The Australian Mediterranean Diet Heart Trial (AUSMED Heart Trial)

Itsiopoulos, Catherine; Kucianski, Teagan; Mayr, Hannah L.; van Gaal, William J.; Martinez-Gonzalez, Miguel Angel; Vally, Hassan; Kingsley, Michael; Kouris-Blazos, Antigone; Radcliffe, Jessica; Segal, Leonie; Brazionis, Laima; Salim, Agus; Tierney, Audrey C.; O’Dea, Kerin; Wilson, Andrew; Thomas, Colleen J.

Published in:
American Heart Journal

DOI:
10.1016/j.ahj.2018.05.010

Published: 01/09/2018

Document Version:
Peer reviewed version

Link to publication in Bond University research repository.

Recommended citation (APA):

General rights
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

For more information, or if you believe that this document breaches copyright, please contact the Bond University research repository coordinator.
The AUStralian MEDiterranean diet heart trial (AUSMED heart trial): A randomized clinical trial in secondary prevention of coronary heart disease in a multi-ethnic Australian population: Study protocol

Catherine Itsiopoulos PhD, Hannah L. Mayr BHSc, William J. van Gaal, Miguel Angel Martinez-Gonzalez, Hassan Vally, Michael Kingsley, Antigone Kouris-Blazos PhD, Jessica Radcliffe, Kerin O'Dea, Andrew Wilson, Colleen J. Thomas

PII: S0002-8703(18)30160-1
DOI: doi:10.1016/j.ahj.2018.05.010
Reference: YMHJ 5700

To appear in:

Received date: 7 May 2017
Accepted date: 21 May 2018

Please cite this article as: Catherine Itsiopoulos PhD, Hannah L. Mayr BHSc, William J. van Gaal, Miguel Angel Martinez-Gonzalez, Hassan Vally, Michael Kingsley, Antigone Kouris-Blazos PhD, Jessica Radcliffe, Kerin O'Dea, Andrew Wilson, Colleen J. Thomas, The AUStralian MEDiterranean diet heart trial (AUSMED heart trial): A randomized clinical trial in secondary prevention of coronary heart disease in a multi-ethnic Australian population: Study protocol. The address for the corresponding author was captured as affiliation for all authors. Please check if appropriate. Ymhj(2018), doi:10.1016/j.ahj.2018.05.010

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.
Trial Design

The AUStralian MEDiterranean Diet Heart Trial (AUSMED Heart Trial):

A randomized clinical trial in secondary prevention of coronary heart disease in a multi-ethnic Australian population: Study Protocol

Catherine Itsiopoulos1 PhD, APD, Teagan Kucianski1 BPharm (Hons), MDiet, APD , Hannah L. Mayr1 BHSc, APD , William J. van Gaal2 MD, Miguel Angel Martinez-Gonzalez1,3 MD PhD, Hassan Vally4 PhD, Michael Kingsley5 PhD, Antigone Kouris-Blazos1 PhD, APD, Jessica Radcliffe1 PhD, Leonie Segal6 PhD, Laima Brazionis1,7 PhD, Agus Salim8 PhD, Audrey C. Tierney1 PhD, APD, Kerin O’Dea1,8 PhD, and Andrew Wilson9 MD PhD; Colleen J. Thomas2 PhD.

1Department of Rehabilitation, Nutrition and Sport, La Trobe University, Bundoora, Australia  
2Department of Cardiology, The Northern Hospital, Epping, Victoria,  
3Department of Preventive Medicine and Public Health, University of Navarra, Pamplona, Spain, 
4Department of Public Health, La Trobe University, Bundoora, Australia,  
5La Trobe Rural Health School, La Trobe University, Bendigo, Australia,  
6School of Health Sciences, University of South Australia, Adelaide, Australia,  
7Department of Medicine, University of Melbourne, Australia,  
8Department of Mathematics and Statistics, La Trobe University, Bundoora, Australia,  
9Department of Cardiology, St Vincent’s Hospital, Melbourne, Australia,  
10Department of Physiology, Anatomy and Microbiology, La Trobe University, Bundoora, Australia,

Running Title: AUSMED Heart Trial Protocol

Address for Correspondence:  
Professor Catherine Itsiopoulos,  
Head of School of Allied Health, Professor of Dietetics and Human Nutrition  
La Trobe University | Bundoora | Victoria 3086 | AUSTRALIA  
Tel: +61 (0) 3 9479 1721 | Fax: +61 (0) 3 9479 2552.  
Email: c.itsiopoulos@latrobe.edu.au
Abstract
The Mediterranean diet was first characterised as a heart-protective diet in the 1960s. The significant cardio-protective effects of the Mediterranean diet in comparison to the standard care low-fat diet have been established in the primary prevention of cardiovascular disease (CVD), however, there is insufficient evidence in secondary prevention research to influence the current standard of care. Opportunity exists to assess the Mediterranean diet as a therapeutic target for secondary CVD prevention within Australia's ethno-culturally diverse communities.

The AUSMED Heart Trial is a multi-site randomized controlled trial that will evaluate the efficacy of the Mediterranean diet for secondary prevention of cardiovascular disease in the Australian healthcare setting. This trial aims to evaluate the effect of a 6-month Mediterranean diet intervention (delivered by dietitians) versus a ‘standard-care’ low-fat diet in reducing the composite incidence of cardiovascular events at 12 months and at trial end in participants with documented evidence of a previous Acute Myocardial Infarct (AMI) at trial entry. The quality of the diet at baseline and follow-up will be assessed using comprehensive dietary questionnaires and diaries as well as relevant dietary biomarkers (such as urinary polyphenols, erythrocyte fatty acids).

Cardiovascular risk markers, including novel measures of immune and inflammatory status, endothelial function, vascular compliance, platelet activity and body composition, will be collected to explore possible mechanisms for treatment effect. Cost-effectiveness will also be estimated to support policy translation.

We plan to recruit 1032 participants (516 per arm) from cardiology clinics in major Australian hospitals in Melbourne, Adelaide and Brisbane.
**Trial registration:** Australia and New Zealand Clinical Trials Register (ANZCTR): ACTRN12616000156482.

**Keywords:** Mediterranean diet, cardiovascular disease, myocardial infarction, secondary prevention, randomized controlled trial
Background

Cardiovascular disease (CVD) is a major cause of disease burden and death in the Australian population (AIHW, 2014) and represents a global healthcare crisis. Modifiable risk factors account for more than 90% of the population attributable risk for acute myocardial ischemia worldwide (McGorrian et al., 2011), and, with the exception of smoking, most of these factors are directly related to food habits. Despite recent efforts to target diet in primary prevention of CVD, these have not been paralleled in secondary or tertiary prevention where the risk of an imminent CVD event is considerably higher.

The cardioprotective properties of the traditional ‘Cretan’ Mediterranean diet were first identified five decades ago in the Seven Countries Study (Keys et al., 1986) and the recent reanalysis of the Seven Countries Study by a panel of experts supports the robust nature of the original study and its findings (Pett, Kahn, Willett, & Katz, 2017). Several recent meta-analyses concluded that greater adherence to a Mediterranean diet was associated with significant improvement in health status and reduction in all-cause, cardiovascular and cancer mortality (Godos et al., 2016; Martinez-Gonzalez & Martin-Calvo, 2016; Martinez-Gonzalez & Bes-Rastrollo, 2014; Sofi, Macchi, Abbate, Gensini, & Casini, 2014).

The recent landmark primary prevention PREDIMED trial in Spain consisted of 7,447 middle-aged people at high risk of CVD. Participants were randomized to three intervention arms: two Mediterranean diet arms; one supplemented with extra virgin olive oil (EVOO) and the other with nuts; and the third arm was allocated to the standard low-fat American Heart Association diet. The trial demonstrated a 30% relative risk reduction in incidence of first major CVD event (non-fatal myocardial infarction, non-fatal stroke or cardiovascular
death) following a median 4.8 years follow-up in the Mediterranean diet arms (Estruch et al., 2013).

The cardioprotective effects of the Mediterranean diet are thought to be mediated via bioactive nutrients that act synergistically with the potential to improve glycaemia (Itsiopoulos et al., 2011), blood pressure and lipid profiles (Hernaez, Castaner, Elosua, et al., 2017; Hernaez, Castaner, Goday, et al., 2017) in conjunction with anti-inflammatory and antioxidant potential. The Mediterranean diet is rich in omega-3 fatty acids from both plants (alpha-linolenic acid (ALA)) and fish and seafood (eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)), polyphenols, carotenoids, and other antioxidants such as Vitamin E and Vitamin C from fruits, vegetables, nuts and EVOO (Medina-Remón et al., 2016; Radcliffe et al., 2016; Ramírez-Anaya, Samaniego-Sánchez, Castañeda-Saucedo, Villalón-Mir, & de la Serrana, 2015; Tresserra-Rimbau et al., 2013). Antioxidant-rich herbs and spices such as garlic, rosemary, oregano, cinnamon and thyme have been shown to have angiotensin converting enzyme (ACE) inhibitor properties with potential antihypertensive effects (Barbosa-Filho et al., 2006). High total polyphenol intake has been reported to reduce all-cause mortality risk (Tresserra-Rimbau et al., 2014) and reduce low grade inflammation (Medina-Remón et al., 2016) in recent re-analyses of the PREDIMED trial. We have shown that flavonols, a group of polyphenols, reduce cardiomyocyte death and inflammation, and improve cardiac function following cardiac injury in animal models (Thomas et al., 2011).

Although observational studies outside the Mediterranean region have demonstrated positive associations between Mediterranean diet adherence and reduced risk of CVD, the most important current challenge is to demonstrate this risk reduction in an intervention study conducted in a non-Mediterranean socio-cultural setting (Martínez-González, 2016).
Multiethnic Australia is one such setting, dominated by Western food choices with its pervasive fast food outlets, highly visible snack foods, highly processed foods, and soft drinks. Moreover, this environment has contributed to the reported 35% of energy intake of Australians derived from discretionary foods (ABS, 2013).

In this paper, our aim is to detail the objectives, design and main aspects of the protocol for the AUSMED Heart trial, the first coronary heart disease (CHD) secondary prevention trial to test the effects of a traditional Mediterranean diet in a multi-cultural population.

**Aims and Hypotheses**

The primary aim of the AUSMED Heart Trial is to evaluate the efficacy of a 6-month Mediterranean diet intervention in reducing the incidence of secondary CVD events at 12 months and trial end (following an acute AMI), compared to patients on a low-fat diet (standard care recommendation in Australian hospital cardiology services).

The primary outcome is the incidence of secondary cardiac events at 12 months and trial end (AMI, cardiac and all-cause mortality, unstable angina requiring either a positive stress test or revascularization, and ischaemic stroke). Secondary outcomes include established and novel cardiovascular clinical biomarkers that may help to explain plausible biological pathways in secondary prevention of cardiac events. In particular, we will investigate changes in status of arterial stiffness, novel immune and inflammatory markers, platelet activity and body composition. The authors hypothesize that, compared to a low-fat (standard care) diet, a Mediterranean diet will reduce the risk of secondary cardiac events in patients who have experienced a cardiac event.
We have shown that a Mediterranean diet intervention is cost-effective using data from the Lyon Diet Heart Study (Dalziel, Segal, & De Lorgeril, 2006) and we therefore plan to evaluate whether the Mediterranean diet intervention is cost-effective in the AUSMED Heart Trial. We will measure net cost per CVD event averted (in 12 months), net cost per quality adjusted life year [QALY] gain, and net budget impact on the health sector, relative to the standard dietary treatment arm.
Methods

Design
The AUSMED Heart Trial (Australia and New Zealand Clinical Trials Register (ANZCTR): ACTRN12616000156482) is a 6-month randomized, controlled, single-blind dietary intervention in a high-risk patient group who have experienced an acute myocardial infarct. The primary endpoint will be the difference in secondary cardiovascular event rates at one year, between the Mediterranean diet and low-fat diet participants. By following participants until 12 months, 6 months after the dietary intervention and staple provisions have ceased, this study design will capture real-world sustainability, feasibility, and adherence data regarding the Mediterranean diet within a non-Mediterranean socio-cultural setting. The pilot of this study is currently being conducted within the cardiology services of two major metropolitan hospitals in Melbourne, Australia; St. Vincent’s Hospital and The Northern Hospital, with anticipated roll out to sites in Adelaide and Brisbane. Patient interviews and clinical, anthropometric, biological and behavioral measurements are currently taking place at La Trobe University, Bundoora, Melbourne.

Study integrity
This trial has been approved by The Human Research Ethics Committee of La Trobe University (#FHEC13/159), The Northern Hospital (HREC/16/Austin/500) and The St. Vincent’s Hospital Melbourne (HREC-A; 016/13) and will be conducted in accordance with the Good Clinical Practice guidelines. The CONSORT guidelines (Schulz, Altman, & Moher, 2010) were consulted to assist in the development of this study. Written informed consent will be obtained from all study participants. Safety protocols have been established to monitor the health and welfare of participants over the course of the study.
Study Population

Participant recruitment

Participants will be recruited across multiple settings at each hospital including; cardiology outpatient clinics, cardiac rehabilitation sessions, cardiac catheter laboratory or inpatients from the coronary care unit. Potentially eligible participants will either be referred by hospital staff or approached by trained study personnel for screening. Screening will be performed by a trained researcher to determine if participants meet the inclusion criteria as noted below.

Participant eligibility

Inclusion criteria

Eligible patients will be aged ≥18 years, English speaking and within one year of acute presentation of AMI to the recruitment sites, as defined by the Cardiac Society Guidelines: a Type 1 myocardial infarction: (STEMI) or non-STEMI presenting with angina pectoris confirmed with elevated cardiac enzymes (troponin levels) or coronary angiography or balloon angioplasty (with or without stent) as defined by the third universal definition of myocardial infarction (UDMI) (Thygesen et al., 2012). Patients with Type 2 Diabetes will be included.

Exclusion criteria

Patients will be excluded if they have: active malignancy; symptomatic chronic heart failure (New York Heart Association Functional Classification II, III & IV); chronic inflammatory disease (e.g. inflammatory bowel disease or rheumatoid arthritis treated with anti-inflammatory or immuno-modulating medications); chronic kidney disease stage 3 or above, decompensated liver disease or taking medications that cause hepatosteatosis;
immunodeficiency or HIV-positive status; body mass index (BMI) >40 kg/m$^2$; are currently breastfeeding, pregnant or trying to fall pregnant; are currently participating in an intervention trial targeting CVD, diet or exercise; are unable to attend all study appointments. Patients with serious food allergies will be managed appropriately by the dietitians on the team to ensure allergens are avoided.

**Study procedure**

*Screening assessment and randomization*

Prospective participants will be assessed for eligibility by a thorough screening questionnaire either administered face-to-face or via telephone by a trained researcher. If the individual meets the inclusion criteria, medical records will be cross-checked to confirm eligibility and the participant is invited to attend a pre-baseline appointment. Participants will be randomized, using a computer-generated stratification approach according to age, and sex, into either the Mediterranean Diet (intervention) group or Low-fat diet (control) group to ensure the assignment to both diets is well balanced. The process of randomization will be performed by the biostatistician (AS).

*Timeline of study consultations*

Following randomization, participants in both groups will receive the same number of consultations for the duration of the study. As shown in Figure 1 and Table 1, all participants will complete face-to-face intensive dietary assessment and counselling; medical and lifestyle assessment; and anthropometry and biochemistry measures at baseline, end-intervention (6 months) appointments, and 12-month follow-up, with a smaller batch of measures at the 3-month appointment. Participants from both groups will also receive five phone call follow-up reviews at weeks 3, 6 and 9 and then at months 4 and 5, to set and assess dietary goals and
identify and address participant issues. The primary outcome of CVD events and CVD composite clinical outcomes will be determined via access of data on major adverse cardiovascular events (MACE), including deaths through the state-based data linkage systems across Australia. Participants will give informed consent for this data access on entry into the trial. An independent committee will be established to review de-identified event data and confirm all CVD events.

Protocol

**Mediterranean Diet intervention:** Participants who are randomized to the intervention group (MedDiet) will receive nutrition assessment and intensive education on the Mediterranean diet. The MedDiet intervention has been explained in detail elsewhere (George et al., 2018). The diet will be based on the key principles of the Mediterranean dietary patterns as published by Martínez-González et al. (2012) in the PREDIMED Trial (www.predimed.es) and using Mediterranean diet intervention models, meal plans and recipes utilized by the Chief Investigators in previous published trials (Itsiopoulos et al., 2011; Papamiltiadous et al., 2016; Ryan et al., 2013). The validated PREDIMED 14-item Mediterranean Diet Adherence Screener (MEDAS) will also be used to assess compliance of this diet at each measurement time-point (Schröder et al., 2011).

Participants will be asked to complete a 7-day food diary in household measures during the week prior to the baseline appointment to determine habitual diet. Individuals will then be interviewed by an Accredited Practising Dietitian (APD) and will receive a 14-day meal plan which incorporates the key principles of the Mediterranean diet and is consistent with the participant’s cultural and religious dietary requirements. A resource kit including a Mediterranean Diet food pyramid, shopping lists, food checklists, and advice on label reading
will be provided (George et al., 2018). Meal plans will be designed to meet current energy requirements for weight maintenance and will be consistent with the macronutrient composition of the Mediterranean diet (15-20% protein; 35-40% fat (18-20% of total energy intake as monounsaturated fatty acids); 40-45% carbohydrate). Food group recommendations will include: daily intake of extra virgin olive oil (EVOO), nuts, vegetables, fruit and wholegrain cereals, regular intake of legumes, fish and yoghurt, and limited intake of commercial sweets or pastries and red or processed meat. Poultry, eggs and feta cheese will be recommended in moderation.

Our team has extensive experience in the design and implementation of this Mediterranean diet model in a number of clinical trials as previously reported (Itsiopoulos et al., 2011; Papamiltiadous et al., 2016; Ryan et al., 2013). Advice on alcohol consumption (only for those who already consume alcohol) will be consistent with current National Health and Medical Research Council of Australia (NHMRC) guidelines which “recommend that healthy men and women drink no more than two standard drinks on any one day” (NHMRC, 2009). A preference for wine (red, when possible) will be suggested to participants who consume alcohol, in line with the Mediterranean Alcohol Drinking Pattern (MADP) associated with lower CVD risk (Gea et al., 2014).

Compliance to this diet will be facilitated by the provision of a food hamper at two time points (start of intervention and at 3 months) which will include samples of the key elements of this diet. Participants will also be provided with a free copy of The Mediterranean Diet (Itsiopoulos, 2013) which includes a 7-day menu plan and traditional Mediterranean recipes used in previous studies the authors have conducted (Itsiopoulos et al., 2011; Ryan et al., 2013). Participants on the Mediterranean diet arm will be provided with 6 months’ supply or
6L of extra virgin olive oil (Cobram Estate) to achieve 60-80ml/day, and 1.2kg nuts, including almonds (Almond Board Australia), and walnuts and hazelnuts (purchased wholesale from local supermarkets) to achieve an intake of 30g nuts per day for the 6 months duration of the trial. In addition, participants will be provided with a hamper at the commencement of the trial including samples of canned legumes and tinned tuna and salmon (Simplot Australia Pty Ltd and HJ Heinz Company Australia Limited), and Low Fat Greek yoghurt (Jalna Dairy Foods Pty Ltd). Participants will receive breakfast on the day of study appointments. Adherence to the intervention will be evaluated at 12 months to assess longer term feasibility.

**Low-Fat Diet Intervention (control):** Participants following the standard care low-fat diet will receive assessment and education according to the standard protocol that is consistent with the dietetic service of the participating hospitals, i.e. a diet based on the National Heart Foundation (NHF) Guidelines (NHF, 2012) and the Australian Dietary Guidelines (ADGs) (NHMRC, 2013). Participants will be provided with existing resources, including relevant NHF and ADGs printed education material and a sample low-fat meal plan modelled on these resources and previous intervention studies of the investigators (Papamiltiadous et al., 2016; Ryan et al., 2013). In terms of contribution to total energy consumption, target macronutrient intakes will be <30% total fat, <7% saturated fat, 45-65% carbohydrate, 15-25% protein and ≤5% alcohol. Food group recommendations will include daily intake of grains and cereals (mostly whole grains, 5-7 serves/day), vegetables (5-6 serves/day), fruit (2 serves/day), protein foods (2-3 serves/day) and low-fat dairy foods (2 serves/day)(NHMRC, 2013). The PREDIMED 9-item low-fat diet Assessment Tool will also be used to assess compliance of this diet at each measurement time-point. The low-fat diet group will have the same number of appointments and support as the intervention group in order to control for the level of
attention received by both groups. This group will be provided with 3 shopping vouchers ($20 value) on entry to the study, and at 3 months and 6 months to facilitate compliance and continuation in the study.

**Primary endpoints** are the following composite clinical endpoints: Secondary AMI (Type 1 myocardial infarction: STEMI or non-STEMI, as defined by the third universal definition of myocardial infarction (Thygesen et al., 2012), death from cardiovascular causes and death from any cause, hospital admission for angina pectoris requiring a positive stress test, and ischaemic stroke). The definition of MI is based on the 3rd Universal definition of MI (UDMI) and is outlined in the Supplementary Appendix. Percutaneous coronary intervention (PCI) related MI, stent thrombosis associated with MI, and Coronary artery bypass grafting (CABG) related MI events, as described in the 3rd UDMI, will be recorded but not included in the definition of spontaneous MI.

**Secondary CVD endpoints** will include the composite CVD endpoints noted for the primary endpoint including unplanned coronary revascularization.

An independent clinical-events committee, whose members will be unaware of the treatment assignments, will adjudicate all CVD end points. To further prevent bias in reporting of events independent researchers who are blinded to the study arms will record all hospital admissions irrespective of admission or discharge diagnosis for patients on the trial within the trial period thus flagging a hospital separation (admission). These researchers will document the detailed admission and discharge diagnoses and all this detail will then be provided for adjudication by an independent committee.
Consent will be obtained at enrolment from participants to access their health records (including hospital admission data and emergency room presentation), Medicare Benefits Schedule (MBS) data and death records. To ensure the event adjudication is blinded, data on major cardiovascular events, including deaths, will be obtained by a researcher unaware of the study-group assignment through the state-based data linkage systems across Australia.

The application for access to Hospital in-patient, emergency department and death records for all trial participants using key identifiers (name, date of birth, gender, current address) will be submitted to the Population Health Research Network (PHRN). The three data sets are the subject of on-going collections across the three jurisdictions. Data items that will be requested to 30th June 2023 are: (1) In-patient hospital separations: Admission date, Separation date, Diagnosis – principal, Diagnoses – additional, Diagnosis-Related Group (DRG), Discharge destination; (2) Emergency Department presentations: Departure date/time, Diagnosis, Presentation date, Presenting problem, Triage category, Visit type and (3) Deaths Register; Date of death, Cause of death type, Cause of death text. Data will be made available at no charge (nominal charge for the death data).

**Secondary outcomes to assess biological pathways**

Secondary outcome measures will include a variety of cardio-metabolic risk markers which will be assessed at baseline, 3 and 6 months, and 12 months and comprise: lipid and metabolic profiles, inflammatory markers, clotting factors, endothelial function, vascular compliance, blood pressure, heart rate variability, anthropometry and body composition and physical activity levels. Table 1 illustrates the study measurements and frequency including a series of measures which will only be taken on a subset of the study population (n=258).
### Table 1. Trial interventions, study measurements and their frequency

<table>
<thead>
<tr>
<th>Item/Measurement</th>
<th>Brief Description/purpose</th>
<th>Month</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eligibility questionnaire</td>
<td>Inclusion/exclusion criteria</td>
<td>1 0 0 0</td>
</tr>
<tr>
<td>Informed Consent</td>
<td></td>
<td>1 0 0 0</td>
</tr>
<tr>
<td>Randomisation (stratified by age, sex)</td>
<td></td>
<td>1 0 0 0</td>
</tr>
<tr>
<td>7-day food diary</td>
<td>Dietary intake</td>
<td>1 1 1 1</td>
</tr>
<tr>
<td>FFQ</td>
<td>Pilot-validated 115-item FFQ*</td>
<td>1 1 1 1</td>
</tr>
<tr>
<td>14-item MedDiet questionnaire</td>
<td>Mediterranean diet adherence tool</td>
<td>1 1 1 1</td>
</tr>
<tr>
<td>9-item low-fat diet questionnaire</td>
<td>Low-fat diet adherence tool</td>
<td>1 1 1 1</td>
</tr>
<tr>
<td>Cardio-Med questionnaire*</td>
<td>Medical history, medications, physical activity, smoking and socio-cultural data</td>
<td>1 1 1 1</td>
</tr>
<tr>
<td>Health-related quality of life</td>
<td>Validated 36-item Short Form Health Survey (SF-36)</td>
<td>1 1 1 1</td>
</tr>
<tr>
<td>Activity questionnaire</td>
<td>Validated 13-item Active Australia Survey</td>
<td>1 1 1 1</td>
</tr>
<tr>
<td>Physical activity</td>
<td>Actigraph; assessment of sedentary vs. moderate to vigorous activity in a subset of participants.</td>
<td>1 0 1 0</td>
</tr>
<tr>
<td>Anthropometry</td>
<td>Height, body weight, circumferences (hip, waist, neck, upper middle arm)</td>
<td>1 1 1 1</td>
</tr>
<tr>
<td>Body composition</td>
<td>Dual-energy x-ray absorptiometry (DXA) in a subset of participants.</td>
<td>1 0 1 0</td>
</tr>
<tr>
<td>Blood sample</td>
<td>All Participants</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Standard biomarkers (cholesterol, glucose, hSCRP, renal and liver markers)</td>
<td>1 1 1 1</td>
</tr>
<tr>
<td></td>
<td>• Inflammatory cytokines and adipokines</td>
<td>1 0 1 1</td>
</tr>
<tr>
<td></td>
<td>• Dietary biomarkers (erythrocyte fatty acids)</td>
<td>1 0 1 0</td>
</tr>
<tr>
<td></td>
<td>Subset of participants:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Adhesion molecules and Clotting factors</td>
<td>1 0 1 0</td>
</tr>
<tr>
<td></td>
<td>• Insulin, homocysteine, oxidative stress, ApoA, ApoB</td>
<td></td>
</tr>
<tr>
<td>Urine sample</td>
<td>Na⁺: K⁺</td>
<td>1 0 1 0</td>
</tr>
<tr>
<td></td>
<td>Dietary biomarkers (polyphenols)</td>
<td></td>
</tr>
</tbody>
</table>
**Laboratory**

A sample of 40 ml whole blood, following an overnight fast of at least 8 hours, will be drawn by a trained phlebotomist from the antecubital vein at baseline and each face-to-face appointment. Blood samples will be processed by the researchers or nominated laboratories according to standard protocol. Once processed, aliquoted samples (both whole blood, plasma/serum and urine) will be stored at -80°C until batch analyses are performed. The samples will be analyzed according to standardized methods. Additional frozen plasma, serum and urine will remain stored at -80 °C for future novel biomarkers of interest.

Samples will be analyzed at each time point for lipid profile (triglycerides, total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), markers of glucose metabolism (C-peptide, insulin, glucose), liver function, renal function (urea, creatinine, electrolytes), homocysteine, and high sensitivity C-reactive protein (hs-CRP). Additionally, the following non-routinely measured markers will be measured at baseline and end of intervention (6 months) in a subset of participants (n=258): cholesterol transport.
(ApoA/ApoB), oxidative stress (F2 Isoprostanes), inflammation and immunity (Interleukin-1, -4, -6, -10 and -17), adipokines (adiponectin, resistin) and endothelial dysfunction (ADMA, nitrate, nitrite, and adhesion molecules, ECAM-1; VCAM-1; ICAM-1).

At each face-to-face appointment, participants will also be instructed to collect a first-morning urine sample in provided containers. Urine will be analyzed for urinary Na⁺:K⁺, adjusted for creatinine concentration, and urinary polyphenols as a dietary biomarker.

**Blood pressure and heart rate variability**

Systolic and diastolic blood pressure will be measured in triplicate using an automated digital blood pressure monitor (OMRON Tp9, IntellisenSense, Australia) after the seated participants have been resting for 5-10 minutes. The average of the second and third measurements will be recorded. Electrocardiogram (ECG) traces will be accessed from the participant’s medical records. ECG recordings will be subsequently analyzed using commercial software (Darwin V2, Medilog, Schiller AG, Switzerland) to determine temporal and spectral outcome measures, as per the recommendations of the Task Force of the European Society of Cardiology and North American Society of Pacing and Electrophysiology, 1996. Sinus RR intervals will be de-trended and windowed using a Hanning window before Fast Fourier Transformation of 5-minute segments with 50% overlap to determine the power spectral density in very low frequency bandwidth (<0.04 Hz), low frequency bandwidth (0.04-0.15 Hz) and high frequency bandwidth (0.15-0.40 Hz), as previously described (Kingsley, Lewis, & Marson, 2005).
Vascular Function

In a subset of participants (n=258), vascular compliance (central aortic blood pressure and arterial stiffness) will be assessed using a Sphygmocor device (Atcor P/L). This system measures central aortic blood pressures and arterial stiffness noninvasively and has been shown to clearly identify the presence of target organ changes. In the same subset of participants, endothelial dysfunction will also be assessed using an EndoPat 2000 (Itamar Medical) which measures endothelium-mediated changes in peripheral arterial tone.

Anthropometry and Body Composition

Anthropometric measures height, weight, waist circumference, hip circumference, upper middle arm, and neck circumference will be measured in all participants using standard techniques (ISAK, 2001), in duplicates by a trained researcher at each face-to-face appointment. Body composition will be measured by dual energy X-ray absorptiometry (DXA) in a subset of participants (n=258) using a Hologic Discovery W© with analysis performed using QDR™ (Quantitative Digital Radiography) for Windows for assessment of fat mass, fat-free mass and visceral adipose tissue (VAT). Hologic scientists developed their method for measuring VAT from DXA (Kelly, Wilson, & Ruth, 2015), which is highly correlated (r=0.93) and linearly related to VAT measurements by computed tomography (Micklesfield, Goedecke, Punyanitya, Wilson, & Kelly, 2012).

DIET AND LIFESTYLE

Dietary intake

Dietary intake will be measured for each participant using 7-day food diaries and a pilot-validated Cardio-Med Questionnaire including a 115-item Food Frequency Questionnaire (FFQ) purpose built by the investigators to capture adherence to the principles of a
Mediterranean diet in a multi-ethnic cohort (Kucianski et al, unpublished). Data from food diaries and FFQs will be validated by the APD and analyzed using a standard nutrient analysis program (FoodWorks™ ver 8.0 Xyris Software Australia).

**Dietary adherence**

Compliance to the two study diets (Mediterranean diet and Low-fat diet) will be monitored in all participants using established biomarkers: erythrocyte fatty acids (omega-3:omega-6 ratio), urinary polyphenols, and urinary Na⁺:K⁺ ratio adjusted for creatinine. Dietary adherence will also be assessed using the validated PREDIMED 14-item Mediterranean Diet Adherence Screener (MEDAS) (Schröder et al., 2011) (MedDiet), and 9-item Low Fat questionnaire (Low-fat diet), and a 7-day food diary at baseline, 6 months and again at 12 months to assess longer term sustainability of the intervention following cessation of supply of key staples after 6 months (EVOO and nuts).

**Health and lifestyle**

Socio-demographic data, health care resource utilization, family medical history, participant medical history and medication use, and smoking status is to be collected via a self-completed questionnaire (Cardio-Med questionnaire, Kucianski et al, unpublished) at each face-to-face appointment. Wherever possible, data will be validated against the participant’s hospital medical records. Quality of life data will be collected at each face-to-face appointment using the Short Form Health Survey, SF-36 Version 2 (Motamed, Ayatollahi, Zare, & Sadeghi Hassanabadi, 2005).

**Physical activity**
Baseline physical activity levels and changes at 3, 6 and 12 months will be assessed via self-report, using the validated 13-item Active Australia Survey (AIHW, 2003). A subset of participants will be instructed to wear a triaxial accelerometer (WGT3X-BT; Actigraph Corp, Florida, United States) on their right hip during waking hours, except when in water, for 7 consecutive days prior to each study appointment. Detailed instructions on how to wear the monitor will be provided to each participant. Additionally, participants will be required to manually record the wear time of the accelerometer. Data from each monitor will be downloaded and analysed using associated software (Actilife v6; Actigraph Corp, Florida, USA). Using established criteria (Freedson, Melanson, & Sirard, 1998), the time undertaking moderate to vigorous physical activity, and time spent as sedentary, will be determined for each wear day. No specific intervention on physical activity will be included beyond that routinely provided in standard care.

Data management

A trial staff member at each site will be responsible for the secure storage of participant data. Hard copies of de-identified case report forms and participant questionnaires will be kept in a secure filing cabinet in a locked room. All electronic data will be saved in a password protected database. Biological specimens will be kept in a locked freezer. All data and biospecimen samples will be stored at La Trobe University, Bundoora, Melbourne. Participants will be entitled to access their individual results by making a request to the clinical trial coordinator. Additionally, dissemination of research results will occur upon the conclusion of the study.

Power calculation for primary endpoint: We assume that at the end of the 12-month follow-up, 12.35% of participants in the treatment arm will experience an event (defined as
any of the following: cardiac death, non-fatal myocardial infarction, unstable angina, stroke, heart failure, pulmonary or peripheral embolism) compared to 19% of participants in the control arm (relative risk = 0.65). The hypothesis that the intervention will reduce the relative event rates by 35% (absolute event rates by 6.65%) is conservative compared to the observed event rate difference of 70% in the Lyon Diet Heart Study (de Lorgeril et al., 1999), and provides a relative reduction similar to results seen in the PREDIMED trial (Estruch et al., 2013), and is supported by current event rates in the statin era in the Australian high risk populations (Chew et al., 2008). To detect this 35% relative difference (6.65% absolute difference) between the two groups with at least 80% power, we need to recruit 465 participants per arm (5% significance level is assumed). We aim to recruit 516 participants per arm (1032 participants in total) to allow for a 10% drop out rate.

**Statistical analyses:** Intention-to-treat (ITT) will be used to analyze the primary and secondary endpoints. Kaplan-Meier (K-M) plots and Cox’s proportional hazard models will be used to analyze and compare rates of events between control and treatment groups. Proportional hazard assumptions will be examined to ensure the validity of the conclusion drawn. For quantitative endpoints (e.g., hsCRP), t-test and linear regression will be used to compare the two groups. If normality assumption is not fulfilled, non-parametric tests such as Wilcoxon rank-sum (Mann-Whitney) test or data transformation methods will be used. Multiple imputation will be used to input missing data for covariates and outcomes. Adjusted results will be calculated using a multiple linear regression model including the stratification factors (gender, age group), recruitment site, and other baseline factors including baseline Mediterranean diet score and physical activity. All statistical analyses will be performed using 5% significance levels. SPSS Version 24 and Stata 14.0 will be used for the statistical analyses.
**Economic evaluation:** This study will also include a formal economic evaluation to assess the cost effectiveness of the Mediterranean diet intervention. This will be completed by investigator Leonie Segal, who was lead investigator on the economic evaluation with Dalziel and de Lorgeril of the Lyon Diet Heart Study (Dalziel et al., 2006). It will follow the same protocol as that study. A Markov Model will be developed based on transition probabilities between event states (including death) observed during the trial and the up to 12-month follow-up period in the intervention and control arms. These transition probabilities will then be applied for a limited period beyond trial end. Each event state (e.g. AMI, or stroke) will carry a quality of life utility score and treatment cost. In sensitivity analysis the length over which outcomes will be modelled will be varied, as well as the extent of maintenance of effect, and program cost. Costs will be based on data on number of dietetic consultations delivered and by whom, to which published salary rates will be applied; and for hospitalizations DRG costs will be applied (latest year).

**Enrolment status and feasibility:** Recruitment for the AUSMED Heart Trial is currently underway with 66 participants enrolled to date from participating hospitals. Of the 66 participants enrolled in the trial, 56 completed the 6 month intervention representing a 15% drop out rate to date. Recruitment is currently on hold awaiting further funding. Participant demographics are representative of a multi-cultural Australian population (38 born in Australia, 28 from overseas). Preliminary analysis of results demonstrate that participants in the MedDiet intervention arm are adhering to the intervention (Mayr et al., 2018) with a mean Mediterranean diet score increase from 5/14 to 10/14 at 3-months (p<0.001) which has been maintained at 6 and 12 months. There have been no adverse reactions to the Mediterranean diet.
Acknowledgements and Study Funding

We thank La Trobe University (Understanding Disease RFA Start-Up Grant, 2014; La Trobe University DVCR support grant, 2018) and the ongoing support of our industry partners (Boundary Bend P/L (Cobram Estate EVOO), the Almond Board of Australia, Jalna Dairy Foods, Simplot Australia, Carman’s and Heinz) for supply of key foods to be provided to participants of this trial. The authors also wish to thank several contributing staff Dr Wolf Marx (protocol updates and revisions to ethics), PhD students Ms. Elena George (development of dietary intervention); Ms. Tania Thodis (survey development) and Nutrition Research Honours students (Ms. Cassandra Bendall and Mr. Dean Monaghan).

Author Contributions

Itsiopoulos C is the Chief Investigator of the Study and has primary responsibility for the final content. Itsiopoulos C, Thomas CJ, Mayr HL and Kucianski T wrote the paper. All authors had a significant input into the design of the protocol. All authors read and approved the final manuscript. The authors declare there is no conflict of interest.
Discussion

The Mediterranean diet was first described as cardioprotective in the 1960s, yet dietary prevention and management strategies have largely focused on reduction of saturated fat and dietary cholesterol for the past 50 years, including in Australia (NHF, 2012). This focus on dietary fat may have inadvertently increased intakes of highly processed carbohydrates, contributing further to the obesity and diabetes epidemics worldwide, which in turn lead to increased CVD morbidity and mortality. There is now a recognized need to promote healthy dietary patterns, including a Mediterranean diet, rather than focus on individual nutrients for reduction of CVD risk (Freeman et al., 2017).

Evidence for the cardioprotective and diabetes-protective effects of the Mediterranean diet is supported by longitudinal cohort studies (Martinez-Gonzalez & Martin-Calvo, 2016; Schwingshackl, Missbach, Konig, & Hoffmann, 2015) and the landmark primary prevention PREDIMED trial which demonstrated an impressive 30% relative risk reduction in CVD events in high risk individuals following the Mediterranean diet arms supplemented with EVOO and nuts (Estruch et al., 2013). The landmark secondary prevention trial, the Lyon Diet Study (de Lorgeril et al., 1999), conducted over two decades ago demonstrated a dramatic protective (70% reduction in relative risk of second AMI) and highly cost-efficient effect of this diet in secondary prevention (Dalziel et al., 2006). Thus, illustrating that a focus on secondary prevention with this dietary approach could in part replace the high cost strategies associated with secondary prevention.

No other findings from secondary prevention trials in AMI patients using a Mediterranean diet have been published since the late 1990s. The CORDIOPREV Study, a secondary prevention trial investigating an olive oil supplemented Mediterranean diet versus low-fat
diet, commenced in 2012 in Spain and results are pending (Delgado-Lista et al., 2016). To our knowledge there are no secondary prevention trials in CHD using a Mediterranean diet model in populations outside southern Europe. One study conducted in the USA (Tuttle et al., 2008), reported a secondary prevention trial of a Mediterranean-style diet intervention versus low-fat diet after first AMI; however, the intervention had no focus on the key Mediterranean foods of EVOO or nuts.

To our knowledge, the AUSMED Heart Trial is the first secondary prevention trial in patients with an AMI investigating the potential of a traditional Mediterranean Diet intervention in preventing secondary CVD events outside a Mediterranean region. A reduction in cardiac (CVD) re-event rates, relative to standard care, would reduce long-term cost of treatment and care of these patients, improve their quality of life and extend life. Further, this trial is extensively focused on investigating the mechanisms of this diet’s cardioprotective effect on the vascular endothelium. In addition to standard CVD risk markers, we are measuring markers of cholesterol transport, oxidative stress, inflammation and immunity, adipokines, and endothelial dysfunction as well as functional outcomes such as flow mediated dilatation in capillaries and body composition changes.

This trial is designed to have a strong translational focus, as the recruitment and interventions are embedded within cardiology clinics across four prominent acute and ambulatory care sites in three jurisdictions across Australia. It is therefore optimally placed to influence clinical practice of health care professionals who engage with patients ‘at the coal face’; dietitians, nurses, physiotherapists, exercise physiologists, medical residents and registrars and consultant cardiologists. Furthermore, by using a sufficiently-powered, high-quality trial
design that captures primary endpoints as well as mechanistic and cost-efficacy data, the results of this trial will provide significant evidence to inform clinical practice.

A potential limitation of this trial is the ability to translate this dietary pattern from the Mediterranean region to an Australian population who are ostensibly consuming a Western dietary pattern. Common to all dietary interventions is the potential for contamination of the control group, a relevant issue to this trial given the widespread popularity of the Mediterranean diet in the media. However, to reduce possible contamination, the AUSMED study is advertised to eligible patients as a dietary intervention study, without placing particular emphasis on the Mediterranean diet or control diet and separate research dietitians will also provide dietary consultations to the intervention and control group. Related to this, are possible issues with adherence to specific foods that may be unfamiliar to an Australian population. However, our pilot data to date (N=66) does not support these concerns with results showing strong adherence to a Mediterranean diet pattern in the intervention group, and significant and expected differences in food intake patterns between the intervention and the low-fat control group.

In summary, scientific evidence for the cardioprotective and diabetes-preventive impacts of a Mediterranean diet has been supported by longitudinal cohort studies (Lairon, 2007; Serra-Majem, Roman, & Estruch, 2006) and the landmark dietary intervention trials that have provided high-level evidence of benefit, conducted solely in Mediterranean populations (de Lorgeril et al., 1999; Estruch et al., 2006). It is now prudent to establish the generalizability of the benefits of the Mediterranean diet in non-Mediterranean populations (Martínez-González, Hershey, Zazpe, & Trichopoulou, 2017). This study will be the first sufficiently
powered RCT to evaluate this in the multi-cultural Australian setting and provide cost efficiency and mechanistic data, compared to the ‘standard care’ diet.
References


Arm 1 – Low Fat diet (prescribed in practice)
Arm 2 - Mediterranean Diet

Pre baseline
0 3 6 12 Follow-up
Time (months)

Eligibility screening
Participant consent
Randomisation

Appt #1  Appt #2  Appt #3

Dietary measures
Cardiology survey
Participant feedback

Appointment measures
- Anthropometry & Body composition
- Medical history
- Self-report dietary intake measures
- Blood sampling
- Cardiology survey

* Provision of Food hamper (Med diet) or Supermarket voucher (Control)

Figure 1