Mediterranean diet- and exercise-induced improvement in age-dependent vascular activity

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Abstract

The aging effect on microvascular integrity, marked by endothelial dysfunction and reduction in exercise tolerance, is a major cause of CVD (cardiovascular disease). Improved dietary habits, known to reduce morbidity and mortality, are also known to attenuate those aging effects. The present study investigated the effects of combined MD (Mediterranean diet) and exercise intervention on lower- and upper-limb cutaneous microvascular functions in an older healthy population. A total of 22 sedentary healthy participants (age, 55 ± 4 years) underwent cardiopulmonary exercise tolerance test, and were assessed for their upper- and lower-limb vascular endothelial CVC (cutaneous vascular conductance) using LDF (laser Doppler fluximetry) with endothelium-dependent [ACh (acetylcholine chloride)] and -independent [SNP (sodium nitroprusside)] vasodilation. Participants were then randomized into two groups: MD and non-MD, and followed an 8-week intervention programme, which included discontinuous treadmill running based on each individual’s exertion, twice per week. Exercise training improved CVC in both groups (e.g. 0.42 ± 0.19 compared with 1.50 ± 1.05 and 0.47 ± 0.26 compared with 1.15 ± 0.59 at 1000 μCb for MD and non-MD respectively; P < 0.001). This was also combined by improvement in the exercise tolerance indicated by increased VT (ventilatory threshold) in both groups (12.2 ± 2.8 compared with 14.8 ± 2.8 ml·(kg of body weight)^−1·min−1 and 11.7 ± 2.7 compared with 14.6 ± 3.2 ml·(kg of body weight)^−1·min−1 for MD and non-MD groups respectively; P < 0.05). However, the MD group showed greater improvement in endothelium-dependent vasodilation than non-MD [ANCOVA (analyses of co-variance), P = 0.02]. The results of the present study suggest that compliance with MD, combined with regular moderate exercise, improves age-provoked microcirculatory endothelial dysfunction and increases exercise tolerance, both responsible for reducing cardiovascular risk in this age group.

Key words: aging, cardio-pulmonary exercise testing, endothelium, microcirculation, nutrition, ventilatory threshold

INTRODUCTION

It has been well-documented that the structural and functional integrity of the microcirculation to maintain blood flow, tissue oxygenation and nutrient delivery are among the factors that affect tissue viability and susceptibility in a range of diseases and conditions [1–4].

Microvascular integrity, however, is not only compromised by a number of diseases and conditions; it is affected by the aging process. Aging is associated with attenuated vasodilator responses of the skin microcirculation, which is largely the result of endothelial dysfunction [3]. Endothelial dysfunction is considered an early and important promoter for atherosclerosis and thrombosis and thus contributes to the occurrence of cardiovascular events [4,5], whereas the skin-microvessel vasodilator dysfunction might also render the aged more vulnerable to heat-related illness and injury during exposure to elevated environmental temperatures and complications [6].

Therefore it appears important to identify strategies that can attenuate these mechanisms and improve our long-term health. Exercise appears to provide a response to the problem. For example, in two previous studies on the effects of aerobic exercise training on lower-limb cutaneous microvascular function in post-surgical varicose-vein patients, we found that both acute [7] and chronic [8] brisk walking exercise improved microvascular endothelial function [ACh (acetylcholine

Abbreviations: ACh, acetylcholine chloride; BP, blood pressure; CRP, C-reactive protein; CVC, cutaneous vascular conductance; CVD, cardiovascular disease; HR, heart rate; LDF, laser Doppler fluximetry; MD, Mediterranean diet; NO, nitric oxide; RER, respiratory exchange ratio; RPE, rating of perceived exertion; SNP, sodium nitroprusside; Tmax, time to reach maximum perfusion; V̇O₂, oxygen uptake; V̇CO₂, CO₂ production; VT, ventilatory threshold.

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chloride)-responsiveness], an adaptation that might be clinically meaningful with respect to risk of venous ulceration, whereas other studies have shown that the exercise improves microvascular function in older populations [9,10].

It is, however, widely agreed that cardiovascular health is the result of a continuous interaction between genetics and environmental factors. Therefore, although exercise can be beneficial, other factors, such as nutrition, can improve well-being and expand lifespan further as well [11].

For example, evidence is clear that following the MD (Mediterranean diet) has a positive effect on heart health, particularly in relation to reduced chronic disease development [12,13]. Results observed in randomized control trials suggest several beneficial physiological mechanisms of action, which include altering lipid profiles and reductions in BP (blood pressure), insulin resistance and systemic markers of inflammation [14,15]. Further studies show that an adherence to the MD has been associated with a significant improvement in health status as seen by a reduction in overall mortality (9%; same amount of reduction in deaths due to cardiovascular causes) [16] and in benefits on cardiovascular risk factors even in the elderly population [17].

It must be noted, however, that these and other ‘healthy-diet’ studies [18,19] fail to adequately capture the multi-dimensional effects that such interventions may bring. That is to say the number of trials studying the microcirculation involving both exercise and diet control has been limited and restricted to specific patient groups, i.e. diabetics [20]. Even in those where the effects on endothelial function has been explored, findings have been conflicting [5,21], whereas the MD concept has been almost completely neglected. Therefore and despite the apparent benefits to the participants, little information has been provided on the combined effect of nutrition and exercise on the mechanisms of endothelial health and vasodilatory activity. This could open new pathways and increase their implementation to a large number of patient populations. Moreover, it could play a role in the attempt to reduce age-related decline in a healthy, but sedentary, population, hence reducing the danger of further complications.

Therefore we investigated the medium-term effects of an intervention that combined a lower-limb exercise training (in the form of treadmill-based exercise) and a dietary management scheme, based on the MD, on both lower- and upper-limb cutaneous microvascular functions in a normal population.

## MATERIALS AND METHODS

### Participants

A total of 22 healthy participants were recruited via posters and word-of-mouth in Lincolnshire, U.K. All participants were required to be over 50 years of age, normotensive, non-smokers, sedentary and not taking any regular medication. Sedentary status was defined as the participants not being engaged in purposeful physical activity with the intention of improving physical fitness for at least 6 months. Participants with past venous ulceration, lower-limb arterial disease, hypercholesterolaemia, peripheral oedema or cardiac failure, and those with major skin changes in the gaiter area, were excluded as well as those undertaking any form of regular exercise. This choice ensured that all participants were similar in age to groups that we have studied previously [7,8], so that the findings of the present study can be comparable. Female participants were studied in days 1–7 of their menstrual cycle to minimize the influence of cyclical changes in female hormones [22]. Participants were asked to refrain from any regular or structured exercise activities outside their supervised exercise sessions, undertaken for the purposes of the study.

As part of the study eligibility, participant dietary habits were initially scrutinized by completing an adapted nine-item questionnaire, as described elsewhere [23]. The questionnaire was used to assess the regularity of consuming olive oil, fruit, vegetables and salad, fish, legumes, meat, wholegrain foods and wine, all of which constitute part of the MD. A point score of <5 was installed as a marker for inclusion (range 0–9) and participants with scores of 5 or more were excluded. As described in the literature, higher scores (5–9) suggest adherence to a cardio-protective diet and a reduced risk of cardiovascular events [23]. Participant characteristics are shown in Table 1. Baseline outcome measures were then assessed (see below). Sample size calculations were based on our previous work on similar study groups for a significance level of 5 and 85% power [24].

The present study was approved by University of Lincoln Ethics Committee. This research was carried out in accordance with the Declaration of Helsinki of the World Medical Association and all participants gave their written informed consent to participate.

### Assessment of cutaneous microvascular function

Outcome measures were assessed at baseline and following the 8-week exercise intervention. Participants were fully habituated with the assessment protocols prior to baseline data collection. Participants were instructed not to perform any exercise in the 24 h before an assessment and to abstain from caffeine for at least 2 h before an assessment.

All microvascular assessments were performed in a temperature-controlled room (range, 22–24 °C) following an acclimatization period ≥10 min. With the participant lying supine, the gaiter area of the left leg was cleaned with an alcohol wipe and allowed to dry before applying two Perspex iontophoresis

<table>
<thead>
<tr>
<th>Variable</th>
<th>Exercise group</th>
<th>MD group</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (n)</td>
<td>3/8</td>
<td>4/7</td>
<td>0.89</td>
</tr>
<tr>
<td>(male/female)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>54 ± 9</td>
<td>54 ± 4</td>
<td>0.89</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>74.1 ± 18.2</td>
<td>79.9 ± 14.5</td>
<td>0.51</td>
</tr>
<tr>
<td>Stature (cm)</td>
<td>168.0 ± 10.3</td>
<td>168.4 ± 7.9</td>
<td>0.94</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>97.2 ± 15.3</td>
<td>92.6 ± 11.5</td>
<td>0.50</td>
</tr>
<tr>
<td>Systolic pressure (mmHg)</td>
<td>128.9 ± 25.7</td>
<td>124.5 ± 9.5</td>
<td>0.61</td>
</tr>
<tr>
<td>Diastolic pressure (mmHg)</td>
<td>81.3 ± 10.4</td>
<td>76.1 ± 12.9</td>
<td>0.41</td>
</tr>
</tbody>
</table>
chambers to the skin surface 4–8 cm proximal to the medial malleolus. The chambers were positioned approximately 4 cm apart, with one containing 80 μl of the endothelium-dependent vasodilator ACh (Miochol-E; Novartis), and the other 80 μl of the endothelium-independent vasodilator SNP (sodium nitroprusside; Rottapharm, S.L.). Drug concentrations of 10 g⁻¹ were used with deionized water as the solvent. The laser Doppler probe was positioned through the centre of each chamber.

LDF (laser Doppler fluximetry) measurements were made using the moorVMS-LDF2 system (Moor Instruments) that included skin temperature, flux and microvascular dose-response curves for each of the four iontophoretic challenges obtained. A battery-powered iontophoresis controller (MIC2; Moor Instruments) was used to provide the charge needed for ACh and SNP delivery. The anodal (positive) current was used to transfer ACh, with the cathodal (negative) current used to transfer SNP [25].

After achieving a stable recording of baseline flux, LDF responses to ACh and SNP were measured using an incremental-dose iontophoresis protocol, as described previously [2]. In brief, dose–response curves for ACh- and SNP-induced vasodilation were characterized using the following procedure to apply incremental charge-stimuli: 25 μA applied for 10 s (i.e. 250 μCb), 50 μA for 10 s (500 μCb), 100 μA for 10 s (1000 μCb) and 100 μA for 20 s (2000 μCb), with a 4-min recording period between each dose. This protocol was chosen as it is sufficient to provide effective ACh and SNP delivery while largely avoiding the non-specific vasodilation observed with higher electrical charges with satisfactory reproducibility both in patient and healthy populations [2,26].

Thereafter, following a 10-min recovery period, the protocol was repeated with the probes attached on the ventral surface of the right forearm of each participant, to establish a baseline for the upper-limb cutaneous microvascular function. The protocol was repeated on visit 2. Skin temperatures were continuously recorded using temperature sensors that are part of the laser Doppler probes to ensure stability in measurement conditions. Additionally, the probes used for each drug were alternated at each test. HR (heart rate) and BP (contra-lateral arm to avoid occlusion-related alterations in skin blood flow for the experimental arm; DinamapDash 2500; GE Healthcare) were recorded throughout the protocol. There were no adverse effects.

Cardiopulmonary exercise assessment
All participants were assessed for their cardiopulmonary response throughout two incremental exercise tests on a treadmill (Cosmos HP Mercury 5.0) before and after the exercise and/or dietary intervention. Participants’ \( \dot{V} \text{O}_2 \) (oxygen uptake), \( \dot{V} \text{CO}_2 \) (CO₂ production) and RER (respiratory exchange ratio) were measured continuously breath-by-breath using an online gas analyser (Metalyzer Cortex 3B). Flow sensor and gas analysers using gases of known concentration (16% for \( \text{O}_2 \) and 5% for \( \text{CO}_2 \)) and a 3 litre gas volume syringe were calibrated prior to each test. The incremental test protocol started with 2.0 km/h and was increased by 1.0 km/h every 3 min until reaching the test termination criteria defined as reaching RER-1, and 85% of age-predicted maximal HR, to avoid any presence of low-peak RER [27].

Randomization and exercise programme
All participants were randomized, using a computer program (nQuery Advisor 6.0; Statistical Solutions), to either Group A (treadmill-exercise group) or Group B (dietary control group). Group A participants were required to attend the facilities for 16 supervised treadmill exercise sessions (undertaken twice a week over eight consecutive weeks), whereas Group B participants were required to follow a customized dietary schedule based on the MD, in addition to the requirement to attend treadmill exercise sessions as in Group A. The groups were well-balanced for demographic variables (Table 1). During each exercise session, participants trained in cycles of 2 min exercise followed by 2 min rest, for a total exercise time of 20 min in a 40-min session. The intensity of the exercise was set to elicit an RPE (rating of perceived exertion) of 11–13 (‘light’ to ‘somewhat-hard’) using Borg’s 6–20 RPE scale [28]. HR and RPE were monitored throughout each training session. Participants were not engaged in regular or structured exercise apart from the one undertaken for the purposes of this study.

Dietary intervention
Within a week of being assessed, each participant completed a baseline 3-day (consecutive) diet record and received a 1 h one-to-one educational and advisory session about the experimental diet by a registered nutritionist. The 3-day record has been advocated as a reasonable period for reporting dietary intake, avoiding response fatigue from participants [29]. Participants were encouraged to increase food and drink items which were traditionally associated with a Mediterranean-style diet, without any recommendations to restrict energy or calorie intake. The nutritionist used a ‘cultural model’, as described in the literature [30], to explain and inform the constitution of the MD and provided relevant materials and guides (sourcing foods, recipes, shopping lists, meal preparation advice, methods of cooking, etc.). The main components of the MD such as vegetables, seasonal fruits, olive oil, tree nuts and fresh oily fish were highly recommended to all participants. These foods have been recognized as containing potential anti-atherogenic effects [15,31]. All participants had access to the nutritionist by phone and email throughout the study for additional advice and guidance. A further progress meeting with the nutritionist at the midway period of the intervention (4 weeks) was arranged with each participant. These sessions were individually tailored and involved counselling with behavioural and psychosocial issues: barrier removed, keeping motivated and setting appropriate goals. In the final week, participants completed a second 3-day diet diary. The procedures stated here are similar to previously published trials studying the effects of the MD on health [14,15].

Data recording and analysis
Peak cutaneous flux responses to ACh and SNP, recorded in conventional PU (perfusion units), were used as the measures of microvascular endothelium-dependent and -independent functions respectively. \( T_{\text{max}} \) (time to reach maximum perfusion) was also measured according to the literature [26].

The breath-by-breath \( \dot{V} \text{O}_2 \) and \( \dot{V} \text{CO}_2 \) data were averaged for the final minute of each stage. VT (ventilatory threshold) was
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Figure 1 comparison between groups for CVC for ACh in the lower limbs prior to (left-hand panel) and following (right-hand panel) the intervention. Values are means ± S.D.; *P < 0.001.

Figure 2 comparison between the groups for CVC for SNP in the lower limbs following the intervention. Values are means ± S.D.; *P < 0.05.

RESULTS

Perfusion in lower limbs

A dose-dependent effect was observed for both ACh and SNP in the lower limbs (P < 0.001, d = 0.79; and P < 0.001, d = 0.78 respectively). Both groups appeared to have an improved raw CVC following the 8-week period (e.g. P = 0.001, d = 0.59 for ACh; and P = 0.002, d = 0.56 for SNP). Additionally, analysis revealed significant interaction between the time-period and the dose delivered, for both agents (P < 0.001, d = 0.46 for ACh; and P < 0.001, d = 0.44 for SNP), suggesting a dose-by-dose difference following the intervention (Figure 1). Exploring the interactions between the time period (e.g. prior and following the intervention) and the group, however, revealed a stronger improvement in the MD group in comparison with the exercise group, for ACh, following the intervention (P = 0.02, d = 0.44). No significant difference was observed between groups in endothelium-independent vasodilation (SNP) following the 8-week period (P = 0.25, d = 0.1) (Figure 2).

determined individually for each test using the V-slope method [32] defined as the first deflection point in \( \dot{V}CO_2 \) as a function of \( \dot{V}O_2 \). Detection of each threshold point was determined visually by an experienced researcher, and verified by another experienced staff.

Outcome measures were first assessed for normal distribution using the Kolmogorov–Smirnov goodness-of-fit test and normalized using logarithmic transformation, if necessary, before further analysis. Cutaneous blood flux data were also divided by mean arterial pressure to calculate CVC (cutaneous vascular conductance) [24]. Differences in group characteristics were assessed using independent Student’s t tests and \( \chi^2 \) tests. Mixed-model (group by time) ANCOVA (analysis of co-variance) were used to detect changes in outcome measures between groups, with baseline data used as the covariate [33]. Effect sizes (Cohen’s d) were calculated for the exercise group data, with 0.2, 0.5 and 0.8 representing small, medium and large effects respectively [24]. Two-way mixed ANOVA model was applied to detect the intervention effects on VT, with training (before and after) as within subjects. Dietary records were analysed by a computer software package: ‘Microdiet’ (Downlee Systems Ltd). A paired Student’s t test was applied to detect changes pre–post diet intervention. Where the assumptions were not met for a parametric test, a suitable alternative non-parametric test was chosen. Statistical significance was set at \( P \leq 0.05 \). Values are means ± S.D. unless otherwise stated.
effects between training and diet.

However, the improvement in VT and VT velocity were not affected by the dietary intervention, and there were no interaction effects for training.

**Dietary intervention**
Participants following the MD intervention had a significantly lower calorific intake ($P = 0.02$) and a reduction in the percentage of energy derived from consuming sugars ($P = 0.01$) at the end of the 8-week period. Daily consumption of olive oil, fruit and vegetables were also significantly higher at 8 weeks when compared with baseline values (e.g. fruit and vegetables, $2.4 \pm 1.1$ compared with $3.3 \pm 0.8$; $P < 0.01$). The relative energy contribution from mono- and poly-unsaturated fats between dietary assessment points was increased, although not significantly (Table 3).

**DISCUSSION**
In the recent past, a number of studies have revealed the prophylactic and therapeutic effects of exercise in the microcirculatory physiology of a number of patients [8,34] and normal, older, sedentary [24] groups. The present study, however, took a step further by exploring the effects of an additional lifestyle change, that of the dietary adaptation. This was in response to recent findings, which, based on epidemiological [11] and interventional [19] studies, identified healthy diet as a source for risk reduction.
of CVDs (cardiovascular diseases) and diabetes, whereas other studies have demonstrated that obesity may impair vascular endothelial function, suggesting at the same a lifestyle change as the key to improvement of vascular function in overweight adults [35].

Comparing studies where a diet adaptation has been instigated is a difficult task, as ‘healthy’ diets cannot be directly comparable due to nutritional variability as well as differences in the implementation group (e.g. older compared with young populations), their target (e.g. BMI and well-being perception improvements compared with endothelial function) and also their duration. Consequently, their implementation may have conflicting results [5,21]. Therefore when deciding the choice of diet type to be used for a study, those elements have to be taken into consideration together with cultural differences, and restrictions in ingredients’ availability may jeopardize the success of a trial.

We decided to implement a MD, slightly modified for a British target group: although it is well documented that it reduces the risk for CVDs and overall mortality [11,17], its benefits on micro- and macro-vascular functions have not been properly explored.

We believe that two main messages can be highlighted in the present study. First, that the combination of a moderate-intensity exercise-training programme and a dietary adaptation that is based on moderate supervision and encouragement is feasible and offers direct benefits to older participants. This is especially important, as with an increasing working age beyond the age of 65 in the Western world, it is crucial to provide ways to improve the well-being and quality of life of people, in a manner that will be easy to adapt to. Although, we could have opted for a higher-intensity training exercise, taking into account its previous potential for alleviating vascular dysfunction [36], and a more closely followed diet intervention, we decided to adopt a more flexible and easier-to-follow approach. This included a medium-intensity, treadmill-exercise regime, leaving at the same time the exact meal choices to the participants, offering however, weekly follow ups and suggestions from the research team along the way. Therefore there was an element of self-selection in the training intensities, applied in order to increase the participation pleasure and ensure more effective long-term compliance than fully imposed intensities [37]. This choice seemed successful as it gave them sufficient motivation to adapt to a healthier lifestyle, without deterring them by being far too hard.

Secondly, it appears that older, previously sedentary and otherwise healthy, people who decide to adapt their diet as well as their exercising habits improve significantly their endothelium-dependent vasodilatory function, in the lower leg (assessed by ACh) when compared with those who only exercise (P = 0.02, d = 0.44). In our study, and although both groups have improved following the intervention (P < 0.001, d = 0.65), the effect was stronger in the MD group. ACh is known to elicit vasodilation through a mainly NO (nitric oxide)-dependent pathway [7], with the contribution of other mediators [such as prostanoids and EDHF (endothelial-derived hyperpolarizing factors)] being also possible [7]. This has been suggested to be especially true in older populations (similar to the ones in the present study), where although other compensatory mechanisms exist to preserve heat-induced vasodilatory responses, these are largely NO-dependent, especially in the higher ACh doses [9] (similar to the ones exerted in our study).

The effects of exercise in the microcirculatory function have been studied extensively and it has been suggested that both endothelial adaptations (in the form of increased NO synthesis [9] and alterations in neural vascular control [38]), may play a role. Additionally, Wang [25] has shown an increased response to ACh as well as an increased level of plasma NO metabolites after 8 weeks of training in healthy subjects. Attributing however, the further improvement, observed in the MD group, appears to be more challenging. It is possible that this is attributable to changes that occur at a molecular level, e.g. in the serum markers of endothelial dysfunction/adhesion molecule [ICAM (intercellular adhesion molecule)] and changes in the inflammatory markers [CRP (C-reactive protein)] or angiogenesis that are findings that have been associated with dietary changes and exercise introduction [5]. The possibility of having decreased inflammatory markers such as serum CRP, IL-6 (interleukin-6), endothelial and monocytary adhesion molecules and chemokines (all related

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### Table 3  Energy intake, relative nutrient contribution and food item selection for the MD group at baseline and post-intervention

<table>
<thead>
<tr>
<th>Energy source/nutrient</th>
<th>Baseline</th>
<th>At 8 weeks</th>
<th>Mean change (+ or −)</th>
<th>Within-group change P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total energy (kcal/day)</td>
<td>1818 ± 497</td>
<td>1478 ± 257</td>
<td>−340</td>
<td>0.02*</td>
</tr>
<tr>
<td>Carbohydrates (%)</td>
<td>48.9 ± 6.2</td>
<td>46.2 ± 9.0</td>
<td>−2.7</td>
<td>0.25</td>
</tr>
<tr>
<td>Sugars (%)</td>
<td>19.7 ± 5.6</td>
<td>15.5 ± 4.3</td>
<td>−4.2</td>
<td>0.01*</td>
</tr>
<tr>
<td>Fibre (g/day)</td>
<td>14.9 ± 5.5</td>
<td>14.1 ± 2.9</td>
<td>−0.8</td>
<td>0.74</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>17.3 ± 2.2</td>
<td>18.0 ± 3.5</td>
<td>+0.7</td>
<td>0.73</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>34.1 ± 6.1</td>
<td>34.7 ± 8.4</td>
<td>+0.6</td>
<td>0.89</td>
</tr>
<tr>
<td>Saturated (%)</td>
<td>11.8 ± 3.3</td>
<td>11.1 ± 4.2</td>
<td>−0.7</td>
<td>0.58</td>
</tr>
<tr>
<td>Monounsaturated (%)</td>
<td>11.9 ± 2.3</td>
<td>13.4 ± 4.7</td>
<td>+1.5</td>
<td>0.34</td>
</tr>
<tr>
<td>Polyunsaturated (%)</td>
<td>5.1 ± 1.8</td>
<td>5.5 ± 2.2</td>
<td>+0.4</td>
<td>0.54</td>
</tr>
<tr>
<td>Fruit and vegetables (serving/day)†</td>
<td>2.4 ± 1.1</td>
<td>3.3 ± 0.8</td>
<td>+0.9</td>
<td>0.01*</td>
</tr>
<tr>
<td>Olive oil (serving/day)‡</td>
<td>0.4 ± 0.4</td>
<td>0.8 ± 0.8</td>
<td>+0.4</td>
<td>0.04*</td>
</tr>
</tbody>
</table>

†Serving based on 80 g estimate (e.g. 4 tablespoons/one large fruit).
‡Serving based on 11 g estimate (e.g. 1 tablespoon of olive oil).
Exercise, Mediterranean diet and the microcirculation in older people

to atherosclerosis), through MD is also viable: these have been particularly attributed to the virgin olive oil and tree nuts as key contents in reducing cardiovascular risk in high-risk groups [39]. Evidence suggests that the functional components of olive oil including fatty acids (oleic acid), phytosterols (β-sitosterol), antioxidants (α-tocopherol) and phenolic compounds could be responsible for the anti-inflammatory and endothelial activity [32,40]. Further studies will be required to establish this relationship.

It would be incorrect to attribute a meaning of this finding solely with regard to the microcirculation. It was previously revealed [41] that the endothelial dilatory response to increased blood flow and to acetylcholine is similar in large arteries [assessed by FMD (flow-mediated vasodilation)] and in the skin microvasculature (assessed by LDF). This suggests that measurements of blood flow changes in the skin microcirculation using LDF coupled with iontophoretically induced ACh may be used to assess endothelial function within a large range of normal and altered endothelium responses, mirroring the conditioning of larger arteries as well. Consequently, the additional improvement in endothelial function observed in the lower legs of the MD group may mean a further risk reduction for lower-limb arterial diseases (e.g. peripheral arterial disease), which is a positive prospect in this older and more vulnerable age group [42].

An improvement was also observed in the upper limb, in both endothelium-dependent and -independent vasodilation function, in partial agreement with previous studies [24,25]. As no differences existed between groups, it can be safely assumed that this improvement can be attributed to exercise, strengthening our previous assumption that treadmill exercises activates a muscle mass that provides a cardiovascular challenge sufficient to induce generalized haemodynamic improvements [24]. At the same time, it can be postulated that treadmill exercise results in a generalized NO production and/or bioavailability. As has been previously suggested [43], this in turn modulates sympathetic activity, by reducing the chronic suppressive influence exerted by sympathetic tone either directly or by enhancing the NO-induced sympatho-inhibitory effect in both upper and lower limbs.

With regard to the exercise regime followed, the present study considered effective training intensities for enhancing several cardiovascular, respiratory, and metabolic markers, thought to correspond to an RPE of 10–13. This intensity range has been considered effective for improved anaerobic and lactate thresholds, enhanced \( V_{\text{O}_2} \) and increased ability to utilize fatty acids [44]. A 20% increase in both VT and velocity at VT found for both groups of the present study is in agreement with the latter finding. It also confirms the positive effects in microcirculatory markers found in this study as a result of training, and established guidelines confirming VT as an established marker for cardiovascular capacity [27]. This adaptation indicates greater oxygen availability through more efficient blood delivery following the training, which has been reported to reflect an enhanced endothelium vasodilation and greater periphery artery distensibility [34]. Enhanced VT may also relate to reducing the arterial stiffness in healthy adults [45]. The similar improvement found in VT for MD and non-MD is unclear. Perhaps the sample size had an effect, as it was calculated based on microcirculatory data, or

VT was less sensitive as a marker for dietary-dependent effects, compared with the LDF measurement, and therefore, accounting for our findings. Nonetheless, the present findings are the first to be reported for an older population.

Experimental considerations
We were unable to compare our diet group with one under stricter dietary management. This was a conscious choice, based on our attempt to make the study as approachable as possible to the average, healthy, middle-aged participant. It would, however, be interesting to see the outcome of such a comparison in a future study, especially taking into consideration our findings that were derived under a less strict MD adherence. Similarly, and taking into account the participants’ age range, we opted not to implement a higher-intensity exercise regimen and chose an exercise protocol that was based on the 60–70% of the participants’ peak capacity. It is therefore possible that a higher-intensity exercise regimen would have elicited more favourable, endothelium-independent, microvascular adaptations, a suggestion that might also be applicable in patient populations [10]. We also did not include a larger variety of microcirculatory tests (e.g. heating, reactive hyperaemia). This would have increased the duration of the measurements significantly by a number of hours and would have affected the number of participants recruited, most of whom were still employed. Additionally, due to the exploratory nature of the study it was not possible to include biological markers that could provide additional information on the assimilation of the influencing dietary components. Such inclusions, however, will be considered in follow-up studies.

In summary, we have found that an adapted MD, combined with moderate-intensity exercise, improves age-provoked, microcirculatory, endothelial dysfunction and thickening in the lower legs of a healthy but sedentary group of participants. This improvement comes in addition to the established exercise benefits in this age group [24,38] and can provide a further risk reduction in a number of diseases and conditions associated with the lower limbs. In this context, improving age-related endothelial dysfunction in middle-aged and older people by diet and exercise should be regarded as an important strategy for modifying vascular risk in this population.

CLINICAL PERSPECTIVES

- Age-dependent vascular endothelial dysfunction and reduced exercise tolerance are major causes of CVD. Improved dietary habits alone including the MD may reverse those effects. However, little is known on whether and how the combination of exercise and MD reverses those risks in an older population.
- The results of the present study show that compliance with MD and regular exercise combined increase exercise tolerance and reverse microcirculatory endothelial dysfunction in this age group.
- Improvement in exercise tolerance and better improvement in vascular dysfunction in the combined MD and exercise group than in the exercise group alone suggest an effective approach to reduce age-dependent cardiovascular risk.
AUTHOR CONTRIBUTION

Markos Klonizakis co-ordinated the study and was responsible for the study concept, design, participant recruitment, data collection and analysis, and writing and revising the paper. Ahmad Alkhatib was involved in the design of the exercise intervention, in data collection and analysis, and in writing and revising the paper. Geoff Middleton was involved in the design of the dietary intervention, and participated in the data collection and analysis, and in writing and revising the paper. Mark Smith participated in the design of the exercise intervention, in data collection, and in writing and revising the paper. All authors approved the paper for publication.

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Exercise, Mediterranean diet and the microcirculation in older people


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