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Mediterranean Diet Increases Endothelial Function in Adults: A Systematic Review and Meta-Analysis of Randomized Controlled Trials

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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 the risk of cardiovascular diseases (CVD) including stroke or coronary heart disease.

Objective: To conduct a systematic review and meta-analysis of randomized controlled trials (RCTs) investigating the 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 the MedDiet, *3*) inclusion of a control group, and *4*) measurements of endothelial function. A random-effects meta-analysis was conducted. Metaregression and subgroup analyses were performed to identify whether effects were modified by health status (i.e., healthy participants versus participants with existing comorbidities), type of intervention (i.e., MedDiet alone or with a cointervention), study duration, study design (i.e., parallel or crossover), BMI, and age of participants.

Results: Fourteen articles reporting data for 1930 participants were included in the meta-analysis. Study duration ranged from 4 wk to 2.3 y. We observed a beneficial effect of the MedDiet on endothelial function [standardized 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 noninvasive, 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, or age of participants ($P > 0.05$).

Conclusions: MedDiet interventions improve endothelial function in adults, suggesting 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. This study has the PROSPERO registration number: CRD42018106188. *J Nutr* 2020;150:1151–1159.

Keywords: Mediterranean diet, endothelial function, flow-mediated dilation, cardiovascular disease, healthy aging, dietary patterns

Introduction

The endothelium plays a key role in the maintenance of vascular health via the secretion of multiple signaling molecules including NO, endothelins, selectins, and adhesion molecules, which in concert, control vasomotor tone and have antiatherogenic and antiproliferative 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 the risk of CVD such as stroke, coronary heart disease, or peripheral arterial disease (4, 5).

The Mediterranean dietary pattern (MedDiet) is characterized by the 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 to be one of the healthiest dietary patterns

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(6, 7). Evidence from randomized 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-mo MedDiet intervention in healthy older individuals induced highly significant improvements in endothelial function measured via flow-mediated dilation (FMD), with the FMD percentage approximately double baseline values (absolute increase of ∼1.3%) (15). Similarly, in patients with prediabetes and diabetes, a 1.5-y 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 Approaches 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 an intervention period lasting ≥12 wk 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 y (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 the effects were modified by health status, type of intervention, study duration, study design, 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 (19).

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 predefined 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 y 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 with control group including exercise alone); *4*) studies reporting changes in endothelial function for intervention and control groups separately; and *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, articles were 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 1 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, randomization procedure, blinding of exposure and outcome measurements, compliance with the interventions, BMI, dietary intervention, weight loss during the study, baseline and postintervention measurements of systolic and diastolic blood pressure (BP), baseline and postintervention measurements of endothelial function. In addition, 2 independent reviewers (CH, IM) utilized 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 (20) and any discrepancy was resolved by consensus with a third reviewer (MS).

Statistical analysis

Statistical analyses were performed using Comprehensive Meta-Analysis Software Version 2 (Biostat). For this purpose, sample size, and 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 the MedDiet and control were used to calculate the effect size (Cohen's d). For studies that reported changes in endothelial function at 2 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 (21). Data not provided in the main text or tables were extracted from the figures. Some trials used >1 method to assess changes in endothelial function (**Table 1**), which may lead to a reduced independence of

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Abbreviations used: BP, blood pressure; CVD, cardiovascular disease; FBF, forearm blood flow; FMD, flow-mediated dilation; MedDiet, Mediterranean dietary pattern; PREDIMED, Prevención con Dieta Mediterránea (Mediterranean Diet Prevention Study); RCT, randomized controlled trial; SMD, standardized mean difference.

TABLE 1 Summary of the 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 (y)	BMI (kg/m^2)	SBP/DBP (mmHg)	Duration (wk)	Type of intervention	Type of control
Ambring et al. (22)	Sweden	Crossover	Healthy	FBF	22	12	43	26.0	—	12	MedDiet	Swedish diet
Buscemi et al. (23)	Italy	Parallel	Obese	FMD	20	0	38	34.2	128/88	8	MedDiet	Atkins low-carbohydrate diet
Ceriello et al. (24)	Spain	Parallel	DM2	FMD	24	17	—	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. (25)	Italy	Parallel	MetS	EFS	180	89	44	28.0	135/86	96	MedDiet	Prudent diet
Fuentes et al. (26)	Spain	Crossover	Hypercholestoleremic	FMD, BVS	22	22	40	—	—	8	MedDiet + MUFA	NCEP-1 diet
Jaacks et al. (27)	USA	Parallel	Overweight	FMD	30	8	51	31.5	—	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	Crossover	Healthy	CM	20	10	67	31.9	—	4	MedDiet	SFA diet
Murie-Fernandez et al. (30)	Spain	Parallel	CVD risk	CIMT	187	91	67	29.4	—	48	G1: MedDiet + EVOO 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 G2: MedDiet + nuts	Low-fat diet
Thomazella et al. (32)	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	—	61	31.8	—	72	MedDiet + EVOO	Low-fat diet
Torres-Peña et al. (16)	Spain	Parallel	pDM2	FMD	289	—	58	30.3	—	72	MedDiet + EVOO	Low-fat diet
Torres-Peña et al. (16)	Spain	Parallel	Healthy	FMD	78	—	56	29.5	—	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 dilation; G1, group 1; G2, group 2; ICA-IMT, internal carotid intima-media thickness; MedDiet, Mediterranean dietary pattern; MetS, metabolic syndrome; *n*, number of subjects; NCEP-1, The National Cholesterol Education Program Diet; pDM2, prediabetes; SBP, systolic blood pressure.

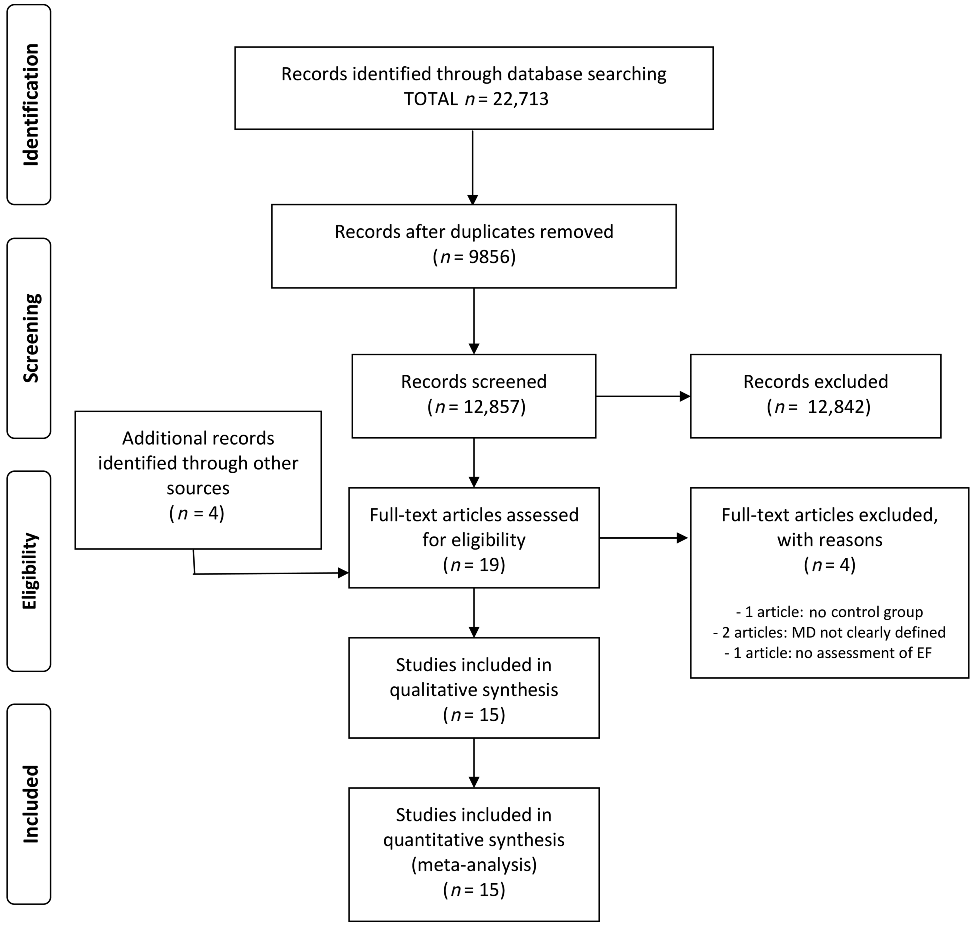

FIGURE 1 Flow diagram of the selection process of the randomized controlled trials included in the meta-analysis. EF, endothelial function; MD, Mediterranean diet.

the measurements and, consequently, to overestimation 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 standardized 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% CIs for the MedDiet interventions were calculated using a weighted DerSimonian-Laird random effects model (33). Forest plots were generated to graphically present 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 plethysmography, or cutaneous microcirculation derived from laser Doppler. Structural measurements include intima media thickness or vessel size, both measured by ultrasound. In addition, we performed sensitivity analyses to test the effects of MedDiet on FMD only, on the basis that this is the reference method for noninvasive, clinical measurement of endothelial function (34) and was used in the majority of studies.

Subgroup analyses were undertaken to investigate variables that may have influenced the effects of MedDiet on endothelial function. These factors included: health status (healthy subjects versus 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 metaregression 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 using the Cochrane Q statistic; $P < 0.1$ indicates significant heterogeneity. The I^2 test was also utilized to assess heterogeneity across trials where a value <25% indicates low risk, 25–75% indicates moderate risk, and >75% indicates high risk (35). Sensitivity analyses were conducted to identify the source of heterogeneity by conducting stratified analyses or selectively removing studies with a 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 summarized in Figure 1. The primary search of the 3 databases produced 12,857 articles, after the removal of duplicates. After title and abstract screening, 15 full-text articles were retrieved for further evaluation. A further 4 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 eligible for inclusion in this systematic review and meta-analysis. Some of these articles reported results from independent studies testing the effects of MedDiet on endothelial function, generating a total of 20 sets of independent

Reference	Std diff in means	95% CI Lower limit	95% CI Upper limit	*P*
Ambring et al. (27)	-0.15	-0.57	0.27	0.495
Buscemi et al. (32)	0.21	-0.67	1.09	0.643
Ceriello et al. (19)	1.22	0.35	2.09	0.006
Davis et al. (15)	0.36	0.06	0.67	0.021
Esposito et al. (33)	1.44	1.11	1.77	0.000
Fuentes et al. (35)	0.37	-0.06	0.80	0.094
Jaacks et al. (20)	0.47	-0.42	1.36	0.304
Klonizakis et al. (28)	-0.09	-0.92	0.75	0.839
Maiorino et al. (17)	0.04	-0.23	0.30	0.786
Marin et al. (29)	0.56	-0.07	1.20	0.080
Murie-Fernandez et al. (30) a	0.09	-0.26	0.44	0.614
Murie-Fernandez et al. (30) b	0.26	-0.10	0.61	0.162
Sala-Vila et al. (31) a	0.24	-0.12	0.60	0.194
Sala-Vila et al. (31) b	0.17	-0.21	0.55	0.385
Thomazella et al. (34)	0.17	-0.46	0.79	0.603
Torres-Pena et al. (16) (DM2)	0.28	0.10	0.47	0.003
Torres-Pena et al. (16) (pDM2)	0.31	0.08	0.54	0.008
Torres-Pena et al. (16) (Healthy)	0.63	0.18	1.09	0.007
Total	0.35	0.17	0.53	0.000

Heterogeneity Test: Q=64.60, I^2=73.68%, P<0.001

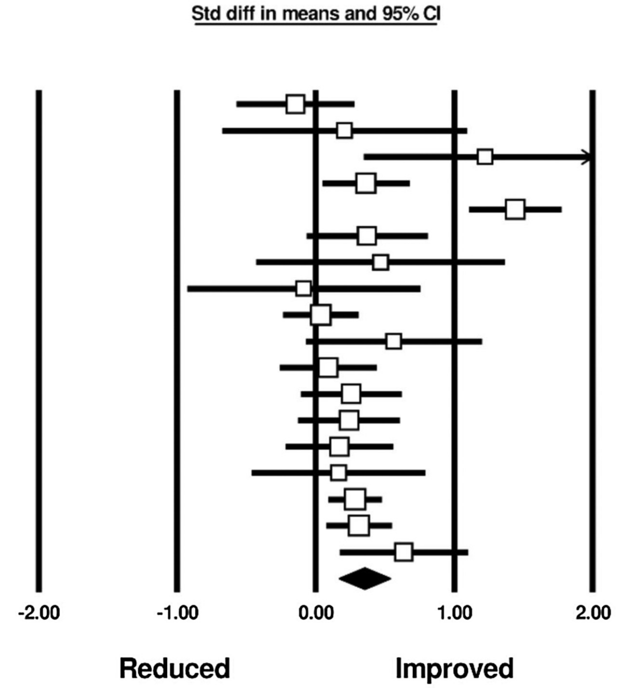

FIGURE 2 Forest plot showing the overall effect of the Mediterranean dietary pattern on endothelial function in adults. Data expressed as standardized mean differences. Horizontal lines denote 95% CIs. The size of the boxes is proportionally scaled to the effect size for each study. DM2, type 2 diabetes; pDM2, prediabetes; a, MedDiet + extra virgin olive oil; b, MedDiet + nuts.

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) y. Eleven of the RCTs included in the meta-analysis were parallel trials with a control group, whereas 3 were crossover studies (20). The paired nature of the crossover trials was taken into account in the meta-analysis to minimize unit-of-analysis errors and underestimation of the effect size. The duration of the interventions ranged from 4 wk to 121 wk (Table 1). Five studies investigated the effect of MedDiet in healthy participants (15, 16, 22, 28, 29), 3 in people with diabetes (16, 17, 24), 2 in patients with elevated risk of CVD (30, 31), 2 in overweight or obese participants (23, 27), 1 in patients with metabolic syndrome (25), 1 in people with prediabetes (16), 1 in patients with acute coronary syndrome (32), and 1 in hypercholesterolemic men (26). 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 MUFAs (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

	Difference in means	95% CI Lower limit	95% CI Upper limit	*P*
Buscemi et al. (32)	1.50	-4.82	7.82	0.642
Ceriello et al. (19)	2.20	0.76	3.64	0.003
Davis et al. (15)	1.30	0.21	2.39	0.020
Fuentes et al. (35)	2.40	0.14	4.66	0.038
Jaacks et al. (20)	2.00	-1.76	5.76	0.298
Thomazella et al. (34)	0.00	-2.75	2.75	1.000
Torres-Pena et al. (16) (DM2)	1.68	0.57	2.79	0.003
Torres-Pena et al. (16) (pDM2)	1.60	0.42	2.78	0.008
Torres-Pena et al. (16) (Healthy)	1.90	0.56	3.24	0.005
Total	1.66	1.15	2.17	0.000

Heterogeneity test: Q = 2.94, I^2 = 0%, P = 0.93

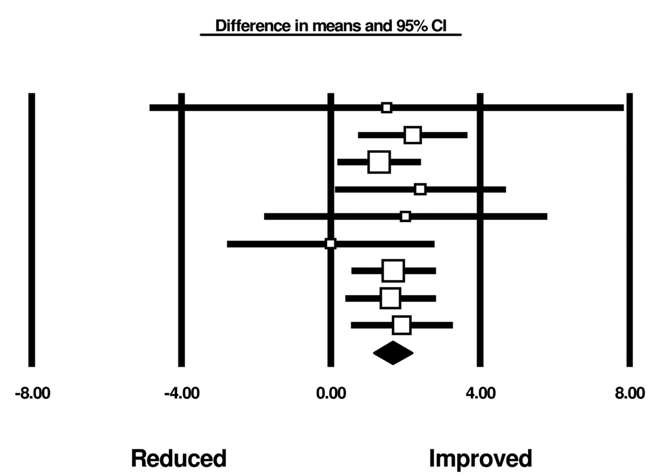

FIGURE 3 Forest plot showing the overall effect of the Mediterranean dietary pattern on flow-mediated dilation in adults (expressed as percentage change). Data showed as percentage differences in means. Horizontal lines denote 95% CIs. The size of the boxes is proportionally scaled to the effect size for each study. DM2, type 2 diabetes; pDM2, prediabetes.

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 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	
• Crossover	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; EF, endothelial function; 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 subgroups within each category.

Education Program Diet ($n = 1$), a non-MedDiet plus exercise ($n = 1$), an SFA 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 FBF, 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 2 studies with wider effect estimates (22, 25) (**Supplemental Figure S1**) 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). 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**). Metaregression analyses demonstrated no modification of the effect size by age, BMI, or study duration (**Table 3**).

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, y	− 0.002	0.008	0.12	0.72
Study duration, wk	0.0008	0.002	0.17	0.67
BMI, kg/m^2	− 0.01	0.04	0.07	0.78

However, a significant association was found between study duration (in weeks) with functional (slope: 0.006; SE: 0.003; P = 0.04; **Figure 4**A) but not structural measurements (slope: −0.001; SE: 0.001; P = 0.39, Figure 4B) of endothelial function.

Study quality and publication bias

Overall, the quality of the trials was modest as the majority of the studies failed to report key information to assess the presence of bias. Attrition bias was present in 50% of the studies (15–17, 25, 27, 31, 32) and few studies reported selection bias (<10% of the studies) (27). Seven studies described the randomization method (15–17, 24, 25, 28, 32), and 3 studies stated the methods of allocation concealment (17, 25, 29). Five studies reported and described participant dropout (22–24, 28, 29), whereas 3 studies described selective reporting of the results (27, 29, 32) (**Supplemental Figure S2**). Visual inspection of the Funnel plot revealed 2 studies with wider effect estimates but overall there was no evidence of publication bias, which was confirmed by the Egger's Regression test (P = 0.71; Supplemental Figure 1).

Discussion

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

In a previous systematic review and meta-analysis, Schwingshackl and Hoffmann (18) reported improvements in endothelial function with MedDiet interventions. In particular, MedDiet interventions increased FMD by 1.86%, which is similar to the 1.66% average improvement in FMD observed in this analysis. Importantly, the pooled effect size reported by Schwingshackl and Hoffmann (18) was based on the results of only 2 studies, whereas our meta-analysis included 7 studies that examined the effects of the MedDiet on FMD, which adds greater confidence to this result. To contextualize these findings, a meta-analysis by Inaba et al. (36) demonstrated

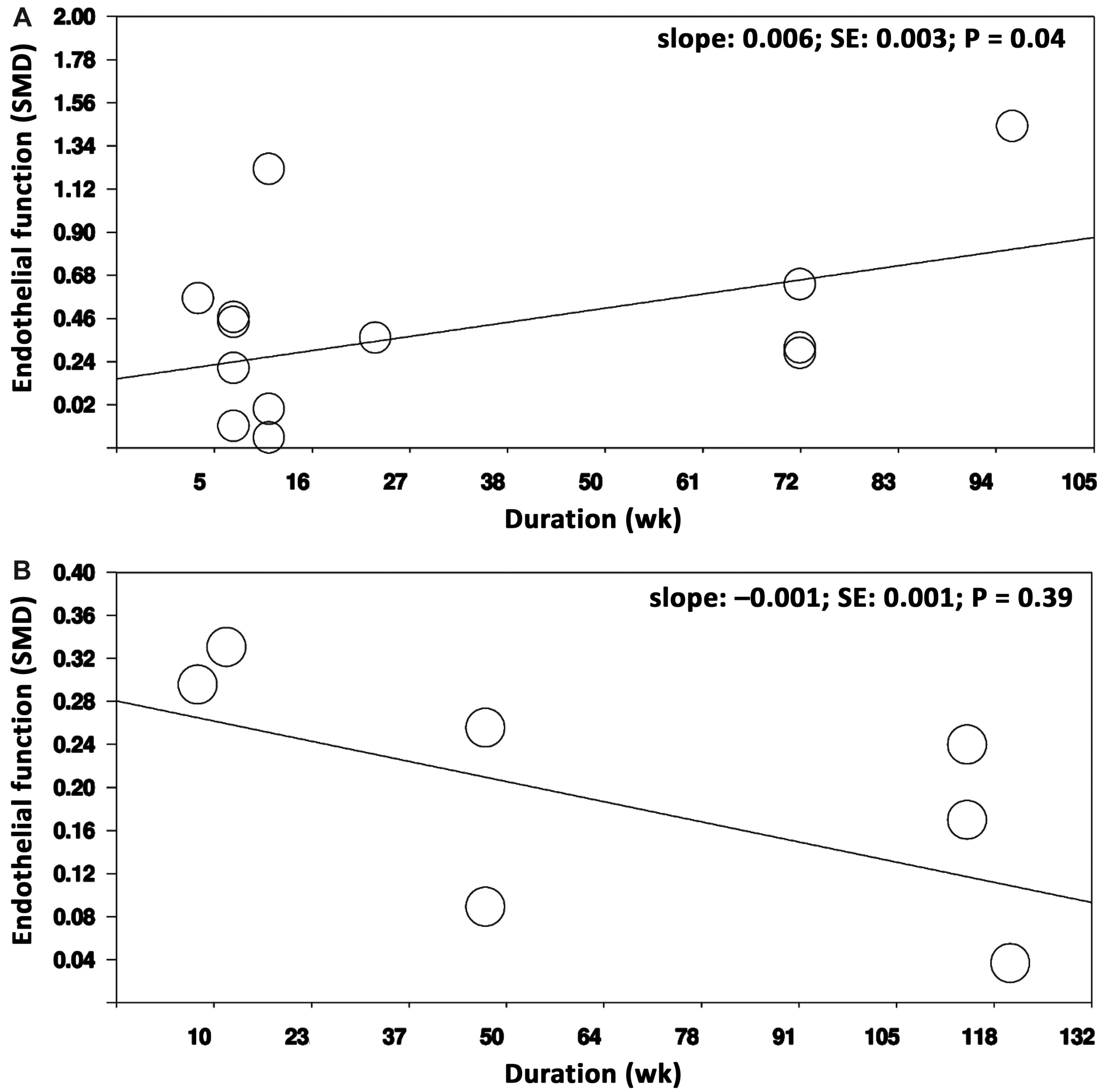

FIGURE 4 Metaregression analysis of the association between study duration and the effect size (expressed as the standardized mean difference [SMD]) of functional (A) and structural (B) alterations in endothelial function in adult subjects following consumption of a Mediterranean dietary pattern.

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 an ∼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 the 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-y 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 metaregression revealed no relation between study duration and the 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 maximize 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 and may account for the beneficial effects observed in this study. Firstly, the MedDiet augments the bioavailability of NO (38), which is essential for healthy endothelial function due to its vasodilatory, antiatherogenic, and antiproliferative actions (5). The NO 'boosting' effects of the MedDiet may be due to antioxidant effects minimizing 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 the oxidation of LDL (42), which plays a major role in endothelial dysfunction and atherogenesis (43, 44). The lower concentrations of oxidized

LDL with the MedDiet are likely mediated by both the antioxidant effects of this dietary pattern and the increased provision of MUFAs 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 IL-6, C-reactive protein (CRP), TNF-α, vascular cell adhesion protein-1 (VCAM-1), and soluble intercellular adhesion molecule-1 (sICAM-1) (47–50). These effects are associated with the 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 3 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 favorable 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 metaregression revealed no influence of age on the effects of the MedDiet, the lack of younger participants may limit our ability to generalize 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 utilized 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 that administered a Mediterranean-type diet that 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.

In conclusion, 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 needed to corroborate the evidence highlighting the positive effects of the MedDiet on endothelial function.

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