Pomace Olive Oil Improves Endothelial Function in Spontaneously Hypertensive Rats by Increasing Endothelial Nitric Oxide Synthase Expression

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Background: The effect of dietary pomace olive oil, which has the same concentration of oleic acid but a higher proportion of oleanolic acid (OA) than olive oil, was examined on animal models of hypertension for the first time.

Methods: During 12 weeks, Wistar Kyoto (WKY) and spontaneously hypertensive rats (SHR) were fed with either a control 2% corn oil diet (BD), or high-fat diets containing 15% of refined olive oil (OL), pomace olive oil (POM), or pomace olive oil supplemented in OA (up to 800 ppm) (POMO). Then, vascular reactivity and endothelial nitric oxide (NO) synthase (eNOS) expression were studied in aortic rings. Plasma nitrite + nitrate levels were also determined.

Results: Diets had no effects on blood pressure (BP). In contrast to the BD and OL dietary groups, POM intake improved relaxation evoked by acetylcholine in SHR aorta. The POMO intake increased vasodilatation to acetylcholine and attenuated phenylephrine-induced contractions in both strains of rats associated with a major NO participation revealed by inhibition of NOS. The enhanced relaxation shown in POM and POMO SHR aorta was attributed to an increased eNOS protein expression. Plasma nitrite levels were also increased in these groups. Although olive and pomace oils used in diets contained similar fatty acid composition, beneficial effects on endothelial function were absent in the OL group. Therefore, these effects must be associated with some minor components from pomace olive oil such as OA.

Conclusions: Chronic intake of diets rich in pomace olive oil improves endothelial dysfunction in SHR aorta by mechanisms associated with enhanced eNOS expression. Important evidence is provided regarding the effects of pomace olive oil and OA on endothelial function in hypertensive animals. Am J Hypertens 2007;20:728–734 © 2007 American Journal of Hypertension, Ltd.

Key Words: Pomace olive oil, spontaneously hypertensive rat, nitric oxide, oleanolic acid, endothelial function.

Major cardiovascular risk factors such as hypertension and diabetes have been found to occur with endothelial dysfunction. In these circumstances, there is an imbalance in the production of relaxing and contracting substances from the endothelium and thus endothelium-dependent vasodilatation is impaired, as manifested in the aorta of hypertensive rats.[1] Epidemiologic studies focused on dietary patterns from Mediterranean countries have demonstrated that olive oil intake decreases the incidence of cardiovascular disease.[2–4] The healthy effects of olive oil on cardiovascular risk factors have been partly attributed to its high content in monounsaturated fatty acids such as oleic acid.[5–7] Nevertheless, several studies propose that the content of oleic acid alone cannot fully explain its healthful properties. In this regard, olive oil contains a variety of minor constituents, such as tocopherols and triterpenoids, that have been related to antiinflammatory, antioxidant, and vasodilator properties.[8–12] Therefore, although they are in low proportion, they confer important biological activities to olive oil.

Pomace or orujo olive oil, as it is called in Spain, is obtained from the residue that remains after virgin olive oil mechanical extraction. As a consequence of the chemical processes needed to obtain both refined olive and pomace olive oils, the hydrosoluble fraction, including phenolic compounds, is lost. In spite of the lack of polyphenols, pomace olive oil contains higher concentrations of triterpenoids than olive oil.[13] Oleanolic acid (OA), one of the main triterpenoids in pomace olive oil, has been associated with a wide range of biological activities (for review, see Herrera et al[14]). Recent research has demonstrated that chronic administration of OA prevents hypertension in salt-sensitive rats.[15] Furthermore, OA is able to induce in vitro endothelium- and nitric oxide (NO)–dependent vasorelaxation of the aorta from normotensive[10] and hypertensive rats.[11] In addition, it has been reported that chronic ingestion of pomace olive oil with a high proportion of OA attenuates lipid peroxidation in rat liver microsomes.[16] Nevertheless, the effects of pomace olive oil intake on endothelial dysfunction due to hypertension have not been analyzed. Therefore, the purpose of this study was to evaluate the preventive effects of long-term intake of pomace olive oil in the development of hypertension and endothelial dysfunction in spontaneously hypertensive rats (SHR) in comparison with its normotensive control, Wistar Kyoto (WKY) rats. In addition, we examined the effects of OA supplementation to pomace olive oil in the parameters evaluated. Finally, we tried to identify the role of NO and endothelial NO synthase (eNOS) expression levels in the protection against endothelial dysfunction after a diet enriched with pomace olive oil with or without additional OA. The involvement of prostanoids derived from the endothelium was also evaluated.

Methods
Animals and Diet Preparation

All experiments were approved by the Institutional Committee on Investigation in Animals (University of Seville, Seville, Spain). Three-week-old male WKY and SHR (Charles River, Barcelona, Spain) were housed at constant temperature ($24° \pm 2°C$) and a 12-h light–dark cycle. During 12 weeks, animals were fed either a control low-fat diet (basal diet, BD) containing 2% (w/w) corn oil, or high-fat diets containing 15% (w/w) refined olive oil, pomace olive oil (POM), and pomace olive oil supplemented with OA up to 800 ppm (POMO). The original POM contains ~200 ppm of OA. Food components were purchased from Musal & Chemical (Granada, Spain) and ICN Nutritional Biochemicals (Cleveland, OH). Olive and pomace oils were provided from Oleotejar (Cordoba, Spain) and corn oil from Koipe (Jaen, Spain). Oleanolic acid was obtained from olive leaves[17] and diets were prepared as described.[18] The fatty (Table 1) and nonfatty acid composition of the oils was analyzed by gas chromatography in Oleotejar. Food intake, corporal weight, heart rate, and systolic blood pressure (BP) of the animals were

Table 1. Fatty acid composition of the oils (g/100 g total fatty acid)

Component	Corn oil*	Olive oil†	Pomace olive oil†²
Myristic (14:0)	0.01	0.02	0.02
Palmitic (16:0)	10.36	10.98	10.29
Palmitoleic (16:1, n-7)	0.12	0.82	0.76
Stearic (18:0)	2.36	3.53	2.95
Oleic (18:1, n-9)	33.05	77.80	74.27
Linoleic (18:2, n-6)	50.89	4.52	8.07
α-Linolenic (18:3, n-3)	0.71	0.62	0.70
Arachidic (20:0)	n.d.	0.42	0.45

n.d. = not detected.
* Corn oil included in a 2% basal diet (BD); † Olive oil (OL diet) and pomace olive oil (POM and POMO diets) are both refined oils.

determined every week. The latter measurements were performed in conscious rats by tail–cuff plethysmography (Niprem 645; Cibertec, Spain). At the end of this period, animals were anesthetized with pentobarbitone (60 mg/kg) and blood was collected by intracardiac puncture.

Arterial Preparation and Vascular Reactivity Studies

Aortic segments were mounted as described.[10] The presence of functional endothelium was determined by the ability of 1 μmol/L acetylcholine (ACh) to induce relaxation of vessels precontracted with 1 μmol/L phenylephrine (Phe). In some arteries, endothelium was removed by gently rubbing the inner surface.

The first series of experiments included the responses evoked by cumulative addition of Phe (1 nmol/L to 100 μmol/L) for aortic rings with or without endothelium and expressed as the percentage of 80 mmol/L KCl-induced contraction. In the second series of experiments, endothelial function was evaluated by adding cumulative concentrations of ACh (1 nmol/L to 10 μmol/L) to arteries precontracted with Phe (0.3 and 1 μmol/L for WKY and SHR, respectively). To evaluate the involvement of NO, curves were performed in the presence of the NO synthase inhibitor N^ω-nitro-L-arginine (L-NAME 300 μmol/L). The ACh-evoked relaxation was also tested in the presence of indomethacin (10 μmol/L) to evaluate the implication of prostanoids derived from cyclooxygenase (COX).

eNOS Detection by Western Blotting

Aortic rings were homogenized in lysis buffer and 30 μg of protein were loaded into 7% sodium dodecyl sulfate (SDS)–polyacrylamide gel to separate eNOS.[19] Immunoblotting was achieved using a specific monoclonal mouse anti-eNOS (1:2500; BD Transduction Laboratories, San Diego, CA) and then with the secondary peroxidase-conjugated antibody (1:5000; Sigma Chemical Co., St. Louis, MO). The blots were detected by chemiluminiscence

(Pierce Chemical Company, Rockford, IL) and evaluated by densitometry. The sample loading was verified by staining membranes with Ponceau red and by immunostaining with monoclonal mouse anti-α-actin (1:5000; Sigma).

Plasma Nitrite Determination

Plasma samples were incubated with nitrate reductase to reduce nitrates to nitrites and final concentration (NOx) was determined by adding Griess reagent to the sample and measuring the absorbance at 540 nm.[20] The NOx concentrations were expressed as μmol/L and calculated using a standard curve of nitrite.

Calculations and Statistical Evaluation

Relaxations were expressed as a percentage from the initial contraction level and as mean $\pm$ SEM for n experiments. The maximum contraction (Emax values) and the concentration of the agonist producing 50% of maximum vasoconstrictor response expressed as negative log molar (pD2 = $-\log EC_{50}$) were calculated by nonlinear regression analysis of each individual concentration–response curve using GraphPad Prism Software 3.0 (San Diego, CA). Data were analyzed using one-way analysis of variance (ANOVA) followed by Fisher's multiple comparison test. Differences were considered significant when $P < .05$.

Results
Hemodynamic Parameters

At the end of the administration period body weight and food intake were not substantially different in WKY or SHR (Table 2). Systolic BP increased in SHR compared with WKY after 12 weeks of treatment (Table 2). Neither BD nor high-oil diets were able to attenuate the systolic BP increase in SHR groups (Table 2). Likewise, heart rate was not different among groups during treatment (data not shown).

Contractile Response to Phenylephrine

Phenylephrine-induced contractions were notably attenuated in SHR intact aortic segments in comparison with those exhibited in WKY groups (Table 3). Feeding normotensive WKY with POMO diet significantly reduced contractile responses evoked by Phe in aortic rings with endothelium compared with the BD group ($P < .01$; Table 3). On the contrary, POM intake increased Phe-induced contractions in WKY aortas ($P < .05$; Table 3). Removal of endothelium augmented Emax to Phe in all WKY groups, whereas incubation with L-NAME only enhanced Emax values in olive oil– and POMO-fed WKY (Table 3). Although either incubation with L-NAME or endothelium removal increased the effect of Phe on POMO-treated WKY, the contraction to Phe was still attenuated compared with the other groups ($P < .05$; Table 3). With regard to SHR, the POMO diet intake also attenuated concentration–response curves to Phe in intact aortic rings in comparison with the BD hypertensive dietary group ($P < .05$; Table 3). Either endothelial removal or NOS inhibition increased maximal contractile response to Phe in all SHR groups compared with intact aortic rings (Table 3). Altogether, these results indicate that POMO intake involves endothelium-dependent mechanisms to decrease Phe-evoked contractions in SHR aortic rings.

Endothelium-Dependent Relaxation

The Ach-induced relaxation in aortic rings with endothelium precontracted with Phe (Fig. 1). Aortas taken from both WKY and SHR fed the POMO diet showed an increased relaxation to ACh compared with those from the strain-matched BD group ($P < .05$; Fig. 1). Likewise, ACh-evoked relaxation was enhanced in aortas taken from POM-treated SHR ($P < .05$; Fig. 1b). In aortas from WKY rats, the relaxant response to ACh was abolished in the presence of the NOS inhibitor L-NAME ($P < .001$; Fig. 2), thus indicating NO involvement in the relaxant response to

Table 2. Body weight, food intake, and systolic blood pressure (SBP) from WKY and SHR rats before (4-week-old) and after 12 weeks of dietary administration (16-week-old)

	Body weight (g)		Food intake (g/day)		SBP (mm Hg)	
	Before	**After**	**Before**	**After**	**Before**	**After**
WKY						
BD	64.5 ± 1.0	337.0 ± 3.1***	15.0 ± 0.3	22.1 ± 1.0***	148.1 ± 4.6	164.4 ± 3.6††
OL	56.5 ± 2.2	336.7 ± 8.1***	15.5 ± 0.2	21.0 ± 1.5***	141.4 ± 5.8	162.4 ± 3.3†††
POM	65.6 ± 3.1	357.0 ± 8.9***	16.0 ± 0.5	21.6 ± 1.3***	137.4 ± 3.9	153.8 ± 2.9†††
POMO	65.0 ± 3.2	348.7 ± 16.9***	16.2 ± 0.7	23.5 ± 1.9***	140.5 ± 1.6	149.9 ± 0.7†††
SHR						
BD	64.0 ± 3.7	328.6 ± 6.7***	16.0 ± 0.8	25.0 ± 0.5***	136.7 ± 5.0†	194.9 ± 8.9***
OL	63.2 ± 2.6	318.0 ± 7.5***	15.0 ± 1.4	26.0 ± 0.4***	125.0 ± 4.5†	207.5 ± 4.5***
POM	58.4 ± 2.1	320.3 ± 8.5***	14.8 ± 0.7	23.2 ± 1.1***	124.3 ± 5.6†	191.9 ± 7.1***
POMO	58.0 ± 2.5	317.0 ± 5.1***	15.0 ± 0.6	22.8 ± 0.4**	131.3 ± 8.4†	193.5 ± 6.9***

BD = basal diet, rich in corn oil; OL = olive oil; POM = pomace olive oil; POMO = pomace olive oil enriched in oleanolic acid.
　Data represented are mean ± SEM.
　** $P < .001$ v values before treatment from the same dietary group; *** $P < .001$; †† $P < .01$; ††† $P < .001$ v diet-matched SHR.

Table 3. Emax and pD_2 values in response to phenylephrine cumulative contractions (1 nmol/L to 100 μmol/L) in isolated rat aortic rings with (E+) or without endothelium (E−), or in the presence of the NO synthase inhibitor N^{ω}-nitro-L-arginine (L-NAME) with functional endothelium

	Emax (% maximal contraction)			pD_2 (-logEC$_{50}$)		
	E+	L-NAME	E-	E+	L-NAME	E-
WKY						
BD	124.8 ± 9.8	149.1 ± 12.9	192.6 ± 30.4#	7.56 ± 0.21	7.19 ± 1.34	7.58 ± 0.59
OL	124.6 ± 9.0	165.1 ± 16.3#	168.3 ± 13.7#	7.73 ± 0.53	7.58 ± 0.47	8.22 ± 0.10
POM	153.8 ± 8.6*	168.4 ± 10.2	187.0 ± 16.8#	7.72 ± 0.56	7.41 ± 0.97	7.81 ± 0.72
POMO	79.6 ± 4.6**	119.3 ± 2.7*#	151.0 ± 8.8*#	7.74 ± 0.25*	7.51 ± 0.54*	8.11 ± 0.07
SHR						
BD	98.0 ± 4.0†	120.9 ± 6.0†#	160.6 ± 12.2##	6.95 ± 3.93	7.22 ± 2.24	7.67 ± 0.34
OL	97.1 ± 9.8†	118.9 ± 6.4††#	142.7 ± 5.0##	7.00 ± 3.13	7.55 ± 0.20	7.63 ± 1.05
POM	100.1 ± 7.3††	122.7 ± 7.9††#	150.3 ± 9.9##	7.12 ± 1.54	7.04 ± 2.55	7.71 ± 0.30
POMO	70.4 ± 2.6*	106.8 ± 6.8###	138.7 ± 9.1##	7.16 ± 0.70	7.20 ± 1.09	7.78 ± 0.19*

Abbreviations as in Table 2.
Data represented are mean ± SEM.
* $P < .05$; ** $P < .01$ v strain-matched BD; † $P < .05$; †† $P < .01$ v diet-matched WKY; # $P < .05$; ## $P < .01$; ### $P < .001$ v strain- and group-matched E+.

ACh. The role of prostanoids was evaluated by incubation with indomethacin. These conditions did not modify endothelial function in normotensive aortas from both BD and olive oil groups (Fig. 2a,b). Interestingly, COX inhibition enhanced vasorelaxation to ACh in POM- and POMO-treated WKY ($P < .05$; Fig. 2c,d). Finally, combination of L-NAME plus indomethacin did not elicit additional inhibition than incubation with only L-NAME (Fig. 2).

In aortas from all groups of SHR, the ACh-evoked relaxation was not completely abolished by L-NAME, suggesting the possible involvement of an NO-independent mechanism (Fig. 3). Because the combination of indomethacin and L-NAME completely blocked the relaxation, this points to the participation of prostanoids on the relaxant effect promoted by ACh (Fig. 3). In addition, in rings from BD SHR, the presence of indomethacin improved the relaxant response to the muscarinic agonist, revealing the involvement of COX-derived constrictor prod-

ucts ($P < .05$; Fig. 3a). In contrast, indomethacin failed to enhance ACh-induced relaxation in SHR aorta from POM and POMO groups compared with the BD group (Fig. 3). This suggests that the POM and POMO diets may have inhibitory effects on contractile prostanoids.

eNOS Expression and NOx Levels

Compared with WKY, BD- and olive oil–treated SHR exhibited a significant reduction of aortic eNOS protein
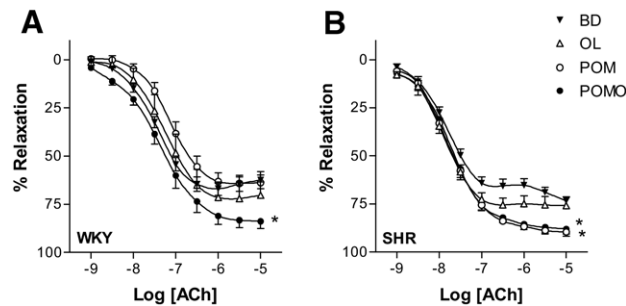
FIG. 1. Relaxation induced by acetylcholine (ACh, 1 nmol/L to 10 μmol/L) of intact aortic rings from either Wistar Kyoto (WKY) rats (**A**) or spontaneously hypertensive rats (SHR) (**B**) fed with 2% corn oil diet (BD) (▼, low-fat, basal diet), 15% of refined olive oil (OL) (△), pomace olive oil (POM) (○), or pomace olive oil supplemented in OA (POMO) (●) for 12 weeks. Values are mean ± SEM, n = 5 to 6 rats. Statistical analysis of concentration–response curves. *$P < .05$ v BD group.
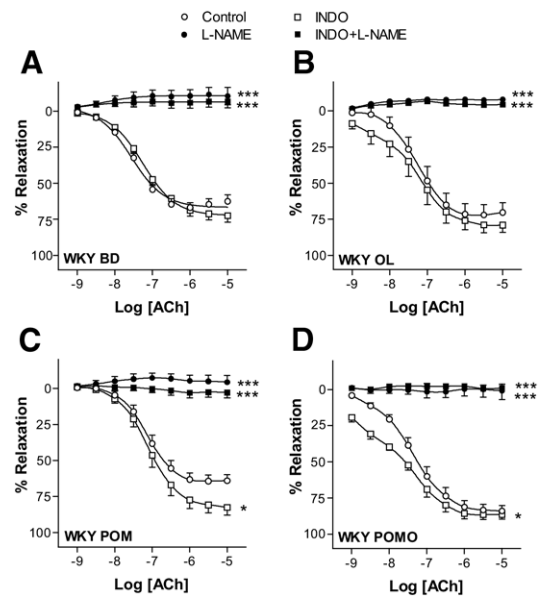

FIG. 2. Relaxation of intact aortic rings induced by ACh (1 nmol/L to 10 μmol/L) in WKY rats fed with BD (low-fat, basal diet; (**A**), OL (**B**), POM (**C**), or POMO (**D**) for 12 weeks. Concentration–response curves were obtained in the absence (○, taken as control) or presence of N^{ω}-nitro-L-arginine (L-NAME) (●) (300 μmol/L), indomethacin (□) (INDO; 10 μmol/L), or INDO plus L-NAME (■). Values are mean ± SEM, n = 5 to 6 rats. Statistical analysis of concentration–response curves. *$P < .05$; ***$P < .001$ v control.
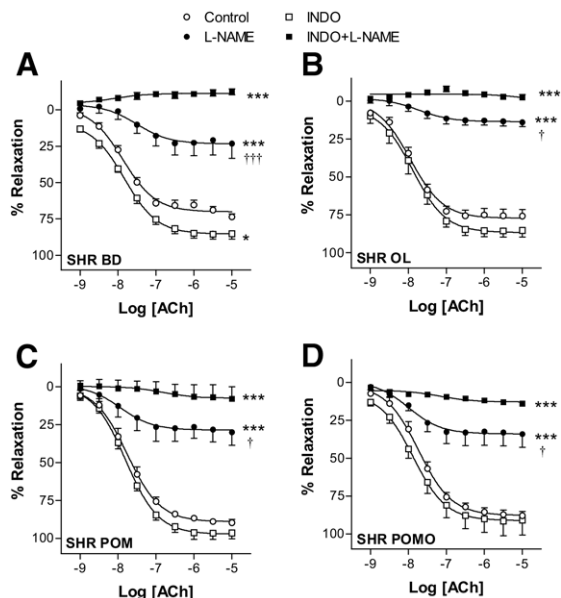
FIG. 3. Relaxation of intact aortic rings induced by ACh (1 nmol/L to 10 μmol/L) in SHR fed with either BD (low-fat, basal diet; (**A**), OL (**B**), POM (**C**), or POMO (**D**) for 12 weeks. Curves were obtained in the absence (○, taken as control) or presence of L-NAME (●) (300 μmol/L), indomethacin (□) (INDO; 10 μmol/L), or INDO plus L-NAME (■). Values are mean ± SEM, *n* = 5 to 6 rats. Statistical analysis of concentration–response curves. *$P < .05$; ***$P < .001$ *v* control; †$P < .05$; †††$P < .001$ *v* INDO + L-NAME.

Table 4. Plasma NOx (nitrites + nitrates) from WKY and SHR rats after 12 weeks of dietary administration

	WKY	**SHR**
NOx (μmol/L)		
BD	4.4 ± 0.3	4.7 ± 0.3
OL	4.5 ± 0.2	5.8 ± 0.6*
POM	5.2 ± 0.02	6.8 ± 0.9*†
POMO	7.3 ± 0.9†	7.1 ± 0.3†

Abbreviations as in Table 2.
　Data represented are mean ± SEM.
　* $P < .05$ *v* diet-matched WKY; † $P < .05$ *v* strain-matched BD.

either WKY or SHR with POMO significantly enhanced plasma NOx levels (48.4% and 65.1%, respectively) compared with the BD group. In contrast, POM intake only enhanced NOx in SHR but not in WKY (43.3% and 17.7%, respectively, versus the BD group).

Discussion

The purpose of this study was to evaluate the effects of pomace olive oil intake on endothelial dysfunction associated with hypertension as well as to determine the influence of higher concentrations of OA on the effects of diets enriched with pomace olive oil. The main result from the present study shows that dietary administration of pomace olive oil enhances endothelium-dependent relaxation to ACh in isolated aorta from SHR and the mechanism primarily involves NO by increasing eNOS protein expression. Oleanolic acid seems to contribute to these beneficial effects.

Cumulative evidence suggests that monounsaturated fatty acids may be the key component in the healthy properties elicited by olive oil intake in endothelial function and vascular inflammatory markers.[5,21] Nevertheless, controversies have been reported regarding the beneficial effects of oleic acid in terms of cardiovascular events such as hypertension.[22,23] A wide range of the cardioprotective effects of virgin olive oil consumption has also been attributed to its high concentration in biologically active polyphenols.[24] However, in our study we used refined olive oils, instead of virgin olive oil, which are lacking in polyphenols (water-soluble fraction) after the refining processes. Both refined olive oil and pomace olive oil presented similar oleic acid proportions but different concentrations in other minor components.

In the present study, hypertensive rats fed pomace olive oil–enriched diets (POM or POMO), but not olive oil–fed animals, showed increased endothelium-dependent ACh relaxations. This finding highlights the fact that other components, different from oleic acid such as minor constituents included in the unsaponifiable fraction of the oils, might be responsible for the long-term response of pomace olive oil intake on endothelium. Triterpenoids, such as

expression (Fig. 4). Interestingly, the expression of eNOS in aortas from POM and POMO-treated SHR was significantly enhanced compared with those from BD and olive oil and reached values that were similar to those from WKY rats (Fig. 4).

As shown in Table 4, there was no difference among plasma NOx levels in BD-fed WKY and SHR. Feeding
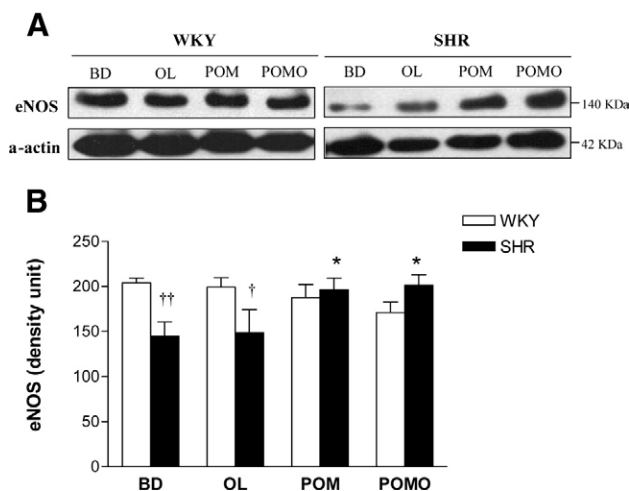
FIG. 4. (**A**) Representative blots of eNOS in aorta homogenates from WKY and SHR fed with BD (low-fat, basal diet), OL, POM, or POMO for 12 weeks. a-actin = -α-actin; eNOS = endothelial nitric oxide synthase. (**B**) Bands showing optic densitometry of *n* = 4 blots. *$P < .05$ *v* strain-matched BD; †$P < .05$; ††$P < .01$ *v* dietary group-matched WKY.

OA, are in higher proportion in pomace olive oil.[16] Our research group has recently demonstrated a novel in vitro vasodilator effect of OA in aortas from hypertensive rats.[11]

In our investigation we have used SHR, which have been characterized by endothelial dysfunction,[1] with an important impairment of NO availability. In addition, a structural feature of this hypertensive rat model is an increased arterial stiffness that reduces contractility in vitro, possibly due to vessel rigidity in vivo,[25] as confirmed in our results. In the present study, we have found that chronic supplementation of pomace olive oil with OA (POMO) improves endothelial function, not only in aorta from SHR, but also in that from WKY. In addition, aortas from SHR fed with POMO, but not with POM, showed a decreased concentration–response to Phe compared with the BD diet, supporting the hypothesis that OA could be responsible, at least partly, for these effects.

Because BP was not modified by any of the diets assayed, we cannot explain the endothelial function improvement associated with POM and OA in diet by a secondary event to antihypertensive effect but by their protective action in the vascular endothelium. In contrast to these results, chronic administration of OA prevented hypertension in another rat model of this disease.[15] However, this antihypertensive effect has been shown with higher dose of OA (60 mg/kg) and after intraperitoneal administration.[15] In comparison, in our study OA was administered as part of the diet and consequently by oral intake and at a lower dose. Therefore, differences in experimental design such as administration routes and measurements of BP could explain the results. We hypothesize that the intake of a diet enriched in POM could protect vascular walls and, consequently, promoting target organs protection independent of BP, which is one of the most important objectives in hypertensive patients. Nevertheless, this therapeutic effect requires further studies.

Regarding the role played by NO in endothelial dysfunction associated with hypertension, it is known that a decreased NO bioavailability as a consequence of an increased superoxide production is a relevant event contributing to the endothelial disturbances in SHR.[1,26] In addition, eNOS expression and activity has been reported to change in hypertensive rats depending on the age and the tissue studied.[27] According to a previous report,[28] at the age of 16 weeks we have found lower eNOS protein expression levels on aortas from BD SHR when compared with diet-matched WKY rats. In the present investigation, exposure of aortic rings to L-NAME revealed higher participation of NO in Ach relaxation of SHR fed with either POM or POMO compared with endothelium curves in the absence of any inhibitor. In contrast, SHR-BD exhibited lower NO involvement compared with L-NAME versus control Ach curves. Furthermore, Phe-induced responsiveness of intact aortas from WKY and SHR fed with POMO was attenuated; this effect was reverted by L-NAME, suggesting partial NO involvement. Accordingly, an increased NO bioavailability in these dietary groups was confirmed by an enhanced plasma concentration of NO end products (NOx). In addition, we found that either the POM or the POMO diet restored eNOS expression in SHR toward values found in WKY. Many hypotheses can explain these results, such as prevention of eNOS degradation, increase of eNOS transcription, or even an increase of mRNA stability by posttranscriptional mechanisms. Nevertheless, further experiments should be carried out to identify the mechanisms underlying the higher eNOS protein levels. In spite of the increased NO participation in endothelium-dependent relaxation observed in arteries from POMO WKY, eNOS expression remains unaltered, suggesting that other mechanisms, such as an increased eNOS activity or a reduction of reactive oxygen species, could be involved in this event. It is important to take into account that, to our knowledge, there are not many studies in the literature regarding the regulation of eNOS expression due to dietary modifications in genetic hypertensive rats. Our investigation provides a novel and relevant upregulation of eNOS in SHR aortic homogenates by long-term intake of POM. This fact may be considered as a reasonable and realistic strategy for the prevention or therapy of cardiovascular diseases including hypertension.

In addition to the decreased NO availability, factors derived from COX play a crucial role in the endothelial dysfunction associated with hypertension.[29] In the present investigation, the enhancement of the ACh-induced relaxation in the presence of the COX inhibitor observed in BD SHR, confirmed the participation of contractile products from this metabolic pathway. On the contrary, high fat intake was able to prevent the implication of contracting COX products in endothelium-dependent relaxation. In addition to contractile prostanoids, the COX pathway generates vasodilator products such as PGI_2. According to previous reports, which showed an increased release of PGI_2 in SHR,[29] our results in the presence of L-NAME revealed the involvement of a relaxant factor different from NO that was inhibited by indomethacin, thus indicating that maybe it is COX derived. The participation of this relaxant COX product, which was masked by NO in basal conditions, remained unaltered in all SHR groups. Contribution of this prostanoid may explain the fact that in POM and POMO SHR, with higher NO availability, a residual relaxation to ACh was found after L-NAME incubation.

Conversely, normotensive rats fed with POM or POMO showed an increase in the resulting COX-derived contracting component. These findings agree with previous reports, where differences in fatty acid composition of dietary oils play an important role in modulating eicosanoids production and lipoprotein peroxidation.[30] We could hypothesize that the presence of monounsaturated fatty acid such as oleic acid could be responsible for contracting prostanoids in SHR fed with high- or low- fat diets, whereas minor constituents such as triterpenoids could contribute to the increased implication of COX products in conductance arteries in WKY rats receiving a

POM-enriched diet. Nevertheless, further studies are required to better characterize the role of endothelial COX products in rats after diet administration with either POM or POMO.

In conclusion, the main finding of this study shows that oral intake of a diet enriched in pomace olive oil, rich in minor constituents such as OA, increased endothelial NO-mediated relaxation of aortic rings through an enhanced eNOS expression in SHR. This is the first report on the cardiovascular properties of pomace olive oil intake and it contributes to the pharmacologic relevance of OA supplementation. The fact that OA improved endothelial function of hypertensive rats without altering BP suggests the potential use of OA as an adjuvant to hypertension therapy. Finally, the information provided in this study is of importance for the traditional use of pomace olive oil by the Spanish population and thus for the Mediterranean diet.

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