A Mediterranean diet supplemented with extra virgin olive oil or nuts improves endothelial markers involved in blood pressure control in hypertensive women

Running title: Endothelium, olive oil, nuts and blood pressure

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Abstract

Purpose Serum nitric oxide (NO) reduction and increased endothelin-1 (ET-1) play a pivotal role in endothelial dysfunction and hypertension. Considering that traditional Mediterranean diet (TMD) reduces blood pressure (BP), the aim of this study was to analyze whether TMD induced changes on endothelial physiology elements such as NO, ET-1 and ET-1 receptors which are involved in BP control.

Methods Non-smoking women with moderate hypertension were submitted for 1 year to interventions promoting adherence to the TMD, one supplemented with extra virgin olive oil (EVOO) and the other with nuts versus a control low-fat diet (30 participants/group). BP, NO, ET-1 and related gene expression as well as oxidative stress biomarkers were measured.

Results Serum NO and systolic BP (SBP) or diastolic BP (DBP) were negatively associated at baseline, as well as between NO and ET-1. Our findings also showed a DBP reduction with both interventions. A negative correlation was observed between changes in NO metabolites concentration and SBP or DBP after the intervention with TMD + EVOO ($p = 0.033$ and $p = 0.044$, respectively). SBP reduction was related to an impairment of serum ET-1 concentrations after the intervention with TMD + nuts ($p = 0.008$). We also observed changes in eNOS, caveolin 2 and ET-1 receptors gene expression which are related to NO metabolites levels and BP.

Conclusions The changes in NO and ET-1 as well as ET-1 receptors gene expression explain, at least partially, the effect of EVOO or nuts on lowering BP among hypertensive women.

Keywords Endothelin-1 · Hypertension · Nitric oxide · PREDIMED study · Oxidative stress

Introduction

Hypertension is one of the most common chronic health problems as it increases the risk for cardiovascular events and renal failure [1]. Conversely, a reduction in blood pressure (BP) among hypertensive subjects prevents or attenuates these complications. Nitric oxide (NO) is a potent relaxing factor [2] whereas endothelin-1 (ET-1) is a potent vasoconstrictor peptide [3], and both are of pivotal importance in maintaining vascular homeostasis. Endothelial dysfunction is the result of an imbalance in the production of these substances among others by the endothelium [4], which is associated to oxidative stress [5]. Thus, numerous studies in animals and humans have implicated NO [6], and ET-1 [7] and their receptors [8] in the pathogenesis and/or maintenance of hypertension.

A healthy diet and lifestyle modification are the first steps for the management of hypertension [9]. Compared with a high-saturated fat diet, the Traditional Mediterranean Diet (TMD), characterized by a high consumption of vegetables, legumes, grains, fruits, nuts and olive oil, is associated with a low BP [10, 11]. The PREvención con DIeta MEDiterránea (PREDIMED) Study is a large-scale, multicenter, parallel, randomized and controlled clinical trial (ISRCTN35739639, www.controlled-trials.com) aimed at assessing the effects of a TMD enriched with extra virgin olive oil (EVOO) or nuts on the primary prevention of cardiovascular disease (CVD) in high risk patients [12]. Among the 772 first recruited participants and after 3 months of intervention, the participants allocated to the TMD + EVOO or TMD + nuts showed significantly lower systolic BP (SBP) than participants allocated to the control group, advised to follow a low-fat diet [13]. Recently, Toledo et al. [14] reported that after 4 years of follow-up, lower values of diastolic BP (DBP) were observed in the two groups that received the TMD + EVOO or TMD + nuts than in the control group. Considering the above mentioned studies, we hypothesized whether the improvement of BP induced by a TMD + EVOO or TMD + nuts would be mediated by the modulation of NO bioavailability and/or ET-1 levels as well as ET-1 receptors gene expression which might be regulated by oxidative stress. This, study was performed after 1 year of the PREDIMED dietary interventions in a subpopulation of non-smoking women with moderate hypertension.

Participants and methods

Subjects

90 non-smoker women (aged 60 to 80 years) not consuming non-steroidal anti-inflammatory drugs without CVD but at high cardiovascular risk participated in this substudy of PREDIMED. Full details of the PREDIMED protocol have been published elsewhere [14]. The presence of type 2 diabetes or at least three or more coronary heart disease risk factors: hypertension (BP ≥ 130/85 mmHg) or treatment with antihypertensive drugs, low density lipoprotein (LDL) cholesterol level ≥ 160 mg/dl or treatment with hypolipidemic drugs, high-density lipoprotein (HDL) cholesterol level ≤ 42 mg/dl, body mass index (BMI) ≥ 25 kg/m^2, or a family history of premature CVD, were considered. Exclusion criteria were history of any severe chronic illness, illegal drug consumption or alcohol abuse, history of allergy or intolerance to olive oil or nuts, or low predicted likelihood of changing dietary habits according to the stages of change model [15].

Participants were recruited in the primary care centres of Reus and Barcelona (Spain) of the PREDIMED study and most of them had moderate hypertension. They provided a written informed consent and the protocol was approved by the institutional review boards of both centres according to the declaration of Helsinki.

Study design

At baseline, the participants completed a validated semiquantitative food-frequency questionnaire with 137 items [16], the validated Spanish version of the Minnesota Leisure Time Physical Activity Questionnaire [17], and a 47-item questionnaire about education, lifestyle, history of illnesses and medication use [12].

After the screening visit, participants were randomly assigned (30 participants/group) to one of the following three dietary intervention groups: a TMD where olive oil was substituted by EVOO (TMD + EVOO consumption of 52 g/d EVOO), a TMD + nuts (15 g/d walnuts, 7.5 g/d hazelnuts, and 7.5 g/day almonds and a consumption of 40 g/d olive oil), or a control low-fat (consumption of 40 g/day of olive oil), advised to follow written recommendations of a low-fat diet according to the American Heart Association guidelines. EVOO (15 l) and nuts (1,350 g walnuts, 675 g hazelnuts and 675 g almonds) were provided every three months to the corresponding TMD group to improve adherence and fulfil the

family needs. We neither advised on total caloric restriction nor promoted physical activity. Trained dieticians were responsible for all aspects of the intervention. Energy intake was derived from Spanish food composition tables.

At baseline and after 1-year follow-up, trained personnel measured BP with a validated semi-automatic oscillometer (Omron HEM-705CP, Hoofddorp, The Netherlands) while the participant was in a seated position after 5 min rest. Arm circumference determined the cuff size and BP was measured in the forearm at heart level. The mean of the three SBP and DBP measurements with a 5-min interval between each reading was recorded.

Serum samples after overnight fast at baseline and 1-year follow-up were coded, shipped to a central laboratory, and stored at -80 ^{o}C until assay.

Assays and chemicals

Serum NO levels were indirectly measured by determining the NO stable metabolites (nitrite + nitrate) using a colorimetric assay kit (Cayman Chem. Co., Ann Arbor, MI, USA). The detection limit was 2.0 µmol/l. The inter-assay and intra-assay coefficients of variation were 2.7 % and 3.4 %, respectively.

Serum ET-1 was analyzed by enzyme immunoassay (R&D Systems, Minneapolis, MN, USA). Minimum detectable concentration of ET-1 was 0.02-0.03 pg/ml. The inter-assay and intra-assay coefficients of variation were 4.5 % and 2.6 %, respectively.

Gene expression analyses were performed by microarray in a subset of the population (10 individuals/group) at baseline, and after the intervention, using the Affymetrix's GeneChip (GeneChip Human genome U133A 2.0) in RNA isolated from peripheral blood mononuclear cells. The microarray data is registered as GSE28358 in GEO (Gene Expression Omnibus), a public functional genomics data repository. Changes in gene expression of eNOS, caveolin 2 and ET-1 receptors after the intervention were calculated by log2ratio (post-intervention value/pre-intervention value).

Serum total antioxidant capacity was measured with a colorimetric test (Cayman Chem. Co., Ann Arbor, MI, USA). It is based on the ability to inhibit the oxidation of ABTS (2,2'-azino-bis-(3-ethylbenzthiazoline-6-sulphonic acid) by methmyoglobin, of both aqueous and lipid-soluble antioxidants by comparison with that of Trolox.

Serum malondialdehyde (MDA) levels were measured by HPLC with fluorescence detection that quantifies genuine MDA-thiobarbituric acid adduct [18] avoiding the total absorbance of several species when using a spectrophotometric detection. Briefly, samples or standards (25 µl) were mixed with 0.44

M H_3PO_4 (375 µl), 40 mM 2-thiobarbituric acid (125 µl) and ultrapure water (225 µl) and placed in a heating cabinet at 97 ^oC for 60 min. After cooling on ice, alkaline methanol was then added 1:1 (v/v) and mixed for 10 s. Samples were centrifuged at 3,000*g* for 3 min and the supernatants were transferred to HPLC vials for analysis. The HPLC system consisted of a Waters 717 plus autosampler, Waters 600 controller pump and Jasco FP-1520 fluorescence detector using a 250 x 4.6 mm Kromasil 100 C18 column with 5 µm particles (Tecknochroma, Barcelona, Spain). Standards were freshly prepared each day using 10 µM 1,1,3,3, tetramethoxypropane in Ringer's solution followed by serial dilutions. The inter-assay and intra-assay coefficients of variation were 10 % and 3.7 %, respectively.

Statistical analyses

Normality of continuous variables was assessed by normal probability plots. Results were expressed as mean ± SEM. General linear models were used to analyze between-group changes. Spearman correlations were estimated between serum NO or ET-1 levels and clinical parameters. A $p \leq 0.05$ (Student's *t*-test) was considered statistically significant. All statistical analyses were performed with the SPSS 12.3 software (SPSS Inc., Chicago, IL, USA) for Windows XP (Microsoft, Redmond, WA, USA).

Results

The three intervention groups were well balanced with respect to anthropometric characteristics, cardiovascular risk factors, metabolic syndrome features and medication use, as non-significant changes between groups were observed (Table 1). More frequent features in this subpopulation were hypertension (97 %), obesity (95 %) and hyperglycemia (61 %). We can not exclude modifications of antihypertensive drugs dosage as this information was not available although no adjustments in the participants' regular prescriptions were part of the intervention.

At baseline, our findings show means in the total of 90 participants of serum nitrite + nitrate and ET-1 of 30.2 ± 1.80 µmol/l and 1.5 ± 0.06 pg/ml, respectively, without differences among the three groups (Table 1). Moreover, we detected a negative correlations between SBP or DBP and serum nitrite + nitrate (*r*=-0.228, *p*=0.031; *r*=-0.221, *p*=0.036), as well as with (nitrite + nitrate)/ET-1 (*r*=-0.257, *p*=0-014; *r*=-0.235, *p*=0.026), and a positive correlation with serum ET-1 concentration at baseline (*r*=0.243, *p*=0.021; *r*=0.252, *p*=0.016). BMI was not correlated with serum nitrite + nitrate or ET-1. We also

observed a negative correlation between serum stable NO metabolites and ET-1 levels at baseline (r = -0.233, p = 0.027).

The consumption of nutrients such as fiber, cereals, fruits, vegetables, legumes, meat and meat products, fish and alcohol as well as physical activity were similar in the three groups at baseline and no changes throughout the study were observed in any intervention group (data not shown).

The main dietary changes recorded at 1-year follow-up were an increase of olive oil and nut consumption and the substitution of olive oil by EVOO and in the corresponding TMD groups. No significant changes in total energy intake between groups (data not shown). These observations together with a similar TMD score in the three groups (around 7.8) suggest that subjects fairly adhered to the dietary interventions.

After 1-year follow-up, SBP and DBP were slightly reduced by the two interventions, whereas DBP was significantly decreased (5 %, $p < 0.0498$) by TMD + nuts intervention (Figure 1A). In addition, serum stable NO metabolites concentration increased in the TMD + EVOO group (63.9 %, $p = 0.009$). The serum ET-1 concentrations decreased (19 %, $p < 0.0492$) in the TMD + nuts group versus their respective baseline values reported in Table 1. These parameters did not appreciably change in the control group. The TMD + EVOO increased 5 fold the serum nitrite + nitrate whereas TMD + nuts decreased ET-1 in the same proportion with respect to variations in the control group (Figure 1B). Interestingly, serum NO metabolites concentration was negatively correlated with SBP or DBP in the TMD + EVOO group (Figure 2), and a positive correlation between ET-1 concentration and SBP was observed. Furthermore, after 1-year of follow-up with TMD + nuts, the ET-1 concentrations were directly correlated with the SBP (Figure 2).

In relation to the changes in serum NO and ET-1 concentrations, we observed an up-regulation of endothelial NO synthase (eNOS) and a down-regulation of caveolin 2 after TMD + EVOO, as well as a down-regulation of ET-1 receptors (ET_AR and ET_BR) after TMD + nuts (Figure 3).

Serum total antioxidant capacity and MDA (Table 2) analysed at baseline did not differ among the three dietary groups. Only an 11 % increase of total antioxidant capacity ($p = 0.122$) was observed in the TMD + EVOO group after 1-year intervention.

DISCUSSION

The imbalance of vasodilation/vasoconstriction molecules release by endothelium is implicated in the etiology and development of hypertension [19] and predicts future cardiovascular events in hypertensive individuals [20]. Thus, recent data indicate that BP in pre-hypertensive subjects is associated with impaired NO-mediated endothelium-dependent vasodilation [21]. Moreover, the role of a decreased NO availability has been reported in hypertensive patients [22]. It has also been described a vasoconstrictor effect of ET-1 in arterial hypertension [23] and in pre-hypertensive adults [24].

The findings of the present study show an increase in nitrite + nitrate and an impairment of ET-1 after 1-year of the TMD + EVOO and TMD + nuts, respectively, suggesting that serum NO and ET-1 could be involved in the control of BP in these dietary interventions. Nitrite + nitrate determination by Griess reaction is a weak surrogate measurement of blood NO concentrations and may not always provide an accurate assessment of NO. We must consider that serum nitrite concentration is a better biomarker of NO production than nitrite + nitrate concentrations. Other methods allow evaluate nitrites by more accurate and sensitive quantification [25]. Moreover, we took into account several factors as sample preparation and foodstuff to minimize possible errors. The three intervention groups had a similar consumption of meat, meat products and vegetables, which are dietary sources of nitrites and nitrates, respectively, and also similar fasting period. Considering the blood half-life of nitrite/nitrate [26], the overnight fasting period could be long enough to reduce serum nitrite/nitrate levels from diet.

Nitrites + nitrates may not reflect biologically active NO solely from endothelium. However, these measurements in combination with functional assessment such as BP will provide more information, In these sense, we have observed at baseline that SBP negatively correlates with serum NO concentrations, and that DBP negatively correlates with serum NO and NO/ET-1, and positively with ET-1 in women with moderate hypertension, in agreement with data obtained in a mice model of hypertension [27], suggesting a link between an enhanced NO bioavailability [28] and reduced ET-1 levels [29]. We have to remark that the correlations observed after the 1-year follow-up were improved (30 participants/group) when compared to those observed at baseline (90 participants), although the reduced number of participants.

Some dietary changes have the potential to decrease BP in nonhypertensive, prehypertensive and hypertensive subjects, with a subsequent reduction in the risk of complications. Our results show that after 1-year follow-up, SBP decreased and DBP increased in control group whereas both TMD interventions slightly decreased SBP and DBP in moderate hypertensive women, being significant the DBP reduction induced by nuts. Findings in agreement with Toledo et al. [14] who observed a DBP

256 decrease in both TMD interventions. The impact of these reductions, even if their magnitudes are
257 small, could be remarkable at the population level. For example, a decrease in 3 mmHg in SBP is
258 associated with reduction of 8 % in stroke mortality and 5 % in coronary heart disease mortality [30]. In
259 this sense, the PREDIMED study recently reported that TMD + EVOO or TMD + nuts reduced the
260 incidence of major cardiovascular events and BP [31]. This result can be explained, at least in part by
261 the correlation between nitrite + nitrate, and ET-1 with BP observed in the present study, which is
262 related to the polyphenol content in these dietary interventions [32].

263 Esposito et al. [33] observed that a TMD might be effective in reducing endothelial dysfunction and
264 vascular inflammation in metabolic syndrome patients, and few clinical trials have assessed the
265 beneficial effect of EVOO or nuts on endothelial dysfunction indirectly ascertained by brachial artery
266 vasodilation in hypercholesterolemic patients [34, 35].

267 Konstantinidou et al. [36] proposed that the benefits associated with a TMD consumption on
268 cardiovascular risk could be mediated though nutrigenomic effects. Our microarray study showed that
269 the TMD + EVOO intervention was associated to an increase in eNOS and consequently in NO release,
270 whereas decreased caveolin 2 gene expression. eNOS contains several putative binding motifs for
271 caveolins that result in stearic inhibition of the enzyme. Thus, in the absence of caveolins, eNOS
272 activity does not respond to negative regulatory signals and consequently NO levels remain
273 constitutively more elevated [37] that suggest a putative mechanism to explain the enhancement of NO
274 levels and the decrease of BP induced by EVOO. It is interesting to remark that TMD also
275 downregulated ET_AR and ET_BR, involved in ET-1 vasoconstriction tone [24] and in the development of
276 hypertension [5]. Considering that the net contractile effect of ET-1 depends mainly on the relative
277 density of ET_A receptors on smooth muscle cells and of ET_B receptors on endothelial cells, the down-
278 regulation of these receptors by nuts together with the impairment of serum ET-1 levels can explain, at
279 least in part, the BP reduction induced by nuts interventions.

280 The impairment of NO bioavailability by the enhancement of vascular oxidative stress plays a critical
281 role in the pathogenesis of hypertension [38]. Considering that the diets used were rich in oleic, linoleic
282 and linolenic acids, we thought interesting to study the global lipid peroxidation status. To these purpose,
283 we focus our study on serum MDA measurement by HPLC with fluorescence detection as MDA is
284 highly associated with cardiovascular risk factors [39]. However, we did not detect changes in serum
285 total antioxidant activity and MDA levels in any intervention. When studying oxidative stress it is
286 commonly accepted that several test related to different aspects of oxidative damage should be used. In
287 these sense, it was observed a decrease in other parameters related to oxidative damage such as F2

isoprostanes, generated predominantly by free radical oxidation of arachidonic acid in membrane phospholipids, and 8 oxo-7,8-dihydro-2'-deoxyguanosine, indicator of DNA damage, after 1-year intervention with both TMD in the same PREDIMED subpopulation [40]. Also, a decrease of oxidized LDL in another subpopulation of the PREDIMED study has been reported [41]. Thus, we can not exclude a beneficial modulation of oxidative stress by these interventions that need a deeper study. All of these events may be related to the effects of specific fatty acids, polyphenols, phytoestrogens or other minor components present in EVOO and/or nuts. In this way, Moreno-Luna et al. [42] reported that olive oil polyphenols decrease BP in young women with mild hypertension and we also observed that olive oil polyphenols have a protective effect on the imbalance of NO/ET-1 induced by hyperglycemia and free fatty acids [43].

Although, our findings suggest that EVOO components mainly affect NO bioavailability whereas nut components modulate ET-1 levels. We must consider that other autacoids might be involved in the effects of both interventions on BP control. Thus, Perona et al. [44] reported that EVOO improves the balance between vasoprotective (PGI_2) and prothrombotic (TxA_2) mediators synthesized by the cyclooxygenase pathway.

Current dietary guidelines emphasize on foods that improve multiple cardiovascular risk factors. The effects we observed in the present paper by the dietary interventions together with the significant reduction in LDL cholesterol and oxidized LDL observed in PREDIMED study [41], would be expected to be related to a low CVD risk. The present study also has several strengths. The adherence to diet was carefully assessed by dieticians to compare the interventions with the control. The participation of only women was very important as the mean value of many of the parameters studied differ between both sexes. Moreover, our population did not consume non-steroidal anti-inflammatory drugs that could interfere with NO/ET-1 generation. Thus, although the size of the population studied was limited its characteristics were enough homogeneous. Finally, we must consider that serum nitrite measurement and additional oxidative stress assays will improve future studies.

In conclusion, the beneficial effects of TMD supplemented with EVOO or nuts on lowering BP among hypertensive women can be partially explained by changes in serum NO/ET-1 as well as ET-1 receptors expression induced by these nutritional interventions. Further studies will be required to explore the specific components of EVOO or nuts involved in BP, NO and ET-1 modulation as well as in the underlying mechanisms.

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Conflict of interest The authors declare that they have no conflict of interest.

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Figure legends

Fig. 1 Effect of 1-year follow-up PREDIMED interventions on variations of systolic and diastolic blood pressure (SBP, DBP, respectively) (A) and variations of nitrite + nitrate and endothelin-1 concentrations (B). Values are expressed as mean ± SEM. (n = 30). *p < 0.05 versus control group (Student's t- test)

Fig. 2 Correlations between systolic and diastolic blood pressure (SBP and DBP) and NO and ET-1 concentrations.

Fig. 3 Gene expression changes after 1-year follow-up of PREDIMED interventions. Relative quantification of endothelial NO synthase (eNOS), caveolin 2 (CAV2), and endothelin-1 receptors (ET_AR and ET_BR) expressed as fold change-log2ratio. Bars are mean ± SEM. (n = 10). *p < 0.05 versus control group.

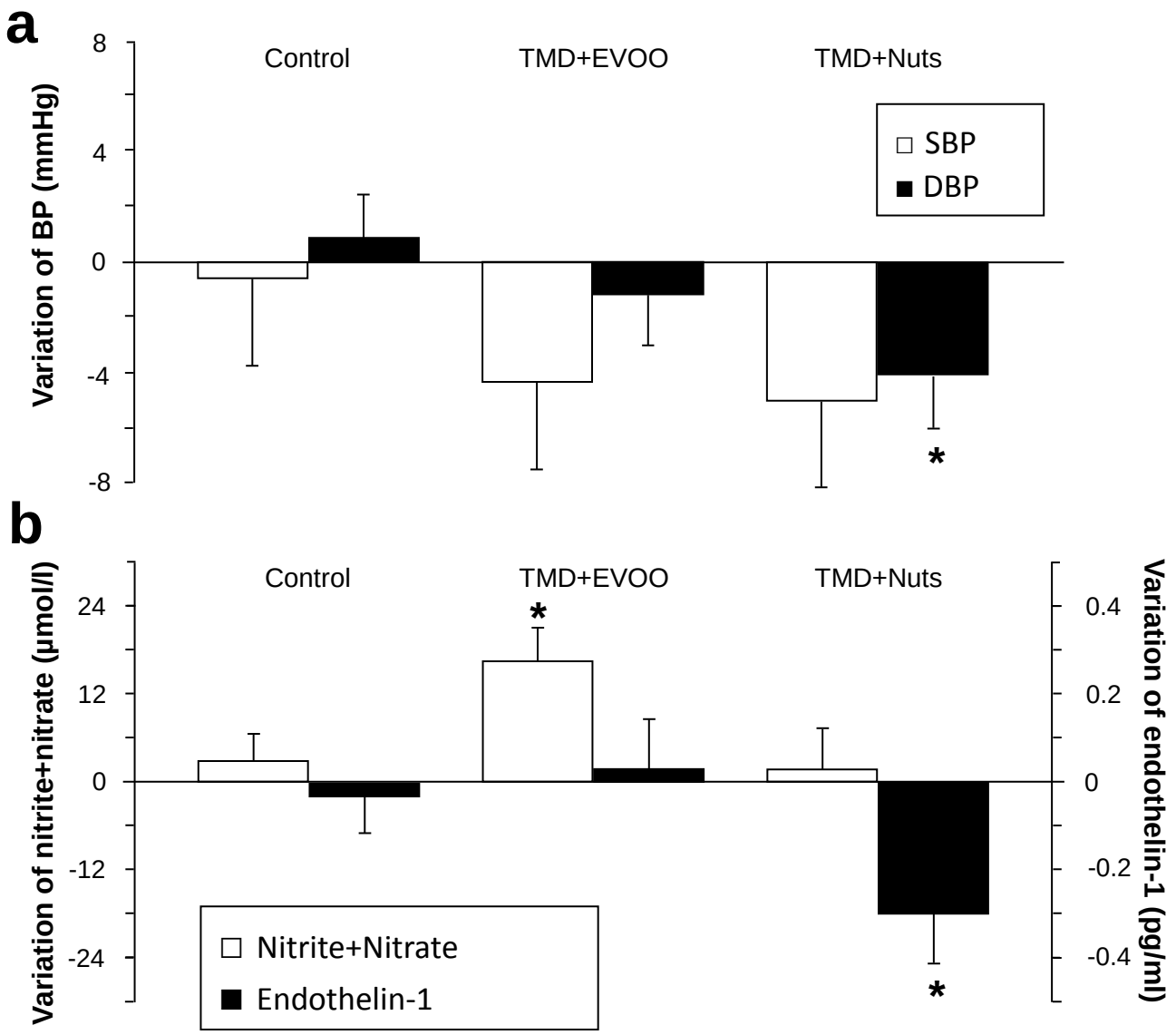
a
Control
TMD+EVOO
TMD+Nuts
Variation of BP (mmHg)
8
4
0
-4
-8
SBP
DBP
*
b
Control
TMD+EVOO
TMD+Nuts
Variation of nitrite+nitrate (µmol/l)
24
12
0
-12
-24
Variation of endothelin-1 (pg/ml)
0.4
0.2
0
-0.2
-0.4
*
*
Nitrite+Nitrate
Endothelin-1

Figure 1

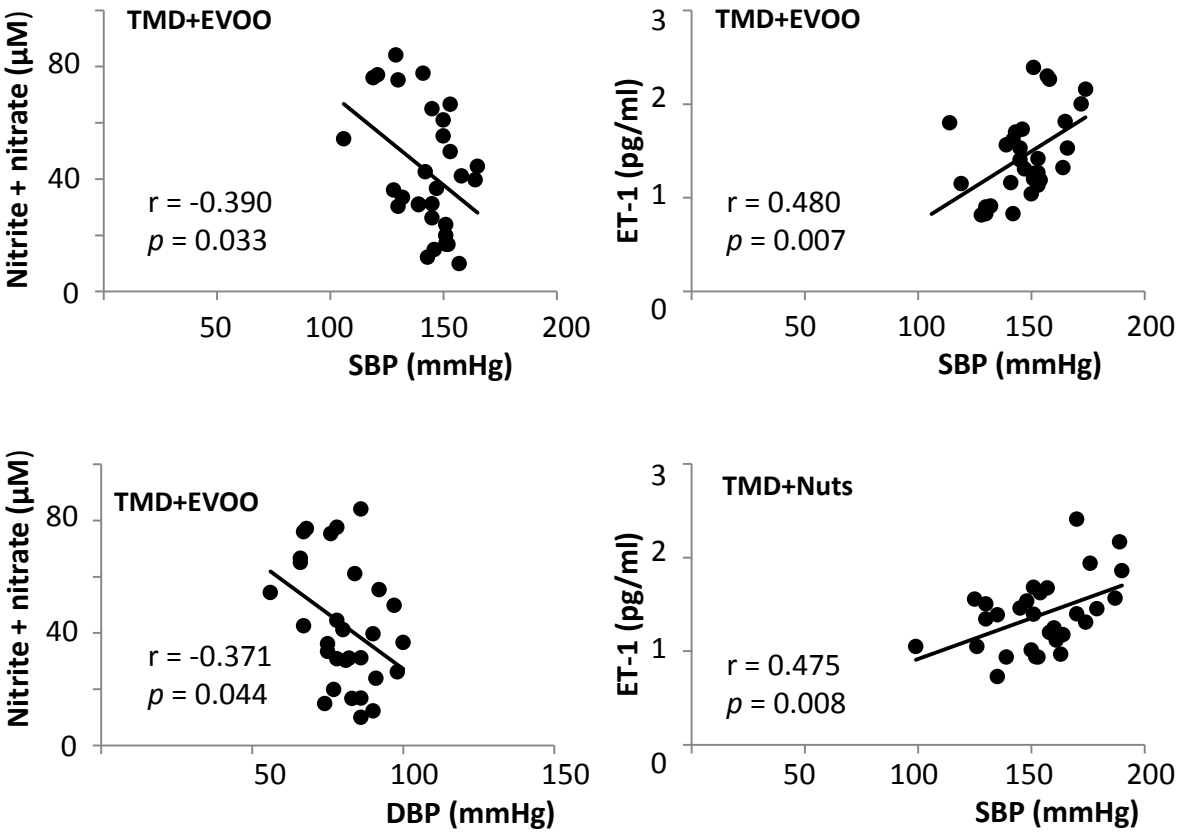
TMD+EVOO
Nitrite + nitrate (µM)
80
40
0
r = -0.390
p = 0.033
50
100
150
200
SBP (mmHg)
TMD+EVOO
ET-1 (pg/ml)
3
2
1
0
r = 0.480
p = 0.007
50
100
150
200
SBP (mmHg)
TMD+EVOO
Nitrite + nitrate (µM)
80
40
0
r = -0.371
p = 0.044
50
100
150
DBP (mmHg)
TMD+Nuts
ET-1 (pg/ml)
3
2
1
0
r = 0.475
p = 0.008
50
100
150
200
SBP (mmHg)

Figure 2

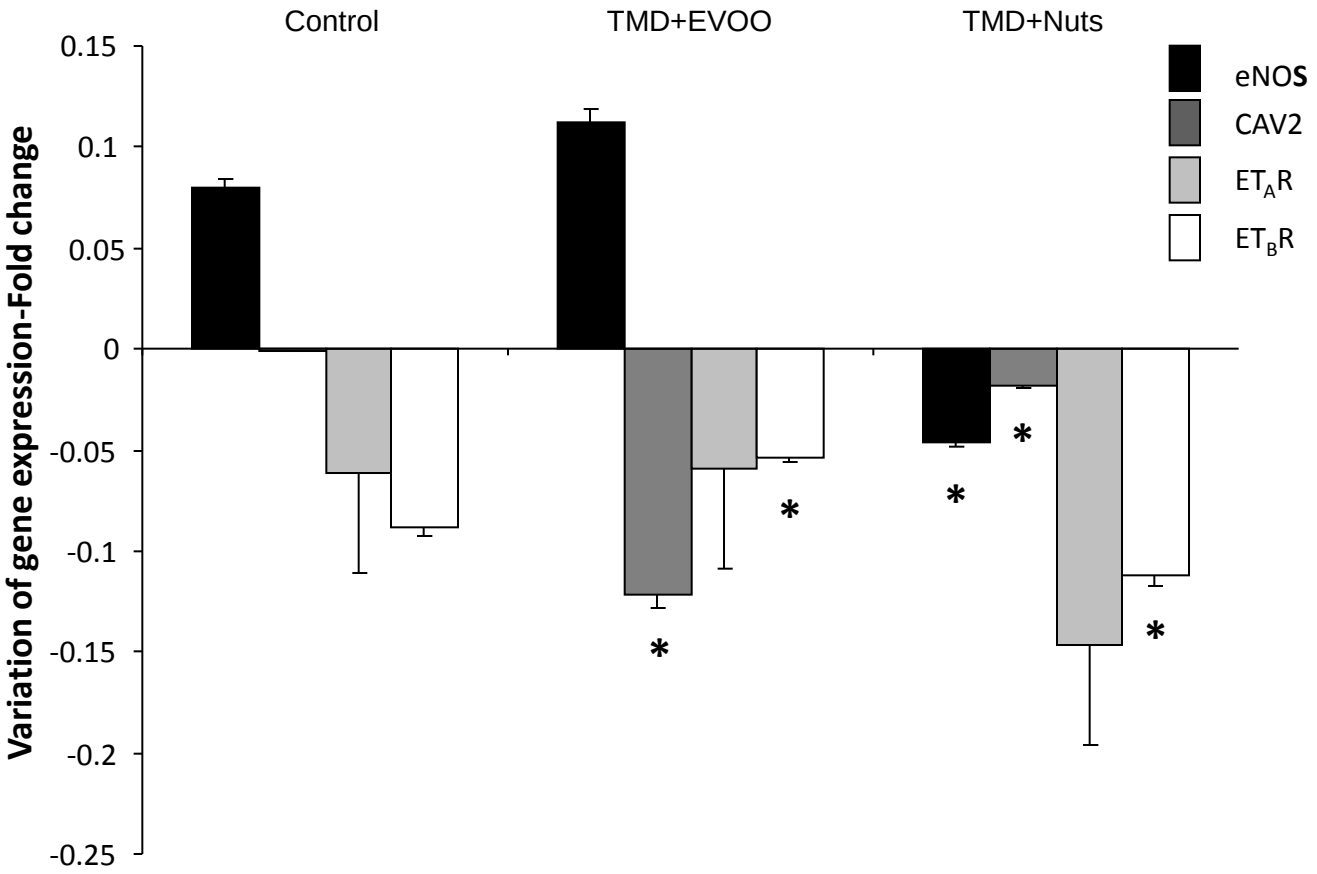
Control
TMD+EVOO
TMD+Nuts
eNOS
CAV2
ET$_A$R
ET$_B$R
Variation of gene expression-Fold change
0.15
0.1
0.05
0
-0.05
-0.1
-0.15
-0.2
-0.25
*
*
*
*
*

Figure 3

Table 1. Baseline characteristics of participants

	Control diet (n = 30)	TMD+EVOO (n = 30)	TMD+Nuts (n = 30)
Age (years)	68.1 ± 0.9	69.1 ± 1.0	68.7 ± 0.9
Body weight (kg)	74.3 ± 1.6	75.7 ± 1.8	77.5 ± 1.8
BMI (kg/m^2)	31.4 ± 0.6	31.9 ± 0.6	31.7 ± 0.6
Waist circumference (cm)	101.2 ± 1.5	102.0 ± 1.4	102.5 ± 1.5
SBP (mmHg)	158.1 ± 3.3	152.5 ± 3.9	156.7 ± 2.6
DBP (mmHg)	83.5 ± 2.1	82.1 ± 2.1	85.2 ± 1.6
Lifestyle			
TMD score	7.8 ± 0.4	8.0 ± 0.4	7.5 ± 0.5
Physical activity (METS)	157.1 ± 21.3	159.7 ± 22.9	154.0 ± 16.9
Serum glucose and lipid profile			
Glucose (mg/dl)	122.4 ± 5.2	129.0 ± 8.2	129.3 ± 8.6
Triglycerides (mg/dl)	136.2 ± 11.6	128.8 ± 9.5	143.2 ± 11.7
LDL-cholesterol (mg/dl)	129.4 ± 5.0	131.9 ± 5.2	124.9 ± 5.7
HDL-cholesterol (mg/dl)	56.1 ± 1.9	57.2 ± 2.8	55.3 ± 2.6
Nitrite+nitrate (μmol/l)	31.0 ± 3.2	27.8 ± 2.6	31.8 ± 3.1
ET-1 (pg/ml)	1.5 ± 0.1	1.5 ± 0.1	1.6 ± 0.1
Metabolic syndrome components (%)			
Abdominal obesity	94.3	97.1	94.3
Low level of HDL cholesterol	22.8	25.7	34.2
Hypertriglyceridemia or receiving treatment for same	20.0	17.1	34.2
High fasting serum glucose or drug treatment for diabetes	51.1	62.8	68.5
High BP (>140/90 mmHg) or antihypertensive treatment	97.1	94.2	100.0
Medication (%)			

Antihypertensive agents	80	77	88
Oral hypoglycemic agents	31	37	44
Insulin	9	11	12

Abbreviations: TMD, traditional Mediterranean diet; EVOO, extra virgin olive oil; SBP, systolic blood pressure; DBP, diastolic blood pressure; METS, metabolic equivalent task; BP, blood pressure; ET-1, endothelin-1. Data are means ± s.e.m. No statistical differences were observed among the three groups.

Table 2. Baseline and 1-year follow-up changes in total antioxidant capacity and malondialdehyde in serum

	TMD (n = 30)	TMD+EVOO (n = 30)	TMD+Nuts (n = 30)
TAC (mEquivalents of Trolox)			
Baseline	1.65 ± 0.11	1.84 ± 0.10	2.04 ± 0.11
Changes	-0.10 ± 0.02	0.20 ± 0.02	-0.04 ± 0.01
MDA (μM)			
Baseline	2.43 ± 0.20	2.50 ± 0.19	2.51 ± 0.21
Changes	-0.17 ± 0.02	0.01 ± 0.01	0.10 ± 0.01

Abbreviations: TAC, total antioxidant capacity; MDA, malondialdehyde; TMD, traditional Mediterranean diet; EVOO, extra virgin olive oil. Data are means ± s.e.m. No statistical differences were observed among the three groups.