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

3

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24

25

26 **Abbreviations**

27

28 CHD; coronary heart disease

29 CRP; C-reactive protein

30 ACS; acute coronary syndrome

31 MI; myocardial infarction

32 TNF- α ; tumor necrosis factor-alpha

33 RCT; randomized controlled trial

34 LDL; low density lipoprotein

35 T2DM; type 2 diabetes mellitus

36 MUFA; monounsaturated fatty acids

37 PUFA; polyunsaturated fatty acids

38 SFA; saturated fatty acids

39 SD; standard deviation

40 CVD; cardiovascular disease

41 BMI; body mass index

42 AHA, American Heart Association

43 EPA; eicosapentaenoic acid

44 DHA; docosahexaenoic acid

45 IL; interleukin

46 EVOO; extra virgin olive oil

47 DASH; Dietary Approaches to Stop Hypertension

48

49

50

51 **Abstract**

52

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

71

72 **Keywords**

73

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

76 1. Introduction

77

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

85

86 Plasma levels of inflammatory biomarkers such as C-reactive protein (CRP), interleukins,
87 and adipokines such as tumor necrosis factor-alpha (TNF- α) and adiponectin, are independent
88 predictors of primary as well as secondary incidence of coronary disease [4-6]. They have
89 emerged as targets for treatment of CHD, beyond the classic risk factor target of cholesterol
90 [7]. Notably, patients who have low CRP levels after statin therapy have better clinical
91 outcomes, regardless of the resultant level of low density lipoprotein (LDL)-cholesterol [8].

92

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

101

102 The Mediterranean diet has the strongest evidence for secondary prevention of CHD. In the
103 1990s, the Lyon Diet Heart study demonstrated a significant reduction in incidence of
104 secondary ACS with a Mediterranean diet intervention compared to control low fat diet [16].
105 The mechanism of dietary influence was not identified by the study investigators and
106 recognized markers of inflammation were not measured. In non-Mediterranean countries, low
107 fat prudent dietary patterns which have less anti-inflammatory potential are still
108 recommended in the management for CHD [17, 18].

109

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

117

118 **2. Approach**

119

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

124

125 **2.1 Search strategy**

126

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

138

139 *2.2 Selection criteria*

140

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

147

148 Studies were required to meet the following inclusion criteria: (1) male and/or female adults
149 (≥ 18 years of age) with diagnosed CHD and/or who have experienced ACS: unstable angina,
150 angiographic diagnosed arterial disease, MI, coronary artery bypass grafting or percutaneous

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

165

166 ***2.3 Study selection***

167

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

174

175 ***2.4 Data extraction***

176

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

186

187 *2.5 Quality assessment*

188

189 Risk of bias in each included study was assessed by two reviewers (HLM and ACT) using the
190 Academy of Nutrition and Dietetics *Quality Criteria Checklist: Primary Research* [22] (see
191 Supplemental Figure S1 for the complete checklist). Studies were assessed on 10 key criteria
192 assessing internal and external validity. Each study was awarded an overall positive (scores
193 $\geq 8/10$), neutral (5 to 7/10) or negative ($< 5/10$) method quality rating. Risk of bias was also
194 assessed across studies, with consideration of the number of studies that achieved high or low
195 risk in each of the 10 key criteria and study design.

196

197 *2.6 Summary measures*

198

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

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

207

208 *2.7 Statistical analyses*

209

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

219

220 **3. Results**

221

222 *3.1 Study selection*

223

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

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

232

233 *3.2 Study and sample population characteristics*

234

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

240

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

250

251 Eligibility criteria varied across the studies. Some studies were restricted to patients
252 undergoing specific cardiac procedures, including candidates for coronary artery bypass
253 grafting [26] or coronary angiograms [27]. Three studies required prior diagnosis of MI [29,
254 33, 34] and the remaining studies included patients with established CHD and/or any ACS.
255 The majority of participants in all studies which reported treatments [26-28, 32, 33, 35] had
256 undergone coronary revascularization. Where reported, time since coronary event ranged
257 between 6 years prior to diagnosis or procedure to immediately post diagnosis or procedure
258 being undertaken. In addition, most studies did not indicate event recurrence, with only two
259 studies specifying restriction to first event [27, 33]. Where cardiac medication use was
260 reported [27, 28, 30-36], statin use was high, ranging from 62 to 100 % of patients, and LDL-
261 cholesterol levels were generally well controlled. No study reported use of nonsteroidal anti-
262 inflammatory drugs. Samples which reported a mean body mass index (BMI) had populations
263 classified as overweight (25 - 30 kg/m², n = 6[26-29, 32, 35]), or obese (>30 kg/m², n = 3 [31,
264 33, 36]). The cohort study measured dietary intake at baseline only and relevant biomarker
265 outcomes from baseline to 9-11 months follow-up [29]. The length of intervention trials
266 ranged from 2 weeks to 24 months.

267

268 3.2.1 *Mediterranean-type diets*

269

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

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

282

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

300

301 Three studies explored the effect of a Mediterranean-type diet with co-intervention. Two
302 studies were conducted in the context of a broader cardiac rehabilitation program with
303 prescribed exercise programs and health education [30, 34] and another employed stress
304 reduction techniques [32]. Two of these controlled trials also had alternative Mediterranean
305 diets as their ‘control’ group. One was a traditional Mediterranean diet without red wine
306 within cardiac rehabilitation [34]. The other differed to its intervention group by receiving
307 less attention and dietary information on the Mediterranean-type diet and stress reduction
308 (short written resources on diet and stress management provided at the start of the study only)
309 [32]. These three studies did not report the effect of the Mediterranean-type diet on
310 biomarkers of inflammation independent of the co-interventions.

311

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

325

326 3.2.2 *Control diets*

327

328 Two observational studies identified comparative dietary patterns. The cross-sectional study
329 which used principal component analysis also identified 'healthy', 'intermediate', 'neo-
330 traditional' and 'Western' patterns of intake [26]. Another study measured a pre-defined
331 Western diet score [28]. Four of the six controlled intervention trials employed low fat diets
332 as the control group [31, 33, 35, 36], as guided by nutrition recommendations of the AHA.
333 Common dietary models included macronutrient targets of: total fat <30 %, carbohydrates
334 >55 %, protein ~15 %, as contribution to energy, and cholesterol <300 mg/day at most.
335 Specific fat recommendations ranged between saturated fat <7 % to <10 %, MUFA 10 - 20%
336 and PUFA 6 - 10 % as contribution to energy. Three studies indicated food group
337 recommendations [31, 33, 35], which all promoted low fat foods and wholegrains and one
338 study specifically promoted daily intake of a phytosterol-rich spread [35]. Three of the four
339 studies with a low-fat diet control group reported on change in nutrient intake [33, 35, 36],
340 whereas change in food groups was not reported. All three achieved a reduction in saturated
341 fat and cholesterol intake from baseline, however there was limited change to other macro- or
342 micronutrients. At end intervention, these three low-fat control diet groups reported
343 significantly lower intakes of PUFA and two reported lower intake of MUFA [35, 36]
344 compared to the Mediterranean-type diet group.

345

346 3.3 *Risk of bias*

347

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

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

363

364 *3.4 Effect of Mediterranean-type diets on study outcomes*

365

366 Table 2 shows inflammatory cytokines or adipokines measured across studies included CRP
367 (10 studies), interleukin (IL)-6 (3 studies) and TNF- α (3 studies). One study also reported
368 measuring IL-1- β , IL-4, IL-8 and interferon- γ .

369

370 *3.4.1 C-reactive protein*

371

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

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

382

383 All intervention trials measured CRP and reported reductions in mean measures in the
384 Mediterranean-type diet intervention group ranging between -0.09 to -2.02 mg/L. However,
385 only two studies demonstrated a statistically significant ($p < 0.05$) reduction in CRP [30, 31].
386 Both had a 12 month follow-up period. One was in a small cohort with a Mediterranean-type
387 diet as part of a cardiac rehabilitation program [30] and the other was the largest RCT
388 included [31] (this publication reported on change in CRP between genotypes of a single
389 nucleotide polymorphism, but data for each study group was pooled for this review). Both of
390 these studies were conducted in Spain, recommended exclusive use of extra virgin olive oil
391 (EVOO) and either did not achieve energy reduction [30] or had an *ad libitum* approach [31].
392 At baseline, these two trials reported mean plasma CRP values of 5.1 [30] and 2.68 [31]
393 mg/L in the Mediterranean-type diet groups. In contrast, three of the five study groups which
394 reported a non-significant reduction in CRP with Mediterranean-type diet had mean baseline
395 plasma CRP values well within the normal reference range of < 3 mg/L, at 0.38 [33], 0.93
396 [32] and 1.65 [35] mg/L. The final two trials reported higher mean baseline plasma CRP
397 levels (4.0 [36] and 8.4 [34] mg/L), but the latter was in an acute cohort hospitalized for
398 recent MI.

399

400 No significant differences were reported between Mediterranean-type diet and low fat diet
401 control groups for effect on CRP. Where calculated, effect sizes were low to medium,
402 ranging between 0.185 to 0.451 for studies where the Mediterranean-type diet had lower
403 mean CRP at end-intervention than a low fat diet [31, 35, 36]. For one study which had lower
404 mean CRP at end-intervention with the low fat diet group [33], the effect size was low at
405 0.272. The meta-analysis pooled the outcomes for these four controlled trials (total n = 1,128
406 participants) reporting the effect of a Mediterranean-type diet versus low fat diet on CRP
407 (Figure 2). Final mean values were used, as no studies reported Mean (SD) for change values.
408 A Mediterranean-type diet was not significantly associated with a greater reduction in CRP
409 compared to low fat diets (weighted mean difference, -0.11 [95% CI: -0.36, 0.15]; p= 0.41).

410

411 3.4.2 *Interleukin-6*

412

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

422

423 3.4.3 *Tumor necrosis factor-alpha*

424

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

431

432 4. Discussion

433

434 This systematic review investigated the effects of a Mediterranean-type diet on inflammatory
435 biomarkers in patients with CHD. A limited amount of literature is reported in the area.

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

449 CRP values at baseline across the included studies. In addition, three studies had small
450 sample sizes (total $n \leq 101$).

451

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

460

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

470

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

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

477

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

486

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

496

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

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

502

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

510

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

519

520 One of the RCTs included in this review [31] reported preliminary results from the
521 CORDIOPREV study [61]. Their included paper, as well as two others, have demonstrated in
522 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

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

560

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

568

569 **5. Future directions**

570

571 More studies are needed to determine whether a Mediterranean diet has an anti-inflammatory
572 effect in a high-risk population with CHD. RCTs are warranted testing the traditional
573 Mediterranean diet with a range of biomarkers of inflammation in addition to CRP, such as
574 inflammatory cytokines (interleukins) and adipokines (TNF- α and adiponectin). These
575 markers should be measured pre- and post-intervention, and at relevant intervals with at least
576 12 months follow up. This effect should also be evaluated in adequately powered studies and
577 in non-Mediterranean populations.

578

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585

586 **Appendix**

587

588 Supplemental Materials

589

590 **Figure Legends**

591

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

594

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

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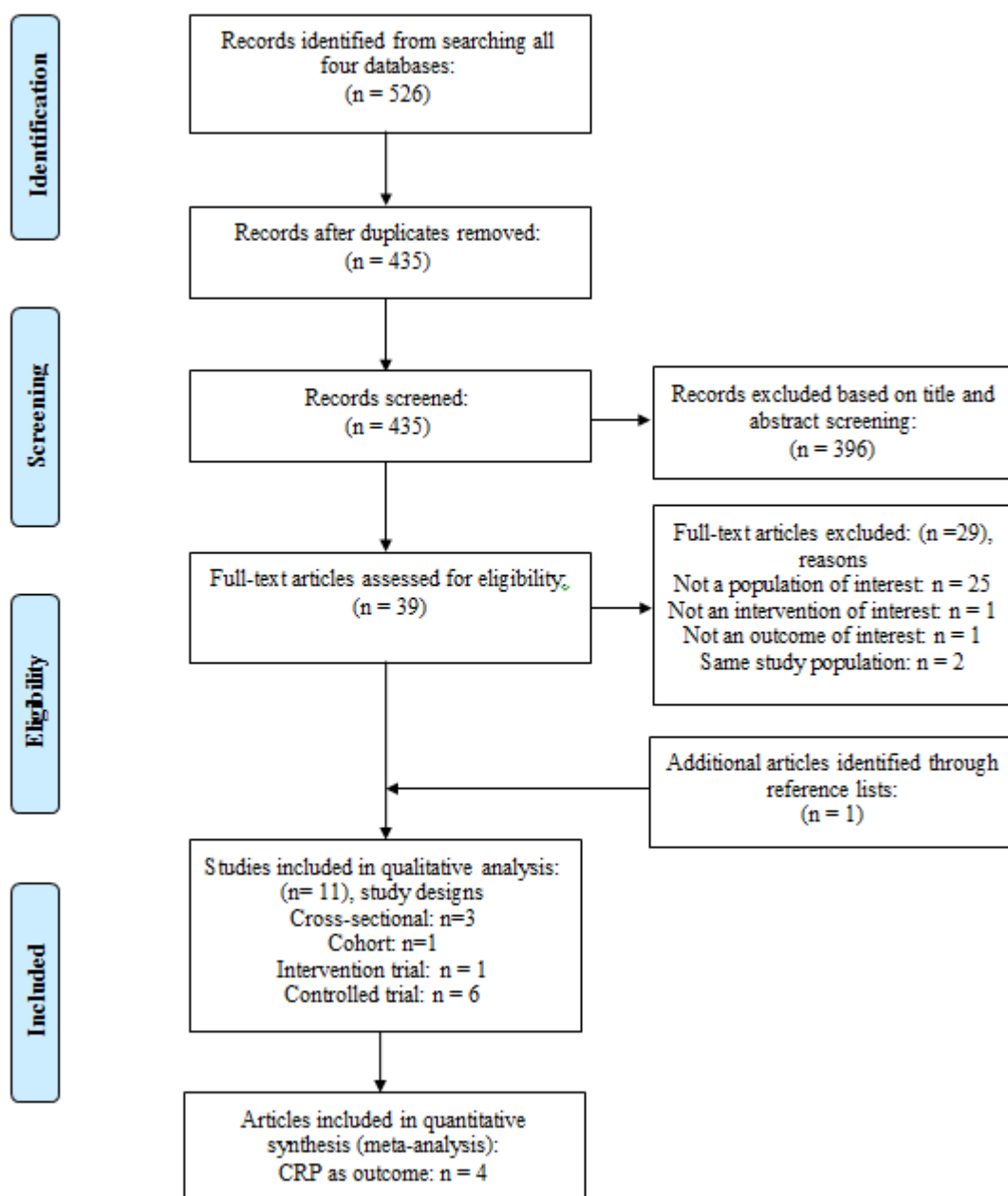
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782

783



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

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789 **Table 2. Summary of the characteristics and main findings of the studies included in**
 790 **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
<i>Observational studies</i>							
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	<i>Plasma CRP (mg/L)</i> , 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	<i>Log CRP</i> , 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) <i>Log IL-6</i> , adjusted for sex, smoking history,

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	regular exercise, BMI, diabetes and medications Diet score (1 unit) $\beta=0.019$ (95% CI -0.033 to -0.005, $p=0.01$) <i>Serum TNF-α</i> (coronary venous blood pg/ml), adjusted for gender, BMI and classical coronary risk factors Mediterranean diet score $\beta = -41.6$ (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 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	<i>High sensitivity-CRP (mg/L)</i> 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	<i>CRP (mg/L)</i> 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 <i>d</i>) = 0.128 <i>TNF-α</i> (<i>pg/mL</i>) Step 1 + almonds diet pre: 1.8 ± 1.8, 6 weeks: 1.8 ± 1.6 (<i>p</i> >0.05), no change Step 1 diet pre: 1.6 ± 1.4, 6 weeks: 1.6 ± 1.4 (<i>p</i> >0.05), no change Effect size (Cohen's <i>d</i>) = 0.133 <i>IL-6</i> (<i>pg/mL</i>) Step 1 + almonds diet pre 3.5 ± 2.2, 6 weeks 3.6 ± 2.5 (<i>p</i> >0.05), mean ↑0.1 Step 1 diet pre- 3.8 ± 2.9, 6 weeks 4.1 ± 2.9 (<i>p</i> >0.05), mean ↑0.3 between group <i>p</i> >0.05 Effect size (Cohen's <i>d</i>) = 0.185 Between groups all outcomes <i>p</i> >0.05
Gomez- Delgado et al. (2015),[31] Spain	Randomiz- ed controlled trial (parallel)	ACS as acute MI, unstable angina or chronic CHD, without events < 6 months prior	n=897 (gender not specified) characteristic s 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 carbohydrat e AHA diet	<i>High</i> <i>sensitivity-</i> <i>CRP</i> (<i>mg/L</i>), adjusted for age, BMI, gender, and smoking status Mediterranea n diet Baseline: 2.68 ±0.19, 12 months: 1.95 ±0.12, mean ↓0.73 (<i>p</i> <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)	<i>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</i>
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	<i>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-α Baseline to 2 weeks in both groups, p>0.05</i>

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 n 8 weeks, total 12 months	Mediterranean diet as part of cardiac rehabilitation with exercise training and health education	Nil	CRP ($\mu\text{g/mL}$) 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 n-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

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

795 ^a Coronary heart disease criteria and time since diagnosis, if reported
796 ^b n= refers to total sample size, data are presented as mean \pm SD, mean (range) or n (%)
797 ^c n= refers to the number of individuals per group, name of diet as indicated by study authors, see Supplemental Table S3 for
798 explanations of the diets
799 ^d Results presented individually for relevant markers measured (with units) pre- and post-intervention and within and
800 between group outcomes indicated. Data are presented as β (95% CI), mean \pm SD or median (IQR), with mean and direction
801 of change and p-values (significance at <0.05). Effect size (Cohen's d) compares final mean values of the study groups and
802 can be interpreted as small (<0.20), medium (2.0 to 0.50) or large (0.5 to 0.80)
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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 (ϕ)	6/10 (ϕ)	6/10 (ϕ)	9/10 (+)	7/10 (ϕ)	3/10 (-)	4/10 (-)	7/10 (ϕ)	9/10 (+)	9/10 (ϕ)	7/10 (ϕ)
Rating (+, ϕ, -)											

806 Abbreviations: +, Yes; -, No; ?, Unclear; NA, not applicable, (+), positive of high method quality with score ≥ 8 and Yes to
807 2,3,6 and 7 where applicable; (ϕ), neutral of moderate method quality with score 5 to 7; (-), negative of low method quality
808 with score < 5 .

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

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HIGHLIGHTS

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

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