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Effect of Dietary High-Oleic-Acid Oils that are Rich in Antioxidants on Microsomal Lipid Peroxidation in Rats

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In contrast to other metabolic functions, the role of dietary antioxidants and oil on microsomal lipid oxidation has been less extensively studied. This study examines ascorbate−Fe^{2+} and NADPH-induced lipid peroxidation of hepatic microsomes of rats that were fed for three weeks high-oleic-acid oils (high-oleic sunflower oil, HOSO; olive oil, OO; or olive pomace oil, OPO) containing different concentrations of the antioxidants α-tocopherol, erythrodiol, and oleanolic acid. The fatty acid composition of hepatic microsomes of Wistar rats that were fed for three weeks with the above-mentioned oils had lower proportions of C16:0, C18:2n6, and C22:6n3 and higher proportions of C18:0 and C18:1n9 than rats fed the control diet. Light emission by hepatic microsomes increased, in the first 180 min, 2-fold after ascorbate-Fe^{2+} addition compared with NADPH addition. Both increases were less pronounced in microsomes of OPO-fed rats and to a smaller extent in microsomes of OO-fed rats. Smaller increases in light emission were associated positively with higher concentrations of dietary α-tocopherol, erythrodiol, and oleanolic acid but were not associated with changes in fatty acid composition of hepatic microsomes. Addition of α-tocopherol, erythrodiol, or oleanolic acid decreased light emission of hepatic microsomes with a greater inhibition in microsomes of rats fed the control diet. Our data suggest that erythrodiol and oleanolic acids partly explain the protective effect of dietary OPO on microsomal lipid peroxidation in rats.

KEYWORDS: Lipid peroxidation; olive oil; high-oleic-acid sunflower oil; olive pomace oil; liver

1. INTRODUCTION

The so-called "orujo" or olive pomace oil (OPO) is obtained by extraction with hexane from the residue formed after obtaining virgin olive oil (VOO). Although this subproduct of VOO is traditionally commercialized in Spain, the nutritional properties have not been studied yet. The new, improved procedures for OPO extraction allow higher concentrations of a number of components from the skin of the olive that are present in low concentration in VOO, including triterpenic acids and alcohols (*1*−*3*). Oleanolic acid (3*β*-hydroxyolean-12-en-28-oic acid) and erythrodiol (3*β*-olean-12-en-28-diol), a pentacyclic triterpenoid acid and alcohol, respectively, have potential therapeutic properties (*4*−*8*) that improve the commercial value of OPO.

Among other biological activities, including antiinflammatory, hepatoprotective, antitumoral, and anti-HIV (*4*), oleanolic acid has been shown to protect low-density lipoproteins (LDL) against oxidation (*5*). Although erythrodiol has also shown antiinflammatory properties (*6*−*8*), to our knowledge, the oxidative activity has not been investigated. The oxidation of LDL is a key factor in the genesis of atherosclerosis and the adherence of monocytes to the wall of the artery, which is enormously relevant to the formation of the atheroma inside the artery. The protective effects of the olive oil (OO)-rich Mediterranean diet against atherosclerosis have been related to the modulation of the cellular oxidative stress/antioxidant status, the modification of lipoproteins, and the down-regulation of inflammatory mediators (*9*).

The high polyunsaturated fatty acid (PUFA) concentration of cellular membranes that have either a dietary origin or are endogenously formed by desaturases makes them vulnerable to oxidation (*10*). There are two broad outcomes to lipid peroxidation: structural damage to membranes and generation of secondary products. These processes combine to produce changes in the biophysical properties of membranes that can have profound effects on the activity of membrane-bound proteins (*11*). It has been reported that ingestion of OO offers greater protection of the mitochondrial and microsomal membranes against damage caused by endogenous peroxidative stress

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Table 1. Fatty Acid Composition of the Dietary Oils[a]

fatty acid mg/100 mg	HOSO	OO	OPO
C16:0	4.2 ± 0.1	13.5 ± 0.4[b]	10.4 ± 0.1[b]
C16:1n-7	0.1 ± 0.0	0.5 ± 0.3	0.8 ± 0.1
C17:0	0.1 ± 0.0	0.3 ± 0.1	0.3 ± 0.1
C18:0	4.4 ± 0.2	2.3 ± 0.3[b]	2.8 ± 0.1[b]
C18:1n-9	79.3 ± 0.6	73.1 ± 0.6	72.7 ± 0.1
C18:1n-7	ND	3.1 ± 0.0	3.6 ± 0.2
C18:2n-6	10.4 ± 0.7	5.6 ± 0.7[b]	7.6 ± 0.1[bc]
C18:3n-3	0.1 ± 0.0	0.7 ± 0.1	0.9 ± 0.1
C18:3n-6	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0
C20:1n-9	0.2 ± 0.0	0.3 ± 0.1	0.2 ± 0.0
C22:0	0.7 ± 0.2	0.2 ± 0.1[b]	0.3 ± 0.1[b]

[a] Data are given as the mean ± SD. ND, not detected. HOSO, high-oleic sunflower oil; OO, olive oil; OPO, olive pomace oil. $n = 3$. Means with different letters within a row differ at $p < 0.05$. [b] $p < 0.05$, vs HOSO. [c] $p < 0.05$, vs OO.

(*12*−*15*). The aim of this study was to examine the effects of dietary high-oleic-acid oils (high-oleic sunflower oil, HOSO; OO; and OPO) that are rich in antioxidants on microsomal lipid peroxidation in rats.

2. MATERIALS AND METHODS

2.1. Materials. Sepharose 4B was from Pharmacia Fine Chemicals, Inc. (Piscataway, NJ). BSA (fraction V, fatty acid free) was obtained from Wako Pure Chemical Industries Ltd. (Japan). Fatty acids and standards of fatty acid methyl esters were from Nu Check Prep, Inc. (Elysian, MN). l(+)-Ascorbic acid and boron trifluoride−methanol reagent 14% were from Merck Quimica (Argentina). All other reagents were of the highest purity available.

2.2. Animals and Treatment. Twelve female 1-month-old Wistar rats (100−110 g) were maintained in a 12-h light−dark cycle (light from 8:00 a.m. to 8:00 p.m.). They were fed a standard diet (Ganave, Pilar, Buenos Aires, Argentina) for 30 days before the studies began. The diet contained 4% of total lipids with a fatty acid composition of 19.14% palmitic acid C16:0, 0.184% palmitoleic acid C16:1n-7, 4.10% stearic acid C18:0, 19.34% oleic acid C18:1n-9, 51.53% linoleic acid C18:2n-6, and 4.83% linolenic acid C18:3n-3. The animals were distributed randomly into four groups of three. Rats were fed either a control diet or diets containing 15% (w/w) HOSO, OO, or OPO. OO and OPO were provided from Oleicola El Tejar (El Tejar, Cordoba, Spain), and HOSO was purchased at a local market. The fatty acid composition of the oils was performed by gas chromatography, as described below, and is shown in Table 1. The diets were prepared by mixing ground pellets with a solution of methyl cellulose (1.5%) without or with the addition of the monounsaturated oils. The pellets were prepared by passage of the mixture through a special device and desiccated overnight at room temperature. Throughout the study, animals were weighed on a daily basis and daily food intake was also measured. These parameters were not affected by the treatment. After three weeks, all the rats were killed by cervical dislocation and the liver was rapidly removed and processed as described below.

2.3. Preparation of Rat Liver Microsomes. Rat liver was cut into small pieces and washed extensively with 0.15 M NaCl. A 30% homogenate (w/v) was prepared in a solution of 0.25 M sucrose, 10 mM Tris-HCl pH 7.4, 1 mM PMFS (Sigma, St. Louis, MO) using a Potter−Elvejhem Homogenizer. The homogenate was centrifuged at 20 000 *g* for 10 min. Three milliliters of the supernatant was applied to a Sepharose 4B column (1.6 cm × 12 cm) equilibrated and eluted with 10 mM Tris-HCl pH 7.4, 0.01% NaN_3. The microsomal fraction appearing in the void volume (10−16 mL) was brought to 0.25 M sucrose by adding solid sucrose as needed. All procedures were done at 4 °C. The quality of this microsomal preparation is of similar composition in terms of concentrations and activities of certain microsomal enzymes to that obtained by ultracentrifugation (*16*).

2.4. Determination of Fatty Acid Composition. Microsomal lipids from control and oil-treated rats were extracted with chloroform/methanol (2:1 v/v) according to the method of Folch et al. (*18*). The extraction solution contained 0.05% (w/v) butylated hydroxytoluene as an antioxidant. The fatty acids were transmethylated with boron trifluoride−methanol (14%) reagent, and their methyl esters (FAME) were analyzed with a GC-14A gas chromatograph (Shimadzu, Kyoto, Japan) equipped with a packed column (1.80 m × 4 mm id, GP 10% DEGS-PS on 80/100 Supelcoport). Nitrogen was used as a carrier gas. The injector and detector temperatures were maintained at 250 °C. The column temperature was held at 180 °C for 12 min, after which the temperature was raised to 205 °C at a rate of 2 °C/min. That temperature was maintained to the end of the analysis. FAME peaks were identified by comparison of retention times with those of standards.

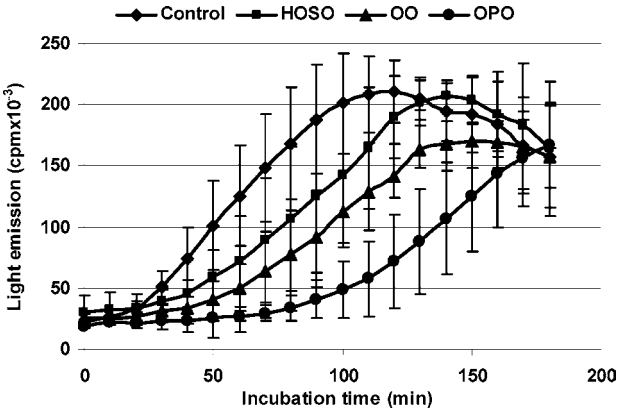

Figure 1. Light emission of hepatic microsomes isolated from rats fed different monounsaturated oils (HOSO, high-oleic sunflower oil; OO, olive oil; or OPO, olive pomace oil) after addition of ascorbate. Data are given as mean ± SD. Three animals per treatment. Control vs HOSO $p < 0.05$, Control vs OO $p < 0.05$, Control vs OPO $p < 0.05$, HOSO vs OPO $p < 0.05$, OO vs OPO $p < 0.05$.

2.5. Determination of Protein Concentration. Protein concentrations were determined by the method of Lowry (*19*) using BSA as standard.

2.6. Determination of Lipid Peroxidation of Rat Liver Microsomes. Lipid peroxidation of rat-liver microsomes was determined by measuring membrane light emission using chemiluminescence as cpm every 10 min over a 180 min period. Lipid peroxidation of rat-liver microsomes was increased by adding ascorbate (nonenzymatic) or NADPH (enzymatic) (*17*). Organelles at a concentration of 1 mg of protein were incubated at 37 °C with 0.05 M phosphate buffer pH 7.4, 0.4 mM ascorbate or 0.2 mM NADPH, final volume 1 mL. The phosphate buffer contained sufficient iron to provide the necessary ferrous or ferric iron for lipid peroxidation, (final concentration in the incubation mixture was 2.15 μM). Microsomal preparations which lacked ascorbate or NADPH were carried out simultaneously. Liver microsomes treated with the different oils were peroxidized after incubation with α-tocopherol, erythrodiol, and oleanolic acid at different concentrations, and the light emission was recorded. The resulting curves were adjusted to a sigmoidal (**Figure 1**) or polynomic (**Figure 2**) models, and the differences were assessed statistically as described below.

2.7. Statistical Analysis. Data are expressed as means ± SD. The significance of the difference among groups was assessed by analysis of variance (ANOVA), with Bonferroni's *t*-test for comparison of the means. Differences among curves for **Figures 1** and **2** were assessed by the Fisher test. Differences were considered significant with a confidence of 95% ($p < 0.05$). The analyses were done using Prism v3.0 (GraphPad software, San Diego, USA).

3. RESULTS

3.1. Fatty Acid Composition and Concentrations of Antioxidants of Dietary Oils. The fatty acid composition, as well as the antioxidant content, of the dietary oils employed in the experimental diets are shown in **Table 1** and **Table 2**, respectively. The fatty acid composition of the oils was very similar. Oleic acid was the main fatty acid in all dietary oil,

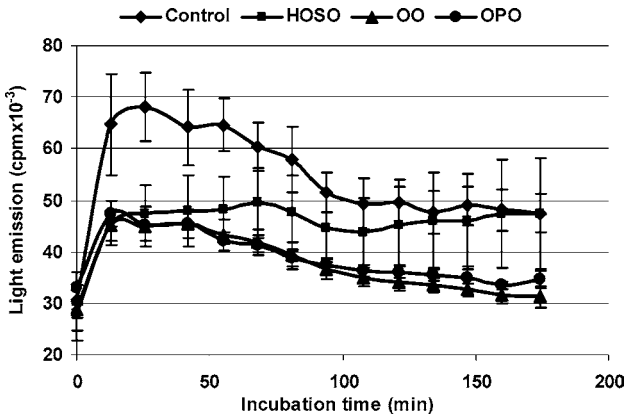

Figure 2. Light emission of hepatic microsomes isolated from rats fed different monounsaturated oils (HOSO, high-oleic sunflower oil; OO, olive oil; or OPO, olive pomace oil) after addition of (NAHPD). Data are given as mean ± SD. Three animals per treatment. Control vs HOSO $p < 0.05$, Control vs OO $p < 0.05$, Control vs OPO $p < 0.05$, HOSO vs OPO $p < 0.05$, HOSO vs OPO $p < 0.05$.

Table 2. Antioxidant Concentrations of Dietary Oils[a]

compounds mg/kg	HOSO	OO	OPO
α-tocopherol	393.2 ± 25.4	207.3 ± 48.1[b]	981.6 ± 82.3[bc]
erythrodiol + uvaol	ND	21.0 ± 1.1[b]	507.6 ± 42.0[c]
oleanolic acid	ND	56.0 ± 7.2[b]	416.3 ± 12.6[c]

[a] Data are given as the mean ± SD. ND, not detected. HOSO, high-oleic sunflower oil; OO, olive oil; OPO, olive pomace oil. $n = 3$. Means with different letters within a row differ at $p < 0.05$. [b] $p < 0.05$, vs Control. [c] $p < 0.05$, vs HOSO. [d] $p < 0.05$, vs OO.

accounting for about 75% of all fatty acids. However, significant differences were found in the content of linoleic and palmitic acids. The former was found at higher concentration in HOSO and the latter in OO and OPO.

OPO had the highest content of α-tocopherol, erythrodiol, and oleanolic acid compared to OO and HOSO. The content of erythrodiol and oleanolic acid was very low in OO, and these compounds were completely absent in HOSO.

3.2. Fatty Acid Composition of Rat Liver Microsomes. The hepatic microsomal fatty acid composition of rats that were fed for three week the high-oleic-acid oils (15 g/100 g food) was lower in C16:0, C18:2n6 and C22:6n3 and higher in C18:0 and C18:1n9 than the microsomal fatty acid composition of control-fed rats (**Table 3**).

3.3. Effects of Ascorbate (Nonenzymatic) or NADPH (Enzymatic) on Lipid Peroxidation of Rat Liver Microsomes. The incubation of microsomes (1 mg of protein) in the presence of ascorbate−Fe^{2+} or NADPH resulted in the lipid peroxidation of membranes as evidenced by the emission of light: chemiluminescence. In the absence of peroxidant, light emission was very low (**Figures 1** and **2**). The light emission produced by rat-liver microsomes, expressed as total cpm during 180 min of incubation at 37 °C, was 2-fold greater in the presence of ascorbate (0.4 mM) when compared with NADPH (0.2 mM). After 120 min of incubation with ascorbate−Fe^{2+}, maximum chemiluminescence of liver microsomes from control-fed rats was reached. When animals were treated with the experimental oils, a delay in the progression of the peroxidation process was observed. Liver microsomes from HOSO-fed rats reached a maximum after 140 min of incubation, OO-fed rats after 150 min, and OPO-fed rats after 180 min or longer (**Figure 1**). The resulting curves were significantly different among all groups, except for HOSO and OO. In the presence of NADPH, a maximum was reached at 25 min in hepatic microsomes of rats regardless of their dietary treatment (**Figure 2**). The highest light emission was produced after peroxidation of control-fed rats. At 180 min, the lowest light emission was found for oxidized liver microsomes of OO- and OPO-fed animals. Ascorbate-stimulated lipid peroxidation was lower in liver microsomes of rats fed OPO in comparison to liver microsomes of rats fed the control diet. NADPH-stimulated lipid peroxidation was lower in liver microsomes of rats fed OPO and OO in comparison to liver microsomes of rats fed the control diet (**Table 4**).

3.4. Effects of α-Tocopherol, Erythrodiol, or Oleanolic Acid on Lipid Peroxidation of Rat Liver Microsomes. Total chemiluminescence decreased when increasing concentrations of α-tocopherol, erythrodiol, or oleanolic acid were added to liver microsome of rats fed the control diet (**Figures 3**−**5**), although the tendency was only significant for α-tocopherol and erythrodiol.

α-Tocopherol inhibited light emission at a concentration as low as 0.25 mM for all animals treated with the experimental oils (**Figure 3**). For the control, 0.50 mM were necessary to achieve the same level of peroxidation inhibition. The highest inhibition of light emission achieved by erythrodiol was found at the highest concentration assayed (60 μM), being the significant difference for Control and HOSO. When inhibition was assessed within erythrodiol concentrations, the influence of the diet was more evident at the lower concentrations (0 and 15 μM). In fact, there was no inhibition of light emission at 30 μM by erythrodiol in the groups fed OO or OPO. The inhibition of light emission by oleanolic acid at 15 μM was relatively homogeneous for all diets studied and significantly higher compared to Control (**Figure 5**). The influence of dietary oils was evident at all concentrations of oleanolic acid studied, although only in some cases, the differences were significant. As found for erythrodiol, the inhibition in the OO and OPO groups at 30 μM was very low, but at 60 μM, it was high in the animals fed OPO.

4. DISCUSSION

The findings of the present study show that administration of monounsaturated oils change the fatty acid composition and lipid peroxidation of rat liver microsomes when compared to a control diet. However, whereas the fatty acid composition is not modified by such oils, there are important differences regarding their antioxidant capacity.

The hepatic microsomal fatty acid composition of rats that were fed for three week the high-oleic-acid oils was similar and lower in C16:0, C18:2n6, and C22:6n3 and higher in C18:0 and C18:1n9 than the microsomal fatty acid compositon of control-fed rats (**Table 3**). The changes in microsomal fatty acid composition are not associated with the antioxidant capacity of liver microsomes isolated from rats because the three oils differed in their effect on lipid peroxidation (**Table 4**).

The incubation of microsomes in the presence of ascorbate−Fe^{2+} or NADPH resulted in lipid-peroxidation of membranes, as evidenced by chemiluminescence in **Table 4**. When rat-liver microsomes isolated from control and oil-fed rats are lipid peroxidized enzymatically (in the precense of NADPH) or nonenzymatically (in the presence of ascorbate-Fe^{2+}), a decrease in the PUFA C20:4n6 and 22:6n3 coincides with increased values of chemiluminescence (*20*−*22*). A strong association between chemiluminescence and lipid peroxidation in rat hepatic microsomes has been demonstrated (*17*). NADPH-dependent

Table 3. Fatty Acid Composition of Hepatic Microsomes Isolated from Rats Fed Different Monounsaturated Oils[a]

fatty acid mg/100 mg	Control	HOSO	OO	OPO
C16:0	21.39 ± 1.95^b	12.56 ± 1.27^c	14.80 ± 0.70^c	12.08 ± 2.02^c
C16:1n7	0.10 ± 0.17^b	0.15 ± 0.20^b	ND	0.22 ± 0.31^b
C18:0	20.45 ± 1.11^b	25.99 ± 1.30^c	25.28 ± 2.94^c	25.22 ± 1.60^c
C18:1n9	11.92 ± 1.82^b	20.78 ± 1.49^c	23.89 ± 0.57^c	21.06 ± 0.17^c
C18:2n6	12.68 ± 1.48^b	7.10 ± 0.45^c	6.98 ± 0.56^c	7.15 ± 0.61^c
C20:4n6	19.95 ± 3.23^b	22.02 ± 1.19^b	20.86 ± 3.92^b	24.12 ± 1.20^b
C22:6n3	11.38 ± 1.74^b	7.34 ± 0.60^c	6.14 ± 1.50^c	6.45 ± 2.92^c

[a] Data are given as the mean ± SD of three independent experiments. ND, not detected. Means with different letters within a row differ at $p < 0.05$. HOSO: high-oleic sunflower oil, OO: olive oil, OPO: olive pomace oil. Three rats were used per dietary treatment. Oils were supplemented in the diet as 15% (w/w) and administrated to rats for three weeks.

Table 4. Total Light Emission over 180 min of Hepatic Microsomes Isolated Form Rats Fed Different Monounsaturated Oils after Addition of Ascorbate or NADPH[a]

	light emission cpm $\times 10^{-3}$			
	Control	HOSO	OO	OPO
no addition	324.80 ± 79.09^b	341.80 ± 80.04^b	339.96 ± 66.52^b	287.28 ± 63.35^b
ascorbate	2655.73 ± 372.60^b	2280.60 ± 484.77^{bc}	1842.10 ± 345.98^{cd}	1225.79 ± 376.83^d
NADPH	1022.44 ± 99.37^b	861.22 ± 63.61^{bc}	709.68 ± 37.02^c	734.40 ± 20.92^c

[a] Data are given as the mean ± SD. Membrane light emission was determined over 180 min at 37 °C and recorded as cpm every 10 min. Three rats were used per dietary treatment. Oils were supplemented in the diet as 15% (w/w) and administrated to rats for three weeks. Means with different letters within a row differ at $p < 0.05$. HOSO, high-oleic sunflower oil; OO, olive oil; OPO, olive pomace oil.

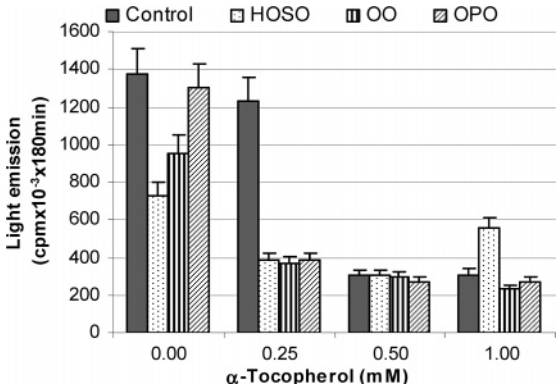

Figure 3. Total light emission inhibition over 180 min of hepatic microsomes isolated from rats fed different monounsaturated oils (HOSO, high-oleic sunflower oil; OO, olive oil; or OPO, olive pomace oil) after addition of α-tocopherol (0, 0.25, 0.5, and 1 mM). Data are given as mean ± SD. Three animals per treatment. Different lowercase letters above the columns indicate differences among experimental diets within α-tocopherol concentrations at $p < 0.05$.

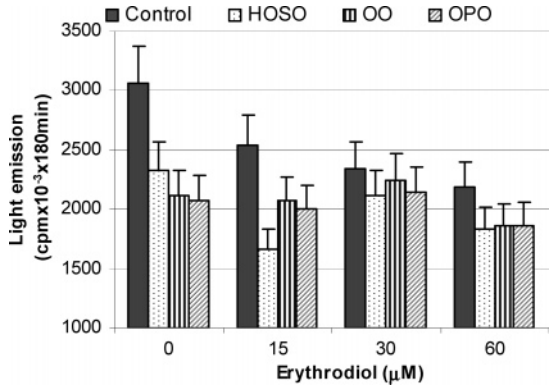

Figure 4. Total light emission inhibition over 180 min of hepatic microsomes isolated from rats fed different monounsaturated oils (HOSO, high-oleic sunflower oil; OO, olive oil; or OPO, olive pomace oil) after addition of erythrodiol (0, 15, 30, and 60 μM). Data are given as mean ± SD. Three animals per treatment. Different lowercase letters above the columns indicate differences among experimental diets within erythrodiol concentrations at $p < 0.05$.

chemiluminescence in our assay system was more active with liver microsomes isolated from control-fed rats than with microsomes isolated from OO- and OPO-fed rats (**Table 4**).

Light emission produced after both enzymatic and nonenzymatic peroxidation of liver microsomes from control rats was significantly higher. Nonenzymatic peroxidation triggered by ascorbate−Fe^{2+} reached a maximum in only 25 min for all dietary groups studied. In contrast, when liver microsomes where incubated with NADPH, the maximum of light emission was found after 120 min (**Figures 1** and **2**). In addition, feeding rats with the experimental oils resulted in a delay of the lag time of the oxidation process. So, the maximum peroxidation occurred after 140, 150, and 180 min for HOSO, OO, and OPO, respectively. The fatty acid composition of the oils would account for the lower oxidation of liver microsomes compared to the control group and for the delay but not for the differences among dietary oils. Previous studies have demonstrated that the two MUFA oils (OO and HOSO), with the same high content of oleic acid but different contents of natural antioxidants, had similar effects on the antioxidant enzyme activities (*23*). Olive oil is the principal source of fat in the Mediterranean diet, which has been associated with a lower incidence of coronary heart disease and certain cancers. Phenolic compounds, e.g., hydroxytyrosol and oleuropein, in OO are responsible for its peculiar pungent taste and for its high stability. Recent findings demonstrate that olive oil phenolics are powerful antioxidants (*20*) and possess other potent biological activities that could partially account for the observed healthful effects of the Mediterranean diet (*24*). Our results here show that OPO, which contains some minor compounds that are absent from OO and HOSO is as effective as OO in preventing lipid peroxidation in a nonenzymatic model and might be even more successful when an enzymatic model is used.

Additionally, the present work shows that erythrodiol and oleanolic acid, two terpenoid compounds present in OPO but found in very low concentrations in OO, are able to prevent

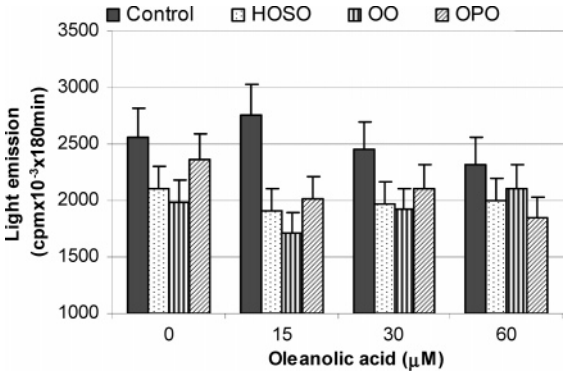

Figure 5. Total light emission inhibition over 180 min of hepatic microsomes isolated from rats fed different monounsaturated oils (HOSO, high-oleic sunflower oil; OO, olive oil; or OPO, olive pomace oil) after addition of oleanolic acid (0, 15, 30, and 60 μM). Data are given as mean ± SD. Three animals per treatment. Different lowercase letters above the columns indicate differences among experimental diets within oleanolic acid concentrations at $p < 0.05$.

lipid peroxidation in rat-liver microsomes to a similar extent as α-tocopherol (**Figures 3**−**5**). α-Tocopherol (0.25 mM) was sufficient to inhibit lipid peroxidation by more than 50% and a greater addition of that vitamin did not exert further effect (**Figure 3**). Diet administration to rats greatly influenced the antioxidant effect of erythrodiol and oleanolic acid. In both cases, at all concentrations studied (0, 15, 30, and 60 μM), the inhibition of light emission was higher when the animal was fed the monounsaturated oils. However, no significant differences were found among dietary groups. Therefore, despite the antioxidant capacity of erythrodiol and oleanolic acid, their content in OO and OPO seem not to have an influence compared to HOSO in the protection of rat liver microsomes. The similar content of oleic acid (C18:1n-9) provided by the three oils might have an important role in this regard. To test this hypothesis, OPO should be compared to another dietary oil poor in that fatty acid.

The pharmacological effects of erythrodiol have not been extensively studied. Only the therapeutic efficiency of erythrodiol on different experimental models of inflammation has been reported (*6*−*8*). In contrast, several therapeutic effects have been attributed to oleanolic acid (*4*). However, very little is known regarding the effects of erythrodiol and oleanolic acid on vascular events. Somova et al. (*25*, *26*) reported that chronic treatment of Dahl salt-sensitive genetically hypertensive rats with erythrodiol and oleanolic acid prevents development of hypertension with significant bradicardia and potent diuretic activity. We have very recently demonstrated for the first time that oleanolic acid and erythrodiol evoke an endothelium-dependent vasorelaxation in rat aorta, suggesting a nitric oxide (NO)-related mechanism (*27*).

Balanehru and Nagarajan (*28*) showed a protective effect of oleanolic acid against lipid peroxidation. Oleanolic acid has been more recently tested for its protective properties against oxidation of LDL, showing effects similar to polyphenols, such as quercetin or luteolin (*5*). To the best of our knowledge, there are no available data concerning the antioxidant properties of erythrodiol, although the effect of its isomer uvaol was comparable to that of oleanolic acid in the study of Andrikopoulos et al. (*5*).

Our data suggest that the potent antioxidant effect exerted by these minor constituents of OO, and specially OPO, might help protect the PUFA present in rat-liver microsomal membranes from oxidation. Although it has been reported that the composition of microsomes is affected by the fatty acid profile of dietary oils and the intake of antioxidant vitamin supplements, nothing is described about the antioxidant capacity of OPO. Therefore, the data presented here seem to be of great interest for future investigations.

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