ORIGINAL ARTICLE

Antithrombotic Potential of Olive Oil Administration in Rabbits with Elevated Cholesterol

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Abstract

Olive oil is the main source of dietary fatty acids in the Mediterranean region. The objective of this study was to evaluate the effect of dietary supplementation with virgin olive oil in an experimental model with rabbits fed an atherogenic diet (saturated fat 48% of total fat). Four different groups of 10 animals each were studied: (1) normolipemic diet (NLD), (2) atherogenic diet or saturated fatty acid-enriched diet (SFAED), (3) NLD with 15% olive oil (NLD+OLIV), and (4) SFAED with 15% virgin olive oil (SFAED+OLIV). The animals were fed the experimental diets for 6 weeks, after which we determined serum lipid profile (total cholesterol, HDL-cholesterol, and triglycerides), platelet aggregation, platelet thromboxane B₂, aortic prostacyclin, and platelet and vascular lipid peroxidation. Scanning electron microscopic images of the vascular endothelium were studied, as were morphometric parameters in the arterial wall and thrombogenicity of the subendothelium (annular perfusion chamber). Animals fed the SFAED showed platelet hyperactivity and increased subendothelial thrombogenicity. Animals fed the SFAED+OLIV showed, compared with the SFAED group, an improved lipid profile with decreased platelet hyperactivity and subendothelial thrombogenicity and less severe morphological lesions of the endothelium and vascular wall. We conclude that supplementation of the SFAED with 15% olive oil reduced vascular thrombogenicity and platelet activation in rabbits. Although the percentage of olive oil in the diet was higher than the amount in the human diet, these results may be helpful in determining the effect of olive oil in the human thrombogenic system. © 2000 Elsevier Science Ltd. All rights reserved.

Key Words: Olive oil; Platelets; Vessel wall; Thromboxane; Prostacyclin

Platelets and the vessel wall are both involved in the genesis and evolution of atherosclerosis, as well as in acute lipid overcharges, participating in the appearance of the initial atheromatous lesion and in the subsequent development of thrombosis, the main complication [1,2]. A number of alterations in platelet function, including hypersensitivity to aggregating agonists and increased thromboxane production, have been reported in animal models and in humans, both in early and late periods of the disease [3–7]. In the arterial wall, decreased endothelial production of prostacyclin and increased thrombogenicity have been described [3–5,8].

Dietary supplementation with unsaturated fatty acids, especially marine oils, has been shown to reduce the alterations found in experimental models of hyperlipidemia and atherosclerosis, decreasing platelet hypersensitivity and attenuating the
elevated synthesis of thromboxane [9–13], as well as decreasing, to a certain extent, thrombogenicity of the arterial wall [9,14]. These changes in platelet function have been considered beneficial in preventing cardiovascular disorders.

In Mediterranean regions olive oil is one of the main sources of dietary fatty acids. Olives and their oil contain oleic acid and a series of polyphenols [15], which have been shown to have an inhibitory effect on platelet function and thromboxane synthesis [16]. The objective of the present study was therefore to evaluate the effects of dietary supplementation with olive oil on platelet–vessel-wall interactions in a model of hyperlipidemia with atherosclerosis in rabbits fed a diet rich in unsaturated fatty acids from olive oil.

1. Materials and Methods

1.1. Animals

A total of 40 male white New Zealand rabbits were used. The animals were 2 months old at the start of the experiment and had a mean body weight of 2498±36 g. The experimental protocol was approved by the Ethical Committee for Animal Care of the University of Málaga; at all times the animals received care in compliance with the criteria in the Guide for the Care and Use of Laboratory Animals prepared by the National Academy of Sciences.

1.2. Experimental Groups

The rabbits were randomly divided into four groups of 10 animals each. Group 1 animals were fed a normolipemic standard diet (NLD) (Lapin Entretien Ref/112, Laboratorios Panlab SL, Barcelona) containing 34% of saturated fatty acid with respect to total fat. Group 2 animals received an atherogenic diet (diet supplemented with 1% cholesterol and saturated fatty acids 48% of saturated fat with respect to total fat [SFAED], Laboratorios Panlab SL). Group 3 animals were given the NLD with 15% virgin olive oil (NLD+OLIV) (Aceites Minerva S.A., Málaga, Spain). Group 4 animals were fed with the atherogenic diet with 15% olive oil (SFAED+OLIV). We used high cholesterol content in the atherogenic diet to obtain high hypercholesterolemia after a short follow-up period (Table 1).

Each group was kept on the diet for 6 weeks. Food intake was recorded periodically to avoid differences between groups in the amount of feed consumed. The OLIV-SFAED diet was prepared fresh daily by mean triturating of the solid food and mixing with olive oil (150 g OLIV in 850 g triturated solid food). Animals from the NLD+OLIV and SFAED+OLIV groups ate 11.8±2.5% less food (NLD: 182±5.2 g/rabbit/day; NLD+OLIV: 161±4.4 g/rabbit/day; SFAED: 199±5.1 g/rabbit/day; SFAED+OLIV: 172±6.0 g/rabbit/day).

1.3. Sample Collection

The animals were anaesthetised with a subcutaneous injection of thalamonal (0.025 mg/kg of phentaeryl and 1.2 mg/kg of dibenhydroperidol), ketamine (50 mg/kg body weight), and midazolam (2.5 mg/kg body weight). A medial laparotomy was done to obtain blood from the inferior vena cava. Trisodium citrate 3.8% at a proportion of 1:10 was used as anticoagulant; moreover, 2 mL of nonanticoagulated blood was kept at 37°C for 45 minutes and centrifuged at 4500×g for 15 minutes at 4°C to obtain serum.

The aorta was dissected from its root at the left ventricle to its iliac bifurcation and divided into five 1.5-cm segments for morphofunctional analyses. In a descending order we used these segments as follows: (1) measured the stable metabolite of prostacyclin (6-keto-PGF1α); (2) measured the fatty acid composition of the arterial wall; (3) observed the endothelium with scanning electron microscopy; (4) studied subendothelial thrombogenicity with a perfusion system in an annular chamber (Baumgartner chamber, Labotron SA, Barcelona, Spain); and (5) observed arterial wall thickness and cell nucleus counts, which were analyzed morphometrically.

1.4. Analytical Techniques

1.4.1. Basic Serum Profile

To determine the basic serum lipid profile, we used standard spectrophotometric techniques to measure the concentration of total cholesterol, high density lipoprotein (HDL) cholesterol, and triglycerides.
1.4.2. Vascular 6-keto-PGF$_{1a}$

To measure 6-keto-PGF$_{1a}$, samples of aortic tissue were placed in 1 mL buffer (composition in mmol/L: 100 NaCl, 4 KCl, 25 NaHCO$_3$, 2.1 Na$_2$SO$_4$, 20 sodium citrate, 50 Tris) and incubated with 100 µM arachidonic acid for 10 minutes at 37°C. After this period two samples of 500 µL each were frozen at −80°C until 6-keto-PGF$_{1a}$ was determined with radioimmunoassay (Amersham International plc, Little Chalfont Buckinghamshire, England).

1.4.3. Platelet Aggregometry

We determined platelet aggregation in whole blood with the electrical impedance method [17] in a Chrono-Log model 540S aggregometer (Chrono-Log Corp., Haverton, PA, USA). Aggregation was induced with different concentrations of adenosine diphosphate (ADP) or collagen (Menarini Diagnóstica, Barcelona, Spain). In each experiment the change in electrical impedance was recorded 10 minutes after the aggregate was added. Concentration–effect curves were drawn from the percentage aggregation obtained at each concentration of inducer, and the concentration of inducer that produced 50% of the maximal aggregation (EC$_{50}$) was calculated.

1.4.4. Serum Thromboxane B$_2$

Thromboxane B$_2$ (a stable metabolite of TxA$_2$) was measured by radioimmunoassay (Amersham International plc, Little Chalfont, Buckinghamshire, England), with (3H)thromboxane B$_2$. The sample of whole blood without anticoagulant was placed in a bath at 37°C for 45 minutes and centrifuged at 4500×g at 4°C for 15 minutes. The serum was removed and kept frozen at −80°C until analysis. To assess the possible influence of platelet number on platelet TxB$_2$ production, we used the formula described by Carter & Hanley [18]: TxB$_2$ (nmol/10$^9$) = TxB$_2$ (nmol/L) × (1 − (hematocrit/100)/platelet number (cells×10$^9$/L)) ×10$^9$.

1.4.5. Lipid Peroxides

Platelet-rich plasma and aortic sections were used to measure enzymatic lipid peroxidation [19] by incubating samples at 37°C with 200 µmol/L arachidonic acid and adding 100 µmol/L indomethacin 5 minutes after the arachidonic acid. Then 500 µL of 0.5% thiobarbituric acid in 20% trichloroacetic acid was added, and the sample was centrifuged at 10,000×g for 5 minutes. The supernatant was separated and incubated at 100°C for 15 minutes, and absorbance was measured spectrophotometrically at 532 nm. Lipid peroxides (main component malondialdehyde) were calculated by comparing the results with a standard curve prepared with malondialdehyde-bis-diethyl-acetal.

1.4.6. Scanning Electron Microscopy

For scanning electron microscopic examination of the vascular endothelium, samples were fixed in 1% glutaraldehyde and dehydrated in a series of alcohol-acetones (50, 70, and 80% ethyl alcohol for 5 minutes each, 96 and 100% ethyl alcohol for 30 minutes each, 100% acetone for 1 hour). Critical-point drying on dry ice was followed by metallization with gold-palladium. The preparations were observed and photographed in a Jeol JSM-840 scanning electron microscope (Jeol Instruments, Tokyo, Japan).

1.4.7. Vascular Wall Morphometry

For morphometric studies of the vascular wall, samples were fixed in 10% formaldehyde and dehydrated in a series of alcohol-acetones (50, 70, and 80% ethyl alcohol for 5 minutes each, 96 and 100% ethyl alcohol for 30 minutes each, 100% acetone for 1 hour). Critical-point drying on dry ice was followed by metallization with gold-palladium. For morphometric analyses of the vessel wall we used the Visilog v. 3.6 program (Microptic, Barcelona, Spain) running on a Sun-Sparc Station (Sun Microsystems, Palo Alto, CA, USA). Images were digitalized at 512×512 pixels with a 256-level gray scale. Once the arterial wall, lumen, and atherosomatous plaque had been identified as different levels of gray, the area of each portion and vessel diameter were measured in five sections from each artery. Cell nuclei were counted in four different zones of five sections per artery (20 zones per rabbit) at a magnification of 500× with a 100-µm$^2$ grid.

1.4.8. Platelet–Subendothelium Interaction

For perfusion in the Baumgartner annular chamber (20), the endothelium was removed with α-chymotrypsin (0.4 mg/ml for 12 hours at 37°C under constant shaking), and the sample was placed in the chamber and perfused with samples of human blood obtained from healthy volunteers (men with a mean age of 38.5±2.2 years, none of whom had taken any drugs during at least 15 days before the
samples were collected). The chamber was coupled to a perfusion pump set at a flow rate corresponding to a shear rate of 800 s⁻¹ for 10 minutes. At the end of the period of blood perfusion the arterial segment was fixed in 10% formaldehyde, embedded in paraffin (modified from [20]), and sectioned lengthwise. Each segment was cut into 120 transverse sections 2 μm in thickness, which were stained with hematoxylin-eosin and used to quantify parameters of platelet–subendothelial interaction (20): Contact (C) was assessed as platelets attached but not spread onto the subendothelium (nonactivated platelets). Spread (A), platelets spread and firmly bound to the subendothelium, measuring less than 5 μm high (representing the first phase of platelet aggregation). Thrombus (T) were considered groups of platelets with clear signs of activation, measuring 5 μm or higher. These structures were examined at 10 different points in each section for a total of 1200 points analyzed in each perfused artery.

1.4.9. Fatty Acids
Fatty acids were quantified by gas chromatography. Samples were previously dried, methylated, and extracted with hexane, then injected into a Hewlett-Packard Model 5890 (Avondale, Pennsylvania, USA) gas chromatograph.

1.4.10. Statistical Analysis
All results were expressed as the mean±SEM. When the variances were equal and the data were normally distributed, the groups were compared with ANOVA analysis and subsequent transformation with the Bonferroni test; if not, the Kruskall-Wallis test was used. All analyses were done with the Statistical Package for Social Sciences (SPSS, version 6.0, SPSS Inc., Chicago, IL, USA).

2. Results

Body weight increased from 2636±36 g before the experimental period to 3028±155 g at the end of the experimental period (+15.5±0.64%) in the normolipemic group. In animals fed the SFAED, the body weight changed from 2495±31 g before the experimental period to 3281±90 g at the end of the experimental period (+31.8±2.1%). Body weight decreased from 2570±35 g before the experimental period to 2553±139 g at the end of the experimental period (−1.3±0.5%) in the normolipemic group with olive oil supplementation. In animals fed the hyperlipidemic diet plus olive oil, body weight changed from 2388±42 g before the experimental period to 2763±71 g at the end of the experimental period (+15.6±2.6%).

In the serum lipid profile (Table 2), the SFAED increased total cholesterol 9.2-fold and increased triglycerides 4.5-fold. The diet decreased HDL-cholesterol by 29.5% and led to a 13.3-fold increase in the total/HDL-cholesterol index. This high triglyceride values may be due to the high fat content of the diet; moreover, the livers of these animals showed steatotic areas (data not shown). In cholesterol-elevated rabbits, dietary supplementation with olive oil significantly reduced total cholesterol (20%) and triglycerides (29.5%) in the serum lipid profile. Serum HDL-cholesterol increased by 67.4%, and the total/HDL-cholesterol index decreased by 50% (Table 2).

In animals fed the SFAED, the endothelial lining had zones of endothelial denudation alternating with abnormal but not denuded zones. In areas where the subendothelium was exposed, erythrocytes, leukocytes, and activated platelets were common. The nuclei were not distributed linearly, as in normolipemic animals, and atheromatous plaques were evident in zones of bifurcation (e.g., suprarenal trunks). Although the endothelial normal pattern was not observed in all of the normolipemic and hyperlipidemic animals fed the olive-oil-supplemented diet (Figure 1, lower left), most of the endothelial lining was intact, and no denuded zones or activated cellular elements were observed (Figure 1, lower right).

Platelet aggregation in whole blood was increased in hyperlipidemic rabbits regardless of whether ADP or collagen was used as the inducer (Figure 2). The EC₅₀ values were significantly lower in hyperlipidemic animals (Table 3). Platelet aggregation in animals fed the SFAED and olive oil was significantly reduced (Figure 2). The EC₅₀ values in these animals were 3.3-fold greater in ADP-induced aggregation and 2.5-fold greater in collagen-induced aggregation in rabbits fed the SFAED and olive oil than in rabbits that were not given olive oil (Table 3).

Platelet production of thromboxane B₂ (Table 3) was nearly 30-fold greater in hypercholesterolemic
animals than in controls. Mean 6-keto-PGF\textsubscript{1\alpha} production was 2.1-fold lower in animals fed the SFAED than in normolipemic controls (Table 3).

In animals with the olive-oil-supplemented diet, platelet production of thromboxane B\textsubscript{2} was significantly reduced (67.8% in hypercholesterolemic rabbits), although it was not as low as in normolipemic animals. In the groups given the normal diet, 6-keto-PGF\textsubscript{1\alpha} production increased 78% after treatment with olive oil. In animals fed the SFAED, 6-keto-PGF\textsubscript{1\alpha} production increased 325% with olive oil treatment (Table 3).

Platelet malondialdehyde equivalents were 9.3-fold greater in hypercholesterolemic animals than in controls, and aortic malondialdehyde equivalents were 2.04-fold greater (Table 3). Platelet lipid peroxidation was inhibited by 25% in normolipemic rabbits and by 57% in rabbits fed the SFAED after treatment with olive oil. Aortic lipid peroxidation was reduced by 14% in normolipemic rabbits and by 75% in hypercholesterolemic rabbits after treatment with olive oil (Table 3).

Morphometric analyses of the wall of the aorta in hypercholesterolemic animals showed thickening and increased nuclear counts in comparison with normolipemic rabbits. In the former group 9.23±0.56% of the vascular wall was occupied by foam cells (Figures 3 and 4). Dietary supplementation with olive oil significantly modified the components of the arterial wall in hypercholesterolemic rabbits, decreasing wall area, foam cell nuclear count, and the percentage of the wall occupied by foam cells in comparison with animals that did not receive dietary olive oil (Figures 3 and 4).

Functional tests of arterial wall-platelet interactions showed that in animals fed the SFAED

Table 2. Basic serum lipid profile in rabbits

<table>
<thead>
<tr>
<th></th>
<th>NLD</th>
<th>NLD+OLIV</th>
<th>SFAED</th>
<th>SFAED+OLIV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>2.11±0.32</td>
<td>2.36±0.41</td>
<td>19.55±3.05*</td>
<td>15.64±2.36**</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>3.02±0.45</td>
<td>3.39±0.45</td>
<td>13.55±2.03*</td>
<td>9.55±1.01**</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/L)</td>
<td>0.61±0.09</td>
<td>0.87±0.09</td>
<td>0.43±0.05</td>
<td>0.72±0.09**</td>
</tr>
<tr>
<td>Total cholesterol/HDL-cholesterol</td>
<td>3.26±0.26</td>
<td>2.86±0.40</td>
<td>43.46±8.80*</td>
<td>21.72±4.12**</td>
</tr>
</tbody>
</table>

NLD=normal lipemic diet, SFAED=saturated fatty acid-enriched diet, OLIV=olive oil.

* p=0.05 (vs NLD); ** p=0.05 (vs SFAED)
thrombogenicity was increased, as reflected by the significant increase in surface screened in the arterial segment mounted in the Baumgartner chamber (32.9% increase in spread, 11.1% increase in thrombus) (Table 4).

The percentage of covered subendothelium was not significantly greater in normolipemic animals treated with olive oil. In hypercholesterolemic animals olive oil significantly decreased the percentage of subendothelium screened by 37%, mainly as a result of the decrease in thrombus (59%) (Table 4).

Table 5 shows the results of chromatographic analyses of the arterial wall to determine fatty acid composition after feeding for 6 weeks with each of the diets.

3. Discussion

Our results show that dietary supplementation with 15% olive oil, in our experimental model of feeding rabbits with an atherogenic diet, reduced thrombogenic factors and increased antithrombotic factors. These effects were seen both in platelets and in the vessel walls. In platelets, dietary olive oil reduced hypersensitivity to aggregation, thromboxane synthesis, and lipid peroxide production. In the arterial wall the supplemented diet increased prostacyclin synthesis and reduced thrombogenicity of the subendothelium. We also observed a reduction in hyperlipidemic status, with increased levels of HDL-cholesterol. All effects were seen especially clearly in animals fed the atherogenic diet and appeared less clearly in normolipemic rabbits.
genic diet led to large increases in cholesterol levels, which are difficult to reduce to normal with dietary measures alone. However, the comparison of the lipid metabolism between humans and rabbits is not so exact.

The platelet hyperactivity found in rabbits that consumed the atherogenic diet was reduced in animals that consumed olive oil. Some phenolic components of olive oil are able to inhibit platelet aggregation in platelet-SFAED plasma in vitro [16], and one study found that sensitivity to collagen-stimulated aggregation was reduced in patients with type II hypercholesterolemia treated with dietary olive oil [23]. Neither normal diet nor SFAED contain polyphenolic substances or a-tocopherol; olive oil contains 10^{2}4 mg/kg of polyphenolic components and 150 mg/kg of a-tocopherol (data obtained from manufacturers). Our results show that in rabbit whole blood, platelets are less sensitive to aggregating agents and show aggregating behavior similar to that in control animals. This inhibitory action may involve inhibition of thromboxane synthesis, which is fundamental for platelet aggregation, or reduced lipid peroxide production. Some antioxidants such as flavonoids are able to inhibit platelet functioning, although this effect does not always correlate with an inhibition of thromboxane synthesis [24]. These antioxidant substances are among many possible components that could play a role in the beneficial effect of olive oil.

An important consideration in analyzing our results is that dietary supplementation with olive oil affected both the platelet and the vascular component of atherosclerosis, reducing subendothelial thrombogenicity and stimulating the endothelial synthesis of prostacyclin. This latter effect does not

Fig. 2. Concentration-dependent curves of platelet aggregation induced by ADP or collagen after 6 weeks on a normolipemic (open symbols) or hyperlipidemic (solid symbols) diet, without (circles) or with (squares) 15% supplementation with olive oil.

The conclusions of earlier studies that investigated reductions in hyperlipidemia in humans have not been unanimous: One study [21] failed to find significant changes in the lipid profile, whereas another [22] reported a decrease in total cholesterol and an increase in the HDL-cholesterol fraction. In the present study of hyperlipidemic rabbits, we found significant changes in cholesterol, triglycerides, and HDL-cholesterol, although the reductions did not return values to those found in control animals that were fed a normal diet. This finding may have resulted from the fact that the atherogenic diet led to large increases in cholesterol levels, which are difficult to reduce to normal with dietary measures alone. However, the comparison of the lipid metabolism between humans and rabbits is not so exact.

The platelet hyperactivity found in rabbits that consumed the atherogenic diet was reduced in animals that consumed olive oil. Some phenolic components of olive oil are able to inhibit platelet aggregation in platelet-SFAED plasma in vitro [16], and one study found that sensitivity to collagen-stimulated aggregation was reduced in patients with type II hypercholesterolemia treated with dietary olive oil [23]. Neither normal diet nor SFAED contain polyphenolic substances or a-tocopherol; olive oil contains 10^{2}4 mg/kg of polyphenolic components and 150 mg/kg of a-tocopherol (data obtained from manufacturers). Our results show that in rabbit whole blood, platelets are less sensitive to aggregating agents and show aggregating behavior similar to that in control animals. This inhibitory action may involve inhibition of thromboxane synthesis, which is fundamental for platelet aggregation, or reduced lipid peroxide production. Some antioxidants such as flavonoids are able to inhibit platelet functioning, although this effect does not always correlate with an inhibition of thromboxane synthesis [24]. These antioxidant substances are among many possible components that could play a role in the beneficial effect of olive oil.

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| Table 3. Aggregometric and prostanoid parameters in the four groups of animals |
|------------------------|-----------------|----------------|-----------------|----------------|
|                       | NLD*            | NLD + OLIV     | SFAED           | SFAED + OLIV   |
| EC_{50} ADP (µmol/L)  | 2.50±0.19       | 2.04±0.54      | 0.84±0.14*      | 2.83±0.35**    |
| EC_{50} Collagen (µg/mL) | 0.55±0.07       | 0.51±0.05      | 0.22±0.04*      | 0.55±0.09**    |
| Thromboxane B_{2} (ng/mL) | 115±15.91      | 127±18.42      | 3095±89.57*     | 997±185**      |
| 6-keto-PGF_{3a} (ng/mg aorta) | 3.49±0.64     | 6.25±0.80*     | 1.68±0.06*      | 7.15±0.51**    |
| Platelet MDA (nmol/10^9 cells) | 0.08±0.01    | 0.06±0.009     | 0.75±0.06*      | 0.32±0.04**    |
| Aortic MDA (nmol/mg protein) | 2.43±0.02     | 2.09±0.16      | 4.96±0.25*      | 1.24±0.09**    |

*a NLD = normolipemic diet; SFAED = saturated fatty acid-enriched diet; OLIV = olive oil; MDA = malondialdehyde equivalents; EC_{50} = concentration of ADP or collagen that produced 50% of maximal aggregation.

* p=0.05 (vs. NLD); ** p=0.05 (vs. SFAED).
appear to have been the result of a direct stimulation of prostacyclin synthase; in animals that consumed the normolipemic diet we found no effect on the synthesis of this prostanoïd. Some components of olive oil have antioxidant effects [25,26], as corroborated by our finding that lipid peroxidation in the vascular wall was inhibited (Table 3). Thus, the decrease in peroxides implies that prostacyclin synthesis was not inhibited, because lipid peroxides reduce the synthesis of this prostaglan- din [27]. This indirect effect has been found with some drugs that show antioxidant properties, such as dipyridamole [28] and S-adenosyl-L-methionine [29]. However, we cannot rule out that the improvement in the morphological lesions in the en-

dotheilial cells allowed them to synthesize more prostaglandin.

The reduced thrombogenicity of the subendothelium (Table 4) reflected the effect of olive oil not only on the rabbit platelet function, but also on the arterial wall, because perfused blood samples were taken from healthy volunteers who did not consume dietary olive oil. Our results are quantita-

tively different from those published by Baumgart-
Table 4. Percentage of vascular subendothelium covered by platelets after 10 minutes of human blood perfusion in the Baumgartner annular chamber (shear rate: 800 s$^{-1}$)

<table>
<thead>
<tr>
<th>Percentages with respect to the surface screened</th>
<th>NLD*</th>
<th>NLD+OLIV</th>
<th>SFAED</th>
<th>SFAED+OLIV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contacts</td>
<td>53.15±4.68</td>
<td>47.09±4.00</td>
<td>14.66±2.57*</td>
<td>30.07±2.05**</td>
</tr>
<tr>
<td>Spread</td>
<td>22.71±2.03</td>
<td>29.91±3.24</td>
<td>55.64±5.48*</td>
<td>49.82±6.77</td>
</tr>
<tr>
<td>Thrombus</td>
<td>23.44±3.12</td>
<td>21.02±3.91</td>
<td>34.58±4.56*</td>
<td>14.12±0.85**</td>
</tr>
<tr>
<td>Covered surface</td>
<td>25.16±3.12</td>
<td>23.87±3.30</td>
<td>38.70±5.13*</td>
<td>24.36±3.84**</td>
</tr>
<tr>
<td>Height of thrombus (μm)</td>
<td>5.61±0.19</td>
<td>5.45±0.18</td>
<td>7.13±0.54*</td>
<td>5.31±0.20**</td>
</tr>
</tbody>
</table>

* NLD=normolipemic diet, SFAED=saturated fatty acid-enriched diet, OLIV=olive oil.
* p=0.05 (vs. NLD); ** p=0.05 (vs. SFAED).

Morphological findings included a thinner vessel wall with fewer foam cells (Figures 3 and 4), i.e., changes that perhaps have been involved in the lower thrombogenicity in this group. Moreover, the fatty acid content in the vessel wall changed in animals that were fed with the olive-oil–supplemented diet (Table 5), as shown by the increase in C18:1 fatty acids. This finding, also found in normolipemic rabbits, was logical in view of the fact that this fatty acid is a major component of olive oil. In general, when olive oil is consumed there is a tendency for the proportion of polyunsaturated fatty acids in the arterial wall to decrease, although this reduction is not statistically significant. Nonetheless, it is important to note that there is a proportional decrease in saturated fatty acids. It has been shown [30] that dietary supplementation with linoleic acid displaces other fatty acids from the vessel wall. In our experimental model, dietary olive oil increased the amount of oleic acid in the arterial wall and also appeared to displace other saturated fatty acids while leaving polyunsaturated fatty acids unaffected. This change in the composition of the vessel wall is notable for three reasons: (1) Saturated fatty acids are atherogenic and favor platelet aggregation, decreasing prostacyclin production and increasing thromboxane production. They can thus be considered prothrombotic substances. (2) Polyunsaturated fatty acids reduce platelet activity and the thrombogenic capacity of the arterial wall [9,11,14]; it is therefore noteworthy that dietary olive oil did not significantly decrease

Table 5. Fatty acid composition (percentages) of aortic vascular tissue from the different groups of rabbits

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>NLD*</th>
<th>NLD + OLIV</th>
<th>SFAED</th>
<th>SFAED + OLIV</th>
</tr>
</thead>
<tbody>
<tr>
<td>C 14:0</td>
<td>4.1±0.4</td>
<td>1.5±0.3*</td>
<td>3.1±0.1</td>
<td>1.8±0.3**</td>
</tr>
<tr>
<td>C 16:0</td>
<td>41.3±2.2</td>
<td>25.5±1.0*</td>
<td>36.5±1.5</td>
<td>24.7±1.3**</td>
</tr>
<tr>
<td>C 18:0</td>
<td>8.6±0.6</td>
<td>5.8±0.4*</td>
<td>9.6±0.8</td>
<td>6.6±0.6**</td>
</tr>
<tr>
<td>C 18:1</td>
<td>28.3±0.5</td>
<td>51.6±2.2*</td>
<td>33.0±2.9</td>
<td>53.0±1.5**</td>
</tr>
<tr>
<td>C 18:2</td>
<td>18.0±1.9</td>
<td>15.3±2.6</td>
<td>15.9±1.6</td>
<td>13.6±1.7</td>
</tr>
<tr>
<td>C 18:3</td>
<td>ND</td>
<td>ND</td>
<td>0.03±0.01</td>
<td>ND</td>
</tr>
<tr>
<td>C 20:0</td>
<td>2.1±0.2</td>
<td>0.8±0.3*</td>
<td>1.3±0.4</td>
<td>1.2±0.1</td>
</tr>
<tr>
<td>MUFAs</td>
<td>26.9±0.8</td>
<td>51.6±2.2*</td>
<td>33.6±2.1</td>
<td>53.0±1.5**</td>
</tr>
<tr>
<td>SFAs</td>
<td>53.8±2.4</td>
<td>33.8±1.4*</td>
<td>50.5±2.1</td>
<td>34.5±1.7**</td>
</tr>
<tr>
<td>PUFAs</td>
<td>18.2±2.0</td>
<td>15.3±2.6</td>
<td>16.2±2.6</td>
<td>13.6±1.7</td>
</tr>
</tbody>
</table>

* NLD=normolipemic diet, SFAED=saturated fatty acid-enriched diet, OLIV=olive oil; ND=not detected; SFAs=saturated fatty acids; PUFAs=polyunsaturated fatty acids; MUFAs=monounsaturated fatty acids.
* p<0.05 vs. NLD, ** p<0.05 vs. SFAED.
the proportion of these fatty acids in the vessel wall. (3) The accumulation of monounsaturated fatty acids provides the arterial wall with an antioxidant agent [25,26]. Moreover, the polyphenols in olive oil also contribute to the effects described above [16].

In summary, dietary supplementation with olive oil in rabbits previously fed an atherogenic diet reduced prothrombotic activity in both of the components involved in thrombus formation. In platelets we found decreases in aggregation, thromboxane synthesis, and lipid peroxide formation; in the arterial wall, we found reduced thrombogenicity of the subendothelium and increased antithrombotic activity in the endothelium (i.e., increased prostacyclin synthesis and reduced lipid peroxide formation). These effects may be the result of a protective effect against the morphological lesions that the atherogenic diet caused in the vessel wall, a modification in the lipid composition of the arterial wall, or a combination of both.

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References


