The effect of soya foods on cognitive function and menopausal symptoms in post-menopausal women

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I confirm that the word count of this thesis is less than 100,000 words

To Mammy and Daddy

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For me, a page of thankyous does not credit the support I have received from family and loved ones throughout the past three years.

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DECLARATION

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PEER-REVIEWED ARTICLES, ABSTRACTS AND CONFERENCE PRESENTATIONS

ARTICLES

Furlong, O.N., Parr H.J., H.J, Hodge, S.J, Slevin, M.M., Simpson E.E., McSorley, E.M McCormack, J.M., Magee, P.J (2018). Consumption of a soy drink has no effect on cognitive function but may alleviate vasomotor symptoms in postmenopausal women.

Furlong, O.N., Simpson E.E., McSorley, E.M., McCormack, J.M., Magee, P.J (2018). Soya Isoflavones: effects on cognitive function and menopausal symptoms in post-menopausal women.

Ellen E Simpson , Orlaith N Furlong , Heather J Parr , Stephanie J Hodge , Mary M Slevin , Emeir M McSorley , Jacqueline M McCormack , Pamela J Magee & Christopher McConville (2018). A Randomised Trial to determine the effects of a 12-week soy drink intervention on everyday mood in postmenopausal women.

ABSTRACTS

Nutrition Society Summer Meeting. Furlong, O.N., Parr H.J., H.J, Hodge, S.J, Slevin, M.M., Simpson E.E., McSorley, E.M McCormack, J.M., Magee, P.J., *et al.* (2018) Dietary patterns and cognitive function in early postmenopausal women. *Proceedings of the Nutrition Society.*

Simpson E.E., Parr H.J., Furlong, O.N., McSorley, E.M., McCormack, J.M., Magee, P.J., et al. (2017) Everyday affect, symptoms and well-being in postmenopause: preliminary findings .

CONFERENCE PRESENTATIONS

Dietary intake and associations with cognitive function in early postmenopausal women

February 2017	Nutrition Society 26th Annual Post-graduate Graduate Conference	Dublin Ireland	Oral

Cognitive performance and dietary patterns in early post-menopausal women

June 2018	Nutrition Society Summer Conference:	Coleraine Ireland	Oral

STATEMENT OF COLLABORATION

The research was conducted at the Nutrition Innovation Centre for Food and Health (NICHE), Ulster University, Coleraine and Psychology Research Institute, Ulster University.

The work detailed in this thesis included collaboration with the following authors:

Author	**Institution**
Dr Liz Simpson	Psychology research Institute Coleraine

Chapter 2. The role of soya isoflavones in cognitive function and menopausal symptoms in post-menopausal women.

Authors: Orlaith N Furlong , Ellen E A Simpson , Emeir M Mc Sorley , Jaqueline M McCormack , Pamela J Magee

The following work was conducted by myself: Preparation of the review paper. All authors reviewed draft manuscript.

Chapter 3. Dietary patterns and cognitive function in postmenopausal women

Authors: Orlaith N Furlong, Heather J Parr, Stephanie J Hodge, Mary M Slevin, , Ellen E Simpson, Emeir M McSorley, Jacqueline M McCormack, Pamela J Magee.

The following work was conducted by the research team, O. Furlong, Dr H. Parr, Dr M. S. Slevin, Dr S. Hodge: Data collection, blood analyses, recruitment. All authors reviewed draft manuscripts prior to publication.

Chapter 4. Consumption of a soy drink has no effect on cognitive function but may alleviate vasomotor symptoms in postmenopausal women.

Authors: Orlaith N Furlong, Heather J Parr, Stephanie J Hodge, Mary M Slevin, , Ellen E Simpson, Emeir M McSorley, Jacqueline M McCormack, Pamela J Magee.

The following work was conducted by the research team, O.Furlong, Dr H. Parr, Dr M. S. Slevin, Dr S. Hodge: Data collection, blood analyses, recruitment. All authors reviewed draft manuscripts prior to publication.

Chapter 5. Attitudes of post-menopausal women towards soya food consumption following an intervention study.

Authors: Furlong O.N, Magee P.J, McSorley E.M, Ellen E Simpson

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ABSTRACT

The menopause is a natural transition that occurs at midlife for a woman and is accompanied by a number of symptoms which can significantly affect quality of life, the most commonly reported being hot flushes. Impaired cognitive function has also been observed in menopausal women and natural alternatives to hormone therapy for the alleviation of menopausal symptoms are being sought. The aim of this thesis was to investigate the effects of soya isoflavones on cognitive function and menopausal symptoms in post-menopausal women.

In an observational study, food diaries were completed by post-menopausal women (*n*=75). Principal component analysis was used to determine dietary patterns and associations with cognitive function, as assessed by CANTAB, were investigated. 5 final dietary patterns were identified. The 'meat and dairy' dietary pattern was associated with greater performance in the spatial working memory cognitive test. 'The social' dietary pattern was associated with negative effects on cognitive performance as was the 'potatoes and poultry' dietary pattern.

A randomised controlled trial in post-menopausal women (*n*=101) was conducted to investigate the effects of soya isoflavones on cognitive function and menopausal symptoms. Participants consumed a volume of soya drink providing a low (10 mg; control group), medium (35 mg) or high (60 mg) dose of soya isoflavones daily for a period of 12 weeks and cognitive function was assessed using CANTAB (spatial working memory, spatial span, pattern recognition memory, 5-choice reaction time and match to sample visual search). Menopausal symptoms were also assessed using a validated questionnaire. Isoflavone supplementation had no effect on the primary outcome measure, cognitive function, although consumption of 350ml of soya drink/day (providing 35mg isoflavones) significantly reduced hot flushes in post-menopausal women with more severe symptoms at baseline.

An observational study was conducted using focus groups to determine overall perceptions towards soya in post-menopausal women and to partly evaluate the soya intervention. Post-menopausal women reported barriers and enablers of consumption as well as attitudes towards soya products in an Irish and Northern Irish population. The main findings from the focus groups include the awareness of soya in the diet and also the benefits that can be attained from consuming soya. Main barriers to soya consumption include price and lack of knowledge about soya products while enablers of consumption include Alpro® being considered a reliable and good brand.

This thesis questions the potential benefits of soya isoflavones on cognitive function in healthy post-menopausal women within 7 years post-menopause. Future studies should

investigate effects in earlier post-menopausal women and/or in those with mild cognitive impairment. Soya isoflavone consumption in post-menopausal women had no effect ocognitive function although may alleviate vasomotor symptoms in those with more severe symptoms at baseline. With the potential risks associated with hormone therapy, soya isoflavones may be a natural alternative for the alleviation of vasomotor symptoms.

ABBREVIATIONS

ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
BMI	Body mass index
BMR	Basal metabolic rate
CANTAB	Cambridge neuropsychological automated test battery
CONSORT	Consolidated Standards of Reporting Trials
DASH	Dietary Approaches to Stop Hypertension
DB,PMP	Double blind
DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic acid
Erα	Oestrogen receptor
ERβ	Oestrogen receptor
FSH	Follicle stimulating hormone
HT	Hormone therapy
IF	Isoflavone
LC-MS/MS	Liquid chromatography tandem mass spectrometry
LH	Luteinizing hormone
LMP	Last menstrual period
MeDi	Mediterranean diet
MIND	Mediterranean-DASH Diet Intervention for Neurodegenerative Delay
MMSE	Mini mental state exam
MTS	Match to sample
MUFA	Monounsaturated
PA	Physical activity

PMW	Post-menopausal women
PRM	Pattern recognition memory
PUFA	Polyunsaturated fatty acid
RDBP	Randomised double blind placebo
RDBP,CO	Randomised double-blind placebo, cross over
RDBP,P	Randomised double blind parallel, placebo
RTI	Reaction time
SERMS	Selective oestrogen receptor modulators
SSP	Spatial span
SWM	Spatial working memory
VMS	Vasomotor symptoms

CHAPTER 1

General introduction with aims and objectives

The Menopause

Previous research has highlighted the importance of oestrogen for normal cognitive processing. The menopause is characterised by the permanent cessation of menses via the depletion of oestrogen levels (Freeman and Sherif, 2007) and is defined as 12 months of amenorrhea associated with a natural decline in ovarian hormone secretion (Freeman and Sherif, 2007). The average age of the menopause differs vastly among women globally (Gold, 2011). As oestrogen levels progressively decline from the onset of the menopause, women experience a number of symptoms which vary in severity and frequency on an individual basis (Bacon, 2017). These symptoms include vasomotor symptoms, more commonly described as hot flushes and night sweats, affecting up to 74% of women in Europe (Archer et al., 2011). Vasomotor symptoms can severely interfere with daily activities including sleep disturbances and are one of the most common symptoms experienced by menopausal women (Nelson et al., 2005). In addition, headaches, and memory problems are often reported, with heightened symptoms occurring <2years prior to and after cessation of the menstrual cycle, although in some women symptoms may continue for several years (Shifren et al., 2014). Due to the persistence of hot flushes during the menopause, treatment in the form of hormone therapy (HT) is often sought to alleviate symptoms. HT is still the most effective treatment for menopausal symptoms, particularly hot flushes (NAMS, 2017, NICE, 2015) albeit there are risks associated with HT use for some women. Women who commence treatment above the age of 60 years, or are more than 10-20 years since menopause onset, are at increased risk of coronary heart disease, stroke, venous thrombosis, and dementia (NAMS, 2017). Furthermore, HT may not be an option for some women due to contraindications e.g. family history of venous thromboembolism (Armeni et al., 2016). Despite HT being a common treatment, some women refrain from use due to these associated side effects. Alternative approaches for the treatment of menopausal symptoms are therefore needed.

Menopause and Cognitive Function

Memory problems are a common complaint in post-menopausal women (Bojar et al., 2015a). During the menopause, the intensity and duration of symptoms can have an effect on cognitive function and progressive declines in episodic memory, working memory and executive function have been observed (Bojar et al., 2015a). Following the menopause, cognitive function is affected potentially owing to the reduction in endogenous oestrogen levels characteristic of this time. Oestrogen is essential for normal brain functioning due to the localisation of oestrogen receptors within the brain. Oestrogen exerts neuroprotective effects on brain regions responsible for working memory and learning, including the hippocampus, hypothalamus, cerebral cortex and pre-frontal cortex

(Fournier et al., 2007, Shaywitz et al., 1999, Spencer et al., 2008). A marked increase in hippocampal grey matter in post-menopausal women was reported in a short-term oestradiol supplementation trial, a finding potentially owing to the abundance of oestrogen receptors in this brain region (Albert et al., 2017).

Soya isoflavones

Soya isoflavones show promise as dietary compounds that may illicit beneficial effects on cognitive function, particularly in post-menopausal women (Cheng et al., 2015). Owing to the structural similarity to 17β-oestradiol (Figure 1), isoflavones have the ability to bind to oestrogen receptors located within the brain exerting oestrogenic/antiestrogenic effects. Isoflavones are classified as selective oestrogen receptor modulators (SERMS), having a higher binding affinity for ER-β than ER-α (Kostelac et al., 2003, Kuiper et al., 1998). Isoflavones bind to the ER much more weakly than oestrogen (Setchell et al., 2005a) and, in addition to their oestrogenic effects, also exert physiological effects that are ER-independent. Soya foods are a unique rich source of isoflavones. The primary soya isoflavones, genistein, daidzein and glycitein represent 50,40 and 10% of total isoflavone content respectively (Messina, 2016). Owing to similarity in structure to 17β- oestradiol, the most abundant circulating female hormone, soya isoflavones mimic many of the physiological actions of oestrogens. Dietary isoflavone intake is approximately 25-50mg/day in Japanese populations but is <3mg/day in European and western populations (Messina et al., 2006). It has been postulated that the lower incidence of menopausal symptoms observed in Japanese populations is attributable to soya consumption and genetic factors (Adlercreutz et al., 1992).

Equol is an isoflavan produced exclusively through the conversion of daidzein in the intestine and is only produced by 30% of western populations (Lampe et al., 1998, Rowland et al., 2000, Setchell and Cole, 2006). In contrast, in Japan, Korea and China, 80% of individuals have the ability to convert daidzein to equol. (Morton et al., 2002, Fujimoto et al., 2008). Equol (7-hydroxy-3-(4,-hydroxyphenyl)-chroman) exists as two isomers, R-(+) equol and S-(-) equol due to its chiral centre. It has been demonstrated that humans produce the S-equol isomer via the bacterial conversion of daidzein following soya consumption (Setchell et al., 2005b). Inter-individual variation in the production of equol has been attributed to differences in genetics, age and gut microflora (Frankenfeld et al., 2004, Roosendaal et al., 1997). It has been postulated that the beneficial effects of soya isoflavones are dependent on equol producer status, a theory known as the “equol hypothesis” (Setchell et al., 2002, Magee, 2011).

Soya Isoflavones and cognitive function

Soya isoflavones may have beneficial effects on cognitive function, predominantly in areas of the brain sensitive to circulating endogenous oestrogen, including those responsible for memory and frontal lobe function (File et al., 2005, Soni et al., 2014, Cheng et al., 2015, Zaw et al., 2017). Isoflavones can cross the blood brain barrier, (Chang et al., 2000, Gu et al., 2005) and influence, areas of the brain susceptible to age-related cognitive decline such as the hippocampus and prefrontal cortex, (Cheng et al., 2015, Kritz-Silverstein et al., 2003) potentially via interaction with oestrogen receptors that are localized within these brain regions (Albert et al., 2017). To date, ten randomized controlled trials have investigated the effects of soya/isoflavones on cognitive function exclusively in post-menopausal women, with only over half of the studies showing beneficial effects (reviewed in Zaw et al., 2017). Contrasting findings are likely due to the wide variation in study design. Well-designed randomised controlled trials that account for covariates such as cognitive measure, age, supplement type/dose, years since menopause and severity of symptoms, are necessary in order to accurately determine the role of soya isoflavones on cognition in post-menopausal women. The ability to produce equol has been postulated to play a role in aiding cognitive function, although evidence in this area is limited (Gleason et al., 2009, Henderson et al., 2012). Equol has the ability to increase cerebral blood flow potentially exerting beneficial effects on cognition (Yu et al., 2016). In a cross-sectional study of older adults, equol producers performed significantly better in tests of cognitive function in comparison to equol non-producers (Igase et al., 2017). Further research is required in this area exclusively investigating the effects of equol producer status on cognitive function as a primary outcome measure to allow firm conclusions to be made.

Soya isoflavones and hot flushes

Numerous studies have investigated the effect of soya isoflavones, in both food and supplement form (Imhof et al., 2018, Tranche et al., 2016), on hot flushes although evidence is inconclusive. Hot flushes are thought to be the consequence of the reduction in oestrogen in post-menopausal women. In a systematic review and meta-analysis, Taku et al., demonstrated that soya isoflavone supplements significantly reduced hot flush frequency and severity in comparison to placebo, with supplements providing more than 18.8 mg of genistein being twice as potent as lower genistein supplements (Taku et al., 2012). A Cochrane review has also reported that high levels of genistein consistently reduce hot flush frequency (Lethaby et al., 2013). The efficacy of isoflavones on hot flushes may be dependent on the severity of symptoms at baseline and on the duration of supplementation. A recent randomised controlled trial reported modest but significant

attenuation in hot flush frequency with 12 weeks supplementation of 100mg isoflavone glycosides, with better effects observed in women with 'severe' symptoms at baseline (Imhof et al., 2018). In addition, a model based meta-analysis (Li et al., 2015) indicated slight but slow effects in attenuating menopausal hot flushes with isoflavone supplementation compared to oestradiol, reporting a 25% decrease in hot flush frequency with a supplementation period of >13.4 weeks required to reach maximal effect. For women that refrain from using HT, isoflavones thus offer an alternative for the alleviation of hot flushes.

Beneficial effects of equol on vasomotor symptoms have also been reported within the literature. The ability to produce equol has been associated with reduced severity in menopausal symptoms. In one observational study, Japanese equol producers reported less severe vasomotor symptoms (Uchyama, 2001). After 12 weeks supplementation with S-equol, a significant improvement in hot flush frequency and other symptoms was reported in a cohort of equol non-producing post-menopausal women (Aso et al., 2012). S-equol derivatives of soya isoflavones are recommended by the North American Menopause Society (NAMS) for the non-hormonal management of vasomotor symptoms, albeit NAMS highlight the need for further research in this area (North American Menopause, 2010).

Diet and cognitive function

Poor dietary intake has been reported in post-menopausal women (Bojar et al., 2015b). Dietary intake has not been directly investigated in relation to cognitive performance in post- menopausal women to date, although there is clear evidence linking several key nutrients and cognitive processes in the aging population. Cognitive studies have mainly focused on individual nutrients such as omega-3 fatty acids (n-3FA) (Gillette-Guyonnet et al., 2013), B-vitamins (Porter et al., 2016), antioxidants (Bowman et al., 2012, Gillette-Guyonnet et al., 2007, Kesse-Guyot et al., 2011, McDaniel et al., 2003), vitamin D (Annweiler and Beauchet, 2013) , zinc (Maylor et al., 2006), and protein (Roberts et al., 2012, Goodwin et al., 1983). Dietary patterns have been investigated in post-menopausal women and associations with sarcopenia (Kaser et al., 2017), obesity (Papavagelis et al., 2018), bone mineral density (Hardcastle et al., 2011, Karamati et al., 2012, Sugiura et al., 2011), cardiovascular disease risk (Papavagelis et al., 2018), and metabolic syndrome (Silva et al., 2015); although few have investigated the link with cognitive function. Nonetheless, the Mediterranean (MeDi), Dietary Approaches to Stop Hypertension (DASH) (Pistollato et al., 2018), and Mediterranean-DASH Intervention for Neurodegenerative Delay (MIND) (Berendsen et al., 2018, Morris et al., 2015) diets are

associated with favourable effects on cognition. Limited data is available in post-menopausal women.

Omega-3 and omega-6 fatty acids (Cooper et al., 2015), folate, vitamin B6 and vitamin B12 (Forbes et al., 2015) are highly reviewed in association with cognitive health (Cooper et al., 2015). Long term supplementation of these vitamins in randomised trials has demonstrated favourable effects on cognitive performance, although these studies primarily focus on elderly populations with little focus in post-menopausal women (de Jager et al., 2012, Douaud et al., 2013, Durga et al., 2007, Smith et al., 2010). Dietary intake in post-menopausal women should be investigated to determine dietary patterns which could illicit beneficial effects in terms of cognitive function.

Consumer attitudes towards soya

As popularity of soya has risen there has been an increased interest in research, moreover favourable effects have been reported in cardiovascular health, cancer and menopausal relief (Messina, 2003, Messina, 2014). As there is clear evidence to suggest the various health benefits associated with the addition of soya to the diet, further investigation into consumer attitudes and opinions of soya is imperative in populations who may benefit from its use. One qualitative method that can be used to collect such information from the public is via focus groups using thematic analysis. In a study investigating attitudes and beliefs towards soya consumption, participants had a healthy perception associated with soya, although were unable to explain why they have this opinion (Schyver and Smith, 2005). Another focus group study has echoed this perception (Tu et al., 2012). These findings highlight a lack of knowledge among the public in relation to the health benefits of soya food consumption. Further research is thus required in this area.

AIMS AND OBJECTIVES

The primary aim of this thesis was to investigate the effects of soya isoflavones on cognitive function and menopausal symptoms in post-menopausal women. Secondary aims were to investigate associations between diet and cognitive function and attitudes towards soya consumption.

Objectives of this thesis are as follows:

1. To critically review the existing literature investigating the effects of soya/isoflavones on cognitive function and hot flushes in post-menopausal women encompassing potential mechanisms (Chapter 2).

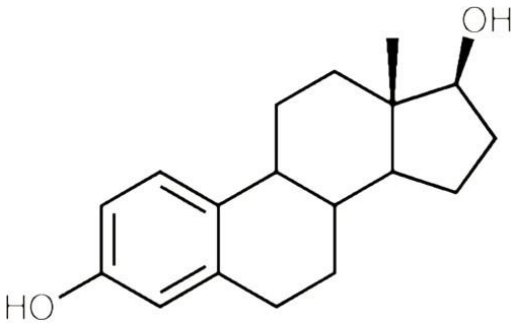
2. To determine if dietary intake in post-menopausal women is associated with cognitive function (Chapter 3).

3. To determine if consumption of a soya drink impacts cognitive function and menopausal symptoms in post-menopausal women (Chapter 4).

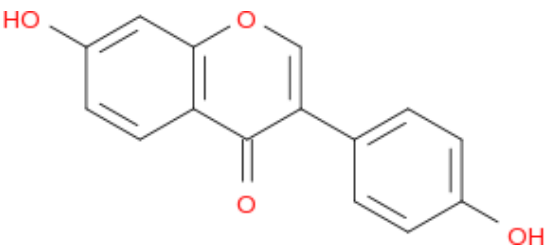
4. To investigate consumer perceptions of soya foods in relation to post-menopausal health and factors influencing soya consumption (Chapter 5).

5. Discuss implications of these studies for future research in this area (Chapter 6).

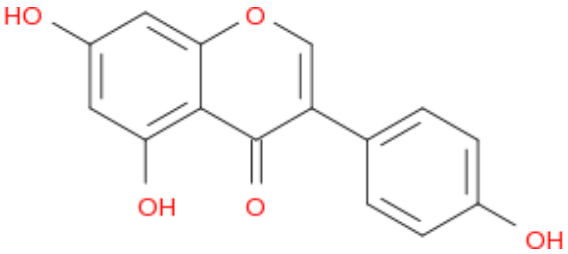
Figure 1: Structure of the female hormone oestradiol and the soya isoflavones genistein and daidzein.



Oestradiol



Daidzein



Genistein

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CHAPTER 2

Soya Isoflavones: effects on cognitive function and menopausal symptoms in post-menopausal women

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Abstract

Hormone fluctuations in women at the time of the menopause can contribute to health-related changes especially in the years closest to the menopausal transition. This decline in oestrogen concentrations can contribute to menopausal symptoms including impairment in cognition, especially memory problems. Isoflavones have been demonstrated to illicit beneficial effects on cognitive domains as well as relief from menopausal vasomotor symptoms. The aim of this review is to determine the effects of soya foods/isoflavones on cognitive function and menopausal symptoms in postmenopausal women. While the focus of the review is on cognitive function, effects on hot flushes will be summarised. Literature demonstrating the effects of isoflavones on cognitive function remains conflicting with over half the studies published in this area showing positive, medium effects on cognitive domains. Greater effects are observed in those who are <10years from their menopausal transition and severity of symptoms at this time may be the cofounding factor.

Summary findings conclude limitations around the literature when summarizing outcome findings, more robust methodology is required in future studies.

Key words: vasomotor symptoms, cognition, isoflavones

Introduction

For the majority of women, the menopause is a natural process that occurs due to ovarian ageing. Menopause is defined as 12 months of amenorrhea and is associated with a natural decline in ovarian hormone secretion (Freeman and Sherif, 2007); as ovarian production of oestradiol diminishes, there is a marked increase in follicle stimulating hormone (FSH) which is used clinically in classifying menopausal status (Bacon, 2017, Shifren et al., 2014). The mean age at which the menopause occurs within the UK population is 51 years (NICE, 2015) albeit it can occur much earlier than this (early/premature menopause) or be induced by medical intervention (Armeni et al., 2016). During the menopausal transition many women experience symptoms which vary widely in severity and incidence on an individual basis. Hot flushes and night sweats are the most commonly reported vasomotor symptoms experienced during menopause, affecting up to 74% of women in Europe (Archer et al., 2011). Other symptoms include headache, sleep disturbance, joint and muscle pain, effects on mood, urogenital symptoms, sexual difficulties and memory problems (Bacon, 2017, NICE, 2015). As endogenous levels of oestrogen progressively decrease with the onset of the menopause a change in severity of symptoms may be observed (Shaywitz et al., 1999, Spencer et al., 2008). Heightened severity of symptoms cluster in the 2 years prior to and after cessation of the menstrual cycle although for some women symptoms may continue for several years (Shifren et al., 2014). Cognitive impairment accompanied with mood disorders are frequently observed in peri- and early postmenopausal women. These symptoms including complaints relating to impaired memory, difficulty in performing tasks as well as depression and anxiety, are more prevalent within the 5 years following the last menstrual cycle (Armeni et al., 2016).

Hormone therapy (HT) remains the most effective treatment for the vasomotor symptoms of the menopause (NAMS, 2017, NICE, 2015), however this treatment is not without risk, dependent on treatment type, dosage, duration of use, route of administration and timing of commencement of treatment (NAMS, 2017). The benefit-risk ratio appears favourable for women <60 years of age who commence treatment within 10 years of onset of the menopause providing they have no contraindications. In contrast, women who commence treatment above the age of 60 years, or more than 10-20 years since menopause onset, are at increased risk of coronary heart disease, stroke, venous thrombosis, and dementia (NAMS, 2017). Although early observational studies suggested HT might benefit cognitive function, two intervention studies, WHIMS-Young (Espeland et al., 2010) and KEEPS-Cog (Wharton et al., 2013) have demonstrated that HT initiated early in menopause has neither beneficial nor harmful effects on cognition.

One large clinical trial, the Women's Health Initiative showed no effect of hormone therapy on cognitive function. This paper was widely criticized for age of the cohort although presented enough evidence for further research into the impact of oestrogens on cognition within a younger cohort of women in a more recent transition into the menopause (Resnick et al., 2009). Despite HT being relatively safe for otherwise healthy young/early post-menopausal women, many refrain from HT use due to the associated side effects outlined above or due to medical history. Alternative approaches for the treatment of menopausal symptoms are therefore needed.

Dietary soya isoflavones may illicit beneficial effects in postmenopausal women. Isoflavones are similar in structure to 17β-oestradiol and thus can bind to the oestrogen receptor (ER) exerting oestrogenic/antioestrogenic effects (Kuiper et al., 1998). Soya isoflavones show promise in the treatment of menopausal symptoms, in particular in alleviating hot flushes (Kurzer, 2008, Messina, 2014b), however research findings have been somewhat controversial and one unequivocal finding among studies is the substantial placebo effect observed in randomised controlled trials, highlighting the need for studies in this area to be sufficiently controlled. The aim of this review is to determine the effects of soya foods/isoflavones on cognitive function and menopausal symptoms in postmenopausal women. While the focus of the review is on cognitive function, effects on hot flushes will be summarized.

Soya Isoflavones

Soya beans are a uniquely rich food source of dietary isoflavones, containing approximately 1.2-4.2 mg/g soya protein, with variables affecting exact concentration including climate, soil, or level of processing (Pilsakova et al., 2010). Isoflavones are classified as selective oestrogen receptor modulators (SERMS), having a higher affinity for ER-β than ER-α (Kostelac et al., 2003, Kuiper et al., 1998). Isoflavones bind to the ER with much less affinity than oestrogen (Setchell et al., 2005) and also exert physiological effects that are ER-independent. Within the soya bean, isoflavones are present as biologically inactive glucosides which are hydrolysed following ingestion to form active aglycones (Soni et al., 2014). Soya foods contain three main isoflavones; genistein, daidzein, and glycitein, representing 50, 40 and 10% of total isoflavone content respectively (Murphy et al., 2002). Daidzein can be further metabolized by intestinal bacteria to form the nonsteroidal oestrogen equol (Setchell et al., 2002), however not everyone has the ability to perform this biotransformation. Approximately 30% of adults among western populations (Lampe et al., 1998, Rowland et al., 2000, Setchell and Cole,

2006) and 60% of Asian adults (Liu et al., 2010, Tanaka et al., 2009) have the ability to convert daidzein to equol following a soya challenge. In 2002 Setchell *et al*. proposed the 'equol hypothesis', i.e. that the beneficial effects of soya/isoflavones may be dependent on equol producer status and this has been the focus of much research in subsequent years (Magee, 2011). Dietary isoflavone intake is approximately 25-50mg/day in Japanese populations but is <3mg/day in European and western populations (Messina et al., 2006).

Cognitive function

During the menopause, the intensity and duration of symptoms can have an effect on cognitive function and progressive declines in episodic memory, working memory, executive function (Bojar et al., 2015), planning and mental flexibility and reductions in processing speed have been observed (Greendale et al., 2010, Schaafsma et al., 2010). Oestrogen is essential for the regulation and utilisation of neurotransmitters essential for cognitive functioning and oestrogen receptors are widely distributed throughout the brain. Oestrogen exerts neuroprotective effects on brain regions responsible for working memory and learning, including the hippocampus, hypothalamus, cerebral cortex and pre-frontal cortex (Fournier et al., 2007, Shaywitz et al., 1999, Spencer et al., 2008). One recent study using short-term oestradiol supplementation observed an increase in hippocampal grey matter in early postmenopausal women, potentially owing to the abundance of oestrogen receptors in this brain region (Albert et al., 2017). Impaired cognitive function during the menopausal transition may thus be attributed to decreased endogenous levels of oestrogen (Tuomisto et al., 2012).

Recent evidence has demonstrated that dietary soya isoflavones may illicit beneficial effects on cognitive function in postmenopausal women. Isoflavones can cross the blood brain barrier (Chang et al., 2000, Gu et al., 2005) and due to their structural similarity to 17β-oestradiol, can exert oestrogenic/antioestrogenic effects on the hippocampus and pre-frontal cortex, areas in the brain susceptible to age-related decline (Cheng et al., 2015, Kritz-Silverstein et al., 2003). Animal and *in vitro* models have demonstrated several potential neuroprotective mechanisms (both ER-mediated and non-ER mediated) for soya/isoflavones and such mechanisms include reduced neuronal loss, inhibition of β-amyloid-induced cell death, facilitation of cholinergic transmission, reduced free radical generation, anti-inflammatory and antioxidant effects (Soni et al., 2014) and via the modulation of mitochondrial function (Yao et al., 2013). Both animal and in vivo studies have demonstrated that oestrogens exert neuroprotective effects within the brain (Georgakis et al., 2016). The hippocampus and prefrontal cortex are both influenced by isoflavones due to the localisation of oestrogen receptors (ERs) within the brain. An

increasing number of studies have presented potential effects of plant compounds on maintaining cognitive function.

Intervention Studies

To date, ten randomized controlled trials have investigated the effects of soya/isoflavones on cognitive function exclusively in postmenopausal women, yielding inconsistent results (Table 1). Six studies have shown beneficial effects of isoflavone consumption on cognitive function, (Casini et al., 2006, Duffy et al., 2003, File et al., 2005, Henderson et al., 2012, Kritz-Silverstein et al., 2003, Santos-Galduroz et al., 2010) with supplementation improving cognitive function tests associated with frontal lobe function including mental flexibility and planning, (Casini et al., 2006, Duffy et al., 2003, File et al., 2005) sustained attention, episodic memory (Santos-Galduroz et al., 2010), visual episodic memory (Henderson et al., 2012) and verbal/semantic memory (Kritz-Silverstein et al., 2003).

In the study by Duffy et al. (2003) soya isoflavone supplementation (60 mg/d for 12 weeks) improved long-term episodic memory, sustained attention and frontal lobe function including mental flexibility and planning in comparison to placebo. When these authors repeated the study over a shorter duration of 6 weeks, isoflavone supplementation again improved frontal lobe function, however no significant effects were observed on sustained attention and long-term episodic memory (File et al., 2005). The duration of supplementation may thus be important in determining the effects of isoflavones on these cognitive domains. These findings are supported by (Casini et al., 2006) reported a significant improvement in mental flexibility with the same dose of isoflavone supplementation (60mg/d) over a period of 12 months, although the source of isoflavone is not clear. In contrast, isoflavone supplementation had no effect on attention in this study, nor in the study by (Ho et al., 2007), albeit different tests were used to assess this domain in comparison to the earlier studies. Soya isoflavone supplementation (110 mg/d for 6 months) significantly improved verbal/semantic memory as assessed by category fluency, with an effect size of 0.54 (Kritz-Silverstein et al., 2003). Although logical memory and recall also improved with isoflavone supplementation in this study, the effect was not significant. Similarly, other studies have observed no significant differences on verbal memory/skills between isoflavone and placebo-treated groups (File et al., 2005, Fournier et al., 2007, Henderson et al., 2012, Kreijkamp-Kaspers et al., 2004).

Variations in study design make it difficult to draw comparisons between the limited number of studies conducted. One study used both soya milk and a soya isoflavone supplement as treatment (Fournier et al., 2007), whereas other studies intervened with

isolated soya isoflavones (Casini et al., 2006, Duffy et al., 2003, File et al., 2005, Ho et al., 2007, Kritz-Silverstein et al., 2003) or isoflavone-rich intact soya protein (Basaria et al., 2009, Henderson et al., 2012, Kreijkamp-Kaspers et al., 2004). Such differences in the form of soya tested may impact on isoflavone bioavailability and pharmacokinetics (Cassidy et al., 2006). The isoflavone dose administered in the above studies has ranged from 60 to 160 mg/day with duration of supplementation ranging from 6 weeks to 2.5 years. Age range and duration of time since menopause, as well as the method used for specifying hormone level varied widely between studies and may partly explain inconsistencies in study findings. Re-analyses of findings from the Women's Health Initiative and Women's Health Initiative Memory Study have demonstrated that oestrogen-containing HT may have beneficial effects on cognition in younger women who commence HT within 10 years of the onset of menopause. In contrast null or detrimental effects were evident in older women who commenced treatment >10 years post-menopause (Zhao and Brinton, 2007). Similarly, evidence indicates that those <10 years post-menopause may gain most cognitive benefit from soya isoflavones. Kritz-Silverstein and colleagues (2003) demonstrated that verbal memory was significantly improved (versus placebo) in postmenopausal women following 6 months supplementation with soya isoflavones (110 mg/day) and in women aged 50-59 years a significant improvement was observed in Trails B (a test of visuomotor tracking and attention) whereas this effect was not apparent in older women (aged 60-74 years). Furthermore, sub-group analysis within the longest (2.5 yrs) intervention trial conducted to date (Henderson et al., 2012) indicated that women <10 years post-menopause were most likely to show cognitive improvement following soya supplementation. Such findings may partly explain why isoflavone supplementation (91 mg/day) had no effect on global cognition in this study given that almost half (42%) of the supplemented cohort were >10 years post-menopause. Methodologies used in the assessment of cognitive function have also been inconsistent between studies and factors such as time of cognitive assessment, time since last meal and whether participants were in a fed/fasted state, or if the subjects had consumed caffeine prior to testing can influence cognitive performance and are not explicit within the current literature. Only a single study to date has investigated the effects of soya foods (rather than supplements) on cognitive function (Fournier et al., 2007). Although a decline in verbal working memory compared to soya supplement and control groups was observed, the study was subject to limitations including: subjective compliance; small sample size, lack of power and factors affecting cognitive function were not controlled. While a recent meta-analysis of the studies cited above concluded that soya isoflavones appear to have a positive effect on improving

summary cognitive function and visual memory in postmenopausal women, a huge knowledge gap exists within this area (Cheng et al., 2015, Zaw et al., 2017). There is a clear need for well-designed controlled trials utilising robust methodologies to assess the effects of soya/isoflavones on cognitive function in postmenopausal women.

Table 1: Human studies investigating the relationship between soya and cognitive function

Reference	*Study design*	*Sample Size/Age*	*Study duration*	*Supplementation*	*Cognitive measure*	*Domains*	*Outcomes*
(Duffy et al., 2003)	RDBP, P	33 50-65yrs	12 weeks	1.Soya supp 30mg/2 times/day 2.Placebo(lactose)	WMS-R: immediate recall DMTS-CANTAB	Short-term episodic memory	Isoflavone group showed improvement in long term recall of pictures and episodic memory
					Picture recall	Long-term episodic memory	
					Category generation	Verbal fluency	
					Reversals IDED-CANTAB SoC-antab	Frontal lobe-mental flexibility Frontal lobe: Planning	
					PASAT-1.2 PASAT-1.6	Sustained attention	
(Kritz-Silverstein et al., 2003)	RDBP	56 55-74yrs	6 months	1.Soya supp 110mg/day 2.Placebo (pills not specified)	Logical memory and recall Category fluency	Verbal/semantic memory Verbal/semantic memory	Isoflavone supplementation showed positive effect on verbal memory, and category fluency(P=0.03)
					Trails A and B	Executive function	
(Kreijkamp-Kaspers et al., 2004)	RDBP	175 60-65yrs	12 months	1.Soya protein 99mg/day 2.placebo (25.6g of total milk protein)	MMS,	Global cognition	No association between cognitive function and isoflavone consumption.
					Auditory Verbal Learning Test, immediate recall, delayed recall and recognition Digit Span test, and the Doors test; complex attention tasks,	Memory	
					Digit Symbol Substitution	Complex attention tasks	
					Trail making test	Executive function	
					Verbal fluency, animals and occupation, Boston naming task	Verbal skills	
(File et al., 2005)	DB, PMP	50 51-56yrs	6 weeks	1.Soya ISF supplement	DMTS-CANTAB	Short term episodic memory	
					WMS-story recall	Logical memory	

				Novasoya,60 mg/day 2.Placebo (capsules not specified)	Delayed story recall Delayed picture recall	Long-term episodic memory	Isoflavone supplementation showed improvement in frontal lobe function
					Category generation	Semantic/verbal fluency	
					Reversals IDED-CANTAB SoC-Cantab	Frontal lobe-mental flexibility Frontal lobe-planning	
					PASAT-1.2 PASAT-1.6	Sustained attention	
(Casini et al., 2006)	RDBP, CO	78 49.5yrs	6 months	1.Aglycone supplement 60mg/day tablet 40– 45% genistein, 40– 45% daidzein, 10–20% glycitein 2.Placebo unspecified	Digit Symbol test	Psychomotor/processing	Isoflavone group showed better incidental learning and mental flexibility and attention
					WAIS Digit span test-back recall	Frontal lobe: mental flexibility	
					Visual Scanning test	Attention	
(Fournier et al., 2007)	RDBP	79 48-65yrs	16 weeks	1.Cow milk and ISF supplement, 70 mg ISF/day) 2.cow milk and a placebo supplement 3. soya drink, 72mg ISF/day and placebo supplement	Stroop task	Selective attention	Isoflavones did not show an increase in cog function. Soya group had worse performance in verbal working memory then supplemented and control group.
					Benton Visual Retention Test	Memory recall and recognition	
					Colour match task	Visuospatial working memory	
					Digit Ordering Task	Verbal working memory	
					Digit span Corsi block tapping	Memory span	
(Ho et al., 2007)	RDBPP	191 56-76y	6 months	Supplement 80mg/day Placebo (capsules not specified)	Hong kong list learning test Complex figure test-RO	Learning and memory	No association of isoflavone consumption on cog function across a number of domains
					WMS-R	Visual construction and memory	
					Trail making test Verbal fluency	Executive function	
					Digit span test Digit vigilance test	Attention concentration	
					Finger tapping	Motor function	

					Rey-osterrieth copy trail	Visual perception	
					MMSE	Global cognitive function	
(Basaria et al., 2009)	RDBP	93 46-76y	12 weeks	1.Supplement 160mg/day Genistein 64mg Daidzein 63mg Glycitein 34mg 2.Placebo powder (20g milk protein)	Trail making Cube comparison Verbal fluency Grooved pegboard Identical pictures	Executive/working Psychomotor/processing	No association of Isoflavone consumption on cog function across a number of domains
(Henderson et al., 2012)	RDBPP	350 45-92y	30 months	Supplement 91mg/day Placebo milk protein	Trail making test B Symbol digit modalities test Shipley abstraction Letter number sequencing Block design Judgement of line orientation Category fluency Boston naming test	Executive function	Isoflavone group showed greater improvement on visual memory factor
					California verbal learning test, Immediate and delayed recall East boston memory test, Immediate and delayed recall	Verbal episodic memory	
					Faces 1 and 2,immediate and delayed recall	Visual episodic memory	
(Santos-Galduroz et al., 2010)	RDBP, CO	38	4 months	Supplement 80mg/day Placebo (tablets ot specified)	Digit span WAIS-3	Visual spatial memory	Increased capacity to integrate information in isoflavone treated group
					Digit Symbol WAIS-3	Agility and attention	
					Similarity WAIS-3	Integrate information	
					Verbal paired associated (WMS-R)	Episodic memory	

RDBP Randomised double blind placebo, RDBP CO Randomised double blind placebo-controlled trial, RDBP P Randomised double blind placebo paralell, DB PMP Double blind double-blind, placebo-matched parallel groups study.

Hot Flushes

The wide variety of symptoms, which are associated with the menopause, can be classified into three different categories known as somatic, physiological and vasomotor. Somatic symptoms are more commonly described as headaches and dizziness, physiological symptoms include mood changes, general malaise and lastly vasomotor symptoms are the well-known hot flushes and night sweats (Kurzer, 2008, Messina, 2014a).

Most prevalent vasomotor symptom more commonly described as hot flushes can severely interfere with daily activities including sleep disturbances (Schmidt et al., 2016) and are one of the core symptoms experienced by menopausal women classified under vasomotor symptoms (Taku et al., 2012). Due to the persistence of hot flushes during the menopause, treatment in the form of hormone replacement is often sought to alleviate symptoms. A hot flush is a transient event consisting of a sensation of warmth often accompanied by sweating, discomfort and anxiety (Zaw et al., 2017). These symptoms are thought to be consequential to the natural reduction in endogenous oestrogen levels at the time of the menopause (Lambert et al., 2017). Currently 50-80% of menopausal women experience episodes of hot flushes, these can occur as frequently as an hourly basis during the day or as infrequently as monthly episodes (Bacon, 2017, Freeman and Sherif, 2007). Owing to the low prevalence of hot flushes among native Japanese women and their high isoflavone consumption, it was proposed that isoflavones may potentially alleviate hot flushes due to their oestrogenic effects (Adlercreutz et al., 1992, Messina, 2014b). A meta-analysis assessing the efficacy of soya isoflavone extracts or synthesised isoflavones on the frequency and severity of hot flushes was conducted in peri and post-menopausal women. Effects were reported to be more pronounced in a subgroup of women who experienced frequent hot flushes up to or more than 5 hot flushes per day, and consistent findings among studies in relation to efficacy associate with isolated genistein (Crisafulli et al., 2004). A systematic review by Li *et al*., 2104 reported a 25% decrease in hot flush frequency with isoflavone supplementation >13.4weeks period to reach maximal effect as well as imhof *et al.* showing a modest but significant attenuation in hot flush frequency with a 100mg supplement of isoflavone glycosides (Imhof et al., 2018, Li et al., 2015). Daily ingestion from 30-80mg/day of isoflavones for a duration of 6weeks to 12 months significantly reduced hot flush frequency (Taku et al., 2012). Efficacy of soya isoflavones on hot flushes have been consistently reported in clinical trials including numerous sources of isoflavones including soya foods, and isolated isoflavones (Imhof et al., 2018, Tranche et al., 2016) . As much as a 20% improvement

has been shown in the reduction of frequency of hot flushes (Taku et al., 2012). Several trials reported statistically significant slow but slight results in reduction of severity and frequency of hot flushes with effect more pronounced in those who suffered >5 hot flushes a day (Crisafulli et al., 2004). With the use of isoflavones as ongoing therapy for relief of vasomotor symptoms there appears to be no negative side effects of isoflavone treatment. Isoflavones provide a safe alternative for PMW.

Equol

As noted above, one potential mechanism attributing to the beneficial effects of soya isoflavones is via equol production (Magee, 2011). The ability to produce equol has been associated with reduced menopausal symptoms. In an observational study Japanese equol producers reported less severe menopausal symptoms and a recent double blind placebo controlled trial has demonstrated that supplementation of 10mg equol for 12 weeks significantly improved hot flush frequency in equol non-producing post-menopausal Japanese women (Aso et al., 2012). To date only two studies have investigated the relationship between equol and cognitive function in PMW following soya/isoflavone supplementation (Gleason et al., 2009, Henderson et al., 2012). In the study of Gleason et al. none of the participants were classified as equol producers and this is likely owing to the older age of the cohort (Frankenfeld et al., 2004). Henderson *et al* reported a trend for postmenopausal women following sub group analysis women within soya supplemented group who were consistent equol producers to have an improved, albeit insignificant, global cognition score compared with placebo. Also, a cross sectional study showed equol producers have a beneficial effect on cognitive function after soya intake vs non producers (Igase et al., 2017). Thus, further studies are required in this area to determine if equol producers derive greater benefits from soya consumption as highlighted within the report of the (North American Menopause, 2010).

Conclusion

Soya isoflavones have potential to exert beneficial effects on cognition, potentially via their interaction with ERs localised within brain regions which play major roles in cognition. It appears that supplementation with soya isoflavones for less than 6 months irrespective of dose can improve cognitive performance as well as longer duration studies. Literature on the effects of isoflavones on cognitive function remains conflicting with only over half the studies showing beneficial effects. `With cognitive measures consistently targeting areas of the brain which are ER sensitive, frontal lobe functioning has received growing attention. Consistent testing of these areas of the brain showing significant improvement would suggest that soya isoflavones may benefit frontal lobe

function in the menopause. Trials varying in duration from 6 weeks to 30 months have both shown to have positive effects on PMW cognitive performance. Greater effects are seen in those who are <10years from their menopausal transition and severity of symptoms at this time may be the cofounding factor. Inconsistent findings among trials may be due to variations in age and time since menopause. There is a prominent reduction of ER-β receptors within the ageing brain, thus areas such as the prefrontal cortex may be less responsive to oestrogenic compounds in older age. Summary findings concluded by only 10 randomised trials has exposed limitations when summarising outcome findings for further studies and therefore enhanced study protocols with more stringent and robust methodology are required.

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CHAPTER 3

Dietary patterns and cognitive function in post-menopausal women.

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ABSTRACT

Background: Dietary intake has been linked with cognitive function, yet few studies have investigated diet and cognition in post-menopausal women.

Objective: This study aimed to investigate associations between dietary patterns and cognitive function in post-menopausal women.

Methods: Dietary intake of post-menopausal women (*n*=75) with an average age of 54y was assessed using a 4-day food diary from which dietary patterns were identified using principal component analysis. Cognitive function was measured using the Cambridge neuropsychological automated test battery (CANTAB®). Multiple linear regression analysis and Mann Whitney U tests were used to investigate potential associations between dietary patterns and cognitive function.

Results: Factor analysis identified five dietary patterns; 'hot beverages and breads', 'meat and dairy', 'potatoes and poultry', 'healthy', and 'social'. The 'meat and dairy' dietary pattern was negatively associated with spatial working memory ($p = 0.009$), measured as total errors made ($\beta = -0.308$; CI = -0.195, -0.029). The 'potatoes and poultry' dietary pattern was positively associated with five-choice reaction time ($p = 0.045$), measured as reaction time latency in milliseconds, ($\beta = 0.245$; CI = 0.000, 0.030). The 'social' dietary pattern showed a negative association with match to sample visual search ($p = 0.017$), measured as mean correct reaction time in milliseconds ($\beta = 0.289$ CI = 0.006, 0.056).

Conclusion: In post-menopausal women, inclusion of alcohol can have negative effects on cognitive performance. Furthermore, intakes of potatoes and poultry can have negative effects of cognitive performance. This finding concludes that high intake of meat and dairy may have a beneficial effect on cognntive function in post menopausal women.

Key words: Dietary patterns; post-menopausal women; cognitive function

INTRODUCTION

Memory problems are commonly reported during the menopause (Etgen et al., 2011, Sturdee and de Villiers, 2011) and reduced cognitive performance has been observed in women at this time (Bojar et al., 2015, Greendale et al., 2010, Schaafsma et al., 2010). Oestrogen is essential for normal cognitive processing (Shaywitz et al., 1999, Spencer et al., 2008). Higher oestradiol levels in older post-menopausal women have been associated with better memory and global cognitive function (Lebrun et al., 2005), as well as greater episodic memory (Hogervorst et al., 2004, Ryan et al., 2012). Furthermore, a significant association between oestradiol concentration and cognitive dysfunction has been reported in healthy post-menopausal women, highlighting a potential benefit of oestradiol replacement therapy (Gholizadeh et al., 2018). Thus, impaired cognitive function may be attributed to decreased endogenous levels of oestrogen during the menopause (Tuomisto et al., 2012).

Adequate nutritional intake may play a role in slowing the onset of cognitive decline (Pistollato et al., 2018). Research has shown the benefits associated with adequate nutritional intake in the prevention of Alzheimer's disease and dementia (Morris et al., 2015, Berendsen et al., 2018). In elderly individuals showing signs of cognitive dysfunction, there occurs an increased risk of Alzheimer's disease (Goodwin et al., 1983, Roberts et al., 2012), in which the Mediterranean diet (MeDi) has shown preventative effects counteracting the onset (Morris et al., 2015). An inadequate nutritional intake defined by insufficient contribution of micronutrients in the diet has been observed in women during the menopausal transition (Bojar et al., 2015), thus dietary intake during the menopause may have important consequences in relation to cognitive health.

Previous studies investigating the relationship between diet and cognition have focused mainly on individual nutrients, particularly, omega-3 polyunsaturated fatty acids (n-3PUFA) (Gillette-Guyonnet et al., 2013), B-vitamins (Porter et al., 2016), antioxidants (Bowman et al., 2012, Gillette-Guyonnet et al., 2007, Kesse-Guyot et al., 2011, McDaniel et al., 2003), vitamin D (Annweiler and Beauchet, 2013), zinc (Maylor et al., 2006), and protein (Roberts et al., 2012, Goodwin et al., 1983). Other diets, associated with a healthier lifestyle, have been linked with a slower rate of cognitive decline and dementia e.g. the Dietary Approaches to Stop Hypertension (DASH) diet, and a hybrid of the DASH (Pistollato et al., 2018), and MeDi (Morris et al., 2015, McEvoy et al., 2017) namely the Mediterranean-DASH Diet Intervention for Neurodegenerative Delay (MIND) diet (Berendsen et al., 2018, Morris et al., 2015). The MeDi has been postulated to play a critical role in cognitive health (Kesse-Guyot et al., 2012). Characterised by a higher intake of green leafy vegetables, fish, and nuts, the MeDi has been associated with

beneficial effects in relation to physical and cognitive health. Adherence to a MeDi diet as investigated by Singh *et al.,* was associated with a 27% reduced risk of developing cognitive impairment (Singh et al., 2014), a finding echoed in a meta-analysis by Psaltopoulou *et al.,* showing that high adherence to the MeDi is associated with less cognitive impairment (Psaltopoulou et al., 2013). Other studies have linked the MeDi with a slower rate of cognitive decline (Alvarez-Alvarez et al., 2018, Gonzalez et al., 2018). Similarly, the original PREDIMED trial, designed to assess the MeDi and health outcomes in a cohort of high-risk vascular individuals, showed small but significant benefits on global cognition (Martinez-Lapiscina et al., 2013). The MIND diet has shown promising results in areas of verbal memory in later life (Berendsen et al., 2018) and has also been positively associated with a slower rate of cognitive decline (Morris et al., 2015). Although evidence consistently demonstrates the beneficial effects of dietary components on cognitive function (Berendsen et al., 2018), there is limited data available in post-menopausal women.

The MeDi is often characterised by the high intake of n-3 and n-6 PUFA, highly reviewed nutrients in association with cognitive health (Grosso et al., 2014a, Grosso et al., 2014b, Hallahan et al., 2016). Nilsson *et al.,* observed an improvement in cognitive function following 5-weeks' supplementation with n-3 PUFA in a cohort of healthy adults aged 51–72 years (Nilsson et al., 2012). Docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA) have also been postulated to have beneficial effects on cognitive health in aging (Bojar et al., 2015, Denis et al., 2013). Folate, vitamin B6 and vitamin B12 are essential for normal brain function (Forbes et al., 2015). Folate is essential for the down regulation of homocysteine through the enzymatic reaction of methionine synthase to produce methyl cobalamin. Methyl cobalamin is required for methylation of homocysteine to methionine. Low vitamin B status is associated with increased blood levels of homocysteine, which could potentially disturb metabolism and cause cognitive impairment (Forbes et al., 2015). Long term supplementation of these vitamins in randomised trials has demonstrated favourable effects on cognitive performance, although these studies primarily focus on elderly populations (de Jager et al., 2012, Douaud et al., 2013, Durga et al., 2007, Smith et al., 2010). It is evident that research is needed in the area of B-vitamins and cognitive health particularly in post- menopausal women.

As the nutrients outlined above are not consumed in isolation, this creates limitations in ascertaining their specific contribution to cognitive health. Dietary pattern analysis overcomes these limitations by accounting for the cumulative effect of nutrients or potential interactions among foods (Hu, 2002, Loy and Mohamed, 2013). Previous

studies have investigated dietary patterns in post-menopausal women in association with sarcopenia (Kaser et al., 2017), obesity (Papavagelis et al., 2018), bone mineral density (Hardcastle et al., 2011, Karamati et al., 2012, Sugiura et al., 2011), cardiovascular disease risk (Papavagelis et al., 2018), and metabolic syndrome (Silva et al., 2015). Few studies have investigated associations with cognitive function. One study explored the relationship between dietary patterns and mental health in a cohort of Chinese post-menopausal women. Three dietary patterns were identified; 'processed foods', 'whole plant foods', and 'animal foods'. A higher intake of whole plant foods and a low intake of processed foods was associated with a reduced risk of depression and perceived stress. A link between depression and a deficit in brain domains responsible for executive function, memory, and attention has been identified (Kaser et al., 2017, Citraro et al., 2015). Furthermore, depression is associated with poorer cognitive function (Murrough et al., 2011), indicating that components of these dietary patterns may attribute to favourable outcomes in relation to cognitive performance. Another study investigated the relationship between dietary patterns and mid-life cognitive performance (Kesse-Guyot et al., 2012). Two dietary patterns were established using factor analysis; a 'healthy' dietary pattern that included fruit, wholegrains, vegetables, fats, nuts and fish and a 'traditional' dietary pattern that included vegetables, vegetable fat, meat and poultry. A better global cognition score was associated with the 'healthy' dietary pattern and adherence to this dietary pattern may help preserve global cognitive function, particularly verbal memory. There are a limited number of studies in this area therefore further studies are required to investigate the relationship between dietary patterns and cognitive function, specifically in post-menopausal women. The aim of this study is to investigate the association between dietary patterns and cognitive function in a convenient sample of post-menopausal women residing in Northern Ireland.

METHODS

Study Characteristics

The present study utilised baseline data from a 12-week randomised controlled trial conducted at Ulster University, Coleraine, Northern Ireland. All procedures were approved by Ulster University's Research Ethics Committee (REC/15/0025) and the study was registered at www.clinicaltrials.gov (NCT03561662). The study was undertaken between the months of October 2015 to June 2018 and was designed to examine the effects of soya on cognitive function and menopausal symptoms in post-menopausal women.

Subjects

Apparently healthy post-menopausal women within 7 years of their last menstrual cycle were considered suitable for inclusion. Exclusion criteria were as follows: not currently taking any HRT or isoflavone supplements, not a habitual soya consumer, not currently using any psychoactive medication, not suffering from cardiovascular disease, cancer, diabetes, thyroid, renal or kidney disease, alcohol or drug abuse and not prescribed any antibiotics within the last 3 months. Women were excluded from the study if they had cognitive impairment as determined by a Mini Mental State Examination (MMSE) score <24; psychiatric distress as determined using a General Health Questionnaire-28 (Goldberg and Hillier, 1979) score of ≥26; red-green colour blind, assessed via the Ishihara test (as CANTAB testing requires colour recognition); abnormal full blood profile (assessed via a Sysmex KX21-N, Sysmex Ltd, UK at Ulster University) and/or insufficient renal/hepatic performance assessed via kidney and liver function tests (assessed photometrically via a Cobas 6000 analyzer [Cobas C501 module], Roche Diagnostics at Causeway hospital).

Anthropometric measures

Trained personnel obtained weight (kg) and height (m) measurements. Standing body height was measured using a calibrated stadiometer (SECA, Model 220, Germany). Body weight was measured to the nearest 0.1kg using Seca 770 electronic weighing scales (Brosch Direct Ltd, Peterborough, United Kingdom), without footwear and heavy clothing. Body mass index (BMI) was calculated as weight (kg)/height (m^2). A validated health and lifestyle questionnaire was used to collect information regarding education, smoking, alcohol and physical activity to assess metabolic equivalent tasks (METs) hours/week. Estimated basal metabolic rate (BMR) (Kcal/d) was calculated according to the following equation for all participants (Henry, 2005).

$$BMR = 8.18\ (weight\ kg) + 502\ (height\ m) - 11.6$$

Dietary Measures

Dietary intake was assessed by completing a prospective 4 day semi-quantitative food diary at baseline (two weekdays and two weekend days; to account for the known variation in day-to-day intake) (Stallone et al., 1997). Comprehensive written and verbal instructions were given to all participants on the method of recording dietary intake and participants were advised not to modify usual dietary habits. Returned food diaries were reviewed in order to clarify errors with participants. The nutritional analysis software package Nutritics was used to calculate energy and nutrient intakes. Total dietary intake

was generated for each participant, including type, quantity of food eaten, as well as energy and nutrient values corresponding to weight of food consumed.

Dietary Patterns: Principal Component Analysis

A total of 19 food groups and their total consumption over the 4-day period (total g) were generated. Foods were grouped according to the types of food or by similarities in nutrient content manually using excel. (see Table 1 for food items included in each group). Factor loadings for dietary patterns are shown in Table 2. The factors were rotated by an orthogonal transformation with the varimax option which maintains uncorrelated factors and produces a simpler structure with easier interpretability. Food loading with greater than 0.3 on a component were considered for the dietary patterns. The criterion for remaining factors included those with an eigenvalue greater than 1 as well as an interpretation of the generated scree plot (see Figure 1) showing a break.

Over/Under Reporting of Energy Intake

Energy intake (kcals/day) for each participant was manually aggregated using SPSS based on data collected from Nutritics. Estimated energy intake for each participant was assessed and used to determine the number of under reporters, over reporters and plausible reporters. The validity of participants' reported energy intake as calculated from the 4 day food diary was assessed using Goldberg *et al,*. (Goldberg et al., 1991) cut offs as outlined by Black (Black, 2000). A physical activity level (PAL) of 1.4 was used as recommended based on the age of the cohort validated by Henry *et al.*, (Henry, 2005). For the current cohort of post-menopausal women, an EI:BMR of over 2.49 and under 0.78 was indicative of over and under-reporting, respectively, based on a PAL of 1.4 (Black, 2000). A total of n=1 were deemed to be 'over-reporters', n=8 were deemed to be 'under reporters' and n=66 were deemed to be 'plausible reporters'. Overall, the mean reported EI for plausible reporters (n=66) was found to be 1607 kcals/day, which was 84% of estimated energy requirements (EER; determined by BMR*1.4). In contrast, the mean reported EI for under-reporters (n=8) was 1115kcals/day, which was 54% of EER (results not shown).

Cognition

Cognitive function was measured using the Cambridge neuropsychological automated test battery (CANTAB), Research Suite; Cambridge Cognition, UK) (CANTAB, 2015). Cognitive measures used were designed to target areas of the brain which are susceptible to oestrogen related decline. CANTAB has been extensively validated for assessing brain-to-behaviour relationships in adult populations (Robbins et al., 1998, Robbins et

al., 1994), has proven test-retest reliability (Louis et al., 1999) and is deemed suitable for use with older adults (Robbins et al., 1994). The following tests were used: spatial working memory (SWM), spatial span (SSP), pattern recognition memory (PRM), 5-choice reaction time (RTI) and match to sample visual search (MTS). The tests chosen activate areas of the brain that are associated with cognitive decline during the menopausal transition and that are sensitive to hormonal changes, including the hippocampus (Albert et al., 2017) and prefrontal cortex (Shanmugan and Epperson, 2014). SWM and SSP activate the temporal and frontal lobe regions of the brain; PRM activates the temporal lobe, hippocampus and amygdala; RTI, and MTS activate the fronto-striatal circuitry (Robbins TW, 1997). The procedure for assessing SSP, RTI and MTS are described in detail elsewhere (Simpson et al., 2005). Spatial working memory (SWM), a sensitive measure of frontal lobe and executive function, requires retention and manipulation of visuospatial information. The test began with 4 coloured squares (boxes) shown on the screen. Participants were required, by selecting boxes and using a process of elimination, to find one blue 'token' in each of 4 boxes (only one token is hidden at a time) and use them to fill up an empty column on the right-hand side of the screen. Touching any box in which a token has already been found is an error. The trial was then repeated three times with 4 boxes and then progressed to four trials with 6 boxes and four trials with 8 boxes. The colour and position of the boxes used are changed from trial to trial to discourage the use of stereotyped search strategies and a practice test was completed prior to testing. The outcome measure was SWM total errors made i.e. the number of times a box is selected that is certain not to contain a token and therefore should have not been visited by the participant. Visual memory was assessed using the Pattern Recognition Memory (PRM) test in a 2-choice forced discrimination paradigm. Participants were asked to remember a series of 12 abstract coloured patterns, each presented for 3s. They were then presented with a series of 12 pairs of old-new patterns and were asked to touch the pattern seen previously in each case. This procedure was repeated with a second set of 12 patterns followed by 12 pairs of patterns for recognition. The outcome measure was mean correct latency i.e. the mean time (milliseconds) to respond correctly.

Statistical Analyses

All analysis was completed using the statistical package for social sciences (SPSS, version 24, IBM, Chicago, IL, USA). Normality tests were carried out for all data, and indicated that food group, nutrient intake and cognitive data were not normally distributed. Descriptive analysis was carried out and all data is expressed as mean and

standard deviation. Non-parametric statistical tests were completed for comparison of differences between all participants (n=75) and plausible reporters only (n=66). Multiple linear regression analysis was used to examine relationships between dietary patterns, nutrients and cognitive function while controlling for factors also known to influence cognitive function (age, education). Following calculation for energy misreporting, principal component analysis was complete.

RESULTS

The general health and lifestyle characteristics and dietary intake of the participants is presented in Table 3. A total of 75 participants successfully completed and returned the 4-day food diary. A total of 9 (12%) participants were classified as under reporters, 1 (1.3%) as an over reporter and 65 (86.67%) as plausible reporters.

Identification of Dietary Patterns

Principal component analysis was carried out with both plausible (n=65) and all participants (n=75) and there was no difference in the dietary patterns generated. Principal component analysis was completed with all participants (n=75) and resulted in 5 dietary patterns, 'hot beverages and breads', 'meat and dairy', 'potatoes and poultry', 'healthy', and 'social'. Using19 food groups the dietary patterns generated accounted for 48.76% of total variance in the diet of this cohort. The scree plot identified the break point of dietary patterns to be retained. The 'hot beverages and breads' pattern explained 14.73% of the variance. The 'meat and dairy' pattern accounted for 10.26% of the variance and was made up of meat and dairy, western foods including snacks such as cakes and biscuits, eggs, and red meat. The 'potatoes and poultry' pattern, characterised by potato, snacks and chicken, accounted for 8.67% of the variance. The 'healthy' pattern explained 7.7% of total variance and consisted of healthier dietary choice snacks and alternative milks. The 'social' pattern accounted for 7.41% of total variance and consisted of alcohol and savoury snacks.

Dietary patterns and cognitive function

Table 4 shows the associations between dietary patterns and cognitive function using multiple regression analysis. The "hot beverages and breads" pattern was the most commonly consumed dietary pattern among post-menopausal women, although this pattern as well as the "healthy" dietary pattern did not present any significant associations with cognitive measures. The 'meat and dairy' dietary pattern was negatively associated with SWM ($p = 0.009$), measured as total errors made ($\beta = -0.308$; CI = -0.195, -0.029). The 'potatoes and poultry' dietary pattern was positively associated with RTI ($p = 0.045$), measured as reaction time latency in milliseconds, ($\beta = 0.245$; CI = 0.000, 0.030), representing an effect size small in magnitude. The 'social' dietary pattern showed a negative association with MTS ($p = 0.017$), measured as mean correct reaction time in milliseconds ($\beta = 0.289$ CI = 0.006, 0.056).

DISCUSSION

Five main dietary patterns were identified in this cohort of post-menopausal women. "Meat and dairy", a dietary pattern associated with intake of protein, was significantly associated with a reduction in errors in spatial working memory, representative of a greater performance in a measure of frontal lobe function. The 'potatoes and poultry' dietary pattern was associated with a slower performance time in a cognitive test measuring cognitive domains associated with attention and psychomotor speed. Alcohol intake represented in the 'social' dietary pattern was associated with slower visual matching ability and poorer performance in short term visual recognition memory.

Our findings support previous literature demonstrating that adherence to a diet rich in dairy protein is linked to greater cognitive health (LaRue et al., 1997, Roberts et al., 2012). These links are associated with activations at the anterior brain region frontal lobe, affecting non-verbal learning and verbal memory domains. Our findings indicate that a diet rich in protein and dairy is associated with a greater ability to retain and manipulate visuospatial information. Similarly, when we considered individual nutrient intake, protein intake was associated with a significant improvement in SWM.

In support of our findings, one longitudinal study (Crichton et al., 2012) demonstrated a significant cognitive improvement in multiple brain domains in individuals that consumed dairy once a day, compared to those who rarely consume dairy. Beneficial effects on global cognitive function, spatial memory, organisation working memory and executive function were observed, although it is yet to be investigated what underlying causal mechanisms are at play. In contrast, a recent randomised controlled trial investigating the association between dairy intake and cognitive function showed no effect, a finding likely owing to the fact that baseline comprehensive cognitive performance data were not available (Kesse-Guyot et al., 2016). One review reported that increased risk of vascular dementia and poorer cognitive function is associated with lower consumption of milk or dairy products. In contrast, whole fat dairy products were potentially associated with cognitive decline in the elderly, likely due to the saturated fat content. While findings suggested that dairy consumption may have a beneficial effect on cognitive function, the review highlighted the need for well-controlled, long-term intervention trials to confirm this theory (Crichton et al., 2010). Interestingly, a MeDi diet supplemented with dairy foods has been shown to improve processing speed in comparison to a low-fat control diet in an ageing Australian population (Wade et al., 2018).

In this study, the "potatoes and poultry" pattern, high in carbohydrates and chicken dishes was significantly associated with a poorer performance in RTI, a measure focused on areas

of the brain associated with attention and psychomotor speed. The "potatoes and poultry" dietary pattern is representative of intakes of carbohydrates and protein (Gibson, 2007, Goodwin et al., 1983, Williams and Rollo, 2015). In contrast to our findings, a recent review concluded that a high intake of fruit and vegetables, whole grains legumes, pulses and potatoes are key for the prevention of cognitive decline (Kiefte-de Jong et al., 2014). As discussed above, protein has also been associated with having favourable effects on cognitive function (Goodwin et al., 1983, Daly et al., 2015) although evidence in this area is novel.

The "social" dietary pattern in this cohort, which loaded highly for alcohol and savoury snacks, was associated with a slower reaction time in MTS, i.e. slower visual matching ability and poorer performance in short term visual recognition memory. It has been clearly established in the literature that alcohol-dependant individuals are subject to age related cognitive decline as seen in neuropathological studies (Harper, 1998). In contrast, there is a lack of conclusive findings showing that small to moderate amounts of alcohol have a negative effect of cognitive function (Hassing, 2018, Vasiliadis et al., 2018); more recent evidence suggests no relationship (Sun et al., 2018). One recent study in a Chinese population has demonstrated that alcohol consumption is associated with poorer episodic memory and reduction in consumption could help preserve cognitive degeneration in this population (Ge et al., 2018).

Studies investigating associations between dietary intake and cognitive function, as dietary patterns or singular nutrient approaches in younger adults are limited. The majority of previous studies in this area have been conducted in participants ≥65y whereas the mean age of participants in our study was 54y. Also, with the average age of the onset of the menopause being 51y of age, findings in elderly cohorts are not representative of early post-menopausal women. Cognitive studies in mid-life adults are required, particularly focused in post-menopausal women (Bojar et al., 2015). Gardener *et al.*, report that executive function and visuospatial brain functions are highly sensitive to dietary intake (Gardener et al., 2015); interestingly, these specific areas of the brain are highly sensitive to reduced function during the menopause (Bojar et al., 2015). One large longitudinal study of generally healthy individuals with no cognitive impairment aged 60-64y identified that a MeDi was not protective of cognitive decline (Cherbuin et al., 2011) , this contradictory finding on efficacy of the MeDi is likely owing to variances within study designs such as age, severity of cognitive impairment within the cohort, dietary and cognitive measures used.

This study has numerous strengths. PCA is the most common method for obtaining dietary patterns. This study also has limitations. This contrast in findings may be attributed to the wide variety of tests used to assess cognitive function, study protocols

differ vastly also contributing to the contrast in findings among studies in this area. The sample size of this cohort is small, dietary data is often representative of a much larger sample size. Nonetheless this study has provided novel findings in the area of dietary patterns and cognitive function in post-menopausal women. These findings present new evidence to consider in further studies which are focused on dietary intake in post-menopausal women and their potential beneficial effects on cognitive decline.

CONCLUSION

In conclusion, among the five dietary patterns identified the ‘meat and dairy” dietary pattern showed favourable effects on cognitive function, the protein content in this dietary pattern is postulated to contribute to these findings. There is a lack of research investingating dietary patterns in relation to cognitive health in post- menopausal women. Considering the importance of dietary intake on cognition in the elderly, further studies should consider the importance of dietary intake and its association within post-menopausal women.

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Table 1: Food groups used in dietary pattern analysis

Food Group	*Food items*
Alcohol	Alcoholic drinks, spirits wines, larger, stout
Beverages	Coffee, fruit juices, soft drinks, teas, squash, cordials
Biscuits & Cakes	Biscuits, cookies, cakes, desserts, candy sweets, chocolate, confectionary, non-chocolate confectionary
Breads	Bread, rolls, bagels, tortilla, flat breads, sandwiches
Cereals & Grains	Cereal bars, cereals, muesli, crackers, crisps breads, flours, pastas, pastry, doughs, pizzas, rice
Dairy	Cheeses, cow's milk, creams, drinking yoghurts, eggs, milkshakes, smoothies, processed milks, powders
Fats & Oils	Oils, spreading fats
Fish	Canned fish, fatty fish, low fat fish, seafood dishes, shellfish
Fruit	Fruit, dried fruit
Meat	Bacon, beef, lamb, meat products, pork
Miscellaneous	Food additives, herbs and spices, salts, sauces, sugars, syrups, preserves
Non-dairy	Oat milk, soya milk, almond milk, rice milk
Nuts & Seeds	Nuts, seeds, trail mix, almond butter, peanut butter
Potatoes	Potato crips, crisps, potato products, potatoes,
Poultry	Chicken, poultry dishes
Savoury snacks	Cous cous, taco shell, prawn crackers, papadums, Yorkshire pudding, stuffing, popcorn, breadsticks
Soup	Packet soups, soups
Vegetables & Legumes	Beans, peas, lentils, leafy vegetables, mushrooms, fungi, roots, tubers, bulbs, vegetable dishes

Figure 1: Scree plot indicating cut off for Dietary Patterns.

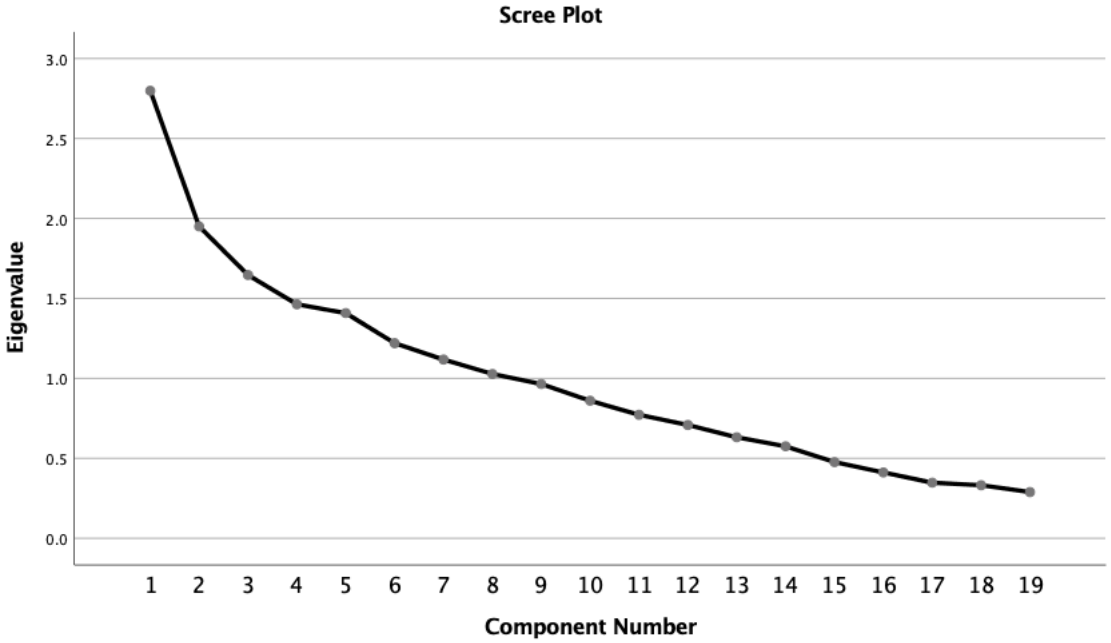

Table 2: Factor loadings for dietary pattern identified by principal component analysis (PCA) for n=75 food diaries

Food Group	Hot beverages and breads	Meat and dairy	Potatoes and poultry	Health	Social
Fats & Oils	.857	-	-	-	-
Breads	.768	-	-	-	-
Beverages	.485	-	-	-	-
Meat	-	.881	-	-	-
Dairy	-	.559	-.377	-	-
Potatoes	-	-	.760	-	-
Poultry	-	-	.722	-	-
Alternative milks	-	-	-	.761	-
Fruit	-	-	-	.633	-
Savoury snacks	-	-	-	-.451	.449
Alcohol	-	-	-	-	.727
Biscuits & Cakes	-	.417	-	-	-.593
Cereals & Grains	-	- - - -			
Miscellaneous	-	-	-	-	-
Nuts & Seeds	-	-	-	.407	-
Sauces	-	-	-	-	-
Vegetables	-	.327	-	-	-
Soup	-	-	-	-	-
Fish	-	-	-.306	-	-
% of variance explained	14.73	10.26	8.66	7.7	7.41

Only food groups with factor loading values of -0.3 or 0.3 were included, some food groups were excluded as they did not load onto any factor retained.

Table 3: Characteristics of all participants and plausible reporters only

Descriptives	[1]All participants n=75	[1]Plausible n=66	*[2]P*
Height, m	1.63 ± .065	1.63 ± 0.06	.816
Weight, kg	70.12 ± 13.13	68.79 ± 11.89	.588
BMI, kg/m^2	26.42 ± 5.24	26.03 ± 4.46	.673
Age, y	54.07 ± 3.8	54.49 ± 3.72	.635
Alcohol, units/wk	8.07 ± 9.81	8.70 ± 10.28	.710
Education, %			
Primary	1	2	
Secondary	41	41	
Tertiary	56	56	.992
PA, METs/week	181.34 ± 66.28	181.11 ± 67.57	.990
LMP, mo	36.96 ± 21.12	38.31 ± 21.14	.840
FSH, IU/ml	87.97 ± 30.46	89.67 ± 29.84	.708

BMI, body mass index, METs, metabolic equivalents, PA, physical activity, LMP, time since last menstrual period, FSH, follicle stimulating hormone

[1]mean ± SD

[2]No significant differences were observed between all reporters and plausible reporters of dietary intake as determined using a Mann Whitney U

Table 4: Multiple regression analysis of dietary pattern predictors of cognitive function in post-menopausal women (all participants).

Cognitive test[2]	*Hot beverages and breads*		*Meat and dairy*		*Potatoes and poultry*		*Healthy*		*Social*	
	β	95%CI	β	95%CI	β	95%CI	β	95%CI	β	95%CI
RTI	.156	-.005, 0.24	-.070	-.018, .010	.245	.000, .030*	-.088	-.019, .009	.136	-.006, .022
SSP	-.036	-.027, 0.020	.112	-.012, .033	.032	-.021, .027	.005	-.022, .023	-.074	-.030, 016
SWM	-.095	-.125, 0.56	-.308	-.195, -.029*	.076	-.065, .122	.169	-.024, .146	-.003	-.090, .087
PRM	-.033	-.025, 0.019	-.206	-.040, .001	-043	-.027, .019	.033	-.018, .024	-.002	-.022, .021
MTS	.043	-.022, 0.031	.062	-.019, .032	.126	-.013, .042	-.052	-.031, .020	.289	.006, .056*

[2]RTI, five-choice reaction time measured as reaction time latency (milliseconds); SSP, spatial span measured as longest sequence length recalled correctly; SWM, spatial working memory measured as total errors made; PRM, pattern recognition memory measured as mean correct latency (milliseconds); MTS, match to sample visual search measured as mean correct reaction time (milliseconds).

*significant results $p<0.05$

CHAPTER 4

Consumption of a soy drink has no effect on cognitive function but may alleviate vasomotor symptoms in postmenopausal women; a randomised trial.

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ABSTRACT

Purpose: Cognitive decline is commonly reported during the menopausal transition, with memory and attention being particularly affected. The aim of this study was to investigate the effects of a commercially available soy drink on cognitive function and menopausal symptoms in postmenopausal women.

Methods: 101 postmenopausal women, aged 44-63 years, were randomly assigned to consume a volume of soy drink providing a low (10 mg/day; control group), medium (35 mg/day) or high (60 mg/day) dose of isoflavones for 12 weeks. Cognitive function (spatial working memory, spatial span, pattern recognition memory, 5-choice reaction time and match to sample visual search) was assessed using CANTAB. Menopausal symptoms were assessed using Greene's Climacteric Scale.

Results: No significant differences were observed between the groups for any of the cognitive function outcomes measured. Soy drink consumption had no effect on menopausal symptoms overall, however when women were stratified according to the severity of vasomotor symptoms (VMS) at baseline, women with more severe symptoms at baseline in the medium group had a significant reduction (P=0.001) in VMS post-intervention (mean change from baseline score: -2.15 ± 1.73) in comparison to those with less severe VMS (mean change from baseline score: 0.06 ± 1.21).

Conclusions: Soy drink consumption had no effect on cognitive function in postmenopausal women. Consumption of ~350ml/d (35mg IFs) for 12 weeks significantly reduced VMS in those with more severe symptoms at baseline. This finding is clinically relevant as soy drinks may provide an alternative, natural, treatment for alleviating VMS, highly prevalent among western women.

Keywords: isoflavones, CANTAB, menopausal symptoms, hot flush, hot flash, equol

INTRODUCTION

Cognitive decline is commonly reported by peri- and post-menopausal women and deteriorations in memory, attention and processing speed have been observed during the menopausal transition (Kok et al., 2006; Schaafsma et al., 2010; Epperson et al., 2013; Maki and Henderson et al., 2016). These effects have been attributed to a reduction in circulating oestrogen concentrations (Shanmugan and Epperson, 2014) albeit, this has been contested (Henderson., 2008). Hormone therapy (HT) remains the most effective treatment for vasomotor menopausal symptoms (VMS) (North American Menopause Society, 2017) and early observational studies supported a beneficial effect of HT on cognitive function (Mc Carrey and Resnick, 2015). Two recent intervention studies have demonstrated that HT initiated early in menopause has neither beneficial nor harmful effects on cognition (Espeland et al., 2013; Wharton et al., 2014). Furthermore, despite HT being commonly prescribed for otherwise healthy young/early post-menopausal women, many refrain from HT use due to the health risks associated with HT use in older women (North American Menopause Society., 2017; Marjoribanks et al., 2017). Alternative, natural approaches for the treatment and prevention of menopausal symptoms are thus being sought.

Dietary soy isoflavones (IFs) have been reported to be efficacious in the treatment of hot flushes (Taku et al., 2012*)* and to have the potential to enhance cognitive function in postmenopausal women, having the ability to cross the blood brain barrier in small amounts (Chang et al., 2000; Gu et al., 2005). Genistein and daidzein, the main IFs present in the soy bean, can bind to oestrogen receptors (ERs), and are classified as selective ER modulators, having a higher affinity for ER-β than ER-α (Kuiper et al., 1998; Oseni et al., 2008). ERs are localised throughout the adult brain including the hippocampus, where ER7 is more highly expressed in comparison to ER777 and are also localised within the prefrontal cortex (Shanmugan and Epperson, 2014). These areas are important for learning, memory, attention and higher-order cognitive function and are particularly susceptible to age-related decline (Burke and Barnes, 2006). Both ER-mediated and non- ER mediated neuroprotective effects have been demonstrated for soy/IFs using *in vitro* and animal models (Yao et al., 2013; Soni et al., 2014;). Nevertheless, randomized controlled trials investigating the effects of soy/IFs on cognitive function in postmenopausal women have yielded inconsistent findings, with some showing positive effects on cognitive function (Duffy et al., 2003; Kritz-Silverstein et al., 2003; File et al., 2005; Casini et al., 2006; Santos-Galduroz et al., 2010) and others demonstrating null/negative effects (Kriejkamp-Kaspers et al., 2004; Fournier et al.,

2007; Ho et al., 2007; Basaria et al., 2009). Two recent comprehensive reviews (Soni et al 2014; Zaw et al., 2017) have highlighted several variations in study design that have likely contributed to these disparate outcomes including: age; time since menopause; habitual soy/IF intake; IF dose, duration, bioavailability and metabolism and cognitive function assessment. To date only one study has investigated the effects of soy/IFs on cognitive function in postmenopausal women using a food (soy milk) (Fournier et al., 2007) rather than a supplement. There is, therefore, a need for additional, well-designed studies to further assess the effects of IFs on cognitive function.

In line with the 'critical window hypothesis' of HT and cognitive function that postulates that optimal effects are evident with early initiation (Maki, 2013), limited evidence suggests that younger postmenopausal women (< 60 y) may gain more cognitive benefit from soy IFs in comparison to their older counterparts (Kritz-Silverstein et al., 2003). It has also been postulated that the effects of soy on health may be determined by an individual's equol producer status (Setchell et al., 2002). S-equol, a potent ligand for ER77 is an isoflavan formed exclusively via the bacterial conversion of daidzein in the intestine (Setchell et al., 2005) and is only produced by ~30% of western populations following a soy challenge (Lampe et al., 1998; Rowland et al., 2000; Setchell and Cole et al., 2006). The ability to produce equol has been associated with reduced VMS (Newton et al., 2015). Furthermore, S-equol supplementation alleviates hot flushes (Aso et al., 2012) and S-equol derivatives of soy IFs are now recommended by the North American Menopause Society for the non-hormonal management of VMS (North American Menopause Society, 2015), who have highlighted the need for further studies in this area. The aim of this study was to investigate the effects of a commercially available soy drink on cognitive function and menopausal symptoms in postmenopausal women.

SUBJECTS AND METHODS

Design

This 12-week parallel group, randomised, controlled trial was conducted between October 2015 and May 2018. All procedures were approved by Ulster University's Research Ethics Committee (REC/15/0025) and the study was registered at www.clinicaltrials.gov (NCT03561662). Participants were recruited throughout the province of Ulster and screening, baseline and post-intervention appointments were conducted before and after the 12-week intervention either at the university, the participant's home or at a location convenient for the participant. The duration of intervention was based on previous intervention studies in postmenopausal women that

have demonstrated that soy IF supplementation (60 mg/d) for 6 weeks significantly improves frontal lobe function (File et al., 2005), with significant improvements in sustained attention and long-term episodic memory additionally observed when supplementation is extended to 12 weeks (Duffy et al., 2003). The primary outcome of the study was effect on cognitive function and the secondary outcome was effect on menopausal symptoms. Sub-analysis investigated the effect of the intervention based on severity of VMS at baseline and also investigated cognitive function and menopausal symptoms according to equol producer status.

Participants

Eligible participants were apparently healthy women within 7 years post menopause (i.e. 1-7 y since last menstrual period). Postmenopausal status was confirmed based on a serum concentration of follicle-stimulating hormone (FSH) >30 mIU/ml (assessed via electrochemiluminescence immunoassay on a Cobas 8000 analyzer [Cobas 602 module], Roche Diagnostics at Antrim Area hospital). Exclusion criteria included: surgically induced menopause; habitual consumers of soya foods (> 2 serves/week); current use of HT or IF supplements; antibiotics use within the previous 3 months; current use of psychoactive medication; presence or history of cardiovascular disease, cancer, diabetes, thyroid, renal or kidney disease, alcohol or drug abuse; cognitive impairment as determined by a Mini Mental State Examination score <24; psychiatric distress as determined using a General Health Questionnaire-28 (Goldberg and Hillier, 1979) score of ≥26; red-green colour blind, assessed via the Ishihara test (as CANTAB testing requires colour recognition); abnormal full blood profile (assessed via a Sysmex KX21-N, Sysmex Ltd, UK at Ulster University) and/or insufficient renal/hepatic performance assessed via kidney and liver function tests (assessed photometrically via a Cobas 6000 analyzer [Cobas C501 module], Roche Diagnostics at Causeway hospital).

Intervention

An independent clinical trials manager used MINIM software (Minim, 2017) to randomise recruited women to one of three treatment groups with an allocation ratio of 1:1:1 Participants were asked to consume soy drinks (Alpro®) providing a low (10 mg/100ml), medium (35 mg/350ml) or high (60 mg/600ml) dose of IFs daily for a period of 12 weeks and could choose from different flavours of drink (original, unsweetened, chocolate or strawberry). Limited evidence suggests that IFs consumed in divided doses may be more effective in alleviating menopausal symptoms than a single dose (Kurzer, 2008; Crawford et al., 2013), thus women were advised to spread their intake throughout the day. The group that consumed the lowest dose of IFs was considered a low dose

control group as beneficial effects of soy IFs on postmenopausal health have previously been observed in intervention studies at much higher doses (Messins, 2014); furthermore, it was not possible to obtain a placebo control drink. Previous soy/IF intervention studies on cognitive function in postmenopausal women have used doses ranging from 60 – 160 mg IFs per day (Duffy et al., 2003; Kritz-Silverstein et al., 2003; File et al., 2005; Casini et al., 2006; Santos-Galduroz et al., 2010; Henderson et al., 2012; Kreijamp-Kaspers et al., 2004; Fournier et al., 2007; Ho et al., 2007; Basaria et al., 2009). This study utilised a dose that was achievable in a commercially available soy drink and at a volume that was easily incorporated into an individual's daily diet. Compliance was monitored by measuring plasma concentrations of soy IFs. Total genistein, daidzein and equol concentrations were assessed using LC-MS/MS by LGC Limited (Cambridgeshire, UK). Equol producers were defined as those with a plasma equol concentration of >20nmol/L (5 7g/L) (Setchell and Cole, 2006).

Dietary intake, anthropometrics and general health and lifestyle

Weight (kg) and height (cm), were measured at baseline and used to calculate BMI [weight (kg)/height $(m)^2$]. Body weight was measured to the nearest 0.1kg using Seca 770 electronic weighing scales (Brosch Direct Ltd, Peterborough, United Kingdom), without footwear and heavy clothing. Standing body height was measured to the nearest 0.1cm using a Seca 220 stadiometer (Seca Ltd, Hamburg, Germany). The participant stood without footwear, with their heels together, hands and arms hanging relaxed, and measurements were taken with the Frankfurt plane in a horizontal position. Dietary intake was assessed at baseline and post-intervention using a four-day food diary. Participants received instructions on how to complete the diary from a trained researcher and dietary intake was analysed using Nutritics nutritional analysis software (Nutritics, Research Edition v5.031). A general health and lifestyle questionnaire was completed by participants at baseline and provided information on age, gender, marital status, education level, occupation, smoking habits, alcohol use, dietary habits and physical activity.

Blood collection and processing

Fasted blood samples were collected by a trained phlebotomist before and after intervention for the analysis of serum FSH and plasma IF concentrations. Participants were instructed to fast from 10pm the night prior to blood sampling and water intake was encouraged. Fasted blood samples were obtained from the antecubital fossa using a 21-gauge butterfly needle and 8mL serum and 9mL ethylenediametetraacetic acid (EDTA) plasma vacutainer tubes (Greiner Bio-One GmbH, Kremsmunster, Austria). Following inversion, serum samples were allowed to clot for >60 min and plasma samples placed in

refrigeration until full blood profile analysis. Following this, all tubes were centrifuged at 2200rpm for 15 min at 4°C to allow separation of whole blood into its respective components. Following separation, serum and plasma samples were divided into aliquots and stored at -80°C until further analysis.

Cognitive function

Pre- and post-intervention cognitive function was assessed in the morning, after participants had consumed a standard, caffeine-free, breakfast. Cognitive function was assessed using the Cambridge Neuropsychological Test Automated Battery (CANTAB Research Suite; Cambridge Cognition, UK) (CANTAB, Cambridge Cognition 2015). CANTAB has been extensively validated for assessing brain-to-behaviour relationships in adult populations (Robbins et al., 1994; Robbins et al., 1998), has proven test-retest reliability (Louis et al., 1999) and is deemed suitable for use with older adults (Robbins et al., 1998). The following tests were used: spatial working memory (SWM), spatial span (SSP), pattern recognition memory (PRM), 5-choice reaction time (RTI) and match to sample visual search (MTS). The tests chosen activate areas of the brain that are associated with cognitive decline during the menopausal transition and that are sensitive to hormonal changes, including the hippocampus (Albert et al., 2017) and prefrontal cortex (Shanmugan and Epperson, 2014). SWM and SSP activate the temporal and frontal lobe regions of the brain; PRM activates the temporal lobe, hippocampus and amygdala; RTI, and MTS activate the fronto-striatal circuitry (Robbins et al., 1997). The procedure for assessing SSP, RTI and MTS are described in detail elsewhere (Simpson et al., 2005). Spatial working memory (SWM), a sensitive measure of frontal lobe and executive function, requires retention and manipulation of visuospatial information. The test began with 4 coloured squares (boxes) shown on the screen. Participants were required, by selecting boxes and using a process of elimination, to find one blue 'token' in each of 4 boxes (only one token is hidden at a time) and use them to fill up an empty column on the right-hand side of the screen. Touching any box in which a token has already been found is an error. The trial was then repeated three times with 4 boxes and then progressed to four trials with 6 boxes and four trials with 8 boxes. The colour and position of the boxes used are changed from trial to trial to discourage the use of stereotyped search strategies and a practice test was completed prior to testing. The outcome measure was SWM total errors i.e. the number of times a box is selected that is certain not to contain a token and therefore should have not been visited by the participant.

Visual memory was assessed using the Pattern Recognition Memory (PRM) test in a 2-choice forced discrimination paradigm. Participants were asked to remember a series of

12 abstract coloured patterns, each presented for 3s. They were then presented with a series of 12 pairs of old-new patterns and were asked to touch the pattern seen previously in each case. This procedure was repeated with a second set of 12 patterns followed by 12 pairs of patterns for recognition. The outcome measure was mean correct latency i.e. the mean time (milliseconds) to respond correctly.

Menopausal symptoms

The Greene Climacteric Scale (Greene, 2008) was used to assess menopausal symptoms at baseline and post-intervention. This 21-item scale provides three main independent measures of psychological, somatic and vasomotor symptoms. Participants were asked to indicate the extent to which they were currently bothered by the list of symptoms on a scale from 1 ‘not at all’ up to 4 ‘extremely’.

Statistical analyses

An a priori power calculation was conducted using spatial working memory total errors data obtained from the study of Thompson et al. (Thompson et al., 2005). Based on the probability of a type 1 error (7) = 0.05 and a Power of 0.9, 41 participants were required in each group to be able to reject the null hypothesis that the population means of the treatment groups are equal. To allow for dropouts, we aimed to recruit 150 women to the study. All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) with significance set at $P < 0.05$ throughout (IBM SPSS Statistics for Windows, version 24.0, IBM Corp, Armok NY). Only those participants that had completed cognitive testing at both baseline and post-intervention were included in the analysis. Intention-to-treat analysis was also performed including all participants randomised at baseline and did not change the primary outcome findings. The Shapiro-Wilk test was used to determine whether data followed a normal distribution and skewed variables were log-transformed to attain a normal distribution prior to analysis or analysed using non-parametric statistical tests. Transformations were applied to SWM, SSP, PRM, RTI and MTS data. Descriptive statistics were used to present participant characteristics at baseline. The effect of intervention on the primary outcome measures of cognitive function (SWM, SSP, PRM, RTI and MTS) were analysed using an analysis of covariance (ANCOVA) with baseline measures included as covariates. The secondary outcome measure of menopausal symptoms was analysed using a Kruskal-Wallis test. IF concentrations were compared between groups using a Kruskal-Wallis test with post-hoc analysis conducted using a Mann-Whitney U test. Two outliers with post-intervention genistein concentrations of > 800 ng/ml in the low dose treatment group and one in the high dose group (baseline genistein of 275 ng/ml) were removed prior to statistical

analysis of IF concentrations between groups. Sub-analysis was conducted to determine if the effect of the intervention on cognitive function and menopausal symptoms was significantly different between equol producers and non-producers using a Mann-Whitney U test. This test was also used in sub-analysis to investigate the effect of the intervention on VMS stratifying women according to severity of VMS at baseline. Dietary intake pre- and post-intervention was analysed using the Wilcoxin Signed Rank test.

RESULTS

A total of 101 postmenopausal women completed the study and were included in the final analysis. Participant progress through the study is illustrated in the CONSORT diagram (Moher et al., 2012) in Figure 1. Baseline demographic characteristics of the study participants are shown in Table 1. There was no significant difference at baseline between the groups for any of the characteristics presented. Table 2 shows the effect of the intervention on cognitive function; no significant differences were observed between the groups for any of the cognitive function outcomes measured (RTI, SSP, SWM, PRM, MTS). The soy drink had no effect on menopausal symptoms overall (Table 3), however, when women were stratified according to the severity of their VMS (hot flushes and night sweats) at baseline by splitting into two groups above/below mean, women with more severe VMS at baseline in the medium group had a significant reduction in symptoms after consuming the soy drink for 12 weeks, in comparison to those with less severe symptoms at baseline (Table 4). Furthermore, this observed reduction in symptoms was significantly greater in comparison to that observed in women with more severe VMS at baseline in the low dose control group (P = 0.015, Mann-Whitney U test) and the high dose group (P=0.044).

Compliance, as assessed via plasma IF concentrations appeared good (Table 5), albeit some women found it difficult to consume the instructed volume of soy drink in the high dose group, with seven women failing to complete the intervention for this reason. Blood samples were available for IF analysis for 95 participants at baseline and 87 post-intervention. IF concentrations were not significantly different between groups at baseline. As expected, post-intervention genistein concentration was significantly higher in the medium (P = 0.007) and high (P = 0.013) dose groups in comparison to the low dose group. Post-intervention daidzein was also significantly higher in the medium (P=0.006) and high (P=0.029) dose groups versus the low group. There was no significant difference in post-intervention IF concentrations between the medium and high dose groups. Some 28.7% of the cohort were classified as equol producers with n=7, n=9 and

n=9 participants classified as equol producers within the low, medium and high dose groups respectively. In sub-analysis cognitive performance was not significantly different according to equol status (Table 6) albeit, within the high dose group, spatial working memory improved in equol producers (change from baseline -9.44 ± 15.69, *n*=9) in comparison to non-producers (2.36 ± 13.24, *n*=23), though this effect did not reach significance (*P* = 0.066). VMS were significantly lower in equol producers in comparison to non-producers at both baseline (3.67 ± 1.01 vs 4.49 ± 1.60, *P* = 0.022) and post-intervention (2.96 ± 1.04 vs 3.86 ± 1.67, *P* = 0.046).

Energy intake, carbohydrate, protein and total fat intake were not significantly different between the low, medium and high dose groups at baseline or post-intervention (Table 7). In the high dose group, protein and total fat intake (expressed as % energy intake) were significantly lower post-intervention in comparison to baseline. Using the Goldberg cut-off technique (57), 12.8% and 15.7% of participants were identified as mis-reporters at baseline and post-intervention respectively. Of these, 3 were within the low, 2 within the medium and 5 within the high dose groups at baseline with 6 within the low, 2 within the medium and 3 within the high dose groups post-intervention.

DISCUSSION

In the current study, consumption of a soy drink for 12 wks, providing 35 or 60 mg of IFs/d, had no effect on visual memory, working memory or attention, in apparently healthy postmenopausal women in comparison to consumption of a low dose control providing 10 mg IFs/d. Menopausal symptoms were unaffected by soy drink consumption albeit, sub-analysis identified a potential beneficial effect of soy IFs in women with more severe VMS at baseline. In contrast to our primary findings, File et al. (File et al., 2005) observed an improvement in frontal lobe function (mental flexibility and planning) when healthy postmenopausal women, of similar age range to the current study, consumed an IF supplement (60 mg/d) for 6 wks in comparison to a placebo. This group previously performed a similar study investigating the effect of 12 wks supplementation (Duffy et al., 2003) and, in addition to improvements in frontal lobe function, long-term episodic memory and sustained attention significantly improved. Using the same dose of IFs as that of our high dose group (60 mg/d, 6 mo), Casini et al. observed an improvement in psychomotor performance as assessed by the Digit Symbol test in a placebo-controlled crossover study (Casini et al., 2006). In contrast, we observed no improvement in psychomotor speed as assessed via RTI albeit, the duration of supplementation in our study was shorter. In line with our findings, Casini et al. observed no significant effect on attention as assessed by the Visual Scanning test or on mental flexibility assessed by the

Digit Span test. Similarly, other studies that have assessed memory/attention using the Digit Span Test have observed no significant effects of IF supplementation (Santos-Galduroz et al., 2010; Kreijkamp-Kaspers et al., 2004; Fournier et al., 2007; Ho et al., 2007). In the only study conducted within an Asian population, Ho et al. found no effect of IF supplementation (80 mg/d for 6 mo) on Digit Span or other standard neuropsychological tests in healthy Chinese postmenopausal women (Ho et al., 2007). A notable difference of this study, however, was the mean habitual soy IF intake of 20 mg/d. Additional studies that report improvements in cognitive function with soy IF supplementation have used much higher doses and have intervened over a longer duration than that used in the current study (Krtiz-Silverstein et al., 2003; Santos-Galduroz et al., 2010; Henderson et al., 2012). Kritz-Silverstein et al. observed a significant improvement in verbal memory, as assessed by category fluency, following supplementation with 110 mg/d IFs for 6 mo (Kritz-Silverstein et al., 2003). In women aged 50-59 y a significant improvement was observed in Trails B, a test of visumotor tracking and attention, whereas this effect was not apparent in older women (60-74 y), suggesting that younger postmenopausal women may especially benefit from soy IFs. This finding is supported by Kreijkamp-Kaspers et al. who observed no significant effects on cognitive function, including Trailmaking B, in a cohort of older postmenopausal women, aged 60 – 75 y, following supplementation with 99 mg IFs daily for 12 mo (Kreijkamp-Kaspers et al., 2004). In the longest intervention trial conducted to date, supplementation of 91 mg IFs daily for 2.5 years had no effect on global cognition, executive function or verbal episodic memory in healthy postmenopausal women albeit, an improvement in a visual memory factor was observed (Henderson et al., 2012). In contrast to our study, there was a wide age range in this study (45 – 92 y) with almost half of the cohort > 10 years post menopause; sub-analysis suggested such women were less likely to show cognitive improvement and this may, therefore, have influenced the null findings in this study. Similar to our findings, Basaria et al. found no effect of 12 wks IF supplementation on cognitive function; albeit they did not assess visuospatial function and the IF dose used was 3 – 5 times higher than that used in the current study (Basaria et al., 2009).

To our knowledge, only one other study has investigated the effects of IFs on postmenopausal cognition in the form of a soy drink (Fournier et al., 2007). In this study, consumption of IFs via a drink (72 mg/d) or a supplement (70 mg/d) over 16 wks did not improve short-term memory, long-term memory, working memory or selective attention as assessed using tests similar to those used in our study. The soy milk group showed a poorer performance in verbal working memory in comparison to the supplement and

control groups; albeit this study was subject to limitations including subjective compliance, lack of power and lack of controls, owing to the quasi-experimental design.

Variations in study design make it difficult to draw direct comparisons between the findings of our study and previous work. Although two studies utilised CANTAB to assess cognitive function (Duffy et al., 2003; File et al., 2005), neither used the same tests as the current study. Given the wide range of methodologies currently available to assess cognitive function, there is a need to identify a standard method and testing suite to enable better comparison between studies and reliably inform scientific knowledge in this area. We did not reach our recruitment target of 41 participants/group, largely due to participants not meeting the stringent inclusion criteria (of note was the substantial number of postmenopausal women currently on antidepressants) however, the study was still adequately powered (80% power) to detect significant effects. Reference ranges are not provided for the CANTAB tests used for healthy populations however, our data concur with a previous study in healthy older women (Lenehan et al., 2016).

In agreement with previous literature, 28.7% of our study cohort were equol producers (Lampe et al., 1998; Rowland et al., 2000; Setchell and Cole, 2006). Limited evidence suggests that the ability to produce equol may confer beneficial effects on cognitive function following soy intake (Igase et al., 2017), potentially via increased cerebral blood flow (Yu et al., 2016). We did not observe any significant differences in cognitive performance following intervention between equol producers and non-producers although, within the high dose group, improvements in spatial working memory in producers versus non-producers was approaching significance. Our findings support those of Henderson et al. (Henderson et al., 2012) who observed a nonsignificant trend towards improved global cognition in consistent equol producers. Similar to Henderson's study, the sample size in the current study was likely too small to adequately investigate the role of equol in cognitive performance and further research in this area is warranted.

In agreement with our findings, previous studies, including 2 that used the same scale as that in the current study (Duffy et al., 2003; File et al., 2005), have reported no effect of IF supplementation on menopausal symptoms (Duffy et al., 2003; File et al., 2005; Ho et al., 2007). Basaria et al. observed an improvement in menopausal symptoms using the Menopause-specific Quality of Life questionnaire however, the dose used in this study was very high (160 mg/d) (Basaria et al., 2009). A recent meta-analysis has demonstrated that soy IF supplementation can significantly reduce hot flush frequency and severity in comparison to placebo, with supplements containing > 18.8 mg of genistein being most effective (Taku et al., 2012). In support of these findings, our study has shown that women

with more severe VMS at baseline showed a significant improvement in symptoms following consumption of 35 mg IFs/d (providing ~18 mg genistein) in comparison to those consuming 10 mg/d. This observation was not replicated in the high dose group, possibly due to the sample size, thus further studies are required to confirm these findings. Our findings in the medium group are in agreement with an open-label crossover study that demonstrated a commercial soy drink (ViveSoy®), providing ~50 mg IFs per day for 12 wks, improved climacteric symptoms including hot flushes in peri/postmenopausal women (Tranche et al., 2016). Previous research on the effects of soy foods on menopausal symptoms is limited (Levis and Griebeler, 2010) and our findings warrant further study in this area. In support of the role of S-equol in the alleviation of VMS (Newton et al., 2015), equol producers in the current study had significantly lower VMS scores throughout in comparison to non-equol producers.

Strengths of this study include the use of digital cognitive assessment, CANTAB. Cognitive assessment was strictly controlled with participants consuming the same standard caffeine-free breakfast prior to testing at both timepoints. Time since menopause was kept within a defined limit of < 7 y and compliance was closely monitored. The main limitations of the study were the absence of a placebo control and the non-blinded study design. Nonetheless, the low dose control drink provided only 10 mg IFs/d, an amount well below that typically consumed in Asian populations (~25 – 50mg/d) (Messina et al., 2006) and well below the lowest dose previously used in intervention studies on cognitive function in postmenopausal women (60 mg/d) (Duffy et al., 2003; File et al., 2005; Casini et al., 2006). Given that we used a commercially available soy drink in the current intervention study, we were limited with regard to the dose of IFs that could be tested. Nonetheless, it is important to investigate the effects of soy foods in addition to IF supplements as consumers may gain additional nutritional benefits from consuming soy food as part of a healthy balanced diet (Messina, 2016). Furthermore, IF pharmacokinetics are similar following ingestion of soy foods and IF supplements (Setchell et al., 2011).

In conclusion, a commercially available soy drink had no effect on cognitive function in postmenopausal women. Consumption of ~350ml/d (providing 35 mg IFs) for 12 wks significantly reduced VMS in those women with more severe symptoms at baseline, a finding with potential clinical relevance that warrants further research given the high prevalence of VMS in western women (Archer et al., 2011; Thurston et al., 2011).

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TABLE 1

Baseline participant characteristics[1,2].

	Soy IF treatment group		
Measure[3]	Low *(n=35)*	Medium (*n=37)*	High (*n=29)*
Age, y	53.69 ± 3.72[4]	53.86 ± 3.28	53.72 ± 4.62
Height, m	1.61 ± .065	1.64 ± .062	1.63 ± .073
Weight, kg	70.32 ± 12.36	71.48 ± 13.85	72.91 ± 14.86
BMI, kg/m^2	26.97 ± 5.22	26.76 ± 5.73	27.37 ± 5.77
Non-Smokers (%)	94.12	100	100
Alcohol, units/wk	7.64 ± 6.58	11.73 ± 12.60	7.44 ± 10.21
Education level (n)			
Primary	0	1	0
Secondary	19	13	12
Tertiary	15	21	16
PA, METs/week	165.17 ± 56.32	184.70 ± 57.48	192.74 ± 76.98
LMP, mo	31.97 ± 19.05	39.33 ± 21.99	39.38 ± 23.25
FSH, IU/ml	83.02 ± 34.44	88.79 ± 28.33	85.84 ± 33.84
GHQ-28	16.91 ± 4.82	14.73 ± 5.48	15 ± 4.6
MMSE	29.17 ± .79	29.03 ± 1.07	29.21 ± .73

[1] Characteristics were not significantly different between treatment groups.

[2]FSH, follicle stimulating hormone; GHQ-28, General Health Questionnaire-28; LMP, time since last menstrual period; METs, metabolic equivalents; MMSE, Mini Mental State Examination; PA, physical activity

[3] Data on smoking, alcohol use, education and physical activity was unavailable for 4 participants (n = 1, n = 2 and n= 1 in the low, medium and high dose groups respectively) as they failed to complete the health and lifestyle questionnaire.

[4] mean ± SD (all such values with the exception of smoking and education)

Table 2

Cognitive function of postmenopausal women at baseline and following 12 weeks' soy drink intervention[1].

Treatment group	Low *(n=35)*			Medium (*n=37)*			High (*n=29)*		
Cognitive test[2]	Baseline	Week12	*change from baseline*	Baseline	Week 12	*change from baseline*	Baseline	Week 12	*change from baseline*
RTI	373.66 ± 51.83[3]	367.06 ± 59.34	-6.60 ± 55.11	345 ± 45.66	354.15 ± 67	9.15 ± 55.07	349.33 ± 61.70	363.99 ± 70.79	14.66 ± 52.64
SSP	5.51 ± 1.06	5.77 ± 1.14	0.25 ± 1.22	5.65 ± 1.18	5.68 ± 1.23	0.03 ± 1.55	5.62 ± 1.18	5.76 ± .83	0.14 ± 1.36
SWM	33.86 ± 19.09	29.11 ± 16.95	-4.74 ± 15.74	28.7 ± 17.59	28.92 ± 17.85	0.22 ± 17.42	30.45 ± 16.04	27.90 ± 16.08	-2.55 ± 15.07
PRM	2054.25 ± 395.87	2083.14 ± 544.32	28.89 ± 417.47	2138.94 ± 587.38	1903.244 ± 434.09	-235.7 ± 557.83	1920.65 ± 458.59	1969.56 ± 568.19	48.91 ± 565.60
MTS	2836.70 ± 732.05	2518.54 ± 735.28	-318.16 ± 585.35	2846.98 ± 796.82	2456.46 ± 606.2	-390.52 ± 814.11	2694.54 ± 597.66	2535.90 ± 607.23	-158.64 ± 572.23

[1] No significant differences were observed between the groups for any of the outcomes measured using an ANCOVA to compare post-intervention (week 12) cognitive function with baseline measures as covariates.

[2]RTI, five-choice reaction time measured as reaction time latency (milliseconds); SSP, spatial span measured as longest sequence length recalled correctly; SWM, spatial working memory measured as total errors made; PRM, pattern recognition memory measured as mean correct latency (milliseconds); MTS, match to sample visual search measured as mean correct reaction time (milliseconds).

[3] mean ± SD (all such values)

Table 3

Menopausal symptoms at baseline and following 12 weeks' soy drink intervention[1].

Treatment group	Low *(n=34)*			Medium (*n=35)*			High (*n=27)*		
Greene's Climacteric scale	Baseline	Week12	*change from baseline*	Baseline	Week 12	*change from baseline*	Baseline	Week 12	*change from baseline*
Psychological score	18.29 ± 4.00[2]	17.47 ± 4.37	-0.83 ± 5.10	18.51 ± 4.92	16.40 ± 4.11	-2.11 ± 4.19	17.02 ± 3.55	15.93 ± 3.59	-1.10 ± 3.89
Vasomotor score	4.46 ± 1.58	3.97 ± 1.73	-0.49 ± 1.18	4.50 ± 1.54	3.49 ± 1.27	-1.01 ± 1.84	3.54 ± 1.22	3.37 ± 1.69	-0.17 ± 2.02
Somatic score	9.79 ± 1.81	9.29 ± 2.28	-0.49 ± 2.50	9.71 ± 2.30	9.49 ± 2.13	-0.23 ± 2.68	9.77 ± 2.64	9.26 ± 3.37	-0.51 ± 3.52
Total score	34.72 ± 5.37	32.76 ± 6.52	-1.96 ± 6.83	34.87 ± 7.31	31.29 ± 6.10	-3.58 ± 6.87	32.52 ± 6.42	30.52 ± 7.91	-2.00 ± 8.62

[1] No significant differences were observed between the groups for any of the symptoms assessed as determined using a Kruskal-Wallis test.

[2] mean ± SD (all such values)

Table 4

Effect of soy drink intervention on vasomotor symptoms stratifying women according to severity of symptoms at baseline.

Treatment group	Low *(n=34)*				Medium (*n=35)*				High (*n=27)*			
	Baseline	Week12	*change from baseline*	*P*[1]	Baseline	Week 12	*change from baseline*	*P*	Baseline	Week 12	*change from baseline*	*P*
Vasomotor symptoms at baseline												
Less severe	3.37± 0.90[2]	3.05 ± 0.97	-0.32 ± 1.06	0.290	3.22 ± 0.73	3.28 ± 1.18	0.06 ± 1.21	0.001	2.64 ± 0.50	3.14 ± 2.11	0.50 ± 1.99	0.096
More severe	5.83 ± 1.10	5.13 ± 1.81	-0.70 ± 1.33[a]		5.85 ± 0.83	3.71 ± 1.36	-2.15 ± 1.73[b]		4.51 ± 0.99	3.62 ± 1.12	-0.89 ± 1.88[a]	

[1] Mann-Whitney U test comparing change from baseline of those women with more severe vasomotor symptoms at baseline to those with less severe symptoms within groups
[2]mean ± SD (all such values)
[a,b] values with different superscript letters across a row are significantly different (Kruskal-Wallis with post-hoc Mann-Whitney U Test)

Table 5

IF concentrations at baseline and following 12 weeks' soy drink intervention.

Treatment group	Low		Medium		High	
	Baseline (*n*=32)	Week12 (*n*=32)	Baseline (*n*=35)	Week 12 (*n*=32)	Baseline (*n*=28)	Week 12 (*n*=23)
genistein (ng/ml)	10.01± 22.07[1]	82.75 ± 124.62[a]	9.83 ± 24.25	168.71 ± 166.81[b]	6.40 ± 9.27	216.71 ± 305.36[b]
daidzein (ng/ml)	5.03 ± 11.88	20.44 ± 30.82[a]	2.29 ± 4.37	39.20 ± 36.9[b]	2.50 ± 3.34	49.82 ± 66.49[b]
equol (ng/ml)	1.76 ± 6.01	3.81 ± 8.17[a]	BLD[2]	9.43 ± 19.84[a]	0.42 ± 1.30	11.69 ± 19.23[a]

[1] mean ± SD (all such values)

[a,b] values with different superscript letters across a row are significantly different (Kruskal-Wallis with post-hoc Mann-Whitney U Test)

[2] BLD, below limit of detection

Table 6

Effect of soy drink intervention on cognitive performance stratifying women according to equol producer status.

Cognitive test[1]	Change from baseline		
	Equol non-producers *n*=62	Equol producers[2] *n*=25	*P*[3]
RTI	6.23 ± 54.98[4]	-6.23 ± 41.30	0.764
SSP	0.21 ± 1.34	0.12 ± 1.42	0.735
SWM	-1.82 ± 15.34	-6.16 ± 16.36	0.245
PRM	-45.58 ± 533.00	-62.66 ± 557.10	0.453
MTS	-363.39 ± 675.51	-163.99 ± 630.38	0.234

[1]RTI, five-choice reaction time measured as reaction time latency (milliseconds); SSP, spatial span measured as longest sequence length recalled correctly; SWM, spatial working memory measured as total errors made; PRM, pattern recognition memory measured as mean correct latency (milliseconds); MTS, match to sample visual search measured as mean correct reaction time (milliseconds).

[2] defined as plasma equol concentration > 20nmol/L (51g/L)

[3]independent samples t-test or Mann-Whitney U test for non-parametric data

[4] mean ± SD (all such values)

Table 7

Dietary intake at baseline and following 12 weeks' soy drink intervention.[1,2]

Treatment group	Low			Medium			High		
	Baseline (n=27)	Week12 (n=28)	*P*[3]	Baseline (n=27)	Week 12 (n=21)	*P*	Baseline (n=24)	Week 12 (n=21)	*P*
Energy (Kcal/d)	1532 ± 451.72[4]	1506 ± 526.37	0.715	1623 ± 549.91	1792 ± 665.34	0.845	1562 ± 501.51	1731 ± 583.89	0.163
Carbohydrate (g/d)	165.52 ± 47.76	154.21 ± 46.65	0.144	165.67 ± 44.26	162.43 ± 50.24	0.500	173.38 ± 63.37	170.00 ± 54.52	0.472
Protein (g)	74.74 ± 42.49	72.39 ± 24.59	0.726	75.85 ± 33.37	71.90 ± 28.28	0.184	77.21 ± 27.46	70.71 ± 32.31	0.139
Fat (g)	61.07 ± 25.96	59.86 ± 18.31	0.935	59.56 ± 16.74	65.40 ± 23.34	0.887	61.21 ± 23.32	59.00 ± 25.08	0.868
Carbohydrate (% EI)	44.32 ± 9.85	45.74 ± 28.91	0.068	42.52 ± 8.99	37.60 ± 7.82	0.184	44.26 ± 5.31	41.00 ± 11.35	0.744
Protein (% EI)	15.55 ± 2.55	18.28 ± 12.52	0.563	15.27 ± 3.42	15.37 ± 5.84	0.500	15.46 ± 2.13	13.59 ± 3.67	0.018
Fat (% EI)	42.76 ± 17.35	47.89 ± 25.98	0.503	42.11 ± 11.52	38.67 ± 12.85	0.112	46.94 ± 19.53	37.71 ± 13.52	0.022

[1]Nutrient intake did not differ between the groups at either baseline or post-intervention (Kruskal-Wallis test).

[2] EI, energy intake

[3] Wilcoxin Signed Rank Test comparing week 12 to baseline within groups

[4] mean ± SD (all such values)

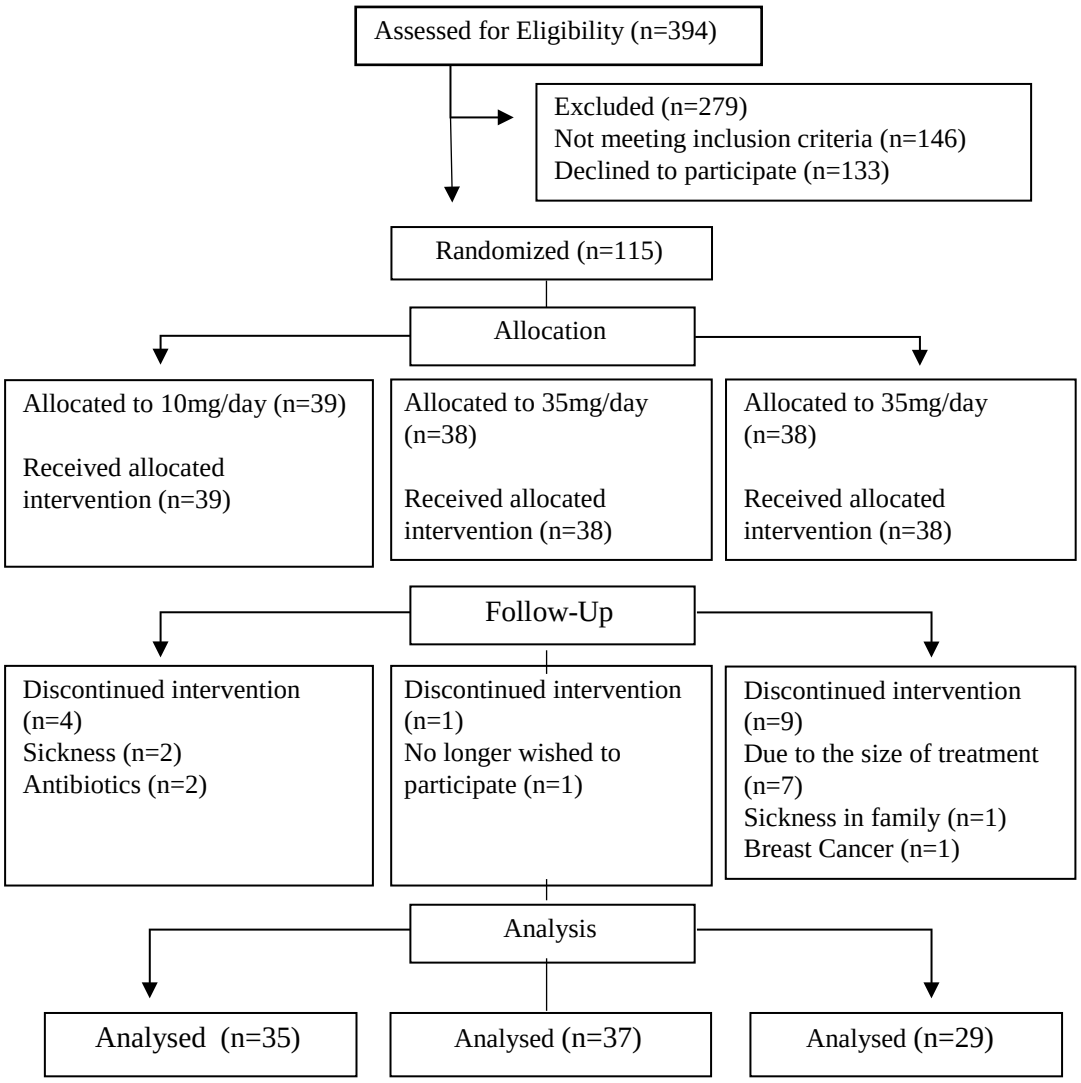

Figure Caption

Fig.1 CONSORT diagram of participant flow. A total of 394 women were assessed for eligibility with 279 excluded due to not meeting the inclusion criteria (n=146) or not wishing to participate in the study (n=133). Remaining postmenopausal women were randomised to receive a soy drink (Alpro®) and asked to consume a volume providing either 10 mg (n=39), 35 mg (n=38) or 60 mg (n=38) IFs daily. A total of 14 participants were lost to follow up owing to illness (n=3) unrelated to the intervention, antibiotic use (n=2), no longer wishing to participate (n=1), family illness (n=1) or being unable to consume the soy drink (n=7). A total of 101 women completed the study and were included in the final analysis

Chapter 5

Attitudes of post-menopausal women towards soya food consumption following an intervention study.

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Abstract

The number of studies evaluating consumer perceptions is increasing as understanding consumer acceptance is a key aspect of consumer choice and attitude. Soya foods have many proven health benefits for the general population, and specifically for postmenopausal women, albeit soya consumption among western populations is low. The aim of this study was to evaluate the attitudes of post-menopausal women to soya consumption following an intervention study. Three focus groups were conducted in post-menopausal women who had recently participated in a randomised trial where they consumed a specified volume of a soya drink (Alpro®) every day for 12 weeks. Focus groups were designed to represent individuals from each of the three treatment groups within the study; group 1 consumed 100 ml/day, group 2 consumed 350 ml/day and group 3 consumed 600ml/day. Focus groups included discussion on overall knowledge of Alpro® soya, attitude towards soya drinks, advantages and disadvantages of adding soya to the diet and enablers/barriers to soya consumption. Eight common themes were identified using thematic analysis. Barriers to soya consumption included the image of soya, lack of familiarity with the product, and sugar content of the products. Advantages of soya included the healthfulness and fortification of micronutrients. Soya consumption was perceived to be associated with allergies, a healthful change in lifestyle or adoption of a vegetarian diet. Educating consumers about soya and improving the image of soya may increase consumption in western populations.

Introduction

Clinical and epidemiological evidence demonstrates beneficial effects of soya foods and isoflavones on post-menopausal health, specifically breast cancer, cardiovascular disease, cognitive function and menopausal vasomotor symptom relief (Casini et al., 2006, Duffy et al., 2003, File et al., 2005, Messina, 2014). The North American Menopause Society recommends S-equol derivatives of soya isoflavones for the non-hormonal management of vasomotor symptoms (North American Menopause, 2010).

As there is clear evidence of health benefits associated with the addition of soya to the diet, further investigation into consumer attitudes and opinions of soya is imperative in populations who may benefit from its use. In spite of health claims and benefits associated with soya consumption, intake still remains low in western populations in comparison to Asian populations. Isoflavone intake is approximately 25-50mg/day in Japanese populations but is <3mg/day in European and western populations (Messina et al., 2006; Bai *et al,* 2014; Zamora Ros *et al* 2012). Therefore, a clear understanding of motivators and barriers to soya consumption is imperative. Investigating barriers and enablers of soya consumption alongside evaluation of a soya intervention with qualitative methods using a systematic approach allows for key information from participants to be utilised.

Focus groups are useful for collecting valuable information from the public/research participants and can be utilised to investigate consumer attitudes and to evaluate interventions in order to produce more effective research protocols (Leung et al., 2019). This method involves using valuable data around methodology and participants' perceptions of research trials. The use of this systematic approach, incorporating thematic analysis, to evaluate human intervention trials is imperative for development of future research trials, particularly randomised trials, in ascertaining the quality of results. Many interventions are complex, made up of multiple components, and delivered on multiple levels (Mackenzie et al., 2018). Understanding the use of a systematic approach may improve evaluation of complexities within study designs (De Silva et al., 2014) thus improving the design of future studies. The evaluation process ensures for the development in the administration of adequate more robust interventions to attain robust results. Intervention evaluations implicate changes such as different methodology, educating people about benefits of soya, awareness of soya in menopause, sample size, study type/design, intervention time period, and methods of data collection, ensuring effective trials to address the relevant clinical question (Fletcher, 2002). Changes to intervention methods, which can be adopted through evaluation, successfully address research question issues that arise.

Evaluating interventions requires sophisticated methods in order to establish what has been achieved and how it has been achieved, uncovering strengths and weaknesses. Further investigating whether the most effective methods were used and whether it was conducted in the most effective way are all outcomes of evaluating interventions and have the potential to have a major impact in future trials adopting similar approaches and outcomes (Richard A. Krueger, 2009). These methods include, observing a change in attitudes, discussing research questions, observing behaviour changes and interpreting written questionnaires. Assessing an intervention process involves comprehensive evaluation of what occurred during implementation and creating a judgement on efficacy. Effectiveness is evaluated through assessing questions; did you achieve your goal/outcome as quickly as possible and cost efficiently (Kitzinger, 1995). A good theoretical understanding is required of how the intervention causes change, therefore weaker components can be addressed and changed (Craig and Petticrew, 2013). Achieving the outcome may for instance not have been carried out as efficiently as possible so therefore this needs to be evaluated so that changes can be implemented. Self-evaluation and feedback are fundamental in assessing interventions. Receiving feedback is essential in monitoring performance and can be carried out in many ways through questions and anonymous feedback (Scriven, 2010, Parry-Langdon et al., 2003, Craig et al., 2008).

This evaluation process is imperative in research interventions influencing future more robust studies. Impacts arising from this process include improving your own practice; helping others improve their practice through breaking down your own evaluation; justifying your methodology and providing evidence to support as well as identify any unplanned or unexpected findings that may be significant for future trials (Scriven, 2010). Evaluating interventions has an impact on public health, medical evidence and future studies which rely heavily on efficacy of intervention impact, therefore this process is imperative and has major impact on research, effectiveness, and implementation of trials.

A limited number of focus group studies have been conducted to date investigating attitudes and opinions towards soya. Schyver et al, 2005., investigated attitudes and beliefs towards soya in consumers *vs* non consumers (Schyver and Smith, 2005). Major themes identified were: barriers to soya consumption; promotors of soya consumption; perceived health attributes of soya and suggestions on how to increase soya consumption. Sub-themes generated highlighted: soya's overall image, availability and cost as well as ways to increase overall soya consumption through consumer attitudes. The main barriers to soya consumption were price, not knowing how to prepare it and the unfavourable

image of soya. The main promoter of consumption was the initiation of a major change in lifestyle of individuals. Participants were aware of the healthfulness perception associated with soya, similar to findings in a focus group study investigating attitudes to soya in French and Vietnamese cohorts. In this study by Tu et al., (Tu et al., 2012) however, individuals were not sure why soya was perceived as healthy. A commonality among focus group studies conducted on soya is the association with the product being produced exclusively for vegetarians or those who have an intolerance to dairy milks. Price was also a determinant of soya consumption among the Vietnamese cohort, particularly in comparison to dairy product prices. Similarities among these focus groups show the importance for more focus groups to be conducted in this area. Furthermore, findings have highlighted the need for education on the health benefits of soya for the population.

This focus group study was designed in order to evaluate the attitudes of post-menopausal women towards consumption of soya and to partly evaluate the overall effectiveness of a 12-week soya drink intervention in which participants had recently participated.

Materials and methods

The present study utilised participants from a 12-week randomised trial conducted at Ulster University, Coleraine, Northern Ireland. All procedures were approved by Ulster University's Research Ethics Committee (REC/15/0025) and the study was registered at www.clinicaltrials.gov (NCT03561662). The study was undertaken between the months of October 2015 to June of 2018 and was designed to examine the effects of soya on cognitive function and menopausal symptoms in post-menopausal women. Women aged 44-63 y, within 7 y post menopause were randomly assigned to consume a volume of Alpro® soya drink providing a low (10 mg isoflavone; control group), medium (35 mg isoflavone) or high (60 mg isoflavone) dose of isoflavones daily for a period of 12 wks. Women that completed the intervention study were invited to participate in the focus group study. Focus groups were designed to represent individuals from each of the three treatment groups from the intervention. Each focus group consisted of 2-5 participants. Focus groups were held in a suitable room in the university and refreshments were provided. Focus groups were led by a trained researcher and assisted by a research assistant. The outline of the focus groups is provided in Table 1. The focus groups were audio-recorded and then transcribed verbatim. All responses were kept anonymous.

Participants

Eligible participants were apparently healthy women within 7 years post menopause

(i.e. 1-7 y since last menstrual period). Baseline characteristics are shown in table 1. Postmenopausal status was confirmed based on a serum concentration of follicle-stimulating hormone (FSH) >30 mIU/ml (assessed via electrochemiluminescence immunoassay on a Cobas 8000 analyzer [Cobas 602 module], Roche Diagnostics at Antrim Area hospital). Exclusion criteria included: surgically induced menopause; habitual consumers of soya foods (> 2 serves/week); current use of HT or IF supplements; antibiotics use within the previous 3 months; current use of psychoactive medication; presence or history of cardiovascular disease, cancer, diabetes, thyroid, renal or kidney disease, alcohol or drug abuse; cognitive impairment as determined by a Mini Mental State Examination score <24; psychiatric distress as determined using a General Health Questionnaire-28 (Goldberg and Hillier, 1979) score of ≥26; red-green colour blind, assessed via the Ishihara test (as CANTAB testing requires colour recognition); abnormal full blood profile (assessed via a Sysmex KX21-N, Sysmex Ltd, UK at Ulster University) and/or insufficient renal/hepatic performance assessed via kidney and liver function tests (assessed photometrically via a Cobas 6000 analyzer [Cobas C501 module], Roche Diagnostics at Causeway hospital). Women who participated in the focus groups were health conscious individuals who maintained a healty lifestyle.

Focus groups were used as the method of data collection as they provide insight into topics and perceptions about soya drinks that otherwise may not have been known. Focus

groups have the ability to produce opinions from similar groups of people at the same time.

Data Collection

Open ended questions were used based on the information we were interested in clarifying about soya products through the focus groups (Table 2). Focus groups included discussing familiarity with soya drinks, advantages/disadvantages, continued consumption likelihood, enabling factors of consumption, barriers to soya consumption and lastly, did soya drinks aid in menopausal symptoms. Audio recorded transcripts were transcribed manually verbatim. Transcriptions were reviewed by a research assistant to verify that recording had been transcribed correctly. Researchers met to debrief on the content discussed to identify emerging themes.

Thematic Analysis

Thematic analysis is a widely-used qualitative analytical method (Roulston, 2001) particularly in psychology, used in identifying, analysing and reporting themes within data (Tuckett, 2005). Qualitative approaches are incredibly diverse, thematic analysis is one of the foundational methods for qualitative analysis. Thematic analysis is carried out through a step by step process considering all elements of data collected in focus groups. This theory driven approach was conducted by two researchers in parallel, coding and identifying concepts based on themes generated, agreeing on interpretation of the material. The thematic analysis process advised by Braun and Clarke (Braun, 2006, Clarke and Braun, 2013) was carried out in the current study. Phases involved in thematic analysis include; familiarising yourself with the data and understand the research question. This is carried out by reviewing data to ensure that initial ideas and prominent themes are recorded in writing. Steps involved in this process include generating initial codes, coding interesting features across the whole data set collating important themes, and lastly, defining these themes. This process allows for ongoing analysis generating clear themes producing the report, this is the final analysis of selected extracts, relating to research question and literature.

The first step of thematic analysis is when the analyst begins to identify, look for reoccurring patterns of meaning, of interest in the data. Unlike statistical analysis, writing ideas and potential coding is integral of thematic analysis, with a constant back and forth between the entire dataset representing the flexibility in this method of data investigation. Qualitative analysis guidelines are not rules, although following basic phases allows application to fit the research question. With this recursive process moving back and forth

across phases of data analysis it allows for the development over time (Clarke and Braun, 2017). Researchers have used this method to explore a wide variety of experiential research questions which vary in sample size, data collection, methods and meaning generation (Clarke and Braun, 2013). By using thematic analysis it generates a link in concepts and opinions among participants and allows data to be comparable to other data collected from other studies (Alhojailan, 2012). Another strength of utilising thematic analysis is allowing full submersion of data and eradicating precipitous conclusions from the onset of analysis. Overall thematic analysis allows you to gather a clear logical understanding over narrative data which allows for interpretation of participants' thoughts and conveys their experience in relation to themes generated and the overall research question. Furthermore, a greater understanding of participants' attitudes and reflections on specific issues through this method prevents calculating unambiguous words or statements for expressing ideas and generates a more robust understanding of attitudes within specific cohorts (Alhojailan, 2012).

Results and discussion

Intervention Evaluation

It was evident through evaluation of the intervention that women had a heightened awareness of soya. Women expressed they will be continuing to consume soya and would even recommend it to a friend. The intervention also successfully encouraged women to incorporate soya into the diet in many different ways, ie. tea/coffee and cereals. Women also expressed they had a relaxed feeling from taking part in the study, therefore the intervention process was successful in terms of generating a further willingness within volunteers to contribute to further research in the future. It was also evident, regardless of dose, through the focus group evaluation that participants felt 12 weeks was a long period of time to be consuming daily doses of soya drink. This may be owing to the fact that larger doses of soya hindered daily consumption and therefore it was concluded that future studies in this area should combine supplements and soya drink to attain the dose required for effect within interventions. The soya drink was also referred to as a "filling" drink and larger doses were difficult to consume on a daily basis, this confirms the need to consider a change in methodology in terms of the supplement matrix used in interventions using soya supplementation.

Attitudes and perceptions of soya

The analysis resulted in 8 final themes (see Table 3); knowledge of Alpro® products; impressions of soya products; advantages of Alpro® soya in diet; disadvantages of

Alpro® soya in diet; continue to consume; enablers to consumption; barriers to consumption and menopausal symptoms. Combined thematic analysis from all three focus groups was carried out and results were amalgamated due to the consistencies that were evident throughout each of the focus groups. The major findings of our study identified consumer attitudes towards soya including barriers to regular consumption, enablers of continued use a healthful impression and an unhealthful impression. There are a limited number of studies utilising focus groups to investigate perceptions towards soya and also little evidence in the area of post-menopausal women. Two focus group studies have been conducted in post-menopausal women investigating perceptions and experience of the menopause (Fox-Young et al., 1999) and women's decision making at the time of the menopause (Alfred and Kazakov, 2006), neither of which mention soya in relation to the menopause.

Knowledge of Soya products

Sub themes generated from this major theme included overall familiarity with soya products. This sub theme represents the overall opinions and attitudes gathered from post-menopausal women and encompasses whether soya was known to participants or not. Women who were aware of soya further mentioned whether they had tasted the product or not. Familiarity of soya milk was low among the three focus groups, see Table 3. Soya was unknown to an equal number of those who were familiar with the product with very few previously tasting the product before. There was some familiarity with soya desserts among the focus groups. There was a small number of women throughout the three focus groups who had tried soya before.

Impressions of Soya products

Two sub themes were generated from overall impressions of soya milk, these include healthful impression and flavour. In addition, women expressed a consistent healthful impression of soya and interestingly soya was perceived as a product which is a "healthy alternative" for vegetarians or those with allergies (see Table 3), an alternative to dairy products, as well as improving women's health, all similar findings which were reported in previous focus group studies reporting attitudes and beliefs about soya (Schyver and Smith, 2005, Tu et al., 2012). Both of these focus group studies express benefits of soya on menopausal health. One participant during the focus groups stated;

"I had some vague awareness of soya being used as an alternative to dairy"

Although there was an awareness that soya products are healthful, no one was able to report benefits associated with cardiovascular disease. Participants had a vague

understanding that soya may be good for menopausal symptoms as well as have a beneficial effect in terms of memory and have lower calories that dairy milk. Participants debated about the flavours of the soya drinks (see Table 3 discussed as sub theme flavour).

Quotes below from participants during focus group discussion about flavour indicated a differing opinion;

"I didn't like it to taste"

"I like those creamy sort of flavours"

Participants agreed that one of the sole enablers to consumption was the flavour of the original soya drink. Although there was concern about the sweetness of strawberry and chocolate flavours available, the "creamy" flavour of the original soya drink was the greatest factor in increasing consumption. Further discussion about the flavoured products identified concerns in relation to content of sugar and an unnatural taste.

Advantages of adding soya to the diet

There were several sub themes generated throughout the focus groups considering advantages of adding to the diet. These included "fortification"; "source of protein", and "reducing dairy farming". A common finding among all of the focus groups was the "feel good factor" (see Table 3) or participation in an intervention which potentially could benefit other women. Contribution to research and taking part in a study was considered one of the main advantages of adding soya to the diet. Soya products were also perceived to be a good source of micro nutrients essential for the diet, such as vitamin-D and calcium, as well as a good source of protein. Although this was mentioned, participants did enquire with researchers what the difference was in protein content between whole milk and the soya drink. In addition, it was eluded to that soya consumption enhanced breast size, prevented cancer, and was a good source of isoflavones for the prevention of menopausal symptoms.

Disadvantages of adding soya to the diet

Many of the participants thought that soya added to the diet was filling and so associated it with weight gain (see Table 3), one response below from a participant highlights this;

"I had said to (name omitted) that I thought I had put on weight"

One of the other main disadvantages of adding soya to the diet was that it was not preferred in tea or coffee over whole milk. As many of the women only consumed dairy milk in tea and coffee, it was mentioned that it was difficult to add a milk alternative to the diet when they did not habitually drink milk.

"I didn't like it in tea and coffee"

Consumption in the diet

Although there was a mixed opinion about soya during the intervention, many of the participants agreed that they would and already have recommended soya products, particularly if they knew it would help with menopausal symptoms (see Table 3). As part of the intervention, women incorporated soya into their diet in many ways. Participants mostly added it into their diet by using it in tea and coffee and breakfast cereals. There are many other ways that soya was introduced into the diet including drinking as a shake, drinking in lattes, or as a snack on the go.

Enablers to consumption

One major theme generated "incorporation into the diet" presented common ways in which women added soya to the diet. Below are several quotes from participants during the focus group that indicate ways in which they incorporated soya into the diet;

"I would use mine as a milky coffee, I actually preferred the soya in coffee now"

"Yea I put it in to make my porridge, half milk half water"

One of the strongest enablers for consumption of soya was the brand itself, participants associated Alpro® soya with a good brand associated with healthy foods. Many of the participants would buy soya specifically when it was on offer. Carton sizes played a crucial role in enabling consumption of soya due to lack of users in a household. If there was a smaller carton size available in local shops they would buy soya more frequently (see Table 3). Buying 1L cartons for single users in homes eluded to increase of waste of the product. When more people are consuming the product in the home it is a massive enabler for consumption. Longer shelf-life products as well as flavour are also enabling consumption of soya.

Barriers to consumption

Sub themes that were generated through thematic analysis under barriers to consumption include cost; sugar content, and a bad impression of soya brand (see Table 2). The identified barriers to consumption are similar to those of Schyver et al., who reported that price, controversy over processing, genetically modified factors and interaction with thyroid stimulation inhibited use of soya products (Schyver and Smith, 2005). One participant stated that she felt that the product was too expensive;

"It's expensive I think; to me I think it's kind of expensive"

A finding echoed in a focus group conducted in Vietnamese and French cohorts (Tu et al., 2012) to investigate cultural influences of soya, with price identified as a barrier. One of the major findings in these focus groups was the price barrier of soya drinks of then eluding to the product as expensive and only even buying when it is on offer. Also, association with the amount of soya milk that was consumed throughout the study became a barrier to consumption, being asked to consume 100-600ml/day proved difficult for some of the participants. Sugar content of the product was a clear barrier to consumption as participants did not want to add sugar to their diet but were unaware as to the content in soya. One particular participant made the following statement in relation to their impression on sugar content;

"Too much sugar I, you know I stayed away from the sugar"

Unfamiliarity of the product was also identified as a barrier to consumption;

"I think the soya puts people off"

Some concern was expressed relating to the controversy over processing of soya and concerns of genetically modified products and interaction with the thyroid, similar to findings from Scyhver et al., (Schyver and Smith, 2005). Although this was a concern, participants were unaware that Alpro® products are not genetically modified.

Menopausal symptoms

Sub themes which appeared included symptom free; unaware if menopausal symptoms were affected and hot flushes returning after the study. In two focus group studies in post-menopausal women and attitudes to soya (Alfred et al., 2006, Fox-Young et al., 1995), recognition of some benefits during the menopause was highlighted, other than knowing it can be of some benefit, participants in neither of the two studies were able to indicate why this might be the case. In this study, although participants understood that soy is a healthful food, (Messina and Messina, 2003) prior to taking part in the study, they had little knowledge of the potential beneficial effects on the menopause. Some of the ladies did report that they were symptom free while consuming the soya milk and equally others were unaware if they could ascertain symptom free spells with the soya drinks (see Table 3), expressed by one participant through the following quote;

"while I was on it yes I was quite I was quite free of menopausal symptoms"

Women felt that soya helped alleviate hot flushes, provided more energy and it was even found that soya made some women feel more relaxed.

To conclude, results reported are very similar to those reported by Tu et al., and Scyhver et al., and generate the following understanding about soya consumption (Schyver and Smith, 2005, Tu et al., 2012). Soya is perceived as a healthier option to many other dairy choices and those who are making lifestyle changes are more likely to change to soya milk than at any other time. Soya is predominately seen as a replacement drink for those who have allergies or intolerances to dairy milks and also for vegetarians (Schyver and Smith, 2005). Prior to an intervention with soya drinks women wouldn't have recommended soya to a friend or even tried it themselves to alleviate menopausal symptoms or as a health choice. Also, participants are more likely to continue with consumption of soya if they knew that it helped with menopausal symptoms. Soya is rising in popularity as an area of focus in dietary interventions and parameters of health.

Product development and Public health

In relation to the themes generated from this thematic analysis there can be a number of influencing factors in realtion to public health and product development. Despite the healthfulness of soya known to the public some of the strongerst influencing factors for consumption were price and familiarity of the product. This finding shows that the development stage of planning and processing in product development requires extensive communication on the beneficial factors associated with soya consumption. On a wider public health standpoint meaningful collaboratve effort for effective communication with the public on the intake of soya is essential, particularly in a cohort of post menopausal women.

Conclusion

Through the evaluation of the intervention it is evident that future interventions should consider using a soya supplement along with a soya food in order to accomplish greater compliance from participants. Consumption of the soya drink was well received in relation to the intervention although it is clear that the higher dose of soya has an implication on satiety and therefore a reduced desire to consume all of the recommended dose. Evaluation of the intervention successfully highlighted awareness of benefits of soya and incorporation of soya into the diet. Soya is continuously being investigated in relation to different parameters of health, however, acceptance as a valuable food in reducing potential health risks has yet to be concluded. Main barriers to soya consumption include price and lack of knowledge about soya products while enablers of consumption include Alpro® being considered a reliable and good brand. Introducing education about soya products is required in relation to nutrient content and sugar content in order to reduce barriers to consumption. Consumption of soya in western populations still remains

low and use is limited to particularly health conscious individuals interested in maintaining a healthy lifestyle. Post-intervention, individuals confirmed they would continue to consume soya due to the health benefits associated with its use that they were unaware of prior to taking part in the study. Therefore, increasing overall awareness of the healthfulness of soya may increase consumption in western populations.

Table 1: Baseline participant characteristics presented for the focus group participnats as mean and standard deviation.

Measure	All participants *(n=10)*
Age, y	55.20 ±3.05 [4]
Height, m	1.63 ± .051
Weight, kg	70.69 ± 14.33
BMI, kg/m	26.51 ± 5.92
Alcohol, units/wk	1 ± 0
Education level (n)	
PA, METs/week[1]	161.17 ± 63.42

[1]METs, metabolic equivalents; MMSE, Mini Mental State Examination; PA, physical activity

Table 2: Showing the line of questioning and probes used in focus group discussions of soya with post-menopausal women.

Q1	Before you joined the women's health study, were you familiar with Alpro® soya products? Had you tasted/bought them previously?
Q2	Before you joined the women's health study what was your overall impression of soya products? What do you think of their healthfulness? Why? Why did you want to/not want to consume them? What encouraged you to take part in the study?
Q3	What do you feel are the advantages of adding Alpro® soya products to your diet? What did you think of the flavours offered on the study? How did you incorporate the soya products into your regular diet? Did you experiment with different ways of adding the Alpro® drinks to your diet? Did the products help in any other way?
Q4	What do you feel are the disadvantages of adding Alpro® soya products to your diet?
Q5	Will you continue to consume these products?
Q6	What factors or circumstances would enable you to use Alpro® soya products in the future?
Q7	Do you feel the Alpro® products helped with your menopausal symptoms? In what way?
Q8	Is there anything further you would like to tell us about your use of Alpro® products as part of this study?

Table 3: Showing major themes and sub themes of the three focus groups conducted in post-menopausal women.

Main theme	F	Quotes from focus groups
Knowledge of Alpro® products		
Familiarity with Soya milk	9	"Soya milk in the shops, you know"
No	9	"Nope never used them"
Familiarity with Soya Desserts	4	"Actually, the wee soya custards and soya desserts"
Yes	3	"Yes"
Previously tasted	3	"I tried it before"
Impressions of Soya products		
Healthful impression		
Dairy alternative	4	"I had some vague awareness of soya being used as an alternative to dairy"
Allergies	4	"vegetarian's sort of ate them" "I would've thought with people who always have allergies and stuff like that"
Improved menopausal symptoms	3	"I think it definitely helped with the hot flushes"
Lower incidence of menopausal symptoms in Japanese women	3	"um it being very big in Japanese, and Japanese ladies don't suffer menopausal symptoms, that they had soya in the rest of their diet"
Low calorie	2	"is the soya milk lower in calories than ordinary milk or not"
Improved memory	1	"I don't know that it helped my memory, I think my memory is particularly bad so maybe that the reason"
Flavour		
Too sweet	8	"and maybe the fact that it was a little bit sweet

Nice creamy flavour	7	"I like those creamy sort of flavours I like, you know I don't know I like, I think there's a nice comforting um, if you had it as a warm drink" "Yea because, the fresh one was much creamier, and a nicer taste"
Unsweetened favourable flavour	5	"And I like the unsweetened and the chocolate" "So no I buy the unsweetened soya milk now"
Disliked strawberry flavour	4	"The strawberry didn't taste very natural, because it was a very strong, very sweet, not as sweet as the chocolate, or sweeter than the chocolate sorry, sweeter than the chocolate"
Disliked chocolate flavour	4	"I didn't go near the chocolate or anything because I thought probably too much sugar"
Disliked unsweetened flavour	4	"I didn't like to taste"
Vanilla favourable flavour	3	"because even the vanilla one was delicious really like a little milkshake but it was good"
Advantages of Alpro® Soya in diet		
Contribution to research	8	"I think if I heard benefits coming out of the research I would certainly tell everybody" "Maybe it's just because you were doing a survey and taking part in something and other people were doing it"
Fortified with vitamin-D	6	"I think it had Vit D, didn't it, did it have vitamin D or calcium?"
Feel good factor	4	"I think that must be a good thing but also, it am it just expands your whole way of thinking about your intake of food you know" "a feel good factor with taking it too"
Good source of protein	3	"well I think, the protein point of view" "its replacing or giving me proteins that I didn't have before then it means that it's worth keeping it up"
Bigger breast	2	"but the size of me here was getting bigger"

		"My bust did get bigger for sure"
Reduced cattle in dairy farms	2	"you know and I think overall that's always going to be a good thing, just in terms of environmentally keeping dairy cattle and beef cattle isn't a great thing"
Prevention of cancer	1	"it was then the introduction to soya, and whether an enzyme was released in the gut and it was for the prevention of cancer"
Good source of isoflavones	1	
Disadvantages of Alpro® Soya in diet		
Filling to consume	7	"It's quite filling anyway"
Weight gain	4	"I had said to (name omitted) that I thought I had put on weight when I was, during the time that I was drinking it"
Dislike taste when added to tea and coffee	4	"I didn't like it in tea and coffee" "it did feel different in tea or coffee; I didn't mind it too much in tea but I didn't like it in coffee"
Continue to consume		
Recommend to a friend	8	"I have recommended it since, told people about the study" "I have recommended it since"
With cereal products	7	"I ended up just drinking it mostly and made porridge and put it in the odd thing" "Yea I put it into, make my porridge half milk/half water"
Drink as a milkshake	5	"and maybe the fact that it was a little bit sweet, I said earlier it's kinda like have a little milkshake, it was a little bit sweet and delicious really"
Chai latte	4	"but a chai latte now I would always have in soya"
Milk substitute	3	"I didn't try any other milk substitutes"

Incorporation into the diet		
Tea/coffee	21	"I did have it in tea as well and coffee"
		"I would use mine as a milky coffee, I actually preferred the soya in coffee now"
Drank in a glass	10	"I didn't find it a problem drinking that amount as such"
		"it was just sorting it out and remembering to drink it"
Drank throughout the day	8	"I just kind of paced it throughout the day"
Handy on the go snack	3	"the wee cartons are handy to have in the car when you're on the move"
		"know I would buy them and they're handy"
Enablers to consumption		
Alpro®; Good brand	10	"I would agree with that, you know that I think Alpro® is a good brand and I associate it with healthy food and a good product"
		"I thought it's the one if I were out and there were lots of brands, I would be looking for the Alpro®"
Price if the product if on offer	9	"I only buy it when it's on offer"
		"I'd be the same"
Choice of different carton sizes available	4	"yea and I think that's really good to see that there is a range of size of the packaging"
Other family members who also consume Soya milks		"also the size of the package, that's a great size (500ml) because you know if you live on your own"
Longer shelf life	3	"I have to say if it's on offer I'd buy a couple of cartons of it you know because it probably has a slightly longer shelf life"
Flavour	3	"You it has that sort of, slight nuttyish flavour which I really liked

Barriers to consumption		
Expensive	22	"It's expensive I think; to me I think it's kind of expensive" "Yes, I only buy it when it's on offer"
A lot to consume	17	"I think, you have an idea what the quantity is going to be, and then when it comes to it the actually reality of taking it, it's slightly different" "It was quite a lot"
Sugar content of flavoured drinks	10	"Too much sugar I, you know I stayed away from the sugar" "The strawberry didn't taste very natural, because it was a very strong, very sweet"
Bad impression of Soya brand	6	"I think the soya puts people off"
GMO product	5	"Well I don't like genetically modified products because it worries me"
Alpro® brand	4	"I don't know maybe it's the name Alpro® that isn't very nice" "I think the soya puts people off"
Weight gain	3	"Huge weight gain"
Association with thyroid function	2	"I have this worry about the thyroid problem so I just thought I'll be a bit cautious"
Sole user in the household	1	"Because I'd be the only one consuming it in our house now buying the big carton, it'll be going off by the time I would get through it"
Menopausal symptoms		
Symptom free	5	"While I was on it yes I was quite free of menopausal symptoms" "Mine's coming to an end anyway I think that I didn't connect it possibly with the previous menopause symptoms that I had"
Unaware	4	"and I didn't know anything at all about soya product"

Hot flushes returned after the study	4	"While I was on it yes I was quite free of menopausal symptoms"
		"Mine's coming to an end anyway I think that I didn't connect it possibly with the previous menopause symptoms that I had"
Hot flushes	2	"I think it definitely helped with the hot flushes"
No difference	2	"I don't see really any difference"
		"I just didn't feel It made a huge difference to me so I didn't sort of feel I could say you must try this it makes a big difference"
More energy	2	"I don't know I just in the day it made me more energetic, or just something you know that"
Feeling more relaxed	1	"I think I did feel more relaxed"

F, Frequency at which the relevant statement was mentioned during all three focus groups

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CHAPTER 6

General discussion and future directions

The effects of soya isoflavones (IFs) on cognitive function and menopausal symptoms has received much attention due to their similarity in structure to the endogenous circulating oestrogen 17β - oestradiol. As highlighted within the literature review presented in **Chapter 2**, there is a lack of well-designed studies investigating the effects of isoflavones on cognitive function in post-menopausal women. This thesis therefore aimed to address this research gap by conducting a robust human intervention study to investigate the effects of a soya isoflavone food intervention on the cognitive performance and menopausal symptoms of post- menopausal women (**Chapter 4**). Food diaries collected during the intervention study were utilised to investigate associations between dietary patterns and cognitive function in post-menopausal women (**Chapter 3**). Post-menopausal women were invited to take part in focus groups to evaluate their attitudes towards consumption of soya during a 12-week intervention and also to partly evaluate the overall effectiveness of this intervention (**Chapter 5**).

Absence of a determined dose which consistently represents an improvement in cognitive function with isoflavone supplementation led to the intervention method used in **Chapter 4.** The majority of studies to date have used varied supplementation doses/types ranging from 60-160mg isoflavones/day, creating difficulty in comparing studies. Therefore, in the intervention study (**Chapter 4)**, 10mg IFs/d 100ml was used as a low dose control group, 35 mg IFs/d 350ml as a medium dose and 60 mg IFs/d 600ml as a high dose, allowing comparison to be made among treatment groups. The quantities of soya consumed throughout the study were considered manageable by participants although it was highlighted that the high dose 60 mg IFs/d (600ml) was perhaps too large of a quantity for some post menopausal women, therefore this could be a limitation to the study as this was the highest treatment group. **Chapter 4** has presented no significant differences among the three groups for any of the cognitive function outcomes measured following a 12- week soya intervention. Soya drink consumption had no effect on overall menopausal symptoms, although when women were stratified based on severity of their vasomotor symptoms at baseline, those who suffered with more severe symptoms in the medium group at baseline had a significant reduction in vasomotor symptoms post intervention in comparison to those with less severe vasomotor symptoms. Therefore, future studies should aim to recruit stratifying women based on severity of vasomotor symptoms and or recruitment of peri menopausal women (Li et al., 2010) who may be more symptomatic as symptoms cluster within the first two years before and after menopausal transition (Shifren et al., 2014). In support of these findings on vasomotor symptoms, a recent meta- analysis has demonstrated that isoflavone supplementation

containing > 18.8 mg of genistein, in comparison to a placebo group, can significantly reduce hot flush frequency and severity (Taku et al., 2012). It is also important to investigate the effects of soya foods in addition to isoflavones supplements as consumers may gain additional nutritional

benefits from consuming soya foods as part of a healthy balanced diet (Messina, 2016). Furthermore, isoflavone pharmacokinetics are similar following ingestion of soy foods and isoflavone supplements (Setchell et al., 2011). The critical window hypothesis in relation to hormone therapy and cognitive function postulates that optimal effects are evident with early initiation (Maki, 2013), limited evidence suggests that younger postmenopausal women (< 60 y) may gain more cognitive benefit from soya isoflavones in comparison to their older counterparts (Kritz-Silverstein et al., 2003). Cognitive assessment was strictly controlled through providing a standard caffeine- free breakfast prior to testing at both timepoints. Post-menopausal status of women was defined and limited to <7 years since last menstrual cycle. Limitations of the study include absence of a placebo control, large quantity of soya in the high dose group and the non-blinded study design. Difficulties in relation to recruitment were also a limitation to the study as there were a large number of women who were not experiencing a natural menopause who were prescribed antidepressants for their vasomotor symptoms. Nonetheless, the control group consumed only 10mg/IF/d, a quantity much lower than amounts consumed in Asian populations. Also the dose of soya isoflavones used was limited due to the use of a commercially available soya drink.

In **Chapter 3**, it was found that high dietary intakes of meat and dairy may be associated with improved frontal lobe function. It was also found that a high intake of alcohol is associated with poorer visual recognition memory function. Consumption of potatoes and poultry is associated with poorer performance in response accuracy. Factor analysis identified five final dietary patterns; ‘hot beverages and breads’, ‘meat and dairy’, ‘potatoes and poultry’, ‘healthy’, and ‘social’. The ‘hot beverages and breads’ and ‘healthy’ dietary patterns were not significantly associated with any of the 5 cognitive outcome measures. Vitamin D, LCPUFA, and B vitamins have been postulated to play a role in cognitive health and performance. These particular micronutrients were associated with beneficial effects on cognitive function being positively associated with reduced errors in SWM, representing frontal lobe function. This study has numerous strengths. The method used to assess cognitive function CANTAB. Additionally, Principal component analysis, PCA, is the most common method for obtaining dietary patterns. This study also has limitations. Study protocols differ vastly in relation to cognitive function measures which contributes to the contrast in findings among studies in this area.

Self-reporting food diaries are prone to bias or misreporting. The sample size of this cohort is small, dietary data is often representative of a much larger sample size. Nonetheless this study has provided novel findings in the area of dietary patterns and cognitive function in post-menopausal women. These findings present new evidence to consider in further studies which are focused on dietary intake in post-menopausal women and their potential beneficial effects on cognitive decline.

In **Chapter 5** a mixed method design is introduced and provides pragmatic advantages when exploring the complex research questions in the focus groups. This qualitative data provides a deep understanding through focus group responses, and statistical analysis which provides detailed analysis. **Chapter 5** has presented evidence on methods which could potentially increase soya consumption in western populations and possible ideas to develop future interventions focusing on investigating soya. Through qualitative method thematic analysis, 8 final themes were generated encompassing: knowledge of Alpro products; impressions of soya products; advantages of Alpro soya in the diet; disadvantages of Alpro soya in the diet; likelihood of continued consumption; enablers to consumption; barriers to consumption and menopausal symptoms.The results observed in the focus group could also explain the observed reduction in protein and fat intake (as %EI) within the high dose group at the end of the intervention as intake of soya was considered by participants to be "very filling" Major contributors to soya consumption was healthfulness of the product as well as impact on nutritional intake through fortification or specific use for vegetarians. Soya is considered a healthy addition to any diet. Barriers to soya consumption included the image of soya, lack of familiarity with the product, and sugar content of the products. Overall, increased awareness of the benefits of soya and educating the public about soya may contribute to increased consumption in western populations. A strength of this study was the use of thematic analysis as a qualitative method to determine themes within the data. The use of focus groups and individuals who recently added soya to their diet was also a strength of this study. Limitations include the choice of questions in order to evaluate the intervention and small number of participants who attended the focus groups.

Limitations and scope for further research

All participants (**Chapter3, 4, and 5**) typically consisted of middle-aged post-menopausal Caucasian women residing in Ireland, at the early stages of menopausal transition therefore it is not possible to generalise these results to other demographics such as non-Caucasian post-menopausal women and those of the same age but not

entering menopause. Aside from limitations discussed within each chapter, general limitations should be acknowledged. With a significant number of women excluded from the intervention based on the stringent exclusion criteria as mentioned in **Chapter 4**, it was discovered that a large number of women were currently administered anti-depressants for both mental health and menopausal symptoms (although not reported in the thesis). These women were excluded based on the exclusion criteria although this generates a concern that our cohort may not be completely representative of post-menopausal women residing in Ireland and Northern Ireland. The exclusion criteria were based on confounding measures of cognitive function. It may have been interesting to recruit these women on anti-depressants and generate a comparison among women on anti-depressants vs those not on anti-depressants, to see if those on anti-depressants gained more benefit from isoflavone supplementation. Indeed, recent literature suggests soya may have a role in the management of depression (Messina and Gleason, 2016). Although, depression is associated with cognitive dysfunction and therefore controlling results of CANTAB assessment may be problematic (Kaser et al., 2017). Also, with the absence of a placebo group and the non-blinded design as well as use of commercially available soya which limited dose alternatives, future studies could investigate a combination of isoflavone supplements and soya foods specifically in western populations. **Chapter 3** examined dietary patterns associated with cognitive function using 4-day cumulative food diaries. Dietary information was collected prior to commencing the intervention, and it has been well established the bias which is associated with self-reported dietary data.

As cognitive function was the primary outcome of the intervention, women were not recruited based on menopausal symptom status; recruitment stratified by severity of menopausal symptoms may have presented with greater benefits from isoflavone supplementation. Future studies should aim to recruit post-menopausal women who suffer from more severe vasomotor symptoms >5 hot flushes/day.

Finally, this thesis has proposed a number of new research questions highlighted in the following areas for future research:

- Do isoflavones have a greater effect on women suffering ≥5 hot flushes/day, do they derive greater benefits on cognitive function?

- Do women taking anti-depressants derive greater health benefits from isoflavone supplementation and effects on cognitive function and menopausal symptoms?

- In early menopausal women, how does earlier commencement of isoflavones/soya drink impact on menopausal symptoms?

Overall, results presented in this thesis suggest that isoflavone supplementation may not impact cognitive function in post-menopausal women within 7 years of menopausal transition. Future studies should investigate effects on early postmenopausal women who report memory problems as a result of the menopause or in those with mild cognitive impairment. Secondary analysis indicates that isoflavone consumption significantly reduced vasomotor symptoms in those with more severe symptoms at baseline. This should be corroborated by additional intervention trials assessing the effects of isoflavone supplementation using vasomotor symptoms as the primary outcome measure. Administration of isoflavones should be considered in food and supplementation form to ascertain their effect on menopausal symptoms in early menopause.

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APPENDIX 1

Confirmation of ethical approval

Chapter 4 confirmation of ethical approval

Ulster University

Memo

To: Dr P Magee, W2064, Biomedical Sciences, CE

From: Elaine McCormick, Research Governance, 1H12, JN

Date: 04 January 2016 **Ref:**

Dear Dr Magee

Research Ethics Committee application number: REC/15/0025

Project Title: The effect of soya foods on cognitive function and menopausal symptoms in postmenopausal women

Amendment Number: 3 (extending recruitment base)

Following submission of Amendment Number 3 for ethical approval, the Research Ethics Committee is pleased to confirm that the amendment should proceed.

The period for which the committee's decision is valid remains unchanged from the original approval.

If you need any further information please do not hesitate to contact me.

Thanks and best wishes.

Elaine McCormick
Admin Officer
Research Governance Section
Ext. 66518
e.mccormick@ulster.ac.uk

APPENDIX 2 Nutritional content of soya drinks used

Typical Values	**Typical Values per 100 ml**
Energy	161 kJ / 39 kcal
Fat	1.8 g
of which	-
Saturates	0.3 g
Carbohydrate	2.5 g
of which	-
Sugars	2.5 g
Fibre	0.5 g
Protein	3.0 g
Salt	0.08 g
Vitamins:	-
D	0.75 µg*
Riboflavin (B2)	0.21 mg*
B12	0.38 µg*
Minerals:	-
Calcium	120 mg*
Iodine	22.5 µg*
* = 15% of the nutrient reference values	-
These values are approximate due to the variations which occur in natural ingredients	-
Lactose	nil

Typical Values	Typical Values per 100 ml
Energy	260 kJ / 62 kcal
Fat	1.8 g
of which	-
Saturates	0.3 g
Carbohydrates	7.6 g
of which	-
Sugars	7.6 g
Fibre	0.5 g
Protein	3.3 g
Salt	0.14 g
Vitamins:	-
D	0.75 µg*
Riboflavin (B2)	0.21 mg*
B12	0.38 µg*
Minerals:	-
Calcium	120 mg*
Lactose	nil
*= 15% of the nutrient reference values	-
These values are approximate due to the variations which occur in natural ingredients	-

Typical Values	Typical Values per 100 ml
Energy	326 kJ / 78 kcal
Fat	2.1g
of which	-
Saturates	0.6g
Carbohydrate	10.9g
of which	-
Sugars	9.3g
Fibre	1.0g
Protein	3.3g
Salt	0.14g
Vitamins:	-
D	0.75µg*
Riboflavin (B2)	0.21mg*
Vitamin B12	0.38µg*
Minerals:	-
Calcium	120mg*
* = 15% of the nutrient reference values	-
These values are approximate due to the variations which occur in natural ingredients.	-
Lactose	nil

APPENDIX 3

Copies of questionnaires

Mini mental State

1.Orientation

What is today's date?	correct	☐	☐ Incorrect
What is the year?		☐	☐
What is the month?		☐	☐
What day is today?		☐	☐
What season is it?		☐	☐
What is the name of this place?		☐	☐
What floor are we on?		☐	☐
What is the name of the town we are in?		☐	☐
What county are we in?		☐	☐
What country are we in?		☐	☐

2.Immediate recall

Ask subject to repeat these words. Allow 1 second per word, and up to 6 trials. Score 3 points if correct first time, 2 if correct the second time, and one if correct the third time. Ask patient to keep the three things in mind.

Ball	recall	☐	☐ not recall
Flag		☐	☐
Tree		☐	☐

3.Attention and concentration.

Ask the patient to take seven from 100, then seven from the result and so on for five subtractions. Score 1 point for each correct answer.

93	Correct	☐	☐ Incorrect
86		☐	☐
79		☐	☐
72		☐	☐
65		☐	☐

4.Memory recall.

Ask the patient to recall the three objects from 2.

Ball	Correct	☐	☐ Incorrect
Flag		☐	☐
Tree		☐	☐

5.Language.

Show two familiar objects and ask to name.

Pen	Correct	☐	☐ Incorrect
Watch	Correct	☐	☐ Incorrect

Ask the patient to repeat the sentence "No ifs, ands or buts"

Correct ☐ ☐ Incorrect

Ask the patient Please take th paper in your left hand.

Correct ☐ ☐ Incorrect

Fold it in half.

Correct ☐ ☐ Incorrect

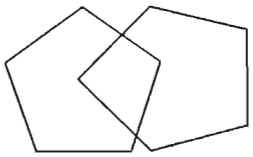
Put the paper on the floor.

Correct ☐ ☐ Incorrect

Ask the patient to read the following instructions "CLOSE YOUR EYES".
Correct ☐ Incorrect ☐

Ask patient to write simple sentence, with a subject, verb and should make sense.
Correct ☐ Incorrect ☐

Visual reconstruction.
Ask patient to copy a picture of intersecting pentagons.
Correct ☐ Incorrect ☐



Close your eyes.

Please read this carefully:

We should like to know if you have had any medical complaints, and how your health has been in general, over the past few weeks. Please answer ALL the questions on the following pages simply by underlining the answer which you think most nearly applies to you. Remember that we want to know about present and recent complaints, not those that you had in the past.

It is important that you try to answer ALL the questions. Thank you very much for your cooperation.

HAVE YOU RECENTLY:

A1. Been feeling perfectly well and in good health?

Not at all	No more than usual	Rather more than usual	Much more than usual

A2. Been feeling in need of a good tonic?

Not at all	No more than usual	Rather more than usual	Much more than usual

A3. Been feeling run down and out of sorts?

Not at all	No more than usual	Rather more than usual	Much more than usual

A4. Felt that you are ill?

Not at all	No more than usual	Rather more than usual	Much more than usual

A5. Been getting any pains in your head?

Not at all	No more than usual	Rather more than usual	Much more than usual

A6. Been getting a feeling of tightness or pressure in your head?

Not at all	No more than usual	Rather more than usual	Much more than usual

A7. Been having hot or cold spells?

Not at all	No more than usual	Rather more than usual	Much more than usual

B1. Lost much sleep over worry ?

Not at all	No more than usual	Rather more than usual	Much more than usual

B2. Had difficulty in staying asleep once you are off?

Not at all	No more than usual	Rather more than usual	Much more than usual

B3. Felt constantly under strain?

Not at all	No more than usual	Rather more than usual	Much more than usual

B4. Been getting edgy and bad-tempered?

Not at all	No more than usual	Rather more than usual	Much more than usual

B5. Been getting scared or panicky for no good reason?

Not at all	No more than usual	Rather more than usual	Much more than usual

B6. Found everything getting on top of you?

Not	No more	Rather more	Much more
at all	than usual	than usual	than usual

B7. Been feeling nervous and strung-up all the time?

Not	No more	Rather more	Much more
at all	than usual	than usual	than usual

C1. Been managing to keep yourself busy and occupied?

Not	No more	Rather more	Much more
at all	than usual	than usual	than usual

C2. Been taking longer over the things you do?

Not	No more	Rather more	Much more
at all	than usual	than usual	than usual

C3. Felt on the whole you were doing things well?

Not	No more	Rather more	Much more
at all	than usual	than usual	than usual

C4. Been satisfied with the way you've carried out your task?

Not	No more	Rather more	Much more
at all	than usual	than usual	than usual

C5. Felt that you are playing a useful part in things?

Not	No more	Rather more	Much more
at all	than usual	than usual	than usual

C6. Felt capable of making decisions about things?

Not	No more	Rather more	Much more
at all	than usual	than usual	than usual

C7. Been able to enjoy your normal day-to-day activities?

Not	No more	Rather more	Much more
at all	than usual	than usual	than usual

D1. Been thinking of yourself as a worthless person?

Not	No more	Rather more	Much more
at all	than usual	than usual	than usual

D2. Felt that life is entirely hopeless?

Not	No more	Rather more	Much more
at all	than usual	than usual	than usual

D3. Felt that life isn't worth living?

Not	No more	Rather more	Much more
at all	than usual	than usual	than usual

D4. Thought of the possibility that you might make away with yourself?

Not	No more	Rather more	Much more
at all	than usual	than usual	than usual

D5. Found at times you couldn't do anything because your nerves were too bad ?

Not	No more	Rather more	Much more
at all	than usual	than usual	than usual

D6. Found yourself wishing you were dead and away from it all?

Not	No more	Rather more	Much more
at all	than usual	than usual	than usual

D7. Found that the idea of taking your own life kept coming into your mind?

Not	No more	Rather more	Much more
at all	than usual	than usual	than usual

The effect of soya foods on cognitive function and menopausal symptoms in postmenopausal women.

Screening Questionnaire

Name: _____ Date of Birth:_____

1. Can you confirm that your last menstrual period was at least 12 months but not longer than 5 years ago?

   Yes ☐ No ☐

2. How long ago (approximately) did you experience your last menstrual cycle (period)?

   _____

3. Did you go become menopausal as the result of surgery (i.e. removal of both ovaries)?

   Yes ☐ No ☐

4. Do you currently consume soya foods?

   Yes ☐ No ☐ (go to Q6)

5. If you answered 'yes' to Q4 how often would you consume soya foods per week?

   < once per week ☐ 1-2 times per week ☐ > 2 times per week ☐

6. Are you currently on hormone therapy?

   Yes ☐ No ☐

7. Are you currently taking soya/isoflavone supplements?

   Yes ☐ No ☐

8. Have you taken an antibiotic within the last 3 months?

   Yes ☐ No ☐

9. Are you currently taking medication for the treatment of a psychiatric condition?

   Yes ☐ No ☐ (go to Q11)

10. If you answered ‘yes’ to Q9 which medication(s) are you currently taking?

_____

11. Do you suffer from or have a history of any of the following conditions:

cardiovascular disease	☐
cancer	☐
diabetes	☐
thyroid disease	☐
renal disease	☐
kidney disease	☐
alcohol or drug abuse	☐

Thank you for taking the time to complete this questionnaire.

Researcher’s signature _____

Date_____

FOOD FREQUENCY QUESTIONNAIRE

This questionnaire asks for some background information about you, especially about what you eat.

Please answer every question. If you are uncertain about how to answer a question then do the best you can, but please do not leave a question blank.

1. YOUR DIET LAST YEAR

For each food there is an amount shown, either a "medium serving" or a common household unit such as a slice or teaspoon. Please put a tick (✓) in the box to indicate how often, **on average**, you have eaten the specified amount of each food **during the past year**.

EXAMPLES:

For white bread the amount is one slice, so if you ate 4 or 5 slices a day, you should put a tick in the column headed "4-5 per day".

FOODS AND AMOUNTS	AVERAGE USE LAST YEAR								
BREAD AND SAVOURY BISCUITS (one slice or biscuit)	Never or less than once/month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day
White bread and rolls								✓	

For chips, the amount is a "medium serving", so if you had a helping of chips twice a week you should put a tick in the column headed "2-4 per week".

FOODS AND AMOUNTS	AVERAGE USE LAST YEAR								
POTATOES, RICE AND PASTA (medium serving)	Never or less than once/month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day
Chips				✓					

For very seasonal fruits such as strawberries and raspberries you should estimate your average use when the fruits are in season, so if you ate strawberries or raspberries about once a week when they were in season you should put a tick in the column headed "once a week"

FOODS AND AMOUNTS	AVERAGE USE LAST YEAR								
FRUIT (1 fruit or medium serving)	Never or less than once/month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day
Strawberries, raspberries, kiwi fruit			✓						

Please estimate your average food use as best you can, and please answer every question - do not leave ANY lines blank. PLEASE PUT A TICK (✓) ON EVERY LINE

FOODS AND AMOUNTS	AVERAGE USE LAST YEAR								
MEAT AND FISH (medium serving)	Never or less than once/month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day
Beef: roast, steak, mince, stew or casserole									
Beefburgers									
Pork: roast, chops, stew or slices									
Lamb: roast, chops or stew									
Chicken or other poultry eg. turkey									
Bacon									
Ham									
Corned beef, Spam, luncheon meats									
Sausages									
Savoury pies, eg. meat pie, pork pie, pasties, steak & kidney pie, sausage rolls									
Liver, liver paté, liver sausage									
Fried fish in batter, as in fish and chips									
Fish fingers, fish cakes									
Other white fish, fresh or frozen, eg. cod, haddock, plaice, sole, halibut									
Oily fish, fresh or canned, eg. mackerel, kippers, tuna, salmon, sardines, herring									
Shellfish, eg. crab, prawns, mussels									
Fish roe, taramasalata									
	Never or less than once/month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day

Please check that you have a tick (✓) on EVERY line

PLEASE PUT A TICK (✓) ON EVERY LINE

FOODS AND AMOUNTS	AVERAGE USE LAST YEAR								
BREAD AND SAVOURY BISCUITS (one slice or biscuit)	Never or less than once/month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day
White bread and rolls									
Brown bread and rolls									
Wholemeal bread and rolls									
Cream crackers, cheese biscuits									
Crispbread, eg. Ryvita									
CEREALS (one bowl)									
Porridge, Readybrek									
Breakfast cereal such as cornflakes, muesli etc.									
POTATOES, RICE AND PASTA (medium serving)									
Boiled, mashed, instant or jacket potatoes									
Chips									
Roast potatoes									
Potato salad									
White rice									
Brown rice									
White or green pasta, eg. spaghetti, macaroni, noodles									
Wholemeal pasta									
Lasagne, moussaka									
Pizza									
	Never or less than once/month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day

Please check that you have a tick (✓) on EVERY line

PLEASE PUT A TICK (✓) ON EVERY LINE

FOODS AND AMOUNTS	AVERAGE USE LAST YEAR								
DAIRY PRODUCTS AND FATS	Never or less than once/month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day
Single or sour cream (tablespoon)									
Double or clotted cream (tablespoon)									
Low fat yogurt, fromage frais (125g carton)									
Full fat or Greek yogurt (125g carton)									
Dairy desserts (125g carton)									
Cheese, eg. Cheddar, Brie, Edam (medium serving)									
Cottage cheese, low fat soft cheese (medium serving)									
Eggs as boiled, fried, scrambled, etc. (one)									
Quiche (medium serving)									
Low calorie, low fat salad cream(tablespoon)									
Salad cream, mayonnaise (tablespoon)									
French dressing (tablespoon)									
Other salad dressing (tablespoon)									
The following on bread or vegetables									
Butter (teaspoon)									
Block margarine, eg. Stork, Krona (teaspoon)									
Polyunsaturated margarine (tub), eg. Flora, sunflower (teaspoon)									
Other soft margarine,dairy spreads (tub), eg. Blue Band, Clover (teaspoon)									
Low fat spread (tub), eg. Outline, Gold (teaspoon)									
Very low fat spread (tub) (teaspoon)									
	Never or less than once/month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day

Please check that you have a tick (✓) on EVERY line

PLEASE PUT A TICK (✓) ON EVERY LINE

FOODS AND AMOUNTS	AVERAGE USE LAST YEAR								
SWEETS AND SNACKS (medium serving)	Never or less than once/month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day
Sweet biscuits, chocolate , eg. digestive (one)									
Sweet biscuits, plain, eg. Nice, ginger (one)									
Cakes eg. fruit, sponge, home baked									
Cakes eg. fruit, sponge, ready made									
Buns, pastries eg. scones, flapjacks, home baked									
Buns, pastries eg. croissants, doughnuts, ready made									
Fruit pies, tarts, crumbles, home baked									
Fruit pies, tarts, crumbles, ready made									
Sponge puddings, home baked									
Sponge puddings, ready made									
Milk puddings, eg. rice, custard, trifle									
Ice cream, choc ices									
Chocolates, single or squares									
Chocolate snack bars eg. Mars, Crunchie									
Sweets, toffees, mints									
Sugar added to tea, coffee, cereal (teaspoon)									
Crisps or other packet snacks, eg. Wotsits									
Peanuts or other nuts									
SOUPS, SAUCES, AND SPREADS									
Vegetable soups (bowl)									
Meat soups (bowl)									
Sauces, eg. white sauce, cheese sauce, gravy (tablespoon)									
Tomato ketchup (tablespoon)									
Pickles, chutney (tablespoon)									
Marmite, Bovril (teaspoon)									
Jam, marmalade, honey (teaspoon)									
Peanut butter (teaspoon)									
	Never or less than once/month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day

Please check that you have a tick (✓) on EVERY line

PLEASE PUT A TICK (✓) ON EVERY LINE

FOODS AND AMOUNTS	AVERAGE USE LAST YEAR								
DRINKS	Never or less than once/month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day
Tea (cup)									
Coffee, instant or ground (cup)									
Coffee, decaffeinated (cup)									
Coffee whitener, eg. Coffee-mate (teaspoon)									
Cocoa, hot chocolate (cup)									
Horlicks, Ovaltine (cup)									
Wine (glass)									
Beer, lager or cider (half pint)									
Port, sherry, vermouth, liqueurs (glass)									
Spirits, eg. gin, brandy, whisky, vodka (single)									
Low calorie or diet fizzy soft drinks (glass)									
Fizzy soft drinks, eg. Coca cola, lemonade (glass)									
Pure fruit juice (100%) eg. orange, apple juice (glass)									
Fruit squash or cordial (glass)									
FRUIT **For seasonal fruits marked *, please estimate your average use when the fruit is in season**									
Apples (1 fruit)									
Pears (1 fruit)									
Oranges, satsumas, mandarins (1 fruit)									
Grapefruit (half)									
Bananas (1 fruit)									
Grapes (medium serving)									
Melon (1 slice)									
* Peaches, plums, apricots (1 fruit)									
* Strawberries, raspberries, kiwi fruit (medium serving)									
Tinned fruit (medium serving)									
Dried fruit, eg. raisins, prunes (medium serving)									
	Never or less than once/month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day

Please check that you have a tick (✓) on EVERY line

PLEASE PUT A TICK (✓) ON EVERY LINE

FOODS AND AMOUNTS	AVERAGE USE LAST YEAR								
VEGETABLES Fresh, frozen or tinned (medium serving)	Never or less than once/month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day
Carrots									
Spinach									
Broccoli, spring greens, kale									
Brussels sprouts									
Cabbage									
Peas									
Green beans, broad beans, runner beans									
Marrow, courgettes									
Cauliflower									
Parsnips, turnips, swedes									
Leeks									
Onions									
Garlic									
Mushrooms									
Sweet peppers									
Beansprouts									
Green salad, lettuce, cucumber, celery									
Watercress									
Tomatoes									
Sweetcorn									
Beetroot									
Coleslaw									
Avocado									
Baked beans									
Dried lentils, beans, peas									
Tofu , soya meat, TVP, Vegeburger									
	Never or less than once/month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day

Please check that you have a tick (✓) on EVERY line

YOUR DIET LAST YEAR, continued

2. Are there any **OTHER** foods which you ate more than once a week? Yes ☐ No ☐

If yes, please list below

Food	Usual serving size	Number of times eaten each week

3. What type of milk did you most often use?

Select one only

Full cream, silver ☐
Skimmed/blue ☐
Dried milk ☐
Other, *specify* ☐
Semi-skimmed, red/white ☐
Channel Islands, gold ☐
Soya ☐
None ☐

4. How much milk did you drink each day, including milk with tea, coffee, cereals etc?

None ☐
Quarter of a pint ☐
Half a pint ☐
Three quarters of a pint ☐
One pint ☐
More than one pint ☐

5. Did you usually eat breakfast cereal (excluding porridge and Ready Brek mentioned earlier)? Yes ☐ No ☐

If yes, which brand and type of breakfast cereal, including muesli, did you usually eat?

List the one or two types most often used

Brand *e.g. Kellogg's*	Type *e.g. cornflakes*

6. What kind of fat did you most often use for frying, roasting, grilling etc?

Select one only

Butter ☐
Lard/dripping ☐
Vegetable oil ☐
Solid vegetable fat ☐
Margarine ☐
None ☐

If you used vegetable oil, please give type eg. corn, sunflower ☐

7. What kind of fat did you most often use for baking cakes etc?

Select one only

Butter ☐
Lard/dripping ☐
Vegetable oil ☐
Solid vegetable fat ☐
Margarine ☐
None ☐

If you used margarine, please give name or type eg. Flora, Stork ☐

8. How often did you eat food that was fried at home?

Daily ☐ 1-3 times a week ☐ 4-6 times a week ☐
Less than once a week ☐ Never ☐

9. How often did you eat fried food away from home?

Daily ☐ 1-3 times a week ☐ 4-6 times a week ☐
Less than once a week ☐ Never ☐

10. What did you do with the visible fat on your meat?

Ate most of the fat ☐ Ate as little as possible ☐
Ate some of the fat ☐ Did not eat meat ☐

11. How often did you eat grilled or roast meat? ☐☐ times a week

12. How well cooked did you usually have grilled or roast meat?

Well done /dark brown ☐ Lightly cooked/rare ☐
Medium ☐ Did not eat meat ☐

13. How often did you add salt to food while cooking?

Always ☐ Rarely ☐
Usually ☐ Never ☐
Sometimes ☐

14. How often did you add salt to any food at the table?

Always ☐ Rarely ☐
Usually ☐ Never ☐
Sometimes ☐

15. Did you regularly use a salt substitute (eg LoSalt)? Yes ☐ No ☐

If yes, which brand? ☐

16. During the course of last year, on average, how many times a week did you eat the following foods?

Food type	**Times/week**	**Portion size**
Vegetables (not including potatoes)	☐☐	medium serving
Salads	☐☐	medium serving
Fruit and fruit products (not including fruit juice)	☐☐	medium serving or 1 fruit
Fish and fish products	☐☐	medium serving
Meat, meat products and meat dishes (including bacon, ham and chicken)	☐☐	medium serving

17. Have you taken any vitamins, minerals, fish oils, fibre or other food supplements during the past year? Yes ☐ No ☐ Don't know ☐

If yes, please complete the table below. If you have taken more than 5 types of supplement please put the most frequently consumed brands first.

Vitamin supplements	**Average frequency** Tick **one** box per line to show how often on average you consumed supplements									
Name and brand Please list full name, **brand** and strength	**Dose** Please state number of pills, capsules or teaspoons consumed	Never or less than once a month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day

Thank you for your help

Life style questionnaire.

The questionnaire is all about your general life style.

The researcher will have a quick look through the questionnaire when you have finished, and will help you with any sections that you have had trouble with.

1- General information.

What is your date of birth? __/__/__. Age_____(in years).

What is your gender? male ☐ ☐ female

1-1 Marital status.

Please specify what your marital status is at present, tick the approprate box?

- Married ☐
- Fulltime relationship ☐
- Separated ☐
- Divorced ☐
- Widowed ☐
- Single ☐

Do you have any children? yes ☐ ☐ no

If yes, how many children do you have?

1-2 Education.

Can you indicate by ticking the appropriate boxes which of the following you have attended?

	Yes	No
No education	☐	☐
Primary education	☐	☐
Secondary education	☐	☐
Tertiary education	☐	☐

(University, Higher or further education college)

At what age did you leave school?

1-3 Occupation.

Please indicate which of the following boxes best desribe your present working situation.

	Yes	No	
Retired	☐	☐	How long is it since you retired? What was your main occupation?
Still working full time	☐	☐	What is your current occupation?
Still working part time	☐	☐	What is your current occupation?
Unemployed	☐	☐	What was your main occupation?

Give a brief description of the duties under taken by you as part of the job named above.

Who else lives in your house with you?

	Yes	No
No one.	☐	☐
Spouse/partner.	☐	☐
Children.	☐	☐
Relatives.	☐	☐
Pets.	☐	☐
Others. (not relatives)	☐	☐

2-Living Habits.

2-1Smoking Habits.

Which of the following statements best describes you? (please tick one box only).

I smoke every day. ☐
I smoke occasionaly, but not every day. ☐
I used to smoke daily, but don't smoke at all now. ☐
I used to smoke occasionally, but don't smoke at all now. ☐
I have never smoked. ☐

If you have never smoked, please go to question 2-2 Alcohol, page 4.

If you used to smoke, go to the next set of questions, page 3.

If you smoke at all, please answer the the following questions.

How old were you when you started to smoke?

What do you usually smoke? (Please tick one box only).

Cigarettes with filter. ☐
Cigarettes without filter. ☐
Hand rolled cigarettes. ☐
Cigars. ☐
Pipe. ☐
Other (Please specify). ☐

How often would you smoke the following?

	never	daily	weekly	monthly
Cigarettes	☐	☐	☐	☐
Cigars	☐	☐	☐	☐
Pipe	☐	☐	☐	☐

Can you indicate how many of the following you would smoke on a weekly basis?

	none	less than 10	11-20	21-30	31-40	41-50	more than 50
Cigarettes.	☐	☐	☐	☐	☐	☐	☐
Cigars.	☐	☐	☐	☐	☐	☐	☐
Pipe.	☐	☐	☐	☐	☐	☐	☐

If you smoke more than 50 of any of the above per week, specify what it is you smoke and how many you smoke per week.

If you used to smoke, but have now stopped, please answer the following questions.

How old were you when you started smoking?

How old were you when you gave up smoking?

What did you usually smoke? (please tick one box only).

- Cigarettes with filter tip. ☐
- Cigarettes with no filter. ☐
- Hand rolled cigarettes. ☐
- Cigars. ☐
- Pipe. ☐
- Other (please specify). ☐

How often did you smoke the following?

	never	daily	weekly	monthly
Cigarettes.	☐	☐	☐	☐
Cigars.	☐	☐	☐	☐
Pipe.	☐	☐	☐	☐

Can you indicate how many of the following you used to smoke on a weekly basis?

	none	less than 10	11-20	21-30	31-40	41-50	more than 50
Cigarettes.	☐	☐	☐	☐	☐	☐	☐
Cigars.	☐	☐	☐	☐	☐	☐	☐
Pipe.	☐	☐	☐	☐	☐	☐	☐

If you used to smoke more than 50 of any of the above per week, specify what you smoked and how many you smoked per week.

2-2 Alcohol.

Are you a non-drinker or a teetotaller? yes ☐ ☐ no If yes, go to section 3 Dietary habits, page 6.

How often do you usually drink alcohol? Please tick only one box.

- everyday ☐
- 4-6 times per week ☐
- 2-3 times per week ☐
- once a week ☐
- once a fortnight ☐
- once a month ☐
- less than once a month ☐

How often would you drink the following drinks? Please give an answer for each type of drink. Each box represents the following answers.

1= everyday.
2= 4-6 times a week.
3= 2-3 times a week.
4= once a week.
5= once a fortnight.
6= once a month.
7= less than once a month.
8= never.

	1	2	3	4	5	6	7	8
Beer	☐	☐	☐	☐	☐	☐	☐	☐
Spirits	☐	☐	☐	☐	☐	☐	☐	☐
Red wine	☐	☐	☐	☐	☐	☐	☐	☐
White wine	☐	☐	☐	☐	☐	☐	☐	☐
Liqueurs	☐	☐	☐	☐	☐	☐	☐	☐
Shandy	☐	☐	☐	☐	☐	☐	☐	☐
Alcopops	☐	☐	☐	☐	☐	☐	☐	☐
Other (please specify)	☐	☐	☐	☐	☐	☐	☐	☐

In relation to the answers you have given in the previous question, about how often you drink particular drinks, could you state if these are usually pub measures, glasses, bottles or cans?

	pub measure	glass	pint	Can	bottle
Beer.	☐	☐	☐	☐	☐
Spirits.	☐	☐	☐	☐	☐
Red wine.	☐	☐	☐	☐	☐
White wine.	☐	☐	☐	☐	☐
Liqueurs.	☐	☐	☐	☐	☐
Shandy.	☐	☐	☐	☐	☐
Alcopops.	☐	☐	☐	☐	☐
Other (Please specify).	☐	☐	☐	☐	☐

How many of these measures would you usually have for each drink, on each occasion you drink it?

	1-2	3-4	5-6	7-8	9 or more
Beer.	☐	☐	☐	☐	☐
Spirits.	☐	☐	☐	☐	☐
Red wine.	☐	☐	☐	☐	☐
White wine.	☐	☐	☐	☐	☐
Liqueurs.	☐	☐	☐	☐	☐
Shandy.	☐	☐	☐	☐	☐
Alcopops.	☐	☐	☐	☐	☐
Other (Please specify).	☐	☐	☐	☐	☐

3 Dietary Habits.

	always	often	sometimes	occasionally	never
1. Do you prepare your own meals?	☐	☐	☐	☐	☐
2. Do you eat alone?	☐	☐	☐	☐	☐

3. How often do you eat a cooked meal?(Please tick one box only)

- Everyday. ☐
- Regularly (once or more a week). ☐
- Occasionally (less than once a week). ☐
- Never (less than once a month). ☐

4. What do you do with the leftovers from a meal?	Yes	No
Throw them away.	☐	☐
Reheat them always.	☐	☐
Reheat them sometimes.	☐	☐
Never reheat them, I eat them cold.	☐	☐
Give to dog.	☐	☐
Other (please specify).	☐	☐

	Yes	No
5. Are you on a special diet?	☐	☐

If yes, answer the following questions.
If no, go to question 6, page 7.

What kind of diet is it?

Low salt.	☐	☐
Low fat.	☐	☐
Low calorie.	☐	☐
Weight reduction.	☐	☐
Other (please specify).	☐	☐

How long have you been on this diet?

less than 6 months.	☐	☐
7-12 months.	☐	☐
1-3 years.	☐	☐
more than 3 years	☐	☐
(please specify how long)		

Who prescribed the diet for you? Please select only one answer.

- Doctor. ☐
- Dentist. ☐
- Dietician/Nutritionist. ☐
- Yourself. ☐
- Other (please specify). ☐

	Yes	No
6. Do you buy ready made meals?	☐	☐
If yes, are they:		
Chilled.	☐	☐
Frozen.	☐	☐
Canned.	☐	☐
Other. (please specify)	☐	☐

	Yes	No
7. Do you have difficulty chewing?	☐	☐
8. Do you have dentures?	☐	☐
9. Do you wear your dentures for eating?	☐	☐

4. Physical Activity.

This part of the questionnaire is divided into three sections.

Section 4-1, asks about your physical activity patterns in and around the house.
Section 4-2, asks about physical activity patterns at work. This section does not have to be completed by people who have not worked at any stage during the past year.
Section 4-3, Asks about recreational activities engaged in during the past year.

4-1 Home activities.

Sleeping scale.

Please select the correct answer for you which describes your sleeping patterns at different times of the night and day.

a. Average time sleeping over the past year.

During the night.

- Less than 6 hours. ☐
- 6-8 hours. ☐
- 8-10 hours. ☐
- More than 10 hours. ☐

Average time sleeping over the past year.

In the afternoon or during the day.

- None ☐
- Less than 30 minutes ☐
- 30mins to 1hr. ☐
- 1-2 hours. ☐
- more than 2 hours. ☐

b. Average time spent sleeping during each day:

	never	0-1 hour	1-2 hours	2-3 hours	3-4 hours	4-5 hours	more than 5 hours
Laying in a bed.	☐	☐	☐	☐	☐	☐	☐
Sitting in an armchair.	☐	☐	☐	☐	☐	☐	☐

Getting about - apart from going to work.

Please put a tick in the correct box.

	Yes	No
I travel by car most or all of the time.	☐	☐
I travel by public transport most or all of the time.	☐	☐
Are you a car owner/driver?	☐	☐
I work from home.	☐	☐

Average number of journeys per week, other than travelling to work - by :- (Please tick the appropriate box).

	none	1-2	3-4	5-6	7-8	9 or more
Bicycle	☐	☐	☐	☐	☐	☐
Walking	☐	☐	☐	☐	☐	☐

TV or video viewing.

Please tick the most appropriate answer for you.

Hours of TV or video watched per day:

	none	less than 1 hour per day	1-2 hours per day	2-3 hours per day	3-4 hours per day	more than 4 hours
Weekdays:						
Before 6pm.	☐	☐	☐	☐	☐	☐
After 6pm.	☐	☐	☐	☐	☐	☐
Weekends:						
Before 6pm.	☐	☐	☐	☐	☐	☐
After 6pm.	☐	☐	☐	☐	☐	☐

Stair climbing at home.

Please tick the box that corresponds to the number of times you climb up a flight of stairs (approximately 10 steps) each day at home.

- None. ☐
- 1-5 times a day. ☐
- 6-10 times a day. ☐
- 11-15 times a day. ☐
- 16-20 times a day. ☐
- More than 20 times a day. ☐

Activities in and around the home.

Give an approximate idea of the number of hours each week you have undertaken the following activities, during the last year. Put a tick in the box that correspond with the correct answers for you.

Activities in the home:	none	less than 1 hour per week	1-3 hours per week	3-6 hours per week	6-10 hours per week	10-15 hours per week	more than 15 hours
Preparing food, cooking and washing up.	☐	☐	☐	☐	☐	☐	☐
Shopping for food and groceries.	☐	☐	☐	☐	☐	☐	☐
Shopping and browsing in shops for other items (eg clothes, toys).	☐	☐	☐	☐	☐	☐	☐
Cleaning the house.	☐	☐	☐	☐	☐	☐	☐
Doing the laundry and ironing.	☐	☐	☐	☐	☐	☐	☐
Caring for pre-school children and babies at home (not as paid employment).	☐	☐	☐	☐	☐	☐	☐
Caring for handicapped, elderly or disables people at home (not as paid employment).	☐	☐	☐	☐	☐	☐	☐

4-2 Activity at work.

Please answer this section **only** if you have been in paid employment at any time during the past year or if you have carried out regular orgainsed voluntary work during the past year.

If you have not worked in the past year, go to section 4-3 dealing with recreation, page 13.

Types of work during the past year.

We would like to know what full or part time jobs you have done in the past year. For example, someone who worked full time for 6 months, then retired and took a voluntary job for 6 hours a week should complete these questions.

Have you worked during the past twelve months? yes ☐ ☐ no

How many hours per week would you normally have worked during the past twelve months?

How many months in the past year did you do this work?

Activity levels at your work.

Now we would like you to take the total number of hours you worked per week and divide them up according to your activity level.
First of all, read each of the following categories and tick either Yes or No. Then go back through the list and record the nurnber of hours per week that you spent on each activity.
The nurnber of hours spent in each activity should add up to the number of hours that you worked in your job, as you stated above.

Activities at work.	**Yes**	**No**	**Hours.**
Sitting light work. (eg. desk work, or driving a car or truck)	☐	☐	_____
Sitting moderate work. (eg. working heavy levers or driving a mower or fork lift truck)	☐	☐	_____
Standing - light work. (eg. lab technician work or working at a shop counter)	☐	☐	_____
Standing - moderate work. (eg. fast rate assembly line work)	☐	☐	_____
Standing - moderate/heavy work. (eg. masonary/painting)	☐	☐	_____
Walking at work - carrying nothing heavier than a briefcase. (eg. moving about a shop)	☐	☐	_____
Walking - carrying something heavy.	☐	☐	_____
Moving, pushing heavy objects weighing over 75lbs.	☐	☐	_____

If you do other activites that are not included here, please tell us about them and state the number of hours spent on these activites.

Stair or step climbing at work.

Please indicate, by ticking the appropriate boxes, the number of times you climb up the following at work.

	none	1-5 times a day	6-10 times a day	11-15 times a day	16-20 times a day	more than 20 times a day
A flight of stairs (10 steps).	☐	☐	☐	☐	☐	☐
Climb a ladder.	☐	☐	☐	☐	☐	☐

Travel to and from work.

How many miles is it from your home to your job?

How many times a week do you travel from home to your job?

How do you normally travel to your job?	always	usually	occasionally	rarely	never
By car.	☐	☐	☐	☐	☐
By works or public transport.	☐	☐	☐	☐	☐
By bicycle.	☐	☐	☐	☐	☐
Walking.	☐	☐	☐	☐	☐

4-3 Recreation.

The following questions ask about how you spend your leisure time. Please indicate how often you did each activity on average over the past year. For activities that are seasonal, eg. cricket or mowing the lawn, please put the average frequency during the season when you did the activity.

Please indicate the average length of time that you spent doing the activity on each occasion.

Example

You would complete the table below:

If you had mowed the lawn every fortnight in the grass cutting season and took 1 hour and 10 minutes on each occassion.

If you went walking for pleasure for 40 minutes once a week.

Please give an answer for the average time you spend on each activity and the number of times you did that activity in the past year.

Activity	**Hours**	**Mins.**	**Number of times you did the activity in the past year.**					
			None	Less than once a month	once a month	2-3 times a month	once a week	2-3 times a week	4-5 times a week
Mowing the lawn.	1	10			/				
Walking for pleasure.		40				/			

Complete question below in the same way.

Please give an answer for the average time you spend on each activity and the number times you did that activity in the past year.

Activity	**Hours**	**Mins.**	**Number of times you did the activity in the past year.**							
			none	less than once a month	once a month	2-3 times a month	once a week	2-3 times a week	4-5 times a week	everyday
Swimming.	_____	_____	☐	☐	☐	☐	☐	☐	☐	☐
Back packing/ mountain climbing.	_____	_____	☐	☐	☐	☐	☐	☐	☐	☐
Walking for pleasure. (donot include walking as means of transportation as this is included in the previous sections)	_____	_____	☐	☐	☐	☐	☐	☐	☐	☐
Walking the dog.	_____	_____	☐	☐	☐	☐	☐	☐	☐	☐
Cycling. (you should not include cycling as a means oftransportation)	_____	_____	☐	☐	☐	☐	☐	☐	☐	☐

Please give an answer for the average time you spend on each activity and the number of times you did that activity in the past year.

Activity.	Hours	Mins.	Number of times you did the activity in the past year.							
			none	less than once a month	once a month	2-3 times a month	once a week	2-3 times a week	4-5 times a week	everyday
Mowing the lawn.	_____	_____	☐	☐	☐	☐	☐	☐	☐	☐
Watering the grass/lawn in summer.	_____	_____	☐	☐	☐	☐	☐	☐	☐	☐
Digging, shovelling or chopping wood.	_____	_____	☐	☐	☐	☐	☐	☐	☐	☐
Weeding/pruning.	_____	_____	☐	☐	☐	☐	☐	☐	☐	☐
DIY (eg. carpentry, home or car maintanance).	_____	_____	☐	☐	☐	☐	☐	☐	☐	☐
High impact aerobics, step aerobics.	_____	_____	☐	☐	☐	☐	☐	☐	☐	☐
Other types of aerobics.	_____	_____	☐	☐	☐	☐	☐	☐	☐	☐
Exercises with weights.	_____	_____	☐	☐	☐	☐	☐	☐	☐	☐
Conditioning exercises. (eg. using an exercise bike or rowing machine).	_____	_____	☐	☐	☐	☐	☐	☐	☐	☐
Floor exercises. (eg. stretching, bending, keep fit).	_____	_____	☐	☐	☐	☐	☐	☐	☐	☐
Dancing.	_____	_____	☐	☐	☐	☐	☐	☐	☐	☐
Jogging.	_____	_____	☐	☐	☐	☐	☐	☐	☐	☐
Bowling.	_____	_____	☐	☐	☐	☐	☐	☐	☐	☐

Please give an answer for the average time you spent on each activity and the number of times you did that activity in the past year.

Activity.	Hours.	Mins.	Number of times you did the activity in the past year.							
			none	less than once a month	once a month	2-3 times a month	once a week	2-3 times a week	4-5 times a week	everyday
Tennis/Badminton.	_____	_____	☐	☐	☐	☐	☐	☐	☐	☐
Squash.	_____	_____	☐	☐	☐	☐	☐	☐	☐	☐
Table tennis.	_____	_____	☐	☐	☐	☐	☐	☐	☐	☐
Golf.	_____	_____	☐	☐	☐	☐	☐	☐	☐	☐
Football, rugby or hockey. (during the season).	_____	_____	☐	☐	☐	☐	☐	☐	☐	☐
Cricket.(during the season).	_____	_____	☐	☐	☐	☐	☐	☐	☐	☐
Rowing.	_____	_____	☐	☐	☐	☐	☐	☐	☐	☐
Netball, volleyball, basketball.	___	_____	☐	☐	☐	☐	☐	☐	☐	☐
Fishing.	_____	_____	☐	☐	☐	☐	☐	☐	☐	☐
Horse riding.	_____	_____	☐	☐	☐	☐	☐	☐	☐	☐
Snooker, billiards, darts.	_____	_____	☐	☐	☐	☐	☐	☐	☐	☐
Play musical instrument/sing.	___	_____	☐	☐	☐	☐	☐	☐	☐	☐
Ice skating.	_____	_____	☐	☐	☐	☐	☐	☐	☐	☐
Sailing, wind surfing, boating.	___	_____	☐	☐	☐	☐	☐	☐	☐	☐
Winter sports.	_____	_____	☐	☐	☐	☐	☐	☐	☐	☐

Please give an answer for the average time you spent on each activity and the number of times you did that activity in the past year.

Activity.	Hours	Mins.	Number of times you did the activity in the past year.							
			none	less than once a month	once a month	2-3 times a month	once a week	2-3 times a week	4-5 times a week	everyday
Martial arts, boxing wrestling.	_____	_____	☐	☐	☐	☐	☐	☐	☐	☐
Playing cards, chess.	_____	_____	☐	☐	☐	☐	☐	☐	☐	☐
Knitting.	_____	_____	☐	☐	☐	☐	☐	☐	☐	☐
Computer.	_____	_____	☐	☐	☐	☐	☐	☐	☐	☐
Reading.	_____	_____	☐	☐	☐	☐	☐	☐	☐	☐
Volunteer activities.	_____	_____	☐	☐	☐	☐	☐	☐	☐	☐
Other exercises/activities. (please specify).	_____	_____	☐	☐	☐	☐	☐	☐	☐	☐

You have now finished this questionnaire - Thank You.

Green's 21-item Menopause Symptom Checklist.

Please indicate the extent to which you are bothered at the moment by any of these symptoms, by placing a tick in the appropriate box.

1 = Not at all.
2 = a little bit.
3 = quite a bit.
4 = extremely.

Symptoms.

1. Feeling tired and lacking in energy.	1[ ]	2 [ ]	3[ ]	4[ ]
2. Heart beating strongly or quickly.	1[ ]	2 [ ]	3[ ]	4[ ]
3. Feeling tense or nervous.	1[ ]	2 [ ]	3[ ]	4[ ]
4. Difficulty in sleeping.	1[ ]	2 [ ]	3[ ]	4[ ]
5. Excitable.	1[ ]	2 [ ]	3[ ]	4[ ]
6. Attacks of panic.	1[ ]	2 [ ]	3[ ]	4[ ]
7. Difficulty in concentrating.	1[ ]	2 [ ]	3[ ]	4[ ]
8. Loss of interest in most things.	1[ ]	2 [ ]	3[ ]	4[ ]
9. Feeling unhappy or depressed.	1[ ]	2 [ ]	3[ ]	4[ ]
10. Crying spells.	1[ ]	2 [ ]	3[ ]	4[ ]
11. Irritability.	1[ ]	2 [ ]	3[ ]	4[ ]
12. Feeling dizzy or faint.	1[ ]	2 [ ]	3[ ]	4[ ]
13. Pressure or tightness in head or body.	1[ ]	2 [ ]	3[ ]	4[ ]
14. Parts of the body feel numb or tingling.	1[ ]	2 [ ]	3[ ]	4[ ]
15. Headaches.	1[ ]	2 [ ]	3[ ]	4[ ]
16. Muscle and joint pain.	1[ ]	2 [ ]	3[ ]	4[ ]
17. Loss of feelings in hands or feet.	1[ ]	2 [ ]	3[ ]	4[ ]
18. Feelings of suffocation.	1[ ]	2 [ ]	3[ ]	4[ ]
19. Hot flushes.	1[ ]	2 [ ]	3[ ]	4[ ]
20. Sweating at night.	1[ ]	2 [ ]	3[ ]	4[ ]
21. Loss of interest in sex.	1[ ]	2 [ ]	3[ ]	4[ ]

Appendix 1: CASP-19 Questionnaire

Here is a list of statements that people have used to describe their lives or how they feel. We would like to know how often, if at all, you think this applies to you.

		Tick one box on each line			
		Often	Some-times*	Not often*	Never
		1	2	3	4
1	My age prevents me from doing the things I would like to				
2	I feel that what happens to me is out of my control				
3	I feel free to plan for the future				
4	I feel left out of things				
5	I can do the things that I want to do				
6	Family responsibilities prevent me from doing what I want to do				
7	I feel that I can please myself what I do				
8	My health stops me from doing things I want to do				
9	Shortage of money stops me from doing things I want to do				
10	I look forward to each day				
11	I feel that my life has meaning				
12	I enjoy the things that I do				
13	I enjoy being in the company of others				
14	On balance, I look back on my life with a sense of happiness				
15	I feel full of energy these days				
16	I choose to do things that I have never done before				
17	I feel satisfied with the way my life has turned out				
18	I feel that life is full of opportunities				
19	I feel that the future looks good for me				

The PANAS

This scale consists of a number of words that describe different feelings and emotions. Read each item and then mark the appropriate answer in the space next to the word. **Indicate to what extent you feel this way right now, that is, at the present moment.** Use the following scale to record your answers.

1	2	3	4	5
very slightly	a little	moderately	quite a bit	extremely

	very slightly/not at all	a little	moderately	quite a bit	extremely
interested	☐	☐	☐	☐	☐
distressed	☐	☐	☐	☐	☐
excited	☐	☐	☐	☐	☐
upset	☐	☐	☐	☐	☐
strong	☐	☐	☐	☐	☐
guilty	☐	☐	☐	☐	☐
scared	☐	☐	☐	☐	☐
hostile	☐	☐	☐	☐	☐
enthusiastic	☐	☐	☐	☐	☐
proud	☐	☐	☐	☐	☐
irritable	☐	☐	☐	☐	☐
alert	☐	☐	☐	☐	☐
ashamed	☐	☐	☐	☐	☐
inspired	☐	☐	☐	☐	☐
nervous	☐	☐	☐	☐	☐
determined	☐	☐	☐	☐	☐
attentive	☐	☐	☐	☐	☐
jittery	☐	☐	☐	☐	☐
active	☐	☐	☐	☐	☐
afraid	☐	☐	☐	☐	☐

Thank you for your cooperation.

PERCEIVED STRESS SCALE

Instructions

The questions in this scale ask you about your feelings and thoughts during the last month. In each case, you will be asked to indicate how often you felt or thought a certain way. Although some of the questions are similar, there are differences between them and you should treat each one as a separate question. The best approach is to answer each question fairly quickly. That is, don't try to count up the number of times you felt a particular way, but rather indicate the alternative that seems like a reasonable estimate.

For each question choose from the following alternatives:

0=never
1=almost never
2=sometimes
3=fairly often
4=very often

	never	almost never	sometimes	fairly often	very often
1. In the last month, how often have you been upset because of something that happened unexpectedly?	☐	☐	☐	☐	☐
2. In the last month, how often have you felt that you were unable to control the important things in your life?	☐	☐	☐	☐	☐
3. In the last month, how often have you felt nervous or stressed?	☐	☐	☐	☐	☐
4. In the last month, how often have you dealt with irritating life hassles?	☐	☐	☐	☐	☐
5. In the last month,how often have you felt that you were effectively coping with important changes that were occurring in your life?	☐	☐	☐	☐	☐
6. In the last month, how often have you felt confident about your ability to handle your personal problems?	☐	☐	☐	☐	☐
7. In the last month,how often have you felt that things were going your way?	☐	☐	☐	☐	☐
8. In the last month, how often have you found that you could not cope with all the things that you had to do?	☐	☐	☐	☐	☐
9. In the last month, how often have you been able to control irritations in your life?	☐	☐	☐	☐	☐
10. In the last month, how often have you felt that you were on top of things?	☐	☐	☐	☐	☐
11. In the last month, how often have you been angered because of things that happened that were outside of your control?	☐	☐	☐	☐	☐
12. In the last month, how often have you found yourself thinking about things that you have to accomplish.	☐	☐	☐	☐	☐
13. In the last month, how often have you been able to control the way you spend your time?	☐	☐	☐	☐	☐
14. In the last month, how often have you felt difficulties were piling up so high that you could not over come them?	☐	☐	☐	☐	☐

COPE

We are interested in how people respond when they confront difficult or stressful events in their lives. There are lots of ways to try deal with stress. This questionnaire asks you to indicate what you generally do and feel when you experience stressful events. Obviously, different events bring out somewhat different responses, but think about what you usually do when you are under a lot of stress.

Then respond to each of the following items by choosing one number for each, using the response choices listed just below.

1=I usually don't do this at all. 2=I usually do this a little bit.
3=I usually do this a medium amount. 4=I usually do this a lot.

Please try to respond to each item separately in your mind from each other item. Choose your answers thoughtfully, and make your answers as true FOR YOU as you can. Please answer every item. There are no right or wrong answers, so choose the most accurate answer for YOU - not what you think "most people" would say or do. Indicate what YOU usually do when YOU experience a stressful event.

	1	2	3	4
1.I turn to work or other substitute activities to take my mind off things.	☐	☐	☐	☐
2. I concentrate my efforts on doing something about the situation I'm in.	☐	☐	☐	☐
3. I say to myself "this isn't real.	☐	☐	☐	☐
4. I use alcohol or drugs to make myself feel better.	☐	☐	☐	☐
5. I get emotional support from others.	☐	☐	☐	☐
6. I give up trying to deal with it.	☐	☐	☐	☐
7. I take action to try and make the situation better.	☐	☐	☐	☐
8. I refuse to believe that it has happened.	☐	☐	☐	☐
9. I say things to let my unpleasant feelings escape.	☐	☐	☐	☐
10. I get help and advice from other people.	☐	☐	☐	☐
11. I use alcohol or other drugs to help me get through it.	☐	☐	☐	☐
12. I try to see it in a different light, to make it seem more positive.	☐	☐	☐	☐
13. I critize myself.	☐	☐	☐	☐
14. I try to come up with a strategy about what to do.	☐	☐	☐	☐
15. I get comfort and understanding from someone.	☐	☐	☐	☐
16. I give up the attempt to cope.	☐	☐	☐	☐
17. I look for something good in what is happening.	☐	☐	☐	☐
18. I make jokes about it.	☐	☐	☐	☐
19. I do something to think about it less, such as going to the movies, watching TV, reading, daydreaming, sleeping or shopping.	☐	☐	☐	☐
20. I accept the reality of the fact that it has happened.	☐	☐	☐	☐

Respond to each of the following items by choosing one number for each, using the response choices listed just below.

1=I usually don't do this at all. **2=I usually do this a little bit.**
3=I usually do this a medium amount. **4=I usually do this a lot.**

	1	2	3	4
21. I express my negative feelings.	☐	☐	☐	☐
22. I try to find comfort in my religion or spiritual beliefs.	☐	☐	☐	☐
23. I try to get advice or help from other people about what to do.	☐	☐	☐	☐
24. I learn to live with it.	☐	☐	☐	☐
25. I think hard about what steps to take.	☐	☐	☐	☐
26. I blame myself for things that happened.	☐	☐	☐	☐
27. I pray or meditate.	☐	☐	☐	☐
28. I make fun of the situation.	☐	☐	☐	☐

APPENDIX 4

Publications

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ORIGINAL CONTRIBUTION

Consumption of a soy drink has no effect on cognitive function but may alleviate vasomotor symptoms in post-menopausal women; a randomised trial

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Abstract

Purpose Cognitive decline is commonly reported during the menopausal transition, with memory and attention being particularly affected. The aim of this study was to investigate the effects of a commercially available soy drink on cognitive function and menopausal symptoms in post-menopausal women.

Methods 101 post-menopausal women, aged 44–63 years, were randomly assigned to consume a volume of soy drink providing a low (10 mg/day; control group), medium (35 mg/day), or high (60 mg/day) dose of isoflavones for 12 weeks. Cognitive function (spatial working memory, spatial span, pattern recognition memory, 5-choice reaction time, and match to sample visual search) was assessed using CANTAB pre- and post-the 12 week intervention. Menopausal symptoms were assessed using Greene's Climacteric Scale.

Results No significant differences were observed between the groups for any of the cognitive function outcomes measured. Soy drink consumption had no effect on menopausal symptoms overall; however, when women were stratified according to the severity of vasomotor symptoms (VMS) at baseline, women with more severe symptoms at baseline in the medium group had a significant reduction ($P = 0.001$) in VMS post-intervention (mean change from baseline score: -2.15 ± 1.73) in comparison to those with less severe VMS (mean change from baseline score: 0.06 ± 1.21).

Conclusions Soy drink consumption had no effect on cognitive function in post-menopausal women. Consumption of ~350 ml/day (35 mg IFs) for 12 weeks significantly reduced VMS in those with more severe symptoms at baseline. This finding is clinically relevant as soy drinks may provide an alternative, natural, treatment for alleviating VMS, highly prevalent among western women.

Keywords Isoflavones · CANTAB · Menopausal symptoms · Hot flush · Hot flash · Equol

Introduction

Cognitive decline is commonly reported by peri- and post-menopausal women and deteriorations in memory; attention and processing speed have been observed during the menopausal transition [1–4]. These effects have been attributed to a reduction in circulating estrogen concentrations [5]; albeit, this has been contested [6]. Hormone therapy (HT) remains the most effective treatment for vasomotor menopausal symptoms (VMS) [7] and early observational studies supported a beneficial effect of HT on cognitive function [8]. Two recent intervention studies have demonstrated that HT initiated early in menopause has neither beneficial nor harmful effects on cognition [9, 10]. Furthermore, despite HT being commonly prescribed for otherwise healthy

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young/early post-menopausal women, many refrain from HT use due to the health risks associated with HT use in older women [7, 11]. Alternatively, natural approaches for the treatment and prevention of menopausal symptoms are thus being sought.

Dietary soy isoflavones (IFs) have been reported to be efficacious in the treatment of hot flushes [12] and to have the potential to enhance cognitive function in post-menopausal women, having the ability to cross the blood brain barrier in small amounts [13, 14]. Genistein and daidzein, the main IFs present in the soy bean, can bind to estrogen receptors (ERs), and are classified as selective ER modulators, having a higher affinity for ER-β than ER-α [15, 16]. ERs are localised throughout the adult brain including the hippocampus, where ERβ is more highly expressed in comparison to ERα [17], and are also localised within the prefrontal cortex [5]. These areas are important for learning, memory, attention, and higher order cognitive function, and are particularly susceptible to age-related decline [18]. Both ER-mediated and non-ER-mediated neuroprotective effects have been demonstrated for soy/IFs using in vitro and animal models [19, 20]. Such mechanisms include reduced neuronal loss, inhibition of β-amyloid-induced cell death, facilitation of cholinergic transmission, reduced free radical generation, anti-inflammatory and antioxidant effects [19, 21], and via the modulation of mitochondrial function [20]. Furthermore, a high soy diet [22] and soy isoflavone supplementation [23] has been shown to improve cognitive function in men. Nevertheless, randomised-controlled trials investigating the effects of soy/IFs on cognitive function in post-menopausal women have yielded inconsistent findings, with some showing positive effects on cognitive function [24–29] and others demonstrating null/negative effects [30–33]. Two recent comprehensive reviews [19, 34] have highlighted several variations in study design that have likely contributed to these disparate outcomes including: age; time since menopause; habitual soy/IF intake; IF dose, duration, bioavailability and metabolism, and cognitive function assessment. To date, only one study has investigated the effects of soy/IFs on cognitive function in post-menopausal women using a food (soy milk) [31] rather than a supplement. There is, therefore, a need for additional, well-designed studies to further assess the effects of IFs on cognitive function.

In line with the 'critical window hypothesis' of HT and cognitive function that postulates that optimal effects are evident with the early initiation [35], limited evidence suggests that younger post-menopausal women (< 60 years) may gain more cognitive benefit from soy IFs in comparison to their older counterparts [25]. It has also been postulated that the effects of soy on health may be determined by an individual's equol producer status [36]. S-equol, a potent ligand for ERβ, is an isoflavan formed exclusively via the bacterial conversion of daidzein in the intestine [37] and is only produced by ~ 30% of western populations following a soy challenge [38–40]. The ability to produce equol has been associated with reduced VMS [41]. Furthermore, S-equol supplementation alleviates hot flushes [42] and S-equol derivatives of soy IFs are now recommended by the North American Menopause Society for the non-hormonal management of VMS [43], who have highlighted the need for further studies in this area.

The aim of this study was to investigate the effects of a commercially available soy drink on cognitive function and menopausal symptoms in post-menopausal women within 7 years postmenopause. A secondary aim was to assess the relationship between equol producer status and cognitive function and menopausal symptoms.

Subjects and methods

Design

This 12-week parallel group, randomised, controlled trial was conducted between October 2015 and May 2018. All procedures were approved by Ulster University's Research Ethics Committee (REC/15/0025) and the study was registered at http://www.clinicaltrials.gov (NCT03561662). Participants were recruited throughout the province of Ulster and screening, baseline and post-intervention appointments were conducted before and after the 12-week intervention either at the university, the participant's home, or at a location convenient for the participant. The duration of intervention was based on previous intervention studies in post-menopausal women that have demonstrated that soy IF supplementation (60 mg/day) for 6 weeks significantly improves frontal lobe function [26], with significant improvements in sustained attention and long-term episodic memory additionally observed when supplementation is extended to 12 weeks [24]. The primary outcome of the study was effect on cognitive function and the secondary outcome was effect on menopausal symptoms. Sub-analysis investigated the effect of the intervention based on severity of VMS at baseline and also investigated cognitive function and menopausal symptoms according to equol producer status.

Participants

Eligible participants were apparently healthy women within 7 year postmenopause (i.e., 1–7 years since last menstrual period). Post-menopausal status was confirmed based on a serum concentration of follicle-stimulating hormone (FSH) > 30 mIU/ml (assessed via electrochemiluminescence immunoassay on a Cobas 8000 analyzer [Cobas 602 module], Roche Diagnostics at Antrim Area hospital). Exclusion

criteria included: surgically induced menopause; habitual consumers of soya foods (> 2 serves/week); current use of HT or IF supplements; antibiotics use within the previous 3 months; current use of psychoactive medication; presence or history of cardiovascular disease, cancer, diabetes, thyroid, renal or kidney disease, alcohol or drug abuse; cognitive impairment as determined by a Mini-Mental State Examination score < 24; psychiatric distress as determined using a General Health Questionnaire-28 [44] score of ≥ 26; red–green colour blind, assessed via the Ishihara test (as CANTAB testing requires colour recognition); abnormal full blood profile (assessed via a Sysmex KX21-N, Sysmex Ltd, UK at Ulster University); and/or insufficient renal/hepatic performance assessed via kidney and liver function tests (assessed photometrically via a Cobas 6000 analyzer [Cobas C501 module], Roche Diagnostics at Causeway Hospital).

Intervention

An independent clinical trial manager used MINIM software [45] to randomise recruited women to one of the three treatment groups with an allocation ratio of 1:1:1. Participants were asked to consume soy drinks (Alpro®) providing a low (10 mg), medium (35 mg), or high (60 mg) dose of IFs daily for a period of 12 weeks and could choose from different flavours of drink (original, unsweetened, chocolate, or strawberry). Limited evidence suggests that IFs consumed in divided doses may be more effective in alleviating menopausal symptoms than a single dose [46, 47], and thus, women were advised to spread their intake throughout the day. The group that consumed the lowest dose of IFs was considered a low-dose control group as beneficial effects of soy IFs on post-menopausal health have previously been observed in intervention studies at much higher doses [48]; furthermore, it was not possible to obtain a placebo control drink. The previous soy/IF intervention studies on cognitive function in post-menopausal women have used doses ranging from 60 to 160 mg IFs per day [24–33]. This study utilised a dose that was achievable in a commercially available soy drink and at a volume that was easily incorporated into an individual's daily diet. Compliance was monitored by measuring plasma concentrations of soy IFs. Total genistein, daidzein, and equol concentrations were assessed using LC–MS/MS by LGC Limited (Cambridgeshire, UK). Equol producers were defined as those with a plasma equol concentration of > 20 nmol/l (5 µg/l) [40].

Dietary intake, anthropometrics, and general health and lifestyle

Weight (kg) and height (cm) were measured at baseline and used to calculate BMI [weight (kg)/height $(m)^2$]. Body weight was measured to the nearest 0.1 kg using Seca 770 electronic weighing scales (Brosch Direct Ltd, Peterborough, United Kingdom), without footwear and heavy clothing. Standing body height was measured to the nearest 0.1 cm using a Seca 220 stadiometer (Seca Ltd, Hamburg, Germany). The participant stood without footwear, with their heels together, hands and arms hanging relaxed, and measurements were taken with the Frankfurt plane in a horizontal position. Dietary intake was assessed at baseline and post-intervention using a 4-day food diary. Participants received instructions on how to complete the diary from a trained researcher and dietary intake was analysed using Nutritics nutritional analysis software [49]. A general health and lifestyle questionnaire were completed by participants at baseline and provided information on age, gender, marital status, education level, occupation, smoking habits, alcohol use, dietary habits, and physical activity.

Blood collection and processing

Fasted blood samples were collected by a trained phlebotomist before and after intervention for the analysis of serum FSH and plasma IF concentrations. Participants were instructed to fast from 10 pm the night prior to blood sampling and water intake was encouraged. Fasted blood samples were obtained from the antecubital fossa using a 21-gauge butterfly needle and 8 ml serum and 9 ml ethylenediametetraacetic acid (EDTA) plasma vacutainer tubes (Greiner Bio-One GmbH, Kremsmunster, Austria). Following inversion, serum samples were allowed to clot for > 60 min and plasma samples placed in refrigeration until full blood profile analysis. Following this, all tubes were centrifuged at 2200 rpm for 15 min at 4 °C to allow the separation of whole blood into its respective components. Following separation, serum and plasma samples were divided into aliquots and stored at − 80 °C until further analysis.

Cognitive function

Pre- and post-intervention cognitive function was assessed in the morning, after participants had consumed a standard, caffeine-free, breakfast. Cognitive function was assessed using the Cambridge Neuropsychological Test Automated Battery (CANTAB Research Suite; Cambridge Cognition, UK) [50]. CANTAB has been extensively validated for assessing brain-to-behaviour relationships in adult populations [51, 52], has proven test–retest reliability [53], and is deemed suitable for use with older adults [51]. The following tests were used: spatial working memory (SWM), spatial span (SSP), pattern recognition memory (PRM), 5-choice reaction time (RTI), and match to sample visual search (MTS). The tests chosen activate areas of the brain that are associated with cognitive decline during the menopausal transition and that are sensitive to hormonal changes,

including the hippocampus [54] and prefrontal cortex [5]. SWM and SSP activate the temporal and frontal lobe regions of the brain; PRM activates the temporal lobe, hippocampus and amygdala; RTI and MTS activate the fronto-striatal circuitry [55]. The procedure for assessing SSP, RTI, and MTS is described in detail elsewhere [56]. Spatial working memory (SWM), a sensitive measure of frontal lobe and executive function, requires retention and manipulation of visuospatial information. The test began with four coloured squares (boxes) shown on the screen. Participants were required, by selecting boxes and using a process of elimination, to find one blue 'token' in each of four boxes (only one token is hidden at a time) and use them to fill up an empty column on the right-hand side of the screen. Touching any box in which a token has already been found is an error. The trial was then repeated three times with four boxes and then progressed to four trials with six boxes and four trials with eight boxes. The colour and position of the boxes used are changed from trial to trial to discourage the use of stereotyped search strategies and a practice test was completed prior to testing. The outcome measure was SWM total errors, i.e., the number of times which a box is selected that is certain not to contain a token and, therefore, should have not been visited by the participant.

Visual memory was assessed using the Pattern Recognition Memory (PRM) test in a two-choice forced discrimination paradigm. Participants were asked to remember a series of 12 abstract-coloured patterns; each presented for 3 s. They were then presented with a series of 12 pairs of old–new patterns and were asked to touch the pattern seen previously in each case. This procedure was repeated with a second set of 12 patterns followed by 12 pairs of patterns for recognition. The outcome measure was mean correct latency, i.e., the mean time (milliseconds) to respond correctly.

Menopausal symptoms

The Greene Climacteric Scale [57] was used to assess menopausal symptoms at baseline and post-intervention. This 21-item scale provides three main independent measures of psychological, somatic, and vasomotor symptoms. Participants were asked to indicate the extent to which they were currently bothered by the list of symptoms on a scale from 1 'not at all' up to 4 'extremely'.

Statistical analyses

An a priori power calculation was conducted using spatial working memory total error data obtained from the study of Thompson et al. [58]. Based on the probability of a type 1 error (α) = 0.05 and a Power of 0.9, 41 participants were required in each group to be able to reject the null hypothesis that the population means of the treatment groups are equal. To allow for dropouts, we aimed to recruit 150 women to the study. All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) with significance set at $P < 0.05$ throughout (IBM SPSS Statistics for Windows, version 24.0, IBM Corp, Armok NY). Only those participants that had completed cognitive testing at both baseline and post-intervention were included in the analysis. Intention-to-treat analysis was also performed including all participants randomised at baseline and did not change the primary outcome findings. The Shapiro–Wilk test was used to determine whether data followed a normal distribution and skewed variables were log-transformed to attain a normal distribution prior to analysis or analysed using non-parametric statistical tests. Transformations were applied to SWM, SSP, PRM, RTI, and MTS data. Descriptive statistics were used to present participant characteristics at baseline. The effect of intervention on the primary outcome measures of cognitive function (SWM, SSP, PRM, RTI, and MTS) was analysed using an analysis of covariance (ANCOVA) with baseline measures included as covariates. The secondary outcome measure of menopausal symptoms was analysed using a Kruskal–Wallis test. IF concentrations were compared between groups using a Kruskal–Wallis test with post hoc analysis conducted using a Mann–Whitney *U* test. Two outliers with post-intervention genistein concentrations of > 800 ng/ml in the low-dose treatment group and one in the high-dose group (baseline genistein of 275 ng/ml) were removed prior to statistical analysis of IF concentrations between groups. Sub-analysis was conducted to determine if the effect of the intervention on cognitive function and menopausal symptoms was significantly different between equol producers and non-producers using a Mann–Whitney *U* test. This test and one-way ANOVA with quadratic contrasts were also used in sub-analysis to investigate the effect of the intervention on VMS stratifying women according to severity of VMS at baseline. Dietary intake pre- and post-intervention was analysed using the Wilcoxin Signed-Rank test.

Results

A total of 101 post-menopausal women completed the study and were included in the final analysis. Participant progress through the study is illustrated in the CONSORT diagram [59] in Fig. 1. Baseline demographic characteristics of the study participants are shown in Table 1. There was no significant difference at baseline between the groups for any of the characteristics presented. Table 2 shows the effect of the intervention on cognitive function; no significant differences were observed between the groups for any of the cognitive function outcomes measured (RTI, SSP, SWM, PRM, and MTS). The soy drink had no effect on menopausal symptoms

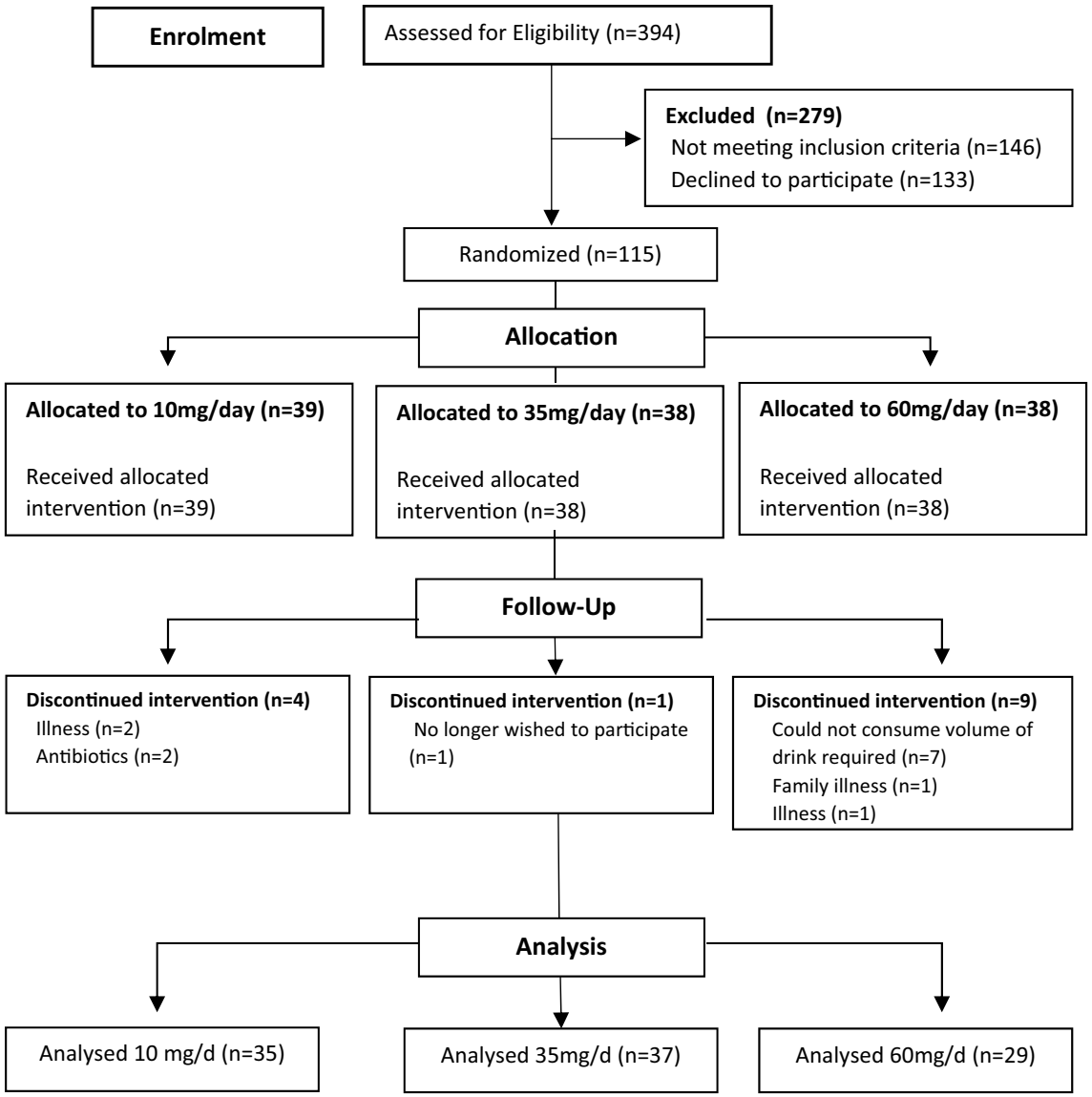

Fig. 1 CONSORT diagram of participant flow. A total of 394 women were assessed for eligibility with 279 excluded due to not meeting the inclusion criteria ($n=146$) or not wishing to participate in the study ($n=133$). Remaining post-menopausal women were randomised to receive a soy drink (Alpro®) and asked to consume a volume providing either 10 mg ($n=39$), 35 mg ($n=38$) or 60 mg ($n=38$) IFs daily. A total of 14 participants were lost to follow up owing to illness ($n=3$) unrelated to the intervention, antibiotic use ($n=2$), no longer wishing to participate ($n=1$), family illness ($n=1$) or being unable to consume the soy drink ($n=7$). A total of 101 women completed the study and were included in the final analysis

overall (Table 3); however, when women were stratified according to the severity of their VMS (hot flushes and night sweats) at baseline by splitting into two groups above/below mean, women with more severe VMS at baseline in the medium group had a significant reduction in symptoms after consuming the soy drink for 12 weeks, in comparison to those with less severe symptoms at baseline (Table 4). There was a significant quadratic trend for dose ($P=0.011$) with the observed reduction in symptoms in the medium-dose group being significantly greater in comparison to that observed in women with more severe VMS at baseline in the low-dose control group ($P=0.018$) and the high-dose group ($P=0.046$).

Compliance, as assessed via plasma IF concentrations, appeared good (Table 5), albeit some women found it difficult to consume the instructed volume of soy drink in the high-dose group, with seven women failing to complete the intervention for this reason. Blood samples were available for IF analysis for 95 participants at baseline and 87 post-intervention. IF concentrations were not significantly different between groups at baseline. As expected, post-intervention genistein concentration was significantly higher in the

Table 1 Baseline participant characteristics

Measure[a]	Soy IF treatment group		
	Low ($n=35$)	Medium ($n=37$)	High ($n=29$)
Age (years)	53.69 ± 3.72[b]	53.86 ± 3.28	53.72 ± 4.62
Height (m)	1.61 ± 0.065	1.64 ± 0.062	1.63 ± 0.073
Weight (kg)	70.32 ± 12.36	71.48 ± 13.85	72.91 ± 14.86
BMI (kg/m^2)	26.97 ± 5.22	26.76 ± 5.73	27.37 ± 5.77
Non-smokers (%)	94.12	100	100
Alcohol (units/week)	7.64 ± 6.58	11.73 ± 12.60	7.44 ± 10.21
Education level (n)			
Primary	0	1	0
Secondary	19	13	12
Tertiary	15	21	16
PA (METs/week)	165.17 ± 56.32	184.70 ± 57.48	192.74 ± 76.98
LMP (months)	31.97 ± 19.05	39.33 ± 21.99	39.38 ± 23.25
FSH (IU/ml)	83.02 ± 34.44	88.79 ± 28.33	85.84 ± 33.84
GHQ-28	16.91 ± 4.82	14.73 ± 5.48	15 ± 4.6
MMSE	29.17 ± 0.79	29.03 ± 1.07	29.21 ± 0.73

Characteristics were not significantly different between treatment groups

FSH follicle-stimulating hormone, *GHQ-28* General Health Questionnaire-28, *LMP* time since last menstrual period, *METs* metabolic equivalents, *MMSE* mini-mental state examination, *PA* physical activity

[a]Data on smoking, alcohol use, education, and physical activity were unavailable for 4 participants ($n=1$, $n=2$ and $n=1$ in the low-, medium-, and high-dose groups, respectively) as they failed to complete the health and lifestyle questionnaire

[b]Mean ± SD (all such values with the exception of smoking and education)

medium- ($P=0.007$) and high-dose ($P=0.013$) groups in comparison to the low-dose group. Post-intervention daidzein was also significantly higher in the medium- ($P=0.006$) and high-dose ($P=0.029$) groups versus the low group. There was no significant difference in post-intervention IF concentrations between the medium- and high-dose groups. Some 28.7% of the cohort was classified as equol producers with $n=7$, $n=9$, and $n=9$ participants classified as equol producers within the low-, medium-, and high-dose groups, respectively. In sub-analysis, cognitive performance was not significantly different according to equol status (Table 6) albeit, within the high-dose group, spatial working memory improved in equol producers (change from baseline −9.44 ± 15.69, $n=9$) in comparison to non-producers (2.36 ± 13.24, $n=23$), though this effect did not reach significance ($P=0.066$). VMS were significantly lower in equol producers in comparison to non-producers at both baseline (3.67 ± 1.01 vs 4.49 ± 1.60, $P=0.022$) and post-intervention (2.96 ± 1.04 vs 3.86 ± 1.67, $P=0.046$).

Table 2 Cognitive function of post-menopausal women at baseline and following 12 weeks' soy drink intervention

Treatment group	Low ($n=35$)			Medium ($n=37$)			High ($n=29$)		
Cognitive test	Baseline	Week 12	Change from baseline	Baseline	Week 12	Change from baseline	Baseline	Week 12	Change from baseline
RTI	373.66 ± 51.83[a]	367.06 ± 59.34	−6.60 ± 55.11	345 ± 45.66	354.15 ± 67	9.15 ± 55.07	349.33 ± 61.70	363.99 ± 70.79	14.66 ± 52.64
SSP	5.51 ± 1.06	5.77 ± 1.14	0.25 ± 1.22	5.65 ± 1.18	5.68 ± 1.23	0.03 ± 1.55	5.62 ± 1.18	5.76 ± 0.83	0.14 ± 1.36
SWM	33.86 ± 19.09	29.11 ± 16.95	−4.74 ± 15.74	28.7 ± 17.59	28.92 ± 17.85	0.22 ± 17.42	30.45 ± 16.04	27.90 ± 16.08	−2.55 ± 15.07
PRM	2054.25 ± 395.87	2083.14 ± 544.32	28.89 ± 417.47	2138.94 ± 587.38	1903.244 ± 434.09	−235.7 ± 557.83	1920.65 ± 458.59	1969.56 ± 568.19	48.91 ± 565.60
MTS	2836.70 ± 732.05	2518.54 ± 735.28	−318.16 ± 585.35	2846.98 ± 796.82	2456.46 ± 606.2	−390.52 ± 814.11	2694.54 ± 597.66	2535.90 ± 607.23	−158.64 ± 572.23

No significant differences were observed between the groups for any of the outcomes measured using an ANCOVA to compare post-intervention (week 12) cognitive function with baseline measures as covariates

RTI five-choice reaction time measured as reaction time latency (ms), *SSP* spatial span measured as longest sequence length recalled correctly, *SWM* spatial working memory measured as total errors made, *PRM* pattern recognition memory measured as mean correct latency (ms), *MTS* match to sample visual search measured as mean correct reaction time (ms)

[a]Mean ± SD (all such values)

Table 3 Menopausal symptoms at baseline and following 12 weeks' soy drink intervention

Treatment group	Low ($n=34$)			Medium ($n=35$)			High ($n=27$)		
Greene's Climacteric Scale	Baseline	Week 12	Change from baseline	Baseline	Week 12	Change from baseline	Baseline	Week 12	Change from baseline
Psychological score	18.29 ± 4.00[a]	17.47 ± 4.37	−0.83 ± 5.10	18.51 ± 4.92	16.40 ± 4.11	−2.11 ± 4.19	17.02 ± 3.55	15.93 ± 3.59	−1.10 ± 3.89
Vasomotor score	4.46 ± 1.58	3.97 ± 1.73	−0.49 ± 1.18	4.50 ± 1.54	3.49 ± 1.27	−1.01 ± 1.84	3.54 ± 1.22	3.37 ± 1.69	−0.17 ± 2.02
Somatic score	9.79 ± 1.81	9.29 ± 2.28	−0.49 ± 2.50	9.71 ± 2.30	9.49 ± 2.13	−0.23 ± 2.68	9.77 ± 2.64	9.26 ± 3.37	−0.51 ± 3.52
Total score	34.72 ± 5.37	32.76 ± 6.52	−1.96 ± 6.83	34.87 ± 7.31	31.29 ± 6.10	−3.58 ± 6.87	32.52 ± 6.42	30.52 ± 7.91	−2.00 ± 8.62

No significant differences were observed between the groups for any of the symptoms assessed as determined using a Kruskal–Wallis test

[a]Mean ± SD (all such values)

Energy intake, carbohydrate, protein, and total fat intake were not significantly different between the low-, medium-, and high-dose groups at baseline or post-intervention (Table 7). In the high-dose group, protein and total fat intake (expressed as % energy intake) were significantly lower post-intervention in comparison to baseline. Using the Goldberg cut-off technique [60], 12.8% and 15.7% of participants were identified as mis-reporters at baseline and post-intervention, respectively. Of these, three were within the low, two within the medium, and five within the high-dose groups at baseline with six within the low, two within the medium, and three within the high-dose groups post-intervention.

Discussion

In the current study, consumption of a soy drink for 12 weeks, providing 35 or 60 mg of IFs/day, had no effect on visual memory, working memory, or attention, in apparently healthy post-menopausal women in comparison to consumption of a low-dose control providing 10 mg IFs/day. We did not observe a significant effect for menopausal symptoms; albeit, sub-analysis identified a potential beneficial effect of soy IFs in women with more severe VMS at baseline. Our findings on cognitive function are in agreement with the previous studies that observed no beneficial effects of isoflavone supplementation on cognition in post-menopausal women [30, 32, 33]. In contrast, others have demonstrated an improvement in frontal lobe function (mental flexibility and planning) [24, 26], long-term episodic memory, sustained attention [24], psychomotor performance [27], verbal/semantic memory [25], and visual memory [29]. Variations in study design make it difficult to draw direct comparisons between the findings of our study and previous work. Mixed results may be reflective of the different cognitive tests used. Although two studies, showing beneficial effects, utilised CANTAB to assess cognitive function [24, 26], neither used the same tests as the current study. Given the wide range of methodologies currently available to assess cognitive function, there is a need to identify a standard method and testing suite to enable a better comparison between studies and reliably inform scientific knowledge in this area. Conflicting results may also be reflective of variation in the dose and duration of isoflavone supplementation used. Three studies that report improvements in cognitive function with soy IF supplementation have used much higher doses and/or have intervened over a longer duration than that used in the current study [25, 27, 29]. Furthermore, age/time since menopause may be a key factor in determining response to soy isoflavones, with Kritz-Silverstein et al. [25] observing significant effects on a test of visumotor tracking and attention in younger (50–59 years) but not older women (60–74 years). Kreijkamp-Kaspers et al. also observed no

Table 4 Effect of soy drink intervention on vasomotor symptoms stratifying women according to severity of symptoms at baseline

Treatment group	Low ($n=34$)			P^{a}	Medium ($n=35$)			P	High ($n=27$)			P
	Baseline	Week 12	Change from baseline		Baseline	Week 12	Change from baseline		Baseline	Week 12	Change from baseline	
Less severe	3.37 ± 0.90^{b}	3.05 ± 0.97	-0.32 ± 1.06	0.290	3.22 ± 0.73	3.28 ± 1.18	0.06 ± 1.21	0.001	2.64 ± 0.50	3.14 ± 2.11	0.50 ± 1.99	0.096
More severe	5.83 ± 1.10	5.13 ± 1.81	-0.70 ± 1.33^{A}		5.85 ± 0.83	3.71 ± 1.36	-2.15 ± 1.73^{B}		4.51 ± 0.99	3.62 ± 1.12	-0.89 ± 1.88^{A}	

[a]Mann–Whitney *U* test comparing change from baseline of those women with more severe vasomotor symptoms at baseline to those with less severe symptoms within groups

[b]Mean ± SD (all such values)

[A,B]Values with different superscript letters across a row are significantly different (one-way ANOVA with quadratic contrast analyses)

significant effects using the same test in a cohort of older post-menopausal women, aged 60–75 years [30]. In the longest intervention trial conducted to date, supplementation of 91 mg IFs daily for 2.5 years had no effect on global cognition, executive function, or verbal episodic memory in healthy post-menopausal women albeit, an improvement in a visual memory factor was observed [29]. In contrast to our study, there was a wide age range in this study (45–92 years) with almost half of the cohort > 10 years postmenopause; sub-analysis suggested that such women were less likely to show cognitive improvement and this may, therefore, have influenced the null findings in this study. Finally, differences in the makeup of the supplement used, in terms of the individual isoflavone constituents, may account for the observed mixed findings on cognition. Genistein and daidzein have distinct biological effects, e.g., genistein is a potent inhibitor of protein tyrosine kinase (PTK) [61], whereas daidzein is not a PTK inhibitor [62]. Genistein is now thought more promising as a treatment for Alzheimer's disease [63–65].

To our knowledge, only one other study has investigated the effects of IFs on post-menopausal cognition in the form of a soy drink [31]. In this study, consumption of IFs via a drink (72 mg/day) or a supplement (70 mg/day) over 16 weeks did not improve short-term memory, long-term memory, working memory, or selective attention as assessed using tests similar to those used in our study. The soy milk group showed a poorer performance in verbal working memory in comparison to the supplement and control groups; albeit this study was subject to limitations including subjective compliance, lack of power, and lack of controls, owing to the quasi-experimental design.

In agreement with the previous literature, 28.7% of our study cohort were equol producers [38–40]. Limited evidence suggests that the ability to produce equol may confer beneficial effects on cognitive function following soy intake [66, 67], potentially via increased cerebral blood flow [68]. We did not observe any significant differences in cognitive performance following intervention between equol producers and non-producers, although, within the high-dose group, improvements in spatial working memory in producers versus non-producers were approaching significance. Our findings support those of Henderson et al. [29] who observed a nonsignificant trend towards improved global cognition in consistent equol producers. Similar to Henderson's study, the sample size in the current study was likely too small to adequately investigate the role of equol in cognitive performance and further research in this area is warranted.

In agreement with our findings, previous studies, including 2 that used the same scale as that in the current study [24, 26], have reported no effect of IF supplementation on menopausal symptoms [24, 26, 32]. Basaria et al. observed an improvement in menopausal symptoms using the menopause-specific quality-of-life questionnaire; however, the

Table 5 IF concentrations at baseline and following 12 weeks' soy drink intervention

Treatment group	Low		Medium		High	
	Baseline ($n=32$)	Week 12 ($n=32$)	Baseline ($n=35$)	Week 12 ($n=32$)	Baseline ($n=28$)	Week 12 ($n=23$)
Genistein (ng/ml)	10.01 ± 22.07[a]	82.75 ± 124.62[A]	9.83 ± 24.25	168.71 ± 166.81[B]	6.40 ± 9.27	216.71 ± 305.36[B]
Daidzein (ng/ml)	5.03 ± 11.88	20.44 ± 30.82[A]	2.29 ± 4.37	39.20 ± 36.9[B]	2.50 ± 3.34	49.82 ± 66.49[B]
Equol (ng/ml)	1.76 ± 6.01	3.81 ± 8.17[A]	BLD	9.43 ± 19.84[A]	0.42 ± 1.30	11.69 ± 19.23[A]

BLD below limit of detection

[a]Mean ± SD (all such values)

[A,B]Values with different superscript letters across a row are significantly different (Kruskal–Wallis with post hoc Mann–Whitney *U* test)

Table 6 Effect of soy drink intervention on cognitive performance stratifying women according to equol producer status

Cognitive test	Change from baseline		P^b
	Equol non-producers	Equol producers[a]	
	$n=62$	$n=25$	
RTI	6.23 ± 54.98[c]	− 6.23 ± 41.30	0.764
SSP	0.21 ± 1.34	0.12 ± 1.42	0.735
SWM	− 1.82 ± 15.34	− 6.16 ± 16.36	0.245
PRM	− 45.58 ± 533.00	− 62.66 ± 557.10	0.453
MTS	− 363.39 ± 675.51	− 163.99 ± 630.38	0.234

RTI five-choice reaction time measured as reaction time latency (ms), *SSP* spatial span measured as the longest sequence length recalled correctly, *SWM* spatial working memory measured as total errors made, *PRM* pattern recognition memory measured as mean correct latency (ms), *MTS* match to sample visual search measured as mean correct reaction time (ms)

[a]Defined as plasma equol concentration > 20 nmol/l (5 µg/l)

[b]Independent samples *t* test or Mann–Whitney *U* test for non-parametric data

[c]Mean ± SD (all such values)

dose used in this study was very high (160 mg/day) [33]. A recent meta-analysis has demonstrated that soy IF supplementation can significantly reduce hot flush frequency and severity in comparison to placebo, with supplements containing > 18.8 mg of genistein being most effective [12]. In support of these findings, our study has shown that women with more severe VMS at baseline showed a significant improvement in symptoms following consumption of 35 mg IFs/day (providing ~ 18 mg genistein) in comparison to those consuming 10 mg/day. This observation was not replicated in the high-dose group, possibly due to the sample size, and thus, further studies are required to confirm these findings. Our findings in the medium group are in agreement with an open-label crossover study that demonstrated a commercial soy drink (ViveSoy®), providing ~ 50 mg IFs per day for 12 weeks, improved climacteric symptoms including hot flushes in peri/post-menopausal women [69]. Previous research on the effects of soy foods on menopausal symptoms is limited [70] and our findings warrant further study in this area. In support of the role of S-equol in the alleviation

Table 7 Dietary intake at baseline and following 12 weeks' soy drink intervention

Treatment group	Low			Medium			High		
	Baseline ($n=27$)	Week 12 ($n=28$)	P^a	Baseline ($n=27$)	Week 12 ($n=21$)	P	Baseline ($n=24$)	Week 12 ($n=21$)	P
Energy (Kcal/day)	1532 ± 451.72[b]	1506 ± 526.37	0.715	1623 ± 549.91	1792.62 ± 665.34	0.845	1562 ± 501.51	1731 ± 583.89	0.163
Carbohydrate (g/day)	165.52 ± 47.76	154.21 ± 46.65	0.144	165.67 ± 44.26	162.43 ± 50.24	0.500	173.38 ± 63.37	170.00 ± 54.52	0.472
Protein (g)	74.74 ± 42.49	72.39 ± 24.59	0.726	75.85 ± 33.37	71.90 ± 28.28	0.184	77.21 ± 27.46	70.71 ± 32.31	0.139
Fat (g)	61.07 ± 25.96	59.86 ± 18.31	0.935	59.56 ± 16.74	65.40 ± 23.34	0.887	61.21 ± 23.32	59.00 ± 25.08	0.868
Carbohydrate (% EI)	44.32 ± 9.85	45.74 ± 28.91	0.068	42.52 ± 8.99	37.60 ± 7.82	0.184	44.26 ± 5.31	41.00 ± 11.35	0.744
Protein (% EI)	15.55 ± 2.55	18.28 ± 12.52	0.563	15.27 ± 3.42	15.37 ± 5.84	0.500	15.46 ± 2.13	13.59 ± 3.67	0.018
Fat (% EI)	42.76 ± 17.35	47.89 ± 25.98	0.503	42.11 ± 11.52	38.67 ± 12.85	0.112	46.94 ± 19.53	37.71 ± 13.52	0.022

Nutrient intake did not differ between the groups at either baseline or post-intervention (Kruskal–Wallis test)

EI energy intake

[a]Wilcoxon signed-rank test comparing week 12 to baseline within groups

[b]Mean ± SD (all such values)

of VMS [41], equol producers in the current study had significantly lower VMS scores throughout in comparison to non-equol producers.

Strengths of this study include the use of CANTAB, a well-validated method in digital cognitive assessment. CANTAB is a sensitive method to detect changes in cognitive domains, including spatial memory, executive function, and information processing, in response to nutrition intervention trials, including soy isoflavone trials [71]. Despite being a more sensitive measure, one limitation of this method, highlighted within a recent International Life Sciences Institute (ILSI) review [71], is the speed/accuracy trade-offs associated with the more complex information processing tasks available within computerized test batteries as well as training/practice effects in comparison to simple information processing tasks. In the current study, cognitive assessment was conducted 12 weeks apart, and thus, training/practice effects would be negligible. Cognitive assessment was strictly controlled with participants consuming the same standard caffeine-free breakfast prior to testing at both timepoints. Time since menopause was kept within a defined limit of < 7 years and compliance was closely monitored. The main limitations of the study were the absence of a placebo control and the non-blinded study design. Nonetheless, the low-dose control drink provided only 10 mg IFs/day, an amount well below that typically consumed in Asian populations (~ 25–50 mg/day) [72] and well below the lowest dose previously used in intervention studies on cognitive function in post-menopausal women (60 mg/day) [24, 26, 27]. Given that we used a commercially available soy drink in the current intervention study, we were limited with regard to the dose of IFs that could be tested. Nonetheless, it is important to investigate the effects of soy foods in addition to IF supplements as consumers may gain additional nutritional benefits from consuming soy food as part of a healthy balanced diet [73]. Furthermore, IF pharmacokinetics are similar following ingestion of soy foods and IF supplements [74]. We did not reach our recruitment target of 41 participants/group, largely due to participants not meeting the stringent inclusion criteria (of note was the substantial number of post-menopausal women currently on antidepressants); however, the study was still adequately powered (80% power) to detect significant effects. Reference ranges are not provided for the CANTAB tests used for healthy populations; however, our data concur with a previous study in healthy older women [75].

Our participants were apparently healthy and relatively early post-menopausal (< 7 years; mean 3.05 ± 1.78 years post-menopausal) and our findings are, therefore, not representative of all post-menopausal women. Women > 7 years post-menopausal are less likely to be prone to fluctuating hormone levels and associated menopausal symptoms [76]. Furthermore, our findings cannot be extrapolated to post-menopausal women suffering mild cognitive impairment; further research in this area is needed.

In conclusion, a commercially available soy drink had no effect on cognitive function in post-menopausal women. Consumption of ~ 350 ml/day (providing 35 mg IFs) for 12 weeks significantly reduced VMS in those women with more severe symptoms at baseline, a finding with potential clinical relevance that warrants further research given the high prevalence of VMS in western women [77, 78].

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical standards This study was approved by Ulster University's Research Ethics Committee (REC/15/0025) and was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendment. All participants gave their informed consent prior to their inclusion in the study.

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