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Influence of Extra Virgin Olive Oil on Blood Pressure and Kidney Angiotensinase Activities in Spontaneously Hypertensive Rats

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Key words
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Abstract

High-fat diets are associated with the development of cardiovascular diseases. The efficacy of the current strategies of treatment is still not entirely satisfactory, and new approaches are being considered. To analyze the beneficial effects of extra virgin olive oil as a major component of the Mediterranean diet, we studied systolic blood pressure and angiotensinase activities, since this enzyme is involved in the metabolism of angiotensins, in the kidney of hypertensive rats fed during 12 weeks with a diet enriched with extra virgin olive oil compared with a standard diet. As a reflex of oxidative stress, 8-isoprostanes and nitric oxide were quantified in urine. Results demonstrated a progressive increase in systolic blood pressure until the end of the feeding period in both groups. However, this increase was delayed in the extra virgin olive oil group until week six, with the systolic blood pressure being always lower in this group. Nitric oxide and 8-isoprostanes were lower in the extra virgin olive oil group. While we can deduce a higher formation of angiotensin 2–10 in the renal cortex, a higher availability of angiotensin II may be presumed in the renal medulla of animals fed an extra virgin olive oil diet than in animals fed a standard diet. Our results support the beneficial influence of extra virgin olive oil on cardiovascular function and suggest that the Mediterranean diet may be beneficial in itself but it may also be an effective tool in the treatment of hypertension.

Introduction

Hypertension is one of the major cardiovascular risk factors affecting around 10% of the population, and its effective treatment reduces cardiovascular morbidity and mortality. Current treatment of hypertension involves diuretics, renin-angiotensin aldosterone system (RAAS) inhibitors or antagonists (angiotensin-receptor blockers, ARB), central or peripheral sympathicolytics, calcium channel blockers, and vasodilators [1,2]. However, the efficacy of these drugs is still not entirely satisfactory, and new approaches should be considered [3].

A diet rich in fruits, vegetables, and low-fat dairy products and reduced in saturated fat, total fat, and cholesterol (the "DASH" diet) significantly lowers blood pressure (BP). This effect has been suggested to be due, in part, to a counter-regulatory response of the RAAS [4]. The reduction of saturated fat in favor of mono and/or polyunsaturated fat attenuated the development of hypertension, with this effect being mediated, at least in part, by interference with the RAAS at the level of the blockade of angiotensin II (Ang II) receptors [5]. The Mediterranean diet (MD), characterized by 35% of energy delivery from fat, has demonstrated a notable cardioprotective and antihypertensive role. The main fat in the MD is extra virgin olive oil (EVOO; from *Olea europaea* L.; Oleaceae), a natural oil with a high monounsaturated fatty acid (MUFA) content and a lot of bioactive components in its minor fractions [6–8]. It is proposed that these protective effects may be due, in part, to the influence of components other than Ang II [9].

Blood pressure control is the result of a balance of closely related factors acting the at central or peripheral level, with some of them having opposite effects. Amongst these factors, the RAAS plays an important role. Until recently, Ang II was considered the main peptide of the RAAS involved in the control of BP. However, other peptides of the system, such as Ang III, Ang IV, Ang 1–9, Ang 1–7, or Ang 2–10, also seem to play an important role in BP control [10,11].

	Standard diet	EVOO diet	p
Body weight increase (g)	235.60 ± 7.42	243.30 ± 4.86	p > 0.05
Total heart weight (g/100 g BW)	0.38 ± 0.01	0.38 ± 0.01	p > 0.05
Left ventricle weight (g/100 g BW)	0.29 ± 0.01	0.30 ± 0.01	p > 0.05
Left kidney weight (g/100 g BW)	0.35 ± 0.01	0.31 ± 0.01	**p < 0.05**
Water intake (6th week, mL/day)	20.80 ± 1.42	22.90 ± 0.93	p > 0.05
Water intake (12th week, mL/day)	19.90 ± 0.61	16.40 ± 1.12	**p < 0.05**
Urine volume (6th week, mL/day)	9.80 ± 0.98	6.10 ± 0.52	**p < 0.01**
Urine volume (12th week, mL/day)	9.00 ± 0.42	9.00 ± 1.24	p > 0.05
Urine NO (6th week, µmol/L)	55.90 ± 6.55	35.40 ± 6.19	**p < 0.05**
Urine NO (12th week, µmol/L)	69.40 ± 16.40	48.80 ± 10.84	p > 0.05
8-isoprostane (12th week, ng/mg creatinine)	15.00 ± 1.01	7.50 ± 0.72	**p < 0.001**

Table 1 Mean values ± standard error of body weight (BW) increase (g), total heart weight (g/100 g BW), left ventricle weight (g/100 g BW), water intake at 6th and 12th weeks (mL/day), urine volume at 6th and 12th weeks (mL/day), urine NO at 6th and 12th weeks (µmol/L), and urine 8-isoprostane at 12th week (ng/mg creatinine) in standard and extra virgin olive oil (EVOO) groups.

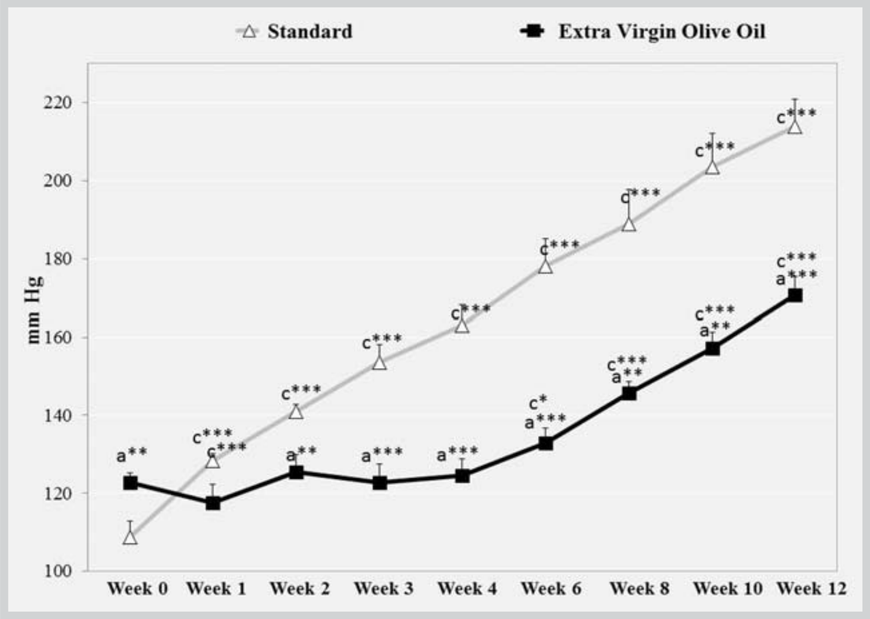

Fig. 1 Mean values ± standard error of systolic blood pressure (mmHg) in standard and extra virgin olive oil groups, throughout the experimental period. **a** Differences with respect to the S group; **c** differences with respect to week 0 in each group; ** p < 0.01, *** p < 0.001.

The formation and inactivation of these angiotensin-related peptides is regulated by various aminopeptidases (AP), called angiotensinases, and by the enzyme vasopressinase. For instance, aspartyl-aminopeptidase (AspAP) is responsible for the metabolism of Ang I to Ang 2–10, glutamyl-aminopeptidase (GluAP) causes the formation of Ang III from Ang II, whereas alanyl-aminopeptidase (AlaAP) is accountable for Ang III metabolism to Ang IV and to Ang 4–8. Cystinyl-aminopeptidase (CysAP), also called insulin-regulated aminopeptidase (IRAP), oxytocinase, or vasopressinase, was identified to be the AT_4 receptor binding site of Ang IV [12–15]. In addition to the systemic RAAS, local systems have been described in different tissues [16], including the kidney which has been directly associated with the development of hypertension [17–20].

In order to analyze the beneficial effects of EVOO as a major component of the MD, in this work we studied systolic blood pressure (SBP) and angiotensinase activities as enzymes involved in the metabolism of angiotensins in the kidney of young spontaneously hypertensive rats (SHR), still with undeveloped hypertension at the beginning of the experiment, fed a diet enriched with EVOO during 12 weeks compared to those fed a standard diet (S). In addition, several physiologic parameters such as body weight, water intake, urine volume, and heart and kidney weight were quantified. As a marker of oxidative stress, 8-isoprostanes and nitric oxide (NO) were also determined in the urine [21, 22].

Results

Results are presented in **Table 1** and **Fig. 1** and **Fig. 2**. Body weight increased progressively, without differences between groups, until the end of the experimental period (results not shown). Therefore, the body weight increase was similar in both groups at the end of the feeding period (**Table 1**). The weight of the total heart and left ventricle did not show differences between the S and EVOO groups. However, the weight of the left kidney of SHR fed a diet enriched in EVOO was significantly lower ($p < 0.05$; 15% lower) than animals fed a standard diet at the end of the experiment (**Table 1**).

Except for the first two weeks of the feeding period (data not shown) and week 12 ($p < 0.05$), in which the EVOO group demonstrated lower water intake levels than the S group (**Table 1**), there were no remarkable differences between groups in the rest of the period. On the other hand, throughout the feeding period, the urine volume was mostly lower in the EVOO group (data not shown). However, at the end of the feeding period (12 weeks), the urine volume did not differ between both groups (**Table 1**). Although the urine levels of NO were lower in the EVOO group at the end of the experimental period, they did not reach statistical significance between groups (**Table 1**). However, in the middle of the feeding period (week six), urine levels of nitric oxide were significantly lower ($p < 0.05$) in the EVOO group than in the S

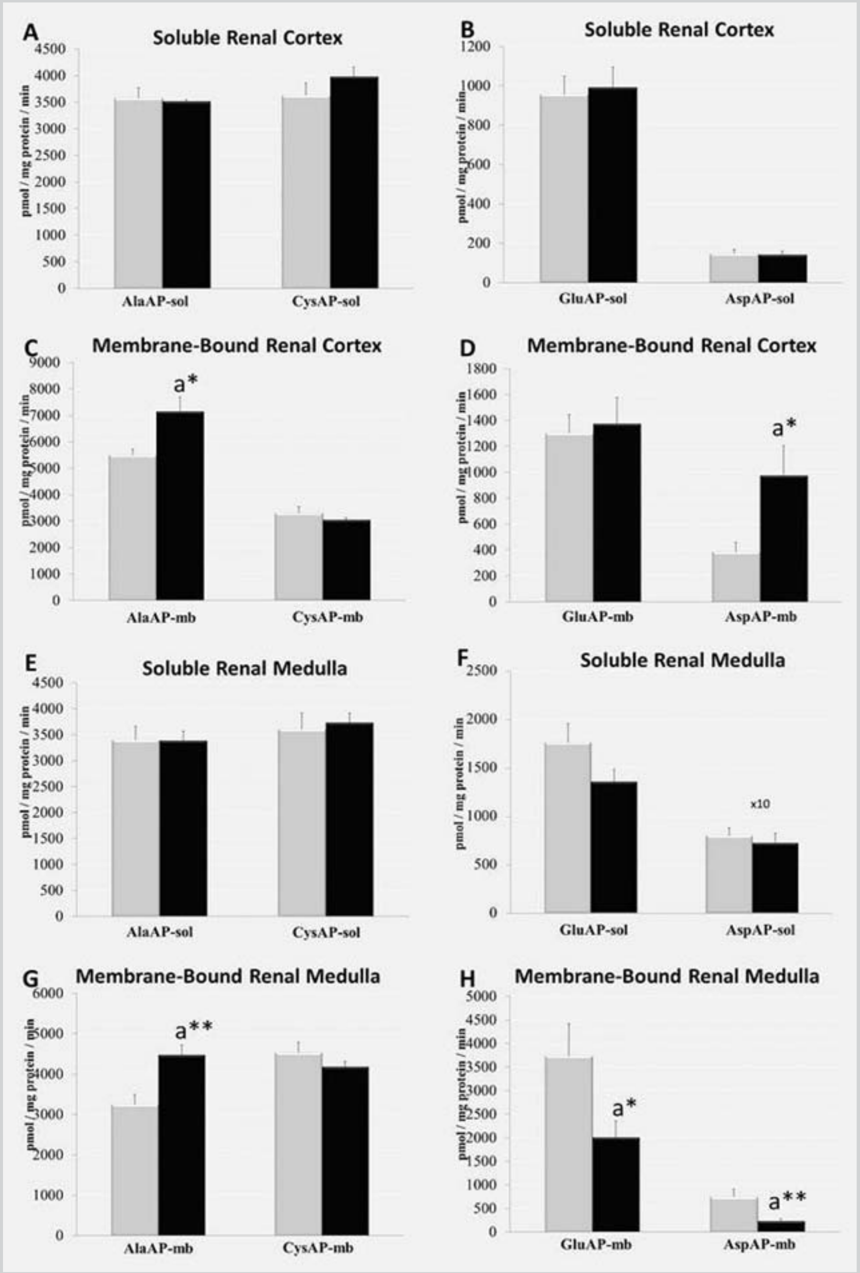

Fig. 2 Mean values ± standard error of alanyl (AlaAP), cystinyl (CysAP), glutamyl (GluAP), and aspartyl (AspAP) aminopeptidase activities, expressed as pmoles of the corresponding aminoacyl-β-naphtylamide hydrolyzed per mg of protein and per minute, in standard and extra virgin olive oil groups. **A** and **B** Soluble (sol) fraction of renal cortex; **C** and **D** membrane bound (mb) fraction of the renal cortex; **E** and **F** soluble (sol) fraction of the renal medulla; **G** and **H** membrane bound (mb) fraction of the renal medulla. **a** Difference between S and EVOO diets; * $p < 0.05$; ** $p < 0.01$.

group (◉ **Table 1**). Moreover, the levels of 8-isoprostanes in the urine were significantly lower ($p < 0.001$) in the EVOO group than in the S group at the end of the experimental period (◉ **Table 1**).
Systolic blood pressure levels, monitored throughout the total experimental period, from week 0 until week 12, are represented in ◉ **Fig. 1**. A progressive significant increase ($p < 0.001$) was observed in the S group compared to the first measurement. However, the levels of SBP in the EVOO group did not show differences compared to the first measurement (week 0) until week six, in which they increased progressively until the end of the feeding period. In addition, except at the beginning of the experiment, in which the S group demonstrated lower SBP levels ($p < 0.01$) than the EVOO group, the levels of SBP were always higher in S than in EVOO animals during the rest of the experimental period, being 20% higher during the most part of the experiment.

No differences were observed between animals fed a standard diet and a diet enriched with virgin olive oil in the aminopeptidase activities assayed from the soluble fractions of the renal cortex and renal medulla (◉ **Fig. 2**). However, remarkably, only significant differences were observed in the renal cortex and in the inner medulla selectively for membrane-bound activities (◉ **Fig. 2**). In the renal cortex, AlaAP ($p < 0.05$) and AspAP ($p < 0.05$) increased significantly. In the renal medulla, while AlaAP also increased significantly ($p < 0.01$), GluAP ($p < 0.05$) and AspAP ($p < 0.01$) decreased.

Discussion

The progressive increase in SBP from the beginning of the experiment observed in the S group is in agreement with previous data from SHR animals [23]. Nevertheless, the levels of SBP in the EVOO group did not show differences compared to the first measurement (week 0) until week six, in which they increased progressively until the end of the feeding period, and the levels of SBP were always higher in the S group during the most part of the experiment. These results clearly demonstrated an influence of dietary fat on the regulation of blood pressure, as was previously reported [5,9].

The lower levels of NO in the urine of EVOO animals were parallel with the lower levels in SBP. Several studies have described higher levels of NO and NO synthase expression in SHR tissues through the development of hypertension in these animals [23–27]. Moreover, urine levels of 8-isoprostane were also lower in the EVOO group. This may suggest a decrease in oxidative stress, probably associated with the effect of different minor components of EVOO, such as hidroxytyrosol and/or oleouropeine [28–30].

Regarding the significant differences observed in the renal cortex and inner medulla membrane-bound aminopeptidase activities, the increase in renal cortex AlaAP ($p < 0.05$) and AspAP ($p < 0.05$) in the EVOO group may suggest a higher metabolism of Ang III and Ang IV and a higher formation of Ang 2–10 than in the standard group. A noticeable intraglomerular conversion of Ang I to Ang 2–10, which may be critical to counterbalance the local actions of Ang II, was reported [31]. Therefore, a diet enriched in EVOO may increase the local production of Ang 2–10 at the glomerular level, compensating the negative actions of Ang II. In the renal medulla, while AlaAP increased significantly ($p < 0.01$), GluAP ($p < 0.05$) and AspAP ($p < 0.01$) decreased. These results suggest an increased metabolism for Ang III and Ang IV, but a lower metabolism for Ang II that would exert a longer effect under these conditions. It has been described that in the inner medulla, urea transport is enhanced by Ang II, contributing to increased sodium and water reabsorption [32], which would be in agreement with the reduced levels of diuresis observed during the feeding period (▶ **Table 1**). However, this argumentation contrasts with the reduced SBP observed during the feeding period (▶ **Fig. 2**). Therefore, the present results suggest an opposite response between the renal cortex and medulla, while in the renal cortex, the Ang II effect may be counterbalanced and in the renal medulla it may be potentiated. In consequence, to explain the beneficial influence on blood pressure of a diet enriched in EVOO, other factors such as modifications in vascular resistance or cardiac output, as well as a reduction in the levels of oxidative stress, may be involved. In conclusion, our results strongly support the beneficial influence of EVOO on cardiovascular function and suggest that the MD may be beneficial in itself but also as an effective tool in the treatment of hypertension.

Materials and Methods

▼

Animals and diets

Male SHR, aged four to five weeks, weighing 100–150 g, still with undeveloped hypertension at the beginning of the study, were used for control (S, n = 8) and for treatment (EVOO, n = 8). All experimental procedures were performed in accordance with the European Communities Council Directive 86/609/EEC and reviewed and approved by the bioethics committee of the University of Jaén (dated January 9, 2008). Diets were administered to each group in *ad libitum* conditions during 12 weeks.

The nutritional composition of the S diet was: 16.1% protein, 3.1% total fat, 80.8% carbohydrates, 5.1% minerals, and 3.9% fiber. The EVOO diet was prepared by adding 20% of EVOO [oleic acid (C18:1) 75.5%, palmitic acid (C16:0) 11.5%, and linoleic acid (C18:2) 7.5%] to the S diet. Nutritional composition of the EVOO diet was: 12.9% protein, 23.1% fat, 64.6% carbohydrates, 4.1% minerals, and 3.1% fiber. EVOO was supplied by "San Isidro Andalusian Cooperative Society" (batch no.: 2308, sample registration no.: 2010–2308).

Blood pressure, water intake, and diuresis quantification

SBP was monitored by the plethysmographic method as previously described [33]. Briefly, SBP was measured by tail-cuff plethysmography in unanesthetized animals. The rats were placed in plastic holders and warmed to 37 °C for each recording session. At least seven determinations were made in every session, and the mean of the stable values within a range of 5 mmHg was recorded as the SBP level. Measurements at the beginning and end were discarded. Since SBP has diurnal variations, all measurements were performed during the same period of the day (between 10.00 a. m. and 12.00 noon).

SBP was measured at the beginning of the experimental period and every week until the end of the experimental period (12 weeks). Body weight, water intake, and urine volume were also measured at the 6th and 12th weeks.

Collection of tissue samples

At the end of the treatment period, after recording SBP, body weight, water intake, and urine volume were measured; blood samples were obtained from the left cardiac ventricle under equithensin anaesthesia (2 mL/kg body weight), and the plasma was isolated by centrifugation for 10 min at 2000 *g* and stored at − 20 °C, then each rat was perfused with saline through the left cardiac ventricle. Total heart, from which the left ventricle was dissected, and the left kidney were obtained, and the weight of all of them was measured. Finally, samples from the renal cortex and inner medulla were quickly removed and frozen in dry ice. They were dissected as previously described [34,35]. Angiotensin components such as some peptides and enzymes have been reported to be differentially located in kidneys [35,36], for example, angiotensinogen and renin are mainly located in the renal cortex and ACE is higher in the renal medulla [36]. Therefore, meticulous care was taken to cut fine strips of the outer cortex and to restrict the medulla to its internal section to limit the presence of juxtamedullar glomerules [36].

Nitric oxide, creatinine, and 8-isoprostane determination

In urine, the levels of NO, creatinine, and 8-isoprostane were determined by a standard routine laboratory test. Briefly, creatinine was determined by the Jaffe procedure, a colorimetric method based on the reaction of creatinine and picric acid in an alkaline solution. The NO levels were estimated on the basis of the total amount of nitrites, which were measured using Griess reagent (Stressgen Biotechnology Corp.). A competitive ELISA kit (Cayman Chemicals) was used to detect the levels of 8-isoprostane and to avoid the effect of urine volume fluctuation; the urinary concentration of 8-isoprostane is expressed as ng/mg creatinine.

Procedures for enzymatic assays

To obtain the soluble fraction, tissue samples (renal cortex and inner medulla) were homogenized in 10 volumes of 10 mM HCl–Tris buffer (pH = 7.4) and ultracentrifugated at 100 000 × *g* for 30 min (4 °C). The resulting supernatants were used to measure soluble enzymatic activity and protein content, and were assayed in triplicate. To solubilize membrane proteins, the pellets were rehomogenized in HCl–Tris buffer (pH = 7.4) plus 1% Triton X-100. After centrifugation (100 000 g, 30 min, 4 °C), the supernatants were used to measure membrane-bound activity and proteins in triplicate. To ensure the complete recovery of activity, the detergent was removed from the medium by adding adsorbent polymeric Biobeads SM-2 (100 mg/mL) (Bio-Rad) to the samples and shaking for 2 h at 4 °C [35].

AP activities (angiotensinases and vasopressinase) were measured fluorometrically using arylamide derivatives as substrates, as formerly described [37, 38]. Briefly, AlaAP and CysAP were measured fluorometrically using aminoacyl-β-naphthylamides (aaNNap) as substrates: AlaNNap and CysNNap. Twenty microliters of each supernatant were incubated during 30 min at 37 °C with 100 µL of the substrate solution: 2.14 mg/100 mL AlaNNap or 5.63 mg/100 mL CysNNap, 10 mg/100 mL bovine serum albumin (BSA), and 10 mg/100 mL dithiothreitol (DTT) in 50 mM of phosphate buffer pH 7.4 for AlaAP, and 50 mM HCl–Tris buffer pH 6 for CysAP. GluAP was determined in a fluorometric assay using GluNNap as the substrate; 20 µL of each supernatant was incubated during 30 min at 37 °C with 100 µL of the substrate solution (2.72 mg/100 mL GluNNap, 10 mg/100 mL BSA, 10 mg/100 mL DTT, and 0.555 g/100 mL $CaCl_2$ in 50 mmol/L HCl–Tris pH 7.4). AspAP was determined fluorometrically with AspNNap as the substrate; 20 µL of each supernatant was incubated for 30 min at 37 °C with 100 µL of the substrate solution (2.58 mg/100 mL AspNNap, 10 mg/100 mL BSA, and 39.4 mg/100 mL $MnCl_2$ in 50 mmol/L HCl–Tris buffer pH 7.4). The sensitivity of the method allows measurements of aminopeptidases in the picomolar range [37].

Statistical analysis

For the statistical analysis, we used one-way analysis of variance to evaluate differences between groups. Post hoc comparisons were made with Tukey's test. P values below 0.05 were considered significant. Statistical calculations were carried out with the Statgraphics Centurion for Windows software package.

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Conflict of Interest

The authors declare no conflict of interest.

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