energy intake, no differences in perceived satiety were observed. The findings suggest peanuts may be a preferred snack food to include in the diet for maintaining a healthy weight.

Keywords: Nuts, Snack food, Satiety, Energy intake

4.2 Introduction

Understanding the relationship between snack foods, satiety and energy balance is important as snacks are being consumed in growing amounts (Astrup, Nackenhorst, Bovy, & Popova, 2006; Piernas & Popkin, 2010). Previous research has indicated that snacking, particularly on highly processed foods, may contribute to higher intakes of energy, which in turn could lead to obesity (Bes-Rastrollo et al., 2010). Epidemiological studies have reported lower rates of obesity and diabetes with higher consumption of nuts (Mozaffarian, Hao, Rimm, Willett, & Hu, 2011; Zizza, Siega-Riz, & Popkin, 2001). However, the effects of nuts on these measures are yet to be clarified in randomised controlled trials.

Current evidence indicates that acute consumption of peanuts and tree nuts can suppress appetite. Ground walnuts (48 g), peanuts or peanut butter (43 g), almonds or almond butter (43 g) added to breakfast meals have been shown to increase satiety compared with control breakfasts (Brennan, Mantzoros, Sweeney, & Liu, 2010; Mori, Considine, & Mattes, 2011; Reis et al., 2013). In the walnut study, the protein content of the walnut breakfast was three times higher and the fibre was one and a half times higher than the control break- fast which may account for the increased satiety. The control break- fasts in the peanut and almond studies were not energy matched, making conclusions more difficult to draw. Energy intake is a primary determinant of satiety, as demonstrated by Alper and Mattes (Alper & Mattes, 2002), indicating that test foods and diets need to be energy matched with controls.

The extant research on the satiating effects of nuts has been largely limited to either comparisons with other nut products or comparing the addition of nuts to a meal or an ad libitum diet (Brennan et al., 2010; Burton-Freeman, Davis, & Schneeman, 2004; Casas-Agustench et al., 2009; Cassady, Hollis, Fulford, Considine, & Mattes, 2009; Devitt et al., 2011; Kendall et al., 2011; Kirkmeyer & Mattes, 2000; Mori et al., 2011). Moreover, not all studies have dem- onstrated satiety benefits, with nut consumption (Burton-Freeman et al., 2004; Casas-Agustench et al., 2009; Devitt et al., 2011; Johnston, Trier, & Fleming,

2013) likely reflecting differences in study design and complexities of the satiety response. Few studies have com- pared the effects of nut consumption relative to consumption of other known popular snacks. A recent randomised crossover trial using a sample of 15 young adults, average age 28 years, unexpectedly demonstrated that acute consumption of a grain snack bar in- creased satiety compared with iso-energetic consumption of peanuts (Johnston et al., 2013). Short term satiety effects of the snack bar were attributed to its high glycaemic load, whereas the low glycaemic response of the peanuts may have elicited a delayed satiety response which was not measured. Comparison of three iso-energetic snack foods (42 g hazelnuts, 50 g potato crisps or 50 g chocolate) added to an ad libitum diet (control) for 12 weeks did not elicit differences in total energy intake, indicating that all three test foods demonstrated satiating properties (Tey, Brown, Gray, Chisholm, & Delahunty, 2012). Other studies have demonstrated reduced energy intake with nut consumption despite their high fat and energy content (Alper & Mattes, 2002). Although epidemiological evidence demonstrates nut consumption is associated with lower body weight (Bes-Rastrollo et al., 2009), intervention trials measuring energy intake indicate conflicting findings.

The purpose of this study was to compare the effects of eating iso-energetic snack foods, viz. unsalted peanuts and low salt potato crisps, on appetite and subsequent energy intake. We hypothesised that participants would be more satiated after consumption of peanuts which are high in protein, fibre and unsaturated fat com- pared with potato crisps (low in protein and fibre, high in carbohydrate and saturated fat) and hence their energy consumption would be reduced. The satiating properties of protein and fibre have been demonstrated in several studies (Bowen, Noakes, & Clifton, 2006; Slavin & Green, 2007). Polyunsaturated fatty acids (PUFA) and monounsaturated fatty acids (MUFA) may also be more satiating than saturated fats due to their higher rates of oxidation (Lawton, Delargy, Brockman, Smith, & Blundell, 2000); however, the role of fatty acids on satiety remains unclear (Casas-Agustench et al., 2009).

Two varieties of peanut with differing proportions of monounsaturated fat were used for this comparison. The effects of high- oleic peanuts on appetite have not been evaluated and to our knowledge no studies have previously measured acute satiety effects of peanuts compared with another savoury snack food. Most nut satiety studies have used young populations; however, the prevalence of overweight and obesity is highest in males over 40 years and females over 60 years in the United States (Flegal, Carroll, Kit,& Ogden, 2012). For this reason, an older overweight/obese group was chosen for the present study. The potential of peanuts to contribute to long term satiety and consequent dietary changes would be valuable in light of health risks associated with obesity.

4.3. Participants and methods

4.3.1 Participants

Twenty-five healthy participants were recruited from July to September 2011 through newspaper advertisements, flyers and word of mouth. The inclusion criteria were healthy males or post-menopausal females (to avoid influences of hormonal cycles on appetite), aged between 50 and 75 years, non-smokers, BMI $\geq$ 25 kg/ m^2 with self-reported stable weight. Exclusion criteria included restrained eaters as determined by a score of $\geq$12 for The Three Factor Eating Questionnaire (Stunkard & Messick, 1985). This questionnaire provides scores for measures of cognitive restraint of eating, disinhibition of eating and hunger and has been validated (Allison, Kalinsky, Gorman, & Butcher, 1992). A score of >12 (out of 21) for restrained eating indicates a tendency to restrict food intake in order to control body weight. Other exclusion criteria included current smokers, regular nut consumers, self-reported consumption of $\geq$1 handful of nuts per week, cardiovascular disease, diabetes, a thyroid condition or a nut allergy. The study was approved by the University of South Australia Human Research Ethics Committee and all participants provided written informed consent prior to participation. The study was registered with the Australian and New Zealand Clinical Trials Registry (ANZCTR N0 12611001072909).

4.3.2 Test products

Roasted, unsalted Hi-oleic (cultivar Middleton ᵇ) and roasted, unsalted regular (cultivar Streeton, normal oleic variety closely related to cultivar Middleton ᵇ) peanuts with skins were provided in 28 g sealed portions by the Peanut Company of Australia (Kingaroy, Queensland, Australia). Unsalted potato crisps (Freedom Foods® 'No Salt' potato crisps, 100 g size) were purchased from a local supermarket as the control food. These food products were stored at room temperature in dark, low humidity conditions. Un- salted potato crisps were chosen as they are similar in energy density (23 kJ/g) and sodium content but lower in protein and fibre content compared with the peanuts (24 kJ/g). See **Table 4.1** for nutrient profile of test foods.

Table 4.1 Nutrient profile of test foods (per 100 g)

	Hi-oleic peanuts	**Regular peanuts**	**Potato crisps**
Energy (kJ)	2374	2486	2278
Total fat (g)	48	49	36
MUFA (g)	38	24	15
PUFA (g)	3	16	4
SFA (g)	5	7	16
Protein (g)	26	26	6
Fibre (g)	9	9	4
Sodium (mg)	16	16	30

MUFA, monounsaturated fatty acid, PUFA, polyunsaturated fatty acid, SFA, saturated fatty acid

4.3.3 Study protocol

The study used a 3 way randomised crossover design to compare the effects of eating Hi-oleic peanuts and regular peanuts with an alternative snack food (low salt potato crisps) on satiety and sub- sequent energy intake. During each of the three experimental visits, participants attended the Nutritional Physiology Research Centre at The University of South Australia after an overnight fast of at least 12 hours. On arrival participants were asked to complete visual analogue scales (VAS) for assessment of appetite and satiety (t = −15 min). Participants were randomised to a treatment order using computer generated software (www.randomization.com). At t = 0 min, participants were provided one of three snacks estimated as 15%–20% of daily energy intake based on

typical energy intake from a previous study in our research centre with a similar population group (Crichton, Murphy, & Bryan, 2010). Females received 56 g of roasted unsalted Hi-oleic peanuts or roasted un- salted regular peanuts or 60 g of low salt potato crisps, males received 84 g of peanuts or 90 g of the potato crisps. Participants were asked to consume the snack over 10 minutes and were provided with a small amount of water to drink. At t = 30, 60, 120 and 180 min VAS were completed. At t = 180 min the participants were offered a cold buffet meal containing a range of pre-weighed foods and beverages, to be freely consumed for up to 30–45 min until fullness was achieved; the amount eaten was recorded. Participants were pro- vided with the same amount of the allocated snack food to be consumed at any time on each of the following 3 days. A set of food scales was also provided with instructions on how to accurately weigh (to the nearest gram), measure and record intake in a food diary. Participants recorded dietary intake and physical activity for the remainder of the buffet meal day and for the next 3 days. They were instructed to maintain usual patterns of physical activity and to consume no nuts, nut based products or crisps other than those provided whilst enrolled in the study. Normal diet was resumed on days 5, 6 and 7 as a washout. This protocol was repeated on two more occasions with the remaining 2 snack foods (**Figure 4.1**)

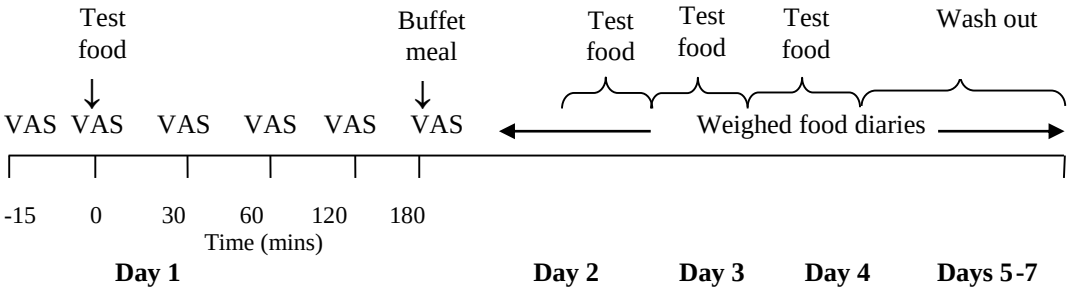


Figure 4.1 Timeline for each study visit showing when visual analogue scales (VAS) and food diaries were completed and when test foods and buffet meal were consumed.

4.3.4 Visual analogue scales (VAS)

VAS responses were recorded on a 100 mm line and measured to the nearest mm as the distance from the left hand anchor, with "not/none at all /no desire" on the left and "extremely/great desire" on the right. Questions were "How hungry are you right now?", "How thirsty are you right now?", "What is your appetite right now?", "What amount of food could you consume right now?" and "What is your feeling of fullness right now?" The validity and reliability of this approach has previously been established (Flint, Raben, Blundell, & Astrup, 2000; Jamison et al., 2002). Completed VAS score sheets were removed so participants did not see their previous scores. Whilst participants were not blinded, the researchers evaluating VAS data were blinded so they did not know which phase the data were from. The code was broken once data extraction was complete. Area under the curve (AUC) for VAS scales (mm) plotted over time was calculated for each satiety/hunger measure using the trapezoidal estimation method (Doucet, St-Pierre, Alméras, & Tremblay, 2003). For each AUC response generated, values which fell below baseline (negative area) were subtracted from the total area.

4.3.5 Buffet and dietary analysis

Participants were offered a test meal after completion of the final VAS, 3 hours post consumption of the snack food, which consisted of a cold buffet (total energy content 15,400 kJ). This meal was to be eaten freely for up to 30 min, consumed alone and without distractions such as TV, radio or reading. The test meal contained various food items which included bread, cheese, cured meats, tuna, spreads, salad items, savoury snack foods (including potato crisps and almonds), sweetened snack foods, yoghurt, fruit (dried and preserved), juice, diet and sugar sweetened soft drink, flavoured milk and water which were pre-weighed to the nearest gram. The total amount of food eaten at the meal was calculated by weighing food items remaining to the nearest gram. Energy intake and macronutrient profiles were calculated from the buffet and over the 4 days using Foodworks® Nutritional software (version 7, Xyris Software, Pty Ltd, Highgate Hill, Queensland; Australia).

4.3.6 Statistical analysis

To estimate sample size the extant literature was reviewed (Mattes, 2007). Eighteen participants provided 85% power with an alpha of 0.05 to detect a 20% difference in energy intake during the buffet following the consumption of Hi-oleic peanuts compared with potato crisps. Twenty-five participants were recruited to allow for dropout. Prior to analysis, data were checked for order effect and normality with no order effect found. Descriptive statistics for variables are presented as means ± standard error of the mean (SEM). A random effects model was used to determine the difference in energy intake between the three snack conditions, both acutely (at the buffet) and over 4 days (including the buffet). Differences in satiety measures (AUC from VAS) between snacks were determined using the same model. The level of significance accepted was 0.05. SPSS version 20S (SPSS Inc., Chicago, IL) and STATA 11.0 (Stata Corp, Texas, USA) were used for all statistical analyses.

4.4 Results

Twenty-four participants completed the study, 11 were women with a mean BMI of 31 ± 3 kg/ m^2 (range 30–36 kg/m^2) and mean age of 61 ± 6 years (range 53–71 years), 13 were men with a BMI of 30 ± 3 kg/m^2 (range 26–36 kg/m^2) and mean age of 62 ± 8 years (range 54–73 years). **Figure 4.2** shows a CONSORT diagram of participants who were screened and enrolled in the study. One participant withdrew due to poor tolerance of the potato crisps, reporting nausea after consumption of the first dose. Participants commented on the high palatability of the peanuts with most enjoying them, despite the relatively large quantities consumed (56 g/ day for women, 84 g/day for men). Compliance with consumption of test foods at home over 3 days was high (99 % for all test foods) as assessed by food diary entries.

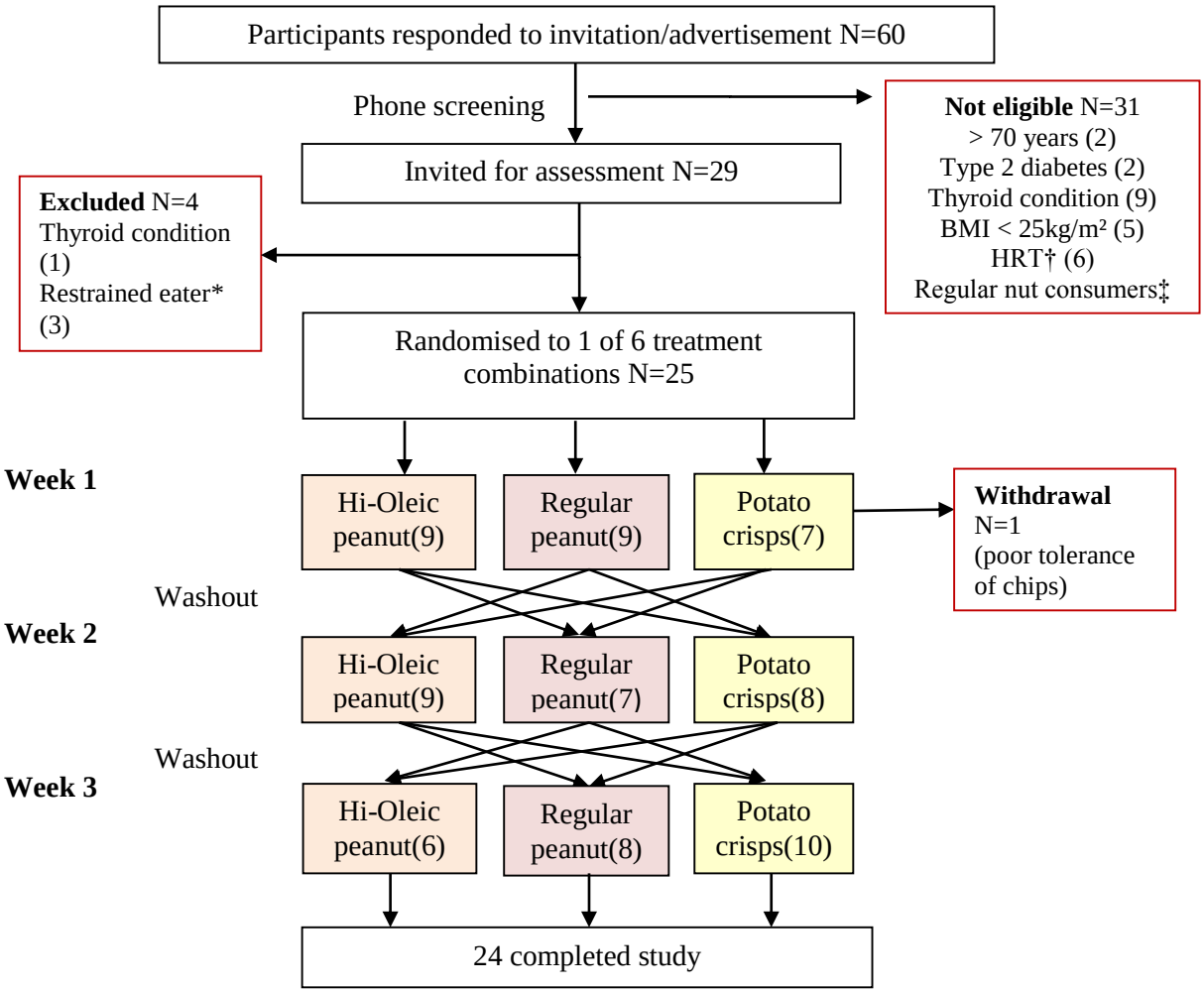


Figure 4.2. Consort diagram of participants who were screened, enrolled and completed the study

*Restrained eater (Three Factor Eating Questionnaire, score cut off >12 excluded), † hormone replacement therapy, ‡consuming ≥ handful (approx. 30g) nuts/week

4.5 Buffet energy intake

Compared with potato crisps, subsequent total energy intake was lower following consumption of Hi-oleic and regular peanuts by −21 % ($p < .001$) and −17 % ($p < .01$) respectively (**Table 4.2, Figure 4.3**). The energy difference was attributed to −26% ($p < .01$) and −31 % ($p < .01$) difference in fat consumption following Hi-oleic and regular peanut consumption respectively and 15 % ($p < .01$) less carbohydrate intake after regular peanuts (**Table 4.2, Figure 4.4**). In addition, compared with crisps, participants ate 11 % less food (g) acutely after the regular peanuts ($p < .05$) but no differences were seen in the amount eaten (based on weight) with Hi-oleic peanuts (**Table 4.2**).

4.5.1 Four day energy intake/expenditure

The mean daily energy intake with Hi-oleic peanut and regular peanut consumption over 4 days was 11 % ($p < .001$) and 9 % ($p < .01$) lower respectively than the potato crisp phase (**Table 4.3, Figure 4.3**). Participants ate 11 % ($p < .05$) and 8 % ($p < .01$) more fat, 23 % ($p < .001$) and 16 % ($p < .001$) more carbohydrate on days when potato crisps were consumed compared with Hi-oleic and regular peanuts respectively, with the crisps accounting for the increase in overall energy intake (**Figure 4.4**). There were no differences in the amount (weight) of food consumed during the 4 day period (**Table 4.3**), resulting in a greater energy density (kJ/g) of foods consumed during the potato crisp phase compared with both peanut phases. No differences were seen in energy expenditure from physical activity diaries during the 4 day period with each of the treatments.

Table 4.2 Macronutrient profile and weight of foods consumed at the buffet meal, 3 hours post consumption of peanuts or crisps[1]

	Crisp	Hi-oleic peanut	Crisp minus Hi-oleic peanut	Effect size	p	Regular peanut	Crisp minus regular peanut	Effect size	p
Weight(g)	1027±41	911±45	116±44	0.54	0.059	932±57	95±47	0.41	**0.022**
Energy(kJ)	3031±241	2380±196	651±196	0.68	**<0.001**	2498±186	533±206	0.53	**0.004**
Total fat(g)	29±15	20±10	9±3	0.64	**0.002**	21±12	8±3	0.60	**<0.001**
Protein(g)	33±12	31±11	2±2	0.20	0.290	30±13	3±3	0.23	0.310
Carbohydrate(g)	80±42	65±37	14±29	0.50	0.076	68±27	11±30	0.40	**0.013**
Fibre(g)	23±2	25±2	1±2	0.50	0.178	24±1	1±2	0.50	0.149

[1]data are presented as mean ± SEM, p<0.05 statistically significant

Table 4.3 Daily energy expenditure and macronutrient intake over 4 days whilst consuming either peanuts or crisps[1]

	Crisp	Hi-oleic peanut	Crisp minus Hi-oleic peanut	Effect size	p	Regular peanut	Crisp minus regular peanut	Effect size	p
Energy intake(kJ)	9233±605	8182±398	1054±344	0.62	**<0.001**	8420±481	814±384	0.44	**0.008**
Energy expenditure(kJ)	8660±165	7950±169	710±77	0.14	0.60	8800±179	140±120	0.24	0.17
Weight(g)	3528±152	3418±144	110±85	0.26	0.72	3522±165	40±129	0.06	0.32
Total fat(g)	95±19	83±17	11±4	0.55	**0.016**	86±18	8±4	0.46	**0.002**
Protein(g)	94±7	99±5	-4±6	0.12	0.127	100±5	-6±6	0.19	0.33
Carbohydrate(g)	221±17	170±9	46±9	0.91	**<0.001**	184±15	40±10	0.77	**<0.001**
Fibre(g)	23±2	24±1	1±0	0.01	0.072	25±2	1±0	0.38	0.58
Alcohol(g)	9±2	10±3	-1±2	0.15	0.67	10±3	-1±2	0.15	0.75

[1]data are presented as mean ± SEM, p<0.05 statistically significant

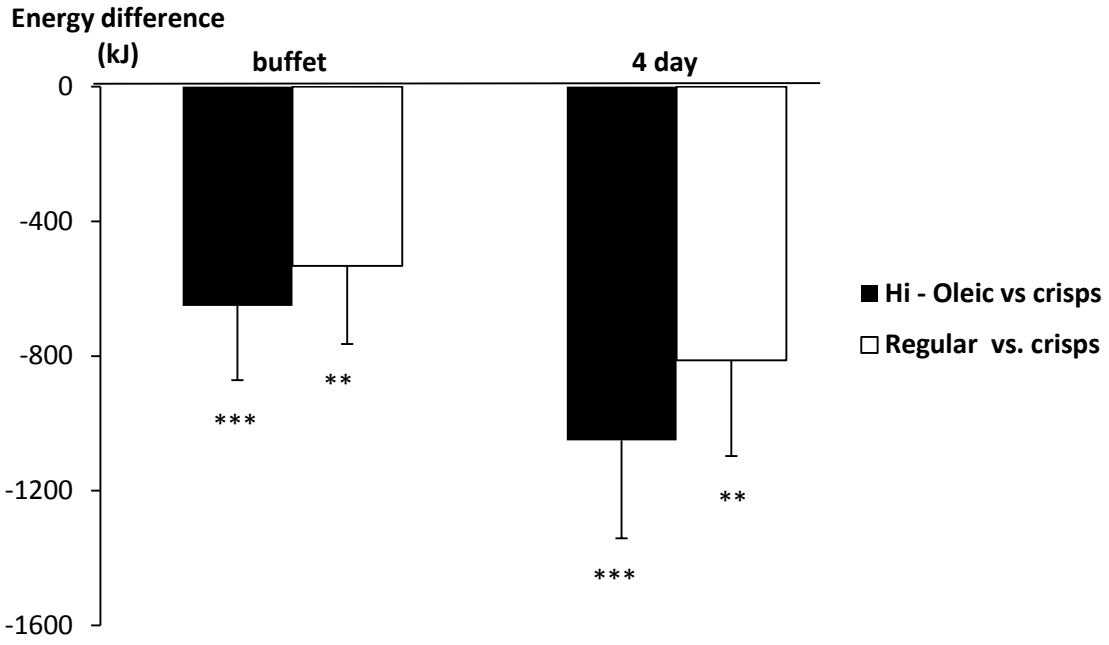


Figure 4.3 Difference in energy intake at the buffet and over 4 days with peanut varieties compared with potato crisps (mean difference ± SEM ** = P<0.01, *** = P<0.001).

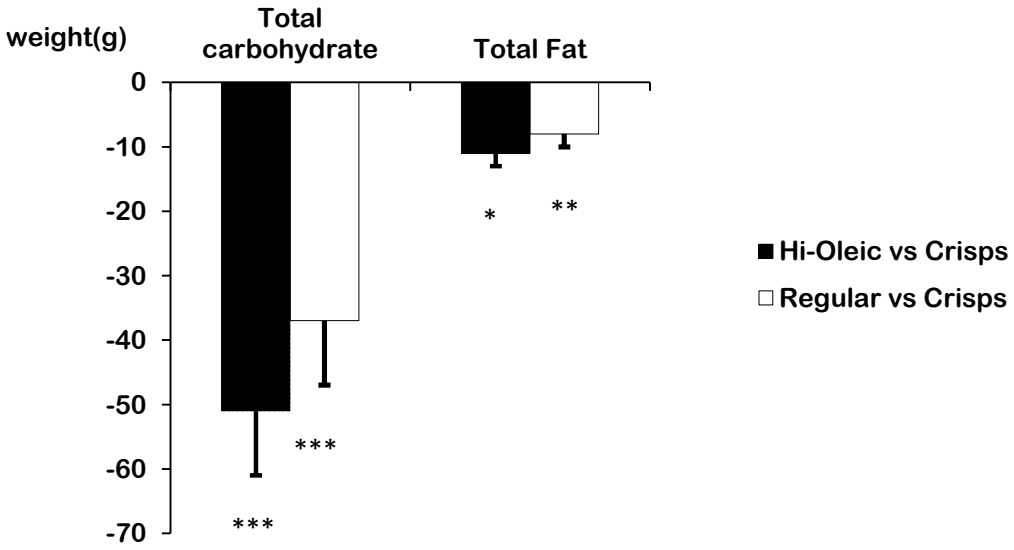


Figure 4.4 Difference in carbohydrate and fat intake (g) over the 4 days when consuming peanuts varieties compared with crisps (mean difference ± SEM * = P<0.05, ** = P<0.01, *** = P<0.001).

4.5.3 Satiety/hunger measures

Following consumption of the test foods, area under the curve (AUC) calculations of repeated visual analogue scale (VAS) measurements demonstrated no significant differences in estimates of hunger, satiety or thirst between the test foods (**Table 4.4**).

Table 4.4 Acute satiety ratings after consumption of peanuts or crisps[1,2,3]

	AUC (mm/min)	Hi-oleic peanuts	Regular peanuts	Potato crisps
Hunger	-2366±751	-2366±751	-1190±648	-1182±649
Thirst	-1132±713	-1132±713	-703±638	-1193±470
Appetite	-2155±764	-2155±764	-1229±689	-1651±685
Amount	-1536±657	-1536±657	-831±604	-1055±511
Fullness	3081±921	3081±921	2859±787	2592±861

the curve (AUC) calculations (mm/min) derived from visual analogue scales (VAS),
[2] mean ± SEM, [3]no significant differences between peanut varieties and crisps

4.6 Discussion

This is the first study to investigate the satiating effects of peanut consumption compared with another savoury snack food. The principal findings were lower total energy intakes, acutely and over a 4 day period with both types of peanuts compared with iso-energetic consumption of potato crisps. These findings align with a previous study that reported ad-libitum consumption of peanuts (89 g/day) for 8 weeks elicited a strong dietary compensatory response resulting in a reduction of energy from other sources (Alper & Mattes, 2002). In contrast, a study investigating snacking on almonds or cereal bars compared with a healthy control diet reported no differences in energy intake with either of the 3 phases, suggesting that energy compensation took place with the addition of both snacks (Zaveri & Drummond, 2009).

Differences in energy intake observed were largely attributable to a reduction of total fat and carbohydrate intakes with peanut consumption, not the amount (g) of food eaten. The energy intake observed over 4 days was substantially

less with peanuts than crisps. This suggests that inclusion of ~60–80 g/day of peanuts or crisps would result in a potential difference of up to 1 kg body weight over one month, with crisp consumers weighing more. Several studies demonstrate that the sensory specific satiety of nuts is maintained compared with other snacks over time (Alper & Mattes, 2002; Tey et al., 2012). Epidemiological evidence has revealed an inverse association between nut consumption and weight gain over a period of 8 years, as demonstrated by the Nurses' Health study (Bes-Rastrollo et al., 2009). The Seguimiento University of Navarra (SUN) study cohort found inverse associations between baseline consumption of nuts and weight gain (Bes-Rastrollo et al., 2007), with lower weight gains observed among participants consuming >2 servings of nuts per week. In addition, a recent meta-analysis of 23 clinical trials investigating the effect of chronic nut consumption on body weight demonstrated a small non-significant decrease (weighted mean 0.47 kg) in body weight. However, in most of the studies, nuts were used in iso-energetic diets so weight changes were not expected (Flores-Mateo, Rojas-Rueda, Basora, Ros, & Salas-Salvadó, 2013). Weight control diets that include nuts have resulted in improved compliance and greater weight loss (Wien et al. 2003).

Interestingly, in the present study the reduction in energy intake with peanut consumption compared with potato crisps occurred despite a lack of difference in subjective satiety ratings, whilst other studies measuring satiety have reported variable outcomes. Peanut butter, and to a lesser extent whole peanuts, enhanced secretion of gut hormones important for satiety and reduced the desire to eat when added to a breakfast meal (Reis et al., 2013). Consumption of a grain bar, one hour prior to meals elicited a greater glycaemic and satiety response than peanuts; however, prolonged satiety measures were not evaluated (Johnston et al., 2013). It is possible that the timing of snacks may be an important factor as well as the macronutrient content, reflecting the complexity of satiety responses. In the present study, apart from during the acute testing phase in the research centre, test foods (both potato crisps and peanuts) were consumed at any time of the day when participants were at home. Many factors affect satiety, including sensory properties of food, physiological and cognitive responses which subjective

satiety ratings may not be sensitive to detect. The satiety observations in the current study may reflect insensitivity of the measures, insufficient timing points, too small a sample or mechanisms other than perceived satiety influencing subsequent energy intake. It is also possible that energy differences observed in the present study (~600 kJ at the buffet and ~900 kJ/d over 4 days) were insufficient to be detected by perceived satiety measures. Other studies have demonstrated differences in subjective satiety ratings with more substantial energy reductions (1500 kJ/day and 1890 kJ/ day) (Johnston et al., 2013; Lee et al., 2006 & Kandeel, 2003), possibly due to their satiating effect. The present study supports evidence that nut consumption is beneficial for weight management. Surprisingly in the present study, no significant correlations were observed between the various satiety measures immediately prior to the buffet (t = 180 minutes) and subsequent energy intake. Similarly, Kirkmeyer and Mattes (2000) found no correlation between satiety measures and subsequent energy intake with preloads of peanuts, peanut butter, chestnuts, chocolate pickles and rice cakes.

This present study supports the hypothesis that observed differences in energy intake may be attributable to the satiety value of protein and fibre, with the peanuts providing an additional 11 g and 19 g of protein for women and men respectively (2–3% of energy intake) and an additional 3–4 g of fibre per day. Previous studies have demonstrated that protein and fibre intake can enhance ratings of satiety which are related to increases in satiety hormones (Burton-Freeman, 2000; Veldhorst et al., 2008). Satiating effects have been noted following consumption of bread enriched with lupin flour (also high in protein and fibre), viz. a 40% reduction in energy intake at the test meal compared with consuming control bread (Lee et al., 2006). In addition it is possible that the additional mastication (not measured) required for the peanuts may also have contributed to their satiety value, as differential appetite responses have been observed with foods requiring different amount of mastication (Zhu, Hsu, & Hollis, 2013). Evidence from human and animal studies indicate that saturation of fatty acids have differential rates of oxidation, with unsaturated fatty acids being more readily oxidised (Kamphuis, Westerterp-Plantenga, & Saris, 2001; Soares, Cummings, Mamo, Kenrick, & Piers, 2004;

Wongsuthavas et al., 2011) and hence may be more satiating than saturated fatty acids (Lawton et al., 2000). The present study partially supports this hypothesis as the higher content of unsaturated fatty acids in the peanuts compared with potato chips may have contributed to the findings. We observed a greater magnitude of change in energy intake for the Hi-oleic peanuts (high in monounsaturated fat) than with regular peanuts but the study was not designed to directly compare the two nuts so clear conclusions cannot be drawn. Reports are inconsistent on the satiety value of different fatty acids (Alfenas & Mattes, 2003), hence more studies are warranted in this area.

A strength of the present study was the cross over design which assessed the test foods in the same individuals, hence reducing between participant variability. Test foods were matched as closely as possible for portion size (g) and energy value, both of which are key determinants of satiety and fullness (Kirkmeyer & Mattes, 2000; Kral, Roe, & Rolls, 2004). Another strength was the age of the population selected. The majority of satiety studies with nuts have been with young healthy population groups; however, the highest prevalence of overweight and obesity is in older adults (Flegal et al., 2012).

A study limitation was our inability to double-blind the intervention; hence the possibility of performance bias cannot be excluded. It is also possible that food intakes were influenced by participants' health perception of the test foods which was another limitation. However, they were informed that food diaries were measuring 'food patterns' (presented as a neutral term) rather than energy intake. Another limitation was the present study did not measure satiety hormones, which may have been able to indicate whether the reduced energy intake observed was attributable to changes in satiety hormones. In addition gastrointestinal symptoms, bowel habits and stool mass were not monitored to determine if additional fibre content of the peanuts increased faecal bulk, hence increasing the feeling of fullness.

Future study recommendations should include trials of longer duration to determine if a difference in energy intake with peanuts is sustained and to assess the effects of peanut consumption on eating behaviors. In conclusion,

the consumption of both types of peanuts resulted in lower energy intakes both acutely and over 4 days compared with the iso-energetic consumption of potato crisps. This is consistent with epidemiological and intervention studies indicating that, rather than increasing energy intake, supplementing the diet with peanuts can reduce overall energy intake compared with consumption of a high energy snack food (potato crisps). However, the findings cannot generalised to other populations, hence studies are warranted in lean, younger population groups and those with restrained eating. Peanuts are an ideal high fibre, high protein, high unsaturated fat snack food and their incorporation into the diet may assist with energy balance and provide a healthy snack choice for weight management.

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Authors' contribution*:* J.A.B (PhD candidate), A.M.C., P.R.C.H., J.D.B., J.B. and G.W. were all involved with the research design, G.W. was responsible for choice of peanuts, J.A.B. conducted the research and prepared the manuscript, J.A.B., A.M.C., P.R.C.H., J.D.B. and J.B. all contributed to data analysis and editing of the manuscript (including responsibility for the final content); all authors have read and approved the final manuscript.

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4.8 Summary

This is the first satiety study with Hi-oleic peanuts; the findings indicated that peanuts could promote satiety and reduce total energy intake acutely and over 4 days, supporting them as an appropriate choice of snack food. While this study demonstrated reduced energy intake over a relatively short time period with peanut consumption compared with a high energy snack food, it was not clear whether such an effect would be sustained over the longer term.

Weight management plays an important role in cardiovascular health and prevention of cognitive decline. The following chapters describe **Study 2**, which measured the effects of sustained Hi-oleic peanut consumption vascular and cognitive function, cardio-metabolic outcomes, dietary intake and body composition. Unlike **Study 1**, the peanut and control phases were intentionally not energy matched, in order to observe the influence of peanut intake on cardio-metabolic, vascular and cognitive measures as well as energy intake and body composition when added to the habitual diet.

Chapter 5: The effect of daily Hi-oleic peanut consumption on vascular and cognitive measures

5.1 Introduction

This chapter will provide a brief literature summary of the effects of nut consumption on vascular and cognitive performance, followed by details of **Study 2** assessing the influence of 12 week Hi-oleic peanut consumption on vascular and cognitive measures. **Chapter 6** will describe the remaining outcome measures from **Study 2**; these include dietary intake, body composition and cardio-metabolic outcomes.

5.2 Background - Can nuts improve cognition and metabolic health by improving endothelial function?

There is emerging evidence that frequent nut consumption beneficially affects cardiovascular risk through a combination of lipid lowering, antioxidant and anti-inflammatory responses, glucose regulation, endothelial function and possible body composition improvements, demonstrated in several studies (de Lorgeril et al. 2001; Jenkins et al. 2008a; Landmesser, Hornig & Drexler 2004). These mechanisms and in particular, endothelial function are also essential for cerebral vascular function to provide adequate blood flow to the brain. It has been proposed that by improving blood flow regulation in the brain, cognitive performance is also improved (Krestin et al. 2008; Sinn & Howe 2008).To date, no studies have investigated the effects of nut consumption on cerebral blood flow.

There is growing evidence that some components of the diet or specific foods may have benefits for cognitive performance. A positive relationship between cognitive performance and bioactive nutrients such as MUFA, vitamins and polyphenols found in nuts has been demonstrated in several dietary studies. (Joshi & Praticò 2012; Kennedy et al. 2010; Macready et al. 2009; Naqvi et al. 2011). Human, animal and in vitro studies have also demonstrated potential benefits of nuts for cognitive function (O'Brien et al. 2014; Willis et al. 2010; Willis et al. 2009). Hi-oleic peanuts are a good source of several bioactive nutrients such as monounsaturated fatty acids, L-arginine and polyphenols (including resveratrol) which have been shown to improve endothelial function which may also influence cognitive function hence, they were an ideal food choice for **Study 2** described below.

5.3 Hypothesis

A hypothesis for this study was introduced in **Sections 1.3 and 3.3** of the scoping review and summarised in **Figure 3.1**. More detailed hypotheses are presented below.

It was hypothesised that in healthy overweight or obese individuals after 12 weeks of Hi-oleic peanut:

i. Cognitive performance would increase. This effect was expected to be the result of increased cerebrovascular reactivity (CVR, a measure of endothelial function in the cerebral blood vessels), allowing greater blood flow and supply of nutrients and oxygen to the brain.
ii. The effect on CVR would be expected to be associated with one or more of the following; lowering of lipids, blood pressure, inflammation, glucose, insulin, and/or body fat.

5.4 Aim

To investigate the effect of adding Hi-oleic peanuts to habitual diets of healthy overweight adults, on vascular measures (blood pressure, arterial compliance and cerebrovascular reactivity) and cognitive measures and to determine if there are any relationships between these outcomes.

5.4.1 Primary outcome

To determine the effect of chronic Hi-oleic peanut consumption on CVR

5.4.2 Secondary outcomes

To determine the:

- Effect of chronic consumption of peanuts on cognitive performance.
- Effect of chronic Hi-oleic peanut consumption on arterial compliance (a measure of peripheral blood vessel elasticity) and blood pressure
- Relationship between cerebral reactivity and cognitive performance

5.5 Method

5.5.1 Participant selection

Participants were recruited from February to May 2012 through, The Nutritional Physiology Research Centre participant data base, newspaper advertisements and word of mouth. The inclusion criteria were healthy overweight males or post-menopausal females aged between 50 and 75 years with self-reported stable weight and body mass index (BMI) ≥ 25 kg/m². Smokers, regular nut consumers and people with cardiovascular disease, a thyroid condition, nut allergy were excluded (**Table 5.1**). Restrained eaters determined by a score of ≥ 12 for the Three Factor Eating questionnaire (Stunkard & Messick 1985) and those with mild cognitive impairment (determined by a score of <10 for the DemTect® test (Kalbe et al. 2004) were also excluded. Participants on stable doses of fish oil lipid lowering or anti-hypertensive medications were included in the study but requested not to alter their doses during the course of the study.

Table 5.1 Participant inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
• Overweight (BMI > 25 kg/m²) < 135kg (upper limit DEXA) • Men 50-75 years • Blood pressure<160/100mmHg • Women 50-75 years post-menopausal (self-reported cessation of menses >12 mths)	• Allergy to peanuts or any other nuts • Suspected mild cognitive impairment • Smoker or nicotine replacement • Medications, disease or condition which may affect the outcome (e.g. thyroid condition, diabetes or cardiovascular disease) • Restrained eaters • Nut consumers (>30g/week)

Abbreviations: BMI, body mass index, **DEXA** Dual X-ray absorptiometry

This group of healthy overweight, middle-aged to older participants were chosen as they are more likely than younger or leaner participants to have reduced endothelial function (measured as cerebrovascular reactivity), hence more likely to benefit from the peanut intervention (Joseph, L et al. 2002). Participants with diabetes or cardiovascular disease were excluded as they were more likely to

have extensive endothelial damage and so less likely to demonstrate reversal of endothelial dysfunction (Forman et al. 2008). The study was approved by the University of South Australia Human Research Ethics Committee (0000026851) and was registered with the Australian and New Zealand Clinical Trials Registry (ACTRN 12612000192886). All participants provided written informed consent.

5.5.2 Pre-commencement: eligibility and dietary assessment

Potential participants were initially screened for eligibility either over the phone or via email and by completing a diet and lifestyle questionnaire (to determine medical history) and The Three Factor Eating questionnaire (to determine restrained eating). Eligible participants were asked to immediately cease all intake of nuts and nut products until completion of the study (except for the Hi-oleic peanuts provided). They were provided with 4 day weighed food and physical activity diaries (Bouchard et al. 1983) along with instructions on how to weigh (to the nearest gram), measure and record intake and physical activity prior to their first visit. At visit one, participants attended the research centre after an overnight fast of at least 12 hours for screening and if successful they commenced the study at the same visit.

5.5.3 Experimental design

Eligible participants were randomised using computer generated software (www.randomization.com) to commence either 12 weeks consumption of Hi-oleic peanuts or habitual diet (devoid of nuts). Participants then crossed over to the alternate diet at week 12 as shown in **Figure 5.1**.

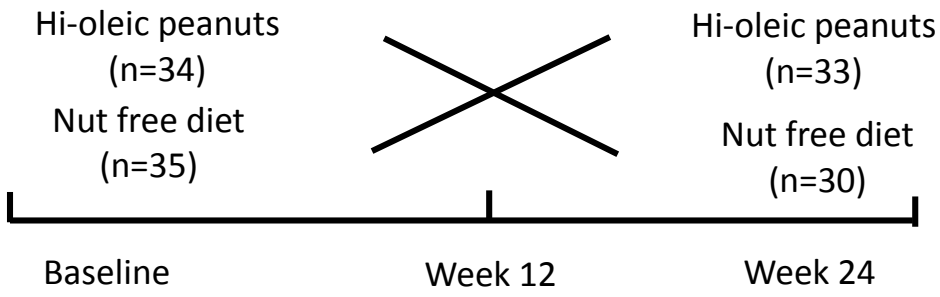


Figure 5.1 Experimental design

5.5.4 Nutrient profile of peanuts

Roasted, unsalted Hi-oleic cultivar Middleton) peanuts with skins on were provided in 28 g sealed foil packets by the Peanut Company of Australia (Kingaroy, Queensland, Australia). Males were provided with 3 packets (84 g) per day and females 2 packets (56 g) per day to be consumed daily for 6 days per week (providing ~ 15 % of energy intake). To assist with compliance participants were given one rest day per week with no peanuts.

Table 5.2 Nutrients provided by Hi-oleic peanuts for males & females

Nutrient	Females (56g/d peanuts)	Males (84g/d peanuts)
Energy (kJ)	1330	1996
Total fat (g)	28	42
MUFA (oleic) (g)	22	34
PUFA (linoleic) (g)	2	3
SFA (palmitic) (g)	3	4
Protein (g)	15	22
Arginine (g)	2	3
Resveratrol (mg)	0.1	0.2
Fibre (g)	5	7
Vitamin E (mg)	5	7
Folate (µg)	134	202
Magnesium (mg)	94	141
Potassium (mg)	395	592
Iron (mg)	2.5	4

Abbreviations: MUFA monounsaturated fatty acid, PUFA, polyunsaturated fatty acid, SFA, saturated fatty acid

The nutrient profile of the peanuts for males and females is shown in **Table 5.2**. The peanuts were stored at room temperature in dark, low humidity conditions. At the mid-point and end of the intervention period, participants returned peanut packaging (including uneaten peanuts) as a measure of compliance.

5.5.5 Study protocol

At visit 1 (day 0) screening assessments were conducted for:

1) Resting supine blood pressure (>160/100 mmHg excluded)
2) Body weight/BMI (>135 kg or <25 kg/m² were excluded)
3) Transcranial Doppler (TCD) measurements (excluded if no clear signal on found either side)
4) Mild Cognitive impairment (score <10 excluded)

After screening, consent documentation and randomisation were completed, baseline measurements were taken. Height, weight, blood pressure and TCD measures used as assessment for study eligibility were included as baseline measures.

Other measures were: waist circumference, fasting blood lipids, glucose, insulin and C-reactive protein. Participants were then provided with a small snack (Uncle Tobys Le Snack®, 357 kJ) and performed a battery of cognitive tests. Baseline measures took ~ 2 hours to complete.

Following baseline testing, the intervention group was provided with 6 weeks supply of peanuts and instructed to consume 56 g (women) or 84 g (men) of Hi-oleic peanuts for 6 out of 7 days each week. A rest day was included to assist with compliance. Participants were reminded not consume any peanuts or other nuts or nut products other than those provided by the study; no other dietary intervention advice was provided. Participants were also asked not changes doses of their medications during the course of the study. The control group was instructed to continue their normal diet devoid of nuts or nut products.

At week 6 participants attended the research centre for visit 2 and measurements of body mass and waist circumference were taken. Participants were provided with a further set of diaries to complete 4 days prior to the next visit and the intervention group were provided with a further 6 week supply of peanuts.

At week 12 (visit 3) measurements taken at visit 1 were repeated with the exception of DemTect® and height. In addition, measurements of body composition (with Dual-Energy X-Ray absorptiometry (DEXA) described in chapter 6) with large and small arterial elasticity (LAE and SAE) were performed. Participants then crossed over to the to the alternate treatment arm and attended the research centre at week 18 and week 24. See **Figure 5.1 and Table 5.3** for experimental design and measures taken at each time point.

Table 5.3 Study measures taken at baseline and the mid and end points of each 12 week phase.

Measure	Base line	Peanut mid-point	Peanut end-point	Control mid-point	Control end-point
Waist circum.	X	X	X	X	X
CVR	X		X		X
Cognitive assessments	X		X		X
Lipids	X		X		X
Glucose	X		X		X
Insulin	X		X		X
CRP	X		X		X
Arterial compliance(LAE/SAE)			X		X
DEXA			X		X
Dietary intake	X	X	X	X	X
Physical activity	X	X	X	X	X

Abbreviations: BMI, body mass index, CVR, cerebrovascular reactivity, CRP, C-reactive protein, DEXA, dual energy X-ray absorptiometry, LAE, large artery elasticity, SAE, small artery elaticity.

5.6 Measurements

5.6.1 Cerebral vascular responsiveness (CVR)

Transcranial doppler (TCD) (Multigon EMS 9A, Multigon Industries, Inc, NY, USA) was used to non-invasively measure increases in blood flow velocity in the middle cerebral artery resulting from downstream dilatation of cerebral arterioles in response to acute hypercapnia (increased pCO_2 in the circulation). - Intraclass correlation coefficients for CVR intra-rater reliability = 0.725 (McDonnell et al. 2013). Participants were fitted with a headband containing automated oscillometer and TCD ultrasound probes that align with both left and right temples. This provides a more direct measure to reflect endothelial function in cerebral arteries than either EndoPAT (peripheral artery tonometry) or flow mediated dilatation (FMD (Harrison & Markus 1992; Markus 2000). After a clear signal was obtained, a 30-second baseline was recorded before the subject inhaled carbogen gas (5 % CO_2/95 % O_2) for 2 ½ minutes. Mean flow velocity was recorded for each cardiac cycle during the whole 150 seconds. To improve test reliability, this assessment was performed 3 times with a 2-minute interval to allow flow velocity to return to baseline levels. Data was stored electronically in the device and on the Nutritional Physiology Research Centre (NPRC) server.

5.6.2 Blood pressure and arterial compliance

Blood pressure measurements were performed in accordance with the Joint National Committee on Prevention, Evaluation and Treatment of High Blood Pressure (VI): US Department of Human Services (Chobanian, Bakris & Black 2003). Arterial compliance is defined as the ability of an artery to expand and recoil with cardiac pulsation and relaxation (Arnett, Evans & Riley 1994). Arterial elasticity can be assessed non-invasively using a cardiovascular profiler (HDI cardiovascular profiler CR 2000, Hypertension Diagnostics, Eagan, Minnesota, USA (Prisant et al. 2002)) which is a computerized radial artery pulse wave analysis. The arterial pulse wave analysis (**Figure 5.2**) of the radial artery allows an evaluation of the elasticity of the large conduit arteries and the small microcirculatory arteries (Cohn et al. 1995; Finkelstein & Cohn 1992). SAE correlation coefficient = 0.38*, LAE correlation coefficient = 0.46*

(*Wave pulse analysis, 10 participants treated twice, Alicia Thorpe, 2003 unpublished data)

Participants were rested in a supine position for 10 minutes before measurements of blood pressure and arterial compliance were performed. The cardiovascular profiler assessed the large and small artery elasticity indexes. Four consecutive readings were taken at five minute intervals by automated oscillometry using standard or large blood pressure cuff (depending on the size of participants' arm) over the left brachial artery and tonometer placed over the right radial artery to assess blood pressure and pulse wave analysis respectively. After discarding the first reading for blood pressure and arterial compliance, an average of the remaining measurements (no more than 10% variance between readings) was recorded for analysis.

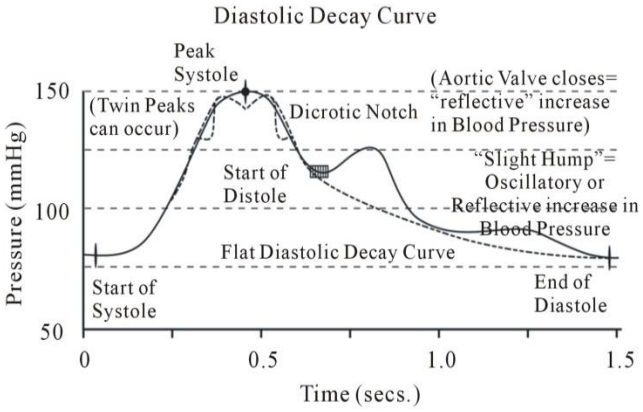


Figure 5.2 Arterial pulse waveform (HDI Diagnostics, Inc., Eagan, MN, USA)

5.6.3 Cognitive performance measures

Participants were initially screened for mild cognitive impairment, to be eligible for the study they were required to obtain a score of >10 on the DemTect® (Kalbe et al. 2004). As part of the study three sets of tests were chosen to cover a broad range of cognitive abilities including memory (immediate and delayed recall of verbal information and working memory), executive function (verbal fluency and inhibition) and processing speed with each test having high test-retest reliability and inter-rater reliability. Cognitive tests were conducted one-to-one with the same investigator at the same time of day, in a quiet room.

5.6.4 Mild cognitive impairment screening

DemTect® (Kalbe et al. 2004) is a short test (5 questions) which has 80% sensitivity to detect mild cognitive impairment. The test covers a wide range of cognitive abilities including immediate and delayed recall of verbal information, working memory, language and number processing and executive function. It has high sensitivity (80%) to detect mild cognitive impairment and high inter-rater reliability (r = 0.99) (**see Appendix C**).

5.6.5 Memory - measured by The Rey Auditory Verbal Learning Task (RAVLT)

The Rey Auditory Verbal Learning test uses a list-learning format to examine verbal memory including immediate recall, recall following short and long delay periods and recognition **(Appendix C)**. Participants were randomised to complete a different RAVLT test at each visit to eliminate a learning effect. They listened to a list of 15 words read aloud at a rate of one word per second by the principal investigator (Jayne Barbour). The test included five presentations of a 15 word list (list A), after each of which participants reported as many of the words they could remember, in any order. These five trials were followed by a second word list (list B) which was recalled, and then a sixth recall trial of the first list (List A). The test also assessed delayed recall with a seventh recall trial of words (list A) administered after a 20-30 minute delay. Recognition was then tested by presenting in random order the 30 words from both lists plus 20 additional words, printed on a sheet of paper. Participants were

required to identify by circling in pencil the words from lists A and B. The test took approximately 15 minutes to administer per participant. A composite memory score was calculated by the addition of z-scores from the 3 RAVLT tests. The RAVLT test-re-test reliability is r = 0.61 - 0.86 (Lezak 1995).

5.6.6 Executive function - measured by the Stroop Colour-Word Test and tests of verbal fluency.

5.6.6.1 The Stroop colour-word test

The Stroop colour-word test measures an individual's ability to suppress an irrelevant response to a task. This test requires participants to supress the tendency to read a word and name the colour of the word (in an incongruent colour), whilst maintaining concentration throughout (**See Appendix C**) (Strauss 2002). The rationale for using this test is other dietary interventions have demonstrated improvements with this measure of executive function (Berry et al. 2011; Wong et al. 2012). This test has a test-retest reliability coefficient of r = 0.86 and its validity has been confirmed with healthy individuals (Graf, Utte & Tuokko 1995).

For this test participants were required to read from a sheet displaying random variations of four colour names (red, yellow, green, blue), printed in an incongruent colour (e.g. the word "yellow" was printed in red ink). During trial 1 participants were required to read the words as written and in order as fast and accurately as possible (word reading trial). In the second trial participants were required to name the colour of each word and ignore the word itself, as quickly and accurately as possible (colour naming trial). The number of corrected and uncorrected errors and the time for each trial was recorded. As is traditional, the Stroop interference score was calculated from the time taken to complete the colour naming trial (trial 2) divided by the time to complete the word naming trial (trial 1). Thus, a higher Stroop interference score typically demonstrates poorer performance as this reflects greater interference when trying to suppress the reading of words in order to name colours. Uncorrected errors reflect failure to inhibit the reading of words in order to name colours. For consistency with

other tests, scores were adjusted by multiplying by -1 such that higher scores reflected better performance.

5.6.6.2 Initial and excluded letter fluency

Initial and excluded letter fluency tests measure the ability to assess the strategic retrieval of verbal material. Successful performance on these tests is thought to depend on the ability to initiate, search and retrieve data from the memory reflecting executive function and attention (Baldo et al. 2001). These tests required participants to produce as many words as possible within 60 seconds either beginning with a designated letter e.g. F, S (initial letter task) or not containing a designated letter e.g. E, A (excluded letter task) (Vakil & Blachstein 1997). A composite score was calculated by the addition of z- scores from each component of the test to provide a verbal fluency score. A composite executive function score was calculated by the addition of z-scores from Initial Letter, Excluded Letter and Stroop Colour-Word Test scores.

5.6.7 Processing speed - measured by Symbol Search and Coding.

The Symbol Search and Coding tests are subtests of the Wechsler Adult Intelligence 4th edition (WAIS IV) (Wechsler 2008)(**Appendix C**)

5.6.7.1 Symbol search

The Symbol Search test is designed to assess information processing speed and visual perception; high scores require rapid and accurate processing of nonverbal visual information (i.e., symbols without *a priori* meaning). This is a self-paced task during which participants are allotted 120 seconds to complete as many symbol discrimination items as possible. Each item is presented sequentially as a row. In each row, two geometric symbols are presented to the left and another set of five symbols to the right. If either symbol of the left matched any symbols on the right, participants were required to circle the matching symbol, if there were no matching symbols then the word "NO" was circled. The test retest reliability coefficient for symbol search is $r = 0.81$and is highly correlated ($r = 0.91$) with the coding task (Wechsler 2008).

5.6.7.2 Coding

This task is a measure of visual-motor coordination, motor and mental speed. Participants were requested to match numbers with a corresponding symbol completing as many in 120 seconds as possible (Wechsler 2008). The reliability co-efficient for this test is $r = 0.86$ (Wechsler 2008). A composite score for processing speed was calculated by the addition of z-scores from Coding and Symbol Search.

5.7 Statistical analysis

It was estimated that 64 participants would provide 80 % power to detect a medium effect size (0.5) in CVR (primary outcome). Seventy five participants were recruited over 2 cohorts to allow for dropouts (see consort diagram **Figure 5.3**). All data were checked for errors, and outliers removed (mean ± 3 standard deviations) before analysis (outliers were found with one peanut and one control phase TCD and blood pressure measure, three baseline TCD recordings and one baseline CRP measure). Data were analysed for study completers only and were checked for normality using frequency histograms. General linear repeated measures analysis of variance was used to analyse the data comparing the 2 treatments, controlling for baseline as a covariate, using SPSS version 21(Chicago, Illinois). Statistical significance was accepted at $p < 0.05$ for the primary outcome (left and right CVR).Comparisons were made between the cohort which commenced peanuts first vs cohort on control first and cohort on peanuts second vs cohort on control second to test for order and time effects. A sequential Bonferroni procedure (Rice 1989) was used to adjust for multiple comparisons of the 11 remaining measures at week 12 (blood pressure, arterial compliance, glucose, insulin, C-reactive protein, lipids, body composition, memory, verbal fluency, executive function, speed of processing) (see **Appendix D** for sequential Bonferroni calculation). Where significant outcomes were observed or where a possible relationship was possible, these measures (changes between intervention and control) were correlated. For these multiple interrelationship comparisons significance was set at $p = 0.05$. Effect sizes were calculated as the change from baseline to end of intervention divided by the

standard deviation of the change. Where appropriate, data are presented as mean ± standard error of mean.

5.8 Results

5.8.1 Participant selection

A flow chart of participants, who were screened, assessed, recruited and completed the study is shown in **Figure 5.3.** A total of 97 people were screened by phone and 82 invited for assessment. Of the 69 participants who commenced, 61 completed the study. Twenty eight participants were excluded with a thyroid condition (6), type 2 diabetes (1), cardiovascular disease (1), BMI < 25kg/m² (2), were regular nut eaters (4), over 75 years (4) or taking hormone replacement therapy (3). A detectable TCD signal could not be found in 6 people. After commencement of the study 5 participants withdrew for either health reasons (2) or due to time constraints (3). Three people were excluded for not complying with the study criteria by either not maintaining physical activity levels (extended trekking holiday), exceeding 4 alcoholic drinks per day or commencing lipid lowering medication during the course of the study.

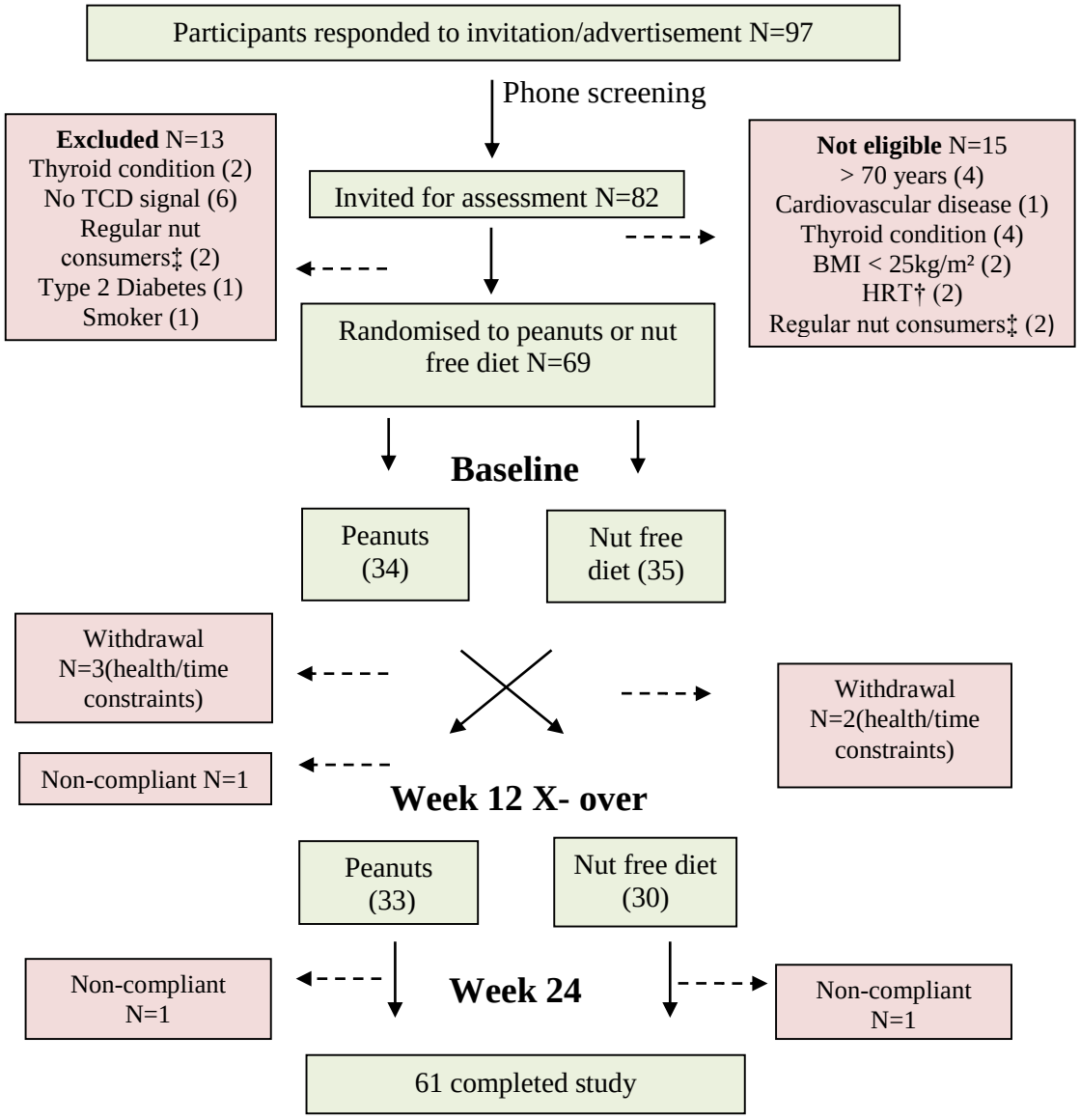


Figure 5.3 Consort diagram of participants who were screened, enrolled and completed the study
† hormone replacement therapy, ‡consuming ≥ 30g nuts/week

5.8.2 Participant baseline measures

Participant characteristics are shown below in **Table 5.4**. There were a similar number of males (29) and females (32) completing the study.

Table 5.4 Participant characteristics at baseline

Participant characteristic	Mean (SD)
Number of participants	61
Age (years)	64.5 (7.3)
Height (cm)	169.4 (8.0)
Body mass (kg)	88.8 (14.7)
BMI (kg/m²)	30.2 (7.0)
Waist circumference (cm)	100.0 (11.0)

Abbreviations: BMI, body mass index, SD standard deviation

5.8.3 Baseline vascular and cognitive measures

Baseline measures of vascular and cognitive measures are reported below in **Table 5.5** DemTect® was measured at baseline only as part of the screening process to exclude any participants with suspected mild cognitive impairment (score of <10). The DemTect® rating for participants was high with a mean score of 16.7 ± 1.8 out of a possible maximum of 18.

Table 5.5 Participant measures of vascular and cognitive function at baseline (data expressed as mean (SD)) (n=61)

Vascular measures	
Left % cerebral vascular reactivity	26.9 (7)
Right % cerebral vascular reactivity	26.3 (7)
Systolic blood pressure (mmHg)	133.2 (15)
Diastolic blood pressure (mmHg)	74.9 (8)
Cognitive measures	
DemTect®	16.7 (1.8)
RAVLT recall	47.7 (10)
RAVLT delayed recall	8.3 (3)
RAVLT distractor	17.4 (3)
Stroop interference	-2.0 (0.7)
Coding task	56.3 (14)
Symbol search task	25.8 (6)
Initial letter task	26.0 (8)
Excluded letter task	25.5 (9)

Abbreviations: RAVLT, Rey auditory verbal learning test, SD, standard deviation

5.8.4 Cognitive and vascular data

All data was checked for normality using frequency histograms and all measures were found to be normally distributed. No diet order or carry over effects were observed for CVR, arterial compliance, blood pressure or cognitive measures. A sequential Bonferroni procedure (Rice 1989) was used to adjust for multiple comparisons of blood pressure, arterial compliance and cognitive tests (**Appendix D**). All % changes indicated are from baseline to end, between treatments.

5.8.4.1 Vascular measures

5.8.4.2 Cerebrovascular reactivity (CVR)

Despite satisfactory image quality of the middle cerebral artery (MCA) using TCD for 61 participants at the time of screening, 53 and 49 complete analysable CVR files were obtained on the left and right sides respectively (files which could not be analysed were eliminated from the analysis). After 12 weeks of peanut consumption a significant 8% and 9% improvement in LCVR (p=0.035) RCVR (p= 0.032) respectively compared with the nut free diet. Both sides demonstrated a small to medium effect size **(Table 5.6, Figure 5.4)** with peanut consumption.

Table 5.6 Effect of 12 week Hi-oleic peanut consumption and nut free diet on cerebrovascular reactivity (CVR)

	12 weeks Hi-oleic peanuts (SD)	12 weeks control diet (SD)	Difference peanuts-control (SEM)	P value treatment	Effect size
% Left CVR (n=53)	26.8(6.8)	25.6(6.8)	1.3(0.7)	**0.035***	0.38
% Right CVR (n=49)	28.0(7.6)	26.7(7.3)	1.7(0.9)	**0.032***	0.48

Abbreviations: SD standard deviation, SEM standard error,* p value < 0.05 reached significance,

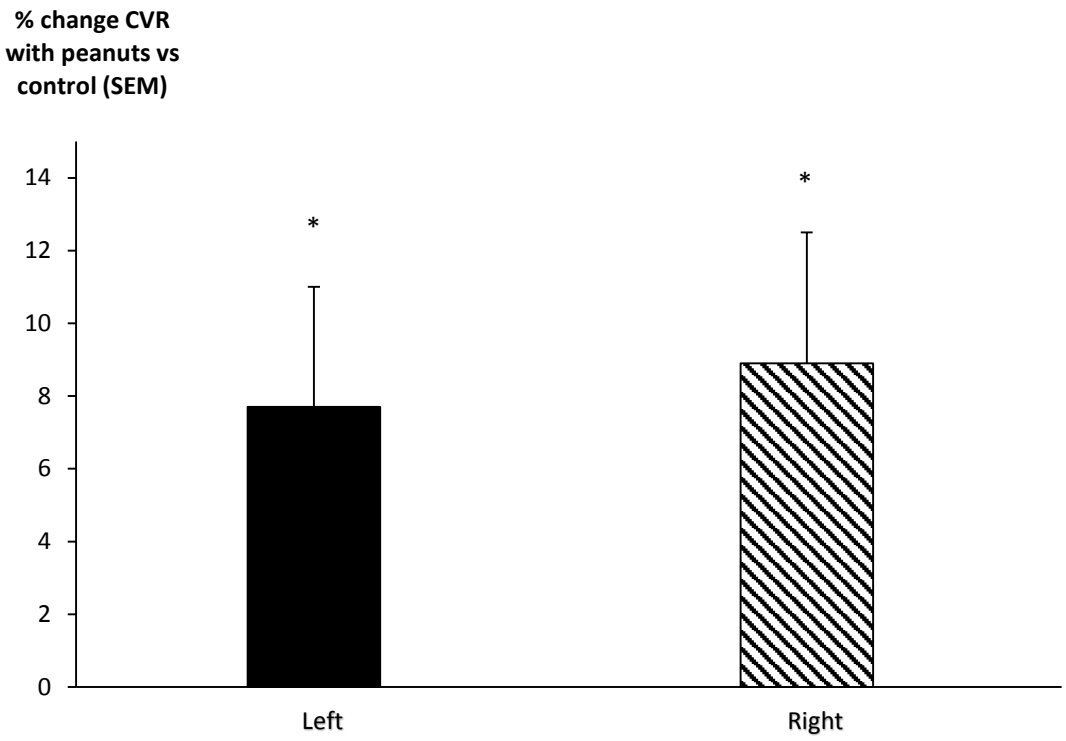


Figure 5.4 Change in cerebral vascular reactivity (CVR) with peanuts compared with control (* denotes $p < 0.05$)

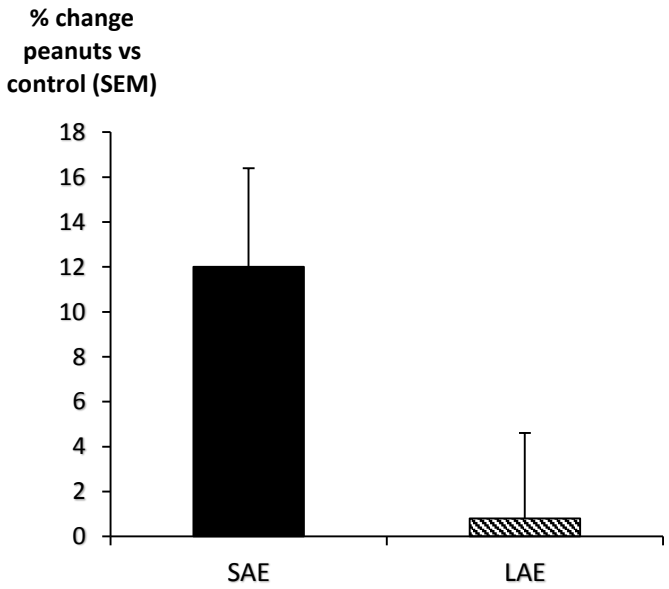


Figure 5.5 Change in small artery elasticity (SAE) and large artery elasticity (LAE) with peanuts compared with control

5.8.4.3 Arterial compliance

A 12% increase in small artery elasticity (SAE) with an effect size 0.4 was observed with peanut consumption compared with a nut free diet (**Table 5.7, Figure 5.5**; however this did not reach significance after sequential Bonferroni correction. Large arterial elasticity demonstrated no change with the peanut intervention (**Table 5.7, Figure 5.5**). There were no significant correlations between arterial compliance and other vascular measures and no correlations between small and large artery elasticity (**Table 5.8**).

5.8.4.4 Blood pressure

No changes in systolic (p=0.71) or diastolic blood pressure (p=0.45) were observed **(Table 5.7)** when comparing participants consuming Hi-oleic peanuts with a nut free diet. Changes in systolic and diastolic blood pressure (between peanut and control phase) were closely correlated (r = 0.66, p<0.001)

Table 5.7 Effect of peanut consumption and nut free diet on blood pressure and arterial compliance

	12 wk peanuts (SD)	12 wk control (SD)	Difference peanuts - control (SEM)	p value	Adjusted p value‡	Effect size
SBP (mmHg) n=61	134.0 (2.1)	134.2 (2.3)	-0.5 (3.1)	0.402	4.800	-0.11
DBP (mmHg) n=61	75.9 (1.4)	75.4 (0.5)	0.5 (1.5)	0.843	9.600	0.12
SAE ml/mmHg x100 n=59	5.5 (3.5)	5.0 (3.0)	0.5 (0.2)	0.015	0.180	0.40
LAE ml/mmHg x10 n=59	14.7 (5.0)	14.6 (5.3)	0.1 (0.6)	0.712	8.400	0.13

Abbreviations: SBP, systolic blood pressure, DBP, diastolic blood pressure, LAE, large artery elasticity, SAE, small artery elasticity, ‡sequential Bonferroni, SD standard deviation, SEM standard error

Table 5.8 Relationships between vascular outcomes

		LAE	SAE	LCVR	RCVR
SBP	Pearson Correlation	-0.077	-0.255	0.100	-0.025
	Sig. (2-tailed)	0.558	0.054	0.514	0.885
DBP	Pearson Correlation	0.045	-0.108	0.185	-0.104
	Sig. (2-tailed)	0.731	0.421	0.224	0.541
LAE	Pearson Correlation	-	0.196	-0.247	-0.086
	Sig. (2-tailed)	-	0.140	0.098	0.606
SAE	Pearson Correlation	0.196	-	-0.293	0.152
	Sig. (2-tailed)	0.140	-	0.056	0.382

Abbreviations: LAE, large artery elasticity, SAE, small artery elasticity, LCVR, left cerebrovascular reactivity, RCVR, right cerebrovascular reactivity

5.8.4.5 Cognitive measures

A range of cognitive tasks were conducted to test for changes in memory, executive function and processing speed. No diet order, time or cognitive test order effects were observed. All cognitive measures demonstrated improvements or a trend towards improvements with small to medium effect sizes after peanut consumption compared with the control diet (**Table 5.9, Figure 5.6)**). The RAVLT recall, a measure of short term memory demonstrated a 5% improvement with an effect size of 0.34 as a trend but did not reach significance ($p = 0.02$). Other measures of memory including delayed memory and retrieval (RAVLT delayed recall, RAVLT target identified) also, did not reach significance. The Stroop colour word test is a test of interference and executive function; this demonstrated an 11 % improvement with peanut consumption with a small effect size (0.26) but did not reach significance ($p = 0.22$). Excluded letter and initial letter tasks are measures of verbal fluency and executive function. Excluded letter demonstrated a statistically significant increase 11% ($p = 0.004$) with an effect size of 0.39. Although the initial letter task did not reach significance, when z scores for these two measures were combined to provide a verbal fluency score this demonstrated a significant, medium effect size (0.46). Coding and symbol search are tests of processing speed; these demonstrated small improvements (effect size 0.34 and 0.25 respectively) with the coding task approaching significance ($p = 0.055$). Combined z scores of memory, executive function and processing speed did not reach significance. Correlations of

cognitive and vascular function with nutrient intake, cardio-metabolic and body composition outcomes are presented in **Chapter 6**.

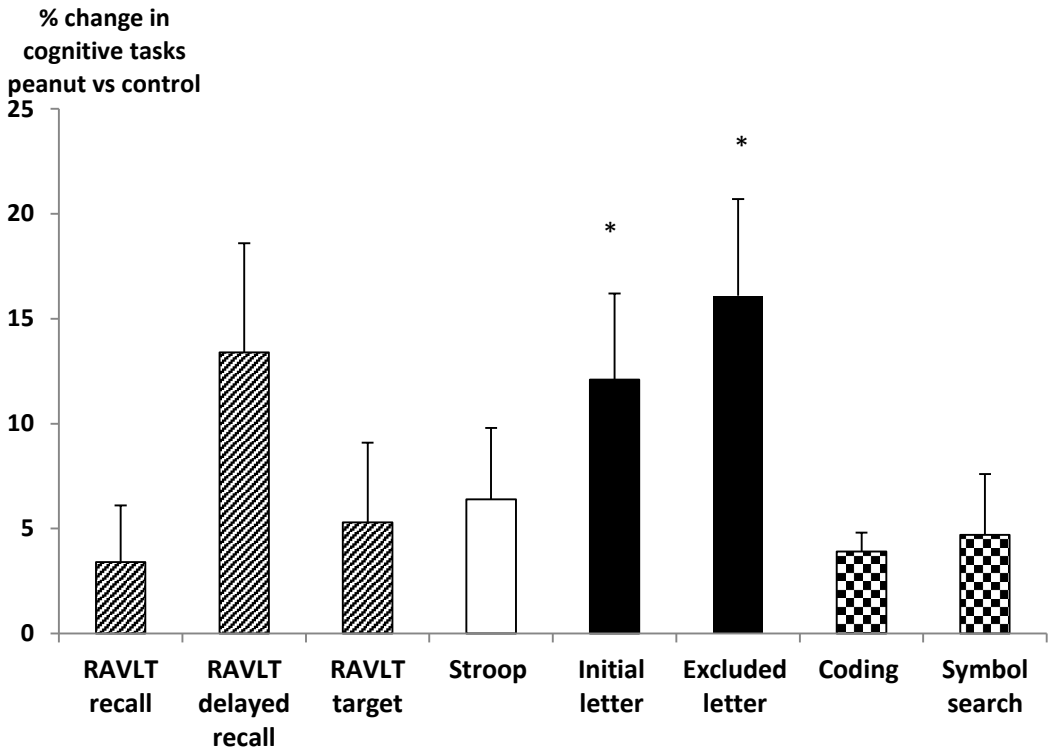


Figure 5.6 Change in cognitive tasks with peanut consumption compared with control diet (* denotes significant treatment, RAVLT = Rey Auditory Verbal Learning Test)

Table 5.9 Effect of 12 week Hi-oleic peanut consumption and nut free diet on cognitive measures (n=61)

	12 week peanut (SD)	12 week control (SD)	Difference peanuts-control (SEM)	P value	Adjusted p value†	Effect size
RAVLT recall	54.0 (10.5)	51.9 (10.4)	1.2 (1.2)	0.020	0.990	0.34
RAVLT delayed recall	10.0 (3.0)	9.5 (3.4)	0.6 (0.3)	0.090	0.990	0.24
RAVLT target	21.0 (5.1)	21.7 (4.9)	0.3 (0.6)	0.580	0.990	0.01
Stroop¶	-1.8 (0.7)	-1.9 (0.9)	0.1 (1.4)	0.090	0.990	0.26
Initial letter	28.6 (1.2)	26.4 (8.7)	2.2 (0.9)	**0.020**	**0.011**	**0.33**
Excluded letter	27.8 (8.9)	24.7(7.8)	2.8 (1.0)	**0.004***	**0.011**	**0.39**
Coding	61.6 (15.2)	59.5 (14.3)	2.2 (0.7)	0.005	0.055	0.34
Symbol search	26.7 (6.6)	26.2 (6.8)	0.6 (0.6)	0.280	0.055	0.25
Memory‡	0.11 (3.4)	-0.13 (3.2)	0.26 (0.28)	0.190	0.990	0.23
Executive function‡	0.16 (1.9)	-0.28 (1.8)	0.49 (0.17)	0.016	0.990	0.35
Processing speed‡	0.12 (1.9)	-0.03 (1.9)	0.18(0.11)	0.047	0.055	0.27
Verbal fluency‡	0.28 (1.8)	-0.28 (1.5)	0.56 (0.14)	**<0.001***	**0.011**	**0.46**

¶data reversed to reflect higher values = improved cognitive performance, ‡ calculated as z-scores, *significant treatment effect, †sequential Bonferroni, RAVLT, Rey verbal learning test, SD standard deviation, SEM standard error

Table 5.10 Relationships between cognitive tasks and vascular measures (shaded values indicate $p < 0.05$)

		SBP	DBP	LAE	SAE	LCVR	RCVR
RAVLT recall	Pearson Correlation	0.04	0.045	-0.146	-0.01	0.115	-0.199
	Sig. (2-tailed)	0.764	0.733	0.261	0.94	0.447	0.232
RAVLT delay	Pearson Correlation	0.233	0.235	-0.023	-0.174	**0.290**	-0.074
	Sig. (2-tailed)	0.073	0.071	0.862	0.191	**0.050**	0.659
RAVLT target	Pearson Correlation	0.144	0.02	-0.18	-0.075	**0.339**	0.105
	Sig. (2-tailed)	0.278	0.878	0.168	0.578	**0.021**	0.537
Stroop	Pearson Correlation	-0.086	-0.027	-0.164	0.134	-0.091	0.061
	Sig. (2-tailed)	0.514	0.836	0.206	0.316	0.547	0.714
Initial letter	Pearson Correlation	0.015	-0.124	-0.221	-0.107	0.064	-0.289
	Sig. (2-tailed)	0.911	0.344	0.087	0.423	0.673	0.078
Excluded letter	Pearson Correlation	0.031	0.106	-0.037	-0.163	0.254	0.048
	Sig. (2-tailed)	0.820	0.428	0.779	0.229	0.092	0.775
Coding	Pearson Correlation	0.148	**0.261**	0.065	-0.072	-0.129	-0.142
	Sig. (2-tailed)	0.266	**0.048**	0.625	0.600	0.397	0.402
Symbol search	Pearson Correlation	0.201	**0.262**	-0.217	-0.149	0.115	-0.084
	Sig. (2-tailed)	0.130	**0.047**	0.099	0.273	0.452	0.619
Memory	Pearson Correlation	0.064	0.171	-0.154	-0.101	0.08	-0.043
	Sig. (2-tailed)	0.632	0.195	0.242	0.453	0.601	0.799
Executive function	Pearson Correlation	-0.062	-0.137	**-0.380**	-0.043	0.067	-0.110
	Sig. (2-tailed)	0.637	0.295	**0.003**	0.746	0.657	0.513
Processing speed	Pearson Correlation	0.231	0.393	-0.246	-0.095	0.004	-0.157
	Sig. (2-tailed)	0.083	0.003	0.062	0.492	0.978	0.359
Verbal fluency	Pearson Correlation	0.02	-0.002	**-0.361**	-0.105	0.264	-0.141
	Sig. (2-tailed)	0.88	0.988	**0.004**	0.432	0.076	0.398

Abbreviations: SBP systolic blood pressure, DBP, diastolic blood pressure, LAE, large artery elasticity, SAE, small artery elasticity, LCVR, left cerebrovascular reactivity, RCVR, right cerebrovascular reactivity, RAVLT Rey Auditory Verbal Learning Test.

5.9 Discussion

5.9.1 Cerebrovascular reactivity

This is the first study to have investigated the effect of nut consumption on cerebral blood flow (a measure of cerebrovascular reactivity) or used a whole food. A significant improvement (8 - 9%) in cerebrovascular responsiveness (CVR) of the middle cerebral artery was demonstrated with Hi-oleic peanut consumption compared with the control. Similarly 2 weeks intake of cocoa polyphenols increased cerebral blood flow by 10% (Francis et al. 2006) as presented in **Table 5.11.** Other dietary studies have observed either no improvement (tea polyphenols) (Wightman et al. 2012) or up to 2-4 fold improvement with acute or chronic ingestion (Jackson et al. 2012; Kennedy et al. 2010; Presley et al. 2011; Wong et al. 2010) (**Table 5.11**). Interestingly, those studies which elicited substantial improvements used either a diet with several foods (high nitrate diet) or food extracts (wild green oats, resveratrol, fish oil). The current study demonstrated CVR benefits with chronic consumption of a single whole food which is encouraging despite smaller improvements than some studies.

Increases in CVR are suggested to reflect improvements in endothelial function of blood vessels in the brain (Coates & Howe 2007). Few studies have measured the dietary effects of cerebral vascular reactivity (CVR), in contrast a large number of dietary intervention studies have measured peripheral endothelial function. Many of these have demonstrated acute and or chronic benefits with food products such as grape seed extract (Clifton 2004), cocoa (Schroeter et al. 2006), soy products (Castillo et al. 2003) and tea (Duffy & Vita 2003) as well as with nutritional supplements (Plantinga et al. 2007) (**Table 5.11**). In addition, studies have demonstrated that chronic walnut (Cortés et al. 2006; Ma et al. 2010; Maranhao et al. 2011) and pistachio (Sari et al. 2010a) intake can benefit endothelial function, with the greatest response for 80-100g/d of pistachios. These effect sizes are similar to that observed for CVR in this study (~0.45) with consumption of ~ 60g of Hi-oleic peanuts.

Table 5.11 Dietary intervention studies measuring effects of cerebral blood flow

Author	Intervention	Test	Outcome
(Kennedy et al. 2010)	Resveratrol (acute)	NIRS	↑ X 2-4 CBF @ rest ↑ 50% CBF + cognitive task
(Jackson et al. 2012)	Fish oil (acute)	NIRS	↑ CBF x 2 @ rest ↑ 25% CBF + cognitive task
(Wightman et al. 2012)	Tea polyphenols (acute)	NIRS	No Δ CBF @ rest ↓ CBF + cognitive task
(Presley et al. 2011)	High nitrate diet (4 days)	MRI	↑24% CBF frontal lobe No Δ CBF globally
(Sorond et al. 2008)	High flavonol cocoa (2 weeks)	TCD	↑10% CBF
(Wong et al. 2012)	Wild green oats (12 weeks)	TCD	↑ 42% CBF

Abbreviations: NIRS, near infrared spectroscopy, TCD, transcranial Doppler, MRI, Magnetic resonance imaging, CBF, cerebral blood flow

It was hypothesised in this study that reductions in body composition and/or improvements in cardio-metabolic outcomes would facilitate improvements in vascular measures. However, there were no observed changes in any cardio-metabolic or body composition outcomes with peanut consumption and no correlations between cardio-metabolic and vascular measures (see **Section 6.6.6** and **6.6.7**). In addition, CVR measurements did not correlate with other vascular measures which may be due to changes of endothelial function in the brain not directly related to changes in blood pressure or blood vessel structure in other parts of the body. Similarly other studies found no correlation (Gardner & Parker 2011) between endothelial function and arterial compliance. It is possible that structural and blood pressure changes may only be observed for periods of time longer than 12 weeks as suggested by findings from the systematic review in **Chapter 3.**

As there was little evidence for cardio-metabolic and body composition measures influencing CVR in this study, it is possible that bioactive nutrients may be responsible for benefits observed. Substantial increases in monounsaturated fatty acids, magnesium, folate and vitamin E intakes were observed with a smaller increase in fibre and a likely increase in arginine intake

(suggested by an increase in protein intake) (see **Table 6.3** and **6.4)**. These bioactive nutrients have all demonstrated endothelial function improvements in other studies (Brock et al. 2006; Fuentes et al. 2001; Heffernan et al. 2010; Pérez-Jiménez et al. 1999; Ros et al. 2006; Skyrme-Jones et al. 2000; Vita 2005; Wong et al. 2010). The influence of fibre intake on vascular function is thought to be via cardio-metabolic improvements (Pal et al. 2012); hence, fibre was less likely to be of importance in the current study than other nutrients, as no cardio-metabolic changes were observed. In contrast, improvements in both peripheral and cerebral vascular function which may act directly on the blood vessels (Ghosh & Scheepens 2009) have been observed with several polyphenols (Kennedy et al. 2010; Vita 2005; Wong et al. 2012; Wong et al. 2010). Over 50 polyphenol compounds have been identified and characterized in peanuts; these include resveratrol, flavonoids and phenolic acids. Flavonoid compounds include catechins, proanthocyanidins and isoflavones and like other polyphenols they are found predominantly in the peanut skins, with concentrations comparable to those found in grape seeds known for their high flavonoid content (Lewis et al. 2013). It is possible that a combination of several bioactive nutrients working together with additive or synergistic effects elicited benefits in CVR.

Mechanisms for the proposed action of bioactive nutrients and polyphenols on endothelial function include augmentation of nitric oxide (NO), anti-inflammatory action or antioxidant effects in endothelial cells (Ganz et al. 2000; Heffernan et al. 2010).These have the potential to enhance endothelium-dependent relaxation thereby increasing cerebral blood flow (CBF) as hypothesised in **Chapter 1**.

5.9.2 Arterial compliance

Despite a 12% increase in small artery elasticity (SAE) with a medium effect size of 0.40 this measure did not reach significance after correcting for multiple comparisons. Small artery compliance is a marker for endothelial function which induces functional or structural changes in the microcirculation and is a predictor of adverse cardiovascular events (Cohn et al. 1995). SAE measures often indicate a loss of arterial elasticity before, and to a somewhat greater degree, than LAE measures and hence are an earlier predictor of cardiovascular disease (Grey et al. 2003; Wilkins et al. 2012). Conversely improvements in SAE are often seen before those in LAE which may explain why no changes in LAE were found. Body fat measures have been found to be the strongest predictors of large arterial stiffness (Wildman et al., 2003) and this study found an inverse association between fat mass and LAE (**Table 6.9**). However, no changes in body composition were observed overall may also partly explain why no changes in LAE were found. Despite the improvement in SAE not reaching significance this is still a promising finding; it is possible that it was underpowered as a secondary outcome. It appears that structural and functional improvements in blood vessel function may take longer than earlier improvements in endothelial function observed (Stoner, Young & Fryer 2012). This may partly explain why improvements in SAE in this study did not reach significance.

Studies which have demonstrated beneficial effects on arterial elasticity include those with Concord grape juice, cranberry juice and red wine (Dohadwala et al. 2011; Krnic et al. 2011; Siasos et al. 2014). To date only two other studies with nuts have investigated the effects of nuts on arterial compliance as described in the scoping review (**Chapter 3**). A small dose of walnuts (15g/d) consumed for four weeks did not demonstrate a benefit for arterial stiffness which may not have been a sufficient dose or not consumed for long enough to elicit benefits (Din et al. 2011). Investigators chose this as a realistic amount likely to be consumed in free living individuals rather than higher doses used in other nut intervention studies. A cross sectional study measured arterial compliance and compared quintiles of a healthy dietary pattern including nuts and found no

association with a healthier diet pattern; however the amount of nuts consumed was not identified so provided limited information.

5.9.3 Blood pressure

No changes in blood pressure were observed with 12 weeks peanut consumption in this study. These findings are consistent with other intervention studies demonstrating no significant change in blood pressure in 25 of 33 studies reviewed in the systematic search (**Chapter 3**). Most studies which demonstrated a beneficial effect on blood pressure used nuts consumed for relatively long periods of twelve weeks and up to two years. Epidemiological studies have demonstrated an inverse relationship between hypertension and nut consumption (Djoussé et al. 2010; O'Neil et al. 2011; Rajaram, Connell & Sabaté 2010; Steffen et al. 2005), although other studies found no association with nut consumption and hypertension or blood pressure (Alvarez León, Henríquez & Serra-Majem 2006; Martínez-Lapiscina et al. 2010; Nettleton et al. 2008). The potential benefit of nut consumption may only be observed only after an extended period of time as indicated by these longer intervention and observational studies with habitual consumption.

5.9.4 Cognitive measures

As is common in nutrition intervention trials, this study examined the effect of Hi-oleic peanut consumption on a number of outcome measures hence, increasing the risk of Type I error. Cognitive measures were secondary outcomes and significance values for these are presented in **Table 5.9**. Sequential Bonferroni (Bonferroni-Holm method (Rice 1989)) was used to adjust for the effect of multiple comparisons (n = 11 dependent variables). With this approach, statistical significance was maintained for excluded letter (a measure of verbal fluency) as well as the composite score of verbal fluency. Despite other cognitive measures not reaching significance with this method, RAVLT recall, excluded letter, coding and executive function tasks achieved effect sizes of 0.34 - 0.39. These effect sizes are comparable to those found in other dietary interventions demonstrating statistically significant cognitive outcomes such as isoflavone supplementation (effect size 0.40 - 0.44) (Thorp et al. 2009), low fat

dairy intake (effect size 0.32)(Crichton, Murphy & Bryan 2010) and Ginko biloba (effect size 0.31) (Le Bars et al. 1997)

Interestingly, improvements in processing speed correlated with an increase in diastolic blood pressure which could indicate that as blood pressure increased there was an accompanied increase in cerebral blood flow which may have resulted in improvements in processing speed (**Table 5.10**). This is likely to be only a temporary effect as prolonged hypertension is associated with impaired endothelial dysfunction, dementia and cognitive decline (Novak & Hajjar 2010). Unexpectedly, improvements in executive function and verbal fluency were inversely correlated with changes in large artery elasticity (**Table 5.10**). There is no plausible explanation; however LAE did not alter with Hi-oleic peanut consumption so this correlation is not likely to be clinically meaningful.

Few other studies have previously assessed the effect of nut consumption on cognitive measures. The only other intervention study found an improvement in inferential verbal reasoning (a measure of critical thinking) following walnut consumption, with a young population of University students with an effect size of 0.40 (Pribis et al. 2012). Tests of non-verbal reasoning and memory did not improve in this study, possibly due to a ceiling effect with students already performing at a high cognitive level.

Epidemiological studies have found higher cognitive performance for nut consumers than non-consumers. Recent results from the Nurses' Health study demonstrated long-term total nut intake was associated with better cognitive status for all outcomes measured including immediate and delayed memory, category fluency and attention (O'Brien et al. 2014). Walnut consumption (but not other nuts) has demonstrated an association with short term and working memory (Valls-Pedret et al. 2012). Furthermore, the Doetinchem study observed that nut intake over 5 years was associated with an improvement in global cognitive function, memory, processing speed and flexibility compared with non-nut consumers in middle aged and older adults with no cognitive decline observed over this period (Nooyens et al. 2011). The current findings are

promising and support previous research indicating the potential for nuts to influence cognitive function.

In addition to improvements in cerebral vascular reactivity (CVR) and cognitive measures, changes in CVR of the left side of the brain correlated with changes in two measures of verbal memory, which is predominantly a function on the left side of the brain. These findings provide some evidence to support the hypothesis of cognitive benefits from improvements in cerebral vascular function with Hi-oleic peanut intake. This research also supports other studies indicating a correlation between CBF and cognitive function in human participants (Fisher, Sorond & Hollenberg 2006) and a decrease in CBF in patients with dementia (Koudstaal et al. 2005). However these results cannot be generalised to other population groups.

In addition to CVR influencing cognitive changes it is possible that bioactive nutrients in Hi-oleic peanuts may have acted directly on the brain. There is emerging evidence from animal and human studies that bioactive compounds including vitamin E (Arzi, Hemmati & Razian 2004; Masaki et al. 2000; Morris et al. 2002) and polyphenols (Macready et al. 2009) may have the potential to improve cognitive function. These compounds may protect against injury induced by neurotoxins, neuro-inflammation and lipid peroxidation (Gómez-Pinilla 2008; Joseph, J, Shukitt-Hale & Willis 2009; Spencer 2010; Van Dyk & Sano 2007; Youdim, Shukitt-Hale & Joseph 2004). This is supported by a Mediterranean diet study demonstrating urinary or plasma levels of polyphenols were associated with higher scores of immediate verbal memory and global cognitive performance (Feart et al. 2009).

5.9.5 Strengths and limitations

A strength of this study was the randomised, controlled cross over design which assessed the effects of Hi-oleic peanuts in the same individuals, hence reducing between participant variability. The study was adequately powered, demonstrating a significant increase in CVR (8-9%). Other strengths included use of a free living healthy overweight, middle-aged to older population group.

They were chosen as being more likely to have some decline in endothelial function; hence more likely to benefit from the peanut intervention than a younger, leaner population. Participants with diabetes or cardiovascular disease were excluded with a greater likelihood of extensive endothelial damage which may be difficult to reverse (Forman et al. 2008).

Another strength of the study was the range of validated cognitive tests used to assess verbal memory, processing speed and executive function. The study also compared outcome measures at the end of each 12 week period for the peanut and the control diets using a repeated linear measures analysis. This allowed for control of baseline (some baseline values were not the same for those starting on peanuts compared with those starting on the control phase). If baseline was not controlled for then some of the changes observed over 12 weeks may have been masked or exaggerated. With the exception of the cognitive measures, all outcomes demonstrated no carry over effects with no significant differences between baseline measures and those at the end of the control period. The cognitive measures demonstrated a learning effect over the 3 time points; however this was controlled for with the statistical analyses.

One of the limitations of the study, as with many dietary studies was the inability to double-blind the intervention; however, data collection and data entry was blinded to minimise investigator bias. Another limitation was the high dose of peanuts chosen as proof of concept. The recent Australian Nutrition survey (Australian Bureau of Statistics 2012) indicated Australians currently consume an average of 5.2 g of nuts per day. Mean intake in this study was 59 g for males and females combined; the American Heart Association recommends daily nut intake of 42 g per day for reduction of cardiovascular disease so a dose of 42 g may still have some cerebrovascular and cognitive benefits. This needs to be confirmed with further investigation. In addition, participants were not surveyed to determine their liking for the peanuts or likely long term acceptability and at what doses. However, other studies have demonstrated that large nut doses can be maintained for prolonged periods as demonstrated by 12 week consumption of hazelnuts (42 g) which was more resistant to sensory specific satiety (reduction in “pleasantness”) than chocolate or crisps (Tey et al. 2012). Chronic

consumption of up to 90 g of peanuts for 12 weeks also did not lead to a decline in pleasantness ratings (Alper & Mattes 2002), indicating that a recommendation to consume nuts regularly is both achievable and sustainable.

Another limitation was that other biomarkers such as plasma Vitamin E and polyphenol levels, red blood cell monounsaturated fatty acid content, inflammatory markers (in addition to C-reactive protein) were not measured which may have provided information on the potential mechanisms for the vascular and cognitive outcomes observed. The polyphenol content and in particular the resveratrol content of the Hi-oleic peanuts was not measured to determine if levels present could be sufficiently potent to induce changes observed.

This study did not include a washout period which may be viewed as a limitation; however, the control phase was an habitual (nut free) diet for an extended period of 12 weeks, which was used to allow sufficient time for reversal of any effects of peanut consumption such as changes in lipids, body weight and endothelial function hence a washout period was not deemed necessary (Wong et al. 2012) .

Another limitation of this research is that it was conducted in older overweight/obese individuals and hence findings cannot be generalised to other population groups.

5.9.6 Conclusion

In summary, there are a range of nutrients and bioactive components both in the Hi-oleic peanut kernel and peanut skin which have the potential to enhance peripheral, cerebral vascular and neuronal function. It is possible that a combination of several of these working together were able to elicit benefits in vascular and cognitive measures observed in this study.

Chapter 6: The effect of daily 12 week Hi-oleic peanut consumption on, energy balance, nutrient intake, body composition and cardio-metabolic risk factors

6.1 Introduction and background

6.1.1 Summary of the literature

Nuts are rich sources of bioactive nutrients with potential to deliver metabolic and cardiovascular health benefits. Hi-oleic peanuts are an excellent source of protein (approximately 25% of energy intake) and of dietary fibre providing 5–10% of daily fibre requirements in one serving (30g), providing potential satiety benefits for weight control. Hi-oleic peanuts are also rich in monounsaturated (providing up to 80% of the fatty acid composition) and contain phytosterols both of which have demonstrated lipid lowering effects (Kris-Etherton & Griel 2006).

The previous chapters have outlined analysis of the literature pertaining to the effects of nut consumption on cardio-metabolic risk factors, body weight and energy balance. Nut consumption has also been associated with a reduced risk of type 2 diabetes (Jiang et al. 2002; Nettleton et al. 2008) and has demonstrated improvement in glycaemic control and insulin sensitivity (Adamsson et al. 2011; Sari et al. 2010). Clinical and epidemiological evidence also indicate that nut consumption can enable improvements in inflammatory markers and lipid profiles with daily doses of 30g most likely to confer benefits (Mena et al. 2009; Solà et al. 2012). The cholesterol lowering, anti-inflammatory and glucose regulating benefits of nuts have been attributed to their high content of unsaturated fat, possible weight reduction effect and bioactive compounds, including plant sterols, dietary fibre and antioxidants (Griel & Kris-Etherton 2006). Whilst previous studies have investigated the effect of peanut intake on body weight (Alper & Mattes 2001; Pelkman et al. 2004), few have measured cardio-metabolic outcomes of peanuts and no research has previously been conducted with Australian Hi-oleic peanuts.

6.2 Hypothesis

The overall hypothesis for this study was introduced in **Section 3.3** of the scoping study and summarised in **Figure 3.1;** more detailed hypotheses are presented below.

It was hypothesised, that 12 weeks of Hi-oleic peanut consumption in healthy overweight or obese individuals would demonstrate:

i. A reduction in fasting lipids, inflammation and glucose regulation.

ii. Maintenance or reduction of body mass, waist circumference and body composition.

Blood lipid reduction was proposed to be the result of the high monounsaturated fat and fibre content of the Hi-oleic peanuts and possible reduction of saturated fat/carbohydrate intake (due to substitution of some foods containing these nutrients with the peanuts). Improvement in inflammation was hypothesised to be due to the fibre and polyphenol content of the peanuts. Improved glucose regulation was proposed as a consequence of a reduced glycaemic response to meals and snacks with Hi-oleic peanut consumption and improvement in insulin sensitivity with increased monounsaturated fatty and fibre intakes or via indirect effect through possible lowering of body weight. The protein, fibre and unsaturated fat content were proposed to increase satiety; thereby maintaining or reducing body mass.

6.3 Aim

To investigate the effect of 12 week intake of Hi-oleic peanuts added to the habitual diets of healthy overweight adults on energy balance, nutrient intake, body composition, fasting blood glucose, insulin, lipids and inflammatory marker (C- reactive protein) and to determine if there are any relationships between cardio-metabolic measures, body composition, nutrient intake, vascular and cognitive measures.

6.3.1 Outcomes

To determine the:

- Effect of Hi-oleic chronic peanut consumption on fasting glucose, insulin, lipids and inflammatory marker (C-reactive protein).
- Effect of chronic Hi-oleic peanut consumption on energy balance and nutrient intake.
- Effect of chronic Hi-oleic peanut consumption on body weight and body composition.
- Relationships between cardio-metabolic measures and body composition.
- Relationships between cardio-metabolic, nutrient intake, vascular and cognitive measures.

6.4 Measurements

6.4.1 Body mass and height

Weight was measured to the nearest 200g using an electronic scale (Tanita Ultimate Scale 2000; Tokyo, Japan) with participants wearing light clothing and without shoes. Two measurements were taken with the average used. Height was measured to the nearest 0.1cm using a wall-mounted telescopic stadiometer (Seca220; Vogel & Halke, Hamburg, Germany) with participants in stockinged or bare feet and two measurements taken and the average used.

6.4.2 Body composition

Body composition measuring total body fat mass, percentage body fat and lean tissue mass was performed by Dual–Energy X-Ray absorptiometry (DEXA) (Luna Corp> Prodigy Advance® Model GE, Madison WI, USA). Participants were required to remove all items of jewellery, over clothes and under garments with metal fixings and any surgical devices or prosthesis were noted. The machine was calibrated prior to each scan with details of participants' body weight, height and date of birth entered. Participants were scanned supine, positioned centrally on the mat and restrained at the ankle to minimise movement. DEXA is limited to participants under 135 kg hence those over this weight were excluded. Previous reports from Nutritional Physiology Research Centre has found the standard error of the measurement for assessment of body composition by DEXA were 0.87 % for percentage body fat, 0.53 kg (1.6 %) for fat mass, 1.05 kg (2.3 %) for lean mass, measured on consecutive days.

6.4.3 Blood sampling

Fasting blood samples were obtained by venepuncture after participants were seated for more than ten minutes using 1 × 9mL EDTA (purple) and 1 x 6mL antiglycolytic (grey) blood collection tubes. Plasma was stored at −80° C until analysis. Fasting plasma triglycerides, total cholesterol, high density lipoprotein (HDL-C) and glucose concentrations were determined using a commercial assay kit with a Konelab 20XT clinical chemistry analyser (Thermo Fisher Scientific, Waltham, MA, USA). These tests have good precision and accuracy with coefficient of variation within and between assays (>5.0 %). Low density cholesterol (LDL-C) will calculated using the Friedewald equation (Friedewald, Levy & Fredrickson 1972). Fasting plasma insulin concentrations were determined by ELISA (Mercodia, Uppsala, Sweden). This assay has good precision and accuracy with coefficient of variation within and between assays (>4.0 %). Measures of insulin sensitivity (homeostatic model assessment (HOMA) of insulin resistance, insulin sensitivity and beta cell function) were calculated from the glucose and insulin levels using the HOMA 2 online calculator: http://www.dtu.ox.ac.uk/homa. High sensitivity CRP was analysed by chemistry analyser (Konelab 20XT) using a commercially available kit (Thermo Fisher 981798, range 0.25-40 mg/l).

6.4.4 Nutrient intake and physical activity measurements

A set of food scales was provided with instructions on how to accurately weigh (to the nearest gram), measure and record intake in a food diary for 3 weekdays and one weekend day prior to the next visit. Weighed or measured food intake was recorded for 4 days prior to each assessment visit at baseline and weeks 6, 12, 18 and 24. Participants were instructed to maintain similar patterns of physical activity throughout the study and 4 day physical activity diaries (Bouchard et al. 1983) were completed at the same time points as the food diaries. Energy intake and nutrient profiles were calculated using Foodworks® Nutritional software (version 7 Xyris Software Pty Ltd Highgate Hill, Queensland; Australia). In addition to energy, fibre, alcohol and macronutrients, dietary intake data were collected and analysed for micronutrients found in Hi-oleic peanuts.

6.5 Statistical analysis

See **Chapter 5**, **Section 5.7** for full details of statistical analyses. For dietary measures, significant differences between the 2 phases were set at $p = 0.05$. A paired t-test revealed no significant differences between food intake at the midpoint (week 6) and end (week 12) of each of the two treatment phases indicating that nutrient intakes and were maintained for 12 weeks with both diet phases ($p = 0.18$ peanut phase, $p = 0.72$ control phase). Hence, an average of measures at week 6 and week 12 were analysed and reported with the exception of energy intake and energy expenditure which is reported including the mid time points at week 6.

6.6 Results

Good compliance with the peanuts was considered above 75%. Reported compliance from the diet diaries was 80 % (45.1 ± 1.1 g) for females and 75 % (62.9 ± 3.3 g) for males, 6 days per week. This was confirmed by return of 89 % of peanut packets for females and 70 % for males with some empty packets forgotten to be returned.

6.6.1 Macronutrient intake and energy balance

Baseline nutrient intakes are reported in **Table 6.1.**

Table 6.1 Baseline mean daily measures of nutrient and energy intake from 4 day diaries† (n=61)

Nutrient/ Energy	Mean (SD)	%NRV	Nutrient	Mean (SD)	%NRV
Energy intake (kJ)	8253 (2061)	-	**Alcohol (g)**	7.5 (7.1)	-
Protein (g)	95.8 (31.1)	118-172	**Iron (mg)**	13.3 (4.3)	166
Carbohydrate (g)	221.2 (61.9)	-	**Zinc (mg)**	12.3 (4.0)	103-189
Total fat (g)	68.3 (23.4)	-	**Sodium (mg)**	2379 (765)	258- 515
PUFA (g)	10.1 (4.1)	-	**Potassium (mg)**	3535 (929)	66-125
MUFA (g)	25.6 (8.3)	-	**Magnesium (mg)**	379.2 (109.1)	79-90
SFA (g)	26.8 (8.4)	-	**Vitamin E (mg)**	9.0 (4.1)	90-131
Fibre (g)	27.2 (7.9)	90-108	**Folate (mcg)**	388.0 (148.3)	97

Abbreviation: PUFA, polyunsaturated fatty acids, MUFA, monounsaturated fatty acids, SFA saturated fatty acids, NRV, nutrient reference ranges, †, average of weeks 6 & 12, SD standard deviation

General linear repeated analysis of variance revealed that consumption of Hi-oleic peanuts increased energy intake by 12 % (857 kJ) compared with the nut free diet (**Table 6.2, Figure 6.1**).The increase could be attributed to a 23 g (~30 %) increase in intake of fat which was predominantly monounsaturated fat (22 g increase), with a small significant increase in polyunsaturated fat intake (**Table 6.3, Figure 6.2**). A 12 % increase in protein intake and 15 % increase in fibre was observed with peanut consumption. No significant changes in carbohydrate, or alcohol intake were observed between the two dietary phases (**Table 6.3)**. No differences were observed in energy expenditure from physical activity diaries between the two dietary phases as indicated below in **Table 6.2** and **Figure 6.1.**

Table 6.2 Effect of peanut consumption and nut free diet on energy intake and physical activity energy expenditure from 4 day food and activity diaries (n=61)

	Wk6 peanut (SD)	Wk12 peanut (SD)	Wk6 control (SD)	Wk12 control (SD)	Mean peanut (SD)	Mean control (SD)	% ↑ with peanut (SEM)	P value
Daily energy intake (kJ)	**9004** (2014)	**9227** (2116)	**8261** (1800)	**8292** (1925)	**9068** (1878)	**8215** (1735)	**12.2 (2.2)**	**0.000**
Daily PA energy expenditure (kJ)	**3626** (273)	**3879** (236)	**3877** (255)	**3689** (236)	**3771** (321)	**3795** (376)	**-1.2 (0.7)**	**0.940**

Abbreviation: PA, physical activity, SD, standard deviation, SEM, standard error

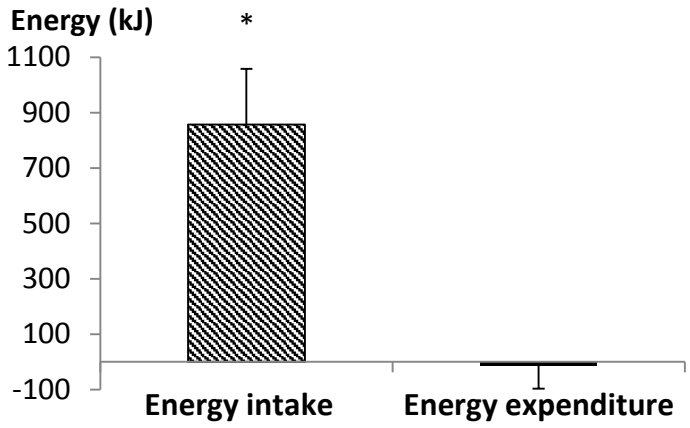


Figure 6.1 Mean (SEM) difference in energy intake and expenditure between peanut and nut free diets, * = $p < 0.05$ (average week 6 and 12)

Table 6.3 Effect of daily Hi-oleic peanut consumption† and nut free diet on macronutrient, fibre, alcohol and % energy intake over 4 days (n=61)

Nutrient	Peanuts (SD)	Control (SD)	Difference peanut-control (SEM)	P value
Protein				
g	104.2 (27.4)	95.2 (25.9)	8.9 (2.7)	**0.005***
% energy intake	19.6 (3.3)	19.8 (3.6)	-0.5 (0.6)	0.850
Carbohydrate				
g	202.0 (47.5)	206.4 (52.7)	-4.3 (4.7)	0.630
% energy intake	38.4 (8.5)	42.7 (6.9)	-4.3 (1.1)	**0.000***
Total fat				
g	93.4 (20.9)	71.0 (20.7)	22.7 (2.4)	**0.000***
% energy intake	37.9 (8.8)	31.8(5.3)	6.1 (1.4)	**0.000***
MUFA				
g	47.5(11.3)	25.2 (7.7)	22.3 (1.2)	**0.000***
% energy intake	19.6 (4.0)	11.3 (2.2)	8.3 (0.6)	**0.000***
PUFA				
g	11.2 (3.3)	9.8 (3.3)	1.5 (0.4)	**0.001***
% energy intake	4.6 (0.1)	4.4 (0.2)	0.2 (0.1)	**0.048***
Saturated fat				
g	31.8 (8.7)	29.6 (9.7)	2.2 (0.9)	0.141
% energy intake	13.1 (3.6)	13.2 (3.0)	0.1 (0.4)	0.883
Alcohol				
g	8.6 (8.9)	9.6 (10.1)	-1.1(14.4)	0.454
% energy intake	2.8 (11.4)	3.6 (15.1)	-0.8 (4.7)	0.337
Fibre				
g	27.0 (6.9)	23.4 (7.0)	3.5 (0.6)	**0.001***

Abbreviation: PUFA, polyunsaturated fat, MUFA, monounsaturated fat, SD, standard deviation, SEM, standard error, †average of 6 & 12 weeks, * indicates reached significance ($p<0.05$)

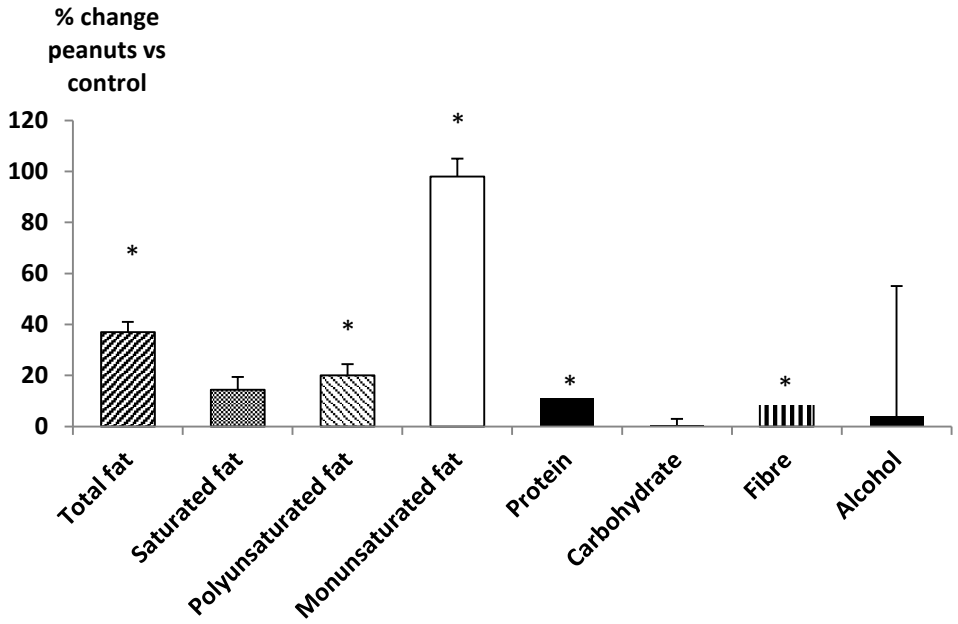


Figure 6.2 Percentage change (SEM) in macronutrient, alcohol and fibre intake with peanuts compared with nut free diet, * = $p < 0.05$ (average 6 & 12 weeks)

6.6.2 Micronutrient intake

Intakes of the micronutrients folate, magnesium, sodium, potassium, vitamin E, magnesium and zinc with peanut consumption and control diet are shown below in **Table 6.4, Figure 6.3**. Consumption of Hi-oleic peanuts increased both vitamin E and magnesium significantly by 77 % and folate by 50 % compared with the nut free diet; no significant differences in other micronutrients analysed were observed (**Table 6.4, Figure 6.3**).

Table 6.4 Effect of Hi-Oleic peanut consumption and nut free diet on daily† micronutrient intake over 4 days (n=61)

Nutrient	Peanut (SD)	Control (SD)	Difference peanut - control (SEM)	p value
Vitamin E (mg)	14.1 (5.0)	8.5 (3.0)	5.6 (0.5)	**0.000***
Folate (mcg)	581.3 (231.4)	451.0 (225.0)	129.1 (25.3)	**0.000***
Magnesium (mg)	433.7 (125.1)	357.2 (124.9)	77.3 (11.4)	**0.000***
Sodium (mg)	2343 (673)	2492 (709)	-63.0 (104.4)	0.372
Potassium (mg)	3427 (632)	3296 (791)	131.3 (63.7)	0.303
Iron(mg)	13.5 (4.6)	12.6 (4.7)	1.1 (0.6)	0.339
Zinc (mg)	13.3 (3.8)	12.1 (4.0)	0.5 (0.5)	0.512

Abbreviation: SD, standard deviation, SEM, standard error, † average 6 & 12 weeks * indicates reached significance ($p<0.05$)

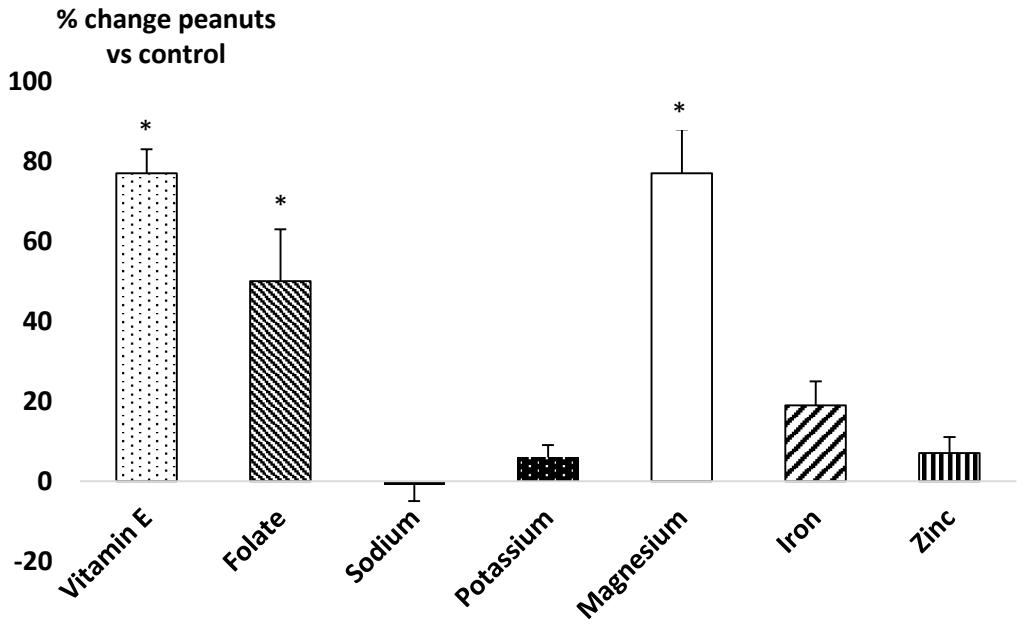


Figure 6.3 Percentage change (SEM) in micronutrient intake with peanuts compared with nut free diet, * = $p < 0.05$ (average 6 & 12 weeks)

6.6.3 Fasting blood lipids, inflammation (C-reactive protein) and glucose regulation

No participants were excluded from the low density lipoprotein (LDL-C) calculation using the Friedwald equation (i.e. no triglycerides > 4.52mmol/l). There were no differences observed in lipids, CRP or glucose regulation between the two groups (**Table 6.5).**

6.6.4 Body composition and anthropometric measures

Consumption of Hi-oleic peanuts demonstrated no significant effects on any anthropometric or body composition measures over 6 weeks or 12 weeks compared with the control phase (**Table 6.6 and Table 6.7**).

Table 6.5 Effect of 12 week Hi-oleic peanut consumption and nut free diet on fasting blood lipids, inflammation (C- reactive protein) and glucose (n=57)

Measure	Target *	Baseline (SD)	12 weeks Peanuts (SD)	12 weeks control (SD)	Difference peanut- control (SEM)	p value	Adjusted p value‡	Effect size
Total chol.(mmol/l)	<5.5	5.1(0.8)	5.2 (0.8)	5.1 (0.8)	0.0 (0.1)	0.662	1.430	-0.1
LDL-C (mmol/l)	<3.5	3.3(0.7)	3.6 (0.8)	3.5 (0.8)	0.0 (0.1)	0.421	1.430	-0.2
HDL-C (mmol/l)	>1.0	1.4(0.3)	1.4 (0.3)	1.4 (0.4)	0.0 (0.0)	0.190	1.430	0.2
Triglyceride(mmol/l)	<2.0	1.2(0.6)	1.2 (0.7)	1.3 (0.6)	-0.1 (0.1)	0.129	1.430	-0.2
LDL/HDL	-	2.6(0.2)	2.6 (0.2)	2.6 (0.2)	-0.0 (0.0)	0.819	1.430	0.0
C-reactive protein (mg/l)	< 3.0	1.8(2.2)	2.1 (1.7)	2.3 (1.9)	-0.3 (0.2)	0.620	6.820	-0.1
Glucose (mmol/l)	4-6	5.5(0.7)	5.5 (0.6)	5.5 (0.5)	0.0 (0.1)	0.614	6.710	0.2
Insulin (mU/l)	0-12	7.9(4.5)	7.8 (5.4)	7.9 (5.1)	-0.1 (0.5)	0.412	4.510	0.2
HOMA IR	-	1.1(0.6)	1.1 (0.7)	1.1 (0.6)	0.1 (0.1)	0.769	4.510	0.1
HOMA IS	-	114.3(54.5)	124.2 (58.1)	119.9 (56.3)	7.3 (9.0)	0.701	4.510	0.2
β cell	-	83.0(31.7)	80.2(30.4)	80.4 (32.1)	1.3 (3.9)	0.900	4.510	0.0

Abbreviation: SD, standard deviation, SEM, standard error *(American Heart Association 2008; Commonwealth Scientific and Industrial Research Organisation 2013; National Heart Foundation of Australia & Cardiac Society of Australia & New Zealand 2005; Royal College of Pathologists), ‡ sequential Bonferroni, HDL-C, high density lipoprotein, LDL-C, low density lipoprotein, HOMA, homeostasis model assessment, IR, insulin resistance, IS, insulin sensitivity

Table 6.6 Effect of 6 and 12 week Hi-oleic peanut consumption and nut free diet on anthropometric measures (n=61)

	6 wk peanut (SD)	6 wk control (SD)	Peanut-control (SEM)	p value	12 wk peanut (SD)	12 wk control (SD)	Peanut-control (SEM)	p value	Adjusted p value‡	Effect size
Body mass (kg)	88.4 (1.9)	88.0 (1.8)	0.4 (0.2)	0.01	88.4 (1.7)	87.8 (2.1)	0.5 (0.2)	0.010	0.11	0.4
Waist circ. (cm)	100.6 (0.9)	101.1 (1.1)	-0.5 (1.0)	0.59	99.4 (1.9)	99.4 (1.8)	0.3 (0.3)	0.780	0.11	0.1
BMI (kg/m^2)	30.9 (0.9)	30.7 (0.8)	0.1 (0.1)	0.05	30.9 (1.1)	30.6 (1.2)	0.2 (0.2)	0.742	0.11	0.1

Abbreviation: SD, standard deviation, SEM, standard error, ‡ sequential Bonferronni

Table 6.7 Effect of 12 week Hi-oleic peanut consumption and nut free diet on body composition (n=61)

	12 week peanut (SD)	12 week control (SD)	peanut-control 12 week (SEM)	p value	Adjusted p value‡	Effect size
Fat mass (g)	35179 (1313)	34729 (1333)	45.2 (201.1)	0.030	0.11	0.4
% body fat	38.9 (9.1)	38.4 (9.3)	0.3 (0.2)	0.144	0.11	0.3
Lean mass (g)	49174 (1344)	49268 (1392)	-97.1 (160.2)	0.611	0.11	0.2
Fat free mass (g)	52236 (1422)	52119 (1452)	117.2 (162.9)	0.550	0.11	0.2
Bone mineral mass (g)	2999 (668)	2998 (658)	1.1 (10.3)	0.856	0.11	0.0

Abbreviation: SD, standard deviation, SEM, standard error, ‡sequential Bonferroni

6.6.5 Relationships between vascular, cardiovascular, body composition, cardio-metabolic measures and nutrient intake.

Where significant outcomes were observed or where a possible relationship may exist these were measures were correlated.

Table 6.8 shows changes in MUFA were negatively correlated with changes in insulin and a positive correlation was found between fibre intake and triglyceride changes.

Table 6.8 Relationships between changes in cardio-metabolic outcomes and nutrient intake (shaded values indicate $P < 0.05$).

		HDL	LDL	Trig	CRP	Glucose	Insulin
Energy	Pearson Correlation	0.047	0.045	0.027	0.132	0.034	0.187
	Sig. (2-tailed)	0.729	0.736	0.843	0.327	0.807	0.160
Total fat	Pearson Correlation	0.159	-0.164	-0.088	-0.057	0.026	-0.19
	Sig. (2-tailed)	0.240	0.223	0.513	0.674	0.854	0.156
SFA	Pearson Correlation	0.171	-0.079	-0.086	-0.131	0.092	-0.025
	Sig. (2-tailed)	0.202	0.555	0.522	0.331	0.509	0.851
PUFA	Pearson Correlation	0.104	-0.230	0.193	0.004	0.001	-0.201
	Sig. (2-tailed)	0.441	0.082	0.148	0.974	0.992	0.131
MUFA	Pearson Correlation	0.244	0.051	-0.029	0.063	0.015	**-0.283**
	Sig. (2-tailed)	0.07	0.705	0.831	0.646	0.912	**0.033**
Fibre	Pearson Correlation	-0.077	0.147	**0.311**	-0.113	-0.099	-0.022
	Sig. (2-tailed)	0.575	0.281	**0.020**	0.411	0.484	0.875

Abbreviation: SFA, saturated fatty acid, PUFA, polyunsaturated fatty acid, MUFA monounsaturated fatty acid, HDL high density lipoprotein, LDL, low density lipoprotein, Trig, triglycerides, CRP, C-reactive protein

Table 6.9 Relationships between dietary intake and body composition outcomes (shaded areas indicate $P \leq 0.05$).

		BMI	Fat mass	% Body fat	Waist circumference
Energy	Pearson Correlation	0.023	-0.239	-0.06	**0.252**
	Sig. (2-tailed)	0.861	0.064	0.649	**0.050**
Total fat	Pearson Correlation	-0.017	0.114	0.000	-0.252
	Sig. (2-tailed)	0.897	0.384	0.990	0.052
SFA	Pearson Correlation	-0.103	0.110	0.127	-0.111
	Sig. (2-tailed)	0.429	0.400	0.333	0.395
PUFA	Pearson Correlation	0.210	0.140	0.057	-0.003
	Sig. (2-tailed)	0.104	0.282	0.664	0.980
MUFA	Pearson Correlation	0.071	**-0.264**	-0.14	-0.037
	Sig. (2-tailed)	0.591	**0.042**	0.290	0.780
Fibre	Pearson Correlation	0.211	-0.215	-0.094	-0.128
	Sig. (2-tailed)	0.109	0.102	0.481	0.335
Protein	Pearson Correlation	0.112	-0.058	-0.042	-0.073
	Sig. (2-tailed)	0.396	0.662	0.752	0.581

Abbreviation: SFA, saturated fatty acid, PUFA, polyunsaturated fatty acid, MUFA monounsaturated fatty acid

Energy intake was associated with waist circumference and MUFA was inversely associated with fat mass (**Table 6.9**).

Table 6.10 presents the correlations between changes vascular and cardio-metabolic measures with no relationships found with any of these.

Table 6.10 Relationships between vascular and cardio-metabolic outcomes.

		LAE	SAE	LCVR	RCVR
Total	Pearson Correlation	0.076	-0.157	-0.013	-0.127
Cholesterol	Sig. (2-tailed)	0.572	0.251	0.935	0.461
LDL	Pearson Correlation	0.067	-0.149	-0.008	-0.111
	Sig. (2-tailed)	0.620	0.278	0.961	0.519
HDL	Pearson Correlation	0.090	-0.072	-0.095	-0.08
	Sig. (2-tailed)	0.507	0.606	0.543	0.647
Trig	Pearson Correlation	-0.056	-0.026	0.011	0.04
	Sig. (2-tailed)	0.677	0.852	0.945	0.816
CRP	Pearson Correlation	-0.017	0.068	0.124	0.093
	Sig. (2-tailed)	0.899	0.621	0.434	0.596
Glucose	Pearson Correlation	0.148	0.184	-0.163	-0.111
	Sig. (2-tailed)	0.284	0.197	0.315	0.532
Insulin	Pearson Correlation	0.253	0.235	-0.145	-0.150
	Sig. (2-tailed)	0.055	0.087	0.353	0.384

Abbreviation: LAE, large artery elasticity, SAE, small artery elasticity, LCVR, RCVR left cerebrovascular reactivity, RCVR, right cerebrovascular reactivity, HDL, high density lipoprotein, Trig, triglycerides, LDL, low density lipoprotein, CRP, C-reactive protein.

Table 6.11 shows changes in MUFA were negatively correlated with changes in insulin and a positive correlation was found between fibre intake and triglyceride changes.

Table 6.11 Relationships between changes in cardio-metabolic outcomes and nutrient intake (shaded values indicate $P < 0.05$).

		HDL	LDL	Trig	CRP	Glucose	Insulin
Energy	Pearson Correlation	0.047	0.045	0.027	0.132	0.034	0.187
	Sig. (2-tailed)	0.729	0.736	0.843	0.327	0.807	0.160
Total fat	Pearson Correlation	0.159	-0.164	-0.088	-0.057	0.026	-0.19
	Sig. (2-tailed)	0.240	0.223	0.513	0.674	0.854	0.156
SFA	Pearson Correlation	0.171	-0.079	-0.086	-0.131	0.092	-0.025
	Sig. (2-tailed)	0.202	0.555	0.522	0.331	0.509	0.851
PUFA	Pearson Correlation	0.104	-0.230	0.193	0.004	0.001	-0.201
	Sig. (2-tailed)	0.441	0.082	0.148	0.974	0.992	0.131
MUFA	Pearson Correlation	0.244	0.051	-0.029	0.063	0.015	**-0.283**
	Sig. (2-tailed)	0.07	0.705	0.831	0.646	0.912	**0.033**
Fibre	Pearson Correlation	-0.077	0.147	**0.311**	-0.113	-0.099	-0.022
	Sig. (2-tailed)	0.575	0.281	**0.020**	0.411	0.484	0.875

Abbreviation: SFA, saturated fatty acid, PUFA, polyunsaturated fatty acid, MUFA monounsaturated fatty acid, HDL high density lipoprotein, LDL, low density lipoprotein, Trig, triglycerides, CRP, C-reactive protein.

Table 6.12 Relationships between vascular, body composition and nutrient intake (shaded values indicate P < 0.05).

		LAE	SAE	LCVR	RCVR
BMI	Pearson Correlation	0.224	0.141	-0.154	0.121
	Sig. (2-tailed)	0.082	0.293	0.307	0.469
Body Weight	Pearson Correlation	-0.237	-0.148	-0.260	**-0.360**
	Sig. (2-tailed)	0.066	0.269	0.081	**0.027**
Fat mass	Pearson Correlation	**-0.278**	0.074	-0.089	-0.070
	Sig. (2-tailed)	**0.030**	0.581	0.555	0.678
% body fat	Pearson Correlation	-0.106	0.042	-0.124	-0.137
	Sig. (2-tailed)	0.420	0.755	0.416	0.418
Waist	Pearson Correlation	-0.093	-0.103	0.004	-0.103
	Sig. (2-tailed)	0.477	0.440	0.978	0.538
Energy	Pearson Correlation	0.036	0.198	0.086	0.146
	Sig. (2-tailed)	0.784	0.136	0.569	0.382
Total fat	Pearson Correlation	0.154	0.097	-0.137	0.160
	Sig. (2-tailed)	0.240	0.471	0.369	0.337
SFA	Pearson Correlation	0.207	0.081	-0.243	0.018
	Sig. (2-tailed)	0.109	0.545	0.104	0.913
PUFA	Pearson Correlation	-0.045	0.102	-0.082	0.171
	Sig. (2-tailed)	0.731	0.446	0.590	0.306
MUFA	Pearson Correlation	0.245	0.082	-0.007	0.228
	Sig. (2-tailed)	0.059	0.544	0.965	0.168
Vitamin E	Pearson Correlation	**.0267**	0.168	-0.219	0.115
	Sig. (2-tailed)	**0.038**	0.208	0.143	0.492
Magnesium	Pearson Correlation	0.061	0.165	0.130	0.107
	Sig. (2-tailed)	0.640	0.216	0.389	0.524

Abbreviation: LAE, large artery elasticity, SAE, small artery elasticity, LCVR, left cerebrovascular reactivity, RCVR, right cerebrovascular reactivity, BMI, body mass index, SFA, saturated fatty acid, PUFA, polyunsaturated fatty acid, MUFA, monounsaturated fatty acid.

Table 6.12 shows relationships between vascular measures, body composition and nutrient intake. RCVR was negatively associated with body weight and LAE was negatively associated with body fat mass. LAE was associated with vitamin E intake.

Table 6.13 Relationships between cognitive and cardio-metabolic measures (shaded values indicate P < 0.05).

		HDL	Trig	LDL	CRP	Glucose	Insulin
	Pearson		-				
RAVLT	Correlation	0.216	0.163	0.11	0.063	0.080	0.043
recall	Sig. (2-tailed)	0.107	0.221	0.411	0.644	0.566	0.749
RAVLT	Pearson		-		-		
delay	Correlation	0.144	0.049	0.213	0.209	-0.096	-0.004
	Sig. (2-tailed)	0.286	0.717	0.109	0.118	0.492	0.978
	Pearson		-		-		
RAVLT	Correlation	0.006	0.047	0.179	0.107	-0.017	-0.035
target	Sig. (2-tailed)	0.965	0.726	0.183	0.433	0.904	0.796
	Pearson						
Stroop	Correlation	0.132	0.064	0.027	0.205	0.043	-0.129
	Sig. (2-tailed)	0.326	0.632	0.843	0.127	0.756	0.334
Initial	Pearson	-	-		-		
letter	Correlation	0.082	0.116	0.012	0.089	0.147	0.069
	Sig. (2-tailed)	0.543	0.385	0.931	0.51	0.288	0.605
	Pearson	-					
Excluded	Correlation	0.048	0.138	-0.169	0.070	-0.135	-0.052
letter	Sig. (2-tailed)	0.726	0.305	0.21	0.607	0.337	0.700
	Pearson						
Coding	Correlation	0.065	-0.03	-0.027	0.029	0.206	0.176
	Sig. (2-tailed)	0.636	0.827	0.842	0.836	0.156	0.194
	Pearson						
Symbol	Correlation	0.032	0.123	-0.035	0.184	0.018	0.009
search	Sig. (2-tailed)	0.814	0.365	0.798	0.179	0.897	0.949
	Pearson		-		-		
Memory	Correlation	0.251	0.137	**0.338**	0.128	0.236	-0.021
	Sig. (2-tailed)	0.062	0.311	**0.012**	0.347	0.089	0.876
	Pearson	-					
Executive	Correlation	0.066	0.149	-0.106	0.084	-0.185	-0.133
function	Sig. (2-tailed)	0.627	0.263	0.429	0.533	0.180	0.319
	Pearson						
Processing	Correlation	0.034	0.112	-0.037	0.167	0.217	0.108
speed	Sig. (2-tailed)	0.805	0.416	0.791	0.227	0.126	0.434
	Pearson	-			-		
Verbal	Correlation	0.136	0.095	-0.111	0.051	-0.028	-0.088
fluency	Sig. (2-tailed)	0.314	0.476	0.407	0.708	0.842	0.513

Abbreviation: RAVLT Rey Auditory Verbal Learning Test, SFA, saturated fatty acid, PUFA, polyunsaturated fatty acid, MUFA, monounsaturated fatty acid.

LDL and memory changes were correlated but no other cardio-metabolic outcomes correlated with cognitive tasks (**Table 6.13**).

Table 6.14 Correlation data for cognitive tasks and nutrient intakes (shaded areas indicate $p < 0.05$).

		Fibre	MUFA	Vitamin E	Folate
RAVLT recall	Pearson Correlation	-0.038	0.131	-0.005	**0.351**
	Sig. (2-tailed)	0.776	0.326	0.971	**0.007**
RAVLT delay	Pearson Correlation	**0.262**	-0.042	0.025	**0.296**
	Sig. (2-tailed)	**0.045**	0.75	0.851	**0.022**
RAVLT target	Pearson Correlation	0.206	-0.13	-0.06	-0.222
	Sig. (2-tailed)	0.120	0.326	0.650	0.091
Stroop	Pearson Correlation	0.017	0.061	0.011	-0.198
	Sig. (2-tailed)	0.897	0.644	0.934	0.129
Initial letter	Pearson Correlation	0.126	-0.182	-0.021	-0.028
	Sig. (2-tailed)	0.342	0.164	0.872	0.833
Excluded letter	Pearson Correlation	-0.046	-0.101	-0.132	0.052
	Sig. (2-tailed)	0.736	0.453	0.318	0.701
Coding	Pearson Correlation	-0.054	-0.186	0.018	-0.069
	Sig. (2-tailed)	0.691	0.161	0.892	0.606
Symbol search	Pearson Correlation	0.054	-0.252	-0.142	0.000
	Sig. (2-tailed)	0.691	0.056	0.283	0.998
Memory	Pearson Correlation	0.040	0.018	0.041	0.059
	Sig. (2-tailed)	0.764	0.892	0.759	0.658
Executive function	Pearson Correlation	0.014	-0.164	-0.155	-0.086
	Sig. (2-tailed)	0.915	0.210	0.233	0.512
Processing speed	Pearson Correlation	-0.02	-0.231	-0.176	0.030
	Sig. (2-tailed)	0.885	0.085	0.186	0.824
Verbal fluency	Pearson Correlation	-0.072	-0.131	-0.181	-0.07
	Sig. (2-tailed)	0.586	0.318	0.164	0.595

Abbreviation; RAVLT Rey Auditory Verbal Learning Test, SFA, saturated fatty acid, PUFA, polyunsaturated fatty acid, MUFA, monounsaturated fatty acid.

Two RAVLT memory tests (RAVLT recall and RAVLT delay) correlated with either folate and/or fibre intakes **Table 6.14**).

6.7 Discussion

The aim of this chapter was to describe the effect of Hi-oleic peanut consumption on cardio-metabolic, energy balance, body composition measures and nutrient intake. It was hypothesised that protein, fibre and unsaturated fat content would increase satiety; thereby reducing or maintain energy intake despite energy intake from peanuts. Fasting lipids was hypothesised to be as a result of high mono-unsaturated fat and fibre content of the Hi-oleic peanuts and possible reduction of saturated fat/carbohydrate intake in the diet. It was also hypothesised that the fibre and polyphenol content of the peanuts would reduce inflammatory markers including C-reactive protein. In addition glucose was hypothesised to be reduced as a consequence of a reduction in glycaemic response to meals and snacks with Hi-oleic peanut consumption and improvement in insulin sensitivity by the monounsaturated fatty content and fibre content of the peanuts or indirectly via a possible lowering of body weight.

6.7.1 Dietary intake, energy balance and body composition

The food diaries revealed ~ 60 g daily intake of peanuts, consumed by both men and women (6 days per week). The expected increase in energy intake from this amount of peanuts was 1400 kJ per day (assuming no other changes were made to the diet). The actual increase in energy intake was 840 kJ (60 % of the expected value) indicating that some of the peanuts were substituted for other foods in the diet. Additional monounsaturated fat consumed during the peanut phase accounted for ~ 90 % of the additional energy consumed. The energy increase with Hi-oleic peanut consumption is in contrast to the acute satiety study (Chapter 4) which demonstrated a ~10% reduction in energy intake over 4 days. However, this study compared peanut intake with an iso-energetic high fat snack food (potato crisps).

The predicted weight gain for this increase in energy reported was 1.6 kg over 12 weeks. However, the actual average weight gain over this period was only 0.5 kg (33% of expected) which did not reach significance, with no changes waist circumference or body composition observed. Energy expenditure from physical

activity remained the same for both phases so this was not a contributor to the findings. Similarly, eight week consumption of 89 g/day of peanuts for 8 weeks with no dietary compensation demonstrated a weight gain of only 1kg which was 28 % of the predicted 3.6 kg (Alper & Mattes 2002).

A possible reason that the predicted weight gain with increased energy intake was not reached is incomplete fat absorption from the peanuts. Fat is contained within walled cellular structures of the peanuts and is incompletely digested in the gut (Traoret et al. 2008), this is possibly compounded by incomplete mastication (Cassady et al. 2009). The energy value of other nuts (almonds (Cassady et al. 2009) and pistachios (Baer, Gebauer & Novotny 2012)) has found to be overestimated which may be explained by limited fat absorption and digestion.

In addition the body fat storage may have been limited as a result of the unsaturated fatty acids being oxidized. Unsaturated fats and have a greater thermo-genic effect (Hermsdorff, Volp & Bressan 2007) and are more readily oxidised (Jones, Pencharz & Clandinin 1985) than saturated fatty acids making them less readily stored in the adipose tissue. Further, peanut consumption for 8 weeks has demonstrated a small (5%) but significant increment in resting energy expenditure in obese individuals (Alper & Mattes 2002). A recent study with high-oleic peanuts has also revealed a greater increase in diet induced thermogenesis compared with conventional peanuts (Alves et al. 2014) with MUFA intake suggested to contribute to the difference. Other studies have demonstrated an increase in diet induced thermogenesis and fat oxidation with MUFA possibly due to a higher stimulation of the sympathetic nervous system by MUFA than other fatty acids (Moussavi, Gavino & Receveur 2008). The findings that MUFA was inversely correlated with fat mass and insulin adds to an emerging literature on the benefits of MUFA on cardio-metabolic risk factors.

It is possible that a combination of these mechanisms contributed to inefficient energy utilisation or an increase in energy expenditure from peanut consumption demonstrating no changes in body composition and less than predicted increase in body weight. Interestingly, in this study MUFA intake was inversely

associated with body fat mass. It has been suggested that fat quality may have a stronger correlation with weight gain than fat quantity (Field et al. 2007) Studies suggest a role for preferential oxidation and metabolism of dietary MUFA, which influences body composition hence ameliorating the risk of obesity (Bergouignan et al. 2009; Gillingham, Harris-Janz & Jones 2011). These mechanisms together may have contributed to the body composition maintenance observed with increase in energy and fat intake. Both the satiety study (Chapter 4) and chronic study suggest that large quantities (up to 60g) of peanuts can be included in the diet without detrimental effects to body weight but only if they are substituted for other foods, not consumed in addition to the diet.

Other observed dietary changes in this study included significant increases in vitamin E, folate, magnesium and fibre. Vitamin E and magnesium intakes both increased by ~ 75%. Vitamin E is a powerful antioxidant and several observational studies have found that supplementation or higher dietary intake of vitamin E is associated with a decreased risk of chronic disease which is associated with oxidative stress and aging (Singh, Devaraj & Jialal 2005). Not all studies, however, have confirmed a relationship and some clinical trials with supplements in high risk groups have shown little benefit (Stein et al. 2000). Magnesium is important for healthy blood pressure and regulation of blood glucose (Song et al. 2005). A systematic review and meta-analysis of prospective studies found that higher serum levels of magnesium were significantly associated with a reduced risk of cardiovascular disease in women (but not in men) (Xu et al. 2013). Marginal-to-moderate magnesium deficiency is thought to contribute to chronic conditions including hypertension, diabetes and cardiovascular disease through exacerbation of chronic inflammation (Nielsen 2010). Serum magnesium levels were not measured in the present study but participants were meeting their recommended intake of magnesium at baseline so additional magnesium intake from peanuts was not likely to play a large role in the improved vascular function observed. Folate increased by 50% in this study. Folate supplementation has demonstrated benefits for individuals with homocysteinaemia, a risk factor for cardiovascular disease particularly in those who have a low intake of folate (Bellamy et al. 1999). As participants in

this the present study were consuming a healthy diet at baseline and meeting their folate requirements, any increase is less likely to be of benefit for this population group. Fibre intake increased by 15% in this study and a meta-analysis has revealed that greater dietary fibre intake is associated with a lower risk of both cardiovascular disease and coronary heart disease (Threapleton, Greenwood & Evans 2013). Small increases in zinc, iron and potassium were also found in the current study which did not reach significance.

Interestingly, an inverse association was observed between body weight and left CVR and fat mass with large artery elasticity, indicating reductions in body fat may have contributed to benefits in vascular function in some individuals. However, as no significant changes in body composition or anthropometric measures were identified for the whole cohort this was unlikely to be a major contributor to the findings. No association with right CVR were found with body composition or anthropometric measures.

6.7.2 Cardio-metabolic measures

Surprisingly, lipid levels were not altered with Hi-oleic peanut consumption contrary to many other tree nut and peanut studies (Sabate, Oda & Ros 2010). However, the cholesterol lowering effects of nuts are shown to be greatest in individuals with higher baseline low density lipoprotein (LDL) and lower body mass index (BMI) (Sabate, Oda & Ros 2010). A small non-significant weight gain and body fat increase was observed, which may have attenuated any small improvements in cardio-metabolic outcomes. The participants in the study were overweight or obese with an average BMI of 30.2 kg/m² and had healthy baseline blood lipid levels. Nut consumption has also been found to lower triglyceride levels predominantly in individuals with hypertriglyceridemia (Sabate, Oda & Ros 2010). Similarly, Mukuddem-Peterson et al. (Mukuddem-Petersen et al. 2007) also reported no changes in blood lipid levels in individuals with obesity with either walnut or cashew consumption. Obese individuals have demonstrated an attenuated cholesterol-lowering response to dietary manipulation of fatty acids compared with lean individuals (Lefevre et al. 2005). In addition, the lipid lowering effects of nuts are greatest when they are

substituted for saturated fat in the diet; in the current study there were no differences in saturated fat intake. These factors combined likely explain why no changes in lipids were observed.

An association was observed between fibre intake and plasma triglyceride levels in this study. Fibre intake is often associated with carbohydrate intake; hence it is possible that those participants consuming a higher intake of fibre were also consuming a higher carbohydrate intake which may have impacted negatively on their triglyceride levels. An increase in triglycerides with higher fibre intake in other studies has also been observed (Riccardi et al. 1984; Ulmius, Johansson & Önning 2009). However, in the current study there was no evidence of peanut consumption directly affecting either total carbohydrate intake or triglycerides.

This study demonstrated no differences in glucose or insulin levels with consumption of Hi-oleic peanuts. Similarly several other studies have not shown benefits with consumption of pistachios, almonds and walnuts on fasting glucose or insulin. (Hongyu et al. 2010; Jenkins et al. 2011; Llorente-Cortés et al. 2010; Lovejoy et al. 2002; Ma et al. 2010; Mercanligil et al. 2007; Mukuddem-Petersen et al. 2007; Tapsell et al. 2004; Thomazella et al. 2011; Wien et al. 2003; Zaveri & Drummond 2009). However, some short-term intervention studies have shown benefits of nut consumption on glucose homeostasis (Estruch et al. 2006; Sari et al. 2010) and insulin secretion (Adamsson et al. 2011; Casas-Agustench et al. 2011a; Estruch et al. 2006; Wien et al. 2010). The effects of nuts on insulin sensitivity are influenced strongly by changes in body weight which may have accounted for the changes observed in one of these studies. As no significant weight changes were observed in this current study, improvements in glucose regulation were less likely.

Improvements in glucose regulation have been demonstrated when nuts are included as part of an intervention diet such as the Nordiet (Adamsson et al. 2011) or a Mediterranean diet (Esposito et al. 2004), with benefits partly attributable to other components of these diets. In the current study an increase in MUFA correlated with a decrease in insulin. A recent review (Gillingham, Harris-Janz & Jones 2011) has outlined studies determining the effect of MUFA

on insulin resistance demonstrating improved insulin sensitivity and glucose regulation following MUFA-rich diets in both healthy (López et al. 2008; Pérez-Jiménez et al. 2001; Vessby et al. 2001) and diabetic individuals (Due et al. 2008; Paniagua et al. 2007; Shah et al. 2007). However, not all studies have demonstrated this effect with benefits more likely to be observed in participants of lower body weight (Lovejoy et al. 2002).

Consumption of Hi-oleic peanuts in the chronic study demonstrated no difference in the inflammatory marker C-reactive protein (CRP) when compared with the nut free diet. Similarly the systematic search outlined in chapter 3 reported approximately half of the studies reviewed (N=15) also demonstrated no significant differences in inflammatory markers with nut consumption. One of the suggested reasons for this was recruitment of healthy individuals who may only demonstrate limited improvements. Participants in this study were healthy with a mean baseline CRP of 1.5 mg/l, well below the cut off of 3.0 mg/l for of cardiovascular disease risk (Pai et al. 2004). In addition, central adiposity is associated with increased CRP levels and it is possible that those with a central adiposity may not demonstrate improvements in inflammatory markers without weight loss (Mathieu, Lemieux & Després 2010). Both male and female participants in this study demonstrated central adiposity with mean baseline waist circumference measurements above the National Heart Foundation classification for risk of increased risk for cardiovascular disease (National Heart Foundation of Australia). Two other nut studies demonstrated no CRP change but did observe reductions in other inflammatory markers, IL-6 and VCAM-1 with large doses (65-100 g) of pistachios and walnuts respectively (Ros et al. 2004; Sari et al. 2010). It is possible in the study that other inflammatory markers were improved with the addition to Hi-oleic peanuts.

Hi-oleic peanuts have similar nutrient composition to other nuts which have shown cardiovascular benefits with a higher content of phytosterols and folate than other nuts; hence, cardio-metabolic benefits were expected in this study. The scoping review (**Chapter 3**) indicated that a minimum nut dose of 30g may be required to elicit benefits for inflammatory markers. The current study used a relatively large dose of nuts (~ 60 g), so this was not likely to be a limiting

factor. A more likely reason is the population used; participants had glucose, insulin, lipid and CRP levels within the normal range for a healthy population at baseline. It may be that in a healthy population group, further improvements in these outcomes are limited; however prolonged nut consumption in this population may help to maintain cardio-metabolic health over time. Few studies have investigated the effects of peanuts on cardio-metabolic other than lipid lowering hence, more studies with different population groups are warranted

6.7.3 Strengths and limitations

One of the strengths of this study was compliance with the consumption of peanuts and with nut free diet was high, as assessed by food diary entries and returned peanut packages. The study also controlled for physical activity levels with participants requested to maintain similar physical activity levels throughout the study. No difference in physical activity was observed from the diaries over the two phases.

Due to budget constraints, it was not possible to measure fat excretion and energy expenditure which may have provided information on a possible mechanism for the observed weight maintenance and body fat despite an increase in energy intake. Mechanistic studies (Mattes 2008) have demonstrated an increase in resting energy expenditure (Alper & Mattes 2002) and diet induced thermogenesis (Alves et al. 2014). In addition other biomarkers such as plasma Vitamin E, polyphenol levels, red blood cell monounsaturated fatty acid content, inflammatory markers (in addition to C-reactive protein) were not measured which may have provided information on the potential mechanisms for vascular and cognitive outcomes observed. The polyphenol content and in particular the resveratrol content of the Hi-oleic peanuts was not measured to determine if levels present could be sufficiently potent to induce changes observed. For further strengths and limitations of the study design see **Section 5.9.5**

6.7.4 Conclusion

Despite being energy dense, Hi-oleic peanuts appear to be a healthy food choice with no detrimental effects on body weight despite additional energy intake. However, prudent dietary advice for including nuts in the diet should include advice not to add them in large amounts without the reduction of other foods (preferably high in energy and low in nutrients). The diet increased in Vitamin E, magnesium and folate with peanut consumption which may have some health benefits particularly in individuals who have low intakes of these nutrients. There were no changes in lipids, glucose regulation or C-reactive protein in this healthy population group however Hi-oleic peanuts have the potential for cardio-metabolic benefits in at risk groups or for maintaining cardio-metabolic health. Few studies have investigated the effects of peanuts on cardio-metabolic effects hence more studies with different population groups are warranted.

Chapter 7: General Discussion

7.1 Summary

Cardiovascular disease, obesity and cognitive impairment are growing worldwide health concerns particularly as populations' age. Interventions that slow or prevent these conditions are valuable and have become a health priority. The current research has demonstrated benefits of peanut consumption for satiety, cerebrovascular and cognitive function, diet quality with no detrimental effects on cardio-metabolic or body composition measures, which are outlined below.

7.2 Hi-oleic peanuts improves cerebrovascular function and cognition

This thesis introduced the hypothesis, that an increase in endothelial function of the cerebral arteries with nut consumption could facilitate improvements in cognitive functioning. The hypothesis was based on findings from the scoping review which concluded that benefits may be found in both endothelial and cognitive function with nut intake. **Study 2** demonstrated a benefit of Hi-oleic peanut consumption on cerebral vasodilator function (CVR) and an improvement in cognitive function hence further supporting this hypothesis. It is possible that a combination of bioactive components in Hi-oleic peanuts including monounsaturated fatty acids, vitamin E, folate, magnesium, fibre, arginine and polyphenols work together to elicit these benefits. Measurement of CVR with dietary interventions is a relatively novel technique; previous studies have predominantly investigated acute or short term effects and few have also measured cognitive outcomes.

These are important study findings adding further to the body of nut research, indicating that Hi-oleic peanuts are a valuable addition to the diet; not only providing nutritional benefits and cardiovascular improvements but also assisting cognitive performance. The study findings also provide evidence for a mechanism of action, that of increased blood flow to the brain for cognitive benefits, which has potential in a range of other nut varieties.

These promising findings indicate that Hi-oleic peanuts could be combined with other functional foods with demonstrated cognitive benefits such as berries and fish to provide a "cognitive health diet". Such a nutritional intervention has the potential to deliver greater cerebral vascular and cognitive benefits than one food alone and could be used for the prevention and treatment of cognitive decline particularly as these become a priority for a growing older population. These findings support the health benefits of peanuts in older overweight adults, beyond cardiovascular health, contributing further evidence for a potential qualified health claim for nuts in Australia, with their increased prominence recommended in future Australian dietary guidelines.

7.3 Satiating effects of peanuts and weight regulation

Study 1 observed that peanuts promoted satiety compared with potato crisps and reduced total energy intake acutely and over 4 days, indicating they are an appropriate choice as a snack food. These findings align with some but not all nut satiety studies, with benefits likely as a result of increased fibre and protein intake. (Alper & Mattes 2001; Zaveri & Drummond 2009). This is the first satiety study with Hi-oleic peanuts and the first acute study to compare nuts an iso-energetic snack with potato crisps. There is evidence that snacking may contribute to long term weight gain (Forslund et al. 2005) hence, it is important to promote healthy alternatives such as peanuts and other nuts which have the potential to promote lower energy intakes when substituted for high energy processed snack foods. However, while this study demonstrated reduced energy intake over a relatively short time period with peanut consumption compared with a high energy snack food, it was not clear whether such an effect would be sustained over the longer term.

During **Study 2**, the two diet phases were intentionally not energy matched in order to observe the influence of Hi-oleic peanuts on energy and nutrient intakes when added to the habitual diet. The addition of Hi-oleic peanuts increased the nutrient intakes of monounsaturated fatty acids, vitamin E, folate, magnesium, fibre and protein, hence increasing diet quality. Of interest, was no observed weight gain or increase in body fat despite an increase in energy and total fat intake with peanuts. A possible reason for this discrepancy is a limited nutrient

bio-accessibility resulting in malabsorption and inefficient energy utilization. This was not tested but other studies have observed increases in faecal fat excretion with nut consumption (Zemaitis & Sabate 2001).

This research supports evidence from other intervention trials that long term nut consumption does not lead to a change in body weight (Flores-Mateo et al. 2013) and observational studies which show higher nut consumption is associated with a lower body mass index (Bes-Rastrollo et al. 2009). Hence, both studies support the use of peanuts as a healthy and nutritious snack to assist with the management of body weight, particularly if substituted for other high energy snack foods. They would be a valuable addition to the diet as obesity becomes a growing health concern.

7.4 Effect of nut consumption on cardio-metabolic measures

This thesis included an extensive scoping review of the cardio-metabolic effects of nut consumption. The review calculated weighted means for study outcomes which added power to several smaller studies, unable to demonstrate changes on their own. Collectively benefits for glucose regulation and inflammation were found; hence these outcomes were chosen in addition to lipid measures identified in other reviews. It was hypothesised that Hi-oleic peanut consumption would influence cardio-metabolic outcomes to facilitate improvements in cerebral vasodilator function (CVR). Despite improvements in CVR, they appeared not to be influenced by any of the cardio-metabolic outcomes (lipids, glucose regulation and C-reactive protein) as these were unchanged. Evidence from the systematic review suggests that improvement in these outcomes with nut consumption is more likely in individuals with hyperlipidaemia or metabolic syndrome (Sabate, Wien & Haddad 2009); hence, for a healthy population it may be difficult to induce further improvements. However, these Hi-oleic peanuts may have cardio-metabolic benefits in other population groups but have yet to be tested. A positive finding from this study was that, despite additional energy and total fat intake with Hi-oleic peanut consumption there were no detrimental effects on any of these cardio-metabolic outcomes.

7.5 Future research

Of particular interest are dietary interventions that have benefits beyond those ascribed to the macro- and micronutrients. Identification of dietary constituents that elicit favourable effects will facilitate the development of diets that are even more effective for both the prevention and treatment of obesity, cardiovascular risk, and cognitive decline. Hi-oleic peanuts contain several bioactive nutrients which have potential benefits beyond their nutritional value. In particular, the vascular and cognitive benefits of Hi-oleic peanuts observed in this current research are novel findings, which pave the way for future research. It would be advantageous to determine whether other nuts are also effective in inducing these changes and the doses and length of time that are required to gain maximum benefit as well as mechanisms for their action. In addition, future cognition studies with other population groups may also be of benefit including adults with hyperlipidaemia or metabolic syndrome who may demonstrate potential cardio-metabolic improvements. Studies with children or adults with cognitive decline may also demonstrate benefits with nut consumption to promote optimal brain and cognitive development or slow cognitive decline. Mechanistic studies observing cerebral blood flow with accompanying changes in structural brain changes using techniques such as proton nuclear magnetic resonance spectroscopy would also provide information on the influence of nuts on brain function.

Nuts are often consumed in a salted form without skins to increase palatability and this may reduce or eliminate their vascular benefits. Of interest would be the testing of Hi-oleic peanuts with skins removed to determine if the findings from this research can be replicated with a substantial reduction in polyphenol content. Future studies using salted or lightly salted nuts would help determine any detrimental effects of adding salt.

Few studies have investigated the effects of peanuts on cardio-metabolic and vascular effects hence more studies with different population groups are warranted. In addition, as few studies have measured the effects of nut consumption on arterial elasticity this warrants more studies with other nut

varieties with arterial elasticity as a primary outcome. This may determine if endothelial function improvements with nut consumption observed can result in longer term structural changes in blood vessel function to benefit vascular health.

The systematic review identified the potential for nut consumption to improve inflammatory markers, blood pressure and glucose regulation; however, they were often measured as secondary outcomes and not adequately powered so not all studies demonstrated benefits. In addition, the review revealed a range of inflammatory markers may be responsive to nut consumption and their effects may be dose dependant. Hence, adequately powered studies measuring the influence of nut consumption in a range of doses on blood pressure, glucose regulation and a range of inflammatory markers are warranted.

The chronic study identified an increase in several nutrients with the addition of Hi-oleic peanuts to the diet. Further research to determine the overall impact on of Hi-oleic and other nuts on diet quality including their influence on the intake of other foods, particularly snack foods or food groups would also be useful to assist with their recommendation in dietary guidelines.

This research has supported the potential benefit of nuts included as part of a weight reduction diet as they may promote satiety and hence increase compliance over an extended period. Future study recommendations should include trials of longer duration to determine if satiety with peanuts is sustained and to assess the effects of peanut consumption on eating behaviours. Mechanisms for satiety action are unclear and future studies with nuts would benefit from assessing satiety hormones in addition to perceived satiety measures. There is evidence that nuts are most commonly consumed as snacks; to date, few satiety studies have compared nut consumption with other snack foods; hence further research comparing nut intake with a range of snack foods such as chocolate, biscuits and savoury snacks would also be beneficial. Knowledge of the most effective timing of nut consumption throughout the day to most benefit satiety would also be beneficial to inform recommendations.

7.6 Conclusion

This research has identified that peanuts are a suitable snack food as an alternative snack to other high fat foods (such as potato crisps). In addition, Hi-oleic peanuts may confer benefits for vascular and cognitive function which are important areas of research especially as chronic disease and dementia are increasing around the world. One of these strategies is the inclusion of foods which improve vascular function and cognitive performance. Hi-oleic peanuts have demonstrated all of these benefits as well as increasing nutrient intake. Hence, they can be defined as a functional food demonstrating physiological benefits above and beyond their nutrient content.

In the United States of America nuts carry a health claim for reduction in cardiovascular risk with the consumption of 45g daily. However, a recent Australian dietary survey (Australian Bureau of Statistics 2012) has shown that average nut consumption for Australian adults is around 5 g/day. One of the reasons for this may be a perception by the general population that nut intake causes weight gain. This research support other studies indicating that inclusion of nut in the diet does not cause weight gain but may assist with weight management.

This research has also identified the potential health benefits of consuming unsalted Hi-oleic peanuts and possibly other nuts with the skins intact. These are normally removed before consumption and in food products such as peanut butter and confectionery products which may reduce their action or potency. With a growing demand for the food industry to provide healthy products there may be in benefits in incorporating peanuts with skins or the skins separately into food or nutraceutical products.

In summary this study has demonstrated that Hi-oleic peanuts are a valuable addition to the diet and the study findings may be used as evidence to promote their intake along with other nuts in the Australian diet and worldwide. This research also provides a foundation for further well designed studies to verify and expand upon these benefits.

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Appendices

Appendix A Statement of authors' contributions to published papers

A.1 Declaration of authorship of Chapter 3

This chapter is a co-authored publication

Jayne A. Barbour, Peter R. C. Howe, Jonathan D. Buckley, Janet Bryan and Alison M. Coates. (2014). Nut consumption for vascular health and cognitive function. Nutrition Research Reviews. 27, 131–158

J.A. Barbour and P.R.C. Howe, J.D. Buckley, J. Bryan and A.M. Coates devised the idea for this manuscript. J.A. Barbour prepared and edited the manuscript. P.R.C. Howe, J.D. Buckley, J. Bryan and A.M. Coates edited the manuscript



J.A. Barbour



P.R.C. Howe



J.D. Buckley



A.M. Coates



J. Bryan

A.2 Declaration of authorship of Chapter 4

This chapter is a co-authored publication

Jayne A. Barbour, Peter R. C. Howe, Jonathan D. Buckley, Janet Bryan, Graeme. C. Wright and Alison M. Coates. (2014). Lower Energy Intake Following Consumption of Hi-oleic and Regular Peanuts Compared with Iso-energetic Consumption of Potato Crisps. Appetite. 82, 124-130.

J.A. Barbour and P.R.C. Howe, J.D. Buckley, J. Bryan and A.M. Coates devised the idea for this manuscript. The study was co-ordinated and data collected and analysed by J.A. Barbour. J.A. Barbour prepared the manuscript and all authors contributed to editing of the manuscript.



J.A. Barbour



P.R.C. Howe



A.M. Coates



G.C. Wright



J. Bryan



J.D. Buckley

Appendix B Materials for satiety study and chronic study

B.1 Flyer for satiety study

University of South Australia | Nutritional Physiology Research Centre

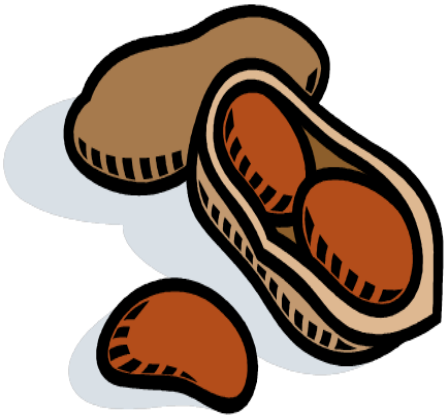
Volunteers Wanted for Peanut Study

If you are aged over 50 years and overweight but otherwise healthy (women must be post-menopausal), we invite you participate to in a study to test the appetite and satiety (fullness) effects of consuming peanuts and potato crisps.

Participants will visit the Nutrition Physiology Research Centre at the University of South Australia (Frome Rd) on three occasions; eat a daily serve of either peanuts or potato crisps for a total of 12 days and record dietary intake and feelings of fullness.

For further information: phone Jayne Barbour on 83022348 or email:jayne.barbour@unisa.edu.au

Approved by University of South Australia Ethics committee



B.2 Flyer for Chronic Study

University of South Australia

Nutritional Physiology Research Centre

VOLUNTEERS WANTED FOR

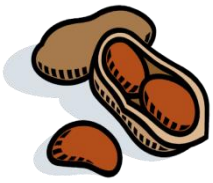
Peanut Study



If you are aged over 50 years and overweight but otherwise healthy (women must be post-menopausal), we invite you to participate in a study to test effects of peanuts on blood vessel health and brain function.

Participants will visit the Nutrition Physiology Research Centre at the University of South Australia (Frome Rd) on 3 occasions, eat a daily serve of either peanuts or potato crisps for a total of 12 days and record dietary intake and complete short questionnaires.

For further information: phone Jayne Barbour on 83022348 or email:jayne.barbour@unisa.edu.au





B.3 Advertisement for chronic study



VOLUNTEERS NEEDED FOR PEANUT STUDY

The Nutritional Physiology Research Centre is investigating the effects of eating peanuts on blood vessel health and brain function.
If you are: **over 50** and **overweight** but **otherwise healthy**
(women must be **post-menopausal) and do not consume peanuts regularly**
we invite you to participate in a 24 week dietary study.
Phone: 8302 2348 for more information
Approved by The University of South Australia Human Research Ethics Committee

B.4 Volunteer information sheet satiety study

University of South Australia | Nutritional Physiology Research Centre

VOLUNTEER INFORMATION SHEET

Project Title: The Effect of "High-Oleic" Peanuts on Appetite and Feeling of Fullness

Investigators:
Ms Jayne Barbour (PhD candidate)
Dr Alison Coates,
Professor Peter Howe
Associate Professor Jon Buckley
Dr Janet Bryan

BACKGROUND Australian dietary guidelines recommend regular consumption of nuts, yet they have an unhealthy image as a high fat, high energy food which promotes weight gain. Evidence suggests that consumption of nuts induces satiety (feeling of fullness) and may lead to reduction of food intake later in the day. In addition there is some evidence that indicates consumption of nuts may assist weight loss especially if unhealthy snacks are replaced with nuts or consumed as part of a weight control diet.

Aim of the study

The aim of this project is to investigate the effect of Hi-Oleic peanuts on feelings of hunger and fullness. We will also observe if there are any changes to food intake whilst consuming peanuts as part of the diet and compare this with a standard snack food.

Conduct of the study

The study is being funded by the Peanut Corporation of Australia and will be conducted in the Nutritional Physiology Research Centre on the City East Campus (Frome Road) of the University of South Australia.

If you agree to participate you will be required to attend the centre on three different occasions (one screening visit to ensure eligibility to participate and two experimental visits).
Prior to your screening visit we will conduct a telephone interview during which we will ask you about your height, weight and medical history to ensure that you are suitable for the study.

At your screening visit we will check your height, weight, blood pressure and ask you to complete a simple questionnaire to look at memory and brain function. If you meet the criteria you will be invited to join the study. We will provide you with a set of scales for weighing food and ask you to measure and record your food and drink intake for the 24 hours prior to your first experimental visit.

For each experimental visits we will ask you to attend our research centre after an overnight fast. We will ask you to complete a short questionnaire about your feelings of wellbeing and hunger. You will then be asked to consume a portion of peanuts or an alternative snack of the same energy content. At regular intervals we will continue to ask you ask you to complete the same questionnaire. After a period of four hours you will be offered a range of foods to eat and we will note the foods eaten.

We will provide you with portion packs of peanuts or an alternative snack food and ask you to consume these for the next 3 days (the amount consumed will depend upon your energy requirements).We will also ask you to continue to complete the same type of questionnaires and record your food and drink intake. There will be a four day gap between the two experimental visits when we will ask you to resume your normal diet but continue to weigh and monitor your food.
The second visit will be 8 days after the first visit and will follow the same format as the first except we will ask you to consume the alternate food (peanuts or snack food). We will then ask you to continue to consume this food, monitor intake and complete questionnaires for the next 3 days.

ELIGIBILITY:

- You must be aged over 50 years.
- If you are female you must be post-menopausal (defined as at least 12 months since last menstruation with no other medical explanation)
- You must be overweight (as categorized by body mass index, we will calculate this for you based on your height and weight at the screening visit)
- Your blood pressure must be less than 160/100mmHg (we will measure this during the screening visit)
- You must **not** have cardiovascular disease, renal disease or diabetes
- You must **not** have an allergy to peanuts or any other nuts
- You must **not** be taking any blood pressure lowering medications
- You must **not** be a smoker or use nicotine replacement therapy

RISKS

There are no known risks associated with taking peanuts (unless you have an unknown peanut allergy). The researcher will have access to an Epipen if you have an allergic reaction after consumption of the first portion of peanuts in the clinic. If you experience any adverse effects you will be asked to notify the researchers immediately and to stop taking the peanuts.

CONFIDENTIALITY

All information collected as part of the study will remain confidential and no information which could lead to identification of any individual will be released. Data will be recorded on data sheets and then transferred to electronic data files. All hardcopies and electronic copies of data will be stored in a secure data storage room within the Nutritional Physiology Research Centre at the University until the research has been published, and will then be stored in the University's archive before being destroyed after 15 years.

WHAT ARE THE BENEFITS OF PARTICIPATING IN THE STUDY?

You will be providing a valuable contribution to the scientific knowledge in this field and will observe any satiety benefits of consuming peanuts. A summary of the group results will be mailed to all volunteers on completion of the study. You will be provided with a supply of peanuts or alternative snack during the study. On completion of the study you will be provided with a peanut hamper as a thank you for your time and effort. Participants may not personally benefit by participating in the study.

Participation in this study is voluntary, and you may withdraw from the study at any time without prejudice.

If you would like to participate in the study, or if you, or any member of your family, require more information to help you arrive at a decision, please contact:
Jayne Barbour; Tel: (08) 83022346, 0422444861; Email: jayne.barbour@unisa.edu.au

This project has been reviewed by the University of South Australia's Human Research Ethics Committee. If you have any ethical concerns about the research or questions about your rights as a participant please contact the Executive Officer of this Committee: Ms Vicki Allen; Tel: (08) 8302 3118; Email: Vicki.allen@unisa.edu.au.

B.5 Volunteer information sheet chronic study

University of South Australia | Nutritional Physiology Research Centre

Project Title: Do Peanuts Improve Blood Vessel Health, Thinking and Memory?
VOLUNTEER INFORMATION SHEET

The study is being funded by the Peanut Company of Australia and will be conducted in the Nutritional Physiology Research Centre on the City East Campus (Frome Road) of the University of South Australia.

Investigators:

Ms Jayne Barbour (PhD candidate)
Dr Alison Coates,
Professor Peter Howe
Associate Professor Jon Buckley
Dr Janet Bryan

Background
Nuts have been shown to be beneficial for cholesterol lowering and reduction of the risk of cardiovascular disease. Nuts may also improve the health of the blood vessels leading to the heart and in the brain, so providing benefit to these parts of the body.

Aim of the Study
The aim of this project is to investigate the effect of peanuts on blood vessel function and see if this leads to improvements in thinking and memory.

Conduct of the Study
If you agree to participate you will be required to attend the centre on five different occasions over 6 months (plus one screening visit to ensure eligibility). Prior to your screening visit we will conduct a brief telephone interview during which we will ask you about your height, weight and medical history to ensure that you are suitable for the study.

At your screening visit we will check your height, weight, waist circumference, blood pressure and ask you to complete 3 questionnaires to ask about lifestyle, medical history, eating habits and ensure you do not have cognitive impairment. If eligible you will be invited to join the study. We will provide you with a set of scales for weighing food and ask you to measure and record your food and drink intake and physical activity for 4 days prior to your first experimental visit.

At the first, third and final experimental visits (week 1 week 12 and week 24) we will ask you to attend our Research Centre after an overnight fast. We will take measurements of;

- Weight and waist circumference
- Blood pressure
- Blood test to measure blood sugar, insulin, cholesterol levels plus red blood cell fat content (to measure if peanuts have been consumed).
- Elasticity of blood vessels using a sensor on the wrist (week 6 and 12 only).
- Blood flow in the brain using ultrasound probe

- Body composition using DEXA body scanner (week 6 and 12 only)
- We will then provide a snack and conduct tests of thinking and memory.

The total time to conduct these tests will be approximately 2½ hours
You will then be asked to **either** a) continue a low nut diet (no nuts as snacks, no peanut butter, foods with a small amount of nuts will be permitted) **or** b) consume a portion of peanuts as and when you wish (amount will depend on your energy requirements) for six days per week, for the next 12 weeks.

At 6 weeks and 12 weeks we will ask you to complete 4 day diet and physical activity diaries and return to the Centre to check diaries, repeat some of the measures and those consuming peanuts will collect their supply for the next 6 weeks. This visit will take approximately 15 minutes.

At week 12 you will attend the Centre to repeat the initial tests (2½ hours) and swap over to consume **either** peanuts **or** your usual diet for the next 12 weeks. At week 18 you will come in for a 15 minute visit to repeat some measures having completed 4 day food and activity diaries. At week 24 you will return to the Centre for the final visit to repeat all the tests (2½ hours) having completed 4 day diet and activity diaries.

Eligibility:
- You must be aged between 50 -75 years.
- If you are female you must be post-menopausal (defined as at least 12 months since last menstruation with no other medical explanation) and not be on hormone replacement therapy (HRT).
- You must be overweight (as categorized by body mass index BMI > 25 kg/m ^{2}), we will calculate this for you based on your height and weight at the screening visit and you must also be below 135kg.
- You must not have cardiovascular disease, renal disease, diabetes or have suffered a stroke.
- You must not have an allergy to peanuts or any other nuts.
- You must not be a regular nut consumer (>30g nuts or peanut butter per week).
- Your blood pressure must not exceed 160/100 mmHg at the screening visit. (You may be on blood pressure medication if your blood pressure is stable)
- You must not be on medications which affect metabolism e.g. thyroid
- You must not be a regular dieter
- You must have adequate memory and cognitive ability to complete the study
- You must not be a smoker or use nicotine replacement therapy

Possible Risks:
There are no known risks associated with taking peanuts (unless you have an unknown peanut allergy). The researcher will have access to an Epipen if you have an allergic reaction after consumption of the first portion of peanuts in the clinic. If you experience any adverse effects you will be asked to notify the researchers immediately and to stop taking the peanuts.

All procedures will be carried out by qualified personnel. However, to assist you in making an informed decision, the risks associated with procedures are set out below:

DEXA Scans
Your level of body fat will be determined by a DEXA scan. In this project you will undergo a DEXA scan on 2 occasions. DEXA machines use X-rays, a form of ionising radiation, to create an image of the body. Each DEXA scan will expose you to a very low dose of radiation at a level posing negligible risk to health. Everyone is exposed to naturally occurring background radiation. The total radiation dose you will receive from the DEXA scans is about 1/10,000th of

the natural background radiation dose that you will receive in Australia in a year. The dose of radiation in this study meets the conditions of the University's Ionising Radiation Policy. More information is available on request from the study co-ordinator.

Blood Sampling
Blood samples will be taken by venepuncture. The associated risks are:
- *Infection* - although all of the needles will be sterile and all reasonable precautions will be taken, in any situation involving penetration of the skin there is a slight risk of infection.
- *Blood Clotting* - insertion of a needle into a blood vessel involves a risk of a blood clot forming which can travel through the circulation and block a smaller blood vessel somewhere else. However, the danger of this occurring is considered to be remote.
- *Bruising* - it is possible that you may experience slight bruising around the area where the needle was inserted. This is nothing to worry about as any such bruising should clear up within a few days. Blood thinning agents, such as aspirin and ginko, should not be taken three days prior to sampling.

Information Collection and Confidentiality
All information collected as part of the study will remain confidential and no information which could lead to identification of any individual will be released. Data will be recorded on data sheets and then transferred to electronic data files. Some information may be retained for future analysis. All hardcopies and electronic copies of data will be stored in a secure data storage room within the Nutritional Physiology Research Centre at the University until the research has been published, and will then be stored in the University's archive before being destroyed after 15 years.

What are the Benefits of Participating in the Study?
You will be providing a useful contribution to the scientific knowledge in this field. Individual results and a summary of the group results will be mailed to volunteers on completion of the study. You will be provided with a set of scales (to keep on completion of the study) and a supply of peanuts for 12 weeks during the study. On completion you will be provided with an honorarium of $200 to compensate for your time and expenses. Participants may not personally benefit by participating in the study.

Participation in this study is voluntary, and you may withdraw from the study at any time without prejudice. If you would like to participate in the study, or if you, or any member of your family, require more information to help you arrive at a decision, please contact:
Jayne Barbour; Tel: (08) 83022348, Email: jayne.barbour@unisa.edu.au

This project has been reviewed by the University of South Australia's Human Research Ethics Committee. If you have any ethical concerns about the research or questions about your rights as a participant please contact the Executive Officer of this Committee: Ms Vicki Allen;
Tel: (08) 8302 3118; Email: Vicki.allen@unisa.edu.au.

B.6 Consent form satiety study

University of South Australia | Nutritional Physiology Research Centre

University of South Australia

PROJECT CONSENT FORM

PROJECT TITLE: The Effect of Peanuts on Appetite and Feeling of Fullness

Ms Jayne Barbour	jayne.barbour@unisa.edu.au
Dr Alison Coates	alison.coates@unisa.edu.au
Prof Peter Howe	peter.howe@unisa.edu.au
Assoc Prof Jon Buckley	jon.buckley@unisa.edu.au
Dr Janet Bryan	janet.bryan@unisa.edu.au

1. I have read the Information Sheet, and the nature and the purpose of the research project and the risks inherent in my participation have been explained to me. I understand and agree to take part.

2. I confirm that I have volunteered all health and medical information to the researchers for determination of my eligibility into this trial and ensure my safety and wellbeing.

3. I understand that while information gained during the study may be published, I will not be identified and my personal results will remain confidential.

4. I understand that this study is voluntary and I can withdraw from the study at any stage and that this will not affect my rights or the responsibilities of the researchers in any respect.

5. I have had the opportunity to discuss taking part in this study with a family member or friend or a GP.

6. I understand that I will receive a peanut hamper as a thank you for my assistance in this research project upon completion of the study.

7. I confirm that I am over 18 years of age.

Name of participant .....

Signed .....

Date .....

I have explained the study to the participant and consider that he/she understands what is involved.

Signed .....

Date .....

B.7 Consent form chronic study

University of South Australia

PROJECT CONSENT FORM

PROJECT TITLE: Do Peanuts Improve Blood Vessel Health, Thinking and Memory

Ms Jayne Barbour	jayne.barbour@unisa.edu.au
Dr Alison Coates	alison.coates@unisa.edu.au
Prof Peter Howe	peter.howe@unisa.edu.au
Assoc Prof Jon Buckley	jon.buckley@unisa.edu.au
Dr Janet Bryan	janet.bryan@unisa.edu.au

1. I have read the Information Sheet, and the nature and the purpose of the research project and the risks inherent in my participation have been explained to me. I understand and agree to take part.

2. I confirm that I have volunteered all health and medical information to the researchers for determination of my eligibility into this trial and ensure my safety and wellbeing.

3. I understand that while information gained during the study may be published, I will not be identified and my personal results will remain confidential.

4. I understand that this study is voluntary and I can withdraw from the study at any stage and that this will not affect my rights or the responsibilities of the researchers in any respect.

5. I have had the opportunity to discuss taking part in this study with a family member or friend or a GP.

6. I understand that I will receive a peanut hamper as a thank you for my assistance in this research project upon completion of the study.

7. I confirm that I am over 18 years of age.

Name of participant .....

Signed .....

Date .....

I have explained the study to the participant and consider that he/she understands what is involved.

Signed .....

Date .....

B.8 Three Factor eating questionnaire satiety and chronic studies (to screen for restrained eating)

Participants with a restrained eating score of ≤ 12 were eligible for **Study 1** and **2** (Stunkard & Messick 1985).

THREE-FACTOR EATING QUESTIONNAIRE
Part I

Please circle the following True (T) or False (F)

1. When I smell a sizzling steak or see a juicy piece of meat, I find it very difficult to keep from eating, even if I have just finished a meal.		
2. I usually eat too much at social occasions, like parties and picnics.		
3. I am usually so hungry that I eat more than three times a day.		
4. When I have eaten my quota of calories, I am usually good about not eating any more.		
5. Dieting is so hard for me because I just get too hungry.		
6. I deliberately take small helpings as a means of controlling my weight.		
7. Sometimes things just taste so good that I keep on eating even when I am no longer hungry.		
8. Since I am often hungry, I sometimes wish that while I am eating, an expert would tell me that I have had enough or that I can have something more to eat.		
9. When I feel anxious, I find myself eating.		
10. Life is too short to worry about dieting.		
11. Since my weight goes up and down, I have gone on reducing diets more than once.		
12. I often feel so hungry that I just have to eat something.		
13. When I am with someone who is overeating, I usually overeat too.		
14. I have a pretty good idea of the number of calories in common food.		
15. Sometimes when I start eating, I just can't seem to stop.		
16. It is not difficult for me to leave something on my plate.		
17. At certain times of the day, I get hungry because I have got used to eating then.		
18. While on a diet, if I eat food that is not allowed, I consciously eat less for a period of time to make up for it.		
19. Being with someone who is eating often makes me hungry enough to eat also.		
20. When I feel blue, I often overeat.		
21. I enjoy eating too much to spoil it by counting calories or watching my weight.		
22. When I see a real delicacy, I often get so hungry that I have to eat right away.		
23. I often stop eating when I am not really full as a conscious means of limiting the amount that I eat.		
24. I get so hungry that my stomach often seems like a bottomless pit.		
25. My weight has hardly changed at all in the last ten years.		
26. I am always hungry so it is hard for me to stop eating before I finish the food on my plate.		
27. When I feel lonely, I console myself by eating		
28. I consciously hold back at meals in order not to gain weight.		
29. I sometimes get very hungry late in the evening or at night.		
30. I eat anything I want, any time I want.		
31. Without even thinking about it, I take a long time to eat.		
32. I count calories as a conscious means of controlling my weight.		
33. I do not eat some foods because they make me fat.		

34. I am always hungry enough to eat at any time. 35. I pay a great deal of attention to changes in my figure. 36. While on a diet, if I eat a food that is not allowed, I often then splurge and eat other high calorie foods.		

Part II

Please answer the following questions by circling the number above the response that is appropriate to you.

37. How often are you dieting in a conscious effort to control your weight?

1	2	3	4
rarely	sometimes	usually	always

38. Would a weight fluctuation of 2kg affect the way you live your life?

1	2	3	4
not at all	slightly	moderately	very much

39. How often do you feel hungry?

1	2	3	4
only at mealtimes	sometimes between meals	often between meals	almost always

40. Do your feelings of guilt about overeating help you to control your food intake?

1	2	3	4
never	rarely	often	always

41. How difficult would it be for you to stop eating halfway through dinner and not eat for the next four hours?

1	2	3	4
easy	slightly difficult	moderately difficult	very difficult

42. How conscious are you of what you are eating?

1	2	3	4
not at all	slightly	moderately	extremely

43. How frequently do you avoid 'stocking up' on tempting foods?

1	2	3	4
unlikely	slightly unlikely	moderately likely	very likely

44. How likely are you to shop for low calorie foods?

1	2	3	4
unlikely	slightly unlikely	moderately likely	very likely

45. Do you eat sensibly in front of others and splurge alone?

1	2	3	4
never	rarely	often	always

46. How likely are you to consciously eat slowly in order to cut down on how much YOU eat?

1	2	3	4
unlikely	slightly likely	moderately likely	very likely

47. How frequently do you skip dessert because you are no longer hungry?

1	2	3	4
almost never	seldom	at least once a week	almost every day

48. How likely are you to consciously eat less than you want?

1	2	3	4
unlikely	slightly likely	moderately likely	very likely

49. Do you go on eating binges though you are not hungry?

1	2	3	4
never	rarely	sometimes	at least once/wk

Part III

50. On a scale of 0 to 5, where 0 means no restraint in eating (eating whatever you want, whenever you want it) and 5 means total restraint (constantly limiting food intake and never 'giving in'), what number would you give yourself ?

0
eat whatever you want, whenever you want it
1
usually eat whatever you want, whenever you want it
2
often eat whatever you want, whenever you want it
3
often limit food intake, but often 'give in'
4
usually limit food intake, rarely 'give in'
5
constantly limiting food intake, never 'giving in'

51. To what extent does this statement describe your eating behaviour ? 'I start dieting in the morning but because of any number of things that happen during the day, by evening I have given up and eat what I want, promising myself to start dieting again tomorrow.'

1	2	3	4
not like me	little like me	pretty good description of me	describes me perfectly

B.9 Physical activity diary (satiety and chronic studies)

(Bouchard et al. 1983)

Physical Activity Record Guidelines

1. For three days following your visit, please record all your activities (including the time you are sleeping) for the whole 24 hours.

There is a separate page for each day of the week

2. Fill in the physical activity record at each meal so that you don't forget any activities completed during the previous few hours.

3. In the table provided there is a box for each 15-min period of the day.

See example on the opposite page.

4. Write in the box provided the category code (from 1-9) which best corresponds to the main activity of each 15-minute period using category table to establish the proper coding (see next page for table of category codes)

5. If you are unable to categorise the activity, please mark the period with an **X** and provide further information regarding the activity in the space provided:

- Type: description of the activity
- Purpose: reason for engaging in the activity (i.e. occupation, household duties, recreation)
- Intensity: perceived effort (light, moderate, or vigorous)

(See example on the opposite page for a completed activity record for one day)

Instructions

Write in the spaces provided below the category code (1-9) which correspond best to the main activity of each 15-minute period.
Please, refer to the activity listing below to establish the proper coding.

Category code	Example of activities
1	**Sleeping,** resting in bed
2	**Sitting:** eating, listening, writing, computer, watching t.v., sexual activity, knitting/stitching/sewing
3	**Light activity standing:** washing, shaving, combing, grooming, cooking, playing instrument
4	Slow walk (less than 4 km/h), driving, dressing, showering, shopping
5	**Light manual work:** floor sweeping, window/car washing, driving a truck, painting, waiting on tables, nursing chores, house work, electrician, barman, light gardening (picking fruit/veg/flowers/tidying yard), child care, walking at 4 to 6 km/h, and walking carrying objects **Light recreational activities:** fishing, darts, billiards, bowls, croquet, sports coaching
6	**Leisure activities and sport in a recreational environment**: baseball, golf, volleyball, canoeing or rowing, archery, cycling (less than 10 km/h), table tennis, dancing (ballroom/modern/ballet), tai chi
7	**Manual work at moderate pace:** mining, carpentry, house building (repair and maintenance), shovelling, loading and unloading goods/boxes (up to 20 kgs), moving furniture, gardening (mowing lawn, planting, weeding, trimming, digging, cutting)
8	**Leisure and sport activities of higher intensity (not formal competition):** canoeing (5 to 8 km/h), bicycling (10-15 km/h), dancing (fast/aerobic), badminton, gymnastics, swimming, tennis, horse riding, walking (greater than 6 km/h), vigorous weight lifting (free-weight, nautilus, or universal-type)
9	**Intense manual work, high intensity sport activities/competition:** carrying heavy loads (greater than 20 kgs), jogging/running (greater than 9 km/h), racquetball, squash, badminton, swimming, tennis, hiking and mountain climbing, martial arts

If you are unable to categorise the activity, please mark the period with an **X** and provide further information regarding the activity in the space provided:

- **Type**: description of the activity
- **Purpose**: reason for engaging in the activity (i.e. occupation, household, recreation)
- **Intensity**: perceived effort (light, moderate, or vigorous)

Record Day 1 Date: _____ Day of the Week_____

Hour	Minutes				Other activities marked with X		
	0-15	16-30	31-45	46-60	type	purpose	intensity
12 am							
1 am							
2 am							
3 am							
4 am							
5 am							
6 am							
7 am							
8 am							
9 am							
10 am							
11 am							
12 pm							
1 pm							
2 pm							
3 pm							
4 pm							
5 pm							
6 pm							
7 pm							
8 pm							
9 pm							
10 pm							
11 pm							

Other comments:

B10. Weighed Food record (satiety and chronic studies)

Weighed Food Record Instructions

ID:

VISIT:

To allow us to assess your dietary intake we will need you to keep a record of everything you eat and drink for four days prior to your visit. It is important that these days be as normal as possible. Please do not change any eating patterns on the days that you record. Remember we are only interested in your usual dietary intake, not the perfect diet!

If you have any question regarding how to complete the food record, please do not hesitate to contact the study team on **83022348**

General Guidelines:

- Please keep the diary for 4 non-consecutive alternate days prior to your visit (i.e. over a period of one week) include 1 weekend day.
- Write down all food and drinks eaten both at home and away from home and include all vitamins, minerals or other supplements taken.
- Beverages of all kinds, including water and alcohol must be recorded. For juices and cordials please record how these were made (strength).
- For packaged foods record the brand name and the weight or volume. Attach product labels with your record if possible, this is very useful.
- For homemade foods please provide the recipe with all the ingredients and number of serves made and the portion or fraction of the recipe you ate
- Record amounts (in grams) as accurately as possible using the scales provided.
- Remember to record the amounts of left overs as well.
- If you are out and unable to measure the food, describe what is eaten as fully as possible and make the best guess you can for the quantity.

- If you do not eat a particular meal or snack, simply draw a line across thepage at this point, to show that you have definitely not eaten anything for this meal.

Guidelines for Describing Foods and Beverages:

- Remember to include any toppings or fillings likes spreads consumed with foods, eg. sauces, dressings, gravy, stuffing, sugar, pesto, margarine, syrups
- When describing foods, provide as many details as possible, for example: cooking methods (eg. fried, steamed, grilled, roasted, poached, boiled), regular or low fat (eg. skim or full cream milk, Diet lite yoghurt, Flora Light Margarine, Salt reduced tomato paste, coke zero),describe if the food has been trimmed of all fat or skin removed (eg. skinless chicken breast, bacon, trimmed)

Guidelines for Describing the Quantity of Foods and Beverages:

Household Measurements – **For foods such as vegetables, cereals and canned fruits, a household measurement (eg. tsp, cup) is adequate. However, state whether the spoons are level, rounded or heaped eg. 1 level cup of cooked rice**

Weight on packaging – **the weights on packaging is a convenient way to determine the amount eaten eg. half of a can of 425g baked beans.**

Bread – **indicate the size of the slice (eg. sandwich, toast slice) and indicate whether it was eaten raw or toasted.**

Cheese, meat and fish – **If possible weigh the portions for these foods. If not possible, please estimate as best you can (use comparisons)**

Use comparisons – **for describing portion sizes by comparing it to objects (eg. potato the same size as an egg, or a square of cheese, the same size as a matchbox)**

DAY ONE

Study ID: Date: Day of the week:

Record ALL food and drink consumed during the date including sweets, snacks, nibbles, sauces and dressings, water and supplements (vitamins).

Please remember to record:

- METHOD OF COOKING (eg. boiled pasta)
- TYPE OF FOOD (eg. wholemeal bread)
- QUANTITY OF FOOD (eg. 6 heaped tsp white sugar or weight (g))
- BRAND NAMES (eg Nestle diet lite) if possible

FOOD CONSUMED	QUANTITY EATEN	DETAILS OF FOOD & DRINK
EARLY MORNING		
BREAKFAST		
DURING MORNING		
MIDDAY		

DAY ONE CONTINUED

Study ID: Date: Day of the week:

FOOD CONSUMED	QUANTITY EATEN	DETAILS OF FOOD & DRINK
DURING AFTERNOON		
EVENING MEAL		
DURING EVENING / SUPPER		

Appendix C Cognitive tests (chronic study)

C.1 DemTect® (Screening test for mild cognitive impairment)

Participants who scored between ≥ 9 points on the DemTect were eligible to continue screening for Study 2 (Kalbe et al. 2004).

Word List

	Apple	Ink	Nail	Bird	Book	Ticket	Tree	Chair	House	Ship
1.	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
2.	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

Correctly remembered words (max.20) ☐

Number conversion

Example 5 - five three – 3

209 = _____

4054 = _____

six hundred and eighty one

two thousand and twenty seven

Correct conversion (max.40) ☐

Supermarket task – 1 minute

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

Named object (max.30) ☐

Series of numbers in reverse

7-2	8-6	☐ 2
4-7-9	3-1-5	☐ 3
5-4-9-6	1-9-7-4	☐ 4
2-7-5-3-6	1-3-5-4-8	☐ 5
8-1-3-5-4-2	4-1-2-7-9-5	☐ 6

Repeat of word list

Apple	Ink	Nail	Bird	Book	Ticket	Tree	Chair	House	Ship
☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

.

Correctly remembered words (max.10) ☐

C.2 RAVLT

The Rey Auditory Verbal Learning Test (RAVLT) (Rey 1964) measures memory. It requires volunteers to recall and recognize lists of 15 words learned over 5 trials, both immediately and after delay.

RAVLT B

Subject ID _____ **VISIT**_____

List A	A1	A2	A3	A4	A5	List B	B1	List A	A6	A7
pipe						bench		pipe		
wall						officer		wall		
alarm						cage		alarm		
sugar						sock		sugar		
student						fridge		student		
mother						cliff		mother		
star						bottle		star		
painting						soap		painting		
bag						sky		bag		
wheat						ship		wheat		
mouth						goat		mouth		
chicken						bullet		chicken		
sound						paper		sound		
door						chapel		door		
stream						crab		stream		
# correct										

Total A1 to A5 = _____ **Intrusions** _____
Trial A6 - A5 = _____

Recognition # targets correctly identified _____

 # distractors correctly identified _____

C.3 Stroop colour word test

For this test participants is required to read the different colour words printed in ink as they are written. Afterwards, the participant is required to *name* the different colours that the words are printed in which are incongruent with the named colour (Dodrill 1978).

yellow red green blue yellow red blue green yellow green blue

yellow green yellow green red yellow red red yellow red yellow

green yellow blue red blue green blue yellow green yellow green

yellow red blue red red yellow red blue green yellow red

blue green red yellow red blue red blue green blue green

green blue red blue red yellow red yellow blue red yellow

blue green red green blue green yellow blue yellow red green

red red blue red green yellow green green green blue yellow

green blue blue green red blue red yellow yellow blue green

green red blue red yellow yellow red red yellow green red

blue blue blue yellow green yellow red yellow green yellow green

blue yellow green yellow blue green red red yellow yellow red

blue blue green red blue red yellow green yellow green green

blue red blue green red blue yellow blue red yellow blue

red yellow red green blue yellow green yellow blue red red

red blue green red yellow blue green yellow red yellow blue

The Stroop Colour-Word Test V-1

C.4 Coding task

This task is a measure of visual-motor coordination, motor and mental speed.

Participants were requested to match numbers with a corresponding symbol completing as many in 120 seconds as possible (Wechsler 2008).

Coding

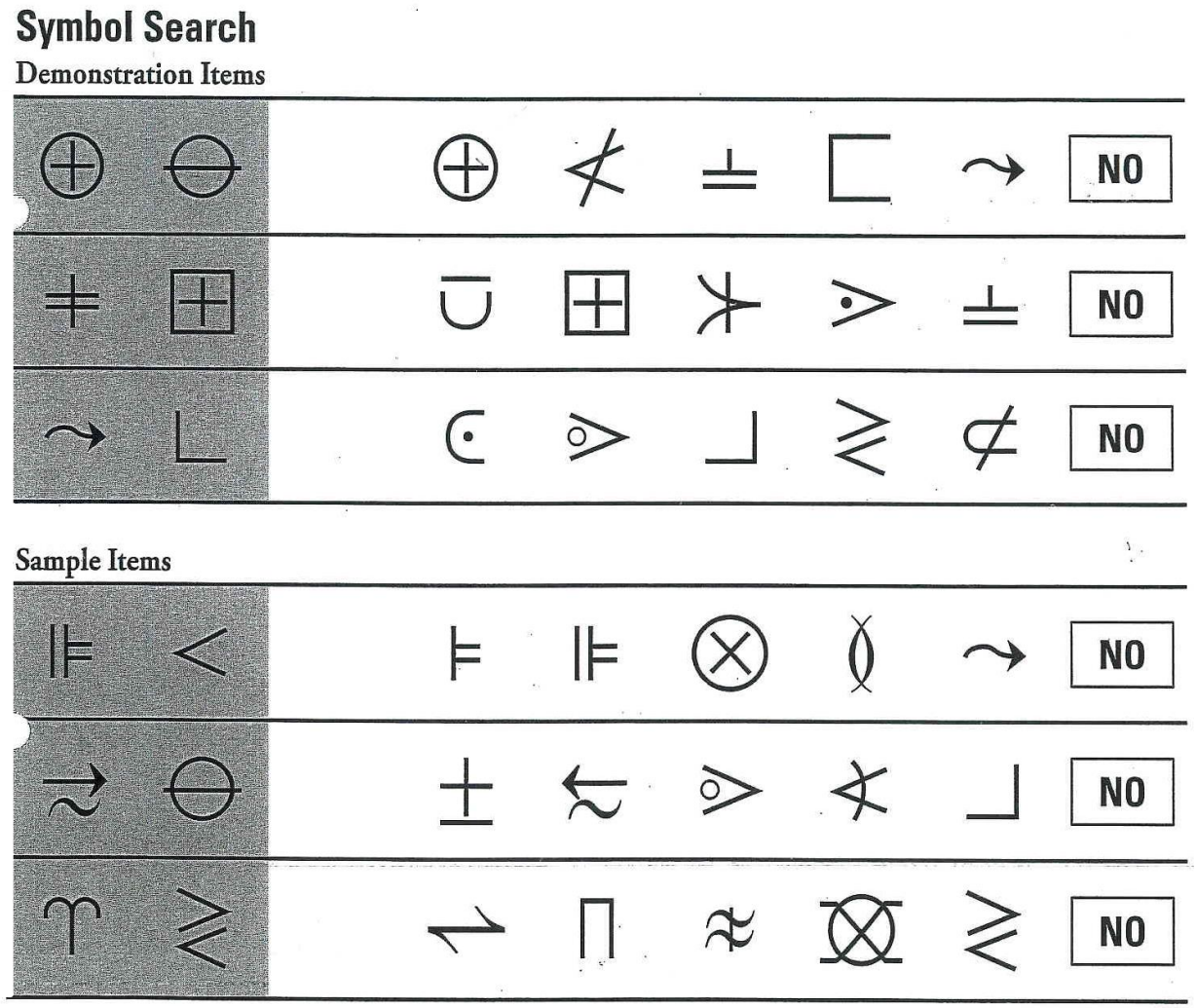


Demo Sample

6	8	3	9	5	4	1	7	2	1	4	8	2	7	6	9	3	5	
8	3	1	9	2	5	6	4	3	7	2	9	8	1	4	7	6	5	
9	1	2	4	7	2	5	6	9	5	8	6	4	3	1	7	8	3	
1	3	9	6	3	9	7	5	1	4	2	8	7	2	8	5	6	4	
7	6	4	1	3	2	8	1	7	9	2	5	3	4	8	6	5	9	
8	1	9	5	1	4	2	6	9	8	7	3	5	6	4	7	2	3	
3	6	8	9	1	8	4	7	5	2	9	6	7	1	5	2	3	4	
6	4	1	9	5	7	3	6	8	3	2	7	5	8	4	2	9	1	

C.5 Symbol search task

The Symbol Search test is designed to assess information processing speed and visual perception; high scores require rapid and accurate processing of nonverbal visual information (i.e., symbols without *a priori* meaning). This is a self-paced task during which participants are allotted 120 seconds to complete as many symbol discrimination items as possible. Each item is presented sequentially as a row. In each row, two geometric symbols are presented to the left and another set of five symbols to the right. If either symbol of the left matched any symbols on the right, participants were required to circle the matching symbol, if there were no matching symbols then the word "NO" was circled (Wechsler 2008).



Appendix D Sequential Bonferroni (chronic study)

Table D1.1 Sequential Bonferroni calculations **Study 2**

(red figures indicate significant values)

Order *	P value	adj p value†	critical value‡	Final p-value¶	Outcome group	Measure	Comment
1	0.001	1.001	0.0454	0.011	Verbal Fluency	Verbal Fluency	(includes excluded letter p=0.004)
2	0.005	1.005	0.045	0.055	Processing Speed	Coding	
3	0.01	1.01	0.045	0.110	Body Composition	Body mass	
4	0.015	1.015	0.045	0.180	Arterial Compliance	SAE	
5	0.09	1.09	0.045	0.990	Memory	RAVLT delay	
6	0.09	1.09	0.045	0.990	Executive Function	Stroop	
7	0.13	1.13	0.046	1.430	Lipids	Triglycerides	
8	0.4	1.4	0.047	4.400	Blood Pressure	SBP	
9	0.41	1.41	0.047	4.510	Insulin	Insulin	
10	0.61	1.61	0.048	6.710	Glucose	Glucose	
11	0.62	1.62	0.048	6.820	CRP	CRP	

Abbreviations: CRP, C-reactive protein, SBP, systolic blood pressure, RAVLT, Rey Auditory Verbal Learning Test, SAE small artery elasticity *lowest to highest p value, †= p value +1, ‡ = 0.5/(11 - (adj p) +1), ¶=11 x critical value,

Appendix E Abbreviations

ACTR	Australian and New Zealand Clinical Trials Registry
ALA	α-linolenic acid
ANZCTR	Australian and New Zealand Clinical Trials Registry
AUC	Area under the curve
BMI	Body mass index
CARDIA	Coronary Artery Risk Development in Young Adults
CBF	Cerebral blood flow
CCK	cholecystokinin
CI	Confidence interval
CVD	cardiovascular disease
CVR	Cerebrovascular reactivity
D	Day
DBP	Diastolic blood pressure
DEXA	Dual-Energy X-Ray absorptiometry
Dm	Diabetes melitis
eNOS	Endothelial derived NO-synthase
FDA	Food and Drug Administration
FFQ	Food frequency questionaire
FFQ	Food frequency questionnaire
FMD	Flow mediated dilatation
G	Gram
GLP-1	Glucagon like peptide -1
HbA1c	Haemoglobin A1c
HDL	High density lipoprotein
HOMA	homeostatic model assessment
hs-CRP	High sensitivity C-reactive protein
ICAM-1	intercellular adhesion molecule-1
Il-6	Interleukin-6
kJ	kilojoule
LAE	Large artery elasticity
LDL	Low density lipoprotein
MCA	Middle cerebral artery
MESA	Multi-Ethnic Study of Atherosclerosis
MUFA	Monounsaturated fatty acid
NHANES	National Health and Nutrition Survey
NO	Nitric oxide
NRV	Nutrient reference range
OO	Olive oil
PCOS	Polycystic ovary syndrome
PP	Pancreatic polypeptide
PREDIMED	PREvencion con DIeta MEDiterranea
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PUFA	Polyunsaturated fatty acid

PYY	Peptide YY
RAVLT	Rey auditory verbal learning test
RCT	Randomised control trial
RR	Relative risk
SAE	Small artery elasticity
SBP	Systolic blood pressure
Sd	Standard deviation
Sem	Standard error of the mean
SFA	Saturated fatty acid
SUN	Seguimiento University of Navarra
TCD	Transcranial Doppler
TNF-α	Tumor necrosis factor
VAS	Visual analogue scales
VCAM-1	vascular cell adhesion molecule-1
WAIS	Wechsler adult intelligence scale