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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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Abstract

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

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

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

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

Trial registration: ACTRN12616000156482.

Keywords: Coronary disease; Mediterranean diet; low-fat diet; adiponectin; oxidative stress; body composition

1. Introduction

The Mediterranean diet (MedDiet) pattern has a strong scientific evidence base for reducing risk of coronary heart disease (CHD) and adverse cardiovascular disease (CVD) events (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 patients with CHD, through increased oxidation of LDL particles and endothelial dysfunction (21). Plasma malondialdehyde (MDA), a non-invasive measure of lipid peroxidation, is a recognised marker of oxidative stress and elevated MDA levels are reported in patients with CHD (22).

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

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

2. Materials and Methods

2.1 Study Design

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

2.2 Recruitment of CHD Patients

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

2.3 Randomisation of Participants and Diet Interventions

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

2.3.1 Mediterranean Diet

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

2.3.2 Low-fat Diet

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

2.4 Study Measures

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

2.4.1 Dietary Intake

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

2.4.2 Cardiometabolic Risk Markers

Our methods for assessment of activity levels, anthropometry, body composition, blood pressure and pathology measures have also been described previously (31). Increased physical activity was not a target of this intervention, however, physical activity levels were assessed to account for any potential confounding effects on outcome markers. Participants wore a triaxial Actigraph accelerometer (WGT3X-BT; Actigraph Corp, Florida, United States) for one week prior to their appointments. Established criteria (39) were used to determine time spent as min /week in moderate-to-vigorous physical activity (MVPA) or as sedentary time. Weight, height and waist circumference measures were performed according to the International Society for the Advancement of Kinanthropometry (ISAK) standards for anthropometric assessment (40). Whole body composition was measured using a fan beam densitometer Dual-energy X-ray Absorptiometry (DXA) machine (Hologic, Discovery W, USA), with analysis performed using QDR™ (Quantitative Digital Radiography) for Windows. Measurements obtained from each scan were total body lean and fat mass, total body and regional fat percentage, subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT) areas. Hologic scientists developed their method for measuring VAT from DXA (41), which is highly correlated (r=0.93) and linearly related to VAT measurements by computed tomography (42). Fat mass index (FMI) was calculated by dividing the total body fat mass (kg) by height (m) squared (43). Systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate (HR) were measured using an automated blood pressure monitor (OMRON Tp9, IntellisenSense, Australia). Hypertension (presence or history of) was classified based on whether the participants were prescribed medication with anti-hypertensive effect (angiotensin converting enzyme [ACE] inhibitor, angiotensin 2 receptor blocker, Beta [β]-blocker or Ca^{2+} channel blocker) and/or mean baseline blood pressure reading of SBP >140 mmHg or DBP >90 mmHg (44). Diagnosis of T2DM was determined by consulting participant medical history records.

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

2.5 Statistical Analyses

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

All outcome measures were analysed based on intention-to-treat with missing data included by bringing baseline or 3-month observations forward, assuming no change (47). Cochran's Q test assessed changes in the proportion of participants taking medication and supplement classes from baseline to 3- and 6-months within each study group. Repeated measures ANOVA (analysis of variance) assessed changes in cardiometabolic risk marker variables from baseline to 6-months between groups. Measures which were non-parametric were log transformed to improve their distribution. The main ANOVA results assessed for effect were (1) group (significant change in one study group compared to the other) and (2) time (significant change in pooled study groups). Post-hoc tests were performed to determine within-group changes (Paired Samples *t*-test). Analyses for all risk markers were adjusted for change in MVPA and haemodynamic and pathology measures were additionally adjusted for baseline BMI. The repeated measures analysis inherently controlled for participant characteristics not subject to change and were not different between study groups at baseline, including sex and T2DM status. The between-group findings for adiponectin and MDA levels were used to perform a sample size calculation to inform future analyses (described in Results).

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

3. Results

3.1 Participants

Randomisation to diet study groups, completion of study appointments and number and reasons for withdrawal have been reported elsewhere (28, 29). Briefly, of 73 randomised participants, 65 attended a baseline appointment and started the intervention. The subsequent attrition rate was 14%, with 2 and 7 participants dropping out from the low-fat diet and MedDiet groups respectively. Participants were lost to follow up (n=2) or discontinued due to relocation (n=2), non-cardiac medical problems (n=3) or family related issues (n=2). There were no significant differences for sociodemographic or clinical characteristics between those participants that dropped out compared to completers.

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

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)

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

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

3.2 Dietary Intake

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

3.3 Activity Levels

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

3.4 Anthropometry and Body Composition

With regards to anthropometry and body composition measures, there was a significant between-group 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-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 diet (+2.4 ± 4.0 cm^2) groups (p=0.58, Figure 1). There were no significant within-group changes for weight, BMI, waist circumference, or waist-hip ratio (Table 2). There was no significant reduction in waist circumference over time in the pooled study groups (-1.1 cm, p=0.07 within the MedDiet and -0.4 cm, p=0.52 within in the low-fat diet group). There was also no significant reduction in total body fat % over time in the pooled study groups (-0.6%, p=0.06 within the MedDiet and -0.4%, p=0.23 within in the low-fat diet group).

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

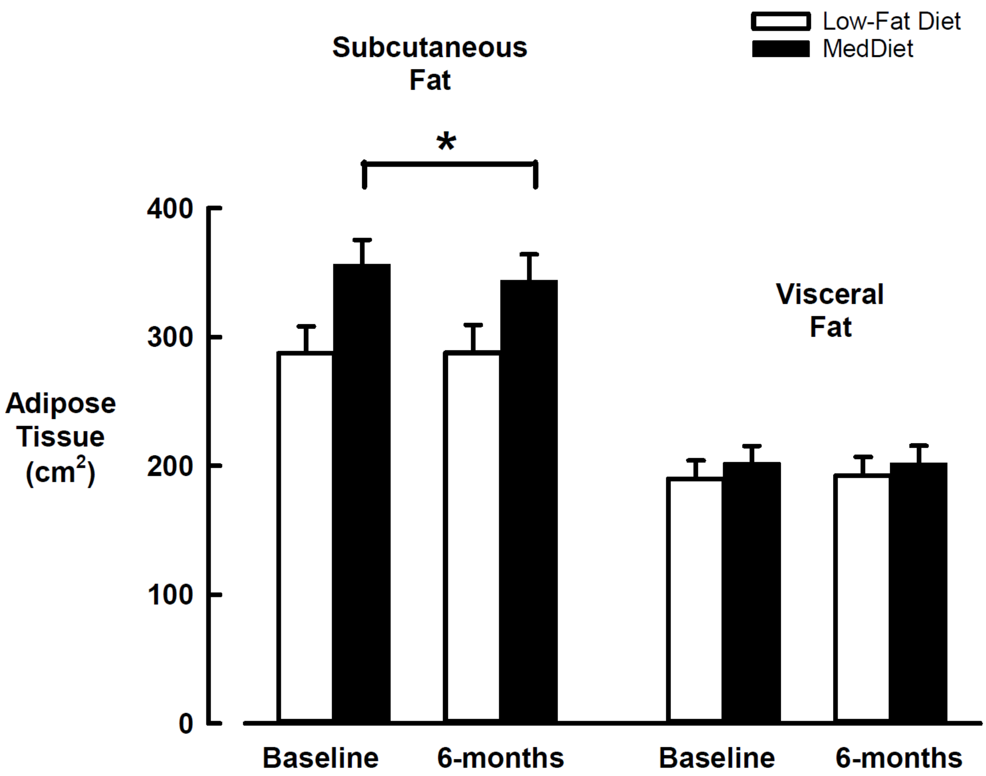

Figure 1. Subcutaneous and visceral adipose tissue measured by dual energy x-ray absorptiometry at baseline and end-intervention in the MedDiet and low-fat diet groups. Data are mean ± SEM with adjustment for change in moderate to vigorous physical activity levels. MedDiet, Mediterranean diet. *Significant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

3.5 Haemodynamic, Cholesterol and Glucose Measures

There were no between-group changes for any of the reported haemodynamic, cholesterol or glucose markers, adjusted for MVPA change and baseline BMI (Table 2). There was a significant reduction in resting HR in the pooled study groups (*p*=0.03), with a trend for greater reduction in the MedDiet (-1.5 bpm, p=0.07) compared to the low-fat diet (-0.8 bpm, p=0.55) group. With regards to lipids, the only significant within-group finding was an increase in LDL cholesterol between baseline and 6-months (+0.22 mmol/L, *p*=0.006) in the low-fat diet group. There were no changes within either study group for triglycerides or fasting glucose levels (also assessed separately for T2DM status).

3.6 Adiponectin

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

3.7 Malondialdehyde

There were no between-group changes for plasma MDA levels (p=0.75) adjusted for MVPA change and baseline BMI (Table 2). There was also no significant change in MDA levels between baseline and 6-months 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 also used to perform a sample size calculation as above. Based on the study group effect size of 0.045 and a correlation value between MDA levels at baseline and 6-months of r=0.775, a sample size of 444 participants would be required to detect a significant effect of the MedDiet on MDA levels compared to the low-fat diet.

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

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

3.8 Association Between Mediterranean Diet Adherence and Risk Markers

All participants were categorised into tertiles of change in MEDAS score from baseline to 6-months. This resulted in tertile 1 (T1) of -2 to +1, tertile 2 (T2) of +2 to 5, and tertile 3 (T3) of +6 to 9. As expected, in T3, with the largest 6-month improvement in MedDiet adherence, 93% of participants were from the MedDiet group. In T2 and T1 the proportion of participants in the MedDiet group was 56% and 22%, respectively. Mean (95% CI) levels for cardiometabolic risk markers at 6-months, adjusted for baseline value, sex, age, T2DM, time since coronary event and change in MVPA, are presented in Table 3. For each of the reported anthropometric, body composition and hemodynamic measures the mean value decreased across tertiles from T1 to T3 (from lowest to greatest MEDAS score improvement), except for VAT area and DBP, which had a higher mean value in T2, followed by T1 and then T3. Compared to T1, T3 participants had a significantly lower mean waist circumference (-2.81 cm, *p*=0.01), waist-hip ratio (-0.022, p=0.047) and SAT area (-27.4 cm^2, p=0.04). Mean levels of other pathology markers did not demonstrate any consistent trends across tertiles. For adiponectin, the mean value increased slightly but with no significant difference across tertiles from lowest to greatest MEDAS score improvement (+0.68 ng/mL from T1 to T3, *p*=0.50). There was also no difference between tertiles for plasma MDA level (-0.31 nmol/mL from T1 to T3, p=0.46).

Table 3. Adjusted means of cardiometabolic risk markers at 6-months by tertiles of change in 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

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

4. Discussion

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

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

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

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

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

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

This was one of first studies to examine the effect of a dietary intervention on oxidative stress marker plasma MDA in patients with CHD and no significant effect of the MedDiet compared to low-fat diet was found. Similarly, a previous study of MedDiet intervention versus control (habitual) diet in patients with Rheumatoid Arthritis and on stable pharmacological treatment (n=51) demonstrated no effect on MDA levels in urine (62). A preliminary study in a subset of PREDIMED participants (n=71), at high risk of but without CHD, did demonstrate a significant improvement in MDA with 3-month MedDiet versus low-fat diet (27). However, MDA was measured from peripheral blood mononuclear cells (rather than circulating levels as measured in our study) and the changes in MDA paralleled improvements in oxidised LDL levels. Recent meta-analysis also demonstrated evidence to support that consumption of extra virgin olive oil reduces MDA levels, however, the four intervention studies each prescribed ~70 g oil per day and were conducted in healthy adults (63).

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

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

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

Conclusions

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

Declarations

Ethics approval and consent to participate

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

Availability of data and materials

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

Competing interests

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

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Authors' contributions

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

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Supplementary Materials

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

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

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

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