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Article

Effect of randomisation to 6-month Mediterranean versus low-fat diet intervention on inflammation and adiposity in patients with coronary heart disease; Preliminary results of the AUSMED Heart Trial

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Abstract: The Mediterranean diet (MedDiet) is recognised to reduce risk of coronary heart disease (CHD), in part, via its anti-inflammatory properties. Diet efficacy via this mechanism is however unclear in patients with diagnosed CHD. This study aimed to determine the effect of MedDiet versus low-fat diet intervention on inflammatory biomarkers and adiposity in a pilot cohort of Australian patients post coronary event. Participants (62±9 years, 83% male) were randomised to the MedDiet (*n*=34) or low-fat diet (*n*=31). At 0-, 3- and 6-months, dietary counselling, anthropometry, body composition (Dual-energy X-ray Absorptiometry) and venepuncture was conducted. Participants adhered well to the MedDiet intervention, however, there were no significant changes in body composition or inflammatory biomarkers hs-C-reactive protein or hs-interleukin-6 in the MedDiet compared to the low-fat diet group after 6-months. Adiponectin, an anti-inflammatory adipokine, tended to increase in response to the MedDiet (+1.1±4.2ng/mL, *p*=0.11) and decrease in response to the low-fat diet (-0.9±3.3ng/mL, *p*=0.20). In the pooled cohort, participants with greatest improvement in MedDiet adherence score had significantly lower waist circumference and subcutaneous fat levels at 6-months. A clinically significant effect of the MedDiet on inflammation and adiposity in CHD patients may require a larger sample, adjunct exercise intervention and/or caloric restriction.

Keywords: Inflammation; coronary disease; Mediterranean diet; Low-fat diet; C-reactive protein; adiponectin; visceral fat; body composition.

1. Introduction

The Mediterranean diet (MedDiet) pattern has a strong scientific evidence base for reducing risk of coronary heart disease (CHD) and adverse cardiovascular disease (CVD) events in both a primary and secondary prevention setting [1,2]. Of note, most studies investigating the MedDiet have been conducted in Mediterranean countries. There is limited evidence that a similar beneficial effect on CHD risk factors and CVD outcomes will occur in non-Mediterranean populations, which explains the reluctance to recommend and endorse the MedDiet for CHD in the multi-ethnic Australian setting [3]. A low-fat diet was the standard care recommendation for prevention and treatment of CHD in Australia for many years [4]. However, there is a lack of evidence supporting the effect of a low-fat

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diet on cardiovascular events and mortality [2,5,6]. A recent position statement from the National Heart Foundation of Australia promotes a variety of healthy dietary patterns, rather than focusing on isolated nutrients, for cardiovascular health [7].

Atherosclerosis is the underlying pathology responsible for CHD. Derangements in lipid levels, blood pressure and insulin homeostasis each lead to endothelial dysfunction, which plays a pivotal role in initiating the atherosclerotic process [8]. A number of studies have demonstrated that the MedDiet improves traditional CVD risk factors, including improvements in triglycerides and high-density lipoprotein (HDL) cholesterol, blood pressure, glucose metabolism and reduced risk of type 2 diabetes mellitus (T2DM) [9-16]. These studies were conducted in patients at risk of, but without, established CHD. In CHD, especially in those who have suffered acute coronary syndrome (ACS), pharmacotherapy is used to achieve recommended lipid, glucose and blood pressure targets [17], hence the possibility to attain additional impact of diet on these risk factors may not be observed in these patients. In fact, the limited published data on the impact of MedDiet on secondary prevention of ACS demonstrated that the diet appears to be operating independently of traditional CVD risk factors [1].

Atherosclerosis is recognised to be an inflammatory condition, which is related to both the chronic development of plaque and its acute rupture [18]. Recognised biomarkers of chronic low-grade inflammation which are associated with increased risk of CHD include C-reactive protein (CRP) and interleukin-6 (IL-6) [19-23]. In addition, obesity, especially increased visceral fat, is causally linked to chronic low-grade inflammation [24,25]. In an obese state, adipose tissue generates pro-inflammatory adipokines, including IL-6, whereas anti-inflammatory adipokines, including adiponectin, are down-regulated [26]. High serum concentrations of adiponectin are associated with decreased risk of CHD [23,27].

To better understand how dietary interventions moderate CHD risk, it is important to ascertain their effect on inflammation and adiposity in addition to classic cardiometabolic risk markers. Meta-analyses of randomised controlled trials (RCTs) have concluded that intervention with the MedDiet improves inflammatory markers [28,29]. However, a recent systematic review of the literature established that in patients with diagnosed CHD an anti-inflammatory effect of MedDiet was unclear, and there were no studies investigating MedDiet effects on adiponectin [30]. There is evidence to show that the MedDiet leads to modest weight loss [31], and a recent review of intervention trials demonstrated that the MedDiet can reduce central obesity; however, most studies measured waist circumference without distinguishing visceral fat and included patients without CHD [32]. Despite evidence for the protective effect of the MedDiet on weight and adiposity, there remains concern that this type of diet, which is high in healthy dietary fats, causes body weight and fat gain [33]. Therefore, the aim of this study was to determine the effect of *ad libitum* MedDiet versus low-fat diet intervention on cardiometabolic risk markers, including inflammatory markers and adiposity, in Australian patients who have experienced an ACS event.

2. Materials and Methods

2.1 Study Design

The AUStralian MEDiterranean Diet Heart Trial (AUSMED Heart Trial) is a multi-centre, parallel design, randomised controlled trial (RCT) for the secondary prevention of CHD in a multi-ethnic Australian population (Australia and New Zealand Clinical Trials Register: ACTRN12616000156482, http://www.anzctr.org.au/). The broader trial involves 6-month MedDiet versus low-fat diet intervention in patients who have experienced a first ACS event, with 12-month follow up to assess the primary outcome of aggregate secondary cardiovascular events [34]. The present study investigated the effect of the two diets on cardiometabolic risk markers in a pilot cohort of participants after 6-months intervention.

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2.2 Recruitment of CHD Patients

Patients for this pilot study were recruited by trained researchers from two teaching hospitals in Melbourne, Australia from October 2014 to November 2016. Eligible patients were adults with CHD, able to read and write in English and who had experienced ACS defined as at least one of the following: acute myocardial infarction (AMI); angina pectoris with documented coronary artery disease on imaging; coronary artery bypass grafting; or percutaneous coronary intervention. Exclusion criteria included: malignant tumour, symptomatic chronic heart failure (New York Heart Association Functional Classification II, III & IV [35]), chronic inflammatory disease requiring anti-inflammatory or immuno-modulating medications, chronic kidney disease stage 3 or above [36], decompensated liver disease, pregnancy or breastfeeding, or current participation in a lifestyle program (including cardiac rehabilitation), drug or supplement trial. The study was conducted in accordance with the Declaration of Helsinki [37] and the CONSORT guidelines [38]. All procedures involving patients were approved by the Human Research Ethics Committees of the Northern Hospital (HREC/16/Austin/500), St Vincent's Hospital Melbourne (HREC-A; 016/13), and La Trobe University (#FHEC13/159), with written informed consent obtained from all enrolled participants before randomisation.

2.3 Randomisation of Participants and Diet Interventions

Participants attended a pre-baseline appointment where consent was obtained and randomisation was conducted by trained researchers. Enrolled participants were randomly assigned in a 1:1 ratio to the MedDiet group or the low-fat diet group. Randomisation tables were developed by the trial statistician using a computer-generated stratified approach based on sex (male/female), age (<55, 55 to 65 and >65 years) and history of AMI (yes/no). Consultation frequency and data collection time points were consistent across the two groups. Baseline, 3- and 6-month face-to-face appointments were conducted to obtain dietary data and for counselling with the dietitian. Five short phone reviews for follow-up dietary counselling with the dietitian also occurred across the 6-months, at weeks 3, 6, and 9 and months 4 and 5. All participants continued to receive standard medical care provided at their respective hospital or primary care settings and their access to outside health services during the study intervention period was recorded at each appointment.

We have published the detail on the diet interventions elsewhere [39]. For both study groups the dietary advice was tailored to each individual through patient-centred counselling and goal setting with the dietitian [40,41]. An active control group receiving the standard (low-fat) diet recommendations for cardiac patients was used in this study, as standard care received by CHD patients is highly variable. Furthermore, this method reduces expectancy bias (i.e., greater expectation of benefit can lead to more favourable outcomes in a sole intervention group) [42]. Both diets were prescribed *ad libitum* with no specific recommendations on energy restriction.

2.3.1. Mediterranean Diet

The rationale and development of our MedDiet intervention, designed for use in chronic disease intervention trials in the Australian setting [34,43], has been explained and published in detail elsewhere [44]. Briefly, it was designed based on the principles of the traditional Cretan MedDiet [45], including information from the Hellenic dietary guidelines [46] and intervention trials [2,16,47,48]. The diet was modelled via a 2-week meal plan which incorporated key dietary components of a MedDiet and a mix of traditional and modified recipes considered to be realistic options in the multi-ethnic Australian setting. Target macronutrient intakes as contribution to total energy consumption were 42% total fat (of which at least 50% was from monounsaturated fatty acids [MUFA] and 25% from polyunsaturated fatty acids [PUFA]), <10% saturated fatty acids, 15% protein, 35% carbohydrate and ≤5% alcohol. Food group recommendations included: daily intake of extra virgin olive oil (EVOO), wholegrain cereals, vegetables, fruit and nuts; regular intake of fish and seafood, legumes and yoghurt; and limited intake of commercial sweets or pastries and red or processed meat. Poultry, eggs and feta cheese were recommended in moderation. For existing alcohol drinkers, red wine was

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suggested to be consumed in moderation (1-2 standard glasses) with meals. Resources provided to participants [44] included the 2-week model meal plan, a recipe book, The Mediterranean Diet by Itsiopoulos (2013) (ISBN 9781742610825), shopping list, food pyramid, weekly food intake checklist, and label reading information. To facilitate dietary compliance and to encourage intake of staple Mediterranean foods less familiar to this Australian population, a hamper was provided to participants at baseline and 3-months. Each hamper included 6L EVOO (to achieve 60-80mL/day) and 1.2kg nuts (almonds, walnuts and hazelnuts to achieve 30g/day) as well as samples of canned legumes, Greek yoghurt, and tinned tuna and salmon.

2.3.2. Low-fat Diet

Participants in the low-fat diet group were instructed to follow the standard diet recommendations provided to cardiac patients in Australia at the time this study was developed (in 2014). Recommendations from the National Heart Foundation [4] and Australian Dietary Guidelines [49,50] were consulted for design of the low-fat diet. Target macronutrient intakes as contribution to total energy consumption were <30% total fat, <7% saturated fat, <1% trans fat, 45-65% carbohydrate, 15-25% protein and ≤5% alcohol. Food group recommendations included daily intake of grains and cereals (mostly whole grains), vegetables, lean meats and alternatives, fruit, and low-fat dairy foods [49]. A 1-week meal plan was created to model a comparative nutrient profile for this diet and to generate a resource for participants. Resources for label reading, low-fat cooking and recommended daily food group serves also were provided. Participants were provided with a supermarket voucher at each of their three face-to-face appointments to aid compliance and encourage continuation in the trial.

2.4 Study Measures

This study reports on measurements collected at the baseline, 3- and 6-month appointments. Data on medical conditions was collected from medical records and in consultation with hospital staff during the screening process, and via a questionnaire at the pre-baseline appointment. Participants completed a self-report survey (Kucianski et al, 2018, Manuscript under review) prior to their baseline appointment which recorded sociodemographic, lifestyle and clinical characteristics, including medication and supplements use. A modified version of the survey was completed at both 3- and 6-month appointments, which re-assessed lifestyle and clinical characteristics.

2.4.1. Dietary Intake

Our methods for assessing dietary intake have been detailed previously [39]. Briefly, the week prior to each face-to-face appointment the participants completed a 7-day food diary in household measures. The diary included quantity, type, brand and cooking methods for consumed foods with unclear details clarified by the dietitian. All food diaries were entered into FoodWorks (Version 8, Xyris software Australia Pty Ltd) for nutrient and food group intake analyses. Food group serve sizes were based on FoodWorks data [51]. The 14-point Mediterranean Diet Adherence Screener (MEDAS), generated and validated for the PREDIMED study [52], was measured at each appointment for both diet study groups. This paper reports on key dietary intake data at baseline and 6-months only, as detail on the dietary changes, including sustainability data at 12-months, has been reported elsewhere (Mayr et al, 2018, Manuscript under review).

2.4.2. Cardiometabolic Risk Markers

Our methods for assessment of activity levels, anthropometry, body composition, blood pressure and pathology measures have also been described previously [34,53]. Increased physical activity was not a target of this intervention and physical activity guidelines were not discussed by the dietitians. However, physical activity levels were assessed to account for any potential confounding effects of changes in physical activity levels on outcome markers. Participants wore a triaxial Actigraph accelerometer (WGT3X-BT; Actigraph Corp, Florida, United States) for one week

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prior to their appointments. Established criteria [54] were used to determine time spent as min /week in moderate-to-vigorous physical activity (MVPA) or as sedentary time.

Anthropometric measures were performed according to the International Society for the Advancement of Kinanthropometry (ISAK) standards for anthropometric assessment [55]. Body weight was measured to the nearest 0.1 kg using calibrated digital scales (WM203, Wedderburn, Willawong, QLD, Australia) after an 8-h fast. Height was measured to the nearest 0.1 cm, while barefoot, using a wall-mounted stadiometer (SE206, SECA, Seven Hills, NSW, Australia). Waist circumference was measured directly over the skin at the level of the narrowest point between the lower costal (10th rib) and top of the iliac crest. Hip circumference was measured over underwear or thin layer of clothing, at the point of greatest posterior protuberance of the gluteal. Two measures of waist and hip circumference were taken to the nearest 0.1 cm, and the average calculated. If the two measures differed by 2% or more a third measure was taken and the average of the 2 closest values calculated. Waist-hip ratio was calculated by dividing waist circumference into hip circumference.

Whole body composition was measured using a fan beam densitometer Dual-energy X-ray Absorptiometry (DXA) machine (Hologic, Discovery W, USA), with analysis performed using QDR™ (Quantitative Digital Radiography) for Windows. Procedures and positioning of participants on the scanning bed were standardised according to recommendations of the Australian and New Zealand Bone and Mineral Society and manufacturer guidelines. Participants were required to be fasted for at least 8 hours, void their bladder immediately prior to scan, wear light clothing free from metal and remove shoes, jewellery and glasses. Participants were instructed to lie supine on the scanning bed with slight internal rotation of legs from the hip, with arms straightened by the sides and palms flat on the bed or placed against thighs. Regions of interest and total body composition analyses were automatically generated by the software. Measurements obtained from each scan were total body lean and fat mass, total body and regional fat percentage, subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT) areas. Hologic scientists developed their method for measuring VAT from DXA [56], which is highly correlated (r=0.93) and linearly related to VAT measurements by computed tomography [57]. Fat mass index (FMI) was calculated by dividing the total body fat mass (kg) by height (m) squared [58].

Systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate (HR) were measured using an automated blood pressure monitor (OMRON Tp9, IntellisenSense, Australia). Measurements were taken at least at 1 min intervals after the participant had been seated for 5 min. At least two measures were performed and then a third measure if either the SBP or DBP differed by 10%. Hypertension (presence or history of) was classified based on whether the participants were prescribed medication with anti-hypertensive effect (angiotensin converting enzyme [ACE] inhibitor, angiotensin 2 receptor blocker, Beta [β]-blocker or Ca^{2+} channel blocker) and/or mean baseline blood pressure reading of SBP >140 mmHg or DBP >90 mmHg [59].

Fasting blood samples were taken by venepuncture and processed immediately into serum/plasma aliquots (as published in detail elsewhere [60]) which were stored at -80 °C until laboratory assays were conducted. Serum low-density lipoprotein (LDL) cholesterol, HDL cholesterol, triglycerides and high sensitivity (hs)-CRP levels were measured at a commercial laboratory (Dorevitch Pathology Pty Ltd, Heidelberg, Australia). Lipids were measured using an automated blood analyzer (ADVIA 2400 Chemistry System, Siemens, Tarrytown, NY, USA) and hs-CRP by chemical analyser (Cobas Integra 400, Roche, Indianapolis, IN, USA). All other biomarkers were measured by trained personnel at La Trobe University. Briefly, serum hs-IL-6 levels were measured by enzyme-linked immunosorbent assay (ELISA) (Abcam, #ab46042, detection sensitivity <0.81 pg/mL) in duplicate. Serum adiponectin levels were measured by ELISA (Invitrogen, Thermofisher Scientific, #KHP0041, detection sensitivity <100 pg/mL) in duplicate. Fasting serum glucose levels were measured using the enzymatic hexokinase method by a chemical analyser (Indiko, Thermofisher Scientific) in duplicate. Laboratory personnel were provided with deidentified samples and were blinded to participant study group. The presence of metabolic syndrome was calculated using the National Cholesterol Education Program ATP III definition [61]. Diagnosis of T2DM was determined by consulting participant medical history records. All risk markers were

assessed at baseline, 3- and 6-months, except for hs-Il-6 which was measured at baseline and 6-months only.

2.4.2. Statistical Analyses

As this study represented a preliminary analysis in a pilot cohort, a sample size calculation was not performed prior to conducting the measures [62]. All statistical analyses were conducted in SPSS® statistical package version 23 (IBM Corp, released 2015). Statistical significance was set at $p<0.05$. Data are presented as means ± standard deviation (SD) or standard error (SEM), medians (interquartile range [IQR]) or n (%), as appropriate. The Kolmogorov–Smirnov test was applied to assess the normality of continuous variables. According to this, an Independent Student's *t*-test or non-parametric Mann-Whitney U test was used to compare continuous variables. Categorical variables were compared using the *Chi-square* test.

All outcome measures were analysed based on intention-to-treat. Missing data were analysed by bringing baseline or 3-month observations forward, assuming no change. This method was used as a conservative method to analyse all participants regardless of study completion [63]. Cochran's Q test assessed changes in the proportion of participants taking medication and supplement classes from baseline to 3- and 6-months within each study group. Change in dietary intake variables from baseline to 6-months was assessed within diet study groups by Paired Samples *t*-test or Wilcoxon Signed Rank test. Repeated measures between-within ANOVA (analysis of variance) assessed changes in cardiometabolic risk marker variables from baseline to 3- and 6-months. Measures which were non-parametric at least 2 out of 3 time-points were transformed (based on log, square root or inverse) to improve their distribution. The main ANOVA results assessed for effect were (1) group (significant change in one study group compared to the other), (2) time (significant change in pooled study groups), and (3) time*group (interaction effect). Post-hoc tests were performed to determine within-group changes (Paired Samples *t*-test) and between-group differences (Independent Student's *t*-test).

In the pooled cohort, tertiles of change in participant MEDAS scores from baseline to 6-months were created in SPSS. Least-squared means (95% confidence interval [CI]) of cardiometabolic risk markers at 6-months were estimated across the tertiles of MEDAS change. Multi-variable general linear models were used to estimate the differences in adjusted means across tertiles. For hs-CRP, participants with serum levels >10 mg/L were excluded from analyses, as these higher concentrations reflect acute rather than chronic inflammation [64].

3. Results

3.1. Participants

Figure 1 illustrates the randomisation to diet study groups and completion of study appointments. Of the 36 participants randomised to the low-fat diet group, 31 commenced and 29 completed the intervention. Of the 37 participants randomised to the MedDiet group, 35 commenced and 27 completed the intervention. Participants were lost to follow up or discontinued due to medical or family related issues. Two of the nine participant dropouts (one from each of the diet groups) were female. There were no significant differences for sociodemographic or clinical characteristics between those participants that dropped out compared to completers.

Baseline characteristics of participants who started the intervention, between the diet study groups, are reported in Table 1. Briefly, the cohort represented a mostly male, middle to late aged group of which close to half were born outside Australia. Most participants were educated above secondary school level and had received previous lifestyle advice through cardiac rehabilitation, with few current smokers and one third having previously seen a dietitian (individually). Participants had highly variable levels of MVPA and their baseline MedDiet adherence was low (score of 5 out of 14). Most participants had experienced an AMI and undergone percutaneous coronary intervention with a median time since ACS event of <6 months prior. Close to one third had diagnosed T2DM and nearly all had current or previous hypertension. Participants were prescribed multiple medications,

of which anti-platelets and statins were the most common. Close to half the participants were using nutrition supplements, of which vitamin D and omega-3 were the most common. There were no significant differences at baseline between the diet study groups for any of these reported sociodemographic, lifestyle or clinical characteristics.

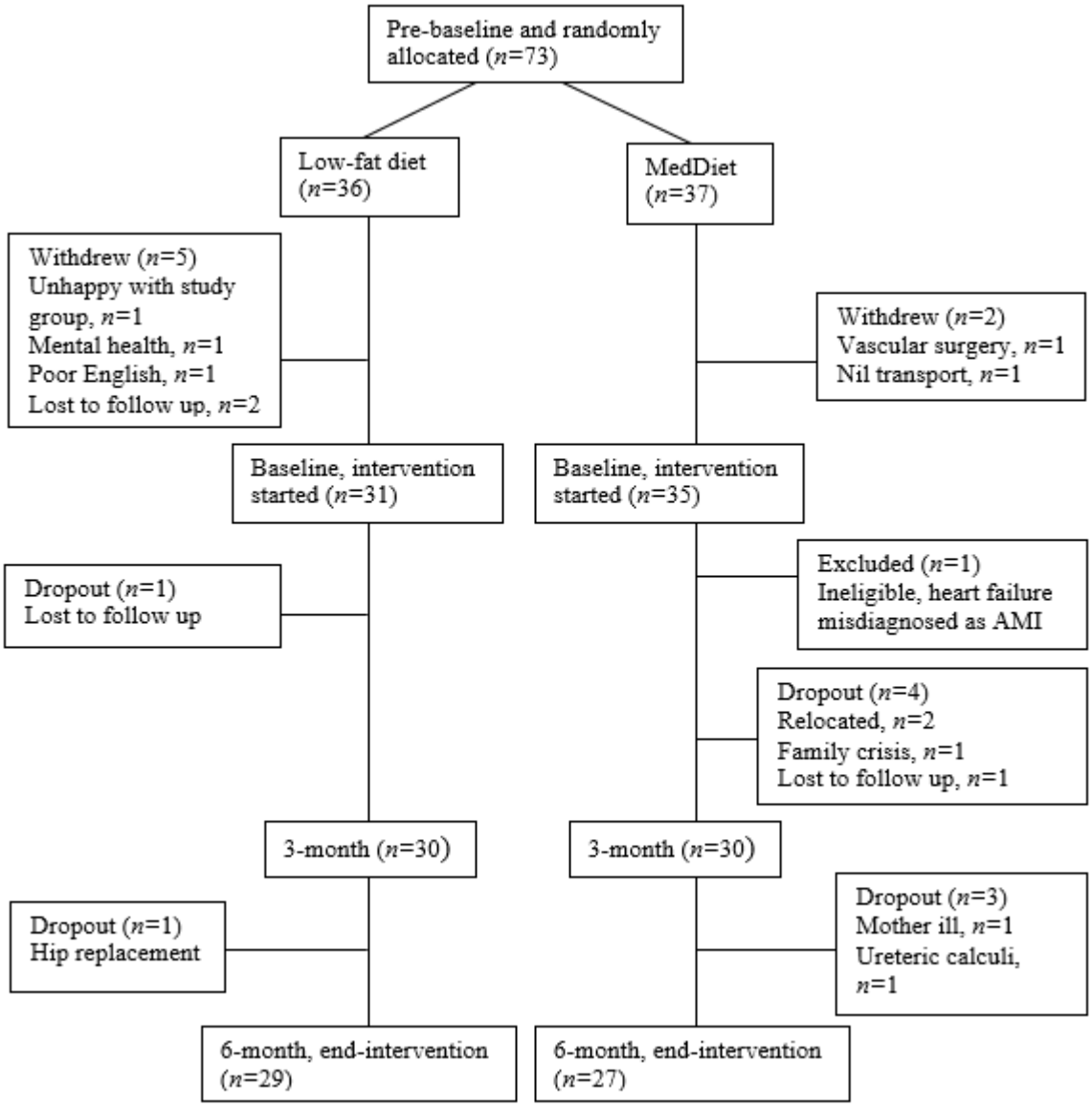

Figure 1. Flow of AUSMED participants through the study, with randomisation to diet study groups, timing of appointments and indication of dropouts. Participants were recruited and completed the intervention between October 2014 to May 2016. AMI, acute myocardial infarction.

Table 1. Participant baseline characteristics in the study groups

Characteristic	Low-fat (*n*=31)	MedDiet (*n*=34)	*p*-value
Sociodemographic			
Male	27 (87.1)	27 (79.4)	0.62
Age (years)	61.8 ± 9.5	61.8 ± 9.2	0.99
Country of birth			
Australia	18 (58.1)	20 (58.8)	1.00
Other	13 (41.9)	14 (41.2)	

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Highest education level			0.63
Primary School	1 (3.2)	3 (9.1)	
Secondary School	7 (22.6)	7 (21.2)	
Trade/ University	23 (74.2)	23 (69.7)	
Lifestyle			
Smoking			
>100 cigarettes in lifetime	18 (58.1)	20 (58.8)	1.00
Current	3 (9.7)	6 (18.2)	0.48
BMI (kg/m^2)	29.1 ± 5.3	30.7 ± 5.0	0.20
Overweight (BMI ≥25 kg/m^2)	25 (80.6)	30 (88.2)	0.50
Sedentary (min /week)	3559 ± 756	3518 ± 735	0.67
MVPA (min /week)†	120.0 (189.5)	153.0 (210.0)	0.35
MEDAS (score out of 14)	4.8 ± 1.8	5.6 ± 2.2	0.12
Cardiac rehabilitation	26 (83.9)	25 (73.5)	0.48
Dietitian (individual consult)	10 (32.3)	11 (32.4)	1.00
Medical History			
Acute coronary syndrome			
Acute myocardial infarction	22 (71.0)	23 (67.6)	0.72
Percutaneous coronary intervention	25 (80.6)	25 (73.5)	0.70
Coronary artery bypass grafting	8 (25.8)	7 (20.6)	0.84
Time since event (months)†	4.5 (6.5)	5.1 (15.2)	0.65
Co-morbidities			
Type 2 diabetes mellitus	9 (29.0)	10 (29.4)	1.00
Metabolic syndrome[a]	11 (35.5)	16 (48.5)	0.42
Hypertension	31 (100)	31 (91.2)	0.24
Depression (diagnosed)	4 (12.9)	6 (17.6)	0.74
Thyroid disorder	2 (6.5)	3 (8.8)	1.00
Medication Use	31 (100)	34 (100)	1.00
Anti-platelet	29 (93.5)	30 (88.2)	0.67
Statin	26 (83.9)	31 (91.2)	0.61
Other lipid-lowering	3 (9.7)	3 (8.8)	1.00
β-blocker	21 (67.7)	24 (70.6)	0.76
ACE inhibitor	15 (48.4)	17 (50.0)	1.00
Angiotensin 2 receptor blocker	6 (19.4)	8 (23.5)	0.92
Ca^{2+} channel blocker	5 (16.1)	4 (11.8)	0.88
Oral hypoglycaemic agent	7 (22.6)	8 (23.5)	1.00
Insulin	2 (6.5)	3 (8.8)	1.00
Anti-reflux	9 (29.0)	9 (26.5)	1.00
Supplement use	13 (41.9)	15 (44.1)	1.00
Omega-3	3 (9.7)	7 (20.6)	0.38
Vitamin D	5 (16.1)	7 (20.6)	0.89
Multivitamin	5 (16.1)	3 (8.8)	0.61
Coenzyme Q10	5 (16.1)	2 (5.9)	0.24
Glucosamine	0 (0.0)	4 (11.8)	0.12
Magnesium	3 (9.7)	3 (8.8)	1.00

Data are N (%), Mean ± SD or Median (IQR)†. BMI, body mass index; MVPA, moderate-to-vigorous physical activity; MEDAS, Mediterranean diet adherence screener; β, Beta; ACE, angiotensin converting enzyme. [a]Unable to calculate presence of metabolic syndrome for one participant due to missing pathology data.

3.2. Attendance at study appointments, other health services and medication/supplement use

There were no significant differences between the groups for frequency of attendance at each of the study appointments and phone call reviews conducted across the diet intervention period (Table S1, Supplementary Materials). The proportion of participants who attended each of the appointments or reviews was 80% or above. The participants reported having accessed a variety of other health services during the intervention period, but there were no significant differences between the study groups (Table S1). There was no change in the proportion of participants taking prescribed medications for most medication types between baseline, 3- and 6-month appointments in either study group (Table S2). The only significant finding was a reduction in the number of participants prescribed β-blockers in the MedDiet group (from 24 to 19 participants) at 3-months and this was maintained at 6-months (p-trend=0.007). High medication compliance self-reported by the participants at baseline remained consistent throughout the study. There were no significant changes within either study group for use of supplements across the intervention period (Table S2).

3.3. Dietary Intake

Daily intake of food group serves, energy and nutrients are shown in Table 2. In the MedDiet group, in line with recommendations, consumption of olive oil, fruit, yoghurt, nuts, legumes and seafood significantly increased, whereas red and processed meats decreased after 6-months. In the MedDiet group intake of energy from total fat, MUFA and PUFA significantly increased and energy from protein, carbohydrates and saturated fats decreased. The MedDiet group also significantly increased intake of fibre and vitamin E and decreased intake of sodium. There were no significant changes for intake of any of the reported food groups or nutrients in the low-fat diet group. Total energy intake tended to increase in the MedDiet group compared to a decrease in the low-fat diet group.

Adherence to the MedDiet is also reported in Table 2. The low-fat diet group significantly improved MEDAS score by 1.2 points (p=0.01), whereas the MedDiet group significantly improved MEDAS score by 4.8 points (p<0.001). This represented a significantly greater improvement in MEDAS score in the MedDiet compared to low-fat diet participants (p<0.001).

Table 2. Dietary intake at baseline and 6-months in the study groups

Intake variable	Low-fat diet (n=31)		MedDiet (n= 33)[a]	
	Baseline	**6-Month**	**Baseline**	**6-Month**
Food group serves /day				
Olive oil (tsp)	0.6 ± 1.1	0.5 ± 0.8	1.4 ± 1.9	7.1 ± 5.1*
Wholegrains	2.8 ± 2.5	2.6 ± 1.8	1.9 ± 1.2	2.2 ± 1.5
Vegetables	3.3 ± 1.2	3.6 ± 1.9	4.0 ± 2.2	4.2 ± 2.1
Fruit	2.0 ± 1.3	1.7 ± 1.1	1.1 ± 1.0	1.5 ± 1.1*
Food group serves /week				
Yoghurt	0.8 ± 1.4	1.4 ± 1.9	1.2 ± 1.7	2.2 ± 2.1*
Cheese	3.0 ± 4.0	2.9 ± 2.9	2.9 ± 2.2	2.6 ± 2.2
Nuts	4.4 ± 6.1	3.6 ± 5.9	2.8 ± 4.0	5.2 ± 4.9*
Legumes	1.8 ± 5.5	1.3 ± 2.3	0.8 ± 1.3	2.6 ± 2.9*
Seafood	3.1 ± 2.9	3.1 ± 2.3	4.7 ± 4.4	6.2 ± 4.8*
Red meat	4.8 ± 4.8	4.3 ± 3.6	5.2 ± 5.6	2.6 ± 1.8*
Processed meat	1.3 ± 1.6	1.5 ± 1.4	1.8 ± 1.3	0.6 ± 0.8*
Wine (100mL)	1.3 ± 3.9	0.8 ± 2.5	3.4 ± 6.9	3.6 ± 6.0
Nutrients /day				

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Energy (kJ)	8049 ± 2195	7531 ± 2265	8156 ± 1798	8427 ± 1929
Protein (%E)	20.5 ± 3.6	21.8 ± 5.8	21.5 ± 4.4	19.4 ± 4.2*
Carbohydrate (%E)	43.1 ± 7.9	42.5 ± 7.1	37.9 ± 6.5	34.8 ± 7.2*
Total fat (%E)	31.0 ± 7.7	30.3 ± 7.2	34.1 ± 6.2	38.7 ± 7.9*
Saturated fat (%E)	10.1 ± 3.4	10.3 ± 3.5	11.5 ± 3.2	9.5 ± 2.4*
MUFA (%E)	14.0 ± 5.0	12.8 ± 3.8	15.2 ± 3.8	20.7 ± 6.2*
PUFA (%E)	5.9 ± 2.6	6.1 ± 2.9	6.2 ± 2.2	7.6 ± 2.9*
LCN3FA (g)	0.56 ± 0.55	0.46 ± 0.49	0.57 ± 0.57	0.74 ± 0.81
Fibre (g)	28.9 ± 11.7	27.6 ± 8.9	24.8 ± 10.6	29.6 ±11.3*
Vitamin C (mg)	137.4 ± 87.9	111.2 ± 70.2	106.7 ± 61.2	116.1 ± 78.2
Vitamin E (mg)	12.3 ± 7.3	11.1 ± 5.8	12.0 ± 4.8	19.3 ± 6.8*
Folate (μg)	657.9 ± 183.5	640.6 ± 212.6	628.0 ± 270.9	594.5 ± 218.6
Sodium (mg)	2182 ± 797	2101 ± 782	2424 ± 839	1970 ±584*
Potassium (mg)	3103 ± 894	3097 ± 859	3224 ± 999	3367 ± 910
Magnesium (mg)	356.7 ± 128.0	335.5 ± 112.3	346.7 ±103.4	376.1 ± 94.1
MedDiet adherence			(n=34)[a]	
MEDAS (score /14)	4.8 ± 1.8	6.0 ± 2.0*	5.6 ± 2.2	10.4 ± 2.3*

MedDiet, Mediterranean diet; %E, percentage contribution to total energy intake; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; LCN3FA, long chain omega-3 fatty acids; MEDAS, Mediterranean Diet Adherence Screener. [a]One participant who dropped out had MEDAS data but no usable food diary data at baseline. *Significant change within group, p<0.05, Paired t-test or Mann Whitney U test.

3.4. Activity Levels

Physical activity was not a target of the interventions; however, activity levels were measured to account for potential confounding on outcome measures. There were no significant changes in time spent as sedentary min /week between baseline and 3-months or 6-months in the MedDiet or low-fat diet groups (Table 3). For MVPA min /week there was a significant reduction over time in the pooled groups (p=0.02). The only significant within-group difference was a reduction between 3- and 6-months in the MedDiet group (p=0.006).

3.5. Anthropometry and Body Composition

There were no significant between-group changes (main effect for group, p>0.05) for any of the reported anthropometric and body composition measures (Table 3). There were also no significant within-group changes for weight, BMI, waist circumference, or waist-hip ratio. With regards to waist circumference, there was a trend for an overall mean reduction over time in the pooled study groups (-1.1 cm in the MedDiet group and -0.4 cm in the low-fat diet group after 6-months, main effect for time, p=0.07). With regards to body composition measures, total fat %, trunk fat % and leg fat % each decreased significantly over time in the pooled study groups (main effects for time, p=0.01, p=0.04 and p=0.003, respectively). However, the only significant within-group changes for these fat % outcomes were a reduction between baseline and 3-months in the MedDiet group. There was also a decrease in each of total fat mass, FMI and arm fat % between baseline and 3-months within the MedDiet group only. There was a trend for a greater reduction in SAT area in the MedDiet group (-12.1 cm^2), compared to the reduction in the low-fat diet group (-8.8 cm^2) after 6-months (main effect for group, p=0.07). There was a significantly higher SAT area in the MedDiet group compared to low-fat diet group participants at baseline, however, the results for change in SAT area did not differ when this was controlled for. From baseline to 6-months there was no significant change in VAT area

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367 in either the low-fat or MedDiet groups; however, within the low-fat diet group there was a (non-
368 significant) reduction in VAT area at 3-months and then a significant increase from 3- to 6-months
369 (the participants returned to the same level as at the start of the study).

370 **Table 3.** Activity levels and anthropometric, body composition, haemodynamic and pathology markers across intervention time points in the study groups

Marker	Low-fat diet (*n*=31)			MedDiet (*n*=34)			*p*-value		
	Baseline	3-month	6-month	Baseline	3-month	6-month	Group	Time	Time* group
Activity levels									
Sedentary (min/week)	3559 ± 756	3534 ± 694	3431 ± 708	3518 ± 735	3455 ± 975	3380 ± 795	0.60	0.50	0.86
MVPA (min/week)	148 ± 144	152 ± 135	135 ± 122	186 ± 164	197 ± 163	144 ± 112[c]	0.30	0.02*	0.59
Anthropometry									
Weight (kg)†	85.4 ± 19.6	84.9 ± 19.3	85.0 ± 18.8	88.8 ± 17.9	88.6 ± 18.2	88.8 ± 17.7	0.34	0.48	0.65
BMI (kg/m^2)	29.1 ± 5.3	28.9 ± 5.4	29.0 ± 5.4	30.7 ± 5.2	30.7 ± 4.9	30.7 ± 5.1	0.18	0.42	0.67
Waist circumference (cm)	102.2 ± 15.1	101.6 ± 13.9	101.8 ± 14.5	104.2 ± 13.9	103.3 ± 13.3	103.1 ± 13.7	0.63	0.07	0.59
Waist-hip ratio	0.978 ± 0.09	0.972 ± 0.09	0.975 ± 0.08	0.974 ± 0.08	0.969 ± 0.08	0.964 ± 0.08	0.78	0.14	0.44
Body composition									
Total lean (kg)	55.7 ± 11.4	55.8 ± 11.3	55.9 ± 11.4	56.0 ± 10.7	56.4 ± 10.5	56.6 ± 10.7	0.85	0.10	0.60
Total fat (kg)	27.9 ± 10.3	27.4 ± 10.2	27.4 ± 9.9	30.9 ± 9.5	30.3 ± 9.4[a]	30.4 ± 9.5	0.23	0.05	0.83
FMI (kg/m^2)	9.50 ± 3.1	9.36 ± 3.1	9.48 ± 3.3	10.79 ± 3.3	10.59 ± 3.3[a]	10.63 ± 3.4	0.13	0.13	0.65
Total fat %	32.8 ± 6.3	32.4 ± 6.5	32.4 ± 6.7	35.2 ± 6.8	34.6 ± 7.2[a]	34.6 ± 7.1	0.18	0.01*	0.79
Trunk fat %	35.1 ± 6.8	34.6 ± 7.1	34.7 ± 7.4	37.8 ± 6.8	37.2 ± 7.2[a]	37.2 ± 7.3	0.14	0.04*	0.82
Arms fat %	32.8 ± 8.3	32.3 ± 8.2	32.3 ± 8.9	36.1 ± 9.1	35.4 ± 9.7[a]	35.7 ± 9.5	0.15	0.06	0.76
Legs fat %	30.6 ± 7.3	30.2 ± 7.3	30.2 ± 7.4	32.6 ± 8.4	31.8 ± 8.4[a]	31.9 ± 8.5[b]	0.38	0.003*	0.67
VAT area (cm^2)	191.6 ± 82.9	179.7 ± 67.7	189.9 ± 76.1[c]	200.8 ± 76.5	198.4 ± 74.6	200.9 ± 77.5	0.49	0.07	0.30
SAT area (cm^2)	298.3 ± 105.9[d]	295.1 ± 125.3	289.5 ± 114.1	354.4 ± 123.4	341.3 ± 123.1[a]	342.3 ± 124.6	0.07	0.35	0.08
Haemodynamic									
SBP (mmHg)	140.3 ± 18.9	139.1 ± 15.5	139.3 ± 15.0	133.7 ± 16.6	133.5 ± 15.5	132.2 ± 15.2	0.09	0.69	0.84
DBP (mmHg)	83.0 ± 7.9	82.8 ± 8.6	82.5 ± 8.2	81.3 ± 9.2	81.2 ± 9.8	81.0 ± 10.0	0.42	0.91	0.99
HR (bpm)	66.8 ± 10.0	65.3 ± 10.6	65.6 ± 10.6	68.7 ± 11.6	63.7 ± 13.0[a]	66.5 ± 11.3[c]	0.78	0.008*	0.16
Pathology									

LDL (mmol/L)†	1.72 ± 0.84[d]	1.83 ± 0.78[a]	1.94 ± 0.94[b]	1.97 ± 0.59	1.96 ± 0.69	2.01 ± 0.72	0.18	0.16	0.11
HDL (mmol/L)†	1.21 ± 0.31	1.25 ± 0.28	1.26 ± 0.30	1.20 ± 0.29	1.24 ± 0.36	1.24 ±0.30	0.77	0.05	0.97
Non-HDL (mmol/L)	2.32 ± 0.90	2.35 ± 0.90	2.49 ± 1.00	2.57 ± 0.67	2.53 ± 0.79	2.65 ± 0.87	0.33	0.10	0.79
Triglycerides(mmol/L)†	1.31 ± 0.63	1.29 ± 0.69	1.27 ±0.74[d]	1.60 ± 0.82	1.54 ± 0.81	1.62 ± 0.82	0.08	0.57	0.36
Glucose (mmol/L)	5.28 ± 1.33	5.41 ± 1.38	5.14 ± 1.08	5.75 ± 1.52	5.73 ± 1.51	5.69 ± 1.73	0.19	0.41	0.57
No T2DM (*n*=44)	4.90 ± 0.83	4.90 ± 0.76	4.72 ± 0.62	4.99 ± 0.61	5.00 ± 0.66	4.95 ± 0.48	0.45	0.25	0.63
Yes T2DM (*n*=19)‡	6.19 ± 1.85	6.62 ± 1.76	6.09 ± 1.35	7.51 ± 1.55	7.40 ± 1.97	7.39 ± 2.33	0.12	0.78	0.72
hs-CRP (mg/L)†**	1.73 ± 2.01	2.11 ± 2.34[d]	1.57 ± 1.65	1.74 ± 2.03	1.23 ± 1.54	1.53 ± 1.74	0.72	0.12	0.25
hs-IL-6 (pg/mL)†	2.06 ± 1.51	-	2.03 ± 1.57	2.63 ± 3.18	-	2.25 ± 2.40	0.59	0.49	0.43

Values are Mean ± SD. One low-fat diet participant did not consent to DXA scan and was excluded from body composition analyses. One MedDiet participant who dropped out and had haemolysed blood sample at baseline was excluded from pathology marker analyses. MedDiet, Mediterranean diet; MVPA; moderate to vigorous physical activity; BMI, body mass index; FMI, fat mass index; VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue; SBP; systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; LDL, low-density lipoprotein; HDL, high-density lipoprotein; T2DM, type 2 diabetes mellitus; hs-CRP, high sensitivity C-reactive protein; hs-IL-6, high sensitivity interleukin-6. †Non-parametric, analyses based on transformed variable. ‡One participant with T2DM had a major increase in insulin dosage and was excluded from analyses. **Two participants excluded for value >10 mg/L. (-)Samples were collected but not measured at 3-months. Significant, $p<0.05$, for: *Main effect of group, time or time x group interaction; [a]difference between baseline and 3-months for that group; [b]difference between baseline and 6-months for that group; [c]difference between 3-months and 6-months for that group; and [d]difference between study groups for that time point.

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3.6. Haemodynamic Measures and Pathology Markers

There were no significant between-group changes (main effect for group, $p>0.05$) for any of the reported haemodynamic or pathology markers (Table 3). There were no significant changes within or between groups for SBP or DBP. There was a significant reduction after 6-months in resting HR for the pooled study groups (-1.2 bpm in the low-fat diet group and -2.2 bpm in the MedDiet group, main effect for time, $p=0.008$). The only significant within-group reduction for HR occurred between baseline and 3-months in the MedDiet group. With regards to lipids, the only significant within-group finding was an increase in LDL cholesterol between baseline and both 3- and 6-months (+0.22 mmol/L in total, $p=0.02$) in the low-fat diet group. There were no significant changes within either study group for triglycerides or fasting glucose levels (also assessed separately for T2DM status).

For hs-CRP there was a trend for reduction in the MedDiet group and increase in the low-fat diet group between baseline and 3-months, which resulted in a significantly higher serum hs-CRP in the low-fat diet group at 3-months (2.1 ± 2.3 vs. 1.2 ± 1.5 mg/L, $p=0.04$). This between-group difference was not present at 6-months. From baseline to 6-months there was no significant change in the pooled cohort or within groups for hs-IL-6. Mean serum adiponectin levels at baseline, 3- and 6-months are presented in Figure 2. There was no significant difference between the low-fat diet and MedDiet groups in adiponectin level at baseline (8.49 ± 4.21 vs. 8.28 ± 3.89 ng/mL, $p=0.84$, respectively). Within the low-fat diet group there was a non-significant trend for mean reduction in adiponectin between baseline and 3-months (-0.23 ± 3.7 ng/mL, $p=0.72$) and 6-months (-0.89 ± 3.3ng/mL, $p=0.20$). Conversely, within the MedDiet group there was a non-significant trend for mean increase in adiponectin between baseline and 3-months (+0.54 ± 3.3 ng/mL, $p=0.39$) and 6-months (+1.08 ± 4.2ng/mL, $p=0.11$). There was no significant main effect on adiponectin for group, time or time*group ($p=0.63$, 0.64 and 0.35, respectively).

Data from this interim analysis on the 6-month between-within group changes for adiponectin were used to perform an *a-priori* sample size calculation in statistical software program G*Power 3.0.10 [65]. Based on the study group effect size (partial eta^2) of 0.063, and a correlation value between adiponectin levels at baseline and 6-months of r=0.689 at 80% power and $\alpha<0.05$, a sample size of 252 participants would be required to detect a significant effect of the MedDiet compared to the low-fat diet.

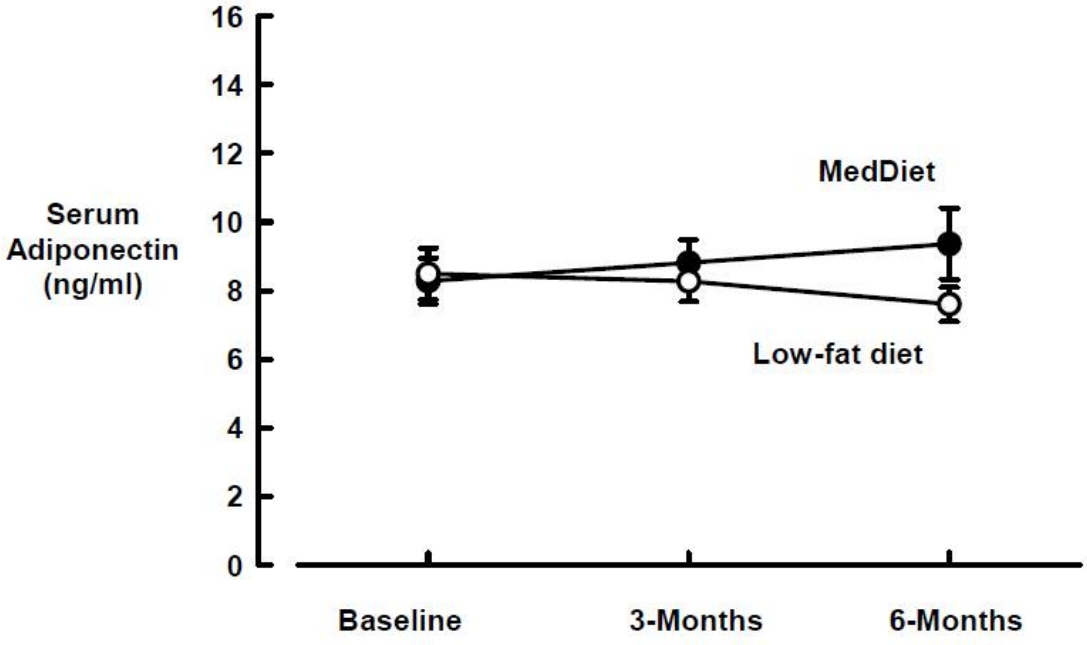

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Figure 2. Effect of 6-month Low-fat diet (*n*=31) and Mediterranean Diet (MedDiet) (*n*=33) on serum adiponectin levels in AUSMED pilot participants. One of the MedDiet participants had a haemolysed blood sample at baseline and was excluded from this dataset. Data are mean ± SEM (standard error).

3.7. Association Between Mediterranean Diet Adherence and Risk Markers

Participants were categorised into tertiles of change in MEDAS score from baseline to 6-months. This resulted in tertile 1 (T1) of -2 to +1, tertile 2 (T2) of +2 to 5, and tertile 3 (T3) of +6 to 9. As expected, in T3, with the largest 6-month improvement in MedDiet adherence, 93% of participants were from the MedDiet group. In T2 and T1 the proportion of participants in the MedDiet group was 56% and 22%, respectively. Mean (95% CI) levels for cardiometabolic risk markers at 6-months, adjusted for baseline value, sex, age, T2DM, time since coronary event and change in MVPA, are presented in Table S3. For each of the reported anthropometric, body composition and hemodynamic measures the mean value decreased across tertiles from T1 to T3 (from lowest to greatest MEDAS score improvement), except for VAT area, which had a higher mean value in T2, followed by T1 and then T3. Compared to T1, T3 participants had a significantly lower mean waist circumference (-2.81 cm, p=0.01), waist-hip ratio (-0.022, p=0.047) and SAT area (- 27.4 cm^2, p=0.04), and a trend for higher fasting glucose level (+0.41 mmol/L, p=0.11). Mean levels of other pathology markers did not demonstrate any consistent trends across tertiles. For adiponectin, the mean value increased slightly across tertiles from lowest to greatest MEDAS score improvement (+0.68 ng/mL from T1 to T3, p=0.50).

4. Discussion

The primary aim of this study was to determine the effect of a 6-month intervention with *ad libitum* MedDiet versus low-fat diet on inflammation and adiposity in a pilot cohort of patients (*n*=65) with CHD. The results demonstrated that despite significantly improved adherence to the Mediterranean dietary pattern, there was no significant effect of the MedDiet on inflammatory markers hs-CRP, hs-IL-6, the anti-inflammatory adipokine adiponectin, body composition, lipids, glucose or blood pressure compared with the low-fat diet. Across tertiles of increasing improvement in MedDiet adherence score in the pooled study cohort at 6-months, a significantly lower waist circumference and SAT area, but not VAT area, was observed.

The MedDiet is world renowned as a healthy dietary pattern which has strong evidence for prevention of CHD [66]. In part, the mechanism proposed for the cardioprotective effect of the MedDiet is its anti-inflammatory properties. Chronic low-grade inflammation is well recognised in the pathogenesis of atherosclerosis and is inversely associated with adherence to the MedDiet [67]. A meta-analysis of RCTs demonstrated that intervention (of 12 weeks to 4 years duration) with a MedDiet significantly reduced levels of circulating pro-inflammatory hs-CRP (14 studies) and IL-6 (6 studies) and increased the anti-inflammatory adipokine adiponectin (2 studies) compared to control diets [29]. The studies included in these meta-analyses were mostly conducted in patients free from CVD.

A recent meta-analysis which explored the effect of the MedDiet on inflammation in studies only recruiting patients with CHD or prior ACS, found that there was no significant effect of Mediterranean-type diets compared to low-fat diets on CRP [30]. In each of the four studies included there was a mean reduction in hs-CRP with MedDiet, however, the only study which produced a significant reduction had the largest sample size (close to 1000 participants [68], compared to 40 to 100 participants in the other studies [69-71]). The review also identified that one of these (low-quality) intervention studies found no effect of a Mediterranean-type diet on IL-6 [69]. The lack of significant effect of the MedDiet on inflammation in this present cohort of patients with CHD is consistent with these previous findings. Current treatment regimens for patients who have experienced ACS include intensive pharmacotherapy [17], which was supported in the clinical description of this cohort. Of relevance, both aspirin and statin medications have pleiotropic anti-inflammatory effects, which have

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been proposed to add to the impact of these drugs on reducing secondary ACS events. For hs-CRP, 80% of this AUSMED cohort were within the normal range (<3 mg/L) [64], with a mean level of 1.7 mg/L at baseline. There may be limited scope for improvement in hs-CRP in the secondary prevention setting and a larger sample size would be required to demonstrate a significant effect of the MedDiet.

This is the first study to examine the effect of MedDiet on the anti-inflammatory marker adiponectin in only patients with diagnosed CHD. Although no significant change was detected in this pilot cohort, we observed an interesting trend for increased serum levels of adiponectin with the MedDiet compared to the low-fat diet. Adiponectin has been reported in previous MedDiet intervention studies that have been conducted in different subject groups. In a study of pre-menopausal obese women adiponectin increased with a calorie-restricted MedDiet compared to general diet/exercise advice [72]. A sub-study of the PREDIMED trial in patients with T2DM also demonstrated an increase in plasma adiponectin, but this increase occurred with all three (Mediterranean + EVOO, Mediterranean + Nuts and low-fat) diet interventions; mean weight loss was significant but less than 1kg in each group [12]. It was also found that a MedDiet in the absence of weight loss can significantly reduce inflammation (composite score of CRP, IL-6 and TNF-α) [73] but not levels of adiponectin [74]. The DIRECT study, which included a MedDiet intervention with 6-month weight loss phase followed by an 18-month weight maintenance phase, demonstrated a continued significant increase in adiponectin for the duration of the trial [75]. Most of these findings suggest that a significant increase in adiponectin with MedDiet is dependent on concomitant weight loss (at least initially), which helps to explain the lack of significant effect on adiponectin in the current study with an *ad libitum* approach and no change in weight. Diet composition is, however, important; the present study observed a trend for reduced adiponectin with the low-fat diet, and a RCT in recently diagnosed T2DM patients demonstrated that despite participants in both MedDiet and low-fat diet arms significantly reducing body weight, only the MedDiet resulted in a significant increase in adiponectin [76].

In this pilot AUSMED cohort, we previously demonstrated that the MedDiet group significantly improved the anti-inflammatory potential of their diet compared to the low-fat diet group, as measured by the dietary inflammatory index (DII) [39]. Hence, it was unexpected that this change in DII did not translate into a more meaningful effect on the inflammatory biomarkers reported in the present analysis. We also found that a reduction in DII (towards a more anti-inflammatory diet score) was significantly associated with lowered levels of hs-IL-6 at 6-months [53]. This differs to the current results that an increase in MEDAS score was not significantly associated with levels of inflammatory markers at 6-months. The DII was formulated based on findings evident in the literature relating diet to inflammatory cytokine signalling pathways and incorporates 45 nutrient/food intake parameters, including flavonoids, herbs and spices [77]. This tool used to categorise individuals' diets on a continuum from maximally anti-inflammatory to maximally pro-inflammatory is fundamentally different to the MEDAS which assesses a pattern of food intake that is associated with the MedDiet, a culinary tradition, and has a finite set of 14 food-based components. There are also parameters in the DII which contribute high anti-inflammatory effect scores, such as green/black tea, saffron, eugenol and turmeric, which are generally not components of the traditional MedDiet.

Chronic low-grade inflammation is more strongly linked to body composition (i.e. higher fat mass) than body weight (which can be confounded by lean tissue) [26]. Our 6-month *ad libitum* MedDiet had no significant effect on weight or body composition measures compared to the low-fat diet. However, within the MedDiet group there was a small but significant reduction in total and regional (trunk, arms and legs) fat % after 3-months, which was generally sustained at 6-months. It is unlikely that engagement in exercise confounded this change in body composition as MVPA levels decreased in the MedDiet participants. In fact, the reduction in exercise observed in MedDiet participants may have reduced the magnitude of the effect of the diet on these outcomes as there is a well-established protective effect of physical activity on body weight and composition [78-80]. The maintenance of weight and small reduction in body fat in the MedDiet group occurred despite the tendency of the group to increase total energy intake. These findings assist to discount the continued belief that the high healthy fat MedDiet is associated with weight and fat gain [33]. These favourable

effects on body composition despite a higher energy intake could be related to the high content of unsaturated fats, particularly MUFA and omega-3 PUFA, in the MedDiet which have been shown to be associated with increased lipid oxidation and thermic effect [81,82]. Furthermore, in a cohort of Australian patients with T2DM (*n*=27) a 12-week *ad libitum* MedDiet intervention resulted in a small reduction in body weight, despite significantly increased energy and MUFA intake [16].

The present study also demonstrated a trend for reduction in waist circumference (-1.1 cm) with the MedDiet, and participants who had greatest improvement in MEDAS score had a significantly lower waist circumference at 6-months. A recent systematic literature review [32] found 18 previous trials which tested the effect of the MedDiet on central obesity, with the majority (16) measuring waist circumference. Twelve of these studies demonstrated a significant reduction in waist circumference (between -10.2 to -0.41 cm) with MedDiet intervention and less than half of those reported that energy restriction was used. One study was conducted in patients with CHD (n=29) and found a significant reduction (-3cm) in waist circumference after 6-week intervention with a Mediterranean-style diet [83]. At the end of the intervention participants had reduced their intake of total fat (to 25% of total energy), which suggests a traditional MedDiet was not recommended and/or achieved. The lower waist circumference observed in participants with greatest MedDiet adherence in this study was associated with a reduction in SAT area, but not VAT area. Two previous studies [84,85] reported a significant reduction in markers of VAT (measured by bioelectrical impedance analysis or ultrasound) following MedDiet intervention. One of these studies also demonstrated that MedDiet intervention did not significantly impact subcutaneous fat [84]. Both of these previous interventions employed energy restriction, which may explain why no reduction in VAT was observed in the current study of an *ad libitum* diet. The reduction in subcutaneous fat in the present study is contradictory to previous findings that intake of MUFA favours deposition as subcutaneous fat [86]. The lack of improvement in VAT with our MedDiet intervention assists to explain the lack of significant effect on inflammatory markers.

The present study demonstrated no significant effect of the MedDiet on LDL cholesterol, triglycerides, blood pressure or glucose, compared to the low-fat diet. These results were generally not unexpected considering that the majority of participants were prescribed statins or other lipid-lowering therapy as well as anti-hypertensives, and nearly all participants with T2DM were taking hypoglycaemic agents. Interestingly, the low-fat diet group significantly increased LDL cholesterol levels after 6-months. This contradicts the premise of the low-fat diet, which was designed to lower LDL cholesterol levels. This finding may be reflective of the lack of improvement in adherence to the low-fat diet principles seen in that group, and their slight increase in saturated fat intake. Additionally, of interest, a significant number (15%) of MedDiet participants stopped taking β-blocker medication during the trial. A potential reduction in need for this medication with the MedDiet is a promising finding as β-blockers have a range of short and long-term side effects [59]. The effect of the MedDiet on medication use will be investigated further in the broader AUSMED Heart Trial.

Our study had a number of strengths. The MedDiet intervention was based on traditional principles of the diet and the control diet was based on Australian nutrition recommendations. The intensity of the dietary counselling was the same in both groups to control for this effect. In both study groups the focus of the intervention was dietary improvement only and the approach was *ad libitum* in order to isolate the effect of diet rather than changes in weight loss or physical activity. We also demonstrated that there were no significant differences in access to other health services or changes in types of medication or supplements taken between the groups, except for a reduction in use of β-blockers in the MedDiet group. Finally, intention-to-treat analyses were performed which meant that dropouts were taken into account in all analyses.

This study was however limited by the small size of a pilot cohort of AUSMED participants, and hence was underpowered. Based on the results in these patients, the reverse power calculation which was performed for adiponectin estimated that a sample size close to four-fold of the current sample would be required to detect a significant effect of the MedDiet compared to low-fat diet in a CHD patient setting. This result will inform future analyses in the broader trial. The patients recruited in

this study represent a lower proportion of females and are potentially more health conscious/motivated than ACS patients in the broader Australian population [87], which may impact the generalisability of the results. A more representative sample needs to be achieved in the broader trial. Nonetheless, the results of this study may be generalisable to other non-Mediterranean, multicultural populations.

5. Conclusions

In a small cohort of patients with CHD a 6-month *ad libitum* MedDiet intervention did not significantly improve inflammatory markers or body composition compared to a low-fat diet. A lack of significant change in weight and a small decline in waist circumference and body fat assists to discount the continued misconception that a diet high in healthy fats, such is the MedDiet, leads to weight or fat gain. Greater improvement in MedDiet adherence was associated with lower waist circumference, however, this was associated with lower SAT and not VAT, which was unexpected. Future studies are needed in larger cohorts. Nonetheless, in CHD patients taking intensive medications, significant clinical effects of the MedDiet on inflammation and adiposity may require adjunct exercise intervention and/or caloric restriction.

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1, Table S1: Frequency of attendance for study appointments and phone reviews and access to other health services during the intervention period in the total cohort and within study groups, Table S2: Proportion of participants taking prescribed medications and supplements across intervention time points in the study groups, Table S3: Adjusted means of cardiometabolic risk markers at 6-months by tertiles of 6-month change in MEDAS score.

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References

1. de Lorgeril, M.; Salen, P.; Martin, J.-L.; Monjaud, I.; Delaye, J.; Mamelle, N. Mediterranean diet, traditional risk factors, and the rate of cardiovascular complications after myocardial infarction. *Circulation* **1999**, *99*, 779-785.
2. Estruch, R.; Ros, E.; Salas-Salvadó, J.; Covas, M.-I.; Corella, D.; Arós, F.; Gómez-Gracia, E.; Ruiz-Gutiérrez, V.; Fiol, M.; Lapetra, J. Primary prevention of cardiovascular disease with a mediterranean diet supplemented with extra-virgin olive oil or nuts. *N. Engl. J. Med.* **2018**, DOI: 10.1056/NEJMoa1800389.
3. Collins, C.; Burrows, T.; Rollo, M. Dietary patterns and cardiovascular disease outcomes: An evidence check rapid review brokered by the Sax institute (www.Saxinstitute.Org.Au) for the National Heart Foundation of Australia. Available online:Https://www.Saxinstitute.Org.Au/publications/evidence-check-library/dietary-patterns-cardiovascular-disease-outcomes/ (accessed on 05 june 2017). 2017.
4. NHF. Reducing risk in heart disease: An expert guide to clinical practice for secondary prevention of coronary heart disease. National Heart Foundation of Australia and the Cardiac Society of Australia and New Zealand: Melbourne, 2012.
5. Howard, B.V.; Van Horn, L.; Hsia, J.; Manson, J.E.; Stefanick, M.L.; Wassertheil-Smoller, S.; Kuller, L.H.; LaCroix, A.Z.; Langer, R.D.; Lasser, N.L. Low-fat dietary pattern and risk of cardiovascular disease: The women's health initiative randomized controlled dietary modification trial. *JAMA* **2006**, *295*, 655-666.
6. Prentice, R.L.; Aragaki, A.K.; Van Horn, L.; Thomson, C.A.; Beresford, S.A.; Robinson, J.; Snetselaar, L.; Anderson, G.L.; Manson, J.E.; Allison, M.A. Low-fat dietary pattern and cardiovascular disease: Results from the women's health initiative randomized controlled trial. *Am. J. Clin. Nutr.* **2017**, *106*, 35-43.
7. NHF. Eating for heart health position statement. National Heart Foundation of Australia: Melbourne, 2017.
8. Kinlay, S.; Libby, P.; Ganz, P. Endothelial function and coronary artery disease. *Curr. Opin. Lipidol.* **2001**, *12*, 383-389.
9. Hernaez, A.; Castaner, O.; Elosua, R.; Pinto, X.; Estruch, R.; Salas-Salvado, J.; Corella, D.; Aros, F.; Serra-Majem, L.; Fiol, M., *et al.* Mediterranean diet improves high-density lipoprotein function in high-cardiovascular-risk individuals: A randomized controlled trial. *Circulation* **2017**, *135*, 633-643.
10. Davis, C.R.; Bryan, J.; Hodgson, J.M.; Woodman, R.; Murphy, K.J. A mediterranean diet reduces f2-isoprostanes and triglycerides among older australian men and women after 6 months. *J. Nutr.* **2017**, *147*, 1348-1355.
11. Estruch, R.; Martínez-González, M.A.n.; Corella, D.; Salas-Salvadó, J.; Ruiz-Gutiérrez, V.; Covas, M.a.I.; Fiol, M.; Gómez-Gracia, E.; López-Sabater, M.C.; Vinyoles, E., *et al.* Effects of a mediterranean-style diet on cardiovascular risk factors: A randomized trial. *Ann. Intern. Med.* **2006**, *145*, 1-11.
12. Lasa, A.; Miranda, J.; Bullo, M.; Casas, R.; Salas-Salvado, J.; Larretxi, I.; Estruch, R.; Ruiz-Gutierrez, V.; Portillo, M.P. Comparative effect of two mediterranean diets

versus a low-fat diet on glycaemic control in individuals with type 2 diabetes. *Eur. J. Clin. Nutr.* **2014**, *68*, 767-772.

13. Vincent-Baudry, S.; Defoort, C.; Gerber, M.; Bernard, M.-C.; Verger, P.; Helal, O.; Portugal, H.; Planells, R.; Grolier, P.; Amiot-Carlin, M.-J. The medi-rivage study: Reduction of cardiovascular disease risk factors after a 3-mo intervention with a mediterranean-type diet or a low-fat diet. *Am. J. Clin. Nutr.* **2005**, *82*, 964-971.
14. Salas-Salvadó, J.; Bulló, M.; Babio, N.; Martínez-González, M.Á.; Ibarrola-Jurado, N.; Basora, J.; Estruch, R.; Covas, M.I.; Corella, D.; Arós, F. Reduction in the incidence of type 2 diabetes with the mediterranean diet. *Diabetes Care* **2011**, *34*, 14-19.
15. Davis, C.R.; Hodgson, J.M.; Woodman, R.; Bryan, J.; Wilson, C.; Murphy, K.J. A mediterranean diet lowers blood pressure and improves endothelial function: Results from the medley randomized intervention trial. *Am. J. Clin. Nutr.* **2017**, *105*, 1305-1313.
16. Itsiopoulos, C.; Brazionis, L.; Kaimakamis, M.; Cameron, M.; Best, J.D.; O'Dea, K.; Rowley, K. Can the mediterranean diet lower hba1c in type 2 diabetes? Results from a randomized cross-over study. *Nutr. Metab. Cardiovasc. Dis.* **2011**, *21*, 740-747.
17. Chew, D.P.; Scott, I.A.; Cullen, L.; French, J.K.; Briffa, T.G.; Tideman, P.A.; Woodruffe, S.; Kerr, A.; Branagan, M.; Aylward, P.E. National heart foundation of australia and cardiac society of australia and new zealand: Australian clinical guidelines for the management of acute coronary syndromes 2016. *Med. J. Aust.* **2016**, *205*, 128-133.
18. Ross, R. Atherosclerosis — an inflammatory disease. *N. Engl. J. Med.* **1999**, *340*, 115-126.
19. Holme, I.; Aastveit, A.H.; Hammar, N.; Jungner, I.; Walldius, G. Inflammatory markers, lipoprotein components and risk of major cardiovascular events in 65,005 men and women in the Apolipoprotein Mortality RIsk Study (AMORIS). *Atherosclerosis* **2010**, *213*, 299-305.
20. Ridker, P.M.; Cannon, C.P.; Morrow, D.; Rifai, N.; Rose, L.M.; McCabe, C.H.; Pfeffer, M.A.; Braunwald, E. C-reactive protein levels and outcomes after statin therapy. *N. Engl. J. Med.* **2005**, *352*, 20-28.
21. Kaptoge, S.; Seshasai, S.R.K.; Gao, P.; Freitag, D.F.; Butterworth, A.S.; Borglykke, A.; Di Angelantonio, E.; Gudnason, V.; Rumley, A.; Lowe, G.D. Inflammatory cytokines and risk of coronary heart disease: New prospective study and updated meta-analysis. *Eur. Heart J.* **2013**, *35*, 578-589.
22. Kim, S.; Kyung, C.; Park, J.S.; Lee, S.-P.; Kim, H.K.; Ahn, C.W.; Kim, K.R.; Kang, S. Normal-weight obesity is associated with increased risk of subclinical atherosclerosis. *Cardiovasc. Diabetol.* **2015**, *14*, 58.
23. Schulze, M.B.; Shai, I.; Rimm, E.B.; Li, T.; Rifai, N.; Hu, F.B. Adiponectin and future coronary heart disease events among men with type 2 diabetes. *Diabetes* **2005**, *54*, 534-539.
24. Nakamura, K.; Fuster, J.J.; Walsh, K. Adipokines: A link between obesity and cardiovascular disease. *J. Cardiol.* **2014**, *63*, 250-259.

25. Samaras, K.; Botelho, N.K.; Chisholm, D.J.; Lord, R.V. Subcutaneous and visceral adipose tissue gene expression of serum adipokines that predict type 2 diabetes. *Obesity* **2010**, *18*, 884-889.
26. Ouchi, N.; Parker, J.L.; Lugus, J.J.; Walsh, K. Adipokines in inflammation and metabolic disease. *Nat. Rev. Immunol.* **2011**, *11*, 85-97.
27. Pischon, T.; Girman, C.J.; Hotamisligil, G.S.; Rifai, N.; Hu, F.B.; Rimm, E.B. Plasma adiponectin levels and risk of myocardial infarction in men. *JAMA* **2004**, *291*, 1730-1737.
28. Neale, E.; Batterham, M.; Tapsell, L.C. Consumption of a healthy dietary pattern results in significant reductions in c-reactive protein levels in adults: A meta-analysis. *Nutr. Res.* **2016**, *36*, 391-401.
29. Schwingshackl, L.; Hoffmann, G. Mediterranean dietary pattern, inflammation and endothelial function: A systematic review and meta-analysis of intervention trials. *Nutr. Metab. Cardiovasc. Dis.* **2014**, *24*, 929-939.
30. Mayr, H.L.; Tierney, A.C.; Thomas, C.J.; Ruiz-Canela, M.; Radcliffe, J.; Itsiopoulos, C. Mediterranean-type diets and inflammatory markers in patients with coronary heart disease: A systematic review and meta-analysis. *Nutr. Res.* **2018**, *50*, 10-24.
31. Esposito, K.; Kastorini, C.-M.; Panagiotakos, D.B.; Giugliano, D. Mediterranean diet and weight loss: Meta-analysis of randomized controlled trials. *Metab. Syndr. Relat. Disord.* **2010**, *9*, 1-12.
32. Bendall, C.L.; Mayr, H.L.; Opie, R.S.; Bes-Rastrollo, M.; Itsiopoulos, C.; Thomas, C.J. Central obesity and the mediterranean diet: A systematic review of intervention trials. *Crit. Rev. Food Sci. Nutr.* **2017**, 1-15.
33. Mozaffarian, D. Food and weight gain: Time to end our fear of fat. *Lancet Diabetes Endocrinol* **2016**, *4*, 633-635.
34. Itsiopoulos, C.; Kucianski, T.; Mayr, H.L.; van Gaal, W.J.; Martinez-Gonzalez, M.A.; Vally, H.; Kingsley, M.; Kouris-Blazos, A.; Radcliffe, J.; Segal, L. 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. *Am. Heart J.* **2018**, DOI: 10.1016/j.ahj.2018.1005.1010.
35. The Criteria Committee of the New York Heart Association. Nomenclature and criteria for diagnosis of diseases of the heart and blood vessels. Little Brown: Boston, 1964.
36. Levey, A.S.; Eckardt, K.-U.; Tsukamoto, Y.; Levin, A.; Coresh, J.; Rossert, J.; Zeeuw, D.D.; Hostetter, T.H.; Lameire, N.; Eknoyan, G. Definition and classification of chronic kidney disease: A position statement from kidney disease: Improving global outcomes (kdigo). *Kidney Int.* **2005**, *67*, 2089-2100.
37. World Medical Association. World medical association declaration of helsinki: Ethical principles for medical research involving human subjects. Available online: Https://www.Wma.Net/what-we-do/medical-ethics/declaration-of-helsinki/ (accessed on 14 february 2018).
38. Schulz, K.F.; Altman, D.G.; Moher, D. Consort 2010 statement: Updated guidelines for reporting parallel group randomised trials. *BMC Med.* **2010**, *8*, 18.

39. Mayr, H.L.; Thomas, C.J.; Tierney, A.C.; Kucianski, T.; George, E.S.; Ruiz-Canela, M.; Hebert, J.R.; Shivappa, N.; Itsiopoulos, C. Randomization to 6-month mediterranean diet compared with a low-fat diet leads to improvement in dietary inflammatory index scores in patients with coronary heart disease: The AUSMED heart trial. *Nutr. Res.* **2018**, *55*, 94-107.
40. Samdal, G.B.; Eide, G.E.; Barth, T.; Williams, G.; Meland, E. Effective behaviour change techniques for physical activity and healthy eating in overweight and obese adults; systematic review and meta-regression analyses. *Int J Behav Nutr Phys Act* **2017**, *14*, 42.
41. Shilts, M.K.; Horowitz, M.; Townsend, M.S. Goal setting as a strategy for dietary and physical activity behavior change: A review of the literature. *Am. J. Health Promot.* **2004**, *19*, 81-93.
42. Staudacher, H.M.; Irving, P.M.; Lomer, M.C.E.; Whelan, K. The challenges of control groups, placebos and blinding in clinical trials of dietary interventions. *Proc. Nutr. Soc.* **2017**, *76*, 203-212.
43. Papamiltiadous, E.S.; Roberts, S.K.; Nicoll, A.J.; Ryan, M.C.; Itsiopoulos, C.; Salim, A.; Tierney, A.C. A randomised controlled trial of a mediterranean dietary intervention for adults with non alcoholic fatty liver disease (medina): Study protocol. *BMC Gastroenterol.* **2016**, *16*, 14.
44. George, E.S.; Kucianski, T.; Mayr, H.L.; Moschonis, G.; Tierney, A.C.; Itsiopoulos, C. A mediterranean diet model in australia; strategies for translating the traditional mediterranean diet into a multicultural setting. *Nutrients* **2018**, *10*, 465.
45. Keys, A.; Mienotti, A.; Karvonen, M.J.; Aravanis, C.; Blackburn, H.; Buzina, R.; Djordjevic, B.; Dontas, A.; Fidanza, F.; Keys, M.H. The diet and 15-year death rate in the seven countries study. *Am. J. Epidemiol.* **1986**, *124*, 903-915.
46. Ministry of Health and Welfare, S.S.H.C. Dietary guidelines for adults in greece. *Archives of Hellenic Medicine* **1999**, *16*, 516-524.
47. Vincent, S.; Gerber, M.; Bernard, M.; Defoort, C.; Loundou, A.; Portugal, H.; Planells, R.; Juhan-Vague, I.; Charpiot, P.; Grolier, P. The medi-rivage study (mediterranean diet, cardiovascular risks and gene polymorphisms): Rationale, recruitment, design, dietary intervention and baseline characteristics of participants. *Public Health Nutr.* **2004**, *7*, 531-542.
48. Ryan, M.C.; Itsiopoulos, C.; Thodis, T.; Ward, G.; Trost, N.; Hofferberth, S.; O'Dea, K.; Desmond, P.V.; Johnson, N.A.; Wilson, A.M. The mediterranean diet improves hepatic steatosis and insulin sensitivity in individuals with non-alcoholic fatty liver disease. *J. Hepatol.* **2013**, *59*, 138-143.
49. NHMRC. Australian dietary guidelines. National Health and Medical Research Council: Canberra, 2013.
50. NHMRC. Nutrient reference values for australia and new zealand; including recommended dietary intakes. Commenwealth of Australia: Canberra, 2006.
51. FoodWorks. What are the serve sizes for the xyris food groups? Available online: Https://support.Xyris.Com.Au/hc/en-us/articles/205716789-what-are-the-serve-sizes-for-the-xyris-food-groups (accessed on 01 august 2017).

52. Schröder, H.; Fitó, M.; Estruch, R.; Martínez‐González, M.A.; Corella, D.; Salas‐Salvadó, J.; Lamuela‐Raventós, R.; Ros, E.; Salaverría, I.; Fiol, M., *et al.* A short screener is valid for assessing mediterranean diet adherence among older spanish men and women. *J. Nutr.* **2011**, *141*, 1140-1145.
53. Mayr, H.L.; Itsiopoulos, C.; Tierney, A.C.; Ruiz-Canela, M.; Hebert, J.R.; Shivappa, N.; Thomas, C.J. Improvement in dietary inflammatory index score after 6-month dietary intervention is associated with reduction in interleukin-6 in patients with coronary heart disease: The AUSMED heart trial. *Nutr. Res.* **2018**, *55*, 108-121.
54. Freedson, P.S.; Melanson, E.; Sirard, J. Calibration of the computer science and applications, inc. Accelerometer. *Med. Sci. Sports Exerc.* **1998**, *30*, 777-781.
55. ISAK. International standards for anthropometric assessment. International Society for the Advancement of Kinanthropometry: South Australia, 2001.
56. Kelly, T.; Wilson, K.E.; Ruth, C. Estimating visceral fat by dual-energy x-ray absorptiometry. Google Patents: 2015.
57. Micklesfield, L.K.; Goedecke, J.H.; Punyanitya, M.; Wilson, K.E.; Kelly, T.L. Dual‐energy x‐ray performs as well as clinical computed tomography for the measurement of visceral fat. *Obesity* **2012**, *20*, 1109-1114.
58. Kelly, T.L.; Wilson, K.E.; Heymsfield, S.B. Dual energy x-ray absorptiometry body composition reference values from nhanes. *PLoS One* **2009**, *4*, e7038.
59. NHF. Guideline for the diagnosis and management of hypertension in adults—2016. National Heart Foundation of Australia: Melbourne, 2016.
60. Mayr, H.L. The effect of a mediterranean diet versus low-fat diet on inflammation and adiposity: An intermediate analysis of the AUSMED heart trial for secondary prevention of coronary heart disease. PhD Thesis, La Trobe University, Melbourne, Australia, Completed 21 June 2018.
61. Grundy, S.M.; Cleeman, J.I.; Daniels, S.R.; Donato, K.A.; Eckel, R.H.; Franklin, B.A.; Gordon, D.J.; Krauss, R.M.; Savage, P.J.; Smith, S.C., *et al.* Diagnosis and management of the metabolic syndrome: An american heart association/national heart, lung, and blood institute scientific statement. *Circulation* **2005**, *112*, 2735-2752.
62. Eldridge, S.M.; Chan, C.L.; Campbell, M.J.; Bond, C.M.; Hopewell, S.; Thabane, L.; Lancaster, G.A. Consort 2010 statement: Extension to randomised pilot and feasibility trials. *Pilot and feasibility studies* **2016**, *2*, 64.
63. Liu-Seifert, H.; Zhang, S.; D'Souza, D.; Skljarevski, V. A closer look at the baseline-observation-carriedforward (BOCF). *Patient Prefer Adherence* **2010**, *4*, 11-16.
64. Pearson, T.A.; Mensah, G.A.; Alexander, R.W.; Anderson, J.L.; Cannon, R.O.; Criqui, M.; Fadl, Y.Y.; Fortmann, S.P.; Hong, Y.; Myers, G.L., *et al.* Markers of inflammation and cardiovascular disease. *Application to Clinical and Public Health Practice: A Statement for Healthcare Professionals From the Centers for Disease Control and Prevention and the American Heart Association* **2003**, *107*, 499-511.
65. Faul, F.; Erdfelder, E.; Lang, A.-G.; Buchner, A. G*power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav. Res. Methods* **2007**, *39*, 175-191.

66. Dinu, M.; Pagliai, G.; Casini, A.; Sofi, F. Mediterranean diet and multiple health outcomes: An umbrella review of meta-analyses of observational studies and randomised trials. *Eur. J. Clin. Nutr.* **2017**, *19*, 1-7.
67. Barbaresko, J.; Koch, M.; Schulze, M.B.; Nöthlings, U. Dietary pattern analysis and biomarkers of low-grade inflammation: A systematic literature review. *Nutr. Rev.* **2013**, *71*, 511-527.
68. Gomez-Delgado, F.; Garcia-Rios, A.; Alcala-Diaz, J.F.; Rangel-Zuniga, O.; Delgado-Lista, J.; Yubero-Serrano, E.M.; Lopez-Moreno, J.; Tinahones, F.J.; Ordovas, J.M.; Garaulet, M., *et al.* Chronic consumption of a low-fat diet improves cardiometabolic risk factors according to the clock gene in patients with coronary heart disease. *Mol. Nutr. Food Res.* **2015**, *59*, 2556-2564.
69. Chen, C.Y.O.; Holbrook, M.; Duess, M.A.; Dohadwala, M.M.; Hamburg, N.M.; Asztalos, B.F.; Milbury, P.E.; Blumberg, J.B.; Vita, J.A. Effect of almond consumption on vascular function in patients with coronary artery disease: A randomized, controlled, cross-over trial. *Nutr. J.* **2015**, *14*, 1-11.
70. Tuttle, K.R.; Shuler, L.A.; Packard, D.P.; Milton, J.E.; Daratha, K.B.; Bibus, D.M.; Short, R.A. Comparison of low-fat versus mediterranean-style dietary intervention after first myocardial infarction (from the heart institute of spokane diet intervention and evaluation trial). *Am. J. Cardiol.* **2008**, *101*, 1523-1530.
71. Thomazella, M.C.D.; Góes, M.F.; Andrade, C.R.; Debbas, V.; Barbeiro, D.F.; Correia, R.L.; Marie, S.K.; Cardounel, A.J.; Laurindo, F.R. Effects of high adherence to mediterranean or low-fat diets in medicated secondary prevention patients. *Am. J. Cardiol.* **2011**, *108*, 1523-1529.
72. Esposito, K.; Pontillo, A.; Di Palo, C.; Giugliano, G.; Masella, M.; Marfella, R.; Giugliano, D. Effect of weight loss and lifestyle changes on vascular inflammatory markers in obese women: A randomized trial. *JAMA* **2003**, *289*, 1799-1804.
73. Richard, C.; Couture, P.; Desroches, S.; Lamarche, B. Effect of the mediterranean diet with and without weight loss on markers of inflammation in men with metabolic syndrome. *Obesity* **2013**, *21*, 51-57.
74. Richard, C.; Royer, M.-M.; Couture, P.; Cianflone, K.; Rezvani, R.; Desroches, S.; Lamarche, B. Effect of the mediterranean diet on plasma adipokine concentrations in men with metabolic syndrome. *Metabolism* **2013**, *62*, 1803-1810.
75. Blüher, M.; Rudich, A.; Klöting, N.; Golan, R.; Henkin, Y.; Rubin, E.; Schwarzfuchs, D.; Gepner, Y.; Stampfer, M.J.; Fiedler, M. Two patterns of adipokine and other biomarker dynamics in a long-term weight loss intervention. *Diabetes Care* **2012**, *35*, 342-349.
76. Maiorino, M.I.; Bellastella, G.; Petrizzo, M.; Scappaticcio, L.; Giugliano, D.; Esposito, K. Mediterranean diet cools down the inflammatory milieu in type 2 diabetes: The médita randomized controlled trial. *Endocrine* **2016**, *54*, 634-641.
77. Shivappa, N.; Steck, S.E.; Hurley, T.G.; Hussey, J.R.; Hébert, J.R. Designing and developing a literature-derived, population-based dietary inflammatory index. *Public Health Nutr.* **2014**, *17*, 1689-1696.

78. Besson, H.; Ekelund, U.; Luan, J.; May, A.; Sharp, S.; Travier, N.; Agudo, A.; Slimani, N.; Rinaldi, S.; Jenab, M. A cross-sectional analysis of physical activity and obesity indicators in european participants of the epic-panacea study. *Int. J. Obes.* **2009**, *33*, 497-506.
79. Lahti-Koski, M.; Pietinen, P.; Heliövaara, M.; Vartiainen, E. Associations of body mass index and obesity with physical activity, food choices, alcohol intake, and smoking in the 1982–1997 finrisk studies. *Am. J. Clin. Nutr.* **2002**, *75*, 809-817.
80. Vissers, D.; Hens, W.; Taeymans, J.; Baeyens, J.-P.; Poortmans, J.; Van Gaal, L. The effect of exercise on visceral adipose tissue in overweight adults: A systematic review and meta-analysis. *PLoS One* **2013**, *8*, e56415.
81. Piers, L.; Walker, K.; Stoney, R.; Soares, M.; O'dea, K. The influence of the type of dietary fat on postprandial fat oxidation rates: Monounsaturated (olive oil) vs saturated fat (cream). *Int. J. Obes.* **2002**, *26*, 814-821.
82. Guebre-Egziabher, F.; Rabasa-Lhoret, R.; Bonnet, F.; Bastard, J.; Desage, M.; Skilton, M.; Vidal, H.; Laville, M. Nutritional intervention to reduce the n− 6/n− 3 fatty acid ratio increases adiponectin concentration and fatty acid oxidation in healthy subjects. *Eur. J. Clin. Nutr.* **2008**, *62*, 1287-1293.
83. Lindeberg, S.; Jönsson, T.; Granfeldt, Y.; Borgstrand, E.; Soffman, J.; Sjöström, K.; Ahrén, B. A palaeolithic diet improves glucose tolerance more than a mediterranean-like diet in individuals with ischaemic heart disease. *Diabetologia* **2007**, *50*, 1795-1807.
84. Buscemi, S.; Verga, S.; Tranchina, M.; Cottone, S.; Cerasola, G. Effects of hypocaloric very ‐ low ‐ carbohydrate diet vs. Mediterranean diet on endothelial function in obese women. *Eur. J. Clin. Invest.* **2009**, *39*, 339-347.
85. Schiavo, L.; Scalera, G.; Sergio, R.; De Sena, G.; Pilone, V.; Barbarisi, A. Clinical impact of mediterranean-enriched-protein diet on liver size, visceral fat, fat mass, and fat-free mass in patients undergoing sleeve gastrectomy. *Surg. Obes. Relat. Dis.* **2015**, *11*, 1164-1170.
86. Calder, P.; Harvey, D.; Pond, C.; Newsholme, E. Site-specific differences in the fatty acid composition of human adipose tissue. *Lipids* **1992**, *27*, 716-720.
87. Chew, D.P.; French, J.; Briffa, T.G.; Hammett, C.J.; Ellis, C.J.; Ranasinghe, I.; Aliprandi-Costa, B.J.; Astley, C.M.; Turnbull, F.M.; Lefkovits, J. Acute coronary syndrome care across australia and new zealand: The snapshot acs study. *Med. J. Aust.* **2013**, *199*, 185-191.