

BMJ Open Study protocol for a double-blind randomised controlled trial investigating the impact of 12 weeks supplementation with a *Fucus vesiculosus* extract on cholesterol levels in adults with elevated fasting LDL cholesterol who are overweight or have obesity

Margaret Murray,¹ Aimee L Dordevic,¹ Katherine H M Cox,² Andrew Scholey,² Lisa Ryan,³ Maxine P Bonham¹

To cite: Murray M, Dordevic AL, Cox KHM, *et al*. Study protocol for a double-blind randomised controlled trial investigating the impact of 12 weeks supplementation with a *Fucus vesiculosus* extract on cholesterol levels in adults with elevated fasting LDL cholesterol who are overweight or have obesity. *BMJ Open* 2018;**8**:e022195. doi:10.1136/bmjopen-2018-022195

► Prepublication history and additional material for this paper are available online. To view these files, please visit the journal online (<http://dx.doi.org/10.1136/bmjopen-2018-022195>).

Received 6 February 2018
Revised 29 August 2018
Accepted 9 October 2018



© Author(s) (or their employer(s)) 2018. Re-use permitted under CC BY-NC. No commercial re-use. See rights and permissions. Published by BMJ.

For numbered affiliations see end of article.

Correspondence to

Prof Maxine P Bonham;
maxine.bonham@monash.edu

ABSTRACT

Introduction Hyperlipidaemia, hyperglycaemia and chronic inflammation are risk factors for chronic diseases cardiovascular disease and type 2 diabetes. Polyphenols are bioactive compounds found in marine algae with potential antihyperlipidaemic, antihyperglycaemic and anti-inflammatory effects. The modulation of these risk factors using bioactive polyphenols may represent a useful strategy for disease prevention and management; research in humans, however, remains limited. This trial aims to determine the impact of a polyphenol-rich brown seaweed extract on fasting hyperlipidaemia, hyperglycaemia and inflammation. Effects on mood and cognition will also be evaluated.

Methods and analysis Fifty-eight hypercholesterolaemic participants who are overweight or have obesity will be randomised to receive either a polyphenol-rich brown seaweed extract (2000 mg dose containing 600 mg polyphenols) or placebo (2000 mg rice flour) daily for 12 weeks. Fasting venous blood samples will be taken at baseline, week 6 and week 12 of the intervention to assess serum cholesterol (total, low-density lipoprotein and high-density lipoprotein) and triglyceride concentrations, plasma glucose and insulin concentrations and markers of inflammation. Mood and cognitive function will be evaluated as exploratory outcomes. Independent t-tests or equivalent will be used to determine differences between the two groups in changes from baseline to week 12. Analysis of variance will be used to assess differences between the groups across the three time points (baseline, week 6 and week 12).

Ethics and dissemination Ethics approval has been granted by the Monash University Human Research Ethics Committee (2017-8689-10379). Results from this trial will be disseminated through publication in peer-reviewed journals, national and international presentations, and a PhD thesis. These results are essential to inform the use of polyphenol-rich brown seaweeds as a functional food or nutritional supplement

Strengths and limitations of this study

- This study will use a double-blind, placebo-controlled randomised trial to investigate the antihypercholesterolaemic effects of a *Fucus vesiculosus* extract.
- This study will be the first to investigate the effect of supplementation with a *Fucus vesiculosus* extract on inflammation in humans at risk of metabolic disease.
- This will be the first study to investigate the anti-hyperlipidaemic, anti-hyperglycaemic and anti-inflammatory effects of a *Fucus vesiculosus* extract in an Australian population.
- This study is powered based on data from a different species of seaweed (*Ecklonia cava*).

ingredients for health promotion and disease prevention and management in humans.

Trial registration number ACTRN12617001039370; Pre-results.

INTRODUCTION

For billions of people worldwide, overweight and obesity is an intractable problem, putting them at increased risk of numerous non-communicable diseases.¹ Weight loss through lifestyle modification has been the primary prevention strategy to date, however, weight loss is challenging to maintain over the long term.^{2,3} Strategies should consider metabolic mechanisms to reduce the onset of obesity-related comorbid diseases, such as cardiovascular diseases (CVD) and type 2 diabetes mellitus (T2DM). Central to this issue is the

identification of bioactive food components that are able to modulate some of the negative metabolic responses associated with obesity (eg, hyperlipidaemia, hyperglycaemia and increased proinflammatory mediators), and thus reduce the risk of chronic disease. The investigation of brown algae (or seaweed), rich in polyphenolic compounds, to modify hyperlipidaemia, hyperglycaemia and inflammation is an emerging area of nutritional science research.⁴

Brown algae or brown seaweeds have long been used as medicinal herbs in Asian countries. They contain a number of bioactive compounds with potential to improve health, including polysaccharides, such as fucoidan ((1,2)- α -L-fucose-4-sulfate with branching or sulfate ester group on C3)⁵ and phytochemicals, such as polyphenols.⁶ Recently, there has been an increased interest in polyphenols for health promotion and disease prevention, particularly the unique class of polyphenols, called phlorotannins, found only in macroalgae.^{4,7,8} Phlorotannins are hydrophilic molecules that contain phenyl (C₆H₅-) and phenoxy (C₆H₅O-) groups. They are formed through the polymerisation of phloroglucinol monomer units (1,3,5-trihydroxybenzene) and vary in structure and size (126 Da to 650 kDa).⁴ Their natural function is as part of the defence system of the alga.⁴

Current scientific evidence for the antihyperlipidaemic effects of brown algae is promising. Research has shown that polyphenols, from the alga *Ecklonia cava*, dose dependently inhibited the activity of 3-hydroxy-3-methylglutaryl-coenzyme A (HMGCoA) reductase, an enzyme involved in cholesterol production, in vitro.⁹ This effect has been translated into reductions in total cholesterol, triglyceride (TG) and low-density lipoprotein (LDL) cholesterol levels after 5 weeks treatment with the same polyphenol extract in high-fat diet-fed mice, compared with high-fat diet-fed mice who received no treatment.⁹ Other animal studies have also demonstrated reductions in total cholesterol, TG and LDL cholesterol levels, and increases in high-density lipoprotein (HDL) cholesterol levels, following treatment with polyphenol-rich algal extracts over 3 days to 12 weeks, in diabetic, obese and hyperlipidaemic mice and rats.¹⁰⁻¹² Similarly, treatment with fucoidan-rich brown seaweed extracts have resulted in reductions in serum TG, total and LDL cholesterol levels and increases in HDL cholesterol levels in hyperlipidaemic rats (28 days treatment)¹³ and hyperlipidaemic mice (acute 24 hours challenge),¹⁴ through modulation of gene expression and enzyme activity.

To date there have been three randomised controlled trials (RCTs) in humans investigating the anti-hyperlipidaemic effects of polyphenol-containing brown seaweed extracts. In an RCT in Korea, 97 adults who were overweight consumed a daily dose of a polyphenol extract (98.5% phloroglucinol equivalents) from *E. cava*, 72 or 144 mg polyphenols, or placebo, for 12 weeks. The treatment groups observed significant reductions in total and LDL cholesterol levels, and total to HDL cholesterol ratio, in a dose-dependent manner, compared with

placebo.¹⁵ Again, in Korea, 63 adults with raised cholesterol (>200 mg/dL total cholesterol or >110 mg/dL LDL cholesterol) consumed a polyphenol-rich *E. cava* extract (400 mg/day, containing 8.2% dieckol (a phlorotannin)) or placebo, for 12 weeks. Reductions in total and LDL cholesterol were observed in the treatment group, compared with placebo.¹⁶ In a third RCT in Mexico, 25 volunteers who were overweight or obese consumed 500 mg of a fucoidan-rich brown seaweed extract (containing 25 mg polyphenols), daily. No differences between active and placebo treatments were reported for total cholesterol, TG or HDL cholesterol levels following 3 months of treatment. However, a reduction in LDL cholesterol levels was observed in the treatment group compared with no change in the placebo group.¹⁷

Polyphenol-rich brown seaweed extracts have demonstrated, in a small number of RCTs, the lowering of total cholesterol, TG and LDL cholesterol levels, likely through the inhibition of the enzyme HMGCoA reductase and other mechanisms yet to be established.^{4,18} However, these studies have been restricted to Asian and Mexican populations and primarily used extracts from the algal species *E. cava*. This study will be the first to investigate a polyphenol-rich *Fucus vesiculosus* extract for cholesterol lowering, and the first to do so in an Australian population. This is particularly relevant now given the rapid growth in the functional food and nutritional supplement market, with particular consumer interest in marine products that are natural, sustainable and nutrient dense.^{8,19-22} However, the claimed health effects of these products remain largely without an evidence base.²³ If polyphenol-rich brown seaweed extracts are effective at lowering cholesterol levels, they have great potential to become a functional food or nutritional supplement ingredient.²³ Therefore, the focus of this study will be to determine the cholesterol lowering effects of a novel, polyphenol-rich brown seaweed extract.

Secondary outcomes of this study include the effects of the polyphenol-rich brown seaweed extract on glycaemia, inflammation, and cognition and mood. There is promising evidence for the antihyperglycaemic^{11,24-28} effects of algal polyphenols in cell and animal models, as recently reviewed by the authors.⁴ However, only three RCTs have investigated the effects of supplementation with a polyphenol-rich brown seaweed extract on fasting glucose and/or insulin levels in humans. After 12 weeks treatment, they found mixed, but largely insignificant results.^{15,17,29} In comparison, more studies have been conducted on the effects of land-based polyphenols on glycaemia, and two reviews of these studies came up with similar conclusions; that their antihyperglycaemic effects are promising, but results remain limited and varied.^{30,31} This RCT will add to the evidence for the antihyperglycaemic effects of polyphenol-rich brown seaweed extracts in humans with elevated LDL cholesterol.

Published evidence for the anti-inflammatory effects of algal polyphenols has shown promise in in vitro and animal models, including the downregulation of

proinflammatory markers interleukin (IL)-10, IL-6, IL-1 β and tumour necrosis factor (TNF)- α .^{11 28 32–35} Similarly, anti-inflammatory effects of numerous land-based polyphenols have also been demonstrated in vivo and in vitro.^{36 37} A 2016 review of land based, specifically fruit, polyphenols indicated that they also show anti-inflammatory effects in humans.³⁸ However, to date, there have been no studies investigating the anti-inflammatory effects of algal polyphenols in humans,¹⁸ despite promising evidence from cell and animal models. This will be the first RCT to investigate the effects of supplementation with a polyphenol-rich brown seaweed extract on markers of chronic inflammation in humans.

A further novel aspect of this study will be the investigation of the effects of a polyphenol-rich brown seaweed extract on cognitive function and mood. The brain is a highly metabolically active organ making it susceptible to oxidative stress, inflammatory processes and fluctuations in blood flow and vascular processes.³⁹ Management of these processes involves multiple biological systems which are influenced by dietary intake, including polyphenols. In healthy older adults, supplementation with polyphenols from curcumin has acutely improved sustained attention and working memory, and improved working memory and mood (alertness and contentedness) over 4 weeks.⁴⁰ In young and middle-aged adults, supplementation with cocoa polyphenols acutely improved self-reported mental fatigue and performance in a serial subtraction cognitive task and, over 30 days, improved mood (calmness and contentedness).⁴¹ There has been one study, to date, that has investigated the effects of algal polyphenols on cognition in humans.⁴² The double-blind, placebo-controlled, randomised parallel trial found that supplementation with algal polyphenols led to improved performance in cognitive tasks relating to attention and reaction time.⁴² These results were observed acutely, following a single dose of polyphenols, there are no human trials that have investigated the effects of algal polyphenols on cognition and mood over an extended period. This will be the first study to investigate the effects of 12 weeks supplementation with a polyphenol-rich brown seaweed extract on cognitive function and mood.

Algal polyphenols show potential as antihyperlipidaemic, antihyperglycaemic and anti-inflammatory agents.⁴ The strongest evidence so far in humans is for the LDL cholesterol-lowering effects of polyphenol-rich brown seaweed extracts.¹⁸ The primary aim of this trial will be to investigate the effects of a polyphenol-rich extract from the brown algae species *F. vesiculosus* on LDL cholesterol in adults with hypercholesterolaemia. Our hypothesis is that the primary outcome, namely fasting LDL cholesterol levels, will be reduced in those taking the extract, compared with those in the placebo group. Secondary outcomes include changes in fasting total and HDL cholesterol levels, TG levels, fasting glucose and insulin levels, and, for the first time in humans, changes in inflammatory markers, mood and cognitive function. Based on current evidence, we hypothesise that taking the

extract will reduce fasting total cholesterol, TG, glucose and insulin levels, increase HDL cholesterol levels, that it will downregulate proinflammatory markers and improve cognitive function. If effective, polyphenol-rich brown seaweed extracts show great potential to be used as a functional food or nutritional supplement ingredients for the prevention and management of chronic disease such as CVD and T2DM.²³

METHODS AND ANALYSIS

Primary aim

To investigate the impact of 12 weeks supplementation with a polyphenol-rich brown seaweed extract on fasting LDL cholesterol levels in hypercholesterolaemic adults.

Secondary aims are to investigate the impact of 12 weeks supplementation on fasting total cholesterol, HDL cholesterol, TG, glucose and insulin levels, as well as inflammatory markers and cognitive function in hypercholesterolaemic adults.

Hypothesis

Taking a polyphenol-rich brown seaweed extract for 12 weeks will result in a reduction in fasting LDL cholesterol levels, compared with placebo.

Taking a polyphenol-rich brown seaweed extract for 12 weeks will result in a reduction in fasting total cholesterol, TG, glucose and insulin levels and an increase in HDL cholesterol levels. It will result in a reduction in proinflammatory markers and improvement in cognitive function, compared with placebo.

Study design

This trial will be a double-blind, placebo-controlled, randomised parallel trial with a 1:1 allocation ratio to the intervention and placebo group. This protocol is reported according to the Standard Protocol Items: Recommendations for Interventional Trials 2013 Statement.⁴³

Setting

The present study will take place in a research clinic at Monash University in Melbourne, Australia. Study participants will be recruited from the local public.

Participants

Eligibility criteria

Participants will be male and female volunteers, aged 18–65 years, with a body mass index (BMI) ≥ 27 to ≤ 35 kg/m² (25–35 kg/m² for participants of an Asian background) and fasting LDL cholesterol level above 2.0 mmol/L. The National Vascular Disease Prevention Alliance recommends an LDL level of < 2.0 mmol/L as a target for primary prevention, to reduce absolute CVD risk.⁴⁴

Exclusion criteria

Participants will be excluded if they have any gastrointestinal issues that may affect the absorption and intestinal actions of the polyphenols; are taking medication

for cholesterol, blood glucose or blood pressure control or any other medications that may influence results; are taking other natural health products known to impact on polyphenols or cholesterol, for example, fish oil or phytosterols; are breast feeding or pregnant; have a serious health condition (eg, liver/thyroid issues or recent major surgery); consume more than 4 standard drinks per day, or 14 standard drinks per week; are a cigarette smoker; or have an implanted cardiac defibrillator. Participants will be excluded from the cognitive and mood testing if they have a history of, or current depression, anxiety or signs of cognitive decline.

Recruitment/screening

Participants will be recruited from the public through advertisements in Monash University networks, local papers, social media, and local health and community centre, through personal contacts, snowballing, participant databases and mail out.

Interested participants will complete an online screening questionnaire (Qualtrics, Provo, UT) with embedded explanatory statement, which collects demographic and medical information. Following researcher review of the screening questionnaire, eligible participants will be invited to attend a screening session at the Be Active Sleep Eat (BASE) facility, Monash University.

During this session, a fasting (≥ 12 hours fast) finger prick blood sample will be taken to assess participants' LDL cholesterol level using the CardioChek PA Analyser (Multipoint Technologies, Preston, Victoria, Australia). Blood pressure will be measured in duplicate in a seated position using a portable sphygmomanometer (Welch Allyn, Skaneateles Falls, New York, USA). To determine BMI, we will measure height, using the Harpenden Stadiometer (Holtain, Crymych) and weight, using the SECA mBCA 515 medical body composition analyser (serial no. 1000000044061, SECA, Hamburg), according to standard procedures. Those who meet the eligibility criteria will provide their written informed consent to participate in the trial, in the presence of the investigator.

In order to participate in the cognitive and mood testing, participants will undergo further screening. The Beck Depression Inventory-II⁴⁵ will be used to screen for clinically significant depression, participants with a score of 10 or below will be eligible to participate. The Mini-Mental State Examination⁴⁶ will be used to screen for cognitive decline, participants with a score 24 or above will be eligible to participate. Participants will be asked whether they have a history of psychiatric disorders. The trait scale of the State-Trait Anxiety Inventory⁴⁷ will be used to assess participants' tendency towards anxiety, but will not be used to exclude potential participants. Participants deemed ineligible for the cognitive and mood testing but who meet the rest of the inclusion criteria will be invited to participate in the main part of the study, without the cognitive and mood testing.

Randomisation, allocation concealment and sequence generation

Participants will be randomised to receive either the intervention or the placebo product. Computer-generated randomisation will be used to determine whether participants receive the polyphenol-rich extract or placebo. Each supplement will be coded with a corresponding letter to conceal its identity. The concealed allocation sequence will be generated by an investigator who will not be involved in participant enrolment or data collection (MPB). A separate investigator who will be blinded to the allocation codes (MM) will carry out participant enrolment and assignment to intervention. All supplements will be encapsulated in identical opaque capsules and given a code to conceal their identity. It will be unknown to the participants, and investigator carrying out participant enrolment, data collection and analysis (MM), which supplement each participant receives. As this supplement has shown no adverse consequences in an acute study, it is highly unlikely that early unblinding of participants will be required. Unblinding will only be permissible once participants have completed the intervention and results have been analysed. The investigator (MM) will remain blinded until analysis of results is complete.

Sample size calculation

Power analyses have been performed to identify the required sample size to detect a change in fasting LDL cholesterol level following intervention, at a power of 80%. Using G*Power V.3.1.9.2,⁴⁸ the sample size was calculated based on data from Shin *et al*,¹⁵ a similar 12-week algal polyphenol intervention study that observed a reduction in LDL cholesterol levels of 0.5 mmol/L (14%) following intervention. A total sample of 52 individuals or 26 per treatment group will be required to detect a change of this size in LDL cholesterol level. To account for a drop-out rate of approximately 10%, we plan to recruit 58 participants.

Preintervention

Prior to the baseline testing visit participants will be asked to complete a 3-day food diary to assess their baseline dietary habits. A Food Frequency Questionnaire (FFQ)⁴⁹ will be used to estimate participants' baseline total polyphenol intake. The International Physical Activity Questionnaire (IPAQ) short version will be used to assess baseline physical activity levels.⁵⁰

Participants taking part in the cognitive and mood testing will be familiarised with all study measures prior to baseline. They will also complete at least two, up to four, practice runs of an abbreviated version of the cognitive test battery prior to baseline testing, in order to minimise any practice effects and errors due to misunderstanding at baseline.

Study intervention

The intervention will be a 12-week protocol, over which time participants take a daily dose of either the placebo

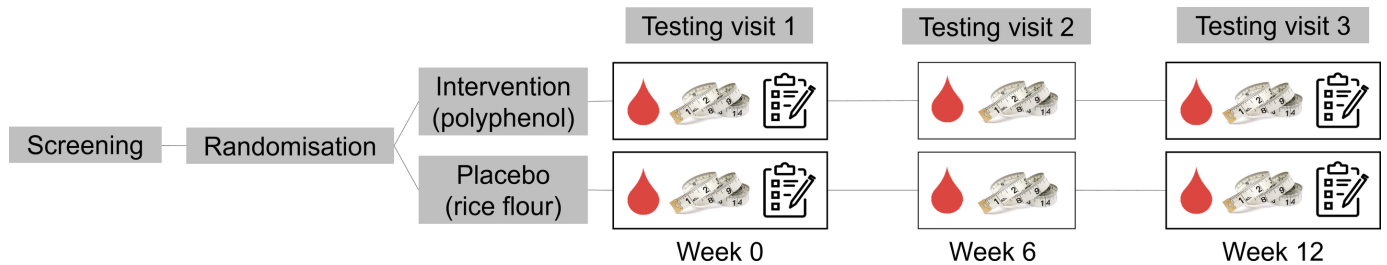


Figure 1 Flow diagram of study design.

or intervention product. The intervention product will be a 2000 mg dose of a commercially available powdered extract from the brown seaweed *F. vesiculosus*, containing 600 mg polyphenols and 1200 mg fucoidan (a complex carbohydrate) (Marinova, Tasmania). The placebo will be a 2000 mg dose of commercially available rice flour (Ward McKenzie, Altona, Victoria, Australia). All test products will be encapsulated in identical opaque size 0 capsules (The Melbourne Food Depot, Australia), to be taken in the form of four oral capsules per dose. Participants will be instructed to consume one dose per day with their evening meal.

Participants will attend three testing sessions over the course of the study, held at baseline (week 0), week 6 and week 12 (figure 1). The testing sessions will begin approximately 1 week after the screening session. The night before each testing session, participants will consume a standardised dinner meal (pasta with tomato-based sauce: energy 3350 kJ; carbohydrate 117 g; protein 23 g; fat 24 g) between 19:00 and 21:00 and then fast overnight.

The selected intervention length is 12 weeks, as polyphenol interventions have previously shown significant changes in cholesterol levels within this period.^{15–17} An additional 6-week testing session will be included to assess whether changes in cholesterol levels occur any earlier than 12 weeks. Participants will be asked to maintain their usual dietary, physical activity and sleeping habits throughout the trial.

Data collection

Biochemical measures

At baseline, week 6 and week 12, fasting (>11 hours) venous blood samples will be taken by a nurse or trained phlebotomist to collect serum (BD Vacutainer SST II Advance Tubes, 8.5 mL, Becton, Dickinson and Company, Franklin Lakes, New Jersey, USA) and plasma (BD Vacutainer K₂EDTA Tubes, 10 mL, Becton, Dickinson and Company) for biochemical measures. LDL, HDL and total cholesterol and TG levels will be measured from serum samples, glucose and insulin levels, and inflammatory markers will be measured from plasma samples.

Plasma samples will be centrifuged immediately for 10 min at 4°C at 1.0 RCF (serial no. 5703BI110739, Eppendorf AG, Hamburg). Serum samples will be given at least 30 min clotting time then centrifuged for 10 min at 1.6 RCF at room temperature (22°C–25°C). The plasma and

serum will then be aliquoted and stored at –80°C until analysis.

Cholesterol, TG, glucose and C reactive protein concentrations will be measured on a Thermo Fisher Indiko clinical chemistry analyser (Thermo Fisher Scientific™, Vantaa, Finland) by enzymatic colorimetric methods using commercially available kits as per the manufacturer's instructions (Thermo Fisher Scientific™). Plasma insulin concentrations will be assessed using Millipore ELISA Kits for Human Insulin (Cat. # EZHI-14K and EZHI-14BK, Merck Millipore, Victoria, Australia) according to manufacturer's instructions, with absorbance read using the Rayto Microplate reader (450 nm wavelength, RT-2100C, Abacus ALS, Queensland, Australia). Inflammatory markers (including TNF- α , IL-1 β , IL-8, IL-10 and adipokines) will be measured on a MagPix (Luminex, Austin, Texas, USA) system using commercially available multiplex magnetic bead panel assays (MILLIPLEX MAP, EMD Millipore Corporation, Billerica, Massachusetts, USA).

Anthropometry

Anthropometric measurements will be taken at baseline, week 6 and week 12. Height will be measured using the Harpenden Stadiometer (Holtain, Crymych) with shoes and socks removed. Weight and body composition (% fat mass, % fat free mass, visceral fat (L)) will be measured using the SECA mBCA 515 medical body composition analyser (SECA, Hamburg), with shoes and socks removed and participants in light clothing. A waist circumference measure will be taken over light clothing or bare skin at the narrowest point around the torso.⁵¹

Blood pressure

Resting blood pressure will be measured at baseline, week 6 and week 12, after 10 min of being seated, using a portable sphygmomanometer (Welch Allyn). Three measurements will be taken to calculate an average at each visit.

Mood measures

Participants taking part in the mood testing will complete mood measures pre and post the cognitive test battery at baseline, week 6 and week 12. State mood will be evaluated using computerised versions of the Bond-Lader Visual Analogue Scales,⁵² which combines scores from 16 individual items to generate three measures of alertness,

contentedness and calmness. Possible scores on these measures range from 0 to 100, with greater scores indicating a more desirable state. Two additional Visual Analogue Scales measure stress and fatigue, also scored from 0 to 100, however, here a lower score indicates lower levels of stress and fatigue and is more desirable. State anxiety will be measured using the state scale of the State-Trait Anxiety Inventory.⁴⁷ Possible scores range from 20 to 80, with higher scores indicating greater anxiety. These measures ask participants to respond according to how they currently feel. Any change in mood following the cognitive array will be interpreted as the impact of undertaking a mental challenge on mood.

Cognitive array

Participants taking part in the cognitive testing will complete a 30 min test battery at baseline, week 6 and week 12. Cognitive assessment will comprise parallel versions of tasks (listed below) from the Computerised Mental Performance Assessment System (Northumbria University, Newcastle upon Tyne, UK).⁵³

Immediate word recall task

Twenty single words will be presented on screen for 1000 ms with an interstimulus interval of 1000 ms. Immediately following the final word participants will be given 60 s to write down as many words as can be recalled. The task will be scored as the number of correctly recalled words.

Two-back letters

Letters will be shown on screen one at a time for 500 ms with an interstimulus time of 2500 ms. The participant must decide if the letter displayed on screen is the same as the one shown two letters previously. The task will be scored for accuracy and response time.

Serial three and serial seven subtraction tasks

A starting number between 800 and 999 will be presented on screen. Participants mentally subtract three/seven and provide their answer using the keyboard number keys. They will continue subtracting three/seven from the previously entered answer for 2 min. Each digit entered will be represented on screen by an asterisk, thus requiring all subtractions to be done mentally, without visual assistance. The task will be scored as the number of correct responses.

Corsi blocks

Nine blue squares on a black background will be displayed on the screen. Some of the blue squares will change to red and back to blue again in a sequence. Participants will be required to remember this sequence and use the cursor to click the blocks in the exact sequence in which they changed colour. The task will be repeated five times at each level of difficulty with the sequence span increasing from four to nine squares in each sequence, as long as participants are making enough correct responses. As soon as they make less than three correct responses (out of the five in one level) the task will end. A span score

will be calculated as the average span of the final three correctly completed trials.

Digit vigilance task

Two single-digit numbers will be presented on screen. The 'target number' will remain steady while the other changes at a rate of 80 numbers per minute for 3 min. Participants will respond with a button press when the changing number matches the target number. The task will be scored for accuracy, response time and false alarm rate.

Delayed word recall task

Participants will be given 60 s to write down as many of the 20 words presented earlier, as they can recall. A delay of approximately 20 min will separate word presentation and the delayed word recall task. The task will be scored as the number of correctly recalled words.

Delayed word recognition task

Participants will be presented with 40 single words, 20 of which will be from the original presentation list. Participants will be required to indicate whether or not they recognise each word as having been presented earlier. Each word will remain on screen until a response is recorded. A delay of approximately 20 min will separate word presentation and the delayed word recognition task. The task will be scored for accuracy and response time.

Intolerance symptoms

An intolerance symptoms questionnaire, as previously used by Paradis *et al.*,⁵⁴ will be completed by participants at their week 12 testing session to assess the occurrence and intensity of any side effects they experienced over the course of the intervention. Participants will indicate whether side effects were absent, or of mild, moderate or severe intensity (scored as 0, 1, 2 or 3, respectively). Side effects included in the questionnaire relate to headache, energy levels, appetite, gastrointestinal symptoms, unusual pain or sensations, cardiac palpitations, balance disorders and depression/anxiety.

Dietary assessment

Participants will complete diaries and questionnaires in the week prior to their baseline and week 12 sessions to assess dietary intake. Food intake data will be collected using a 3-day food diary (over 2 weekdays and 1 weekend day) to establish participants' usual dietary intake, and assessed using FoodWorks 8 (Xyris Software (Australia), Queensland). An FFQ, adapted from a British FFQ⁴⁹ used to assess polyphenol intake, will be used to estimate participants' usual total polyphenol intake, based on information from the online databases Phenol-Explorer V.3.6 Database on polyphenol content in foods,⁵⁵ and the US Department of Agriculture Database for the Flavonoid Content of Selected Foods, Release V.3.1 (December 2013).⁵⁶

Physical activity

Participants will complete the IPAQ short version⁵⁰ in the weeks prior to their baseline and week 12 sessions, to assess physical activity habits.

Compliance

Compliance will be assessed through the collection of supplement check sheets and the return of any unused capsules. Participants will also receive weekly emails to encourage compliance and follow-up on any issues that may arise. Participants will be given their first 6 weeks supply of capsules at the baseline visit, as well as a supplement check sheet to tick off each time they take their supplement. At the week 6 visit, participants will return their first supplement check sheets along with any unused capsules, and be given their next 6 weeks worth of supplement and a new supplement check sheet. At the week 12 visit, any remaining capsules will be collected, along with participants' second supplement check sheets. The capsules will be provided to participants in pill boxes, measured out in daily doses.

Data management

All participant identifiers will be removed and replaced with a unique code, which will be used to identify their biological samples, and electronic and paper-based data, over multiple data collection points. A separate, password-protected computer database, accessible only by the researchers, will store participant identifiers (eg, name, email address, phone number) and their associated code, to ensure the confidentiality of any identified data.

Paper-based data will be stored in a locked filing cabinet at the Monash University BASE facility, Notting Hill, Victoria, Australia for 5 years, after which it will be destroyed. All questionnaire and paper-based data will be entered into an electronic database for statistical analysis. The electronic database will be stored in a password-protected computer file to ensure its security and the confidentiality. Following completion of the study, this data will be kept for 5 years in a password-protected location.

Biological samples will be stored in a swipe card secured laboratory within an -80°C freezer, with an alarm system that alerts a staff member if the temperature rises above a predetermined temperature. Samples will be stored for 5 years from collection date, and disposed of accordingly after that time. Only the named researchers on the project will have access to the samples. All samples will be de-identified and identified by a code known only by the researchers.

Protocol deviations

Protocol deviations will be communicated via an update of the Australian and New Zealand Clinical Trials Registry as well as through a letter to the editor of this Journal.

Statistical analysis plan

All results will be assessed for normality. Where possible, skewed distributions will be log transformed before analysis. The level of significance will be accepted at $p \leq 0.05$.

Data will be expressed as mean \pm SD (parametric) or median \pm IQR (non-parametric) unless otherwise stated. Analyses will be performed using SPSS V.24 (SPSS). A per-protocol analysis will be carried out, in which only participants who complete the full study protocol will be included. Any participants with less than 80% compliance will be considered as not completing. A modified intention-to-treat (ITT) analysis will also be carried out, in which all randomised participants will be analysed in their randomisation group, with the last observed value carried forward for missing data.^{57 58} The modification to the ITT protocol will be the exclusion of participants who were randomised but did not complete baseline measures.⁵⁸ Independent samples t-tests or Mann-Whitney U tests for non-parametric variables will be used to determine differences between the groups in the changes in outcomes from baseline to week 12 (primary outcome). A mixed between-within subjects analysis of variance (ANOVA) will be used to assess the differences between the groups across the three time points (baseline, week 6 and week 12). ANOVAs for the biochemical measures will be repeated including the variables of age, gender, change in weight status and usual polyphenol intake added as covariates. These variables have been chosen as covariates as they may influence levels of cholesterol, TG, glucose, insulin and inflammatory markers in the blood.

Patient and public involvement

There was no involvement of patients or the public in the development of the research question, outcome measures or study design. Study participants will not be directly involved in recruitment, however, snowballing through word of mouth is a part of the recruitment strategy. Furthermore, given that the intervention will involve taking a daily supplement, with no other lifestyle change, participants will not be asked to assess the burden of the intervention. Findings from this research will be disseminated to study participants as detailed below.

ETHICS AND DISSEMINATION

Consent process

Prior to enrolment in the study, all participants will be given access to details of the study, specifically the explanatory statement (online supplementary file 1), via email and the online screening questionnaire. Participants who meet the inclusion criteria will be invited to a screening session where they will have a chance to ask any questions and have the study explained to them verbally. Written informed consent will be attained from all participants who choose to participate, in the presence of the researcher. See online supplementary file 2 for participant informed consent form. All participants will be informed of their right to withdraw from the study at any time.

Data access

There are no contractual agreements that require the data from this trial to be shared.

Confidentiality

Personal information (eg, name and contact details) will be collected by MM and stored in a password-protected electronic database. Only the study's researchers will have access to this information. Only de-identified data will be presented via publications.

Dissemination

The results of this trial will be disseminated in a grouped, de-identified form and there will be no way to identify individual participants. The findings of this trial will be disseminated through peer-reviewed journal articles, national and international conference presentations, and a PhD thesis. Outcomes will be reported to stakeholder and funder, Marinova. Participants will be provided with an individual body composition report, as well as their cholesterol levels as recorded at the screening session. An overall summary of the results of the trial (de-identified) will be disseminated to participants, on request. Participants will be offered monetary compensation (\$50 voucher each) as a gesture of reimbursement for their time and inconvenience.

DISCUSSION

Polyphenol-rich brown seaweed extracts may be useful as a nutrition supplement or functional food ingredient for the management of hyperlipidaemia, hyperglycaemia and chronic inflammation in humans, to help prevent diseases like CVD and T2DM. Evidence in mice and rat models has shown that these products can be as effective as current drug treatments for reducing serum total and LDL cholesterol and TG levels.^{12 59} They also reduce fasting blood glucose,^{10 24 28} and levels of proinflammatory markers in cell and animal models in a dose-dependent manner.^{11 32 34 35} Our 2017 systematic review indicated that polyphenol-rich brown seaweed extracts may be effective at reducing total cholesterol and LDL cholesterol levels in humans, with the most promising research involving polyphenols from the brown macroalgae *E. cava*. However, effects on fasting glycaemia were mixed and no studies to date have examined the anti-inflammatory effects of these extracts in humans.¹⁸ Furthermore, a recent chapter by Cox and Scholey indicated that polyphenols from terrestrial sources (eg, tea, cocoa) had positive impacts on mood and cognition, however, no studies to date have examined the long-term impact of a polyphenol-rich brown seaweed extract on these outcomes.³⁹

Strengths

There are a number of novel features in this study design. This trial will be the first to examine the effects of supplementation with a polyphenol-rich *F. vesiculosus* extract in an Australian population for all of the aforementioned outcomes. It will also be the first to investigate the effects of a polyphenol-rich brown seaweed extract on inflammatory markers in humans, though not necessarily

powered for this outcome. There are also a number of features of this study designed to add to the existing literature. There have been four previous 12-week RCTs that have investigated the effects of polyphenol-rich brown seaweed extracts on fasting cholesterol and/or blood sugar levels.^{15–17 29} This study has been designed as a double-blind, placebo-controlled randomised parallel trial, the gold standard for determining the effect of an intervention,⁶⁰ with a similar duration and population to previous studies, in order to add to this growing evidence base.

Limitations

In order to be included in this study, participants must have a fasting LDL cholesterol level greater than 2.0 mmol/L. While this is based on recommendations for primary prevention of CVD from The National Vascular Disease Prevention Alliance,⁴⁴ we acknowledge that this cut-off (2.0 mmol/L) is classified within the healthy range by other organisations,⁶¹ and therefore, the magnitude of change seen following intervention may be less than if the cut-off was higher. However, the included criterion ensures that participants have a fasting LDL cholesterol level of above 2.0 mmol/L, therefore, by definition, the sample mean LDL level will be higher than 2.0 mmol/L. Another limitation of this study is the, potentially confounding, presence of fucoidan in the extract. There is evidence from animal models that fucoidan itself lowers TG, total and LDL cholesterol levels and increases HDL cholesterol.^{13 14} However, the only RCT to investigate this effect in humans used a fucoidan-rich brown seaweed extract that also contained polyphenols.¹⁷ Therefore, it is unclear whether the fucoidan or polyphenols were responsible for the observed reduction in LDL cholesterol following intervention.¹⁷ Similarly in the observed outcomes from this trial, the role of the fucoidan and polyphenols may be difficult to differentiate. Given there is in vitro and animal model evidence that both brown seaweed polyphenols and fucoidan lower cholesterol levels,^{9–14} the efficacy of the extract as a whole may be more important than determining the effect of its individual components.

Polyphenol-rich brown seaweed extracts are presently of interest given consumer preferences for natural and sustainable health products,^{20 62} alongside the ongoing rise in obesity and chronic disease prevalence.^{1 63 64} With the rapidly expanding global functional food market showing increasing consumer interest in marine products,^{8 22} it is of interest to population health that research is carried out to investigate the efficacy of brown seaweed extracts for use in health promotion and disease prevention and management in humans. This RCT will add to the growing literature for the potential antihyperlipidaemic, antihyperglycaemic, anti-inflammatory, and cognitive and mood enhancing effects of polyphenol-rich brown seaweed extracts in humans.

Author affiliations

¹Department of Nutrition, Dietetics and Food, Monash University, Notting Hill, Victoria, Australia

²Centre for Human Psychopharmacology, Swinburne University of Technology, Hawthorn, Victoria, Australia

³Department of Natural Sciences, Galway-Mayo Institute of Technology, Galway, Ireland

Acknowledgements The authors would like to thank Marinova for providing funding towards this research and for the kind donation of the polyphenol-rich extract. The study team would also like to thank Emma Shaw for her efforts in recruiting for this study and assistance in data collection. Thank you also to the participants who have agreed to participate in this research to date, and to those yet to enrol and complete. We thank Marinova for the donation of the brown seaweed extract and for providing funding to contribute to the analysis.

Contributors MM, MPB, ALD and LR were involved in conceiving and designing the trial. AS and KHMC contributed to the design and development of the cognitive testing. MM drafted the article. MPB, ALD, LR, AS and KHMC were involved in critical revision of the article. All authors were involved in final approval of the article.

Funding This work is supported by an Australian Government Research Training Program Scholarship (MM).

Disclaimer Marinova had no role in developing the study design or in data collection, management or analysis. Marinova will have no involvement in running the study, interpretation of data, writing of the report or the decision to submit the report for publication.

Competing interests None declared.

Patient consent Not required.

Ethics approval Ethical approval has been obtained from the Monash University Human Research Ethics Committee (MUHREC), project number 2017-8689-10379.

Provenance and peer review Not commissioned; externally peer reviewed.

Open access This is an open access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited, appropriate credit is given, any changes made indicated, and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>.

REFERENCES

- World Health Organization. Obesity and overweight Fact sheet No311, 2015. <http://www.who.int/mediacentre/factsheets/fs311/en/>. (cited 14 Jul 2015).
- Jeffery RW, Drewnowski A, Epstein LH, *et al*. Long-term maintenance of weight loss: current status. *Health Psychol* 2000;19:5–16.
- Wing RR, Phelan S. Long-term weight loss maintenance. *Am J Clin Nutr* 2005;82:222S–5.
- Murray M, Dordevic AL, Ryan L, *et al*. An emerging trend in functional foods for the prevention of cardiovascular disease and diabetes: Marine algal polyphenols. *Crit Rev Food Sci Nutr* 2018;58:1342–58.
- Jiménez-Escrig A, Sánchez-Muniz FJ. Dietary fibre from edible seaweeds: Chemical structure, physicochemical properties and effects on cholesterol metabolism. *Nutr Res* 2000;20:585–98.
- Hamed I, Özogul F, Özogul Y, *et al*. Marine bioactive compounds and their health benefits: a review. *Compr Rev Food Sci Food Saf* 2015;14:446–65.
- Li Y-X, Wijesekara I, Li Y, *et al*. Phlorotannins as bioactive agents from brown algae. *Process Biochem* 2011;46:2219–24.
- Murugan AC, Karim MR, Yusoff MB, *et al*. New insights into seaweed polyphenols on glucose homeostasis. *Pharm Biol* 2015;53:1087–97.
- Yeo AR, Lee J, Tae IH, *et al*. Anti-hyperlipidemic Effect of Polyphenol Extract (Seapolynol™) and Dieckol Isolated from Ecklonia cava in vivo and in vitro Models. *Prev Nutr Food Sci* 2012;17:1–7.
- Lee SH, Min KH, Han JS, *et al*. Effects of brown alga, Ecklonia cava on glucose and lipid metabolism in C57BL/KsJ-db/db mice, a model of type 2 diabetes mellitus. *Food Chem Toxicol* 2012;50:575–82.
- Eo H, Jeon YJ, Lee M, *et al*. Brown Alga Ecklonia cava polyphenol extract ameliorates hepatic lipogenesis, oxidative stress, and inflammation by activation of AMPK and SIRT1 in high-fat diet-induced obese mice. *J Agric Food Chem* 2015;63:349–59.
- Yoon NY, Kim HR, Chung HY, *et al*. Anti-hyperlipidemic effect of an edible brown algae, Ecklonia stolonifera, and its constituents on poloxamer 407-induced hyperlipidemic and cholesterol-fed rats. *Arch Pharm Res* 2008;31:1564–71.
- Huang L, Wen K, Gao X, *et al*. Hypolipidemic effect of fucoidan from Laminaria japonica in hyperlipidemic rats. *Pharm Biol* 2010;48:422–6.
- Park J, Yeom M, Hahm DH. Fucoidan improves serum lipid levels and atherosclerosis through hepatic SREBP-2-mediated regulation. *J Pharmacol Sci* 2016;131:84–92.
- Shin HC, Kim SH, Park Y, *et al*. Effects of 12-week oral supplementation of Ecklonia cava polyphenols on anthropometric and blood lipid parameters in overweight Korean individuals: a double-blind randomized clinical trial. *Phytother Res* 2012;26:363–8.
- Chae S-W, Choi E-K, Park S-H, *et al*. Clinical trial of the hypolipidemic effects of a brown alga ecklonia cava extract in patients with hypercholesterolemia. *International Journal of Pharmacology* 2015;11:798–805.
- Hernández-Corona DM, Martínez-Abundis E, González-Ortiz M. Effect of fucoidan administration on insulin secretion and insulin resistance in overweight or obese adults. *J Med Food* 2014;17:830–2.
- Murray M, Dordevic AL, Bonham MP, *et al*. Do marine algal polyphenols have antidiabetic, antihyperlipidemic or anti-inflammatory effects in humans? A systematic review. *Crit Rev Food Sci Nutr* 2018;58:2039–54.
- Mouritsen OG, Dawczynski C, Duelund L, *et al*. On the human consumption of the red seaweed dulse (*Palmaria palmata* (L.) Weber & Mohr). *J Appl Phycol* 2013;25:1777–91.
- Wijesekara I, Yoon NY, Kim SK. Phlorotannins from Ecklonia cava (Phaeophyceae): biological activities and potential health benefits. *Biofactors* 2010;36:408–14.
- Global Nutraceuticals Market - Growth, Trends and Forecasts (2016-2021)*. United States of America: Research and Markets, 2016.
- Abu-Ghannam N, Cox S. Seaweed-based Functional Foods. Herrero M, ed. *Bioactive Compounds from Marine Foods: Plant and Animal Sources*. Madrid, Spain: Wiley-Blackwell, 2014.
- Murray M, Dordevic A, Ryan L, *et al*. Phlorotannins and macroalgal polyphenols: Potential as functional food ingredients and role in health promotion. In: Rani V, Yadav U, *Functional Food in Human Health*. India: Springer, 2018.
- Kang C, Jin YB, Lee H, *et al*. Brown alga Ecklonia cava attenuates type 1 diabetes by activating AMPK and Akt signaling pathways. *Food Chem Toxicol* 2010;48:509–16.
- Kang MC, Wijesinghe WA, Lee SH, *et al*. Dieckol isolated from brown seaweed Ecklonia cava attenuates type II diabetes in db/db mouse model. *Food Chem Toxicol* 2013;53:294–8.
- Min KH, Kim HJ, Jeon YJ, *et al*. Ishige okamuraa ameliorates hyperglycemia and insulin resistance in C57BL/KsJ-db/db mice. *Diabetes Res Clin Pract* 2011;93:70–6.
- Nwosu F, Morris J, Lund VA, *et al*. Anti-proliferative and potential anti-diabetic effects of phenolic-rich extracts from edible marine algae. *Food Chem* 2011;126:1006–12.
- Park EY, Kim EH, Kim MH, *et al*. Polyphenol-rich fraction of brown alga ecklonia cava collected from gijang, korea, reduces obesity and glucose levels in high-fat diet-induced obese mice. *Evid Based Complement Alternat Med* 2012;2012:1–11.
- Lee SH, Jeon YJ. Efficacy and safety of a dieckol-rich extract (AG-dieckol) of brown algae, Ecklonia cava, in pre-diabetic individuals: a double-blind, randomized, placebo-controlled clinical trial. *Food Funct* 2015;6:853–8.
- Coe S, Ryan L. Impact of polyphenol-rich sources on acute postprandial glycaemia: a systematic review. *J Nutr Sci* 2016;5(e24):1–11.
- Kim Y, Keogh J, Clifton P. Polyphenols and glycemic control. *Nutrients* 2016;8:17.
- Kang NJ, Han SC, Kang GJ, *et al*. Diphlorethohydroxycarmalol inhibits interleukin-6 production by regulating NF-κB, STAT5 and SOCS1 in lipopolysaccharide-stimulated RAW264.7 cells. *Mar Drugs* 2015;13:2141–57.
- Kellogg J, Esposito D, Grace MH, *et al*. Alaskan seaweeds lower inflammation in RAW 264.7 macrophages and decrease lipid accumulation in 3T3-L1 adipocytes. *J Funct Foods* 2015;15:396–407.
- Mhadhebi L, Mhadhebi A, Robert J, *et al*. Antioxidant, anti-inflammatory and antiproliferative effects of aqueous extracts of three mediterranean brown seaweeds of the genus cystoseira. *Iran J Pharm Res* 2014;13:207–20.
- Yang EJ, Moon JY, Kim SS, *et al*. Jeju seaweeds suppress lipopolysaccharide-stimulated proinflammatory response in RAW 264.7 murine macrophages. *Asian Pac J Trop Biomed* 2014;4:529–37.

36. González R, Ballester I, López-Posadas R, *et al.* Effects of flavonoids and other polyphenols on inflammation. *Crit Rev Food Sci Nutr* 2011;51:331–62.
37. Hussain T, Tan B, Yin Y, *et al.* Oxidative stress and inflammation: what polyphenols can do for us? *Oxid Med Cell Longev* 2016;2016:1–9.
38. Joseph SV, Edirisinghe I, Burton-Freeman BM. Fruit polyphenols: a review of anti-inflammatory effects in humans. *Crit Rev Food Sci Nutr* 2016;56:419–44.
39. Cox K, Scholey A. Polyphenols for Brain and Cognitive Health. In: Yoshida Y, Cheynier V, Quideau S, *Recent Advances in Polyphenol Research*: John Wiley & Sons, Ltd, 2017.
40. Cox KH, Pipingas A, Scholey AB. Investigation of the effects of solid lipid curcumin on cognition and mood in a healthy older population. *J Psychopharmacol* 2015;29:642–51.
41. Pase MP, Scholey AB, Pipingas A, *et al.* Cocoa polyphenols enhance positive mood states but not cognitive performance: a randomized, placebo-controlled trial. *J Psychopharmacol* 2013;27:451–8.
42. Haskell-Ramsay CF, Jackson PA, Dodd FL, *et al.* Acute post-prandial cognitive effects of brown seaweed extract in humans. *Nutrients* 2018;10:85.
43. Chan A-W, Tetzlaff JM, Altman DG, *et al.* Defining standard protocol items for clinical trials. *Ann Intern Med* 2013;158:200–7.
44. National Vascular Disease Prevention Alliance, *Guidelines for the management of absolute cardiovascular disease risk*, 2012.
45. Beck A, Steer R, Brown G. *BDI-II Manual*. San Antonio: TX: Psychological Corporation, 1996.
46. Folstein M, Folstein S, Mchugh P. The pharmacology of human working memory. *Int J Neuropsychopharmacol* 1975;4:299–313.
47. Spielberger C, Gorsuch R, Lushene R. *STAI: Manual for the State-Trait Anxiety Inventory*. Palo Alto, CA: Consulting Psychologists Press, 1969.
48. Faul F, Erdfelder E, Lang AG, *et al.* G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav Res Methods* 2007;39:175–91.
49. Wootton-Beard PC, Brandt K, Fell D, *et al.* Effects of a beetroot juice with high neobetanin content on the early-phase insulin response in healthy volunteers. *J Nutr Sci* 2014;3(e9):1–9.
50. Craig CL, Marshall AL, Sjöström M, *et al.* International physical activity questionnaire: 12-country reliability and validity. *Med Sci Sports Exerc* 2003;35:1381–95.
51. International Society for the Advancement of Kinanthropometry, *International standards for anthropometric assessment*. Australia: International Society for the Advancement of Kinanthropometry, 2001.
52. Bond A, Lader M. The use of analogue scales in rating subjective feelings. *Br J Med Psychol* 1974;47:211–8.
53. Scholey A, Ossoukhova A, Owen L, *et al.* Effects of American ginseng (*Panax quinquefolius*) on neurocognitive function: an acute, randomised, double-blind, placebo-controlled, crossover study. *Psychopharmacology* 2010;212:345–56.
54. Paradis ME, Couture P, Lamarche B. A randomised crossover placebo-controlled trial investigating the effect of brown seaweed (*Ascophyllum nodosum* and *Fucus vesiculosus*) on postchallenge plasma glucose and insulin levels in men and women. *Appl Physiol Nutr Metab* 2011;36:913–9.
55. Neveu V, Perez-Jimenez J, Vos F, *et al.* Phenol-Explorer: an online comprehensive database on polyphenol contents in foods. *Database* 2010;2010:bap024 <http://phenol-explorer.eu/>.
56. Agricultural Research Service and United States Department of Agriculture. USDA database for the flavonoid content of selected foods, Release 3.1. 2013 <https://www.ars.usda.gov/northeast-area/beltsville-md/beltsville-human-nutrition-research-center/nutrient-data-laboratory/docs/usda-database-for-the-flavonoid-content-of-selected-foods-release-31-december-2013/>.
57. Gupta SK. Intention-to-treat concept: A review. *Perspect Clin Res* 2011;2:109–12.
58. Yelland LN, Sullivan TR, Voysey M, *et al.* Applying the intention-to-treat principle in practice: Guidance on handling randomisation errors. *Clin Trials* 2015;12:418–23.
59. Zhang J, Tiller C, Shen J, *et al.* Antidiabetic properties of polysaccharide- and polyphenolic-enriched fractions from the brown seaweed *Ascophyllum nodosum*. *Can J Physiol Pharmacol* 2007;85:1116–23.
60. National Health and Medical Research Council., *NHMRC additional levels of evidence and grades for recommendations for developers of guidelines*: Australian Government, 2009.
61. The Royal College of Pathologists of Australasia. *The RCPA Manual*. Australia: Surry Hills NSW, 2010.
62. Lee SH, Jeon YJ. Anti-diabetic effects of brown algae derived phlorotannins, marine polyphenols through diverse mechanisms. *Fitoterapia* 2013;86:129–36.
63. World Health Organization. Diabetes fact sheet no312. 2015 <http://www.who.int/mediacentre/factsheets/fs312/en/> (cited 14 Jul 2015).
64. World Health Organization. Cardiovascular diseases (cvds) fact sheet No317. 2015 <http://www.who.int/mediacentre/factsheets/fs317/en/> (cited 14 Jul 2015).