Mediterranean-type diets and inflammatory markers in patients with coronary heart disease: a systematic review and meta-analysis

Mayr, Hannah L.; Tierney, Audrey C.; Thomas, Colleen J.; Ruiz-Canela, Miguel; Radcliffe, Jessica; Itsiopoulos, Catherine

Published in:
Nutrition Research

DOI:
10.1016/j.nutres.2017.10.014

Mediterranean-type diets and inflammatory markers in patients with coronary heart disease: a systematic review and meta-analysis

Hannah L Mayr, Audrey C Tierney, Colleen J Thomas, Miguel Ruiz-Canela, Jessica Radcliffe, Catherine Itsiopoulos

PII: S0271-5317(17)30231-2
DOI: doi: 10.1016/j.nutres.2017.10.014
Reference: NTR 7818

To appear in: *Nutrition Research*

Received date: 27 March 2017
Revised date: 27 September 2017
Accepted date: 6 October 2017

Please cite this article as: Mayr Hannah L, Tierney Audrey C, Thomas Colleen J, Ruiz-Canela Miguel, Radcliffe Jessica, Itsiopoulos Catherine, Mediterranean-type diets and inflammatory markers in patients with coronary heart disease: a systematic review and meta-analysis, *Nutrition Research* (2017), doi: 10.1016/j.nutres.2017.10.014

Mediterranean-type diets and inflammatory markers in patients with coronary heart disease: a systematic review and meta-analysis

Hannah L Mayr[a,d], Audrey C Tierney[a], Colleen J Thomas[b], Miguel Ruiz-Canela[c], Jessica Radcliffe[a], Catherine Itsiopoulos[a].

[a] Department of Rehabilitation, Nutrition and Sport, School of Allied Health, and [b] Department of Physiology, Anatomy and Microbiology, School of Life Sciences, La Trobe University, Melbourne, Victoria, Australia, 3086. [c] Department of Preventive Medicine and Public Health, University of Navarra, Navarra, Pamplona, Spain, 1, 31008. [d] Department of Nutrition and Dietetics, Northern Health, Melbourne, Victoria, Australia, 3076.

Email contacts:

HLM: h.mayr@latrobe.edu.au, ACT: A.Tierney@latrobe.edu.au, CJT: colleen.thomas@latrobe.edu.au, MRC: mcanela@unav.es, JR: J.Radcliffe@latrobe.edu.au; CI: C.Itsiopoulos@latrobe.edu.au

Corresponding author:

Ms. Hannah L Mayr

Nutrition and Dietetics, Level 4, Health Sciences 3 Building, Kingsbury Drive, La Trobe University, Bundoora, Victoria, 3086 Australia.

Phone: +61 - 3-9479 5953

Email: h.mayr@latrobe.edu.au

Abbreviations

CHD; coronary heart disease

CRP; C-reactive protein

ACS; acute coronary syndrome

MI; myocardial infarction

TNF-α; tumor necrosis factor-alpha

RCT; randomized controlled trial

LDL; low density lipoprotein

T2DM; type 2 diabetes mellitus

MUFA; monounsaturated fatty acids

PUFA; polyunsaturated fatty acids

SFA; saturated fatty acids

SD; standard deviation

CVD; cardiovascular disease

BMI; body mass index

AHA, American Heart Association

EPA; eicosapentaenoic acid

DHA; docosahexaenoic acid

IL; interleukin

EVOO; extra virgin olive oil

DASH; Dietary Approaches to Stop Hypertension

Abstract

The health benefits of a Mediterranean diet are thought to be mediated via its anti-inflammatory effects; however, the anti-inflammatory effect of this diet is unclear in patients who have already developed coronary heart disease (CHD). This systematic review and meta-analysis assessed the effect of Mediterranean-type diets on cytokines and adipokines in patients with CHD. An electronic search of the literature was conducted up to October 2016 using PubMed, Scopus, Web of Science and Cochrane Library. Eleven of the 435 articles identified met eligibility criteria. Four observational studies reported significant inverse associations between Mediterranean-type diet scores and inflammatory cytokines. Five clinical trials (four in non-Mediterranean countries) demonstrated non-significant reductions and two trials conducted in Spain demonstrated significant reductions in C-reactive protein (CRP) with a Mediterranean-type diet. Random effects meta-analysis of four controlled trials detected a non-significant difference in final mean value of CRP with Mediterranean-type diet versus low fat diet. Despite promising findings from observational studies, this review demonstrated mostly non-significant effects of Mediterranean-type diet interventions on inflammatory cytokines and no effect in comparison to low fat diets in controlled trials conducted primarily in Mediterranean populations. Therefore, randomized controlled trials of a traditional Mediterranean diet in non-Mediterranean populations and with multiple inflammatory biomarkers are needed in the high risk CHD patient group.

Keywords

Mediterranean diet, coronary disease, inflammation, C-reactive protein, adipokines, meta-analysis

1. Introduction

Atherosclerosis is an inflammatory disease [1]. Circulating biomarkers of inflammation lead to endothelial damage which initiates the infiltration of macrophages and plaque formation at the vessel wall [1]. Furthermore, inflammatory cytokines increase the activity of macrophages and vascular smooth muscle cells at the site of arterial plaque and this increased activity renders the plaque more vulnerable to rupture [2, 3]. Chronic inflammation is therefore involved in the progression of coronary heart disease (CHD) and occurrence of acute coronary syndrome (ACS), including myocardial infarction (MI) [3].

Plasma levels of inflammatory biomarkers such as C-reactive protein (CRP), interleukins, and adipokines such as tumor necrosis factor-alpha (TNF-α) and adiponectin, are independent predictors of primary as well as secondary incidence of coronary disease [4-6]. They have emerged as targets for treatment of CHD, beyond the classic risk factor target of cholesterol [7]. Notably, patients who have low CRP levels after statin therapy have better clinical outcomes, regardless of the resultant level of low density lipoprotein (LDL)-cholesterol [8].

Dietary patterns influence CHD risk via multiple mechanisms including reducing inflammation [9]. Western dietary patterns which are typically high in red meats, full-fat dairy products and refined grains are considered pro-inflammatory [10] and healthy dietary patterns (such as low-fat, plant-based or Mediterranean diets) are considered anti-inflammatory [11-13]. The healthy dietary pattern which has the strongest evidence for reduction in markers of inflammation in randomized controlled trials (RCTs) is a Mediterranean diet [14, 15]. Importantly, most of the dietary interventions have been conducted in populations without CHD and in Mediterranean countries.

The Mediterranean diet has the strongest evidence for secondary prevention of CHD. In the 1990s, the Lyon Diet Heart study demonstrated a significant reduction in incidence of secondary ACS with a Mediterranean diet intervention compared to control low fat diet [16]. The mechanism of dietary influence was not identified by the study investigators and recognized markers of inflammation were not measured. In non-Mediterranean countries, low fat prudent dietary patterns which have less anti-inflammatory potential are still recommended in the management for CHD [17, 18].

A Mediterranean diet has potential to significantly impact clinical outcomes in patients with diagnosed CHD through its anti-inflammatory properties. It is, however, unclear whether a Mediterranean diet has additional anti-inflammatory effects in this patient group who are on intensive medications, and whether this diet improves inflammatory markers more than standard care diets prescribed. The aim of this systematic review and meta-analysis was to evaluate the literature on the influence of a Mediterranean-type diet on biomarkers of inflammation in patients with diagnosed CHD and/or who have experienced ACS.

2. Approach

This systematic literature review followed the requirements of the PRISMA statement (Supplemental Table S1) [19]. The review was registered in PROSPERO, the international prospective register of systematic reviews (http://www.crd.york.ac.uk/ PROSPERO; registration number: CRD42016050970).

2.1 Search strategy

One study author (HLM) conducted a systematic search of the electronic databases: PubMed, Scopus, Web of Science and Cochrane Library (all years to 22 October 2016). The search strategy included terms relating to the population, intervention/exposure and the outcome. Terms included ("coronary heart disease" OR "acute coronary event" OR "acute coronary syndrome" OR "myocardial infarct*" OR "coronary artery bypass" OR "coronary intervention") in combination with ("Mediterranean diet" OR OR "Mediterranean-style diet" OR "Mediterranean-type diet" OR "Med-diet" OR "high monounsaturated fat*" OR "MUFA diet") and in combination with ("inflammation" OR "cytokine" OR "adipokine" OR "c-reactive protein" OR "CRP" OR "interleukin" OR "IL-" OR "adiponectin" OR "tumor necrosis factor" OR "TNF"). The publications were limited to English language. The complete search strategies for all four databases are provided in Supplemental Table S2.

2.2 Selection criteria

The inclusion and exclusion criteria were developed using a PICOS (population, intervention, comparison, outcomes, study design) structure (Table 1). To be included in this review, studies needed to report on the effect of a Mediterranean-type diet score or intervention on recognized biomarker(s) of inflammation in a CHD population. Letters, reviews, conference proceedings and abstract only publications were excluded. Individual studies were not limited according to study design as few publications combined the three elements of criteria.

Studies were required to meet the following inclusion criteria: (1) male and/or female adults (≥18 years of age) with diagnosed CHD and/or who have experienced ACS: unstable angina, angiographic diagnosed arterial disease, MI, coronary artery bypass grafting or percutaneous

coronary intervention. Populations with both CHD and Type 2 Diabetes Mellitus (T2DM) were included. (2) Application of a Mediterranean-type diet as a diet adherence measure or intervention, classified as encompassing at least three of these key components of the traditional Greek Mediterranean diet pattern [20, 21]: high in plant foods including wholegrain cereals, fruit, vegetables and/or legumes; high in monounsaturated fatty acids (MUFA) including olives/olive oil and/or nuts; high in omega-3 polyunsaturated fatty acids (PUFA) including fish or other seafood and/or nuts high in omega-3; low in food rich in saturated fatty acids (SFA) reducing margarine/butter and/or commercial goods and/or meat. Studies were included regardless of whether red wine with meals was included. Studies were not excluded if the Mediterranean-type diet intervention was part of a broader cardiac rehabilitation program or with co-intervention such as exercise or stress management. Studies reporting only postprandial effects of the diet were excluded. (3) Cytokine or adipokine measure(s) reflecting systemic inflammation, where the association with or effect of the diet could be isolated.

2.3 Study selection

Articles were initially screened based on title and abstract by one reviewer (HLM). The full text of these potentially eligible studies was retrieved and independently assessed for complete eligibility by two review team members (HLM and ACT). Any disagreements between reviewers were resolved by consensus. In addition, reference lists of all screened full text articles were reviewed to identify additional relevant articles that were also assessed against eligibility criteria.

2.4 Data extraction

Data was extracted using a standardized form by one reviewer (HLM) and cross-checked for accuracy by the review team. Extracted information included: citation, location and study design; eligibility criteria; population sample characteristics including size, demographics and relevant medical history; follow-up length; details of the Mediterranean-type diet adherence score or intervention and any control/comparison score or intervention; change in dietary intake if reported in intervention trials; inflammatory biomarker outcomes and times of measurement; and information for assessment of the risk of bias. Prior publications reporting on the same study, such as protocol papers, were consulted for missing data on eligibility criteria or sample characteristics. Missing outcome data was requested from study authors.

2.5 Quality assessment

Risk of bias in each included study was assessed by two reviewers (HLM and ACT) using the Academy of Nutrition and Dietetics *Quality Criteria Checklist: Primary Research* [22] (see Supplemental Figure S1 for the complete checklist). Studies were assessed on 10 key criteria assessing internal and external validity. Each study was awarded an overall positive (scores ≥8/10), neutral (5 to 7/10) or negative (<5/10) method quality rating. Risk of bias was also assessed across studies, with consideration of the number of studies that achieved high or low risk in each of the 10 key criteria and study design.

2.6 Summary measures

For observational studies, no summary of outcome measures was performed since studies reported results differently. For intervention trials, the mean change in inflammatory markers

pre- and post- Mediterranean-type diet intervention and in comparison to the control diet, if present, was the principal summary measure. A p-value <0.05 was set as the level of significance for reported between or within group differences in individual studies. If the inflammatory marker mean ± standard deviation (SD) post-intervention was reported for the Mediterranean-type diet and an alternative control diet, an effect size (Cohen's *d*) was calculated and classified as small (<0.20), medium (2.0 to 0.50) or large (0.5 to 0.80) [23].

2.7 Statistical analyses

Study outcomes with sufficient controlled trial data were pooled using the software program Review Manager (RevMan Version 5.3, Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014). A random effects meta-analysis [24] was conducted to assess the weighted mean differences (with 95% confidence intervals) in outcome mean values. Of studies included in the meta-analysis the heterogeneity between studies was assessed using the Cochran Q test and the I^2 statistic. The I^2 was calculated using the formula: $I^2 = 100\% \times (Q - df)/Q$, where Q is the chi-squared statistic, and df is the degrees of freedom [25]. An I^2 value of 75% or greater was deemed to indicate a high level of heterogeneity [25]. Significance was set at the level of p-value <0.05.

3. Results

3.1 Study selection

Figure 1 displays the process of study selection. Review of titles and abstracts revealed 39 relevant articles for full text review, and one additional article was identified through

screening of reference lists (Figure 1). The exclusion of reviewed full-text articles was largely based on the study population criteria, with many cohorts at high risk of cardiovascular disease (CVD) without diagnosed CHD. Following application of the inclusion and exclusion criteria, 11 articles were eligible. Four articles reported on controlled trials of a Mediterranean-type diet versus low fat control diet with CRP as an outcome and were included in the quantitative synthesis.

3.2 Study and sample population characteristics

Detailed characteristics of included studies are displayed in Table 2. Four studies were observational; three were cross-sectional studies [26-28] and one was a multi-center prospective cohort study [29]. One study was an uncontrolled clinical trial [30]. The remaining six studies were controlled trials; three studies employed randomization [31-33], two studies did not [34, 35] and one study was a cross-over design [36].

The majority of studies were conducted in one location and spanned a range of countries: Spain (n=3), France (n=1), Germany (n=1), Iran (n=1), Brazil (n=1) and the USA (n=2). The cohort study (n=1) was conducted across cities from multiple European countries (Greece, Spain, Italy, Germany, Finland and Sweden) and the baseline cross-sectional analysis (n=1) involved 39 countries, across multiple continents. Final sample numbers ranged between 24 and 15,482 participants in observational studies, and between 39 and 926 participants in intervention trials. One study restricted inclusion criteria to males [35] and all others included males and females with eight studies recruiting mostly males (53 to 71 % of those samples) and one with less males (40 % of the sample) [36].

Eligibility criteria varied across the studies. Some studies were restricted to patients undergoing specific cardiac procedures, including candidates for coronary artery bypass grafting [26] or coronary angiograms [27]. Three studies required prior diagnosis of MI [29, 33, 34] and the remaining studies included patients with established CHD and/or any ACS. The majority of participants in all studies which reported treatments [26-28, 32, 33, 35] had undergone coronary revascularization. Where reported, time since coronary event ranged between 6 years prior to diagnosis or procedure to immediately post diagnosis or procedure being undertaken. In addition, most studies did not indicate event recurrence, with only two studies specifying restriction to first event [27, 33]. Where cardiac medication use was reported [27, 28, 30-36], statin use was high, ranging from 62 to 100 % of patients, and LDL-cholesterol levels were generally well controlled. No study reported use of nonsteroidal anti-inflammatory drugs. Samples which reported a mean body mass index (BMI) had populations classified as overweight (25 - 30 kg/m^2, n = 6[26-29, 32, 35]), or obese (>30 kg/m^2, n = 3 [31, 33, 36]). The cohort study measured dietary intake at baseline only and relevant biomarker outcomes from baseline to 9-11 months follow-up [29]. The length of intervention trials ranged from 2 weeks to 24 months.

3.2.1 Mediterranean-type diets

Detailed characteristics of the diet measures, interventions and adherence are summarized in Supplemental Table S3. The four observational studies [26-29] assessed Mediterranean-type diet adherence through application of different scores. Each study first employed a food frequency questionnaire to measure dietary intake. One cross-sectional study employed principal components analysis to determine dietary patterns of consumption, which identified a semi-Mediterranean diet pattern score that was high in fruit, vegetables, legumes, nuts,

olives and sugars, and low in SFA [26]. The remaining observational studies calculated Mediterranean diet scores based on the level of participant intake of pre-defined healthy or protective foods, such as wholegrains, vegetables, fruit, olive oil, fish and moderate alcohol, and unhealthy or high risk foods, such as meat or refined cereals [27-29]. One study [27] utilized a previously validated diet score [37], whilst two studies developed their own diet score [28, 29].

All intervention diets were described as "Mediterranean" except one which was a low-fat American Heart Association (AHA) Step 1 diet supplemented with almonds (Step 1 + almonds) [36]. However, this intervention diet met the inclusion criteria for a Mediterranean-type diet as it incorporated three of the eligible components, which were: high in plant foods (wholegrain cereals, fruit and vegetables); high in MUFA (nuts) and low in food rich in SFA. Nutrient targets were provided in some studies, with common themes including total energy from fat of 30 to 40% (high MUFA and PUFA with low SFA), protein ~15% and carbohydrate ~50% of total energy content. The majority of interventions did not employ energy restriction, with only one noting the diet was low energy [30] and another indicating reduced energy prescription for overweight participants [33]. Specific food group recommendations included increased fish (n=6 [30-35]), fruit, vegetables and wholegrains (n=6 [31-36]), nuts (n=4 [31, 32, 35, 36]) and legumes (n=2 [31, 35]); moderate red wine (n=4 [31, 32, 34, 35]); and decreased discretionary foods or beverages (n=5 [31, 32, 34-36]) and red or processed meat (n=4 [31, 32, 34, 35]). Promotion of oils varied, with some exclusively recommending olive oil (n=3 [30, 31, 35]), others recommending olive oil with other plant oils such as canola, flaxseed or nut oils (n=3 [32-34]), or no recommendation for olive oil in the study which focused on high MUFA from almonds (n=1 [36]).

Three studies explored the effect of a Mediterranean-type diet with co-intervention. Two studies were conducted in the context of a broader cardiac rehabilitation program with prescribed exercise programs and health education [30, 34] and another employed stress reduction techniques [32]. Two of these controlled trials also had alternative Mediterranean diets as their 'control' group. One was a traditional Mediterranean diet without red wine within cardiac rehabilitation [34]. The other differed to its intervention group by receiving less attention and dietary information on the Mediterranean-type diet and stress reduction (short written resources on diet and stress management provided at the start of the study only) [32]. These three studies did not report the effect of the Mediterranean-type diet on biomarkers of inflammation independent of the co-interventions.

Five of the seven intervention trials reported on diet change in the Mediterranean-type diet group (Supplemental Table S3) [30, 32, 33, 35, 36]. Two of these studies demonstrated high adherence to one of two previously validated Mediterranean diet scores [38, 39] post-intervention. The five intervention studies reporting nutrient intakes indicated change in nutrient intake. Two studies reported reduced energy intake [32, 35], however not in the intended study groups. As a % of energy contribution, only one group increased total fat (Step 1 + almonds) [36], two increased MUFA (Step 1 + almonds [36] and a group provided with olive oil and nuts daily [35]) and one increased PUFA (Step 1 + almonds [36]). Two studies that measured plasma fatty acids as an objective marker of compliance both demonstrated significant increases in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) but not oleic acid [32, 33]. Both studies recommended increased fish consumption and olive oil was not exclusively recommended. Carbohydrate intake was reduced in two studies that increased fat intake [30, 36] and protein intake generally did not change.

3.2.2 Control diets

Two observational studies identified comparative dietary patterns. The cross-sectional study which used principal component analysis also identified 'healthy', 'intermediate', 'neo-traditional' and 'Western' patterns of intake [26]. Another study measured a pre-defined Western diet score [28]. Four of the six controlled intervention trials employed low fat diets as the control group [31, 33, 35, 36], as guided by nutrition recommendations of the AHA. Common dietary models included macronutrient targets of: total fat <30 %, carbohydrates >55 %, protein ~15 %, as contribution to energy, and cholesterol <300 mg/day at most. Specific fat recommendations ranged between saturated fat <7 % to <10 %, MUFA 10 - 20% and PUFA 6 - 10 % as contribution to energy. Three studies indicated food group recommendations [31, 33, 35], which all promoted low fat foods and wholegrains and one study specifically promoted daily intake of a phytosterol-rich spread [35]. Three of the four studies with a low-fat diet control group reported on change in nutrient intake [33, 35, 36], whereas change in food groups was not reported. All three achieved a reduction in saturated fat and cholesterol intake from baseline, however there was limited change to other macro- or micronutrients. At end intervention, these three low-fat control diet groups reported significantly lower intakes of PUFA and two reported lower intake of MUFA [35, 36] compared to the Mediterranean-type diet group.

3.3 Risk of bias

Assessment of each included study against the quality criteria recommended by the Academy of Nutrition and Dietetics [22] is presented in Table 3. Two studies received a positive (+) quality rating, seven studies received a neutral (Φ) quality rating and two studies received a

negative (-) quality rating for methods. Risk of bias across the studies was generally low with regards to clear research questions, selection of subjects, outcome measures, statistical analyses, appropriate conclusions and limitations and declaration of funding or sponsorship. Bias was high for the category of comparable study groups, which was related to the inclusion of observational studies (n=4) and lack of control group or random allocation in some intervention trials (n=3). The majority of studies sufficiently described withdrawals (n=6) and the intervention/exposure (n=7). None of the studies employed blinding to the intervention/exposure (an inherent issue when diet-focused) and most studies also did not employ blinding of outcome measures and analyses (n=7). The two studies which received negative quality ratings [30, 34] were both interventions conducted in a hospital setting. Studies were not excluded based on method quality rating, as the aim of this review was to evaluate all of the limited available literature in the specific CHD patient group.

3.4 Effect of Mediterranean-type diets on study outcomes

Table 2 shows inflammatory cytokines or adipokines measured across studies included CRP (10 studies), interleukin (IL)-6 (3 studies) and TNF-α (3 studies). One study also reported measuring IL-1-β, IL-4, IL-8 and interferon-γ.

3.4.1 C-reactive protein

CRP was measured in three observational studies with consistent findings observed throughout. High-sensitive CRP was significantly lower in participants with a higher Mediterranean diet score (3.0 ±7.0 mg/L in the highest scoring category compared to 3.3 ±7.0 mg/L in the lowest scoring category as noted in Table 2) in the largest cross-sectional sample

(n=15,482) [28]. Another study demonstrated that higher semi-Mediterranean diet pattern score was significantly associated with lower plasma CRP levels in women (5.80 ± 0.38 mg/L in the 4th quartile compared to 6.87 ± 0.38 mg/L in the 1st quartile of diet scores) but not in men, and no other diet pattern showed associations with CRP [26]. Another study highlighted that for each unit of increase in Mediterranean diet score there was 3.1 % reduction in the average CRP levels when controlling for potential confounders [29].

All intervention trials measured CRP and reported reductions in mean measures in the Mediterranean-type diet intervention group ranging between -0.09 to -2.02 mg/L. However, only two studies demonstrated a statistically significant ($p<0.05$) reduction in CRP [30, 31]. Both had a 12 month follow-up period. One was in a small cohort with a Mediterranean-type diet as part of a cardiac rehabilitation program [30] and the other was the largest RCT included [31] (this publication reported on change in CRP between genotypes of a single nucleotide polymorphism, but data for each study group was pooled for this review). Both of these studies were conducted in Spain, recommended exclusive use of extra virgin olive oil (EVOO) and either did not achieve energy reduction [30] or had an *ad libitum* approach [31]. At baseline, these two trials reported mean plasma CRP values of 5.1 [30] and 2.68 [31] mg/L in the Mediterranean-type diet groups. In contrast, three of the five study groups which reported a non-significant reduction in CRP with Mediterranean-type diet had mean baseline plasma CRP values well within the normal reference range of <3 mg/L, at 0.38 [33], 0.93 [32] and 1.65 [35] mg/L. The final two trials reported higher mean baseline plasma CRP levels (4.0 [36] and 8.4 [34] mg/L), but the latter was in an acute cohort hospitalized for recent MI.

No significant differences were reported between Mediterranean-type diet and low fat diet control groups for effect on CRP. Where calculated, effect sizes were low to medium, ranging between 0.185 to 0.451 for studies where the Mediterranean-type diet had lower mean CRP at end-intervention than a low fat diet [31, 35, 36]. For one study which had lower mean CRP at end-intervention with the low fat diet group [33], the effect size was low at 0.272. The meta-analysis pooled the outcomes for these four controlled trials (total n = 1,128 participants) reporting the effect of a Mediterranean-type diet versus low fat diet on CRP (Figure 2). Final mean values were used, as no studies reported Mean (SD) for change values. A Mediterranean-type diet was not significantly associated with a greater reduction in CRP compared to low fat diets (weighted mean difference, -0.11 [95% CI: -0.36, 0.15]; p= 0.41).

3.4.2 Interleukin-6

One cross-sectional study highlighted that for each unit of increase in Mediterranean diet score there was 1.9 % reduction in the average plasma IL-6 levels when controlling for potential confounders [29] (Table 2). The Step 1 + almonds diet had no effect on plasma IL-6 levels and this did not differ significantly to the low fat diet control group, however for this control group there was a trend observed with an increase in IL-6 of 0.3 mg/L [36]. The effect size (Cohen's *d)* for mean IL-6 at end-intervention was low, at 0.185. The trial investigating a 2-week traditional Mediterranean diet with or without wine in hospitalized patients also indicated no effect on IL-6 or other interleukins (IL-1-β, IL-4, IL-8), or cytokine interferon-γ (data unable to be obtained) in either group [34].

3.4.3 Tumor necrosis factor-alpha

One cross-sectional study highlighted Mediterranean diet score was significantly inversely associated with coronary venous blood TNF-α levels when adjusting for potential confounding (β = –41.6; 95% CI –76.2 to –7.1) [27]. The Step 1 + almonds diet had no effect on plasma TNF-α levels and this did not differ to the low fat diet control group [36]. The trial investigating a 2-week traditional Mediterranean diet with or without wine in hospitalized patients saw no effect on plasma TNF-α (data unable to be obtained) in either group [34].

4. Discussion

This systematic review investigated the effects of a Mediterranean-type diet on inflammatory biomarkers in patients with CHD. A limited amount of literature is reported in the area. Observational studies consistently demonstrated that this dietary pattern is inversely associated with a range of recognized biomarkers of inflammation. Of the Mediterranean-type diet interventions, two (both conducted in Spain) demonstrated significant reductions in CRP and five showed a trend in CRP reduction that did not reach significance. The majority of studies were underpowered and some employed diets or achieved diet change that was not necessarily reflective of a traditional Mediterranean diet. Only two interventions measured both TNF-α and IL-6, which saw no improvement. Plasma CRP levels were the only outcome with sufficient data to pool and undertake a meta-analysis, which demonstrated a non-significant favorable effect of a Mediterranean-type diet versus low-fat diet with mean CRP values. The main distinction achieved between the intervention and control diets in these studies was higher intake of PUFA and/or MUFA versus reduction in SFA. There was a high level of heterogeneity between studies (I^2 = 90 %) which may be related to the range in intervention lengths, time since or possible inclusion of secondary coronary events, and mean

CRP values at baseline across the included studies. In addition, three studies had small sample sizes (total n ≤ 101).

A Mediterranean diet is recognized to be anti-inflammatory. Other systematic reviews and meta-analyses of both observational studies [40] and RCTs [14, 15] have shown a significant reduction in CRP and IL-6 with a Mediterranean-type diet. However, the majority of included studies employed cohorts at risk of, but without, established CVD, such as overweight, metabolic syndrome or T2DM. These populations receive less intensive therapy (surgical, pharmacological and lifestyle) than patients with prior ACS and/or established CHD, and hence have greater scope for improvement in markers of inflammation and other cardiometabolic risk factors.

Few trials have considered other healthy diet interventions in CHD patients and their impact on inflammatory markers. The low fat Step 1 diet and the Portfolio Diet (low fat with high intake of viscous fibers, soy protein and almonds) both had no effect on inflammatory markers in CHD study cohorts [41, 42]. Both of these diets were primarily designed to lower LDL-cholesterol levels. The Dietary Approaches to Stop Hypertension (DASH) diet, a well-researched low fat dietary pattern, has no trial evidence in the context of established CHD. This literature is consistent with the lack of effect of three of the four low fat diets employed in the control groups of studies in this review. The evidence is stronger for a Mediterranean-type diet in CHD patients but this is yet to be demonstrated with significance.

The definition of a Mediterranean diet has become very broad [43]. This review aimed to capture all Mediterranean-type diets with high anti-inflammatory potential. Therefore, some of the trials in this review [32, 33, 36] employed diet interventions that would be considered

healthy but not necessarily reflective of all the components of a traditional Mediterranean diet pattern, which may in part explain the lack of significant effects on inflammatory cytokines with these interventions.

EVOO is the main culinary fat consumed in a traditional Mediterranean diet [21] and is a core component of this dietary pattern that differs to other diets prescribed for CVD prevention (Step 1, DASH, or Portfolio). Daily intake of 50 mL of EVOO, but not refined olive oil, significantly reduced CRP and IL-6 in patients with CHD [44]. Its anti-inflammatory effect is likely related to its high polyphenol content [45]. Three of the four observational studies in this review considered olive oil as part of their Mediterranean diet score. The two Mediterranean-type diet interventions with significant improvement in CRP exclusively recommended EVOO, whereas the use and type of olive oil varied in others.

In established CHD, long-chain omega-3 fatty acids EPA and DHA have been associated with improved plasma CRP, IL-6 and TNF-α levels [46, 47]. Two studies in this review that reported significant increases in plasma levels of EPA and DHA with Mediterranean-type diets saw reduced CRP, but this was not significant [32, 33]. Fish or plant sources of omega-3 were recommended in most interventions but amounts, type and adherence varied across studies. Other key components of the traditional diet pattern that were inconsistently recommended across the trials, were legumes, nuts and red wine. These foods can reduce levels of inflammatory markers [48-50] and hence their exclusion could have influenced the anti-inflammatory potential of the interventions.

One of the intervention studies recommending a Mediterranean-type diet, which demonstrated significant improvement in CRP, was part of a broader cardiac rehabilitation

program that included exercise training and no control group was employed [30]. Exercise training can improve multiple inflammatory cytokines in patients with CHD [51], hence it is difficult to ascertain what effect the diet component had.

The lack of significant effect on CRP with Mediterranean-type diets may be related to low baseline levels associated with intake of statins [52]. Studies have demonstrated that statin treatment does not impact IL-6 despite reducing CRP [53, 54]. This lack of effect of statins on IL-6 may be related to location of mechanism. IL-6 is a pleiotropic cytokine released from activated cells at the vascular endothelium, whereas CRP is released from the liver, where statins act [55]. The Mediterranean diet can directly improve endothelial function [15] and therefore has the potential to reduce inflammatory cytokines that are not impacted by statins.

In the context of secondary CHD prevention there is limited evidence reporting whether IL-6 (and other interleukins) or TNF-α are impacted by a Mediterranean-type diet, and there is no evidence for other markers, such as adiponectin. As an anti-inflammatory adipokine, adiponectin is related to atherosclerosis, quantity and function of adipocytes and insulin sensitivity [56]. Doubling plasma levels of adiponectin has been shown to be associated with a reduced relative risk of incident CHD by up to 30 % in males with T2DM [57]. RCTs have demonstrated a Mediterranean diet increases plasma adiponectin levels, albeit in populations without CHD [58-60].

One of the RCTs included in this review [31] reported preliminary results from the CORDIOPREV study [61]. Their included paper, as well as two others, have demonstrated in a cohort with CHD that the impact of diet on CRP is dependent on genotyping of metabolic

523 genes [31, 62, 63]. Genotyping could therefore identify individuals at higher risk of
524 inflammation, and thus most likely to benefit from dietary intervention.
525
526 The studies included in this review were limited by a portion being observational, cross-
527 sectional or without a control group, which cannot indicate causation between the diet and
528 inflammation. Positively, two of these observational studies employed populations of diverse
529 ethnicity with large sample sizes. For the intervention trials, most employed small sample
530 sizes, some employed short interventions and not all the trials employed a control group or
531 randomization. In addition, three intervention trials employed co-interventions involving
532 cardiac rehabilitation or stress management from which the effect of diet was not isolated.
533 The risk of bias assessment identified a neutral method quality rating for the majority of
534 included studies, highlighting the need for higher quality research in this area. Two
535 intervention trials that were conducted in a hospital setting received negative method quality
536 ratings which is perhaps due to not being designed for research at the outset.
537
538 The strength of this review is that it specifically investigated the effect of Mediterranean-type
539 diets on patients with established CHD, receiving standard surgical and pharmacological
540 treatment. This systematic review was comprehensive and used both narrative and
541 quantitative summary measures. The search strategy was however limited to English
542 language since no translation facilities were available. It is unknown whether additional
543 relevant studies have been published in another language. This review considered whether the
544 dietary interventions employed traditional Mediterranean diet principles and whether these
545 were adhered to by participants.
546

There was limited available literature on the topic and a sufficient number of trials were retrieved to conduct a meta-analysis for the effect of the Mediterranean-type diets versus low fat AHA diets on CRP only. This data pooled final mean values, and hence could not take into account baseline differences between study groups. Moreover, for most of the studies included in this review, markers of inflammation were not the primary outcome and hence they were unlikely to be adequately powered to report a change in these measures. Our meta-analysis had a total sample size of 1,128 participants, whereas a previous meta-analysis [15] which found a significant effect of Mediterranean-type diets compared to control diets on CRP had a total sample size of 1942 participants, of which only one included study [32] was in a CHD population. Based on this previous meta-analysis, potentially more than double the sample size of our meta-analysis would be required to detect a significant difference in CHD patient groups. Finally, this review was limited to cytokines and adipokines as markers of inflammation.

In conclusion, despite the anti-inflammatory potential of the Mediterranean diet pattern, most studies reported a reduction in CRP with a Mediterranean-type diet that was not significant. The difference in final mean CRP values for Mediterranean-type diet interventions compared to low fat diets was also not significant. Due to small sample sizes, inconsistency between diet interventions and a paucity of other outcome measures, it is unclear whether a traditional Mediterranean diet leads to clinically significant improvements in markers of inflammation in patients with CHD on medications.

5. Future directions

More studies are needed to determine whether a Mediterranean diet has an anti-inflammatory effect in a high-risk population with CHD. RCTs are warranted testing the traditional Mediterranean diet with a range of biomarkers of inflammation in addition to CRP, such as inflammatory cytokines (interleukins) and adipokines (TNF-α and adiponectin). These markers should be measured pre- and post-intervention, and at relevant intervals with at least 12 months follow up. This effect should also be evaluated in adequately powered studies and in non-Mediterranean populations.

Acknowledgment

Funding and sponsorship: This research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors. This work was supported by an Australian Government Research Training Program Scholarship (HLM).

Declaration of interest: The authors have no relevant interests to declare.

Appendix

Supplemental Materials

Figure Legends

Figure 1. Flow chart illustrating the literature search and selection process. CRP, C-reactive protein.

Figure 2. Forest plot of the meta-analysis for C-Reactive Protein (mg/L) between Mediterranean-type (experimental) and low fat (control) diets. The individual effect sizes are presented as difference in mean final values with 95% CIs, z value, and p-values (significance at <0.05) provided for each study. Diamond indicates weighted mean difference with 95% CIs. IV, inverse variance.

600 **References**

601 [1] Ross R. Atherosclerosis — An Inflammatory Disease. N Engl J Med 1999;340:115-26.

602 [2] Moreno PR, Falk E, Palacios IF, Newell JB, Fuster V, Fallon JT. Macrophage infiltration
603 in acute coronary syndromes. Implications for plaque rupture. Circulation 1994;90:775-8.

604 [3] Fuster V, Badimon L, Badimon JJ, Chesebro JH. The pathogenesis of coronary artery
605 disease and the acute coronary syndromes. N Engl J Med 1992;326:310-8.

606 [4] Tanaka A, Shimada K, Sano T, Namba M, Sakamoto T, Nishida Y, et al. Multiple plaque
607 rupture and C-reactive protein in acute myocardial infarction. J Am Coll Cardiol
608 2005;45:1594-9.

609 [5] Ridker PM, Rifai N, Pfeffer M, Sacks F, Lepage S, Braunwald E, et al. Elevation of tumor
610 necrosis factor-α and increased risk of recurrent coronary events after myocardial infarction.
611 Circulation 2000;101:2149-53.

612 [6] Inoue T, Kotooka N, Morooka T, Komoda H, Uchida T, Aso Y, et al. High molecular
613 weight adiponectin as a predictor of long-term clinical outcome in patients with coronary
614 artery disease. Am J Cardiol 2007;100:569-74.

615 [7] Kaptoge S, Seshasai SRK, Gao P, Freitag DF, Butterworth AS, Borglykke A, et al.
616 Inflammatory cytokines and risk of coronary heart disease: new prospective study and
617 updated meta-analysis. Eur Heart J 2013;35:578-89.

618 [8] Ridker PM, Cannon CP, Morrow D, Rifai N, Rose LM, McCabe CH, et al. C-reactive
619 protein levels and outcomes after statin therapy. N Engl J Med 2005;352:20-8.

620 [9] Mozaffarian D. Dietary and policy priorities for cardiovascular disease, diabetes, and
621 obesity: A comprehensive review. Circulation 2016;133:187-225.

622 [10] Fung TT, Rimm EB, Spiegelman D, Rifai N, Tofler GH, Willett WC, et al. Association
623 between dietary patterns and plasma biomarkers of obesity and cardiovascular disease risk.
624 Am J Clin Nutr 2001;73:61-7.

[11] Fung TT, McCullough ML, Newby PK, Manson JE, Meigs JB, Rifai N, et al. Diet-quality scores and plasma concentrations of markers of inflammation and endothelial dysfunction. Am J Clin Nutr 2005;82:163-73.

[12] Smidowicz A, Regula J. Effect of nutritional status and dietary patterns on human serum c-reactive protein and interleukin-6 concentrations. Adv Nutr 2015;6:738-47.

[13] Ahluwalia N, Andreeva V, Kesse-Guyot E, Hercberg S. Dietary patterns, inflammation and the metabolic syndrome. Diabetes & metabolism 2013;39:99-110.

[14] Neale E, Batterham M, Tapsell LC. Consumption of a healthy dietary pattern results in significant reductions in C-reactive protein levels in adults: a meta-analysis. Nutrition research 2016;36:391-401.

[15] Schwingshackl L, Hoffmann G. Mediterranean dietary pattern, inflammation and endothelial function: A systematic review and meta-analysis of intervention trials. Nutr Metab Cardiovasc Dis 2014;24:929-39.

[16] De Lorgeril M, Salen P, Martin J-L, Monjaud I, Delaye J, Mamelle N. Mediterranean diet, traditional risk factors, and the rate of cardiovascular complications after myocardial infarction. Circulation 1999;99:779-85.

[17] Eckel R, Jakicic J, Ard J. 2013 AHA/ACC Guideline on lifestyle management to reduce cardiovascular risk: A report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. J Am Coll Cardiol 2014;63:3027-8.

[18] National Heart Foundation of Australia and the Cardiac Society of Australia and New Zealand. Reducing risk in heart disease: an expert guide to clinical practice for secondary prevention of coronary heart disease. Melbourne: National Heart Foundation of Australia; 2012.

[19] Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. Ann Intern Med 2009;151:264-9.

[20] Willett WC, Sacks F, Trichopoulou A, Drescher G, Ferro-Luzzi A, Helsing E, et al. Mediterranean diet pyramid: a cultural model for healthy eating. Am J Clin Nutr 1995;61:1402S-6S.

[21] Trichopoulou A, Lagiou P. Healthy traditional Mediterranean diet: an expression of culture, history, and lifestyle. Nutr Rev 1997;55:383-9.

[22] Academy of Nutrition and Dietetics. Evidence Analysis Manual: Steps in the Academy Evidence Analysis Process. United States of America: Academy of Nutrition and Dietetics; 2012. p. 90-2.

[23] Thalheimer W, Cook S. How to calculate effect sizes from published research articles: a simplified methodology, http://worklearning.com/effect_sizes.html; 2002 [accessed 01 November 2016].

[24] DerSimonian R, Laird N. Meta-analysis in clinical trials. Control Clin Trials 1986;7:177-88.

[25] Higgins JPT, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. BMJ 2003;327:557-60.

[26] Farhangi MA, Ataie-Jafari A, Najafi M, Foroushani GS, Tehrani MM, Jahangiry L. Gender differences in major dietary patterns and their relationship with cardio-metabolic risk factors in a year before Coronary Artery Bypass Grafting (CABG) surgery period. Arch Iran Med 2016;19:470-9.

[27] Serrano-Martinez M, Palacios M, Martinez-Losa E, Lezaun R, Maravi C, Prado M, et al. A Mediterranean dietary style influences TNF-alpha and VCAM-1 coronary blood levels in unstable angina patients. Eur J Nutr 2005;44:348-54.

[28] Stewart RAH, Wallentin L, Benatar J, Danchin N, Hagström E, Held C, et al. Dietary patterns and the risk of major adverse cardiovascular events in a global study of high-risk patients with stable coronary heart disease. Eur Heart J 2016;37:1993-2001.

[29] Panagiotakos DB, Dimakopoulou K, Katsouyanni K, Bellander T, Grau M, Koenig W, et al. Mediterranean diet and inflammatory response in myocardial infarction survivors. Int J Epidemiol 2009;38:856-66.

[30] Roca-Rodriguez MM, Garcia-Almeida JM, Ruiz-Nava J, Alcaide-Torres J, Saracho-Dominguez H, Rioja-Vazquez R, et al. Impact of an outpatient cardiac rehabilitation program on clinical and analytical variables in cardiovascular disease. J Cardiopulm Rehabil Prev 2014;34:43-8.

[31] Gomez-Delgado F, Garcia-Rios A, Alcala-Diaz JF, Rangel-Zuniga O, Delgado-Lista J, Yubero-Serrano EM, et al. Chronic consumption of a low-fat diet improves cardiometabolic risk factors according to the CLOCK gene in patients with coronary heart disease. Mol Nutr Food Res 2015;59:2556-64.

[32] Michalsen A, Lehmann N, Pithan C, Knoblauch NT, Moebus S, Kannenberg F, et al. Mediterranean diet has no effect on markers of inflammation and metabolic risk factors in patients with coronary artery disease. Eur J Clin Nutr 2006;60:478-85.

[33] Tuttle KR, Shuler LA, Packard DP, Milton JE, Daratha KB, Bibus DM, et al. Comparison of low-fat versus Mediterranean-style dietary intervention after first ,myocardial infarction (from The Heart Institute of Spokane Diet Intervention and Evaluation Trial). Am J Cardiol 2008;101:1523-30.

[34] Rifler JP, Lorcerie F, Durand P, Delmas D, Ragot K, Limagne E, et al. A moderate red wine intake improves blood lipid parameters and erythrocytes membrane fluidity in post myocardial infarct patients. Mol Nutr Food Res 2012;56:345-51.

[35] Thomazella MCD, Goes MFS, Andrade CR, Debbas V, Barbeiro DF, Correia RL, et al. Effects of high adherence to Mediterranean or low-fat diets in medicated secondary prevention patients. Am J Cardiol 2011;108:1523-9.

[36] Chen CYO, Holbrook M, Duess MA, Dohadwala MM, Hamburg NM, Asztalos BF, et al. Effect of almond consumption on vascular function in patients with coronary artery disease: a randomized, controlled, cross-over trial. Nutr J 2015;14.

[37] Martínez-González MA, Fernández-Jarne E, Serrano-Martínez M, Marti A, Martinez JA, Martín-Moreno JM. Mediterranean diet and reduction in the risk of a first acute myocardial infarction: an operational healthy dietary score. Eur J Nutr 2002;41:153-60.

[38] Trichopoulou A, Costacou T, Bamia C, Trichopoulos D. Adherence to a Mediterranean Diet and Survival in a Greek Population. N Engl J Med 2003;348:2599-608.

[39] Martinez-Gonzalez M, Fernandez-Jarne E, Serrano-Martinez M, Wright M, Gomez-Gracia E. Development of a short dietary intake questionnaire for the quantitative estimation of adherence to a cardioprotective Mediterranean diet. Eur J Clin Nutr 2004;58:1550-2.

[40] Barbaresko J, Koch M, Schulze MB, Nöthlings U. Dietary pattern analysis and biomarkers of low-grade inflammation: a systematic literature review. Nutr Rev 2013;71:511-27.

[41] Keith M, Kuliszewski MA, Liao C, Peeva V, Ahmed M, Tran S, et al. A modified portfolio diet complements medical management to reduce cardiovascular risk factors in diabetic patients with coronary artery disease. Clin Nutr 2015;34:541-8.

[42] Koh KK, Ahn JY, Choi YM, Han SH, Kim DS, Kim HS, et al. Vascular effects of Step I diet in hypercholesterolemic patients with coronary artery disease. Am J Cardiol 2003;92:708-10.

[43] Radd-Vagenas S, Kouris-Blazos A, Singh MF, Flood VM. Evolution of Mediterranean diets and cuisine: concepts and definitions. Asia Pac J Clin Nutr 2016:1-32.

[44] Fitó M, Cladellas M, De la Torre R, Marti J, Munoz D, Schröder H, et al. Anti-inflammatory effect of virgin olive oil in stable coronary disease patients: a randomized, crossover, controlled trial. Eur J Clin Nutr 2008;62:570-4.

[45] Martin-Pelaez S, Covas MI, Fito M, Kusar A, Pravst I. Health effects of olive oil polyphenols: Recent advances and possibilities for the use of health claims. Mol Nutr Food Res 2013;57:760-71.

[46] Rangel-Huerta OD, Aguilera CM, Mesa MD, Gil A. Omega-3 long-chain polyunsaturated fatty acids supplementation on inflammatory biomakers: a systematic review of randomised clinical trials. Br J Nutr 2012;107:S159-S70.

[47] Farzaneh-Far R, Harris WS, Garg S, Na B, Whooley MA. Inverse association of erythrocyte n-3 fatty acid levels with inflammatory biomarkers in patients with stable coronary artery disease: The Heart and Soul Study. Atherosclerosis 2009;205:538-43.

[48] Salehi-Abargouei A, Saraf-Bank S, Bellissimo N, Azadbakht L. Effects of non-soy legume consumption on C-reactive protein: a systematic review and meta-analysis. Nutrition 2015;31:631-9.

[49] Ros E. Nuts and CVD. Br J Nutr 2015;113:S111-S20.

[50] Torres A, Cachofeiro V, Millan J, Lahera V, Nieto ML, Martin R, et al. Red wine intake but not other alcoholic beverages increases total antioxidant capacity and improves pro-inflammatory profile after an oral fat diet in healthy volunteers. Rev Clin Esp 2015;215:486-94.

[51] Goldhammer E, Tanchilevitch A, Maor I, Beniamini Y, Rosenschein U, Sagiv M. Exercise training modulates cytokines activity in coronary heart disease patients. Int J Cardiol 2005;100:93-9.

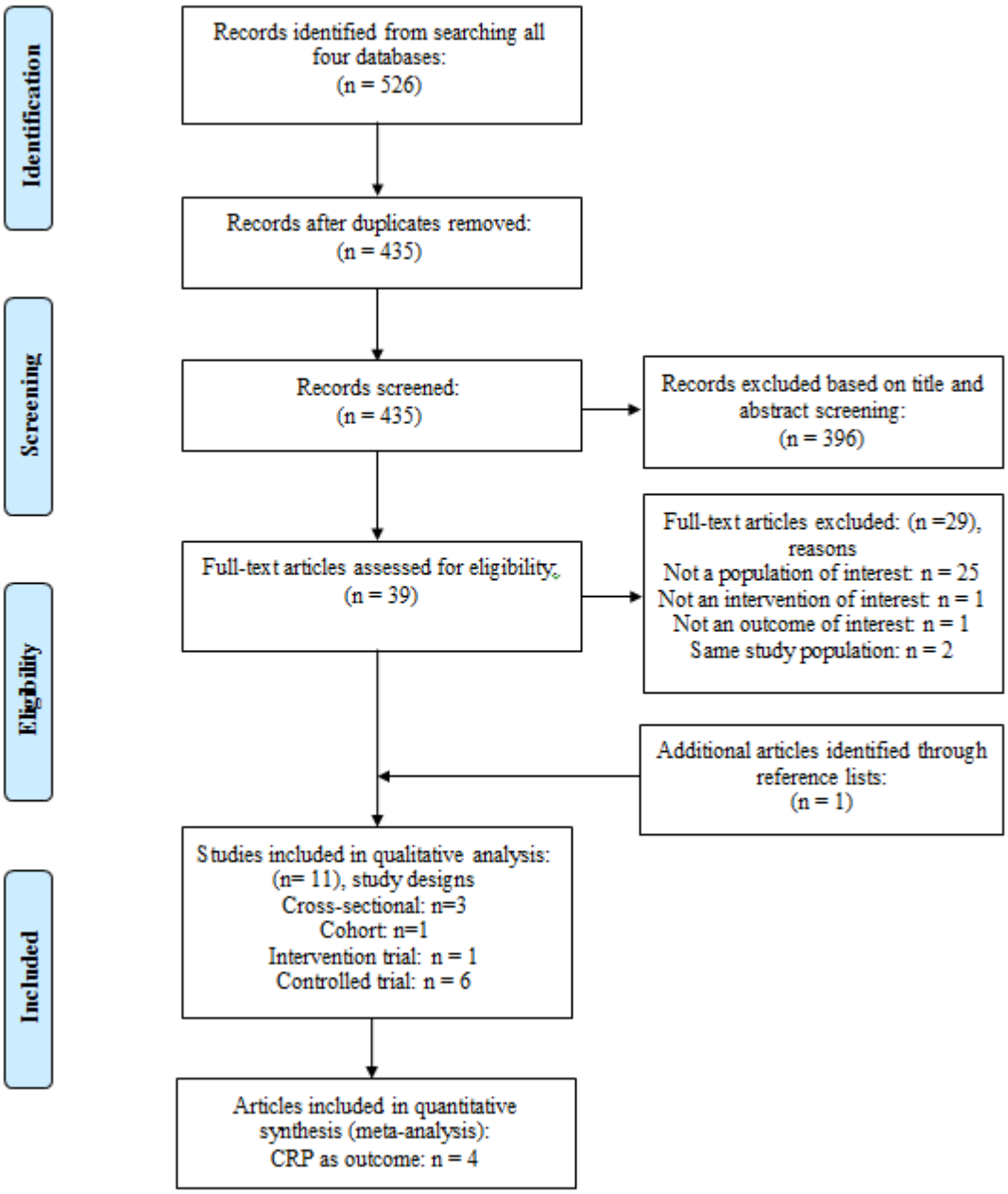
[52] Balk EM, Lau J, Goudas LC, Jordan HS, Kupelnick B, Kim LU, et al. Effects of statins on nonlipid serum markers associated with cardiovascular disease: a systematic review. Ann Intern Med 2003;139:670-82.

747 [53] März W, Winkler K, Nauck M, Böhm BO, Winkelmann BR. Effects of statins on C-
748 reactive protein and interleukin-6 (the Ludwigshafen Risk and Cardiovascular Health study).
749 Am J Cardiol 2003;92:305-8.
750 [54] Jialal I, Stein D, Balis D, Grundy S, Adams-Huet B, Devaraj S. Effect of hydroxymethyl
751 glutaryl coenzyme a reductase inhibitor therapy on high sensitive C-reactive protein levels.
752 Circulation 2001;103:1933-5.
753 [55] Gabay C, Kushner I. Acute-phase proteins and other systemic responses to
754 inflammation. N Engl J Med 1999;340:448-54.
755 [56] Kishida K, Funahashi T, Shimomura I. Adiponectin as a routine clinical biomarker. Best
756 Pract Res Clin Endocrinol Metab 2014;28:119-30.
757 [57] Schulze MB, Shai I, Rimm EB, Li T, Rifai N, Hu FB. Adiponectin and future coronary
758 heart disease events among men with type 2 diabetes. Diabetes 2005;54:534-9.
759 [58] Maiorino MI, Bellastella G, Petrizzo M, Scappaticcio L, Giugliano D, Esposito K.
760 Mediterranean diet cools down the inflammatory milieu in type 2 diabetes: the MÉDITA
761 randomized controlled trial. Endocrine 2016;54:634-41.
762 [59] Lasa A, Miranda J, Bullo M, Casas R, Salas-Salvado J, Larretxi I, et al. Comparative
763 effect of two Mediterranean diets versus a low-fat diet on glycaemic control in individuals
764 with type 2 diabetes. Eur J Clin Nutr 2014;68:767-72.
765 [60] Richard C, Royer M-M, Couture P, Cianflone K, Rezvani R, Desroches S, et al. Effect
766 of the Mediterranean diet on plasma adipokine concentrations in men with metabolic
767 syndrome. Metabolism 2013;62:1803-10.
768 [61] Delgado-Lista J, Perez-Martinez P, Garcia-Rios A, Alcala-Diaz JF, Perez-Caballero AI,
769 Gomez-Delgado F, et al. CORonary Diet Intervention with Olive oil and cardiovascular
770 PREVention study (the CORDIOPREV study): Rationale, methods, and baseline
771 characteristics: A clinical trial comparing the efficacy of a Mediterranean diet rich in olive oil

versus a low-fat diet on cardiovascular disease in coronary patients. Am Heart J 2016;177:42-50.

[62] Gomez-Delgado F, Delgado-Lista J, Lopez-Moreno J, Rangel-Zuniga OA, Alcala-Diaz JF, Leon-Acuna A, et al. Telomerase RNA component genetic variants interact with the Mediterranean diet modifying the inflammatory status and its relationship with aging: CORDIOPREV Study. J Gerontol A Biol Sci Med Sci 2016;Series A:glw194.

[63] Gomez-Delgado F, Alcala-Diaz JF, Garcia-Rios A, Delgado-Lista J, Ortiz-Morales A, Rangel-Zuniga O, et al. Polymorphism at the TNF-alpha gene interacts with Mediterranean diet to influence triglyceride metabolism and inflammation status in metabolic syndrome patients: From the CORDIOPREV clinical trial. Mol Nutr Food Res 2014;58:1519-27.



784

785 Figure 1

786 **Table 1. Summary of PICOS criteria for the inclusion of studies**

Parameter	Description
Population	Male and female adults with diagnosed coronary heart disease and/or who have experienced acute coronary syndrome
Intervention/exposure	Mediterranean-type diet adherence measure or intervention
Comparison	Control/comparison diet adherence measure or intervention (not required)
Outcomes	Association or effect of diet on inflammatory cytokines or adipokines
Study design	No restrictions on study design

787

788

Table 2. Summary of the characteristics and main findings of the studies included in the present review.

Reference, Location	Study design	Inclusion criteria[a]	Population sample characteristics[b]	Follow up length	Mediterranean-type diet assessment or intervention[c]	Control assessment or intervention[c]	Inflammatory cytokine or adipokine outcomes[d]
Observational studies							
Farhangi et al. (2016),[26] Iran	Cross-sectional	Candidates for CABG	n= 454 (333 males, 121 females) Age: 59 (35 – 80) years BMI: 27.44 kg/m^2 Current smoker: 160 (35.2) T2DM: 192 (42.3) Medications - nil reported	Nil	Semi-Mediterranean diet pattern score	Healthy, intermediate, neo-traditional and western diet pattern scores	*Plasma CRP (mg/L)*, adjusted for age Semi-Mediterranean diet pattern (men) 1st quartile: 6.13 ± 0.19, 4th quartile: 6.32 ± 0.55 (p=0.44) Semi-Mediterranean pattern (women) 1st quartile: 6.87 ± 0.38, 4th quartile: 5.80 ± 0.38 (p=0.05), 4th quartile ↓1.07 than 1st quartile All other dietary patterns CRP measures between 1st and 4th quartiles within gender, p>0.05
Panagiotakos et al. (2009),[29] Greece, Germany, Spain, Finland Italy, Sweden	Multi-center prospective cohort study	MI <6 years and >3 months prior	n=967 (762 males, 205 females) Age: 62 years BMI: 28.4 kg/m^2 Current smoker: 79 (8.2) T2DM: 103 (10.7) Medications - recorded but use not reported	9 to 11 months (average repeated visits ranged 4.5 to 6.6)	Mediterranean diet score	Nil	*Log CRP*, adjusted for sex, smoking history, regular exercise, BMI, diabetes and medications Diet score (1 unit) β=0.031 (95% CI -0.055 to -0.005, p=0.02) *Log IL-6*, adjusted for sex, smoking history,

							regular exercise, BMI, diabetes and medications Diet score (1 unit) β=0.019 (95% CI -0.033 to -0.005, p=0.01)
Serrano-Martinez et al. (2005),[27] Spain	Cross-sectional	Patients scheduled for coronary angiogram with recent ACS, nil prior CHD	n=24 (14 males, 10 females) Age: 61.4 ± 12.6 years BMI: 26.1 ±4.3 kg/m^2 Current smoker: 8 (33.3) T2DM: 9 (37.5) Statins: 15 (62.5)	Nil	Mediterranean diet score	Nil	*Serum TNF-α (coronary venous blood pg/ml),* adjusted for gender, BMI and classical coronary risk factors Mediterranean diet score β = –41.6 (95% CI –76.2 to –7.1; p=0.021), R^2= 0.23
Stewart et al. (2016),[28] 39 Countries	Cross-sectional (baseline measures of medication trial)	Stable CHD, defined as prior MI, prior coronary revascularization, or multi-vessel CHD	n=15,482 (12,556 males, 2926 females) Age: 64.2 ±9.5 years BMI ≥30 kg/m^2= 5837 (37.7) Current smoker: 3019 (19.5) T2DM: 6084 (39.3) Statins: 15,018 (97)	Nil	Mediterranean diet score	Western diet Score	*High sensitivity-CRP (mg/L)* Comparisons by Mediterranean diet score ≤12: 3.3 ±7.0, 13-14: 2.9 ±5.6 , ≥15: 3.0 ±7.0, p< 0.001, highest scoring group ↓0.3 than lowest scoring group Western diet score no comparisons reported
Intervention studies							
Chen et al. (2015),[36] USA	Randomized controlled trial (Crossover)	Angiographically proven CHD	n=45 (27 females, 18 males) Age: 61.8 ± 8.6 years BMI: 30.2 ± 5.1 kg/m^2 Statins: 43 (96)	6 week run in, 6 week intervention, 4 week washout period, 6 week intervention, total 22 weeks	n=45 AHA NCEP Step 1 diet with almonds	n=45 Low fat, the AHA Step 1 diet	*CRP (mg/L)* Step 1 + almonds diet pre: 4.0 ± 6.4, 6 weeks: 3.3 ± 4.2 (p>0.05), mean ↓0.7 Step 1 diet pre: 4.5 ± 5.7, 6 weeks: 3.9 ± 5.1 (p>0.05), mean ↓0.6

							Effect size (Cohen's *d*) = 0.128 *TNF-α (pg/mL)* Step 1 + almonds diet pre: 1.8 ± 1.8, 6 weeks: 1.8 ± 1.6 (p>0.05), no change Step 1 diet pre: 1.6 ± 1.4, 6 weeks: 1.6 ± 1.4 (p>0.05), no change Effect size (Cohen's *d*) = 0.133 *IL-6 (pg/mL)* Step 1 + almonds diet pre 3.5 ± 2.2, 6 weeks 3.6 ± 2.5 (p>0.05), mean ↑0.1 Step 1 diet pre- 3.8 ± 2.9, 6 weeks 4.1 ± 2.9 (p>0.05), mean ↑0.3 between group p>0.05 Effect size (Cohen's *d*) = 0.185 Between groups all outcomes p>0.05
Gomez-Delgado et al. (2015),[31] Spain	Randomized controlled trial (parallel)	ACS as acute MI, unstable angina or chronic CHD, without events < 6 months prior	n=897 (gender not specified) characteristics reported across genotypes, on average: Age: 60 years BMI: 31 kg/m^2 statins= 86%	12 months	n= 444 Mediterranean diet supplemented with extra virgin olive oil	n= 453 Low-fat, high–complex carbohydrate AHA diet	*High sensitivity-CRP (mg/L)*, adjusted for age, BMI, gender, and smoking status Mediterranean diet Baseline: 2.68 ±0.19, 12 months: 1.95 ±0.12, mean ↓0.73 (p<0.05) Low fat diet Baseline: 2.86 ±0.18, 12 months 2.15±0.13,

							mean ↓0.71 (p<0.05)
Michalsen et al. (2006),[32] Germany	Randomized controlled trial (parallel)	Established CHD, with coronary angiography performed <3 months prior	n=101 (78 males, 23 females) 77.2% Mediterranean diet group; control group Age: 59.0±8.7; 59.8±8.6 years BMI: 26.1 ±3.2; 27 ±2.8 kg/m^2 Current smoker: 8, 4 T2DM: 3 (6.3); 5 (9.4) Statins: 41 (85.4); 42 (79.2)	12 months	n=48 Mediterranean diet plus practical stress management program	n=53 Mediterranean diet and stress management (both with minimal written advice only)	*High sensitivity-CRP (mg/L)* median (IQR) Mediterranean diet + stress management Baseline: 0.93 (0.48–2.0), 12 months: 0.72 (0.38–1.81) (p>0.05), mean ↓0.21 Advice only Baseline: 0.83 (0.42–2.34) , 12 months : 0.74 (0.45–2.06) (p>0.05), mean ↓0.09 Mean between group difference of change 0.89 (95% CI 0.61 - 1.31) p=0.442
Rifler et al. (2011),[34] France	Non-randomized controlled trial (nil indication of how allocated to groups)	Hospitalized patients with ischaemic cardiopathies and previous MI; majority operated with coronary bridge or stent	n=39 (32 males, 7 females) Age: 65±7.9 years Nil other reported	2 weeks	n=15 ‘‘Western prudent’’ diet, inspired by Mediterranean diet principles of the Lyon Heart study, with wine plus physiotherapy as part of rehabilitation	n=14 ‘‘Western prudent’’ diet, inspired by Mediterranean diet principles of the Lyon Heart study, without wine plus physiotherapy as part of rehabilitation	*CRP (g/L)* Mediterranean diet with wine Baseline: 8.36 ± 8.51, 2 weeks: 6.34 ± 6.69, p>0.05, mean ↓2.02 Mediterranean diet without wine Baseline to 2 weeks p>0.05 IL-1-β, IL-4, IL-6, IL-8, IFN-γ, TNF-a Baseline to 2 weeks in both groups, p>0.05

Roca-Rodríguez et al. (2014),[30] Spain	Intervention trial	Patients enrolled in cardiac rehabilitation after acute cardiac event	n=59 (52 male, 7 female) 88.1% Age: 54.8 ± 8.9 years BMI obese: 20 (34.5) Current smokers: 5 (6.8) T2DM: 12 (21) Statins: 59 (100)	End intervention 8 weeks, total 12 months	Mediterranean diet as part of cardiac rehabilitation with exercise training and health education	Nil	*CRP (μg/mL)* Baseline: 5.1 ± 8.7, 12 months: 4.6 ± 4.5 p=0.008, mean ↓0.5
Thomazella et al. (2011),[35] Brazil	Non-randomized controlled trial (allocated based on previous cultural and diet habits)	≥1 coronary event (MI or unstable angina) <24 and >4 months prior	n=40 (40 males) Mediterranean diet group; control group Age: 55.0 ± 4.6; 54.6 ± 5.0 years BMI: 26.5 ± 1.9; 26.3 ± 2.5 kg/m^2 Ex-smoker: 7 (81); 10 (53) Statins: 17 (81); 16(84)	3 months	n=21 Mediterranean diet	n=19 Low fat, Therapeutic Lifestyle Changes Diet (TLCD)	*Plasma high sensitivity-CRP (mg/L)* Mediterranean diet Baseline: 1.65 ± 1.50, 3 month: 1.07 ± 0.93, mean ↓0.58 TLCD Baseline: 1.38 ± 1.07, 3 Month: 2.07 ± 2.99, mean ↑0.69 p = baseline 0.510, time 0.874, group 0.437, time x group 0.058, Effect size (Cohen's *d*) = 0.451
Tuttle et al. (2008),[33] USA	Randomized controlled trial (parallel)	<6 weeks after first MI	n=101 (75 males, 26 females) 74.3% Mediterranean diet group; control group Age: 58 ± 10; 58 ± 9 years BMI: 30 ± 5; 31 ±6 kg/m^2 Current smoker: 13 (25); 15 (30) T2DM: 10 (20); 10 (20) Statins: 42 (82); 41 (82)	3, 6, 12, 18 month reviews, total 24 months	n=51 Mediterranean-style diet	n=50 Low fat, the AHA Step 2 diet	*High sensitivity-CRP (mg/L)* Mediterranean-style diet Baseline: 0.38 ± 0.39, 24 months: 0.29 ± 0.31 (p>0.05), mean ↓0.09 Step 2 diet Baseline: 0.44 ± 0.46, 24 months 0.22 ± 0.19 (p>0.05), mean ↓0.22 between group p>0.05 Effect size (Cohen's *d*) = 0.272

Abbreviations: ACS, Acute coronary syndrome; MI, Myocardial infarction; CABG, Coronary artery bypass grafting; CHD, Coronary heart disease; BMI, Body mass index; T2DM, Type 2 Diabetes Mellitus; AHA, American Heart Association; CRP, C-reactive protein; IL, Interleukin; TNF, Tumor necrosis factor; IFN, Interferon; ↑, mean change increase; ↓, mean change decrease.

[a] Coronary heart disease criteria and time since diagnosis, if reported
[b] n= refers to total sample size, data are presented as mean ± SD, mean (range) or n (%)
[c] n= refers to the number of individuals per group, name of diet as indicated by study authors, see Supplemental Table S3 for explanations of the diets
[d] Results presented individually for relevant markers measured (with units) pre- and post-intervention and within and between group outcomes indicated. Data are presented as β (95% CI), mean ± SD or median (IQR), with mean and direction of change and p-values (significance at <0.05). Effect size (Cohen's d) compares final mean values of the study groups and can be interpreted as small (<0.20), medium (2.0 to 0.50) or large (0.5 to 0.80)

804 **Table 3. Compliance of the studies included in the present review with the Quality**
805 **Criteria Checklist: Primary Research[22]**

Criteria	Reference										
	Chen et al. (2015) [36]	Farhangi et al. (2016) [26]	Gomez-Delgado et al. (2016) [31]	Michalsen et al. (2006) [32]	Panagiotakos et al. (2009)[29]	Rifler et al. (2011) [34]	Roca-Rodriguez et al. (2014) [30]	Serrano-Martinez et al. (2005) [27]	Stewart et al. (2016) [28]	Thomazella et al. (2011) [35]	Tuttle et al. (2008) [33]
1. Was the research question clearly stated?	+	+	+	+	+	?	+	+	+	+	+
2. Was the selection of study subjects/patients free from bias?	+	+	+	+	+	-	?	+	+	+	?
3. Were study groups comparable?	+	NA	+	+	?	?	NA	NA	NA	?	+
4. Was method of handling withdrawals described?	?	+	-	+	-	?	?	+	+	+	+
5. Was blinding used to prevent introduction of bias?*	-	-	-	-	-	+	-	-	+	+	+
6. Were intervention/therapeutic regimens/ exposure factor or procedure and any comparison(s) described in detail? Were intervening factors described?	?	?	+	+	+	?	?	+	+	+	+
7. Were outcomes clearly defined and the measurements valid and reliable?	+	?	?	+	+	+	?	+	+	+	+
8. Was the statistical analysis appropriate for the study design and type of outcome indicators?	?	+	+	+	+	?	+	+	+	+	+
9. Are conclusions	+	+	+	+	+	+	+	?	+	+	?

supported by results with biases and limitations taken into consideration?											
10. Is bias due to study's funding or sponsorship unlikely?	+	+	?	+	+	?	+	+	+	+	?
Total (out of 10)	6/10	6/10	6/10	9/10	7/10	3/10	4/10	7/10	9/10	9/10	7/10
Rating (+, ϕ, -)	(ϕ)	(ϕ)	(ϕ)	(+)	(ϕ)	(-)	(-)	(ϕ)	(+)	(ϕ)	(ϕ)

Abbreviations: +, Yes; -, No; ?, Unclear; NA, not applicable, (+), positive of high method quality with score ≥8 and Yes to 2,3,6 and 7 where applicable; (ϕ), neutral of moderate method quality with score 5 to 7; (-), negative of low method quality with score <5.

*Trials which employed blinding for outcome assessment only were awarded 'Yes' for this question, as blinding of participants and clinicians cannot be expected in interventions that employ a dietary pattern.

812 **HIGHLIGHTS**

813

814 **Mediterranean diet and inflammatory markers in patients with coronary heart disease:**

815 **a systematic review and meta-analysis**

816

817

818 - Improving levels of circulating inflammatory cytokines and adipokines has been
819 proposed as a therapeutic target for reducing risk of CHD.
820 - This is the first systematic review of literature which reports the impact of a
821 Mediterranean diet on inflammatory markers strictly in patients with CHD and/or who
822 have experienced acute coronary syndrome.
823 - This review found limited literature investigating the effect of a Mediterranean diet on
824 inflammatory cytokines in patients with CHD.
825 - Despite promising findings from observational studies, Mediterranean diet
826 interventions demonstrated mostly non-significant effects on inflammatory cytokines
827 and no effect in comparison to low fat diets in controlled trials.
828 - There is a need for randomized controlled trials using traditional a Mediterranean diet
829 in non-Mediterranean populations and with multiple inflammatory biomarkers in
830 high-risk CHD patients.