

# ULRR

## **Ad libitum Mediterranean diet reduces subcutaneous but not visceral fat in patients with coronary heart disease: a randomised controlled pilot study**

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1 **Ad libitum Mediterranean diet reduces subcutaneous but not visceral fat in patients with coronary**  
2 **heart disease: a randomised controlled pilot study**

3

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27

28 **Abstract**

29 **Background & aims:** The Mediterranean diet (MedDiet) is recognised to reduce risk of coronary heart disease  
30 (CHD), in part, via its anti-inflammatory and antioxidant properties, which may be mediated via effects on  
31 body fat distribution. Diet efficacy via these mechanisms is however unclear in patients with diagnosed CHD.  
32 This study aimed to determine: (1) the effect of *ad libitum* MedDiet versus low-fat diet intervention on  
33 adiposity, anti-inflammatory marker adiponectin, oxidative stress marker malondialdehyde (MDA) and  
34 traditional CVD risk markers, and (2) whether improvement in MedDiet adherence score in the pooled cohort  
35 was associated with these risk markers, in a pilot cohort of Australian patients post coronary event.

36 **Methods:** Participants (62±9 years, 83% male) were randomised to 6-month *ad libitum* MedDiet ( $n=34$ ) or  
37 low-fat diet ( $n=31$ ). Pre- and post-intervention, dietary adherence, anthropometry, body composition (Dual-  
38 energy X-ray Absorptiometry) and venepuncture measures were conducted.

39 **Results:** The MedDiet group reduced subcutaneous adipose tissue (SAT) area compared to the low-fat diet  
40 group (12.5cm<sup>2</sup> more,  $p=0.04$ ) but not visceral adipose tissue or other body composition measures. In the  
41 pooled cohort, participants with greatest improvement in MedDiet adherence score had significantly lower  
42 waist circumference (-2.81cm,  $p=0.01$ ) and SAT area (-27.1cm<sup>2</sup>,  $p=0.04$ ) compared to participants with no  
43 improvement in score at 6-months. There were no changes in adiponectin, MDA or other risk markers in the  
44 MedDiet compared to low-fat diet group, and no differences in 6-month levels between categories of  
45 improvement in MedDiet score ( $p>0.05$ ). Within the MedDiet group only, the proportion of participants taking  
46 beta-blocker medication reduced from baseline to 6-months (71% vs. 56%,  $p$ -trend=0.007).

47 **Conclusions:** Adherence to 6-month *ad libitum* MedDiet reduced subcutaneous fat and waist circumference  
48 which discounts the misconception that this healthy but high fat diet leads to body fat gain. The effect of  
49 MedDiet on body fat distribution and consequent anti-inflammatory and antioxidant effects, as well as need  
50 for medications, in patients with CHD warrants exploration in larger studies. Clinically significant effects on  
51 these markers may require adjunct exercise and/or caloric restriction.

52 **Trial registration:** ACTRN12616000156482.

53  
54 **Keywords:** Coronary disease; Mediterranean diet; low-fat diet; adiponectin; oxidative stress; body  
55 composition

56

57

## 1. Introduction

58

59 The Mediterranean diet (MedDiet) pattern has a strong scientific evidence base for reducing risk of coronary  
60 heart disease (CHD) and adverse cardiovascular disease (CVD) events (1, 2). Nonetheless, the majority of  
61 studies investigating the MedDiet have been conducted in Mediterranean countries. A low-fat diet was the  
62 standard care recommendation for prevention and treatment of CHD in Australia for many years (3), however,  
63 a recent position statement from the National Heart Foundation of Australia promotes a variety of healthy  
64 dietary patterns, rather than focusing on isolated nutrients, for cardiovascular health (4).

65

66 Atherosclerosis is the underlying pathology responsible for CHD. Derangements in lipid levels, blood pressure  
67 and insulin homeostasis each lead to endothelial dysfunction, which plays a pivotal role in initiating the  
68 atherosclerotic process (5). A number of studies, including in the Australian setting, have demonstrated that  
69 the MedDiet improves CVD risk factors, including improvements in triglycerides and high-density lipoprotein  
70 (HDL) cholesterol, blood pressure, glucose metabolism and reduced risk of type 2 diabetes mellitus (T2DM)  
71 (6-13). These studies were conducted in patients at risk of, but without, established CHD. In CHD, especially  
72 in those who have suffered acute coronary syndrome (ACS), pharmacotherapy is used to achieve recommended  
73 lipid, glucose and blood pressure targets (14), hence the possibility to attain additional impact of diet on these  
74 risk factors may not be observed in these patients. In fact, the limited published data on the impact of MedDiet  
75 on secondary prevention of ACS demonstrated that the diet may be operating independently of traditional CVD  
76 risk factors (1).

77

78 Atherosclerosis is recognised to be an inflammatory condition, which is related to both the chronic  
79 development of plaque and its acute rupture (15). In addition, obesity, especially increased visceral fat, is  
80 causally linked to chronic low-grade inflammation (16, 17). In an obese state, adipose tissue generates pro-  
81 inflammatory adipokines, including interleukin-6 (IL-6), whereas anti-inflammatory adipokines, including  
82 adiponectin, are down-regulated (18). High serum concentrations of adiponectin are associated with decreased  
83 risk of CHD (19, 20). Oxidative stress has also been recognised to increase risk of cardiovascular events in

84 patients with CHD, through increased oxidation of LDL particles and endothelial dysfunction (21). Plasma  
85 malondialdehyde (MDA), a non-invasive measure of lipid peroxidation, is a recognised marker of oxidative  
86 stress and elevated MDA levels are reported in patients with CHD (22).

87

88 To better understand how dietary interventions moderate CHD risk, it is important to ascertain their effect on  
89 novel markers such as adiposity, inflammation and oxidative stress in addition to classic cardiometabolic risk  
90 markers. A recent review of intervention trials demonstrated that the MedDiet can reduce central obesity;  
91 however, most studies measured waist circumference without distinguishing visceral and subcutaneous fat and  
92 included patients without CHD (23). Meta-analyses of randomised controlled trials (RCTs) have also  
93 concluded that intervention with the MedDiet improves a range of established inflammatory and oxidative  
94 stress markers (24, 25). However, a recent systematic review of the literature established that no studies have  
95 investigated the effect of MedDiet on adiponectin in patients with diagnosed CHD (26). Moreover, with respect  
96 to lipid peroxidation, MedDiet intervention improved MDA levels in patients at risk of but without CHD (27).

97

98 We have previously reported results from this pilot MedDiet intervention, showing no improvement in the  
99 inflammatory markers high sensitivity C-Reactive Protein (hs-CRP) or hs-IL-6, despite significant  
100 improvement in MedDiet adherence and dietary anti-inflammatory potential (measured by the dietary  
101 inflammatory index) in Australian patients who have experienced an ACS event (28, 29). Therefore, the  
102 primary aim of the present analysis was to determine the effect of *ad libitum* MedDiet versus low-fat diet  
103 intervention on additional cardiometabolic risk markers, including compartmental adiposity, anti-  
104 inflammatory marker adiponectin, and MDA levels in the same pilot cohort. A secondary aim was to determine  
105 whether improvement in MedDiet adherence score in the pooled cohort was associated with resultant  
106 improvement in risk marker levels. Results from this pilot will be used to inform feasibility and sample size  
107 requirements for future analyses.

108

## 109 **2. Materials and Methods**

110

### 111 *2.1 Study Design*

112 The data reported in this study was collected in the pilot of the AUStralian MEDiterranean Diet Heart Trial  
113 (AUSMED Heart Trial), a multi-centre, parallel design, randomised controlled trial (RCT) of 6-month  
114 MedDiet versus low-fat diet intervention for the secondary prevention of CHD at 12-months in a multi-ethnic  
115 Australian population (Australia and New Zealand Clinical Trials Register: ACTRN12616000156482) (30).  
116 As noted above, this pilot study and methodology, including results for nutritional intake and diet quality (29),  
117 the dietary inflammatory index, hs-CRP and hs-IL-6 (28, 31) has previously been reported.

118

## 119 *2.2 Recruitment of CHD Patients*

120 Patients for this pilot study were recruited from two tertiary hospitals in Melbourne, Australia between October  
121 2014 and November 2016. Eligible patients were adults with CHD, able to read and write in English and who  
122 had experienced ACS defined as at least one of the following: acute myocardial infarction (AMI); angina  
123 pectoris with documented coronary artery disease on imaging; coronary artery bypass grafting; or percutaneous  
124 coronary intervention. The study was conducted in accordance with the Declaration of Helsinki (32) and the  
125 CONSORT guidelines (33). All procedures involving patients were approved by the Human Research Ethics  
126 Committees of The Northern Hospital, St Vincent's Hospital Melbourne, and La Trobe University, with  
127 written informed consent obtained from all enrolled participants before randomisation.

128

## 129 *2.3 Randomisation of Participants and Diet Interventions*

130 At a pre-baseline appointment, enrolled participants were randomly assigned in a 1:1 ratio to the MedDiet  
131 group or the low-fat diet group using a stratified approach (based on sex, age and prior AMI). Baseline, 3- and  
132 6-month face-to-face appointments were conducted to obtain dietary data and for counselling with the dietitian.  
133 Five short phone reviews for follow-up dietary counselling with the dietitian also occurred across the 6-months,  
134 at weeks 3, 6, and 9 and months 4 and 5. Both diets were prescribed *ad libitum* with no specific  
135 recommendations on energy restriction. All participants continued to receive standard medical care provided  
136 at their respective hospital or primary care settings and their access to outside health services during the study  
137 intervention period was recorded at each appointment.

138

### 139 *2.3.1 Mediterranean Diet*

140 The rationale, development and resources provided with our MedDiet intervention, designed for use in chronic  
141 disease intervention trials in the Australian setting (30, 34), has been explained and published in detail  
142 elsewhere (35). Briefly, the diet was modelled via a 2-week meal plan which incorporated key dietary  
143 components of a MedDiet and a mix of traditional and modified recipes considered to be realistic options in  
144 the multi-ethnic Australian setting. Food group recommendations included: daily intake of extra virgin olive  
145 oil (EVOO), wholegrain cereals, vegetables, fruit and nuts; regular intake of fish and seafood, legumes and  
146 yoghurt; and limited intake of commercial sweets or pastries and red or processed meat. Poultry, eggs and feta  
147 cheese were recommended in moderation. For existing alcohol drinkers, red wine was suggested to be  
148 consumed in moderation (1-2 standard glasses) with meals. To facilitate dietary compliance and to encourage  
149 intake of staple Mediterranean foods less familiar to this Australian population, a hamper was provided to  
150 participants at baseline and 3-months, including 6L EVOO (to achieve 60-80mL/day) and 1.2kg nuts (almonds,  
151 walnuts and hazelnuts to achieve 30g/day).

152

### 153 *2.3.2 Low-fat Diet*

154 Participants in the low-fat diet group were instructed to follow the standard diet recommendations provided to  
155 cardiac patients in Australia at the time this study was developed (in 2014). Recommendations from the  
156 National Heart Foundation (3) and Australian Dietary Guidelines (36, 37) were consulted for design of the  
157 low-fat diet. Food group recommendations included daily intake of grains and cereals (mostly whole grains),  
158 vegetables, lean meats and alternatives, fruit, and low-fat dairy foods (36). Participants were provided with a  
159 supermarket voucher at each of their three face-to-face appointments to aid compliance and encourage  
160 continuation in the trial.

161

### 162 *2.4 Study Measures*

163 This study reports on outcome measurements collected at the baseline and 6-month appointments. Data on  
164 medical conditions was collected from medical records and in consultation with hospital staff during the  
165 screening process, and via a questionnaire at the pre-baseline appointment. Participants completed a self-report  
166 survey prior to their baseline appointment which recorded sociodemographic, lifestyle and clinical

167 characteristics, including medication and supplement use. A modified version of the survey was completed at  
168 both 3- and 6-month appointments, which re-assessed lifestyle and clinical characteristics.

169

#### 170 *2.4.1 Dietary Intake*

171 The week prior to each face-to-face appointment the participants completed a 7-day food diary which was  
172 entered into FoodWorks (Version 8, Xyris software Australia Pty Ltd) for nutrient and food group intake  
173 analyses. The 14-point Mediterranean Diet Adherence Screener (MEDAS), generated and validated for the  
174 PREDIMED study (38), was measured at each appointment for both diet study groups.

175

#### 176 *2.4.2 Cardiometabolic Risk Markers*

177 Our methods for assessment of activity levels, anthropometry, body composition, blood pressure and pathology  
178 measures have also been described previously (31). Increased physical activity was not a target of this  
179 intervention, however, physical activity levels were assessed to account for any potential confounding effects  
180 on outcome markers. Participants wore a triaxial Actigraph accelerometer (WGT3X-BT; Actigraph Corp,  
181 Florida, United States) for one week prior to their appointments. Established criteria (39) were used to  
182 determine time spent as min /week in moderate-to-vigorous physical activity (MVPA) or as sedentary time.  
183 Weight, height and waist circumference measures were performed according to the International Society for  
184 the Advancement of Kinanthropometry (ISAK) standards for anthropometric assessment (40). Whole body  
185 composition was measured using a fan beam densitometer Dual-energy X-ray Absorptiometry (DXA) machine  
186 (Hologic, Discovery W, USA), with analysis performed using QDR™ (Quantitative Digital Radiography) for  
187 Windows. Measurements obtained from each scan were total body lean and fat mass, total body and regional  
188 fat percentage, subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT) areas. Hologic scientists  
189 developed their method for measuring VAT from DXA (41), which is highly correlated ( $r=0.93$ ) and linearly  
190 related to VAT measurements by computed tomography (42). Fat mass index (FMI) was calculated by dividing  
191 the total body fat mass (kg) by height (m) squared (43). Systolic blood pressure (SBP), diastolic blood pressure  
192 (DBP) and heart rate (HR) were measured using an automated blood pressure monitor (OMRON Tp9,  
193 Intellisense, Australia). Hypertension (presence or history of) was classified based on whether the  
194 participants were prescribed medication with anti-hypertensive effect (angiotensin converting enzyme [ACE]

195 inhibitor, angiotensin 2 receptor blocker, Beta [ $\beta$ ]-blocker or  $Ca^{2+}$  channel blocker) and/or mean baseline blood  
196 pressure reading of SBP >140 mmHg or DBP >90 mmHg (44). Diagnosis of T2DM was determined by  
197 consulting participant medical history records.

198  
199 Fasting blood samples were taken by venepuncture and processed immediately into serum/plasma aliquots (as  
200 published in detail elsewhere (45)) which were stored at  $-80\text{ }^{\circ}\text{C}$  until laboratory assays were conducted. Serum  
201 low-density lipoprotein (LDL) cholesterol, HDL cholesterol, triglycerides and hs-CRP levels were measured  
202 at a commercial laboratory (Dorevitch Pathology Pty Ltd, Heidelberg, Australia). Other biomarker measures  
203 were performed by trained personnel at La Trobe University, except for MDA which was performed at the  
204 University of Newcastle. Enzyme-linked immunosorbent assay (ELISA) kits were used to measure serum hs-  
205 IL-6 levels (Abcam Australia Pty Ltd, #ab46042), serum adiponectin levels (Invitrogen, Thermofisher  
206 Scientific, #KHP0041) and plasma MDA levels (Abcam Australia Pty Ltd, #ab118970). Fasting serum glucose  
207 levels were measured using the enzymatic hexokinase method by a chemical analyser (Indiko, Thermofisher  
208 Scientific). Personnel performing laboratory analyses were blinded to study group allocation of samples.

209

## 210 *2.5 Statistical Analyses*

211 This study represented a preliminary analysis in a pilot cohort and therefore a sample size calculation was not  
212 performed prior to conducting the measures (46). The broader AUSMED Heart Trial is powered to detect a  
213 significant effect of MedDiet on secondary cardiovascular endpoints and will recruit 1000 participants (30).  
214 All statistical analyses were conducted in SPSS<sup>®</sup> statistical package version 25 (IBM Corp, released 2015).  
215 Statistical significance was set at  $p < 0.05$ . Data are presented as means  $\pm$  standard deviation (SD) or standard  
216 error (SEM), medians (interquartile range [IQR]) or n (%), as appropriate. The Kolmogorov–Smirnov test was  
217 applied to assess the normality of continuous variables. According to this, an Independent Student's *t*-test or  
218 non-parametric Mann-Whitney U test was used to compare continuous variables. Categorical variables were  
219 compared using the *Chi-square* test.

220

221 All outcome measures were analysed based on intention-to-treat with missing data included by bringing  
222 baseline or 3-month observations forward, assuming no change (47). Cochran's Q test assessed changes in the

223 proportion of participants taking medication and supplement classes from baseline to 3- and 6-months within  
224 each study group. Repeated measures ANOVA (analysis of variance) assessed changes in cardiometabolic risk  
225 marker variables from baseline to 6-months between groups. Measures which were non-parametric were log  
226 transformed to improve their distribution. The main ANOVA results assessed for effect were (1) group  
227 (significant change in one study group compared to the other) and (2) time (significant change in pooled study  
228 groups). Post-hoc tests were performed to determine within-group changes (Paired Samples *t*-test). Analyses  
229 for all risk markers were adjusted for change in MVPA and haemodynamic and pathology measures were  
230 additionally adjusted for baseline BMI. The repeated measures analysis inherently controlled for participant  
231 characteristics not subject to change and were not different between study groups at baseline, including sex  
232 and T2DM status. The between-group findings for adiponectin and MDA levels were used to perform a sample  
233 size calculation to inform future analyses (described in Results).

234

235 To account for any cross-over in improvement towards the MedDiet pattern in participants of the low-fat diet  
236 group, analyses were also performed in the pooled cohort (with inclusion of hs-CRP and hs-IL-6 which have  
237 not previously been analysed in this way). Tertiles of change in participant MEDAS scores from baseline to  
238 6-months were created in SPSS. Least-squared means (95% confidence interval [CI]) of cardiometabolic risk  
239 markers at 6-months were estimated across the tertiles of MEDAS change. Multi-variable general linear  
240 models adjusted for baseline value, sex, age, T2DM, time since coronary event and change in MVPA were  
241 used to estimate the differences in means across tertiles. For hs-CRP, participants with serum levels >10 mg/L  
242 were excluded from analyses, as these higher concentrations reflect acute rather than chronic inflammation  
243 (48).

244

### 245 **3. Results**

246

#### 247 *3.1 Participants*

248 Randomisation to diet study groups, completion of study appointments and number and reasons for withdrawal  
249 have been reported elsewhere (28, 29). Briefly, of 73 randomised participants, 65 attended a baseline  
250 appointment and started the intervention. The subsequent attrition rate was 14%, with 2 and 7 participants

251 dropping out from the low-fat diet and MedDiet groups respectively. Participants were lost to follow up (n=2)  
 252 or discontinued due to relocation (n=2), non-cardiac medical problems (n=3) or family related issues (n=2).  
 253 There were no significant differences for sociodemographic or clinical characteristics between those  
 254 participants that dropped out compared to completers.

255

256 As reported in Table 1, the cohort represented a mostly male, middle to late aged group of which close to half  
 257 were born outside Australia (18% were born in the Mediterranean region). Participants had highly variable  
 258 levels of MVPA (total range 3 to 665 min /week), their baseline MedDiet adherence was low (mean MEDAS  
 259 score of 5 out of 14) and 80% had previously attended a cardiac rehabilitation program. Most participants had  
 260 experienced an AMI and undergone percutaneous coronary intervention with a median time since ACS event  
 261 of <6 months prior. Close to one third had diagnosed T2DM and nearly all had current or previous  
 262 hypertension. Participants were prescribed multiple medications, of which anti-platelets and statins (both  
 263 >90%) were the most common (Supplementary Materials, Table S1). Close to half the participants were taking  
 264 nutrition supplements, of which vitamin D (19%) and omega-3 (15%) were the most common (Table S1).  
 265 There were no significant differences at baseline between the diet study groups for any of these reported  
 266 sociodemographic, lifestyle or clinical characteristics.

267

268 **Table 1. Participant baseline characteristics in the study groups**

Characteristic	Low-fat (n=31)	MedDiet (n=34)
<i>Sociodemographic</i>		
Male	27 (87.1)	27 (79.4)
Age (years)	61.8 ± 9.5	61.8 ± 9.2
Country of birth		
Australia	18 (58.1)	20 (58.8)
Other	13 (41.9)	14 (41.2)
Mediterranean country	7 (22.6)	5 (14.7)
<i>Lifestyle</i>		
Current smoker	3 (9.7)	6 (18.2)

>100 cigarettes in lifetime	18 (58.1)	20 (58.8)
BMI (kg/m <sup>2</sup> )	29.1 ± 5.3	30.7 ± 5.0
MVPA (min /week)†	120.0 (189.5)	153.0 (210.0)
MEDAS (score out of 14)	4.8 ± 1.8	5.6 ± 2.2
Cardiac rehabilitation	26 (83.9)	25 (73.5)
<i>Medical History</i>		
Acute myocardial infarction	22 (71.0)	23 (67.6)
Percutaneous coronary intervention	25 (80.6)	25 (73.5)
Coronary artery bypass grafting	8 (25.8)	7 (20.6)
Time since event (months)†	4.5 (6.5)	5.1 (15.2)
Type 2 diabetes mellitus	9 (29.0)	10 (29.4)
Hypertension	31 (100)	31 (91.2)
Depression (diagnosed)	4 (12.9)	6 (17.6)

269 Values are n (%), Mean ± SD or Median (IQR)†. MedDiet; Mediterranean diet; BMI, body mass index; MVPA,  
 270 moderate-to-vigorous physical activity; MEDAS, Mediterranean diet adherence screener.

271

272 There were no significant differences between the groups for frequency of attendance at each of the study  
 273 appointments and phone call reviews conducted across the diet intervention period (Supplementary Material,  
 274 Table S2). The proportion of participants who attended each of the appointments or reviews was 80% or more.  
 275 The participants reported having accessed a variety of other health services during the intervention period, but  
 276 there were no significant differences between the study groups (Table S2). There was a reduction in the  
 277 proportion of participants prescribed β-blockers in the MedDiet group between baseline and at 3-months (from  
 278 24 to 19 participants) and this was maintained at 6-months (*p*-trend=0.007). There were no other changes in  
 279 the proportion of participants taking prescribed medications in either study group (Table S1). Participants  
 280 reported high medication compliance at baseline and this remained consistent throughout the study. There were  
 281 no significant changes within either study group for use of nutrition supplements across the intervention period  
 282 (Table S1).

283

284 *3.2 Dietary Intake*

285 Daily intake of food group serves, energy and nutrients have been reported previously (29). Briefly, in  
286 the MedDiet group, in line with recommendations, consumption of olive oil, fruit, yoghurt, nuts, legumes and  
287 seafood significantly increased, whereas red and processed meats decreased after 6-months. There were no  
288 significant changes for dietary intake of individuals nutrients or foods in the low-fat diet group. There was a  
289 significantly greater improvement in mean MEDAS score in the MedDiet group (+4.8 points from a baseline  
290 score of 5.6 out of 14) compared to low-fat diet group (+1.2 points from a baseline of 4.8 out of 14) ( $p<0.001$ ).  
291 The small improvement in MedDiet score in the low-fat diet participants was related to their improved  
292 adherence to score components for vegetable intake and use of butter/cream. There was no significant  
293 difference for change in MEDAS score between participants born in a Mediterranean country versus not, as  
294 assessed separately within the study groups and with the study groups pooled. No participants reported harmful  
295 side effects or adverse events directly related to the dietary interventions.

296

### 297 *3.3 Activity Levels*

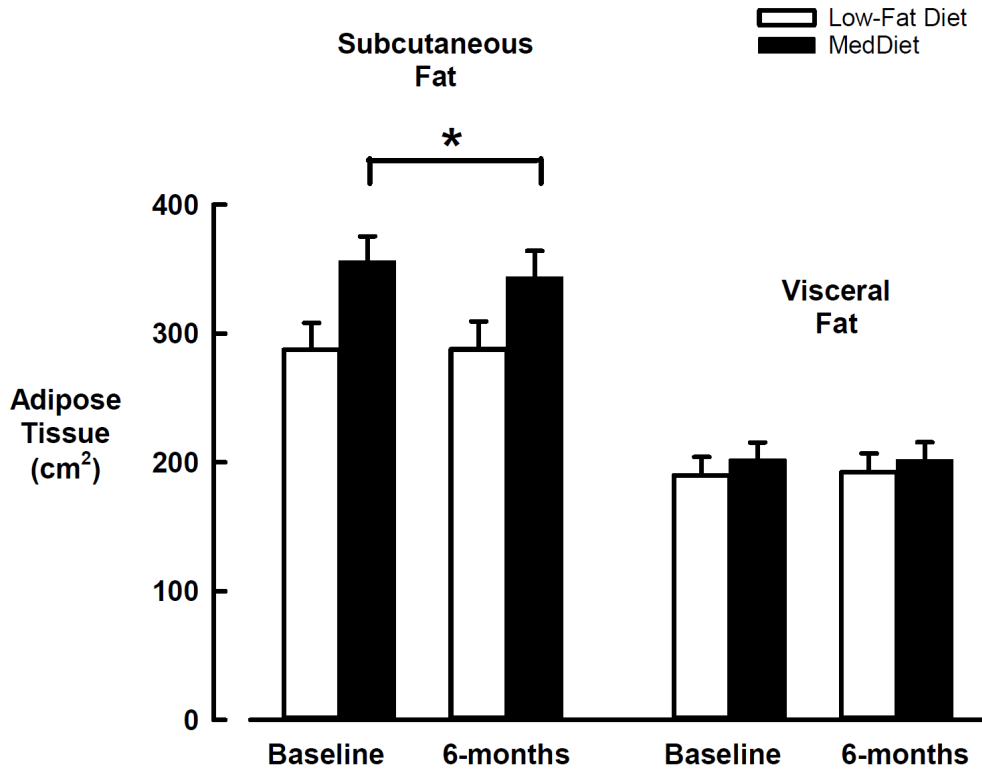
298 There were no significant changes in time spent as sedentary or in MVPA activity between baseline and 6-  
299 months in the MedDiet or low-fat diet groups (Table 2, all variables reported as mean  $\pm$  SEM). However, there  
300 was a high level of individual variability for these measures and participants in the MedDiet group tended to  
301 reduce their MVPA level (by 42 min /week,  $p=0.20$ ), hence this justified controlling for any activity changes  
302 in the risk marker analyses.

303

### 304 *3.4 Anthropometry and Body Composition*

305 With regards to anthropometry and body composition measures, there was a significant between-group  
306 difference for 6-month change in SAT area only ( $-12.1 \pm 6.5$  cm<sup>2</sup> in the MedDiet vs.  $+0.4 \pm 6.9$  cm<sup>2</sup> in the low-  
307 fat diet group,  $p=0.04$ ; Figure 1). VAT area did not change within the MedDiet ( $-0.1 \pm 3.8$  cm<sup>2</sup>) or the low-fat  
308 diet ( $+2.4 \pm 4.0$  cm<sup>2</sup>) groups ( $p=0.58$ , Figure 1). There were no significant within-group changes for weight,  
309 BMI, waist circumference, or waist-hip ratio (Table 2). There was no significant reduction in waist  
310 circumference over time in the pooled study groups ( $-1.1$  cm,  $p=0.07$  within the MedDiet and  $-0.4$  cm,  $p=0.52$   
311 within in the low-fat diet group). There was also no significant reduction in total body fat % over time in the  
312 pooled study groups ( $-0.6\%$ ,  $p=0.06$  within the MedDiet and  $-0.4\%$ ,  $p=0.23$  within in the low-fat diet group).

313 Leg fat %, however, decreased significantly over time in the pooled study groups ( $p=0.04$ ) with a significant  
314 reduction within the MedDiet group ( $-0.6\%$ ,  $p=0.03$ ) but not the low-fat diet group ( $-0.5\%$ ,  $p=0.10$ ).  
315



316  
317 **Figure 1. Subcutaneous and visceral adipose tissue measured by dual energy x-ray absorptiometry at**  
318 **baseline and end-intervention in the MedDiet and low-fat diet groups.** Data are mean  $\pm$  SEM with  
319 adjustment for change in moderate to vigorous physical activity levels. MedDiet, Mediterranean diet.  
320 \*Significant reduction in MedDiet compared to low-fat diet participants,  $p=0.04$ .

321  
322 *3.5 Haemodynamic, Cholesterol and Glucose Measures*

323 There were no between-group changes for any of the reported haemodynamic, cholesterol or glucose markers,  
324 adjusted for MVPA change and baseline BMI (Table 2). There was a significant reduction in resting HR in the  
325 pooled study groups ( $p=0.03$ ), with a trend for greater reduction in the MedDiet ( $-1.5$  bpm,  $p=0.07$ ) compared  
326 to the low-fat diet ( $-0.8$  bpm,  $p=0.55$ ) group. With regards to lipids, the only significant within-group finding  
327 was an increase in LDL cholesterol between baseline and 6-months ( $+0.22$  mmol/L,  $p=0.006$ ) in the low-fat

328 diet group. There were no changes within either study group for triglycerides or fasting glucose levels (also  
329 assessed separately for T2DM status).

330

### 331 *3.6 Adiponectin*

332 There were no between-group changes for serum levels of the anti-inflammatory marker adiponectin ( $p=0.45$ )  
333 adjusted for MVPA change and baseline BMI (Table 2). There was also no significant change in adiponectin  
334 between baseline and 6-months within the low-fat diet ( $-0.91$  ng/mL,  $p=0.23$ ) or MedDiet ( $+1.10$  ng/mL,  
335  $p=0.37$ ) groups. Data from this interim analysis on the 6-month between-within group changes for adiponectin  
336 were used to perform a reverse sample size calculation in statistical software program G\*Power 3.1.94 (49).  
337 Based on the study group effect size (derived from the partial  $\eta^2$  of the repeated measures group comparison)  
338 of 0.101, and a correlation value between adiponectin levels at baseline and 6-months of  $r=0.689$  at 80% power  
339 and  $\alpha<0.05$ , a sample size of 124 participants would be required to detect a significant effect of the MedDiet  
340 on adiponectin compared to the low-fat diet.

341

### 342 *3.7 Malondialdehyde*

343 There were no between-group changes for plasma MDA levels ( $p=0.75$ ) adjusted for MVPA change and  
344 baseline BMI (Table 2). There was also no significant change in MDA levels between baseline and 6-months  
345 within the low-fat diet ( $+0.02$  nmol/mL,  $p=0.93$ ) or MedDiet ( $-0.25$  nmol/L,  $p=0.24$ ) groups. MDA data were  
346 also used to perform a sample size calculation as above. Based on the study group effect size of 0.045 and a  
347 correlation value between MDA levels at baseline and 6-months of  $r=0.775$ , a sample size of 444 participants  
348 would be required to detect a significant effect of the MedDiet on MDA levels compared to the low-fat diet.

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356 **Table 2. Cardiometabolic risk markers at baseline and end-intervention in the study groups**

Marker	Low-fat diet (n=31)		MedDiet (n=34)		p-value	
	Baseline	6-month	Baseline	6-month	Group	Time
<i>Activity levels</i>						
Sedentary (min/week)	3559 ± 135	3431 ± 138	3477 ± 133	3380 ± 135	0.70	0.18
MVPA (min/week)	148 ± 27	135 ± 21	186 ± 26	144 ± 20	0.33	0.37
<i>Anthropometry</i>						
Weight (kg)	85.0 ± 3.3	84.7 ± 3.3	89.1 ± 3.2	89.1 ± 3.1	0.36	0.51
Body mass index (kg/m <sup>2</sup> )	29.0 ± 0.9	28.9 ± 0.9	30.8 ± 0.9	30.8 ± 0.9	0.15	0.58
Waist circumference (cm)	101.9 ± 2.6	101.5 ± 2.5	104.5 ± 2.5	103.4 ± 2.4	0.53	0.07
Waist-hip ratio	0.976 ± 0.02	0.973 ± 0.01	0.977 ± 0.01	0.966 ± 0.01	0.89	0.10
<i>Body composition</i>						
Total lean (kg)	55.6 ± 2.0	55.9 ± 2.0	56.1 ± 1.0	56.6 ± 1.9	0.83	0.22
Total fat (kg)	27.7 ± 1.8	27.2 ± 1.7	31.1 ± 1.7	30.6 ± 1.6	0.16	0.11
Fat mass index (kg/m <sup>2</sup> )	9.45 ± 0.6	9.42 ± 0.6	10.84 ± 0.5	10.68 ± 0.6	0.10	0.42
Total fat %	32.7 ± 1.2	32.3 ± 1.3	35.3 ± 1.1	34.7 ± 1.2	0.13	0.05
Trunk fat %	34.9 ± 1.2	34.6 ± 1.3	37.9 ± 1.1	37.3 ± 1.2	0.10	0.08
Arm fat %	32.7 ± 1.6	32.2 ± 1.7	36.2 ± 1.5	35.8 ± 1.6	0.12	0.21
Leg fat %	30.6 ± 1.4	30.1 ± 1.5	32.6 ± 1.3	32.0 ± 1.4 <sup>a</sup>	0.31	0.04*
<i>Haemodynamic</i>						
SBP (mmHg)	140.6 ± 3.3	139.5 ± 2.8	133.4 ± 3.1	132.0 ± 2.6	0.07	0.84
DBP (mmHg)	83.3 ± 1.6	83.0 ± 1.6	81.0 ± 1.5	80.6 ± 1.5	0.25	0.09
HR (bpm)	66.9 ± 2.0	66.1 ± 2.0	68.5 ± 1.9	66.0 ± 1.9	0.77	0.03*
<i>Pathology</i>						
LDL (mmol/L)†	1.73 ± 0.13	1.95 ± 0.15 <sup>a</sup>	1.96 ± 0.13	1.99 ± 0.15	0.48	0.35
HDL (mmol/L)†	1.20 ± 0.05	1.24 ± 0.05	1.21 ± 0.05	1.25 ± 0.05	0.83	0.47
Triglycerides (mmol/L)†	1.35 ± 0.13	1.30 ± 0.14	1.57 ± 0.12	1.60 ± 0.13	0.12	0.65
Glucose (mmol/L)	5.27 ± 0.26	5.18 ± 0.27	5.76 ± 0.25	5.65 ± 0.26	0.17	0.13
No T2DM (n=44)	4.92 ± 0.16	4.78 ± 0.12	4.98 ± 0.15	4.91 ± 0.11	0.60	0.17
T2DM (n=19)‡	6.20 ± 0.47	6.11 ± 0.67	7.49 ± 0.45	7.38 ± 0.63	0.08	0.31

Adiponectin (ng/mL) <sup>†</sup>	8.40 ± 0.71	7.49 ± 0.83	8.36 ± 0.68	9.47 ± 0.81	0.45	0.65
Malondialdehyde (nmol/mL)	6.96 ± 0.31	6.98 ± 0.33	6.96 ± 0.30	6.71 ± 0.32	0.75	0.11

357 Values are Mean ± SEM with anthropometry/body composition adjusted for MVPA change, and  
358 haemodynamic/pathology markers adjusted for MVPA change and baseline BMI. One low-fat diet participant did  
359 not consent to DXA scan and was excluded from body composition analyses. One MedDiet participant who dropped  
360 out and had haemolysed blood sample at baseline was excluded from pathology marker analyses. MedDiet,  
361 Mediterranean diet; MVPA; moderate to vigorous physical activity; SBP; systolic blood pressure; DBP, diastolic  
362 blood pressure; HR, heart rate; LDL, low-density lipoprotein; HDL, high-density lipoprotein; T2DM, type 2 diabetes  
363 mellitus; <sup>†</sup>Non-parametric, analyses based on transformed variable. <sup>‡</sup>One participant with T2DM had a major  
364 increase in insulin dosage and was excluded from analyses. Significant,  $p < 0.05$ , for: \*Main effect of group or time;  
365 <sup>a</sup>difference between baseline and 6-months for that group.

366

### 367 3.8 Association Between Mediterranean Diet Adherence and Risk Markers

368 All participants were categorised into tertiles of change in MEDAS score from baseline to 6-months. This  
369 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,  
370 with the largest 6-month improvement in MedDiet adherence, 93% of participants were from the MedDiet  
371 group. In T2 and T1 the proportion of participants in the MedDiet group was 56% and 22%, respectively. Mean  
372 (95% CI) levels for cardiometabolic risk markers at 6-months, adjusted for baseline value, sex, age, T2DM,  
373 time since coronary event and change in MVPA, are presented in Table 3. For each of the reported  
374 anthropometric, body composition and hemodynamic measures the mean value decreased across tertiles from  
375 T1 to T3 (from lowest to greatest MEDAS score improvement), except for VAT area and DBP, which had a  
376 higher mean value in T2, followed by T1 and then T3. Compared to T1, T3 participants had a significantly  
377 lower mean waist circumference (-2.81 cm,  $p = 0.01$ ), waist-hip ratio (-0.022,  $p = 0.047$ ) and SAT area (-27.4  
378 cm<sup>2</sup>,  $p = 0.04$ ). Mean levels of other pathology markers did not demonstrate any consistent trends across tertiles.  
379 For adiponectin, the mean value increased slightly but with no significant difference across tertiles from lowest  
380 to greatest MEDAS score improvement (+0.68 ng/mL from T1 to T3,  $p = 0.50$ ). There was also no difference  
381 between tertiles for plasma MDA level (-0.31 nmol/mL from T1 to T3,  $p = 0.46$ ).

382

383 **Table 3. Adjusted means of cardiometabolic risk markers at 6-months by tertiles of change in**  
 384 **Mediterranean Diet Adherence Screener (MEDAS) score<sup>a</sup>**

Marker	Tertile 1 (-2 to +1 points)		Tertile 2 (+2 to 5 points)		Tertile 3 (+6 to 9 points)	
	<i>n</i> =22		<i>n</i> =27		<i>n</i> =15	
	Adjusted	95% CI	Adjusted	95% CI	Adjusted	95% CI
	mean		mean		mean	
<i>Anthropometry</i>						
Weight (kg)†	85.9	84.7, 87.3	85.3	84.1, 86.5	84.1	82.6, 85.7
Body mass index (kg/m <sup>2</sup> )	30.1	29.6, 30.5	30.0	29.5, 30.4	29.6	29.1, 30.2
Waist circumference (cm)	103.5	102.1, 104.9	102.6	101.3, 103.9	100.7	99.0, 102.3*
Waist-hip ratio	0.981	0.967, 0.995	0.965	0.952, 0.978	0.959	0.942, 0.976*
<i>Body composition</i>						
Fat mass index (kg/m <sup>2</sup> )	10.2	9.82, 10.57	10.2	9.85, 10.53	9.8	9.31, 10.21
Total body fat (%)	33.9	33.1, 34.7	33.7	32.9, 34.4	32.9	32.0, 33.9
Trunk fat (%)	36.6	35.6, 37.6	36.0	35.1, 37.0	35.3	34.1, 36.4
VAT (cm <sup>2</sup> )	196.0	186.0, 206.0	200.0	190.9, 209.0	195.3	183.5, 207.1
SAT (cm <sup>2</sup> )	331.7	315.0, 348.4	313.4	298.3, 328.6	304.3	284.3, 324.3*
<i>Haemodynamic</i>						
SBP (mmHg)	136.5	132.1, 140.8	136.1	132.1, 140.8	133.4	128.2, 138.5
DBP (mmHg)	82.0	79.5, 84.4	82.1	79.9, 84.4	80.8	77.9, 83.7
HR (bpm)	66.0	62.9, 69.1	66.2	63.4, 69.1	65.9	62.2, 69.6

*Pathology*<sup>†</sup>

LDL (mmol/L)	1.69	1.51, 1.90	1.97	1.77, 2.17	1.70	1.49, 1.95
HDL (mmol/L)	1.26	1.19, 1.33	1.19	1.14, 1.25	1.19	1.11, 1.27
Triglycerides (mmol/L)	1.16	1.01, 1.33	1.37	1.22, 1.55	1.30	1.11, 1.53
Glucose (mmol/L) <sup>‡</sup>	5.09	4.79, 5.41	5.32	5.06, 5.61	5.50	5.12, 5.87
hs-CRP (mg/L)**	0.69	0.43, 1.09	0.83	0.54, 1.27	0.87	0.50, 1.50
hs-IL-6 (pg/mL)	1.42	1.09, 1.99	1.46	1.09, 1.96	1.46	0.99, 2.15
Adiponectin (ng/mL)	7.19	6.08, 8.51	7.62	6.44, 9.59	7.87	6.44, 9.59
Malondialdehyde (nmol/mL)	6.77	6.24, 7.30	7.11	6.63, 7.58	6.46	5.84, 7.08

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385 T, tertile; CI; confidence interval; VAT visceral adipose tissue; SAT, subcutaneous adipose tissue; SBP, systolic blood  
386 pressure; DBP, diastolic blood pressure; HR, heart rate; LDL, low-density lipoprotein; HDL, high-density lipoprotein;  
387 hs-CRP, high sensitivity C-reactive protein; hs-IL-6, high sensitivity interleukin-6. <sup>a</sup>Adjusted average indices as least-  
388 square means with 95%CI adjusted for baseline value, sex, age, type 2 diabetes mellitus, time since coronary event  
389 and change in moderate-to-vigorous activity levels. \*Significant difference between T1 and T3,  $p<0.05$ . <sup>†</sup>Variable  
390 log-transformed and data are presented as adjusted geometric means and confidence intervals have been backwards  
391 logged, except for MDA. <sup>‡</sup>One participant with T2DM had a major increase in insulin dosage and was excluded from  
392 analyses. \*\*Two participants excluded for value  $>10\text{mg/L}$ .

393

394

395 **4. Discussion**

396

397 This study has reported on the effect of a 6-month intervention with *ad libitum* MedDiet versus low-fat diet on  
398 adiposity, anti-inflammatory marker adiponectin and oxidative stress marker MDA in a cohort of patients with

399 CHD. The results demonstrated that the MedDiet significantly reduced SAT but not VAT area compared to  
400 the low-fat diet. Despite significantly improved adherence to the Mediterranean dietary pattern (29), there was  
401 no significant effect of the MedDiet on adiponectin, MDA or classic CVD risk markers of lipids, glucose or  
402 blood pressure compared with the low-fat diet. Notable within group findings were a reduction in the  
403 proportion of MedDiet participants prescribed  $\beta$ -blockers and increased LDL cholesterol levels in the low-fat  
404 diet group. Across tertiles of increasing improvement in MedDiet adherence score in the pooled study cohort  
405 at 6-months, a significantly lower waist circumference, WHR and SAT area was observed.

406

407 The significant improvement in the SAT but not the VAT area following MedDiet compared to the low-fat  
408 diet was unexpected. Two previous studies (50, 51) reported a significant reduction in markers of VAT  
409 (measured by bioelectrical impedance analysis or ultrasound) following MedDiet intervention. One of these  
410 studies also demonstrated that MedDiet intervention did not significantly impact subcutaneous fat (50). Both  
411 previous interventions employed energy restrictions and were conducted in patients without CVD, which may  
412 explain why no reduction in VAT was observed in the current study of an *ad libitum* MedDiet and the first in  
413 CHD patients. The reduction in subcutaneous fat in the present study is contradictory to previous findings that  
414 intake of MUFA (which AUSMED participants substantially increased) favours deposition as subcutaneous  
415 rather than visceral fat (52). The lack of improvement in VAT with our MedDiet intervention assists to explain  
416 the lack of significant effect on inflammatory markers given VAT represents more metabolically dysfunctional  
417 tissue (16, 17). There is an established protective effect of exercise on visceral fat (53) and chronic  
418 inflammation in patients with CHD (54). Therefore, whilst changes in MVPA were controlled for, it cannot be  
419 ruled out that a lack of improvement in VAT area and inflammatory biomarkers was related to an observed  
420 reduction in MVPA levels in some MedDiet participants.

421

422 The maintenance of weight and trend for reduction in total body fat in the MedDiet group occurred despite the  
423 tendency of the group to increase total energy intake (29). These findings assist to discount the belief that the  
424 high healthy fat MedDiet is associated with weight and fat gain (55) and could be related to the high content  
425 of unsaturated fats, particularly MUFA and omega-3 PUFA, in the MedDiet. These unsaturated fats have been  
426 shown to be associated with increased lipid oxidation and thermic effect (56, 57). Furthermore, in a cohort of

427 Australian patients with T2DM ( $n=27$ ) a 12-week *ad libitum* MedDiet intervention resulted in a small reduction  
428 in body weight, despite significantly increased energy and MUFA intake (13).

429

430 This is the first study to examine the effect of MedDiet on the anti-inflammatory marker adiponectin in patients  
431 with diagnosed CHD. No significant change was detected in this pilot cohort, with only a trend for reduction  
432 in the MedDiet compared to low-fat diet group observed. Adiponectin has been reported in previous MedDiet  
433 intervention studies that have been conducted in subject groups without CHD diagnosis. In a study of pre-  
434 menopausal obese women adiponectin increased with a calorie-restricted MedDiet compared to general  
435 diet/exercise advice (58). A sub-study of the PREDIMED trial in patients with T2DM also demonstrated an  
436 increase in plasma adiponectin, but this increase occurred with all three (Mediterranean + EVOO,  
437 Mediterranean + Nuts and low-fat) diet interventions; mean weight loss was significant but less than 1kg in  
438 each group (9). It was also found that a MedDiet in the absence of weight loss can significantly reduce  
439 inflammation (composite score of CRP, IL-6 and tumour necrosis factor- $\alpha$ ) (59) but not levels of adiponectin  
440 (60). The DIRECT study, which included a MedDiet intervention with 6-month weight loss phase followed by  
441 an 18-month weight maintenance phase, demonstrated a continued significant increase in adiponectin for the  
442 duration of the trial (61). Most of these findings suggest that a significant increase in adiponectin with MedDiet  
443 is dependent on concomitant weight loss (at least initially), which helps to explain the lack of significant effect  
444 on adiponectin in the current study with an *ad libitum* approach. Our results estimated that without weight loss  
445 (and no change in VAT), twice the sample size would be required to demonstrate a significant improvement  
446 in adiponectin with MedDiet compared to low-fat diet.

447

448 This was one of first studies to examine the effect of a dietary intervention on oxidative stress marker plasma  
449 MDA in patients with CHD and no significant effect of the MedDiet compared to low-fat diet was found.  
450 Similarly, a previous study of MedDiet intervention versus control (habitual) diet in patients with Rheumatoid  
451 Arthritis and on stable pharmacological treatment ( $n=51$ ) demonstrated no effect on MDA levels in urine (62).  
452 A preliminary study in a subset of PREDIMED participants ( $n=71$ ), at high risk of but without CHD, did  
453 demonstrate a significant improvement in MDA with 3-month MedDiet versus low-fat diet (27). However,  
454 MDA was measured from peripheral blood mononuclear cells (rather than circulating levels as measured in

455 our study) and the changes in MDA paralleled improvements in oxidised LDL levels. Recent meta-analysis  
456 also demonstrated evidence to support that consumption of extra virgin olive oil reduces MDA levels, however,  
457 the four intervention studies each prescribed ~70 g oil per day and were conducted in healthy adults (63).

458

459 Of particular interest, a significant number of MedDiet participants (15%) stopped taking  $\beta$ -blocker medication  
460 during the trial. Despite cessation of this antihypertensive medication, for the MedDiet cohort no significant  
461 change in mean SBP, DBP or HR was detected. A potential reduction in need for this medication with the  
462 MedDiet is a promising finding as  $\beta$ -blockers have a range of short and long-term side effects (44). This finding  
463 warrants for the effect of the MedDiet on cardiac medication to be investigated further. The present study also  
464 demonstrated no significant effect of the MedDiet on LDL cholesterol, triglycerides or glucose, compared to  
465 the low-fat diet. These results were not unexpected considering that most participants were prescribed statins  
466 or other lipid-lowering therapy as well as anti-hypertensives, and nearly all participants with T2DM were  
467 taking hypoglycaemic agents. Interestingly, the low-fat diet group significantly increased LDL cholesterol  
468 levels after 6-months. This contradicts the premise of the low-fat diet, which was designed to lower LDL  
469 cholesterol levels. This finding may be reflective of the lack of improvement in adherence to the low-fat diet  
470 principles seen in that group, and their slight increase in saturated fat intake (29).

471

472 Our study had a number of strengths. The intensity of the dietary counselling was the same in both groups to  
473 control for this effect. In both study groups the focus of the intervention was dietary improvement only and  
474 the approach was *ad libitum* in order to isolate the effect of diet rather than changes in weight loss or improved  
475 physical activity. The secondary analyses in the pooled cohort allowed for the potential effect of greater  
476 magnitude of improvement in adherence to the MedDiet pattern, including within the low-fat diet group, to be  
477 explored. We also demonstrated that there were no significant differences in access to other health services or  
478 changes in types of medication or supplements taken between the groups, except for a reduction in use of  $\beta$ -  
479 blockers in the MedDiet group. Finally, intention-to-treat analyses were performed which meant that dropouts  
480 were accounted for in all analyses.

481

482 This study was however limited by the small size of a preliminary cohort of AUSMED participants, and hence  
483 was underpowered. Based on the results in these patients, the reverse power calculations which were performed  
484 for novel markers adiponectin and MDA estimated that a sample size double and close to seven times that of  
485 the current sample would be required to detect a significant effect of the *ad libitum* MedDiet compared to low-  
486 fat diet on these markers respectively in a CHD patient setting. These results will inform future studies and  
487 analyses. The patients recruited in this study represent a lower proportion of females and are potentially more  
488 health conscious/motivated than ACS patients in the broader Australian population (64), which may impact  
489 the generalisability of the results. Nonetheless, the results of this study may be generalisable to other non-  
490 Mediterranean, multicultural populations. Finally, whilst Hologic DXA measurement for VAT and SAT area  
491 are validated, they provide an estimate only.

492

#### 493 *Conclusions*

494 In a small cohort of Australian patients with CHD a 6-month *ad libitum* MedDiet nor low-fat diet intervention  
495 led to significant improvement in adiponectin or plasma MDA levels. A lack of significant change in weight  
496 and trends for improved body fat and waist circumference assists to discount the continued misconception that  
497 a diet high in healthy fats, such is the MedDiet, leads to weight or fat gain. Greater improvement in MedDiet  
498 adherence was associated with lower waist circumference, however, this was associated with lower SAT and  
499 not VAT area, which was unexpected and may explain a lack of significant effect on measured cardiometabolic  
500 risk markers. Future studies are needed in larger cohorts. Nonetheless, in CHD patients taking intensive  
501 medications, significant clinical effects of the MedDiet on these markers may require adjunct exercise  
502 intervention and/or caloric restriction.

503

#### 504 **Declarations**

505

#### 506 *Ethics approval and consent to participate*

507 This study was approved by the Human Research Ethics Committees of the Northern Hospital  
508 (HREC/16/Austin/500), St Vincent's Hospital Melbourne (HREC-A; 016/13), and La Trobe University  
509 (#FHEC13/159). Written informed consent was obtained from all participants.

510

511 *Availability of data and materials*

512 The datasets used and/or analysed during the current study are available from the corresponding author on  
513 reasonable request.

514

515 *Competing interests*

516 The Mediterranean Diet by Itsiopoulos (2013) (ISBN 9781742610825) was provided to the Mediterranean diet  
517 group participants as a dietary resource. Otherwise the authors have no conflicts of interest to declare.

518

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525

526 *Authors' contributions*

527 TK, CI and ACT conceptualised and designed the research. HLM collected the presented data, analysed data  
528 and interpreted results (with support from, CI, ACT, JR, CJT, MG and JW). HLM, JR and MG were involved  
529 in laboratory analyses of pathology markers. HLM wrote the draft manuscript. All authors critically reviewed,  
530 edited and approved the manuscript.

531

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545 her assistance with recruitment of participants and conducting appointments.

546

#### 547 **Supplementary Materials**

548 CONSORT 2010 checklist of information to include when reporting a randomised trial. Completed for current  
549 AUSMED study.

550 **Table S1.** Proportion of participants taking prescribed medications and supplements across intervention time  
551 points in the study groups.

552 **Table S2.** Frequency of attendance for study appointments and phone reviews and access to other health  
553 services during the intervention period in the total cohort and within study groups.

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