Oleic acid content is responsible for the reduction in blood pressure induced by olive oil

S. Terés*, G. Barceló-Coblijn*, M. Benet*, R. Álvarez*, R. Bressani†, J. E. Halver‡§, and P. V. Escribá*§

*Laboratory of Molecular Cell Biomedicine, Department of Biology, Institut Universitari d’Investigacions en Ciències de la Salut, University of the Balearic Islands, Carretera de Valldemossa Km 7.5, E-07122 Palma de Mallorca, Spain; †Centro de Ciencia y Tecnología de Alimentos, Instituto de Investigación, University of Guatemala, Guatemala City, Guatemala; and ‡School of Aquatic and Fishery Sciences, University of Washington, Seattle, WA 98195

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Numerous studies have shown that high olive oil intake reduces blood pressure (BP). These positive effects of olive oil have frequently been ascribed to its minor components, such as α-tocopherol, polyphenols, and other phenolic compounds that are not present in other oils. However, in this study we demonstrate that the hypotensive effect of olive oil is caused by its high oleic acid (OA) content (~70–80%). We propose that olive oil intake increases OA levels in membranes, which regulates membrane lipid structure (HII phase propensity) in such a way as to control G protein-mediated signaling, causing a reduction in BP. This effect is in part caused by its regulatory action on G protein-associated cascades that regulate adenylyl cyclase and phospholipase C. In turn, the OA analogues, elaidic and stearic acids, had no hypotensive activity, indicating that the molecular mechanisms that link membrane lipid structure and BP regulation are very specific. Similarly, soybean oil (with low OA content) did not reduce BP. This study demonstrates that olive oil induces its hypotensive effects through the action of OA.

A
drenergic receptors (α- and β-adrenoceptors) are key elements in the central and peripheral control of blood pressure (BP). Recent studies showed that the activity of the adrenoreceptor signaling pathway can be regulated by oleic acid (OA) (cis-18:1n-9) (1), specifically because of the effect of this fatty acid on the structure of cell membranes (1, 2). However, similar modulation of G protein-coupled receptor signaling is not induced by structurally different but closely related fatty acids with identical or similar chemical composition, such as elaidic acid (trans-18:1n-9) or stearic acid (18:0). Nevertheless, a structural analogue of OA, 2-hydroxyoleic acid, can regulate membrane lipid structure and cell signaling in a similar manner as OA, evidence of the high structural specificity (3–5).

Mediterranean areas have a significantly lower incidence of cardiovascular heart disease when compared with other European countries. This phenomenon has been associated with dietary habits (6, 7), which improve parameters associated with major risk factors for cardiovascular disease, such as the lipoprotein profile (8, 9), BP (10), endothelial function (11), and inflammation and oxidative stress (12). Virgin olive oil (VOO) is one of the main components of the Mediterranean diet, and it contains high levels of monounsaturated fatty acids (MUFA), mainly OA (70–80%) that is incorporated into triacylglycerides (TGs). Long-term intake of high doses of VOO reduces BP and the risk of developing hypertension (13–16). At the molecular level, OA and VOO regulate G protein-associated signaling both in vivo (in humans) and in cell culture (1, 17). Interestingly, the hypotensive effect of 2-hydroxyoleic acid involves changes in the same signaling pathways as those affected by OA (18, 19). However, some studies associate the cardioprotective activity of VOO with minor components characteristic of olive oil, such as α-tocopherol, polyphenols, and other phenolic compounds (16, 20–23). In this study, we demonstrate that the high OA content is responsible for the normotensive effects of olive oil.

We have recently shown that OA, but not its structural analogues elaidic and stearic acid, regulates the activity of the α2A/D-adrenoreceptor/G protein/adenyl cyclase-cAMP/PKA system by modulating the structure of plasma membrane lipids (1). Free or esterified, OA can modify the biophysical properties of membranes, specifically increasing the nonlamellar (HII) phase propensity of the membrane (2, 3). In turn, this modification affects the docking of important membrane-associated signal transduction proteins involved in controlling BP, such as G proteins (1, 24, 25). In fact, altered levels and function of G proteins have been reported in both hypertensive humans (26, 27) and experimental models of hypertension (28, 29).

The present study was designed to investigate the molecular bases of the hypotensive effect of VOO. For this purpose, we compared the effects of VOO, triolein (TO; the main constituent of VOO, consisting of a TG with three OA moieties) and OA (the main fatty acid present in VOO) on BP. All of these treatments induced similar hypotensive effects in rats. In contrast, elaidic acid, stearic acid, and soybean oil (with low OA content) had little effect on BP.

Results

Effects of Soybean Oil, VOO, and TO on BP. The effects of soybean oil, VOO, and TO treatments on BP were investigated in Sprague–Dawley rats. Chronic administration of VOO and TO
over 14 days significantly reduced the BP in treated rats when compared with rats that received vehicle alone (26 ± 4.0 and 21 ± 3.4 mm Hg, respectively, *P < 0.001; Fig. 1). Similarly, acute (2 h) exposure to both VOO and TO also reduced systolic BP (20 ± 0.3 mm Hg, *P < 0.001, and 14 ± 1.7 mm Hg, *P < 0.05, respectively; Fig. 2). Although diastolic BP changed in a similar manner to systolic BP, these reductions were not significant. In contrast, soybean oil, which contains little OA, did not induce a reduction in BP after chronic administration (Fig. 1). Furthermore, heart rate was not significantly affected by chronic or acute administration of these products when compared with vehicle-treated rats (Tables 1 and 2).

**Effects of OA, Elaidic Acid, and Stearic Acid on BP.** Whereas TGs constitute the major component of VOO, OA is the main metabolite of this oil in the body. Therefore, we studied the effect of this cis-MUFA on BP in rats. Like VOO and TO, chronic oral administration of OA induced a marked reduction in systolic BP in Sprague–Dawley rats (Fig. 3), which was 17 ± 1.9 mm Hg lower than in vehicle-treated rats after 14-day treatment (Fig. 3; *P < 0.05). The hypotensive effect of OA was also observed after acute treatments, which reduced systolic BP by 13.0 ± 0.3 mm Hg (Fig. 2; *P < 0.001). In contrast, treatment with the trans-MUFA s elaidic or stearic acid (saturated fatty acid) did not significantly affect BP (Fig. 3 and Table 2).

**Effects of VOO on BP in Spontaneously Hypertensive Rats (SHRs).** VOO (2 g kg⁻¹) was also able to reduce the elevated BP in an animal model of hypertension (Fig. 4A), inducing a significant and progressive sustained reduction in systolic BP from as soon as 4 days after the onset of the treatment. Thus, there was a 26-mm Hg reduction (*P < 0.001, Student’s t test) in the BP of SHRs after 14 days of treatment when compared with the animals that received the vehicle alone (Fig. 4A). Similarly, OA induced marked and significant reductions of BP in these hypertensive animals (*P < 0.001), whereas stearic acid and elaidic acid did not significantly change the BP of SHRs (Fig. 4B).

**Effects of VOO, TO, and OA on Signaling Proteins in the Aorta.** Because OA regulates G protein-mediated signaling in 3T3 cells in vitro (4), we investigated whether this molecular mechanism was also involved in the in vivo activity of OA, TO, and VOO. Hence, we measured the levels of important signaling proteins involved in the control of BP in the aorta. The concentrations of G proteins and phospholipase C β1 (PLCB1α/β) were determined by quantitative immunoblotting in aorta tissue from Sprague–Dawley rats treated with OA, TO, VOO, or the vehicle alone for 14 days. After treatment with these lipids, there was a down-regulation of the aortic G proteins that inhibit adenylyl cyclase (AC), Gα₁ (Fig. 5A) and Gα₁ (Fig. 5B), when compared with vehicle-treated rats (note that Gα₁ is not expressed in rat aorta) (30). When compared with vehicle-treated rats, the overall levels of Gα₁ and Gα₁ proteins in the aorta fell by 28% and 34% after OA administration, by 40% and 28% after exposure to TO, and by 34% and 34% after VOO treatment (Fig. 5 A and B). Similarly, the amounts of aortic Gaq/11 decreased significantly by 26%, 35%, and 31% after OA, TO, and VOO treatments, respectively (Fig. 5C). Thus, we investigated whether proteins downstream Gaq/11 might also be affected by the administration of these lipids. Like Gaq/11, chronic OA, TO, and VOO induced significant decreases in the total PLCβ1 levels in the aorta (52%, 33%, and 24%, respectively; Fig. 5D).

**Effects of VOO and Soybean Oil on Membrane Structure.** We have studied the effects of OA and TGs on model membrane structure, specifically on the lamellar-to-hexagonal (Lₐ-to-H₁) transition temperature of 1,2-<i>eladyl</i> phosphatidylethanolamine.

![Table 2. Effect of acute VOO, TO, and OA administration on diastolic BP and heart rate values in rats](image-url)

<table>
<thead>
<tr>
<th>BP/heart rate</th>
<th>Vehicle</th>
<th>VOO</th>
<th>TO</th>
<th>OA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diastolic BP (mm Hg)</td>
<td>99.4 ± 3.6</td>
<td>91.0 ± 2.6</td>
<td>97.1 ± 3.1</td>
<td>91.4 ± 4.6</td>
</tr>
<tr>
<td>Heart rate, beats per min</td>
<td>348 ± 9</td>
<td>347 ± 1</td>
<td>341 ± 8</td>
<td>327 ± 8</td>
</tr>
</tbody>
</table>

Sprague–Dawley rats received a single dose (p.o.) of vehicle, VOO (2 g kg⁻¹), TO (1 g kg⁻¹), and OA (1 g kg⁻¹). Diastolic BP and heart rate values were measured as described in Materials and Methods. Each value represents the mean ± SEM (n = 10).

![Fig. 2. Acute effects of VOO, TO, and OA on systolic BP.](image-url)

![Fig. 3. Chronic effects of OA, elaidic acid and stearic acid on systolic BP.](image-url)

![Fig. 4.](image-url)
(DEPE) by differential scanning calorimetry (2–4). We assessed the effect of VOO and soybean oil on membrane lipid structure, and we found that, in agreement with the effect exerted by their major TGs constituents, both oils induced marked reductions in the L-to-H\textsubscript{II} phase transition (3). The outcome of these measurements was compared with results obtained for a series of structurally and chemically related fatty acids and TO (Fig. 6) (3, 4). We found that there was a tendency for the reduction induced by these lipids on BP to be correlated with the in vitro effect on the L-to-H\textsubscript{II} phase transition \( (r = 0.67; \ P = 0.07; n = 8) \) (Fig. 6A). The failure to identify a significant relationship was mainly caused by the anomalous behavior of soybean oil, which did not reduce BP to the same extent as it regulated the membrane structure. However, the digestion by lipases of both olive and soybean oils results in the release of free fatty acids. Therefore, the differences in the hypotensive effects of olive and soybean oil were most likely caused by their different OA content. In agreement with this hypothesis, the correlation between the reduction in BP and the dose of cis-MUFAs administered to animals was highly significant \( (r = 0.94; \ P < 0.001; n = 8) \) (Fig. 6B). Moreover, we also found significant correlation between the dose of cis-MUFA fatty acids received and the in vitro effect on the L-to-H\textsubscript{II} phase transition \( (r = 0.83; \ P < 0.01; n = 8) \) (result not shown).

**Table 3. Effect of chronic administration of OA, elaidic acid, and stearic acid on diastolic BP and heart rate values in rats**

<table>
<thead>
<tr>
<th>BP/heart rate</th>
<th>Vehicle</th>
<th>OA</th>
<th>Elaidic acid</th>
<th>Stearic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>102.4 ± 3.1</td>
<td>96.2 ± 1.7</td>
<td>106.4 ± 6.5</td>
<td>109.6 ± 0.9</td>
</tr>
<tr>
<td>Heart rate, beats per min</td>
<td>358 ± 9</td>
<td>388 ± 13</td>
<td>348 ± 13</td>
<td>378 ± 1</td>
</tr>
</tbody>
</table>

Sprague–Dawley rats received vehicle, OA (1 g kg\textsuperscript{-1}), elaidic acid (1 g kg\textsuperscript{-1}), and stearic acid (1 g kg\textsuperscript{-1}) p.o. every 12 h for 14 days. Diastolic BP and heart rate values were measured as described in Materials and Methods. Each value represents the mean ± SEM (\( n = 10 \)).

**Discussion**

The interest in the potential cardiovascular health benefits of VOO has increased since Mediterranean diets with high olive oil intake were shown to improve the serum lipoprotein profile (HDL-to-LDL ratio) and reduce BP, insulin resistance, and systemic markers of inflammation in cardiovascular-risk patients (14, 16, 31, 32). Most of these studies have involved months of VOO intake or other oils (13, 16), on the hypothesis that metabolic molecular mechanisms are associated with long adaptive cellular and physiologic processes. However, we demonstrated here that short (2 h and 2 wk) treatments can also lower systolic BP, through a mechanism of action based on the rapid regulation of membrane lipid structure and cell signaling (2–4 h) and rather quick (3–4 days) adaptive processes.

**OA Is Responsible for the Hypotensive Effects of Olive Oil.** In a study of a human population with controlled diets, which only varied in the type of oil used, VOO induced marked and significant reductions in BP with respect to sunflower seed oil (17). Numerous studies suggest that this and other beneficial effects of VOO on cardiovascular health are caused by minor components present in this oil (23). However, we demonstrate here that OA is responsible for the hypotensive effects of olive oil. In this context, VOO induced marked and significant reductions of BP after both acute (2 h) and chronic, yet short (2 wk), administration, unlike soybean oil. Although the hypotensive effects of acute (single dose) olive oil were transient (with a peak at 2–4 h after treatment), reductions in BP were not only marked but they were also stable after 3 or 4 days of high olive oil intake. This rapid effect after acute intake of free fatty acids and related lipids is most likely caused by their easy transfer from the small intestine to blood vessels, where they can regulate cell signaling in vascular cells. VOO is composed of TGs, mainly TO (with three OA moieties), which comprises >50% of all TGs in VOO, followed in abundance by TGs with two OA moieties. Indeed, TO itself also induced marked and significant reductions in BP, although it is unlikely that TGs alone are responsible for the hypotensive effects of olive oil. Indeed, these lipids are the substrates of lipases that readily digest TGs to release the free fatty acid moieties and hence, cells receive mainly the processed lipids. Furthermore, although soybean oil has a high TGs content it has little OA and it was unable to reduce BP in rats. Finally, the MUFA OA (the main component in both VOO and TO) also induced a marked and significant decrease in systolic BP, and the dose of MUFA administered to animals was closely correlated with the reduction in BP. Accordingly, it appears that the major fatty acid found in olive oil, OA, would account for the hypotensive effects of this nutrient.

By contrast, elaidic acid (trans 18:1n-9 isomer of OA) and stearic acid (18:0) do not induce significant changes in BP despite their chemical similarity with OA, which would argue against the involvement of other metabolic factors. It indicates that the fatty acid structure is critical to produce hypotension. Moreover, elaidic and stearic acids appeared to be ineffective to lower BP in SHRs, whereas both OA and VOO exerted normotensive (hypotensive) effects in these hypertensive animals, further...
supporting the beneficial action of high-OA-containing diets on human health (Fig. 4).

**Molecular Mechanisms Involved in the Hypotensive Effects of Olive Oil.** The intensive intake of VOO increases the cis-MUFA levels in cell membranes (16, 17), which augments the nonlamellar-phase propensity of the membranes (2, 3). By contrast, trans-MUFA and saturated fatty acids do not significantly change the membrane curvature strain. The “molecular shape” of lipids (33) in part explains their different effects on membrane structure and cell physiology. The presence of the nonlamellar-prone lipid, OA, in the lipid bilayer induces a decrease in the surface packing and cell physiology. The presence of the nonlamellar-prone lipid, OA, in the lipid bilayer induces a decrease in the surface packing and cell physiology. The presence of the nonlamellar-prone lipid, OA, in the lipid bilayer induces a decrease in the surface packing and cell physiology. Moreover, we have shown that this signaling pathway can also be regulated in vivo, because the structural analogue of OA (2-hydroxyoleic acid) reduces BP. Indeed, this hypotensive effect can be reversed in rats by administration of the PKA blocker Rp-8-BrcAMP, which affects the last protein in this canonical signaling cassette (19). Together, these results indicate a direct relationship between the composition and structure of lipid membranes and BP regulation, which is supported by the relationships we have shown here. In addition, the results provide a rational bases for the positive (in the case of cis-MUFA) or negative effects (in the case of trans-MUFA and saturated fatty acids) that diets enriched in different lipids might have on cardiovascular health.

It was previously shown that human hypertensive subjects have altered levels of membrane lipids and G proteins (27), and that long-term olive oil intake reduces the membrane concentrations of G proteins (17). In the present study, we observed a similar effect in rats treated with VOO, TO, and OA, indicating that the adaptive mechanism is ultimately triggered by OA. All of these lipids induced reductions in the levels of Ga12, Ga13, Goq11, and PLCβ. Moreover, this down-regulation of vascular smooth muscle of vasoconstrictory proteins, Goq11 and PLCβ, is associated with a decrease in the levels of the second messengers, inositol phosphate, diacylglycerol, and Ca2+ (36). Interestingly, OA inhibits inositol-(1, 4, 5)-triphosphate and diacylglycerol production, with a concomitant blockage of Ca2+-mediated cell signaling (37). These second messengers are produced from phosphatidylinositol by PLCβ, and their reduced concentration in cells would be in agreement with a decrease in the levels of the enzyme, as shown in this work. Similarly, rats treated with OA, TO, and VOO express significantly less inhibitory Goi proteins (Gq1 and Gq3) in the aorta. The net result is an enhancement of the vasodilator pathway α2-adrenoreceptor/G protein/adenylyl cyclase-cAMP/PKA (1).
The present work partially unravels the molecular mechanisms involved in the beneficial effects of olive oil in the control of BP and the molecular basis of its favorable effects on human health. Although other minor component of VOO could also have some positive action on cardiovascular health, we have demonstrated that the beneficial effects of OA-enriched diets can be explained by the principles of “membrane-lipid therapy” (43). These principles would not only apply to cis-MUFA but also to the beneficial effects of ω-3 fatty acids and the detrimental action of excessive intake of saturated and transunsaturated fats.

Materials and Methods

Animals, Treatments, and BP Measurements. Female Sprague–Dawley rats or SHRs weighing 230–250 g (Charles River Laboratories) were kept at a constant temperature (24 ± 1°C) with a 12-h dark/light cycle. All rats were fed during the experiments with chow A01 purchased from Panlab, which contains 0.04% palmitic acid, 0.13% palmitoleic acid, 0.65% OA, 1.39% linoleic acid, and 0.13% linolenic acid. Animals were randomly divided into seven groups (n = 10 animals per group), and they received per oral (p.o.) administration every 2 days for 14 days of soybean oil (2 g/kg; R.P. Sol Natural), VOO (2 g/kg; 1% Battle Hermanos), TO (1 g/kg), OA (1 g/kg), elaidic acid (1 g/kg), stearic acid (1 g/kg; Sigma), or vehicle (water). In a second series of experiments, male SHRs (250–300 g; Charles River Laboratories) were treated with VOO (2 g/kg) or vehicle, and BP was measured before treatment (day 0, basal values) and on days 4, 6, 8, 11, and 14 of the treatments. In a different series of experiments, SHRs were treated with OA, stearic acid, elaidic acid (1 g/kg; Sigma), or vehicle (control), and BP was measured before treatment (basal BP) and at the end of 14-day treatments. We always used 10 SHRs per group.

Preparation of Aortic Homogenates. Frozen aortas were ground in a glass mortar by using liquid nitrogen. The resulting powder was homogenized in a glass pestle by using a tissue blender (Ultra-Turrax; Janke & Kunkel) in ice-cold 10 mmol/liter Tris-HCl buffer, pH 7.5, containing 1 mmol/liter EDTA, 1% SDS, 1 mmol/liter PMSF, and 0.1% iodoacetamide. This homogenate allows repeated measurements throughout the treatment period (44). In a second series of experiments, Sprague–Dawley rats received a single p.o. administration of compound at the doses indicated above (acute treatments), and BP and heart rate were measured 2 h after each administration. BP and heart rate were measured by using a tail-cuff device connected to a computerized oscillometer (Nyprem system 645; Cibertec). This noninvasive technique was used in the present work to determine whether VOO might be useful to control high BP in human hypertensive subjects. The animals’ body weight, BP, and heart rate were measured 2 h after each administration. BP and heart rate were measured by using a tail-cuff device connected to a computerized oscillometer (Nyprem system 645; Cibertec). This noninvasive technique was used in the present work to determine whether VOO might be useful to control high BP in human hypertensive subjects. The animals’ body weight, BP, and heart rate were measured 2 h after each administration. BP and heart rate were measured by using a tail-cuff device connected to a computerized oscillometer (Nyprem system 645; Cibertec).
chronic treatments as described (18). The polyclonal antisera against Gα12 and Gα13 were obtained from PerkinElmer, and the anti-PLCγ1 antisera was from BD Biosciences/Transduction Laboratories. Quantification of the immunoblots was performed by image analysis, using standard curves with four points of different protein content loaded on the same gels (i.e., total protein loaded vs. integrated optical density) as described (45). The quantification procedure was repeated at least three times for each sample on different gels. Values were normalized to the protein content of vehicle-treated rats (control, 100%).

Differential Scanning Calorimetry. Differential scanning calorimetry was performed on an MCR-DS-C microcoulometer (OriginLab) at a scan rate of 0.5 K/min as described (46). In brief, DEPE (5 mM) was dissolved in chloroform in the presence or absence of different concentrations of soybean oil and olive oil in a proportion of 20:1 (DEPE/oil, mole/mole assuming that the oils had the same molecular weight of TO). The solvent was removed under an argon flux and then vacuum-dried for at least 3 h. Lipid films were subsequently resuspended in 10 mM Hepes buffer, pH 7.4, containing 100 mM NaCl, and 1 mM EDTA, by vortex shaking for 2 min at 50°C. These suspensions were degassed by stirring under vacuum for 10 min and immediately used in calorimetry experiments.

Data Analysis. The data are the mean ± SEM from the number of animals indicated. The statistical significance was calculated by using GraphPad software. One-way ANOVA followed by a Bonferroni's test or two-tailed t test (where indicated) was used for statistical evaluations. Differences were considered statistically significant at P < 0.05.

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