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Including pork in the Mediterranean diet for an Australian population: Protocol for a randomised controlled trial assessing cardiovascular risk and cognitive function

Alexandra T. Wade¹, Courtney R. Davis¹, Kathryn A. Dyer¹, Jonathan M. Hodgson²,³, Richard J. Woodman⁴, Hannah A. D. Keage⁵ and Karen J. Murphy¹*

Abstract

Background: The Mediterranean diet is characterised by the high consumption of extra virgin olive oil, fruits, vegetables, grains, legumes and nuts; moderate consumption of fish, poultry, eggs and dairy; and low consumption of red meat and sweets. Cross sectional, longitudinal and intervention studies indicate that a Mediterranean diet may be effective for the prevention of cardiovascular disease and dementia. However, previous research suggests that an Australian population may find red meat restrictions difficult, which could affect long term sustainability of the diet.

Methods: This paper outlines the protocol for a randomised controlled trial that will assess the cardiovascular and cognitive benefits of a Mediterranean diet modified to include 2-3 weekly serves of fresh, lean pork. A 24-week cross-over design trial will compare a modified Mediterranean diet with a low-fat control diet in at-risk men and women. Participants will follow each of the two diets for 8 weeks, with an 8-week washout period separating interventions. Home measured systolic blood pressure will be the primary outcome measure. Secondary outcomes will include body mass index, body composition, fasting blood lipids, C-reactive protein, fasting plasma glucose, fasting serum insulin, erythrocyte fatty acids, cognitive function, psychological health and well-being, and dementia risk.

Discussion: To our knowledge this research is the first to investigate whether an alternate source of protein can be included in the Mediterranean diet to increase sustainability and feasibility for a non-Mediterranean population. Findings will be significant for the prevention of cardiovascular disease and age-related decline, and may inform individuals, clinicians and public health policy.

Trial registration: ACTRN12616001046493. Registered 5 August 2016.

Keywords: Mediterranean diet, CVD, Cognitive function, Randomised controlled trial

Background

As the population ages the prevalence and impact of chronic age-related diseases and disorders is predicted to increase dramatically [1]. In the elderly, dementia and cardiovascular disease (CVD) are amongst the greatest contributors to death and disability worldwide, and their costs are expected to double and triple respectively over the next 20 years [2–4]. Mediterranean populations in southern Europe, such as Greece and Italy, exhibit significantly lower rates of mortality from CVD and dementia than populations in northern Europe and the United States [5]. It has been suggested that this disparity is due to diverse dietary patterns across populations, and that a Mediterranean diet may be protective against CVD [6, 7]. As cardiovascular health is a significant predictor of dementia [8, 9], the Mediterranean dietary pattern may also be responsible for lower rates of dementia.
A traditional Mediterranean diet is characterised by a high intake of extra virgin olive oil (EVOO), vegetables, fruits, cereals, nuts, pulses and legumes; a moderate intake of fish, poultry, dairy and red wine; and a low intake of eggs, red and processed meat, and sweet and processed foods [10]. The Mediterranean dietary pattern is therefore rich in bioactive nutrients and phytochemicals such as monounsaturated fatty acids, polyunsaturated fatty acids (including omega-3 s), polyphenols and flavonoids, vitamins, minerals, antioxidants and fibre. Individually, these dietary components are associated with improved cardiovascular health [11–14]. However, single nutrients are not consumed in isolation, but collectively as a dietary pattern. As such, synergistic relationships between nutrients may enhance these benefits [15–17].

When compared with other dietary patterns a Mediterranean diet has consistently improved indicators of cardiovascular health. For example, a Mediterranean diet has been shown to improve blood pressure, insulin sensitivity, lipid profiles and markers of inflammation [18–20], while reducing risk of cardiovascular events by 30% [21].

Table 1 World Health Organisation Trial Registration Data Set

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<td>Dr Karen J Murphy</td>
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<td>Secondary sponsors</td>
<td>Alexandra T Wade, Courtney R Davis, Kathryn A Dyer, Jonathan M Hodgson, Richard J Woodman, Hannah A.D. Keage</td>
</tr>
<tr>
<td>Contact for public queries</td>
<td>Phone: +61 8202 2097. Email: <a href="mailto:Karen.Murphy@unisa.edu.au">Karen.Murphy@unisa.edu.au</a>. GPO Box 2471, Adelaide SA 5001</td>
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<td>Contact for scientific queries</td>
<td>Dr Karen J Murphy, Senior Research Fellow, Dietitian, Alliance for Research in Exercise, Nutrition and Activity, University of South Australia. Phone: +61 8202 1033. Email: <a href="mailto:Karen.Murphy@unisa.edu.au">Karen.Murphy@unisa.edu.au</a>. GPO Box 2471, Adelaide SA 5001</td>
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<td>Intervention(s)</td>
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</table>
While Mediterranean populations who follow a Mediterranean diet exhibit lower risk of CVD and dementia [15, 22–25], few studies have investigated whether the diet can be successfully adopted in populations beyond the Mediterranean Sea. Notably, the MedLeY study examined the effects of a Mediterranean diet over 6 months and found that an older Australian population was capable of adopting the Mediterranean diet [26]. However, participants indicated that one of the most difficult aspects of following the diet was restricting red meat intake [27].

A traditional Mediterranean diet is typically low in red meat products and Mediterranean dietary guidelines recommend consuming less than two serves of red meat per week [28, 29]. A recent review has reported that a traditional Mediterranean diet includes 105 g, or 0.5 to 0.75 standard serves of meat and meat products per day [10]. This figure is inclusive of all processed and unprocessed red and white meat products. In contrast, Australians over the age of 19 consume an average of 184 g of meat per day, or 1.7 serves of lean meats and alternates, with red meat contributing the largest proportion of this (38%) [30]. Australians are amongst the highest consumers of meat in the world [31]. Therefore, it is not surprising that restricting red meat may prove difficult. Countries with comparatively high meat intakes, such as the United States and United Kingdom, may experience similar difficulties (OECD, 2016). As socio-cultural norms and palatability are key determinants of a dietary intervention’s sustainability [32], non-Mediterranean populations may then be more likely to adhere to a diet containing more red meat.

Prospective cohort studies have drawn attention to significant associations between red meat and poor health outcomes, including bowel cancer and CVD [33]. Proposed mechanisms point to the pro-oxidative properties of heme iron found in red meat. However, recent investigations highlight the mediating effects of other dietary components on oxidation [34]. For example, consuming EVOO with red meat has been shown to attenuate lipid oxidation and the production of free radicals [35]. When vitamin E, found in green leafy vegetables and nuts, is consumed in combination with EVOO and red meat, lipid oxidation is further inhibited [35]. Moreover, the European Prospective Investigation into Cancer and Nutrition (EPIC) study reported significant interactions between red meat and dietary fibre [36]. Specifically, individuals consuming red meat every day had a reduced risk of developing colorectal cancer if they also consumed at least 26 g of dietary fibre each day – that is, less dietary fibre than is provided by the Mediterranean diet [10]. The potentially harmful effects of red meat may then be mitigated when consumed within a Mediterranean dietary pattern.

Australia’s most frequently consumed red meat products are beef and pork [31]. In terms of nutritional value, fresh lean beef and pork have similar nutrient profiles. However, fresh lean pork contains considerably less heme iron than beef [37] and less saturated fat per 100 g. Pork may then be an appropriate addition to a Mediterranean diet, which is typically low in saturated fat and heme iron sources [10]. Our previous research indicates that substituting beef and chicken with pork may be effective for improving cardiometabolic health markers, including weight, body mass index (BMI), percentage body fat, fat mass and abdominal fat, without negatively affecting blood lipids, insulin or glucose [38]. Further, when compared with ruminant meats such as beef and some methods of aquaculture, pork production is associated with significantly less agricultural greenhouse gas emissions [39].

At present, there are no studies to our knowledge that have investigated the substitution of meat protein sources in the Mediterranean diet to increase variety and sustainability for the target population and environment. The current study therefore aims to evaluate the cardiovascular and cognitive benefits of a Mediterranean diet, supplemented with fresh, lean pork against a low-fat diet. A low-fat control diet has been chosen due to its continued recommendation for the clinical management and reduction of cardiovascular risk [40]. Further, including a low-fat control will enable comparisons of our findings against larger studies of the same design, such as the Prevención con Dieta Mediterránea (PRE-DIMED) trial. Due to the pressing need to reduce the impact of CVD and dementia, the modified Mediterranean diet will be evaluated for its potential to improve cardiovascular health and cognitive risk in an at-risk population.

**Methods**

**Recruitment**

Details of the current study protocol are provided in Table 1. Volunteers aged between 45 and 80 who are at risk of developing CVD will be recruited via electronic and paper advertisements.

To meet eligibility criteria, volunteers must have elevated systolic blood pressure above 120 mmHg and at least two other risk factors for CVD, including: a BMI ≥ 25 kg/m2; elevated fasting total cholesterol (≥ 5.5 mM), triglycerides (≥ 2.0 mM), low-density lipoprotein (LDL) (≥ 3.5), or low levels of high-density lipoprotein (HDL) (≤ 0.9 for men and ≤ 1.0 for women); impaired fasting glucose tolerance (between 6.1 and 7.8 mmol/L); and/or a family history (up to
one generation) of CVD or type 2 diabetes mellitus (T2DM). Exclusion criteria include: antihypertensive medication; smoking; current CVD or angina; current or recent (within 6 months) malignancies; respiratory disease; gastrointestinal disease; kidney disease; T2DM; a current or previous traumatic head or brain injury; a current neurological or psychiatric condition; antidepressant or anxiety medication; a current diagnosis of Alzheimer’s disease or dementia; or supplemental omega-3 > 1000 mg daily.

Eligibility will be assessed through a diet and lifestyle questionnaire (DLQ) and screening visit at the Sansom Institute for Health Research Clinical Trial Facility (SIHR CTF), Adelaide, South Australia. The DLQ includes medical history, medications and supplements, family medical history, and dietary aversions, allergies and intolerances. Volunteers deemed eligible on the basis of their DLQ will be screened to measure systolic blood pressure, diastolic blood pressure, heart rate, height and weight, fasting cholesterol and fasting glucose. The Addenbrooke’s Cognitive Exam-Revised (ACE-R) will also be administered to detect pre-existing dementia and mild cognitive impairment (MCI).

Design
A 24-week cross-over design trial will compare a Mediterranean diet intervention with a low-fat diet intervention. Each participant will complete both dietary interventions for 8 weeks and the two intervention phases will be separated by an 8-week washout period (See Fig. 1). An independent staff member of the SIHR will perform the block randomisation procedure. Participants will be allocated to one of two groups using, stratified by age and gender, to determine the order in which they undertake the interventions. Allocation concealment will ensure that study personnel and volunteers are not aware of the starting intervention prior to enrolment. During the washout period participants will return to their habitual diet to prevent cross-contamination of effects between dietary phases.

By reducing potential between-subject differences a cross-over design reduces the effects of potential confounding variables, and therefore enhances internal validity of the design. Further, performing repeated measures on the same individual allows for an estimation of an individual’s true value, measurement error and treatment effect, meaning that fewer participants will be required to detect a treatment effect. An 8-week intervention period has been chosen as randomised controlled trials (RCTs) of similar length have found that nutrients contained within the Mediterranean diet can improve both cardiovascular and cognitive function [41–43]. Eight weeks is expected to be an adequate washout period [44]. However, potential carryover effects will be assessed by testing for intervention-phase interactions.

Dietary interventions
Neither diet will restrict energy intake. Instead, participants will be advised to self-regulate their food intake and consume foods ad-libitum.

Low-fat diet
Guidelines for the low-fat diet are based on the PRE-DIMED study, which compared two variations of a Mediterranean diet with a low-fat control diet [45]. Participants will be advised to make adjustments to their habitual diet in order to reduce total fat intake. Specifically, high fat foods, including all types of oil, butter, margarine, processed and high fat meats, nuts, chocolates, cakes, pastry, and high or full fat dairy, will be replaced with low-fat alternatives, such as breads and cereals, legumes, rice, fruits and vegetables and low-fat varieties (e.g. low-fat dairy, low-fat sauces). Daily limits will be set for oil (no more than 20 ml), butter and margarine (no more than two teaspoons), and participants will be instructed to remove visible fat and skin from meat and fish before cooking.

Mediterranean diet
Guidelines for the Mediterranean diet are adapted from Estruch et al. (2013) for an Australian food supply:
• Minimum of one tablespoon (20 mL) of EVOO per day;
• ≥2–3 daily servings of fresh fruit (one serve = 150 g fresh, 30 g dried, or one cup canned in natural juice);
• ≥3 weekly servings of legumes (one serve = 75 g);
• 2–3 weekly servings of fresh, lean pork (one serve = 100 g cooked);
• ≥3 weekly servings of fish and seafood (at least one serving of oily fish) (one serve = 100 g cooked);
• ≥5 weekly servings of raw or roasted nuts or seeds, without added salt, sugar or chocolate (one serve = 30 g; 7.5 g hazelnuts, 15 g walnuts, 7.5 g almonds supplied for each serve);
• Ad-libitum consumption of wholegrain cereal products (bread, pasta, rice, cereal) and dairy
• Ad-libitum consumption of eggs, not to exceed 6 serves per week (1 serve = approx. 70 g or 1 egg)
• Select white meats (poultry without skin) instead of red meats or processed meats;
• Limit consumption of red and cured meat (remove all visible fat) to ≤1 serve/week (one serve of red meat/cured ham = 100 g);
• Limit consumption of chocolate to ≤1 serve/week (one serve of chocolate = 50 g);
• Use EVOO for cooking and dressing vegetables and salad;
• Cook regularly (at least twice a week) with a tomato based sauce (EVOO, tomato, garlic and onion);
• Dress vegetables, pasta, rice and other dishes with EVOO, tomato, garlic and onion sauce;
• Eliminate or limit the consumption of cream, butter, margarine, cold meat, pate, duck, carbonated and or sugared beverages, pastries, commercial bakery products (cakes, donuts, cookies), desserts (puddings), French fries, potato crisps, sweets;
• For usual drinkers, red wine is recommended as the main source of alcohol with a maximum of two standard drinks per day (200 mL = two standard drinks) [46]

Participants will be advised to consume pork in place of chicken and red meat ensure that total meat consumption does not exceed 400 g per week.

To assist with adherence, the following foods will be provided each week: 375 ml EVOO; 250 g of fresh, lean pork; 150 g raw, unsalted almonds, walnuts and hazelnuts; 225 g (net weight) of canned chickpeas, red kidney beans, 4-bean mix and lentils; 95 g of canned tuna and 95 g of canned salmon.

Dietetic counselling
To increase adherence and retention participants will have regular contact with a dietitian. At the beginning of each phase, participants will meet with the dietitian to discuss dietary guidelines in detail. For the Mediterranean diet, participants will be provided with a set of resources, including Mediterranean dietary guidelines (Appendix 1), recommendations on how to include pork in the diet (Appendix 2), education on serving sizes and a Mediterranean diet recipe book. For the continual assessment of adherence, and to encourage familiarity of foods and serving sizes associated with a Mediterranean diet, participants will also be given a semi-quantitative checklist (Appendix 3) to be filled in daily, using a tick system (1 tick = 1 serve), and returned at bi-weekly dietetic visits. While on the low-fat diet, similar dietetic resources will be provided, including a set of low-fat diet guidelines (Appendix 4), education on fat content and a food label reading guide (Appendix 5) to consider when selecting packaged foods.

Throughout each intervention phase participants will attend bi-weekly dietetic visits to discuss their progress, food intake, challenges and any adverse effects. These visits will also include weight measurement to determine body mass, weight loss or weight gain. In the case of weight loss or gain the dietitian will ensure that participants are eating until satiated and discuss mindful eating and portion sizes. If a participant is having trouble adhering to the guidelines the dietitian will discuss strategies and work with the participant to set SMART goals (S, specific; M, measurable; A, achievable; R, realistic; T, time based) for the next 2 weeks.

Outcome measures
Home blood pressure
Home measured systolic blood pressure will be the primary outcome measure. For the reliable assessment of hypertension, home measured blood pressure is comparable to ambulatory blood pressure, and has stronger predictive power than clinic blood pressure [47–49].

Participants will receive training on self-administered blood pressure measurement and will be instructed to measure their systolic and diastolic blood pressure and heart rate every morning, afternoon and evening for 6 days at the four assessment points over the course of the trial. Participants will be provided with a clinically validated A&D Company Ltd. digital blood pressure monitor (model UA-767). Measurements will be taken at a consistent time each day after a five-minute rested period in the seated position. Three consecutive readings are to be taken spaced at least 1 min apart, as per Bondonno et al. (2015) and participants will be advised to avoid caffeine and alcohol for 1 h prior to measurement, and food and exercise for 30 min prior.
Secondary outcome measures and covariates

Over the four assessment time points, home measured diastolic blood pressure and heart rate, clinic blood pressure and heart rate, BMI, waist-to-hip ratio, body composition, fasting blood lipids, C-reactive protein (CRP), fasting plasma glucose, fasting serum insulin, erythrocyte fatty acids, cognitive function, psychological health and well-being, and dementia risk will be examined as secondary outcomes.

Clinic blood pressure will be measured using an Omron Healthcare Co. digital blood pressure monitor (model 1A1B Hem-7000-CIL). The same protocol as for home blood pressure measurement will be followed, where participants will be seated for 5 min and three measurements will be taken at least 1 min apart. Waist and hip circumference will be measured to determine waist-to-hip ratio. Body composition, including percentage body fat, lean mass and abdominal adiposity will be measured using dual-energy x-ray absorptiometry (DEXA). Fasting venous blood will be collected through venepuncture and analysed at an external NATA accredited laboratory using standard procedures. Erythrocyte fatty acids will be measured using direct transesterification as described by Tu et al. (2013) once further funding has been secured. Whole blood will be centrifuged (4 °C, 4000 rpm, 10 min) to separate erythrocytes from spare plasma, both of which will be frozen at −20 °C and then stored at −80 °C. APOEε4, an indicator of increased risk for AD, will be determined using the TaqMan® SNP Genotyping assay kit (Applied Biosystems, Warrington, UK) [50]. For explorative purposes, a faecal sample will also be collected to examine the effects of a Mediterranean diet on gut microbiota. Faecal samples will be frozen at −20 °C and then stored at −80 °C until further funding has been secured.

Cognitive function will be assessed across memory, processing speed and executive function using a selection of tests from the Cambridge Automated Neuropsychological Test Automated Battery (CANTAB). These particular cognitive domains will be examined due to their vulnerability to aging and cardiovascular health, and their demonstrated sensitivity to short term nutritional interventions [51–56]. The battery of tests will include Motor Orientation Task (MOT), Paired Associates Learning (PAL), Delayed Matching to Sample (DMS), Verbal Recognition Memory (VRM), Reaction Time (RTI), Rapid Visual Information Processing (RVIP), Spatial Working Memory (SWM), One Touch Stockings of Cambridge (OTS) and Attention Switching Task (AST).

A computerised mode of testing has been chosen to increase accuracy and reliability, especially where sensitivity to processing speed is critical [57]. To limit the effect of learning over repeated measures, the CANTAB offers parallel versions of each test. For the chosen tests, test re-test reliability (r) ranges from $r = 0.54$ (RTI) to $r = 0.87$ (PAL) [58]. To examine potential measurement error, especially where the test-retest coefficient is less than $r = 0.7$, learning effects can be determined using standardised scores and standard errors provided by Cambridge Cognition [58].

The Karolinska Sleepiness Scale (KSS) will be administered prior to cognitive testing to gain insight into sleep quality and sleepiness, which may impair cognitive function. The KSS is a 9-point scale that asks participants to rate their current level of sleepiness where 1 = Extremely alert, 2 = Very alert, 3 = Alert, 4 = Rather alert, 5 = Neither alert nor sleepy, 6 = Some signs of sleepiness, 7 = Sleepy, but no effort to keep awake, 8 = Sleepy, some effort to keep awake, 9 = Very sleepy, great effort to keep awake, fighting sleep. The KSS has been validated against electroencephalography (EEG) and behavioural measures of sleepiness [59]. A score of 6 and above on the KSS has been linked to significant increases in reaction time and lapses on a psychomotor vigilance task, and decreased levels of arousal as measured by the alpha attenuation test [59].

To screen for dementia and MCI, and to detect change in cognitive function associated with dementia and MCI (attention, orientation, memory, fluency, language and visuospatial abilities), the ACE-R will be administered during screening visits and at the end of each dietary phase [60]. The ACE-R is frequently used in clinical and research settings, with high sensitivity and specificity for detecting both dementia and MCI.

Future risk of dementia will be calculated using the Cardiovascular Risk Factors, Aging and Dementia (CAIDE) score, and the Framingham vascular risk score (FRS) [61]. The CAIDE predicts 20-year dementia risk based on middle age profiles with high sensitivity (0.81) and moderate specificity (0.61). The CAIDE score is based on age, sex, education, total cholesterol, systolic blood pressure, physical activity and APOEε4 genotype. Similarly, the FRS determines risk of CVD on the basis of age, sex, systolic blood pressure, hypertension, HDL cholesterol, total cholesterol, smoking status and diabetes [62]. Although originally developed to predict risk of vascular disease, the FRS reliably predicts decline in cognitive function and has demonstrated stronger associations than the CAIDE with 10-year risk of cognitive decline [10].

Psychological well-being, an outcome and determinant of health behaviours, will be evaluated through the SF-36 Health Survey Version II adapted for use in Australia [63] and the Profile of Mood States (POMS). The SF-36 and POMS have been chosen for their
relevance to a non-clinical population. The SF-36 was
designed to measure subjective perceptions of health
status across the domains of vitality, physical functioning,
body pain, general health perceptions, physical role function,
emotional role functioning, social role function and mental health [64]. The Australian SF-
36 has demonstrated high internal consistency and reli-
ability, adequate test-retest reliability and sensitivity to
change, including sensitivity to nutritional interventions
[65, 66]. The POMS measures mood states of tension,
anxiety, anger, hostility, vigour, activity, fatigue, inertia,
depression, dejection, confusion and bewilderment by
asking participants to rate the extent to which they
have recently felt different emotions [67]. For the pur-
pose of the proposed study, participants will be asked
about the extent to which they have felt emotions over
the past month. The POMS has demonstrated good
internal consistency (Cronbach’s alpha = 0.63 to 0.96)
and test re-test reliability of $r = 0.61$ to $r = 0.69$ at
6 weeks [68].

**Dietary adherence**

A 15-item Mediterranean diet adherence survey and a 9-
item low-fat diet adherence survey (Appendix 6) will be
administered to participants bi-weekly to capture gener-
alised patterns of food consumption during each of the
intervention phases. Each survey has been adapted from
the PREDIMED study to reflect an Australian food sup-
ply and national guidelines for alcohol consumption.
While on the Mediterranean diet, participants will
also complete a semi-quantitative weekly checklist
(Appendix 3, described above). Adherence to the diet-
ary phase will be determined at each bi-weekly visit, where
participants must meet a minimum adherence requirement
of 75%, as determined by checklists. If participants scoring
below 75% are unable to increase their adherence in the
next 2 weeks they will be excluded from the study.

To determine the impact of dietary adherence on out-
comes an overall Mediterranean diet score [15, 26] will
be calculated using data from a 3-day weighed food rec-
ord (WFR) completed by participants before and at the
end of each intervention phase. Further, erythrocyte
fatty acids will provide a biological measure of dietary
adherence at the end of each intervention phase.

**Exit survey**

At the end of each intervention an exit survey will be
administered to capture further insight and qualitative
information about participant perceptions of the diets.
The surveys will ask participants to elaborate what they
enjoyed and found challenging about the diet they have
just completed. To measure hunger and satiety experi-
enced during each intervention the survey will also include
five questions based on the 5-Factor Satiety Questionnaire
[69] with corresponding visual analogue scales (VAS). Par-
ticipants will be asked to indicate 1) “How hungry did you
feel after you finished a meal?”, 2) “How full did you feel
after you finished a meal”, 3) “How satisfied did you feel
after you finished a meal”, 4) “How strong was your desire
to eat after you finished a meal?” and 5) “How much more
could you have eaten after you finished a meal?”. For
questions 1-4, the VAS will range from “Not at all” to
“Extremely”, and for question 5 the VAS will range from
“Nothing at all” to “A very large amount”.

**Procedure**

Data will be collected by participants at home and by
study personnel during clinic assessment visits at the
SIHR CTF across the course of the trial (See Fig. 2).
Participants will attend a pre-baseline appointment at
the SIHR CTF where the study will be explained and
informed consent will be obtained by study personnel
(Appendix 7). Instructions and equipment will be given
for the collection of home measures, including blood
pressure, SF-36, POMS and WFR. Participants will then
return 1 week later for week 0 assessments.

Clinic assessment visits will take place at baseline of the
first diet (week 0), at the end of the first diet (week 8), at
baseline of the second diet, after the 8-week washout
period (week 16), and at the end of the second diet
(week 24). Data for home measures will be collected
by participants in the week prior to each of these visits.

At clinic assessment visits participants will be fasted
from food, beverages (excluding water), alcohol and
caffeine for 12 h. Clinic assessment visits will include
measurement of blood pressure, body anthropometry,
DEXA and collection of fasted blood samples. At weeks
0 and 16 participants will be allocated to their diet and
meet with a dietitian to discuss the dietary guidelines.
Participants will then be given a continental breakfast
before cognitive tasks are administered. To minimise the
impact and influence of external variables, cognitive test-
ing conditions will be rigorously controlled and standar-
dised as outlined by [70]. All cognitive testing sessions
will take place between 9:30 am and 12:00 pm. To limit
the impact of food intake on cognitive performance, par-
ticipants will consume the same breakfast before each
cognitive session.

If participants have performed a strenuous level of
non-habitual exercise in past 10 h, not had an adequate
amount of sleep, or are unusually tired cognitive assess-
ments will be rescheduled. Temperature and noise of
the cognitive testing environment will be controlled in
order to reduce distraction and test administration will
be standardised through the use of a testing script
provided by Cambridge Cognition. Participants will
commence the dietary intervention on the day following
week 0 and week 16 visits, and will commence the washout period on the day of the week 8 visit.

Between weeks 0 and 8, and 16 and 24 participants will attend bi-weekly dietetic visits at the SIHR CTF. In the final dietetic visit of each diet phase, and at week 15, participants will be provided with equipment for the collection of home measures to be returned at their next clinic assessment visit.

Volunteers will be instructed to continue any habitual exercise and medications for the duration of the trial. Changes to exercise and medications will be monitored and recorded at bi-weekly dietetic visits and clinic assessment visits by the investigators.

Statistical analysis

Statistical analyses will be conducted using SPSS for Windows, version 21.0 (SPSS Inc., Chicago, IL, USA) and Stata (version 14.2, StataCorp, College Station, TX, USA). Data will be presented as means ± standard deviation (SD) for descriptive statistics and as means ± standard error (SEM) for reporting estimated effects. All tests will be 2-tailed with \( p \)-values <0.05 deemed statistically significant.

Based on power calculations, a sample size of 31 volunteers is required to detect a clinically relevant difference of 2.5 mmHg in home blood pressure, the primary outcome measure, with power of 90%. This calculation accounts for a total of 54 readings (three readings taken three times per day for 6 days) at each time point, a within-group standard deviation of 14 mmHg, a within-subject repeated measures correlation of \( r = 0.6 \) and a between-phase within-subject correlation of \( \rho = 0.5 \). The correlation (\( \rho \)) and use of a cross-over design reduces the number of required participants by a factor of \( (1 - \rho)/2 = 4 \) i.e., from approximately \( n = 124 \) for a parallel group design using ANCOVA \( (n = 62 \) per group) to \( n = 31 \) subjects in total.

Baseline demographic, cardiometabolic, cognitive, well-being and dietary characteristic data will be compared for those who complete the study and those who withdraw using independent t-test and chi-square tests, depending on the type of variable and distribution of data.
The primary analysis will be performed on a per protocol basis including only those subjects who complete both trial phases. A linear mixed effects model will determine the difference in each outcome between dietary interventions and will measure both between and within subject effects for cardiometabolic and cognitive outcomes. If a significant change is detected for cognitive outcomes a hierarchical regression will include cardiometabolic outcomes to assess their mediating effects. A sensitivity analysis will include weight loss, change in physical activity, medications and supplements as these may cause time-varying confounding should they differ between groups in the second dietary phase. Further, carryover and treatment-period effects will be assessed by including treatment*phase and treatment*order interaction terms. A diet*period*energy intake interaction term will be included to determine any significant changes between diets in relation to total energy intake, macronutrient intake and micronutrient intake. As an additional sensitivity analysis we will also include subjects that did not complete the trial or were not included in the per protocol analysis due to inadequate adherence (<75%). For missing data the linear mixed effects models will estimate unbiased effects as opposed to multiple imputation.

**Trial and data management**

To ensure privacy and confidentiality each participant will be assigned a unique study identification (ID) number. All source documents will be de-identified (re-identifiable by coded ID numbers) and stored in an individual file for each study participant. All information collected as part of the study will remain confidential and no information that could lead to identification of any individual will be released. Access to trial data will be limited to the team of investigators named as authors on this publication.

Unintended effects and adverse events of the trial intervention will be identified during bi-weekly dietetic visits. All adverse and serious adverse events will be recorded and serious adverse events will be reported by the Chief Investigators to the University of South Australia Human Research Ethics Committee. Participants will be advised to consult with their General Practitioner regarding any notable symptoms identified throughout the trial. If the instance of adverse events a decision to continue, modify or cease the intervention will be made on a case-by-case basis.

Due to the size of the trial and nature of the trial intervention a Data Monitoring Committee is not deemed necessary. The Chief Investigator will conduct quality control checks of data values twice during each dietary intervention phase to ensure data quality.

Electronic data entry will be completed throughout the trial by study personnel. Electronic data files will be stored on a secure password protected network and backed up regularly on an external hard drive. Once the trial is complete and results have been published electronic files will be stored on a DVD in the Sansom Clinical Trials facility secure data store in the Bonython Jubilee Building, City East Campus. Study documentation will be kept in locked filing cabinets within in the Sansom Clinical Trials facility in the Bonython Jubilee Building, City East Campus. Once the study is complete and results have been published all data will be archived and stored for a total of 15 years.

Any changes to the trial protocol will require approval by all authors and the University of South Australia Human Ethics Committee. Once approved, modifications to the trial protocol will be communicated to the funding sponsors and the journal wherein the protocol is published.

Results from this trial will be disseminated through peer-reviewed international journals, presentations at national and international meetings and the lead author’s doctoral dissertation. Participants will be provided with a copy of their personal results within 6 months of completing the study and a summary of the research findings will be sent to all participants once data analysis has been completed.

**Discussion**

To reduce the impact of both dementia and CVD health and lifestyle interventions with the potential to mitigate multiple risk factors have been recommended above reactive, costly treatments [71, 72]. Risk factors such as hypertension, dyslipidaemia, insulin sensitivity are shared by both dementia and CVD [73–76]. Interventions targeting each of these simultaneously may be effective in reducing the prevalence of both CVD and dementia. However, it is necessary to ensure that such interventions are sustainable, both for the target population and the environment.

The Mediterranean diet has demonstrated efficacy for reducing risk of CVD and cognitive decline. Yet an Australian population, who typically consumes a high quantity of meat, may find adhering to the meat restrictions of the diet difficult. This paper outlines the protocol of a randomised controlled trial that endeavours to compare a Mediterranean diet with an additional source of meat protein, against a low-fat control diet. Findings of the forthcoming study will be relevant at a policy, clinical and individual level and will provide insight as to whether consuming pork as a part of a Mediterranean diet can improve health outcomes while simultaneously increasing variety and sustainability for the target population.
Appendix 1

Mediterranean Diet Guidelines

Recommendations to Follow the Mediterranean-Pork Diet

Specific Guidelines:

- ≥1 tablespoon of extra virgin olive oil per day. Use abundantly!
- ≥3 serves of vegetables per day (1 serve = 75g, half a cup cooked veg, or 1 cup salad)
- 2 – 3 serves of fresh fruit per day including 100% natural juices (1 serve = 150g fresh fruit or 125 ml juice)
- Usual drinkers have no more than 2 standard drinks per day (mostly as red wine) (1 serve = 100 ml)

- ≥3 serves of legumes per week (1 serve = 75g, or half a cup cooked)
- ≥5 serves of nuts/seeds per week (1 serve = 30g, or a handful)
- 2-3 serves of pork per week (1 serve = 100 g cooked)
- 3 serves of fish per week (1 serve = 100 g cooked). Fish can include fresh or canned fish, but not battered and deep fried (this counts towards discretionary foods)
- Limit to ≤1 per week small goods (deli meats, smoked meats, salami etc), red meat, chocolate (dark – min. 50% cocoa)

General Guidelines:

- Select chicken/pork instead of red meat or processed meats
- Cook minimum twice per week with tomato, garlic and onion (Sofrito sauce).
- Dress pasta and vegetables and rice with Sofrito sauce
- Ad libitum consumption of eggs, dairy, wholegrain cereals, wholegrain rice, pasta and bread, couscous
- Include lots of variety, different kinds of fruits, vegetables, breads, fish, and nuts and try new recipes and foods!

- Avoid refined carbohydrate including white rice, white bread, white flour
- Eliminate or drastically limit discretionary foods including: cream, butter, margarine, ice cream, pate, duck, sugary beverages (soft drinks, fruit drinks, energy drinks), pastries, puddings, cakes and baked goods, sweet biscuits, fried foods, takeaway/fast foods, and confectionary (lollies, chocolate bars, chocolate coated nuts and fruit etc.).

Fig. 3 Guidelines for the MedPork intervention
Appendix 2

Pork and other meats

Pork is a healthy lean meat which is part of the Spanish Mediterranean diet. Pig farming is more sustainable and environmentally friendly than cow farming as well.

A Mediterranean diet typically is low to moderate in meat intake. We would like you to aim for approximately 400 grams of meat (not including fish) per week:

- At least 200 g as lean, fresh pork
- No more than 100g as red meat, including beef, lamb, veal, mutton, kangaroo, goat etc.
- No more than 100g as poultry, including chicken and turkey

If you’d like to have 3 servings of pork instead of 2, you’ll need to replace either red meat or poultry with pork so as not to exceed the 400 g per week meat limit.

In addition, you are required to have 300 g of fish per week, and 225 g of legumes per week. These can be incorporated into lunch or dinner. Finally nuts provide protein also, and you are required to consume at least 5 servings a week (150 g).

Here is an example of a weeks’ worth of lunch and dinner meals incorporating the meat guidelines.

<table>
<thead>
<tr>
<th></th>
<th>Monday</th>
<th>Tuesday</th>
<th>Wednesday</th>
<th>Thursday</th>
<th>Friday</th>
<th>Saturday</th>
<th>Sunday</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lunch</td>
<td>Legumes x1</td>
<td>nil</td>
<td>Legumes x1</td>
<td>Fish x1</td>
<td>nil</td>
<td>Fish x1</td>
<td>Legumes x1</td>
</tr>
<tr>
<td>Dinner</td>
<td>Pork x1</td>
<td>Fish x1</td>
<td>Pork x1</td>
<td>Chicken x1</td>
<td>Fish x1</td>
<td>Legumes x1</td>
<td>Red meat x1</td>
</tr>
</tbody>
</table>

Remember you can have more fish and legumes than the minimum 3 serves, and in this example menu fish and legumes appear 4 times, helping to create filling lunches and dinners.

There are many other ways you can incorporate these servings into your week. For example, you can have 2x a serve in one sitting (e.g. have 200g of fish). Or you can spread them out, for example try having 50g of red meat on 2 days.

Pork can be used like chicken or beef in many recipes. You can bake, stir fry or grill to prepare pork in a number of ways. It can be used in tacos, sandwiches and burgers, as a steak or as the protein centre in stir fry.

Fig. 4 Recommendations for including pork in the Mediterranean diet
### Appendix 3

#### Semi-quantitative weekly checklist to encourage adherence to the MedPork intervention

<table>
<thead>
<tr>
<th>Serving size</th>
<th>FOODS TO EAT DAILY</th>
<th>DAY 1</th>
<th>DAY 2</th>
<th>DAY 3</th>
<th>DAY 4</th>
<th>DAY 5</th>
<th>DAY 6</th>
<th>DAY 7</th>
<th>Total for week</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>EXAMPLE</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 cup salad</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 tbsp</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 lb</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 cal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### FOODS TO EAT WEEKLY

<table>
<thead>
<tr>
<th>DAY 1</th>
<th>DAY 2</th>
<th>DAY 3</th>
<th>DAY 4</th>
<th>DAY 5</th>
<th>DAY 6</th>
<th>DAY 7</th>
<th>Total for week</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 g cooked</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pork</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Legumes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nuts</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### FOODS TO DRASTICALLY LIMIT

<table>
<thead>
<tr>
<th>DAY 1</th>
<th>DAY 2</th>
<th>DAY 3</th>
<th>DAY 4</th>
<th>DAY 5</th>
<th>DAY 6</th>
<th>DAY 7</th>
<th>Total for week</th>
</tr>
</thead>
<tbody>
<tr>
<td>200 g cooked</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red meat</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chocolate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Small goods</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

---

**Fig. 5** Semi-quantitative weekly checklist to encourage adherence to the MedPork intervention.
Appendix 4

The Low-Fat Diet - General Recommendations

The purpose of following a low-fat diet is to reduce overall amount of fat you're consuming.

There are 3 main ways you can achieve this:

1. Avoid or greatly restrict high-fat foods
2. Replace high-fat foods with low-fat versions (e.g., reduced fat dairy)
3. Alter preparation/cooking methods to reduce total fat content of your meal

Combining these strategies will give you the best results! You can add fat-free (as much as you like!) of low-fat,
nutritious foods such as fruits, vegetables, legumes, and whole grain products such as breakfast cereals, breads, rice
and pasta.

AVOID OR GREATLY RESTRICT THE FOLLOWING HIGH-FAT FOODS:

- Oils and oil-based dressings
- Butter, margarine, lard
- Regular fat dairy products, heavy cream, ice cream
- Saturated fats, deep-fried, high-fat meats, crumbled
- Liver, kidney and offal in general
- Processed foods, salted, take-away foods
- Commercial sauces, mayonnaise
- Commercially cooked foods
- Tree nuts and peanuts
- Sausage products, smoked hams and other salty meats
- Cakes, pies, pastries, croissants, high-fat crackers (e.g., Ritz, Ritz, Wheaties)

PREPARE AND COOK WITH LESS FAT:

- Do not smear bread or toast with butter, margarine, oil or other fat spreads
- Remove all visible fat from meat before cooking
- Remove all fat released from meat until cooking
- Cold soups and broths to remove fat layer on top before heating
- Avoid using oil, butter or fat-based sauces
- Dress salads with the least possible oil
- Emphasize simple cooking methods, such as boiling, baking or broiling. Avoid stewing, frying, crumbs
- Use the least possible amount of oil in the frying pan, enough only to avoid sticking of food

BUY LOW-FAT FOODS:

- Low-fat milk, and other dairy products
- Bread, cereals and pasta
- Rice
- Low-fat crackers (rice crackers, water crackers)
- Fruits and vegetables
- Pulses including beans, lentils, chick-peas
- Lean fish and seafood
- Chicken with the skin removed
- Lean cuts of meat and minced

(Adapted from Sainsbury et al. (2008) 2012)

Low Fat Cooking and Recipe Modification

There are several ways you modify recipes. Here are just a few suggestions:

- Use lean meats, fish, poultry, or lean cuts of beef. This can apply to curries, pasta sauces, sales, lasagnas and other sweet and savoury dishes
- Use lower fat choices like feta, cottage and ricotta in dishes like lasagne instead of cheddar
- Use less oil in your muffins, cupcakes and cakes. Use vegetables instead, they provide moisture and are much less energy dense. Good ones include carrots, zucchini and beetroot
- Make the oil and canola oil instead of olive oil
- Use a higher percentage of vegetables, legumes and oil or canola oil in your dishes compared to meat – vegetables, legumes, pasta and rice contain almost no fat
- Leave out high-fat ingredients and replace with low-fat ingredients whenever possible – i.e. leave out nuts and replace with dried fruit. Or if a seeded sauce for added texture, nuts, cheese or oil dressing use other low-fat ingredients like vageen, lemon juice, vinegar, coriander/cress, egg, or add more vegetables
- In general, “make your own and make it at home!” Even high-fat foods, like homemade cakes and muffins, casseroles, pasta dishes etc are likely to have less fat that store bought varieties.

Low Fat foods and snacks

Here is a list of some low-fat foods and snacks you can try:

- Chicken breast and leg (thighs) instead of hamburgers/whole chicken
- Higher grade mincemeat (pork, smart, p wszyst)
- Kangaroo meat – it is naturally very lean compared to beef or lamb
- Legumes instead of meat
- Low-fat sausages and sausages such as beef
- Lean cheese, milk and yoghurt
  - Full fat cheese is around 30% fat. Low fat cheese is around 25% fat, still a high fat product. Other choices such as milk, cow’s milk and cheese do have less fat than cheddar cheese.
  - Milk comes in several varieties:
    - Whole milk: full fat cream. Low-fat milk: reduced fat, Ultra Slim, Skimmed etc. As a general rule, whole or full cream is about 3.5% fat, which will be lower for reduced fat milk, often called light or lite, usually about 2% of the fat removed, so they are around 2.5% fat. Skim milk has 0.02% fat. Any one of these is a good choice.
    - If milk is called skim, or slimmed, it will usually be 5% or 9.5% fat free. If milk is called light, lite, or reduced fat, it will usually have between 1 and 2% fat. But if unsure, check the label.
  - Yoghurt comes in several variations:
    - Natural plain full fat yoghurt has the same fat content as full cream milk, about 3.5%.
    - Greek or natural plain yoghurt has about 8% fat (Greek yogurt is thicker and creamier).
    - Reduced fat versions of these will usually have about half the fat, e.g. reduced fat plain yoghurt might have around 3.9% fat, while reduced fat Greek yoghurt might have around 4% fat.
    - You can also get no-fat yoghurts, just like slimmed milk, and also Greek yoghurts which have had most of the fat removed.
  - We recommend always choosing either reduced fat or no fat yoghurt while on the low-fat diet. If you find Greek yoghurt, look for one that is 2.4%.

Fig. 6 Guidelines for the LF intervention
Appendix 5

Reading Food Labels

- Nutrition panels can be used to compare the nutrient content of different food products.

<table>
<thead>
<tr>
<th>Nutrition Information Panel</th>
<th>Quantity per serving</th>
<th>Quantity per 100g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy</td>
<td>479kJ</td>
<td>1595kJ</td>
</tr>
<tr>
<td>Protein</td>
<td>3.0g</td>
<td>10g</td>
</tr>
<tr>
<td>Fat - total</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- saturated</td>
<td>2.0g</td>
<td>6.5g</td>
</tr>
<tr>
<td>- mono</td>
<td>0.2g</td>
<td>0.7g</td>
</tr>
<tr>
<td>- unsaturated</td>
<td>1.4g</td>
<td>4.4g</td>
</tr>
<tr>
<td>- poly</td>
<td>0.4g</td>
<td>1.4g</td>
</tr>
<tr>
<td>- trans</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Carbohydrate - total</td>
<td>22.2g</td>
<td>74g</td>
</tr>
<tr>
<td>- sugars</td>
<td>8.2g</td>
<td>27.3g</td>
</tr>
<tr>
<td>Fibre</td>
<td>2.8g</td>
<td>9.2g</td>
</tr>
<tr>
<td>Sodium</td>
<td>4.0mg</td>
<td>14mg</td>
</tr>
<tr>
<td>Potassium</td>
<td>98.0mg</td>
<td>327mg</td>
</tr>
</tbody>
</table>

Ingredients: Rolled oats, sugar, rolled wheat, rolled triscake, rye, sultanas, dried paw paw, almonds, dried apple, orange juice concentrate, currants, yoghurt powder, vegetable oil, honey, maize starch, vegetable gum (414), food acid (270), lecithin.

Serve sizes vary between products so it is best to compare products by looking at the per 100g column.

Total Fat: Look for products with less than 10g fat per 100g. When comparing 2 products, choose the one which is lower in fat. For dairy, look for foods less than 2g per 100g.

Saturated Fat: Aim for as low as possible.

Trans Fat: Less than 1g per 100g.

Carbohydrate: Look for sugars less than 10g per 100g. In foods containing fruit (eg. cereal), aim for less than 25g per 100g.

Dietary Fibre: Look for more than 5g per 100g. Recommended daily intake is 30g.

Salt (sodium): Low salt foods contain no more than 120mg per 100g. Reduced salt foods contain 120-400mg per 100g. Look for ‘no added salt’ or ‘salt reduced’.

Fig. 7 Dietetic resource for reading food labels while following the LF intervention.
### Appendix 6

#### 9-item Low-fat Diet Questionnaire

For Low-fat group only

<table>
<thead>
<tr>
<th>Foods and frequency of consumption</th>
<th>Y / N</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Do you exclusively consume low-fat dairy products? (please circle Y or N)</td>
<td>Y / N</td>
</tr>
<tr>
<td>2. Do you remove visible fat and skin off meats before cooking and the fat off soups, broths and cooked meat dishes before consumption (please circle Y or N)?</td>
<td>Y / N</td>
</tr>
<tr>
<td>3. How much oil do you consume per day including oil used for frying, salads, out of home meals, etc.? (please answer in tablespoons (1 Tbsp ≈ approximately 20mL))</td>
<td>Y / N</td>
</tr>
<tr>
<td>4. How many servings of butter, margarine, lard, and mayonnaise do you consume per day? (1 serving = 2 tsp. = 22g)</td>
<td>Y / N</td>
</tr>
<tr>
<td>5. How many servings of fat-rich meats, such as hamburger, manure, sausage, pate, canned ham, bacon, sausage, or effid do you consume per week? (1 serving of meat serving = 100g, 1 serving of ham, sausage or bacon = 70g)</td>
<td>Y / N</td>
</tr>
<tr>
<td>6. How many servings of cream and ice-cream do you consume per week? (1 serving of cream = 1 Tbsp = 44g, 1 serving of ice-cream = 75g, 1 small scoop)</td>
<td>Y / N</td>
</tr>
<tr>
<td>7. How many times per week do you consume fatty fish or fish or seafood caused in oil?</td>
<td>Y / N</td>
</tr>
<tr>
<td>8. How many servings of commercial gravies or industrial bakery products (not homemade), such as cakes, cookies, biscuits or custard do you consume per week? (1 serve cake = 44g, 1 small slice = 80 g, 1-4 pieces Biscuits)</td>
<td>Y / N</td>
</tr>
<tr>
<td>9. How many times per week do you consume nuts (including peanuts), potato chips, French fries, or commercial snacks?</td>
<td>Y / N</td>
</tr>
</tbody>
</table>

#### 15-item Mediterranean Diet Questionnaire

For MedPork group only

<table>
<thead>
<tr>
<th>Foods and frequency of consumption</th>
<th>Y / N</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Do you use olive oil as your main cooking oil? (please circle Y or N)</td>
<td>Y / N</td>
</tr>
<tr>
<td>2. Do you preferentially consume pork, chicken, fish, turkey or wild meat instead of veal, hamburger steak or sausage</td>
<td>Y / N</td>
</tr>
<tr>
<td>3. How much olive oil do you consume per day including oil used for frying, salads, out of home meals, etc.? (please answer in tablespoons (1 Tbsp ≈ approximately 20mL))</td>
<td>Y / N</td>
</tr>
<tr>
<td>4. How many vegetable servings do you consume per day? (1 serving = ½ cup cooked or 1 cup salad or 1 mean)</td>
<td>Y / N</td>
</tr>
<tr>
<td>5. How many fruit servings (including pieces of fruit and 100% fruit pieces) do you consume per day?</td>
<td>Y / N</td>
</tr>
<tr>
<td>6. How many servings of butter, margarine or cream to you consume per day? (1 serving = 2 Tbsp = 20g)</td>
<td>Y / N</td>
</tr>
<tr>
<td>7. How many servings of red meat, hamburger, or meat products (lunch, sausage etc.) do you consume per week? (1 serving = 100g)</td>
<td>Y / N</td>
</tr>
<tr>
<td>8. How many servings of lentils, beans, peas, or other peas do you consume per week? (1 serving = 100g)</td>
<td>Y / N</td>
</tr>
<tr>
<td>9. How many servings of beverages do you consume per week?</td>
<td>Y / N</td>
</tr>
<tr>
<td>10. How many glasses of red wine do you drink per week? (Consider 1 glass as 160mL)</td>
<td>Y / N</td>
</tr>
<tr>
<td>11. How many servings of legumes do you consume per week? (1 serving = 40g)</td>
<td>Y / N</td>
</tr>
<tr>
<td>12. How many servings of fish or shellfish do you consume per week? (1 serving = 100-200g)</td>
<td>Y / N</td>
</tr>
<tr>
<td>13. How many times per week do you consume commercial soups or pastries (not homemade), such as cakes, cookies, biscuits?</td>
<td>Y / N</td>
</tr>
<tr>
<td>14. How many servings of nuts (including study nuts, other nuts you buy e.g. peanuts and nut products) do you consume per week? (1 serving = 40g)</td>
<td>Y / N</td>
</tr>
<tr>
<td>15. How many times per week do you consume soft drinks inside of pastries, tomatoes and garlic sauntered with olive oil with vegetables, posts, rice, or other dishes? (1 serving = 30g)</td>
<td>Y / N</td>
</tr>
</tbody>
</table>

---

Fig. 8 9-item Low Fat diet adherence survey and 15-item Mediterranean diet adherence survey
Appendix 7

Abbreviations
ACE-R: Addenbrooke’s cognitive examination – revised; ATS: Attention switching task; BMI: Body mass index; CAIDE: Cardiovascular risk factors, aging and dementia; CANTAB: Cambridge automated neuropsychological test automated battery; CRP: C-reactive protein; CVD: Cardiovascular disease; DEXA: Dual-energy x-ray absorptiometry; DLQ: Diet and lifestyle questionnaire; DMS: Delayed matching to sample; EEG: Electroencephalography; EPIC: European prospective investigation into cancer; EVOO: Extra virgin olive oil; FRS: Framingham risk score; HDL: High-density lipoprotein; ID: Identification; KSS: Karolinska sleep scale; LDL: Low-density lipoprotein; MCI: Mild cognitive impairment; MOT: Motor orientation task; NATA: National Association of Testing Authorities, Australia; OTS: One touch stockings of cambridge; PAL: Paired associates learning; POMS: Profile of mood states; PREDIMED: Prevención con Dieta Mediterránea; RCT: Randomised controlled trial; RTI: Reaction time; RVP: Rapid visual information processing; SF-36: Short form-36 health survey; SIHR CTF: Sansom Institute for Health Research Clinical Trial Facility; SWM: Spatial working memory; T2DM: Type 2 diabetes mellitus; VAS: Visual analogue scale; VRM: Verbal recognition memory; WFR: Weighed food record

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