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Article *in* American Journal of Hypertension · August 2012

DOI: 10.1038/ajh.2012.128 · Source: PubMed

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Olive Oil Polyphenols Decrease Blood Pressure and Improve Endothelial Function in Young Women with Mild Hypertension

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BACKGROUND

Olive oil polyphenols have been associated with several cardiovascular health benefits. This study aims to examine the influence of a polyphenol-rich olive oil on blood pressure (BP) and endothelial function in 24 young women with high-normal BP or stage 1 essential hypertension.

METHODS

We conducted a double-blind, randomized, crossover dietary-intervention study. After a run-in period of 4 months (baseline values), two diets were used, one with polyphenol-rich olive oil (~30 mg/day), the other with polyphenol-free olive oil. Each dietary period lasted 2 months with a 4-week washout between diets. Systolic and diastolic BP, serum or plasma biomarkers of endothelial function, oxidative stress, and inflammation, and ischemia-induced hyperemia in the forearm were measured.

RESULTS

When compared to baseline values, only the polyphenol-rich olive oil diet led to a significant ($P < 0.01$) decrease of 7.91 mm Hg in systolic and 6.65 mm Hg of diastolic BP. A similar finding was found for serum asymmetric dimethylarginine (ADMA) (−0.09 ± 0.01 µmol/l, $P < 0.01$), oxidized low-density lipoprotein (ox-LDL) (−28.2 ± 28.5 µg/l, $P < 0.01$), and plasma C-reactive protein (CRP) (−1.9 ± 1.3 mg/l, $P < 0.001$). The polyphenol-rich olive oil diet also elicited an increase in plasma nitrites/nitrates (+4.7 ± 6.6 µmol/l, $P < 0.001$) and hyperemic area after ischemia (+345 ± 386 perfusion units (PU)/sec, $P < 0.001$).

CONCLUSIONS

We concluded that the consumption of a diet containing polyphenol-rich olive oil can decrease BP and improve endothelial function in young women with high-normal BP or stage 1 essential hypertension.

Keywords: *blood pressure; endothelial dysfunction; endothelial function biomarkers; essential hypertension; hypertension; ischemia-reactive hyperemia (IRH); olive oil polyphenols*

American Journal of Hypertension, advance online publication 23 August 2012; doi:10.1038/ajh.2012.128

Endothelial dysfunction is one of the phenomena associated with the development of atherosclerosis and cardiovascular disease in patients with hypertension.[1] It is mainly characterized by decreased bioavailability of nitric oxide (NO)[2] and increased levels of oxidized-low-density lipoprotein (ox-LDL).[3] One of the predominant mechanisms in NO inactivation is a perturbation of the L-arginine–NO pathway by oxidative stress leading to elevations of plasma asymmetric dimethylarginine (ADMA), which in turn exacerbates oxidative stress.[4] Decreases in the activity of dimethylarginine dimethylaminohydrolases by reactive oxygen species may also promote intracellular ADMA accumulation and contribute to ADMA export.[5] Both a pro-oxidant status and increased ADMA are common features of disease states associated with atherosclerosis[4,6] and hypertension.[7]

Food intake is an important factor affecting vascular reactivity. Short-term feeding trials have shown the potential for certain foods to improve endothelial function, either as isolated nutrients or as healthy food patterns.[8] Several studies suggest that some antioxidant compounds in food may limit oxidative damage and restore endothelial function, thus slowing atherogenic development.[9] Polyphenol intake has been associated with low coronary heart disease mortality rates.[10] Prior studies have reported that antioxidant and anti-inflammatory polyphenols in the diet improve endothelial function and lipid profile.[11] The minor components in virgin olive oil, particularly the phenolic compounds, may contribute to the health benefits derived from the Mediterranean diet. In experimental studies, the phenolic compounds of virgin olive oil showed strong antioxidant properties.[12,13]

Although there are a large number of studies linking the consumption of phenolic compounds with a decrease in vascular events, recent evidence suggests that the effects of food

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Received 9 January 2012; first decision 19 March 2012; accepted 2 July 2012.

vary according to gender[14] and that women are underrepresented in biomedical studies.[15] Moreover, most of the studies on healthy properties of polyphenols were conducted on high-risk populations, thus underestimating the potential benefits of polyphenols on low-risk populations.[16]

In this study, we enrolled young women with high-normal blood pressure (BP) or stage 1 essential hypertension and hypothesized that consumption of polyphenol-rich olive oil would decrease BP and improve endothelial function. Additionally, we examined which variables correlate with BP values and endothelial function biomarkers.

METHODS

Patients and design. Prior to the study, the Human Investigation Review Committee of the Virgen del Rocio University Hospital approved all protocols, and all participants provided written informed consent. The study was conducted according to the guidelines of good clinical practice and principles expressed in the Helsinki Declaration by the World Medical Association.

The study was conducted as double-blind, randomized, crossover. For randomization, we used a random number generation method. The sampling methods was of cases-consecutives. We consecutively asked to enter the study to forty Caucasian women that were newly diagnosed with high-normal BP ($n = 11$) (a systolic pressure of 120–139 mm Hg or diastolic pressure of 80–89 mm Hg) or stage 1 essential hypertension ($n = 13$) (a systolic pressure of 140–159 mm Hg and diastolic pressure of 90–99 mm Hg). Six women refused to do so, and ten more abandoned after the first dietary intervention because of protocol violation (6), intolerance to the oils (3), or change of address (1). Therefore, 24 women completed the study. Their average age was 26 years (range: 24–27 years) and body mass index was 25.4 kg/m^2 (range: 23.5–27.1 kg/m^2). They had values of plasma TC between 3.88–4.14 mmol/l and TG between 0.51–0.56 mmol/l. All participants completed a comprehensive health-related questionnaire that included lifestyle information (i.e., physical activity, tobacco, alcohol, tea, and coffee consumption, and dietary habits), medical and family history (especially those related to premature cardiovascular disease), and the use of medications, nutritional supplements, and vitamins. The exclusion criteria included previous history of cardiovascular disease, any severe chronic illness, treatment with antihypertensive or lipid-lowering drugs. Smokers or ex-smokers were also excluded.

Before randomization and the crossover, the participants were subjected to a run-in period of 4 months to homogenize in the consumption of a Mediterranean-style diet;[17] a set menu plan, which contained the same calories to the habitual diets, was provided to the participants. The duration of this period was to ensure adequate experience in protocol adherence. Afterwards, women who met inclusion criteria were randomly allocated to the aforementioned Mediterranean-style diet, but with polyphenol-rich olive oil or polyphenol-free olive oil for 2-month period followed after a 4-week washout period by the second dietary 2-month period of the alternate diet. The diet in the washout period was equal to the run-in period. Participants were instructed to avoid the adherence to any special foods or supplements affecting BP, lipid metabolism or highly rich in polyphenols. The only nutritional difference between the run-in/washout period and the intervention periods was the type of dietary fat. Sunflower or corn oil was permitted for the run-in/washout period, whereas a virgin olive oil rich in polyphenols and the same oil without polyphenols (refined olive oil) were provided for the study diets, respectively. During each dietary-intervention period, participants consumed 60 ml of the corresponding dietary fat daily. This study design ensured that each participant consumed comparable energy from dietary fats and amounts of polyphenols from virgin olive oil on the polyphenol-rich olive oil diet. Despite the investigators were aware of which diet the participants received, we do not rule out the possibility that a participant could recognize the taste of virgin olive oil. Participants were required to complete daily food records, which were used to analyze their nutrient intake and compliance. The aim was to minimize metabolic perturbations between test diets focusing on comparison of polyphenol-rich olive oil versus polyphenol-free olive oil. More than 20 different virgin olive oils were analyzed for the polyphenol content, which was measured by reverse-phase high-performance liquid chromatography-diode array detection as previously described.[18] Simple phenols such as hydroxytyrosol, tyrosol, and vanillic acid were found in most of these oils. We selected a virgin olive oil containing 564 mg/kg polyphenols. Therefore, participants consumed ~30 mg/day of polyphenols from olive oil during the period on the polyphenol-rich olive oil diet. A portion of this virgin olive oil underwent oil refining at the Instituto de la Grasa (CSIC, Seville) to produce a polyphenol-free olive oil.[19] Participants were asked to maintain their usual levels of exercise for the duration of the study.

Data on brachial BP and ischemia-reactive hyperemia (IRH), and blood samples were obtained after the run-in period (baseline values) and after the polyphenol-rich and polyphenol-free olive oil diets.

BP measurement. Brachial systolic and diastolic BP was measured using an automated oscillometric device (Omron M6 Comfort; Omron Healthcare, Amsterdam, Netherlands) in the right arm, with participants lying in the supine position for 10 min by a trained observer. Three BP readings were taken at 2-min interval, and the mean was used for data analysis.

Assessment of endothelial function, oxidative stress, and inflammation biomarkers. Participants were required to fast and avoid heavy exercise 12 h before the morning of the examination. Plasma and serum were obtained from blood samples, stored at −80 °C, and analyzed at the Clinic Laboratory Service of University Hospital Virgen del Rocio. Details on analytical methods for each biomarker are provided below. NO via total plasma nitrites/nitrates was measured by a colorimetric assay (NO, Colorimetric Assay; Roche Applied Science, Indianapolis, IN) using a modular analyzer Power Wave XS (Biotek, Winooski, UT). Serum endogenous ADMA (ADMA-ELISA; DLD Diagnostika, Hamburg, Germany), ox-LDL (Human Ox-LDL ELISA Kit; Biomedica Medizinprodukte, Vienna, Austria), and plasma high-sensitivity C-reactive protein (CRP) (CRP ELISA Kit; Immundiagnostik AG, San Diego, CA) were measured with enzyme immunoassays.

IRH measurement. Laser-Doppler linear Periflux System 5000 (Perimed SA, Järfälla, Sweden) was used to measure IRH. The participant was taken into a quiet room at our Day Hospital with only the researcher and a nurse present. The room temperature was maintained at 22 °C. The technique and possible symptoms were explained in detail. With the participant in the supine position and after a 15-min resting, the BP cuff was placed on the patient's arm, and the receptor probe was attached on the forearm at 15 cm from the wrist. The BP cuff was then inflated to 40 mm Hg above the systolic BP and maintained at this pressure for 4 min. During this period, the monitoring system showed how perfusion units (PU) fell steadily to reach the biological zero. Afterwards, the BP cuff was rapidly deflated and how quickly PU rose above the pre-ischemic PU values was monitored. The data were recorded and stored using PeriSoft for Windows. The values of hyperemic response (HA) after the ischemia were automatically calculated. The same researcher to avoid variability performed all measurements.

Statistical analysis. We compared the changes after the polyphenol-rich and polyphenol-free olive oil diets with respect to the run-in period (baseline values) using Student's t-test for paired samples. The Kolmogorov–Smirnov test was used to assess normality of distributions. When samples were not normally distributed, a Wilcoxon paired rank test was used. Pearson correlations were computed to explore the linear relationship between variables at baseline and changes relative to baseline values in response to dietary intervention. Differences were considered to be significant when $P < 0.05$. Data are expressed as mean ± SD. All calculations were performed with the SPSS 15.0 software for Windows (SPSS, Chicago, IL).

RESULTS

The participants reported 100% compliance in terms of polyphenol-rich and polyphenol-free olive oil intake. Neither body weight, nor other demographic and clinical characteristics change over the periods on different diets.

Response in BP

Systolic and diastolic BP decreased after the polyphenol-rich olive oil, but were not affected after the polyphenol-free olive oil diet compared with baseline values after the run-in period (Table 1). Only after the polyphenol-rich olive oil diet, all of the participants had a systolic BP of 140 mm Hg or less, and 22 of 24 participants had a diastolic BP of 90 mm Hg or less.

Response in endothelial function markers

Compared with baseline values, plasma nitrites/nitrates were significantly increased and serum or plasma levels of ADMA, ox-LDL, and CRP were significantly decreased after the polyphenol-rich olive oil diet (Table 1). Changes in these endothelial function, oxidative stress, and inflammation biomarkers did not differ between the baseline values and those after the polyphenol-free olive oil diet. Plasma nitrites/nitrates was the unique variable non-normally distributed and values expressed as median (interquartile range) were as follows: 19.4 (21.6–17.7) μmol/l at baseline, 24.3 (25.6–22.6) μmol/l after the polyphenol-rich diet ($P = 0.002$), and 20.1 (23.0–18.5) μmol/l after the polyphenol-free olive oil diet ($P = 0.54$).

Response in physiological test for endothelial function

The total HA after 4-min arterial occlusions was increased after the polyphenol-rich olive oil diet, but was not significantly affected after the polyphenol-free olive oil diet compared with baseline values after the run-in period (Table 1).

Predictive factors for outcome after polyphenol-rich olive oil diet

The decreases in systolic and diastolic BP, serum ADMA, ox-LDL, and plasma CRP and the increases in plasma nitrites/nitrates and HA after the polyphenol-rich olive oil diet were not related to age or body mass index. However, BP changes were strongly related to BP values at baseline, indicating that participants with higher BP at baseline were the participants with higher decreases in BP after the polyphenol-rich olive oil

Table 1 | Endothelial function, oxidative stress, and inflammation biomarkers in young women with high-normal BP or stage 1 essential hypertension after 4 months on a Mediterranean-style diet (run-in period) and changes after 2 months on the polyphenol-rich or the polyphenol-free olive oil diets

		Changes from baseline		*P* value*
Biomarker	Baseline	Polyphenol-rich olive oil	Polyphenol-free olive oil	
Nitrites/nitrates (μmol/l)	19.7 ± 2.6	+4.7 ± 6.6	+0.8 ± 4.1	<0.001
ADMA (μmol/l)	0.82 ± 0.04	−0.09 ± 0.01	−0.04 ± 0.03	<0.01
Ox-LDL (μg/l)	153.0 ± 51.0	−28.2 ± 28.5	−6.9 ± 22.2	<0.01
CRP (mg/l)	1.6 ± 0.9	−1.9 ± 1.3	−0.6 ± 0.9	<0.001
Blood pressure (mm Hg)				
Systolic	134.14 ± 9.32	−7.91 ± 9.51	−1.65 ± 8.22	<0.001
Diastolic	84.64 ± 8.52	−6.65 ± 6.63	−2.17 ± 7.24	<0.001
IRH measurement (PU)				
HA	1,084 ± 266	+345 ± 386	+36 ± 367	<0.001

Table values are mean ± SD, ***n*** = 24.
ADMA, asymmetric dimethylarginine; BP, blood pressure; CRP, C-reactive protein; HA, hyperemic area; IRH, ischemia-reactive hyperemia; ox-LDL, oxidized low-density lipoprotein; PU, perfusion units.
**P* value for the comparison across the intervention groups by ANOVA.

diet (Figure 1). Similarly, changes in plasma nitrites/nitrates, serum ADMA, ox-LDL, and plasma CRP, and HA after the polyphenol-rich olive oil diet were strongly related to values at baseline (Figure 2a–e).

DISCUSSION

The present study in 24 young women with high-normal BP or stage 1 essential hypertension investigated the potential of olive oil polyphenols to decrease BP and improve endothelial function in a single-blind, randomized, crossover setting. Our study was conducted comparing two olive oils; a polyphenol-rich olive oil and the same one underwent oil refining (polyphenol-free olive oil). The strength of this study is its design and that compliance with consuming test diets, including the dose of olive oil polyphenols during the polyphenol-rich olive oil diet, was high. This enabled us to isolate the effects of olive oil polyphenols without complications of additional nutrient differences. One of the most relevant findings was a marked decrease in systolic and diastolic BP after 2 months on the polyphenol-rich olive oil diet. For the first time, we show that a daily low amount of polyphenols in olive oil had similar BP-lowering effects in early forms of high BP to those obtained by commonly-prescribed first-line drugs for established high BP,[20] such as thiazides of 6 mm Hg, beta-blockers of 5 mm Hg, or angiotensin converting enzyme inhibitors of 8 mm Hg for systolic BP, and calcium-channel blockers of 6 mm Hg or angiotensin II receptor blockers of 10 mm Hg for diastolic BP. These findings agree with our previous study on the effects of virgin olive oil in lowering BP in hypertensive women when compared to high-oleic sunflower oil[21] and have implications at a population level, because reductions of 5 mm Hg in systolic BP and 3 mm Hg in diastolic BP have been shown to predict reduced cardiovascular morbidity and mortality risk up to 20%.[22] Recent human intervention studies also reported significant BP reductions after diets supplemented with polyphenols from orange juice in moderately overweight men[23] and blueberries in obese men and women with metabolic syndrome.[24] However, while polyphenol-rich dark chocolate had BP-lowering effects in healthy overweight and obese subjects,[25] these effects were not apparent in a population with BP in the prehypertensive range.[26]

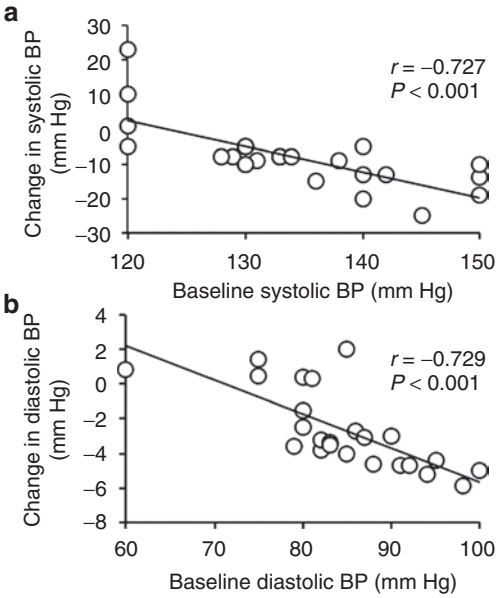

Figure 1 | Changes in (**a**) systolic and (**b**) diastolic blood pressure (BP) relative to baseline values in young women with high-normal BP or stage 1 essential hypertension after 2 months on the polyphenol-rich olive oil diet. The line is the best-fit line, $n = 24$.

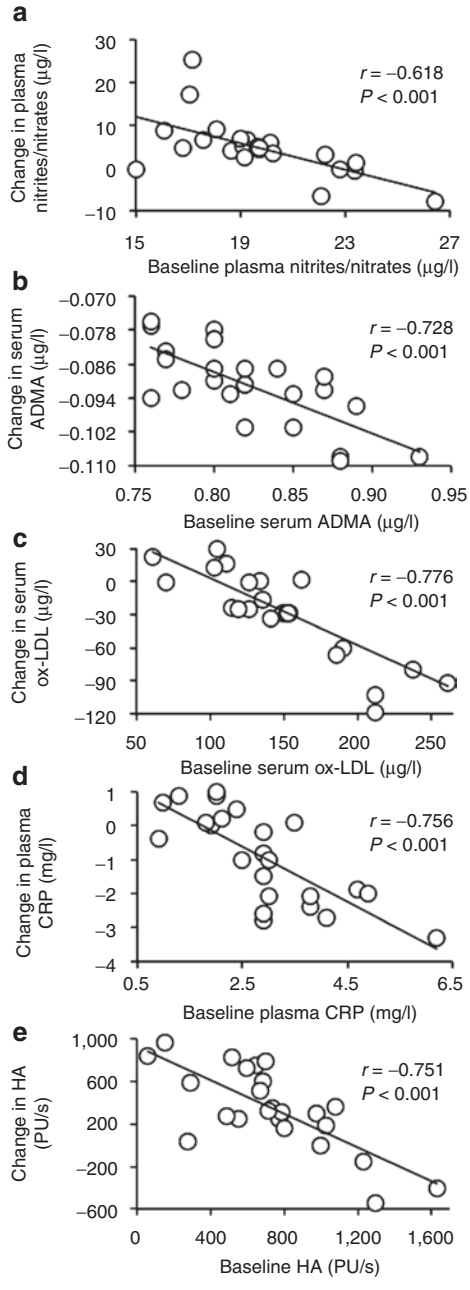

Figure 2 | Changes in (**a**) plasma nitrites/nitrates, (**b**) serum ADMA, (**c**) serum ox-LDL, (**d**) plasma CRP, and (**e**) HA relative to baseline values in young women with high-normal blood pressure (BP) or stage 1 essential hypertension after 2 months on the polyphenol-rich olive oil diet. The line is the best-fit line, $n = 24$. ADMA, asymmetric dimethylarginine; CRP, C-reactive protein; HA, hyperemic area; ox-LDL, oxidized low-density lipoprotein.

We found that the polyphenol-rich olive oil diet increased plasma nitrites/nitrates and decreased serum ADMA. Nitrites and nitrates are NO metabolites and the nitrites/nitrates ratio results from the stepwise reduction of nitrate to nitrite to NO.[27] The mechanism by which plasma levels of nitrites/nitrates increase is not well-known, but NO synthase and diet are the two major sources of nitrates in plasma. It might be hypothesized that polyphenols-rich diet improves endothelial function, thus stimulating NO synthase system and increasing plasma nitrites/nitrates. On the other hand, dietary intake of nitrates can increase systemic levels of nitrates dramatically, and vegetables are by all means the dominant source of nitrates in diet. In this respect, the traditional Japanese diet is naturally very rich in nitrate, and this is reflected in an increased levels of nitrites and nitrates in plasma, and also in a BP reduction when comparing with the control diet.[28] To our knowledge, the effect of polyphenols on plasma nitrites/nitrates concentrations has not yet been studied. Therefore, our data supporting that during diet supplementation with polyphenol-rich olive oil, plasma nitrites/nitrates increased and BP decreased, may be of interest (for review, see ref. [29]).

ADMA is an endogenously produced molecule that inhibits NO synthesis.[30] Therefore, our data conforms to the mechanistic studies linking NO and hypertension[31] and shows that olive oil polyphenols could ameliorate BP levels by improving the bioavailability of NO.

The state of hypertension is often associated with increased vascular oxidative stress and inflammation. For example, it is known that ox-LDL activates renin–angiotensin system and angiotensin II via its type 1 receptor activates ox-LDL receptor LOX-1.[32] CRP is also implicated in inducing endothelial dysfunction by a decline in NO synthase mRNA stability and uncoupling[33] and is considered a novel ligand for LOX-1.[34] After the polyphenol-rich olive oil diet, we observed a decrease in serum ox-LDL and plasma CRP. Most likely, the antioxidant potency of olive oil polyphenols,[35] mainly hydroxytyrosol and derivatives thereof, would be responsible for lowering lipoprotein oxidation. In humans, polyphenol metabolites from olive oil polyphenols can be incorporated into LDL.[36] Many *in vivo* and *in vitro* studies have suggested that phenolic groups in polyphenols can form relatively stable phenoxyl radicals, thereby disrupting chain oxidation reactions.[37] While this evidence of direct antioxidant effects of polyphenols remains to be firmly established,[38] our study also suggests anti-inflammatory effects for the polyphenol-rich olive oil diet, which links with recent findings of direct correlation between levels of ox-LDL and CRP.[39] Several molecular pathways that converge in vascular inflammation have been shown affected by olive oil polyphenols, including the arachidonic acid pathway and nuclear factor-κB.[40] These prototypical biological properties of olive oil polyphenols would contribute to an improvement in vasodilatation, as we observed by the increase in HA in response to arterial ischemia after the polyphenol-rich olive oil diet, which is consistent with previous studies in healthy subjects[41] and hypercholesterolemic patients.[42] Therefore, at least part of the mechanism by which olive oil polyphenols reduce BP includes an amelioration of endothelial function, a suppression of oxidative stress and inflammation, and thereby a restoring of vascular reactivity.

We found that BP-lowering effects of the polyphenol-rich olive oil diet were greater in participants with higher BP at baseline. This may be of interest as we selected participants having a low cardiovascular risk (young women with high-normal BP or stage 1 hypertension) and it is conceivable that BP-lowering effects mediated by olive oil polyphenols could be even more pronounced in severe hypertensive patients. After the polyphenol-rich olive oil diet, reductions in the levels of nitrites/nitrates, ADMA, ox-LDL, and CRP were also correlated with starting serum or plasma values, thus the highest benefit was observed in participants with the worst baseline values. Identical trend was observed for changes in HA relative to baseline values after the polyphenol-rich olive oil diet. These observations are probably due to physiological control of the response to olive oil polyphenols rather than to regression to the mean, because similar observations did not occur with another diet that was not the polyphenol-rich olive oil diet. Furthermore, our data suggest that values for baseline BP, nitrites/nitrates, ADMA, ox-LDL, CRP, and HA could be predictive indicators for the degree of change in response to olive oil polyphenols.

A possible limitation of the study is that the beneficial effect obtained in a group of young women with low cardiovascular risk score, might be meaningless in other groups of risk. The strong taste of the virgin olive oil may also limit its consumption in other nonselected populations. In addition, the small sample size ($n = 24$) is also a limitation of our study.

In conclusion, we report that the consumption of a diet containing polyphenol-rich olive oil can significantly decrease BP and improve endothelial function in young women with high-normal BP or stage 1 essential hypertension. Our data also support the notion that benefits of olive oil polyphenols on BP and endothelial function would be most prominent on a background of hypertension and endothelial dysfunction.

Acknowledgments: This work was supported by CITOLIVA Foundation, Instituto de Salud Carlos III (RD06/0014/0035), and Junta de Andalucía (CVI-4352) grants.

Disclosure: The authors declared no conflict of interest.

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