## TITLE

Mediterranean diet increases endothelial function in adults: A systematic review and meta-analysis of randomized controlled trials


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Mediterranean diet and endothelial function

**ABBREVIATIONS:**

| | |
|---|---|
| BP | Blood pressure |
| BMI | Body mass index |
| CRP | C-reactive protein |
| CVD | Cardiovascular disease |
| DASH | Dietary approach to stop hypertension |
| EVOO | Extra virgin olive oil |
| FBF | Forearm blood flow |
| FMD | Flow mediated dilation |
| IL-6 | Interleukin-6 |
| LDL | Low density lipoprotein |
| MedDiet | Mediterranean dietary pattern |
| MUFA | Monounsaturated fatty acids |
| NO | Nitric oxide |
| PREDIMED | Prevención con Dieta Mediterránea |
| RCT | Randomised controlled trial |
| sICAM-1 | Soluble intercellular adhesion molecule-1 |
| TNF-α | Tumour necrosis factor-α |
| VCAM-1 | Vascular cell adhesion protein-1 |

**ABSTRACT**

Background: The endothelium plays a key role in the maintenance of vascular health, and represents a potential physiological target for dietary and other lifestyle interventions designed to reduce risk for cardiovascular diseases (CVD) including stroke or coronary heart disease.

Objective: To conduct a systematic review and meta-analysis of randomized controlled trials investigating effects of the Mediterranean dietary pattern (MedDiet) on endothelial function.

Methods: Medline, Embase and Scopus databases were searched from inception until January 2019 for studies that met the following criteria: 1) RCTs including adult participants, 2) interventions promoting a Mediterranean dietary pattern, 3) inclusion of a control group and 4) measurements of endothelial function. A random-effects meta-analysis was conducted. Meta-regression and sub-group analyses were performed to identify whether effects were modified by health status (i.e., healthy participants *vs* participants with existing comorbidities), type of intervention (i.e., MedDiet alone or with a co-intervention), study duration, study design (i.e., parallel or crossover), body mass index (BMI), and age of participants.

Results: Fourteen articles reporting data for 1930 participants were included in the meta-analysis. Study duration ranged from 4 weeks to 2.3 years. We observed a beneficial effect of the MedDiet on endothelial function (standardised mean difference (SMD): 0.35 95% CI: 0.17, 0.53, $P<0.001$, $I^2 = 73.68\%$). MedDiet interventions improved flow mediated dilation (FMD) - the reference method for non-invasive, clinical measurement of endothelial function - by 1.66% (absolute change; 95% CI: 1.15, 2.17, $P<0.001$, $I^2 = 0\%$). Effects of the MedDiet on endothelial function were not modified by health status, type of intervention, study duration, study design, BMI, and age of participants ($P>0.05$).

Conclusions: MedDiet interventions improve endothelial function in adults, which suggest that the protective effects of the MedDiet are evident at early stages of the atherosclerotic process with important implications for the early prevention of CVD.

**PROSPERO registration number:** CRD42018106188.

**KEY WORDS:** Mediterranean diet, endothelial function, flow mediated dilation, cardiovascular disease, healthy ageing, dietary patterns

## INTRODUCTION

The endothelium plays a key role in the maintenance of vascular health via the secretion of multiple signalling molecules including nitric oxide (NO), endothelins, selectins and adhesion molecules which, in concert, control vasomotor tone, and have anti-atherogenic and anti-proliferative actions (1,2). Loss of functional and structural integrity of the endothelium is thought to be an early pathogenic step in the development of atherosclerotic lesions and the subsequent onset of cardiovascular diseases (CVD) (3). Therefore, the endothelium has been identified as a tractable physiological target for therapeutic interventions designed to reduce risk for CVD such as stroke, coronary heart disease or peripheral arterial disease (4,5).

The Mediterranean dietary pattern (MedDiet) is characterised by high consumption of olive oil, fruits, vegetables, legumes, nuts and seeds, and unrefined grains, moderate-to-high consumption of fish, and low consumption of red meat and sugar-sweetened products such as sweets, cakes, and pastries and is considered as one of the healthies dietary patterns (6,7). Evidence from randomised controlled trials (RCTs) such as the Lyon Diet Heart Study in France (8) and the Prevención con Dieta Mediterránea (PREDIMED) trial in Spain (9) demonstrates that the MedDiet is effective in both primary and secondary prevention of CVD. Improvements in endothelial function with the MedDiet may be one of the key mechanisms underpinning these beneficial effects (10). Evidence from RCTs demonstrates that, in isolation, a number of components of the MedDiet, particularly olive oil (11), nuts (12), and oily fish (13,14), improve endothelial function. In addition, there is evidence that the composite MedDiet enhances endothelial function in both healthy subjects and patients with cardiovascular and metabolic diseases. For example, a 6 month MedDiet intervention in healthy older individuals induced highly significant improvements in endothelial function measured via flow mediated dilation (FMD), with the percentage FMD approximately double

baseline values (absolute increase of ~1.3%) (15). Similarly, in patients with pre-diabetes and diabetes a 1.5 year MedDiet intervention increased FMD by 1.1% and 1.4%, respectively (16). In addition, markers of endothelial structure such as carotid artery intima media thickness have also been shown to be improved with MedDiet interventions (17)

A previous systematic review and meta-analysis in 2014 reported improved endothelial function and decreased inflammation with MedDiet versus control interventions (18). However, that review also included MedDiet-like interventions such as the Dietary Approach to Stop Hypertension (DASH) in the same analysis, which may elicit differential effects on endothelial function compared with the traditional MedDiet. Moreover, in that systematic review, only studies with intervention period lasting ≥12 weeks were included, and the effects of shorter MedDiet interventions were not evaluated. These limitations, and the emergence of considerable new research in this area over the past 5 years (e.g. 14,15,17–19), provide the rationale for an updated systematic review and meta-analysis of RCTs. Therefore, the aim of this study was to undertake a systematic review and meta-analysis of published RCTs exploring the effects of MedDiet interventions on structural (e.g., intima media thickness) and/or functional (e.g., FMD) measures of endothelial function in humans. In an attempt to understand potential differences in findings, we also investigated whether effects were modified by health status, type of intervention, study duration, study design, body mass index (BMI), and age of participants.

## METHODS

The present systematic review was conducted according to the Preferred Reporting Items for Systematic Review and Meta-analyses (PRISMA) guidelines (21).

**Literature search**

Three databases (Medline, Embase and Scopus) were used to search for articles from inception until January 2019. In addition, a manual search of reference lists of relevant reviews and articles included in the systematic review was performed. The search was conducted based on the pre-defined search terms (Mediterranean AND diet*) AND ("endotheli*" [All Fields] OR "endothelial function" [All Fields] OR "endothelial dysfunction" [All Fields] OR "vascular function" [All Fields] OR "blood flow" [All Fields] OR "vascular reactivity" [All Fields] OR "vasodilation" [All Fields]) and (Mediterranean AND diet*) AND ("Flow-mediated dilatation" OR "Flow-mediated dilation" OR FMD OR "Venous occlusion plethysmography" OR "Peripheral arterial tonometry" OR "Nitric oxide" OR "Endothelial function" OR "Endothelial dysfunction" OR "Carotid Intima-Media Thickness" OR "Pulse Wave Velocity" OR "Augmentation Index"). Further details of the search strategy are provided in **Supplemental Methods 1**.

**Study selection**

The following criteria were applied to identify articles to be included in this systematic review and meta-analysis: 1) RCTs (no further exclusion criteria were applied in relation to study design or blinding); 2) studies involving adults aged ≥18 years and no exclusion criteria were applied for health status or smoking history; 3) MedDiet (which was defined as a MedDiet by the authors of each study) administered alone or with other clinical, pharmaceutical or lifestyle interventions if a comparable and valid control group was included (for example, MedDiet plus exercise compared to control group including exercise alone); 4) studies reporting changes in endothelial function for intervention and control groups separately; 5) no language or time restrictions were applied in searching the databases.

Two investigators (CH, IM) independently screened the titles and abstracts of the articles to evaluate eligibility for inclusion. If consensus was reached, articles were either excluded or moved to the next stage (full-text). If consensus was not reached the articles was moved to the full-text stage. The full-texts of the selected articles were appraised critically to determine eligibility for inclusion in the systematic review. Disagreements were resolved by discussion between the reviewers (including MS) until consensus was reached.

**Data extraction and quality assessment**

Data extraction was completed by one investigator (CH) and data entries were checked for accuracy by a second investigator (IM). The following information was extracted from the eligible articles: author, year of publication, country, study design, inclusion and exclusion criteria, study duration, run-in phase, intention to treat analysis, sample size, type of intervention (control and MedDiet), age, sex, ethnicity, randomisation procedure, blinding of exposure and outcome measurements, compliance with the interventions, BMI, dietary intervention, weight loss during the study, baseline and post-intervention measurements of systolic and diastolic blood pressure (BP), baseline and post-intervention measurements of endothelial function. In addition, two independent reviewers (CH, IM) utilised the Cochrane risk of bias tool to assess the risk of bias of the included studies which was classified as 1) high risk, 2) low risk or 3) unknown risk of bias (22) and any discrepancy was resolved by consensus with a third reviewer (MS)

**Statistical analysis**

Statistical analyses were performed by using Comprehensive Meta-Analysis Software Version 2 (Biostat, Englewood, NJ, USA). For this purpose, sample size, the mean and SD of the endothelial function measurements before and after the intervention period (for both MedDiet

intervention and control) were extracted and used in the analyses. When no baseline measurements were reported, the sample size, means and SDs after the intervention were used. If the mean and SD were not given, the sample size and the *P* value of the difference between MedDiet and control were used to calculate the effect size (Cohen's d). For studies that reported changes in endothelial function at two or more time-points, the last endothelial function measurement was used in the meta-analysis. The calculation of the effect sizes using different sets of data is performed automatically by the software using integrated algorithms (23). Data not provided in the main text or tables were extracted from the figures. Some trials used more than one method to assess changes in endothelial function (Table 1) which may lead to a reduced independence of the measurements and, consequently, to over-estimation of the effect size derived from the meta-analysis. This potential confounding factor was taken into account during analysis by estimating the mean of the standardised effect sizes derived from each endothelial function measurement within each such study to provide a more conservative estimate of the effect size.

Effect sizes and 95% confidence intervals for the MedDiet interventions were calculated using a using a weighted DerSimonian-Laird random effects model (24). Forest plots were generated to present graphically the cumulative effect of MedDiet on endothelial function. Analyses were conducted on all endothelial function measurements but also stratified by type of endothelial function measurement (structural and functional). Functional measurements include FMD derived from ultrasound, forearm blood flow (FBF) derived from phlethysmography or cutaneous microcirculation derived from laser Doppler. Structural measurements include intima media thickness or vessel size measured both by ultrasound. In addition, we performed a sensitivity analyses to test the effects of MedDiet on FMD only, on the basis that this is the

reference method for non-invasive, clinical measurement of endothelial function (25) and was used in the majority of studies.

Subgroup analyses were undertaken to investigate variables which may have influenced the effects of MedDiet on endothelial function. These factors included: health status (healthy subjects *vs* patients with existing comorbidities), type of intervention (MedDiet alone or administered with other clinical or pharmaceutical interventions), type of endothelial function measurement (functional versus structural) and study design (parallel or crossover). Random-effect meta-regression analyses were used to determine whether participant baseline characteristics (age, BMI) and duration of the study influenced the effect of the MedDiet on endothelial function. Funnel plots and Egger's regression tests were performed to evaluate the risk of publication bias. Heterogeneity was assessed by using Cochrane Q statistic; $P<0.1$ indicates significant heterogeneity. The $I^2$ test was also utilised to assess heterogeneity across trials where a value < 25% indicates low risk, 25-75% indicates moderate risk, and >75% indicates a high risk (26). Sensitivity analyses were conducted to identify the source of heterogeneity by conducting stratified analyses or selectively removing studies with larger effect size. All of the data used in the meta-analysis can be found in **Supplemental Tables 1-5**.

## RESULTS

### Search results

The process of screening and selection of the studies is summarised in **Figure 1.** The primary search of the three databases produced 12857 articles, after removal of duplicates. After title and abstract screening, 15 full-text papers were retrieved for further evaluation. A further four studies were found by manual searching of references of relevant reviews and studies.

Examination of the full text of the 19 included articles yielded 14 studies which were eligible to be included in this systematic review and meta-analysis. Some of these papers reported results from independent studies testing the effects of MedDiet on endothelial function generating a total of 20 sets of independent measures of endothelial function using different methods that were included in the meta-analyses.

**Study characteristics**

The total number of participants from the 14 articles included in this systematic review was 1930 with a median of 131 (range 20 - 438) participants per study. The median participant age was 55 (range 38 - 71) years. Eleven of the RCTs included in the meta-analysis were parallel trials with a control group, while 3 were crossover studies (22). The paired nature of the cross-over trials was taken into account in the meta-analysis to minimise unit-of-analysis errors and underestimation of the effect size. The duration of the interventions ranged from 4 weeks to 121 weeks (**Table 1**). Five studies investigated the effect of MedDiet in healthy participants (15,16,27–29), three in people with diabetes (16,17,19), two in patients with elevated risk of CVD (30,31), two in overweight or obese participants (20,32), one in patients with metabolic-syndrome (33), one in people with pre-diabetes (16), one in patients with acute coronary syndrome (34), and one in hypercholesteraemic men (35). Various permutations of the MedDiet were prescribed, including: a MedDiet (n=8), a MedDiet plus supplementary nuts (n=2), a MedDiet plus supplementary extra virgin olive oil (n=5), a MedDiet plus mono-unsaturated fatty acids (n=2), and a MedDiet plus exercise (n=1). Additionally, a variety of different control groups were employed. These were: a low-fat diet (n=8), a typical Swedish diet (n=1), the Atkins low-carbohydrate diet (n=1), the participants habitual diet (n=2), the National Cholesterol Education Program Diet (n=1), a non-Mediterranean diet plus exercise (n=1), a saturated fatty acid diet (n=1), and a prudent diet (n=1). Several methods were used

to assess endothelial function in the included trials. The most commonly used methods were FMD and carotid intima-media thickness. Other methods included forearm blood flow, cutaneous microvascular function, baseline vessel size, and calculation of an endothelial function score (**Table 1**).

**Meta-analysis**

Meta-analysis of the 20 sets of independent results showed that, overall, MedDiet improved endothelial function (SMD: 0.35, 95% CI: 0.17, 0.53, $P$<0.001; **Figure 2**). Heterogeneity between studies was significant ($Q$=64.60, $I^2$=73.68%, $P$<0.001). However, the removal of two studies with wider effect estimates (27,33) (**Supplemental Fig. 1)** explained the heterogeneity of the results ($Q$=13.82, $I^2$=0%, $P$=0.53) while still confirming a significant effect of MedDiet on endothelial function (SMD: 0.27, 95% CI: 0.18, 0.36, $P$<0.001). The subgroup analyses showed that the effect was stronger on functional (SMD: 0.44, 95% CI: 0.19, 0.69, $P$<0.001; $I^2$=78.2%) compared with structural (SMD: 0.16, 95% CI: 0.02, 0.30, $P$=0.01; $I^2$=0%) measurements of endothelial function. MedDiet increased FMD by 1.66% (95% CI: 1.15, 2.17, $P$<0.001; **Figure 3)**. Subgroup analyses showed that MedDiet improved endothelial function significantly in healthy participants (SMD: 0.29, 95% CI: 0.05, 0.53, $P$=0.01; $I^2$=26.5%) and in those with increased risk of CVD (SMD: 0.36, 95% CI: 0.15, 0.58, $P$=0.001; $I^2$=79.1%). The effects of the MedDiet on endothelial function were not modified significantly by the type of study design (crossover or parallel) or type of intervention (MedDiet alone or combined; **Table 2**). Meta-regression analyses demonstrated no modification of the effect size by age, BMI or study duration (**Table 3**). However, a significant association was found between study duration (in weeks) with functional (slope: 0.006; SE: 0.003; $P$=0.04; **Figure 4A**) but not structural measurements (slope: -0.001; SE: 0.001; $P$=0.39, **Figure 4B**) of endothelial function.

255 **Study quality and publication bias**

256 Overall, the quality of the trials was modest as the majority of the studies failed to report key
257 information to assess the presence of bias. Attrition bias was present in 50% of the studies (15–
258 17,20,31,33,34) and few studies reported selection bias (<10% of the studies) (20). Seven
259 studies described the randomisation method (15–17,19,28,33,34), and three studies stated the
260 methods of allocation concealment (17,29,33). Five studies reported and described participant
261 dropout (19,27–29,32), while three studies described selective reporting of the results
262 (20,29,34) (**Supplemental Fig. 2**). Visual inspection of the Funnel plot revealed two studies
263 with wider effect estimates but overall there was no evidence of publication bias, which was
264 confirmed by Egger's Regression test ($P$=0.71; Supplemental Fig. 1).

265

266 **DISCUSSION**

267 Overall, the results of this meta-analysis demonstrate that a MedDiet improves endothelial
268 function. The beneficial effects are evident for both functional and structural measures of
269 endothelial function, although effects were stronger for functional measures. In addition, the
270 effects of a MedDiet were similar in both healthy participants and those at increased risk of
271 CVD and, overall, were not modified by the study design or duration, type of intervention, BMI
272 or age of participants.

273

274 In a previous systematic review and meta-analysis, Schwingshackl and Hoffmann (18) reported
275 improvements in endothelial function with MedDiet interventions. In particular, MedDiet
276 interventions increased FMD by 1.86%, which is similar to the 1.66% average improvement in
277 FMD observed in this analysis. Importantly, the pooled effect size reported by Schwingshackl
278 and Hoffmann (18) was based on the results of only two studies, where as our meta-analysis
279 included seven studies which examined the effects of the MedDiet on FMD, which adds greater

confidence to this result. To contextualise these findings, a meta-analysis by Inaba et al (36) demonstrated a 13% decrease in the risk of cardiovascular events per 1% increase in FMD. Thus, a 1.66% improvement in FMD with a MedDiet could potentially translate into a ~22% reduction in cardiovascular events; however, these results require a cautious interpretation and need corroboration in future, more robust studies. As the MedDiet may also reduce CVD risk via a range of other mechanisms, some of which may be independent of effects on endothelial function (e.g. reduced BP, decreased oxidative stress and inflammation, altered gut microbiome (37)), the overall effects of this dietary pattern on CVD risk may be even greater. Indeed, in the large-scale PREDIMED trial, CVD incidence was reduced by 31% and 28% with an average 4.8 year MedDiet intervention supplemented with additional olive oil or nuts, respectively (9). A novel finding of the present analysis was that MedDiet interventions also improved structural measures of endothelial function (e.g. carotid intima-media thickness). However, effects were less pronounced than for functional changes and meta-regression revealed no relationship between study duration and effects of the MedDiet on structural outcomes. By contrast, there was a positive association between study duration and improvement in functional measures of endothelial function. This suggests that longer term consumption of the MedDiet may maximise the effects of this dietary pattern on functional measures of endothelial function, whilst structural changes appear to be relatively modest irrespective of the duration of exposure to this dietary pattern.

There are several mechanisms through which the MedDiet could improve endothelial function, which may account for the beneficial effects observed in this study. Firstly, the MedDiet augments the bioavailability of nitric oxide (NO) (38), which is essential for healthy endothelial function due to its vasodilatory, anti-atherogenic and anti-proliferative actions (5). The NO 'boosting' effects of the MedDiet may be due to antioxidant effects minimising superoxide

scavenging of NO (38,39), the provision of the NO precursors inorganic nitrate (green leafy vegetables) and L-arginine (nuts, grains, legumes, and fish) (39), and/ or the upregulation of endothelial NO synthase (oily fish) (40,41). In addition, the MedDiet may improve endothelial function by reducing oxidation of low-density lipoprotein (LDL) (42), which plays a major role in endothelial dysfunction and atherogenesis (43,44). The lower levels of oxidised LDL with the MedDiet are likely mediated by both the antioxidant effects of this dietary pattern and the increased provision of monounsaturated fatty acids which enhance the resilience of LDL to oxidation (45). Finally, a strong link has been reported between inflammation and endothelial dysfunction (46), and several studies have demonstrated beneficial effects of the MedDiet on inflammatory markers including interleukin-6 (IL-6), c-reactive protein (CRP), tumour necrosis factor-α (TNF-α), vascular cell adhesion protein-1 (VCAM-1), and soluble intercellular adhesion molecule-1 (sICAM-1) (47–50). These effects are associated with downregulation of the NF-kB pathway (51) and altered methylation of inflammation-related genes (50), and may further contribute towards improvements in endothelial function with a MedDiet.

**Limitations**

The overall quality of the studies included was modest. The majority of investigations did not blind participants to the intervention arm, with only three studies reporting methods of allocation concealment. This is a notable limitation, given the risk of expectation bias whereby the expectation of beneficial effects could result in more favourable outcomes in the intervention group (52). Nevertheless, it is acknowledged that blinding participants is very difficult in dietary intervention studies, particularly those advocating dietary pattern changes, and in many cases this may be unfeasible (53). Some studies had multiple assessments of

endothelial function over the duration of the trial and, for studies with longer duration, this may result in a decline of the effects on endothelial function due to a gradual decrease of the adherence to the interventions. However, we included the last measurement in the meta-analysis to standardize the approach across studies and remove any bias related to the selection of the intermediate measurements to be included in the analysis. A further limitation is that most studies were conducted in older subjects. Although meta-regression revealed no influence of age on the effects of the MedDiet, the lack of younger participants may limit our ability to generalise our conclusions, and further research is warranted to determine if the results are applicable to individuals at different life stages. Additionally, since there is no universal definition of what constitutes a MedDiet, details of the dietary interventions differed between studies, and it is possible that certain permutations of the MedDiet may be more effective than others in improving endothelial function as was evident from the high heterogeneity in our analysis. Likewise, the control group utilised was highly variable, such that there was no uniform benchmark against which the MedDiet was compared, which may further contribute towards the high heterogeneity in our analysis. Finally, there are also certain methodological limitations of this review which warrant discussion. Notably, we decided to only include studies where the authors identified their intervention as a MedDiet. This means that we may have missed some studies which administered a Mediterranean-type diet but which was not defined using this specific terminology. In addition, given the small number of studies included in this review, our analysis may have been underpowered to detect differences in intervention effectiveness based on health status, type of intervention, type of measurement, and study design.

## Conclusions

The present systematic review and meta-analysis demonstrates that the MedDiet improves both functional and structural measures of endothelial function, which likely make a large contribution towards the consistently observed beneficial effects of this dietary pattern on cardiovascular health. However, the overall quality of the evidence was modest and more robust and well-designed trials are need to corroborate the evidence highlighting positive effects of the MedDiet on endothelial function.

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The systematic review was designed by OS and MS. IM, CK and MS searched, collected and analysed the data. OS, IM, CK, MM, AW, SR, AMM, JCM and MS contributed to data interpretation. OS and MS drafted the manuscript. IM, CK, MM, AWA, SR, AMM and JCM further contributed towards the writing and critical revision of the paper. All authors have read and approved the final manuscript. OS and MS had primary responsibility for the final content. All authors have read and approved the final manuscript.

**REFERENCES**

1. Quyyumi AA. Endothelial function in health and disease: new insights into the genesis of cardiovascular disease. Am J Med. 1998;105:S32-S39.

2. Deanfield JE., Halcox JP., Rabelink TJ. Endothelial Function and Dysfunction. Circulation. 2007;115:1285–95.

3. Vanhoutte PM, Shimokawa H, Feletou M, Tang EHC. Endothelial dysfunction and vascular disease - a 30th anniversary update. Acta Physiol. 2017;219:22–96.

4. Versari D, Daghini E, Virdis A, Ghiadoni L, Taddei S. Endothelial dysfunction as a target for prevention of cardiovascular disease. Diabetes Care. 2009;32:S314–21.

5. Siervo M, Scialò F, Shannon OM, Stephan BCM, Ashor AW. Does dietary nitrate say NO to cardiovascular ageing? Current evidence and implications for research. Proc Nutr Soc. 2018;77:112–23.

6. Bach-Faig A, Berry EM, Lairon D, Reguant J, Trichopoulou A, Dernini S, Medina FX, Battino M, Belahsen R, Miranda G, et al. Mediterranean diet pyramid today. Science and cultural updates. Public Health Nutr. 2011;14:2274–84.

7. Shannon OM, Stephan BCM, Granic A, Lentjes M, Hayat S, Mulligan A, Brayne C, Khaw K-T, Bundy R, Aldred S, et al. Mediterranean diet adherence and cognitive function in older UK adults: the European Prospective Investigation into Cancer and Nutrition-Norfolk (EPIC-Norfolk) Study. Am J Clin Nutr. 2019; 110:938-948.

8. de Lorgeril M, Salen P, Martin JL, Monjaud I, Delaye J, Mamelle N. Mediterranean diet, traditional risk factors, and the rate of cardiovascular complications after myocardial infarction: Final report of the Lyon Diet Heart Study. Circulation. 1999;99:779–85.

9. Estruch R, Ros E, Salas-Salvadó J, Covas M-I, Corella D, Arós F, Gómez-Gracia E, Ruiz-Gutiérrez V, Fiol M, Lapetra J, et al. Primary prevention of cardiovascular disease with a

Mediterranean diet supplemented with extra-virgin olive oil or nuts. N Engl J Med. 2018; 378:e34.

10. Estruch R. Anti-inflammatory effects of the Mediterranean diet: The experience of the PREDIMED study. Proc Nutr Soc. 2010;69:333–40.

11. Schwingshackl L, Christoph M, Hoffmann G. Effects of olive oil on markers of inflammation and endothelial function - A systematic review and meta-analysis. Nutrients. 2015;7:7651–75.

12. Xiao Y, Huang W, Peng C, Zhang J, Wong C, Kim JH, Yeoh E-K, Su X. Effect of nut consumption on vascular endothelial function: A systematic review and meta-analysis of randomized controlled trials. Clin Nutr. 2018;37:831–9.

13. Kondo K, Morino K, Nishio Y, Kondo M, Nakao K, Nakagawa F, Ishikado A, Sekine O, Yoshizaki T, Kashiwagi A, et al. A fish-based diet intervention improves endothelial function in postmenopausal women with type 2 diabetes mellitus: A randomized crossover trial. Metab Clin Exp. 2014;63:930–40.

14. Xin W, Wei W, Li X. Effect of fish oil supplementation on fasting vascular endothelial function in humans: A meta-analysis of randomized controlled trials. PLoS ONE. 2012;7:e46028.

15. Davis CR, Hodgson JM, Woodman R, Bryan J, Wilson C, Murphy KJ. A Mediterranean diet lowers blood pressure and improves endothelial function: Results from the MedLey randomized intervention trial. Am J Clin Nutr. 2017;105:1305–13.

16. Torres-Peña JD, Garcia-Rios A, Delgado-Casado N, Gomez-Luna P, Alcala-Diaz JF, Yubero-Serrano EM, Gomez-Delgado F, Leon-Acuña A, Lopez-Moreno J, Camargo A, et al. Mediterranean diet improves endothelial function in patients with diabetes and prediabetes: A report from the CORDIOPREV study. Atherosclerosis. 2018;269:50–6.

17. Maiorino MI, Bellastella G, Petrizzo M, Gicchino M, Caputo M, Giugliano D, Esposito K. Effect of a Mediterranean diet on endothelial progenitor cells and carotid intima-media thickness in type 2 diabetes: Follow-up of a randomized trial. Eur J Prev Cardiol. 2017;24:399–408.

18. 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.

19. Ceriello A, Esposito K, La Sala L, Pujadas G, De Nigris V, Testa R, Bucciarelli L, Rondinelli M, Genovese S. The protective effect of the Mediterranean diet on endothelial resistance to GLP-1 in type 2 diabetes: A preliminary report. Cardiovasc Diabetol. 2014;13:140.

20. Jaacks LM, Sher S, Staercke CD, Porkert M, Alexander WR, Jones DP, Vaccarino V, Ziegler TR, Quyyumi AA. Pilot randomized controlled trial of a Mediterranean diet or diet supplemented with fish oil, walnuts, and grape juice in overweight or obese US adults. BMC Nutr. 2018;4:26.

21. Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gøtzsche PC, Ioannidis JPA, Clarke M, Devereaux PJ, Kleijnen J, Moher D. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: Explanation and elaboration. BMJ. 2009;339:b2700.

22. Higgins JPT, Green, S.E. Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0. London, UK: The Cochrane Collaboration; 2011.

23. Borenstein M, Hedges L., Higgins JP., Rothstein H. Introduction to Meta-Analysis. New Jersey, US: Wiley; 2019.

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

25. Korkmaz H, Onalan O. Evaluation of endothelial dysfunction: Flow-mediated dilation. Endothelium. 2008;15:157–63.

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

27. Ambring A, Friberg P, Axelsen M, Laffrenzen M, Taskinen M-R, Basu S, Johansson M. Effects of a Mediterranean-inspired diet on blood lipids, vascular function and oxidative stress in healthy subjects. Clin Sci. 2004;106:519–25.

28. Klonizakis M, Alkhatib A, Middleton G, Smith MF. Mediterranean diet- and exercise-induced improvement in age-dependent vascular activity. Clin Sci. 2013;124:579–87.

29. Marin C, Ramirez R, Delgado-Lista J, Yubero-Serrano EM, Perez-Martinez P, Carracedo J, Garcia-Rios A, Rodriguez F, Gutierrez-Mariscal FM, Gomez P, et al. Mediterranean diet reduces endothelial damage and improves the regenerative capacity of endothelium. Am J Clin Nutr. 2011;93:267–74.

30. Murie-Fernandez M, Irimia P, Toledo E, Martínez-Vila E, Buil-Cosiales P, Serrano-Martínez M, Ruiz-Gutiérrez V, Ros E, Estruch R, Martínez-González MÁ, et al. Carotid intima-media thickness changes with Mediterranean diet: A randomized trial (PREDIMED-Navarra). Atherosclerosis. 2011;219:158–62.

31. Sala-Vila A, Romero-Mamani E-S, Gilabert R, Núñez I, de la Torre R, Corella D, Ruiz-Gutiérrez V, López-Sabater M-C, Pintó X, Rekondo J, et al. Changes in ultrasound-assessed carotid intima-media thickness and plaque with a Mediterranean diet: A sub-study of the PREDIMED trial. Arterioscler Thromb Vasc Biol. 2014;34:439–45.

32. Buscemi S, Verga S, Tranchina MR, Cottone S, Cerasola G. Effects of hypocaloric very-low-carbohydrate diet vs. Mediterranean diet on endothelial function in obese women. Eur J Clin Invest. 2009;39:339–47.

33. Esposito K, Marfella R, Ciotola M, Di Palo C, Giugliano F, Giugliano G, D'Armiento M, D'Andrea F, Giugliano D. Effect of a Mediterranean-style diet on endothelial dysfunction and markers of vascular inflammation in the metabolic syndrome: a randomized trial. JAMA. 2004;292:1440–6.

34. Thomazella MCD, Góes MFS, Andrade CR, Debbas V, Barbeiro DF, Correia RL, Marie SKN, Cardounel AJ, daLuz PL, Laurindo FRM. Effects of high adherence to Mediterranean or low-fat diets in medicated secondary prevention patients. Am J Cardiol. 2011;108:1523–9.

35. Fuentes F, López-Miranda J, Sánchez E, Sánchez F, Paez J, Paz-Rojas E, Marín C, Gómez P, Jimenez-Perepérez J, Ordovás JM, et al. Mediterranean and low-fat diets improve endothelial function in hypercholesterolemic men. Ann Intern Med. 2001;134:1115–9.

36. Inaba Y, Chen JA, Bergmann SR. Prediction of future cardiovascular outcomes by flow-mediated vasodilatation of brachial artery: a meta-analysis. Int J Cardiovasc Imaging. 2010;26:631–40.

37. Tosti V, Bertozzi B, Fontana L. Health benefits of the Mediterranean diet: Metabolic and molecular mechanisms. J Gerontol A Biol Sci Med Sci. 2018;73:318–26.

38. Medina-Remón A, Tresserra-Rimbau A, Pons A, Tur JA, Martorell M, Ros E, Buil-Cosiales P, Sacanella E, Covas MI, Corella D, et al. Effects of total dietary polyphenols on plasma nitric oxide and blood pressure in a high cardiovascular risk cohort. The PREDIMED randomized trial. Nutr Metab Cardiovasc Dis. 2015;25:60–7.

39. Shannon OM, Stephan BCM, Minihane A-M, Mathers JC, Siervo M. Nitric oxide boosting effects of the Mediterranean diet: A potential mechanism of action. J Gerontol A Biol Sci Med Sci. 2018; 73:902-904.

40. Armah CK, Jackson KG, Doman I, James L, Cheghani F, Minihane AM. Fish oil fatty acids improve postprandial vascular reactivity in healthy men. Clin Sci. 2008;114:679–86.

41. Balakumar P, Taneja G. Fish oil and vascular endothelial protection: Bench to bedside. Free Radic Biol Med. 2012;53:271–9.

42. Fitó M, Guxens M, Corella D, Sáez G, Estruch R, Torre R de la, Francés F, Cabezas C, López-Sabater M del C, Marrugat J, et al. Effect of a traditional Mediterranean diet on lipoprotein oxidation: A randomized controlled trial. Arch Intern Med. 2007;167:1195–203.

43. Ross R. Atherosclerosis - an inflammatory disease. N Engl J Med. 1999;340:115–26.

44. Valente AJ, Irimpen AM, Siebenlist U, Chandrasekar B. OxLDL induces endothelial dysfunction and death via TRAF3IP2: Inhibition by HDL3 and AMPK activators. Free Radic Biol Med. 2014;70:117–28.

45. Bonanome A, Pagnan A, Biffanti S, Opportuno A, Sorgato F, Dorella M, Maiorino M, Ursini F. Effect of dietary monounsaturated and polyunsaturated fatty acids on the susceptibility of plasma low density lipoproteins to oxidative modification. Arterioscler Thromb. 1992;12:529–33.

46. Zhang C. The role of inflammatory cytokines in endothelial dysfunction. Basic Res Cardiol. 2008;103:398–406.

47. Estruch R, Martínez-González MA, Corella D, Salas-Salvadó J, Ruiz-Gutiérrez V, Covas MI, Fiol M, Gómez-Gracia E, López-Sabater MC, Vinyoles E, et al. Effects of a Mediterranean-style diet on cardiovascular risk factors: a randomized trial. Ann Intern Med. 2006;145:1–11.

48. Mena M-P, Sacanella E, Vazquez-Agell M, Morales M, Fitó M, Escoda R, Serrano-Martínez M, Salas-Salvadó J, Benages N, Casas R, et al. Inhibition of circulating immune cell activation: A molecular antiinflammatory effect of the Mediterranean diet. Am J Clin Nutr. 2009;89:248–56.

49. Casas R, Sacanella E, Urpí-Sardà M, Chiva-Blanch G, Ros E, Martínez-González M-A, Covas M-I, Rosa Ma Lamuela-Raventos, Salas-Salvadó J, Fiol M, et al. The effects of the

Mediterranean diet on biomarkers of vascular wall inflammation and plaque vulnerability in subjects with high risk for cardiovascular disease. A randomized trial. PLoS ONE. 2014;9:e100084.

50. Arpón A, Riezu-Boj JI, Milagro FI, Marti A, Razquin C, Martínez-González MA, Corella D, Estruch R, Casas R, Fitó M, et al. Adherence to Mediterranean diet is associated with methylation changes in inflammation-related genes in peripheral blood cells. J Physiol Biochem. 2016;73:445–55.

51. Perez-Martinez P, Lopez-Miranda J, Blanco-Colio L, Bellido C, Jimenez Y, Moreno JA, Delgado-Lista J, Egido J, Perez-Jimenez F. The chronic intake of a Mediterranean diet enriched in virgin olive oil, decreases nuclear transcription factor kappaB activation in peripheral blood mononuclear cells from healthy men. Atherosclerosis. 2007;194:e141-146.

52. Staudacher HM, Irving PM, Lomer MCE, Whelan K. The challenges of control groups, placebos and blinding in clinical trials of dietary interventions. Proc Nutr Soc. 2017;76:203–12.

53. Schulze MB, Martínez-González MA, Fung TT, Lichtenstein AH, Forouhi NG. Food based dietary patterns and chronic disease prevention. BMJ. 2018;361:k2396.

**Table 1:** Summary of main characteristics of randomized clinical trials investigating the effects of Mediterranean Diet on endothelial function in adults

| Author | Country | Study Design | Health Status | Outcome | Sample Size | Male (*n*) | Age (years) | BMI (kg/m$^2$) | SBP/DBP (mmHg) | Duration (Weeks) | Type of Intervention | Type of Control |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Ambring et al. (27) | Sweden | Cross-over | Healthy | FBF | 22 | 12 | 43 | 26.0 | NR | 12 | MedDiet | Swedish Diet |
| Buscemi et al. (32) | Italy | Parallel | Obese | FMD | 20 | 0 | 38 | 34.2 | 128/88 | 8 | MedDiet | Atkins Low-carbohydrate |
| Ceriello et al. (19) | Spain | Parallel | DM2 | FMD | 24 | 17 | NR | 29.5 | 116/78 | 12 | MedDiet + MUFA | Low Fat Diet |
| Davis et al. (15) | Australia | Parallel | Healthy | FMD | 166 | 72 | 71 | 26.9 | 124/71 | 24 | MedDiet | Habitual Diet |
| Esposito et al. (33) | Italy | Parallel | MetS | EFS | 180 | 89 | 44 | 28.0 | 135/86 | 96 | MedDiet | Prudent Diet |
| Fuentes et al. (35) | Spain | Cross-over | Hypercholesteremic | FMD, BVS | 22 | 22 | 40 | NR | NR | 8 | MedDiet + MUFA | NCEP-1 Diet |
| Jaacks et al. (20) | USA | Parallel | Overweight | FMD | 30 | 8 | 51 | 31.5 | NR | 8 | MedDiet | Habitual Diet |
| Klonizakis et al. (28) | UK | Parallel | Healthy | CM | 22 | 7 | 55 | 30.5 | 127/79 | 8 | MedDiet + Exercise | Non-MedDiet + Exercise |
| Maiorino et al. (17) | Italy | Parallel | DM2 | CIMT | 215 | 106 | 52 | 29.6 | 140/87 | 121 | MedDiet | Low Fat Diet |
| Marin et al. (29) | Spain | Cross-over | Healthy | CM | 20 | 10 | 67 | 31.9 | NR | 4 | MedDiet | Saturated Fatty Acid Diet |
| Murie-Fernandez et al. (30) | Spain | Parallel | CVD risk | CIMT | 187 | 91 | 67 | 29.4 | NR | 48 | G1: MedDiet + EVOO<br>G2: MedDiet + Nuts | Low Fat Diet |
| Sala-Vila et al. (31) | Spain | Parallel | CVD risk | ICA-IMT | 175 | 42 | 66 | 29.6 | 150/81 | 115 | G1: MedDiet + EVOO<br>G2: MedDiet + Nuts | Low Fat Diet |
| Thomazella et al. (34) | Brazil | Parallel | ACS | FMD, BVS | 42 | 42 | 55 | 26.4 | 136/84 | 12 | MedDiet | Low Fat Diet |
| Torres-Peña et al. (16) | Spain | Parallel | DM2 | FMD | 438 | NR | 61 | 31.8 | NR | 72 | MedDiet + EVOO | Low Fat Diet |
| Torres-Peña et al. (16) | Spain | Parallel | pDM2 | FMD | 289 | NR | 58 | 30.3 | NR | 72 | MedDiet + EVOO | Low Fat Diet |
| Torres-Peña et al. (16) | Spain | Parallel | Healthy | FMD | 78 | NR | 56 | 29.5 | NR | 72 | MedDiet + EVOO | Low Fat Diet |

ACS, Acute Coronary Syndromes; BVS, Baseline Vessel Size; CIMT, Carotid Intima-Media Thickness; CM, Cutaneous Microvascular Function; CVD risk, Risk of Cardiovascular Disease; DBP, Diastolic Blood Pressure; DM2, Type 2 Diabetes; EFS, Endothelial Function Score; EVOO, Extra Virgin Olive Oil; FBF, Forearm Blood Flow; FMD, Flow Mediated Dilatation; ICA-IMT, Internal Carotid Intima-Media Thickness; MedDiet, Mediterranean Diet; MetS, Metabolic Syndrome; MUFA, Monounsaturated Fatty Acids; *n* = number of subjects; NCEP-1, The National Cholesterol Education Program Diet; pDM2, prediabetes; SBP, Systolic Blood Pressure.

**Table 2**: Sensitivity analysis to evaluate the influence of health status, type of intervention, type of measurement, and study design on the effects of the Mediterranean dietary pattern on endothelial function (EF) in adults.

| Category | No of EF measurements per subgroup | Effect size | 95% CI | *P* | *P* between Groups | $I^2$ |
|---|---|---|---|---|---|---|
| **Health status** | | | | | 0.66 | |
| • Healthy | 7 | 0.29 | 0.05 - 0.53 | 0.01 | | 26.5% |
| • Increased CVD Risk | 13 | 0.36 | 0.15 - 0.58 | 0.001 | | 79.1% |
| **Type of Intervention** | | | | | 0.71 | |
| • MedDiet | 9 | 0.37 | 0.03 - 0.77 | 0.04 | | 85.7% |
| • MedDiet + other | 11 | 0.29 | 0.19 - 0.39 | <0.001 | | 0% |
| **Type of Measurement** | | | | | 0.05 | |
| • Functional | 13 | 0.44 | 0.19 - 0.69 | <0.001 | | 78.2% |
| • Structural | 7 | 0.16 | 0.02 - 0.30 | 0.01 | | 0% |
| **Study Design** | | | | | 0.55 | |
| • Cross-over | 4 | 0.25 | -0.05 - 0.56 | 0.10 | | 41.7% |
| • Parallel | 16 | 0.36 | 0.16 - 0.56 | <0.001 | | 74.9% |

CVD risk = Risk of Cardiovascular Disease. MedDiet = Mediterranean dietary pattern. *P* refers to the effect sizes of the subgroups in each category. *P* between Groups refers to the comparison of the effect sizes between sub-groups within each category.

**Table 3:** Meta-regression analysis to evaluate potential modifiers of the effects of Mediterranean dietary pattern on endothelial function in adults.

| | **Slope** | **SE** | **Q (df=1)** | ***P*** |
|---|---|---|---|---|
| Age (years) | -0.002 | 0.008 | 0.12 | 0.72 |
| Study Duration (weeks) | 0.0008 | 0.002 | 0.17 | 0.67 |
| Body mass index ($kg/m^2$) | -0.01 | 0.04 | 0.07 | 0.78 |

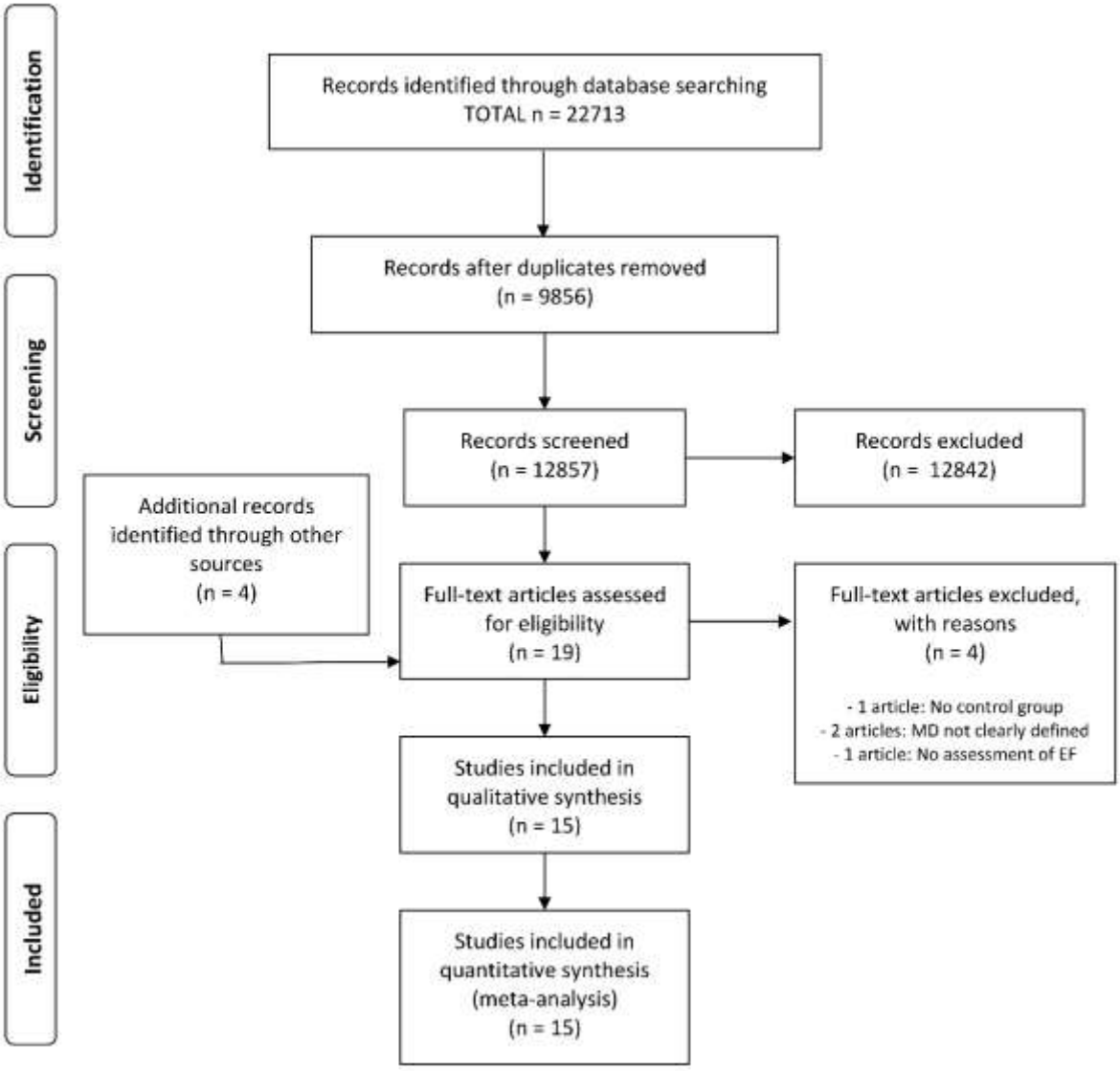


**Figure 1:** Flow diagram of the selection process of the randomized controlled trials included in the meta-analysis.

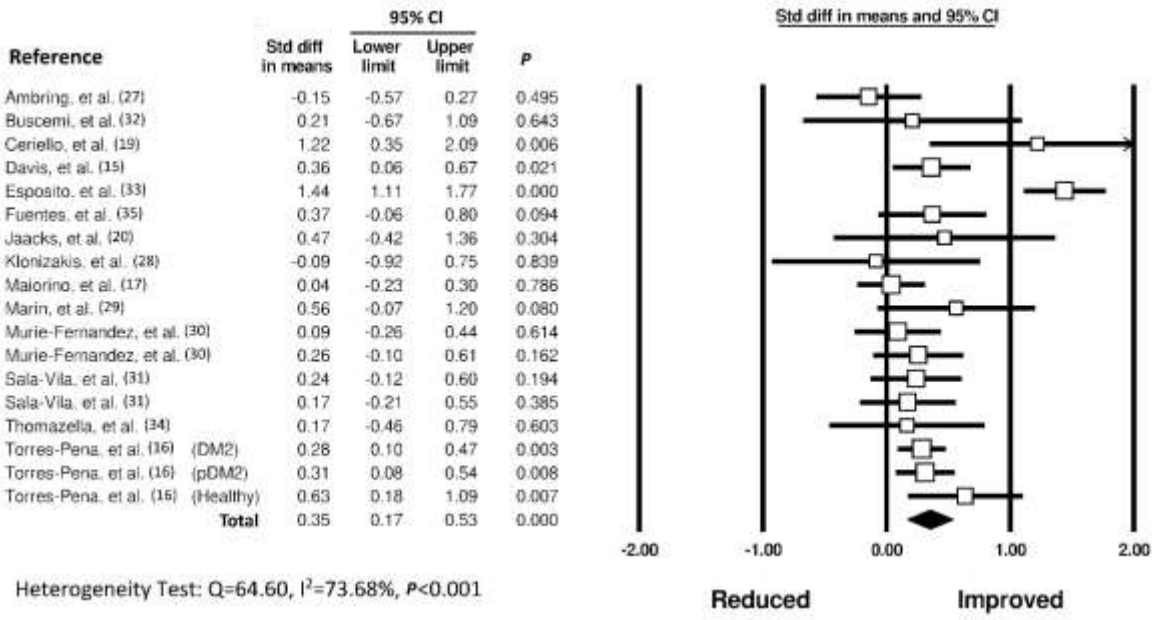


**Figure 2:** Forest plot showing the overall effect of the Mediterranean dietary pattern on endothelial function in adults. DM2 = type 2 diabetes; pDM2 = pre-diabetes; a = MedDiet + extra virgin olive oil; b = MedDiet + nuts. Data showed as standardised differences in means. Horizontal lines denote 95% confidence intervals (CI). The size of the boxes is proportionally scaled to the effect size for each study.

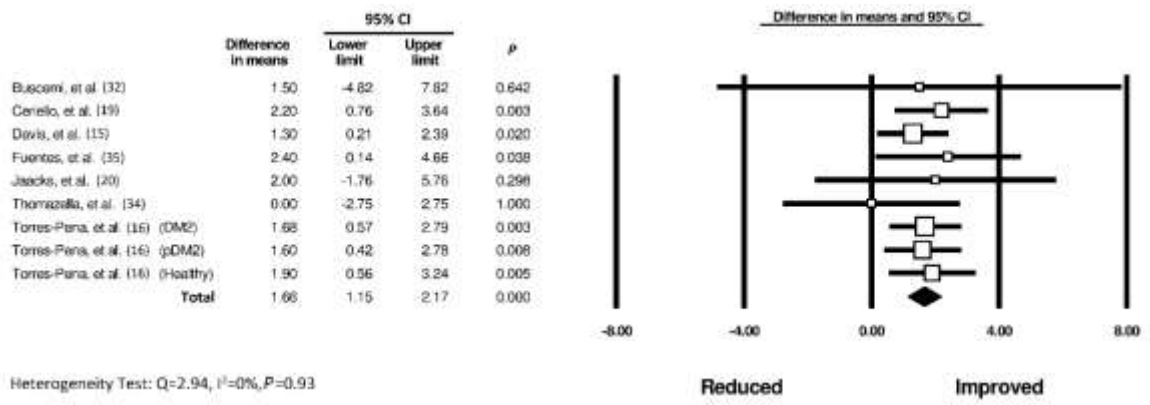


**Figure 3:** Forest plot showing the overall effect of the Mediterranean dietary pattern on flow mediated dilation in adults (expressed as percent change). DM2 = type 2 diabetes; pDM2 = pre-diabetes. Data showed as percent differences in means. Horizontal lines denote 95% confidence intervals (CI). The size of the boxes is proportionally scaled to the effect size for each study.

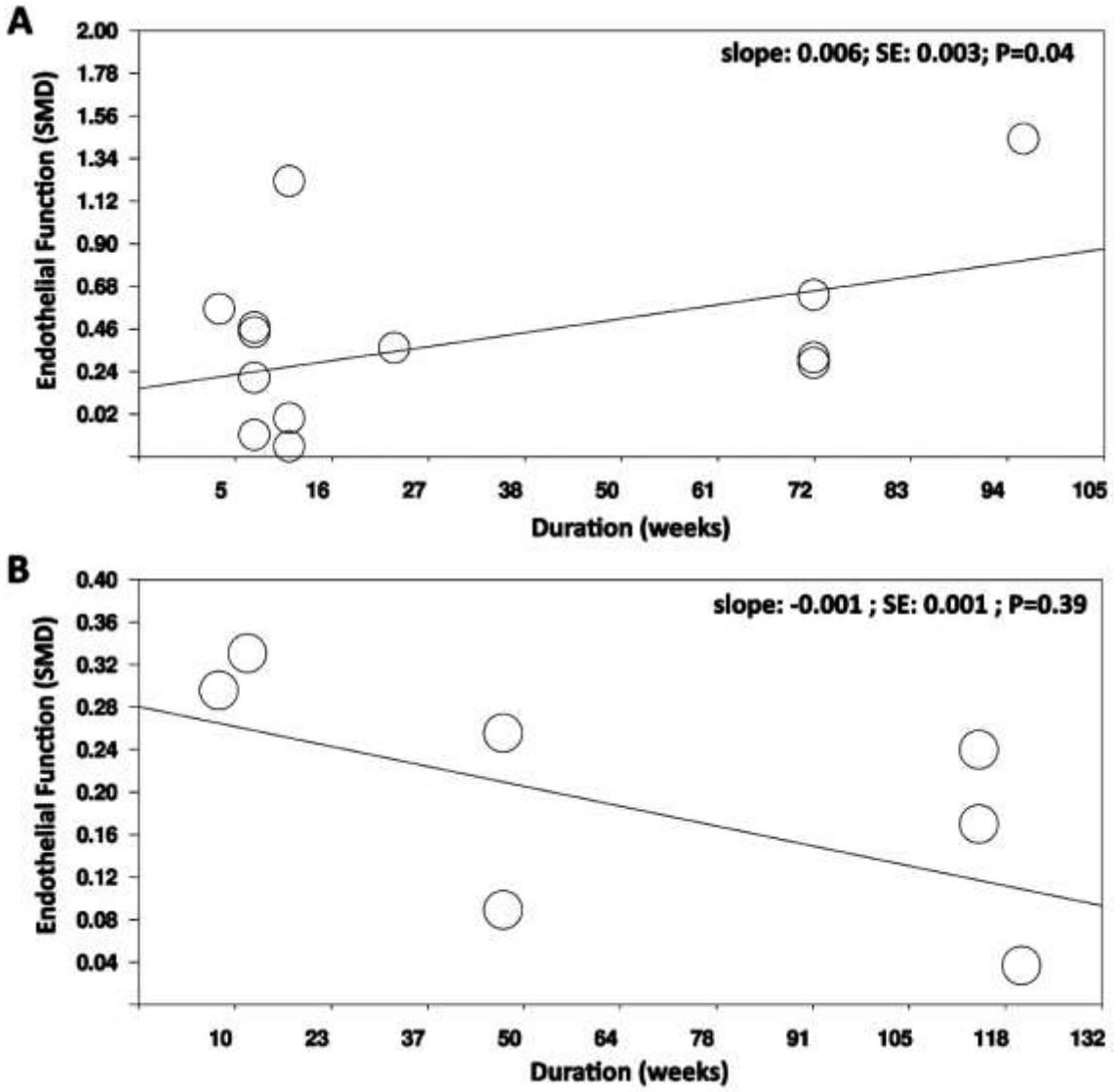


**Figure 4:** Meta-regression analysis of the association between study duration and the effect size (expressed as standardised mean difference [SMD]) of functional (A) and structural (B) alterations in endothelial function in adult subjects following consumption of a Mediterranean dietary pattern.