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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**

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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^2 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^2, 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

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 (1, 2). Nonetheless, the majority of studies investigating the MedDiet have been conducted in Mediterranean countries. A low-fat diet was the standard care recommendation for prevention and treatment of CHD in Australia for many years (3), however, 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 (4).

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 (5). A number of studies, including in the Australian setting, have demonstrated that the MedDiet improves 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) (6-13). 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 (14), 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 may 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 (15). In addition, obesity, especially increased visceral fat, is causally linked to chronic low-grade inflammation (16, 17). In an obese state, adipose tissue generates pro-inflammatory adipokines, including interleukin-6 (IL-6), whereas anti-inflammatory adipokines, including adiponectin, are down-regulated (18). High serum concentrations of adiponectin are associated with decreased risk of CHD (19, 20). Oxidative stress has also been recognised to increase risk of cardiovascular events in

3

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 characters, 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 IntellisenSense, 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 [β]-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 °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® statistical package version 25 (IBM Corp, released 2015).

215   Statistical significance was set at *p*<0.05. Data are presented as means ± 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)
Sociodemographic		
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)
Lifestyle		
Current smoker	3 (9.7)	6 (18.2)

>100 cigarettes in lifetime	18 (58.1)	20 (58.8)
BMI (kg/m^2)	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)
Medical History		
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 ± 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 ± 6.5 cm^2 in the MedDiet vs. +0.4 ± 6.9 cm^2 in the low-

307   fat diet group, $p=0.04$; Figure 1). VAT area did not change within the MedDiet (-0.1 ± 3.8 cm^2) or the low-fat

308   diet (+2.4 ± 4.0 cm^2) 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
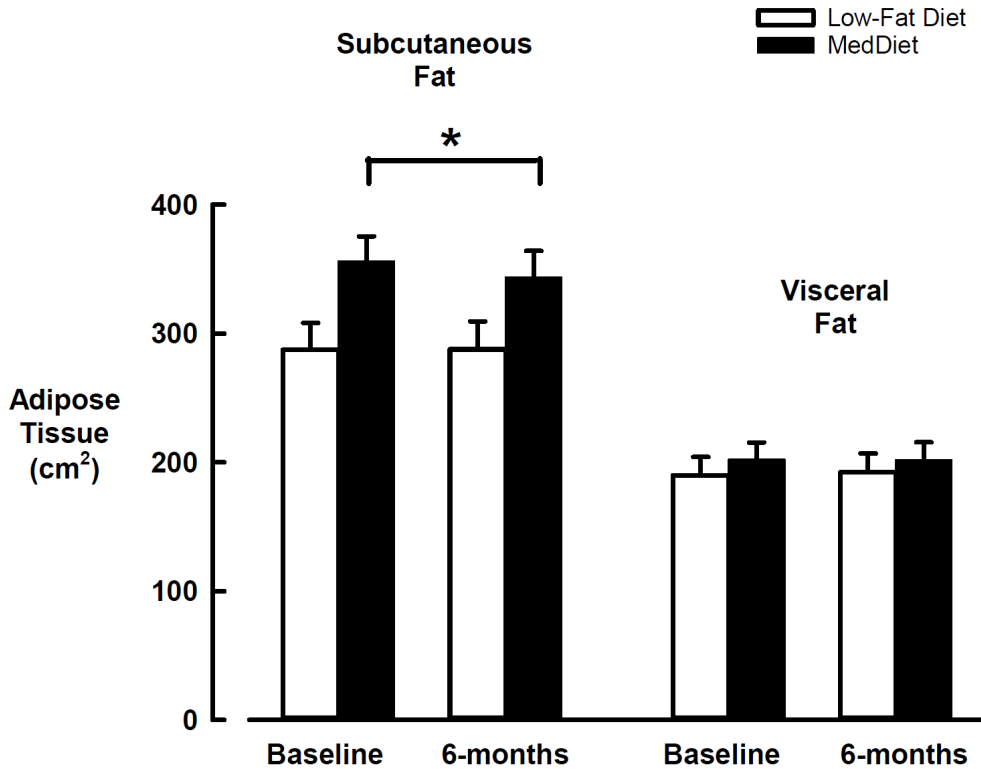
316

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 ± 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 α<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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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**
Activity levels						
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
Anthropometry						
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^2)	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
Body composition						
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^2)	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[a]	0.31	0.04*
Haemodynamic						
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*
Pathology						
LDL (mmol/L)†	1.73 ± 0.13	1.95 ± 0.15[a]	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)†	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; †Non-parametric, analyses based on transformed variable. ‡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 ªdifference 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^2, 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[a]**

Marker	Tertile 1 (-2 to +1 points) n=22		Tertile 2 (+2 to 5 points) n=27		Tertile 3 (+6 to 9 points) n=15	
	Adjusted mean	95% CI	Adjusted mean	95% CI	Adjusted mean	95% CI
Anthropometry						
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^2)	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*
Body composition						
Fat mass index (kg/m^2)	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^2)	196.0	186.0, 206.0	200.0	190.9, 209.0	195.3	183.5, 207.1
SAT (cm^2)	331.7	315.0, 348.4	313.4	298.3, 328.6	304.3	284.3, 324.3*
Haemodynamic						
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†

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)‡	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

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. [a]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, $p<0.05$. †Variable

390 log-transformed and data are presented as adjusted geometric means and confidence intervals have been backwards

391 logged, except for MDA. ‡One participant with T2DM had a major increase in insulin dosage and was excluded from

392 analyses. **Two participants excluded for value >10mg/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 β-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-α) (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 β-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 β-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 β-

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.

537 in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to

538 publish the results.

539

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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