A Preliminary Study on Platelet Aggregation in Postmenopausal Women Consuming Extra-Virgin Olive Oil and High-Oleic Acid Sunflower Oil

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ABSTRACT: The purpose of the present study was to examine the effects of two monounsaturated fatty acid-rich oils, extra-virgin olive oil (EVOO) and high-oleic sunflower oil (HOSO), on platelet aggregation in 14 postmenopausal women (aged 62.9 ± 1.8 yr) with high-fat dietary habits. Both oils contained oleic acid as the major compound (≈76% of total fatty acids), but the content of palmitic and linoleic acids and many minor compounds was significantly different. These oils were used as the only culinary fats during two 28-d periods, and represented ≈62% of the total lipid intake (≈46% of total energy consumption). Other dietary components were matched. The daily energy contribution of saturated, monounsaturated, and polyunsaturated fatty acids to the total energy consumption was 11.8, 28.5, and 2.8%, respectively, during the EVOO dietary period and 10.3, 27.8, and 4.6%, respectively, with HOSO. Aggregation in platelet-rich plasma was measured after addition of ADP. Platelet aggregation (expressed as cm/5 min) was significantly lower after the EVOO diet than after HOSO (2.1 ± 1.1 and 3.0 ± 1.4, respectively; $P < 0.05$). Although maximal aggregation time was 40.2% higher in HOSO than in EVOO, the difference was not significant. Independent of serum cholesterol level, platelet aggregation tended to be different on the EVOO diet when women were classified according to cholesterol levels: <220 mg/dL or ≥220 mg/dL. Results suggest that other compounds present in the oils aside from the fatty acids may play an important role in modulating platelet aggregation in these postmenopausal women.

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KEY WORDS: ADP, aggregation, cholesterol, diet, minor compounds, monounsaturated fatty acids, postmenopausal women.

Dietary fatty acids have been related to the occurrence of atherosclerosis and thrombosis, the pathological substrate for coronary heart disease (CHD) (1). A high intake of dietary saturated fatty acids (SFA) is associated with a high incidence of CHD. Saturated fats promote arterial thrombi formation in a dose-dependent manner; oleic acid and other monounsaturated fatty acids (MUFA) probably have no effect on arterial thrombosis, and linoleic acid is antithrombotic (2).

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Mediterranean populations are characterized by a low prevalence of CHD (3,4), despite consumption of diets in which about 40% of total energy derives from fat sources (5). It has been postulated that the apparent protection from CHD may be due to the high proportion of dietary MUFA, relative to SFA, consumed by these populations (6–8). Although the traditional source of dietary MUFA in these countries has been olive oil, other sources are now becoming available [i.e., low-erucic acid rapeseed oil (canola oil) and oleic acid-rich variants of sunflower or safflower oils]. Despite the fact that the fatty acid composition of these oils is quite similar, the oils do differ in the amount and type of plant sterols and other substances (e.g., polyphenols and vitamins).

Although these compounds may play a significant role in cholesterol metabolism and the peroxidative status of plasma and lipoproteins [some plant sterols reportedly compete with cholesterol for intestinal absorption, and polyphenols may protect low-density lipoproteins (LDL) against oxidation] (9), few studies in humans have examined the effect of compounds like sterols and polyphenols on platelet aggregation. Moreover, menopause is accompanied by an increase in the risk of CHD (10,11).

In the present study we compared the effects on platelet aggregation of two high-fat diets prepared with two different plant oils: extra-virgin olive oil (EVOO) and refined high-oleic sunflower oil (HOSO), both rich in MUFA but with a different palmitic and linoleic acid content. Because hypercholesterolemic patients present hyperaggregability and hypercoagulability (12), this study assessed whether the effects on platelet aggregation of both MUFA-rich diets depended on serum cholesterol levels.

MATERIALS AND METHODS

Subjects. A female religious order population was studied because of its methodical lifestyle and high-fat dietary habits. Fifteen women were initially enrolled in the study, but one woman was subsequently excluded because she was premenopausal. None of the participants presented previous cardiovascular, metabolic, or systemic disease or was taking any drugs that potentially affect lipid or platelet metabolism. The

TABLE 1
Population Characteristics: Religious Order of Postmenopausal Women[a]

Age (yr)	62.9 ± 11.2
Body weight (kg)	54.3 ± 9.3
Body mass index (kg/m^2)	23.2 ± 3.4
Serum cholesterol level (mmol/L)	6.4 ± 1.2
Serum triglycerides (mmol/L)	0.8 ± 0.2

[a]Values are the mean ± SD; n = 14.

study was performed in accordance with the Helsinki Declaration (13). All participants gave informed consent, and the protocol was approved by the Committee for Human Studies at the Universidad Complutense de Madrid. Some characteristics of this population are shown in Table 1. Participants were requested to live as they had before the study, maintaining their normal patterns of activity, and they were urged not to add any extra food to their usual meals. In addition, all diet trials were performed before Christmas (October–December) to avoid any extra food and to ensure temporal consistency. A peculiarity of their dietary habits was to exclude meat intake. Protein was derived from other sources, as we describe later. Another peculiarity was the relatively high intake of cholesterol from eggs and whole milk.

Methods. Dietary assessment. Before the experimental dietary period started, the regular diet in the community was assessed for 4 wk by using the precise weighing method (14). All ingredients used in the preparation of the dishes were weighed, as well as the inedible wastage. The cooked weight of the individual portions and table waste was also recorded by two investigators who were present daily in the community's kitchen during the preparation of the meals. Energy and nutrient intakes were calculated from food composition tables for the raw weights of the foods (15). Menus changed daily, were repeated every 2 wk, and consisted of common solid foods.

During the month the women's diet included fish (11 times), whole eggs (24 times), rice (3 times), pasta (9 times), and legumes (beans, lentils, etc.) (12 times). Fruit, fresh vegetables, bread, and whole milk were consumed daily. The absence of meat, meat products, and alcohol in the diet (Fig. 1), and the relatively high intake cholesterol should be noted. The average daily intake of cholesterol was 400 mg and of fiber, 16.5 g. About 55 g/d (62% of the total dietary lipids) of vegetable oil (a homogeneous mix of nonvirgin olive oil and conventional sunflower oil) was consumed. Lipids accounted for 90.1 g/d and represented 46% of the total energy consumption (Table 2).

Experimental design. The study design included an initial period in which the participating women were studied and divided in two groups of seven individuals each. Each group was assigned to one of the two 28-d dietary periods in a randomized crossover design. During the two consecutive experimental dietary periods, menus and individual ratios were kept constant with respect to the regular diet. The single distinguishing feature of the diets was the oil used: EVOO or HOSO.

Either EVOO or HOSO (Koype Co., Andujar, Spain) was

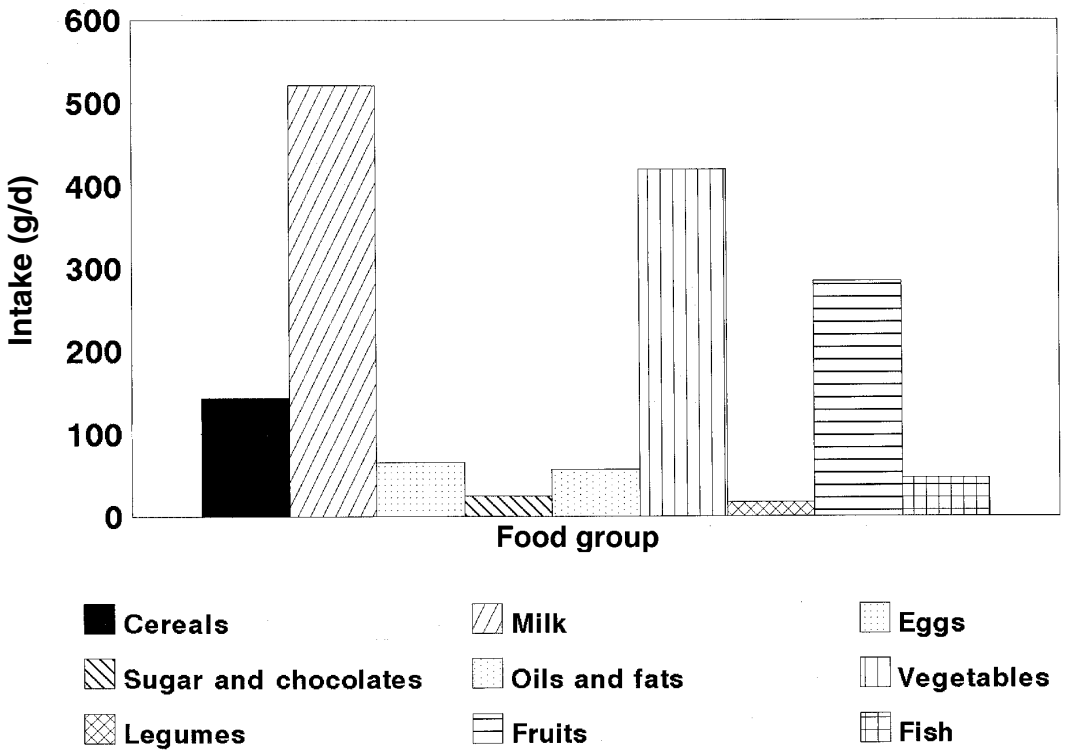

FIG. 1. Daily food intake of different food groups.

TABLE 2
Composition of Diets and Oil Used in the Study[a]

	Extra-virgin olive oil diet	High-oleic acid sunflower oil diet	Extra-virgin olive oil (% total fatty acids)	High-oleic sunflower oil (% total fatty acids)
Energy (KJ/d)	7328 ± 773	7328 ± 773		
Protein (% of energy)	11.7 ± 0.4	11.7 ± 0.4		
Carbohydrates (% of energy)	42.0 ± 3.0	42.0 ± 3.0		
Lipids (% of energy)	46.4 ± 3.0	46.4 ± 3.0		
Saturated fatty acids (% of energy)	11.8 ± 1.7	10.3 ± 1.7	13.4	11.0
Monounsaturated fatty acids (% of energy)	28.5 ± 1.7	27.8 ± 1.6	79.1	77.5
Polyunsaturated fatty acids (% of energy)	2.8 ± 0.1	4.6 ± 0.2	7.5	11.5
Cholesterol (mg/d)	401.4 ± 33.9	401.4 ± 33.9		
Fiber (g/d)	16.5 ± 2.1	16.5 ± 2.1		
Linoleic acid (g/d)	4.2 ± 0.4*	7.8 ± 0.7*	6.8	11.5
(% of energy)	2.2 ± 0.1*	4.0 ± 0.2*		
Linolenic acid (g/d)	0.7 ± 0.1	0.6 ± 0.1		
(% of energy)	0.4 ± 0.0	0.3 ± 0.0		
Oleic acid (g/d)	53.3 ± 4.6	52.4 ± 4.5	76.3	76.4
(% of energy)	27.5 ± 1.6*	27.0 ± 1.6*		
Palmitic acid (g/d)	12.8 ± 1.6*	9.7 ± 1.5*	9.6	4.7
(% of energy)	6.6 ± 0.7*	5.0 ± 0.7*		
Stearic acid (g/d)	5.2 ± 0.7	5.5 ± 0.7	3.2	4.5
(% of energy)	2.7 ± 0.3	2.8 ± 0.3		
α-Tocopherol (mg/kg)	ND	ND	193.0	538.0
Total polyphenols (mg/kg)	ND	ND	108.0	25.0
Δ^5-Avenasterol (mg/kg)	ND	ND	120.2	66.6
Squalene (g/kg)	ND	ND	4.6	0.5

[a]Values are means ± SD. ND, not determined. Values in the same row bearing an asterisk are significantly different ($P < 0.05$).

used for cooking and salad dressing. In both experimental periods the dietary assessment was performed as already described.

Both experimental diets had the same energy contents and the same amounts of cholesterol and macronutrients. Lipids accounted for 46% of the total energy consumed daily (Table 2). Although the fatty acid profile of both diets can be considered rather similar, linoleic acid consumption was 84.9% higher in the HOSO period. MUFA account for a high proportion of fatty acids in both diets (≈28% of total energy).

Body weight was measured twice a week, and energy intake was adjusted as needed to maintain the initial body weight.

Fatty acid analysis. Oil samples were saponified with 0.5 M NaOH (40 mL/g), and then methylated with boron trifluoride by following the IUPAC method (16). The fatty acid content of the oils was analyzed in a Hewlett-Packard 5890 Series II gas chromatograph (Palo Alto, CA), equipped with a 50-m, i.d. 0.22 mm capillary column, BPX70, 0.25 μm film thickness (SGE, Austin, TX). The fatty acid composition of both oils is shown in Table 2.

Minor compound analysis. α-Tocopherol was determined by high-performance liquid chromatography on a reverse-phase column, following the method of Hess *et al*. (17). Total polyphenols were determined by the method of Folins-Denis, described by AOAC (18). The concentration of other compounds, such as squalene and Δ^5-avenasterol, was determined in the unsaponifiable fraction of the oils by gas chromatography (16).

Blood sampling and biochemical determinations. At the conclusion of each period, blood samples were taken, following a 12-h overnight fast, by venipuncture into 3.8% trisodium citrate [9:1 (vol/vol), blood/citrate]. The anticoagulated blood was centrifuged at 200 × *g* for 10 min to prepare platelet-rich plasma (PRP). Platelet counts on PRP samples were done in a hemocytometer, diluting PRP with saline solution. No platelet adjustment was needed before analysis because differences between platelet counts on PRP samples from both dietary periods were nonsignificant. Platelet aggregation was determined by the Cardinal and Flower technique (19) with an electronic aggregometer (model 500, Chronolog Corporation, IZASA-Coulter, Havertown, PA). One aggregation measurement per subject in PRP was performed after addition of 50 μL of 10 μM ADP (Chromopag ADP, IZASA) as aggregating agent. The data were expressed as the extent or rate of aggregation at 5 min (cm/5 min), and the time of the maximal aggregation (min) (19).

Serum cholesterol was determined by the enzymatic cholesterol esterase–cholesterol oxidase method (Boehringer Mannheim, Mannheim, Germany). Triglycerides were determined according to the enzymatic glycerol–phosphate oxidase method (Boehringer Mannheim).

Statistical analysis. The data, presented in text and tables as mean ± SD, were analyzed by a paired Student's *t*-test to evaluate the level of statistical significance. Differences in aggregation parameters between normo- and hypercholesterolemic women were studied with the unpaired Student's *t*-test. Differences in fatty acid contribution between diets

were evaluated with the nonparametric rank sum test of Wilcoxon.

RESULTS AND DISCUSSION

Platelet aggregation (expressed as cm/5 min) was significantly lower ($P < 0.05$) in the women consuming EVOO than in the HOSO group: 2.1 ± 1.1 for EVOO and 3.0 ± 1.4 for HOSO, respectively. A detailed picture of the results from each woman is also shown (Fig. 2). The differences in individual response between the two study periods show that a large proportion (about 80%) of the women showed lower aggregation at 5 min after the EVOO period than after the HOSO.

The maximal aggregation time shows great variability, although in the women consuming HOSO the mean level was 40.2% higher than in those who consumed EVOO. A detailed picture of the results from each woman is also shown (Fig. 3). Results suggest a tendency of platelets to aggregate earlier in the EVOO consumers than after HOSO consumption.

Linoleic acid accounted for 2.2% of total energy intake on the EVOO diet, whereas it represented 4.0% on HOSO. The effect of polyunsaturated fatty acids on platelet aggregation seems to be controversial and highly linoleic acid dose-dependent. In rabbits, Galli *et al.* (20) found that, up to a certain percentage of the total energy intake, from 0 to 5 or 6%, linoleic acid enhances the formation of eicosanoids derived from arachidonic acid. Burri *et al.* (21) observed no differences in platelet aggregation in humans that were fed diets containing linoleic acid at 7.2 or 4.2% of total energy intake. In addition, it has been shown that, by feeding rabbits high levels of linoleic acid (around 10% of the total energy intake), the formation of eicosanoids is depressed. According to Galli (22), as a consequence of high linoleic acid intake, arachidonic acid is replaced in platelets by the excess linoleic acid. Thus, the arachidonic/linoleic acid ratio in platelet phospholipids is lower than under conditions of low dietary linoleic acid. This is due to lack of the desaturase–elongase–desaturase system in platelets, which transforms linoleic acid in arachidonic acid, and to the replacement of arachidonic acid by linoleic acid in the platelet membrane phospholipids. In a related publication (23) we described how the same women under the same experimental conditions displayed an 18.8% higher thromboxane B_2 (TXB_2) level on the HOSO diet than on EVOO. Srivastava (24) indicated that linoleic acid at low concentrations decreased the *in vitro* cyclooxygenase enzyme, TXB_2, and prostaglandin production by human platelets, whereas, at high linoleic acid concentrations (400–500 μM), the cyclooxygenase and thromboxane synthetase activities were decreased but prostacycline (PGI_2) synthesis was increased. Nevertheless, Judd *et al.* (25) observed significant reductions in the excretion of 13,14-dihydro-15-keto-$PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$, but not of TXB_2, by decreasing the linoleate intakes of the subjects from about 6.5% of energy to 3.2%.

Moreover, the aggregation was lower in the EVOO period with respect to HOSO, despite the fact that the contribution to total energy of palmitic acid was higher in the EVOO diet because saturated fats promote arterial thrombi formation in a dose-dependent manner (2).

These results probably cannot be attributed only to differences in the fatty acid composition of the oils and diets. Our findings suggest that other minor constituents in the oils may account for some of the differences in platelet aggregation observed. We have selected two minor constituents, vitamin E and polyphenols, because we found some related data in the literature. Many clinical studies aimed at increasing the resis-

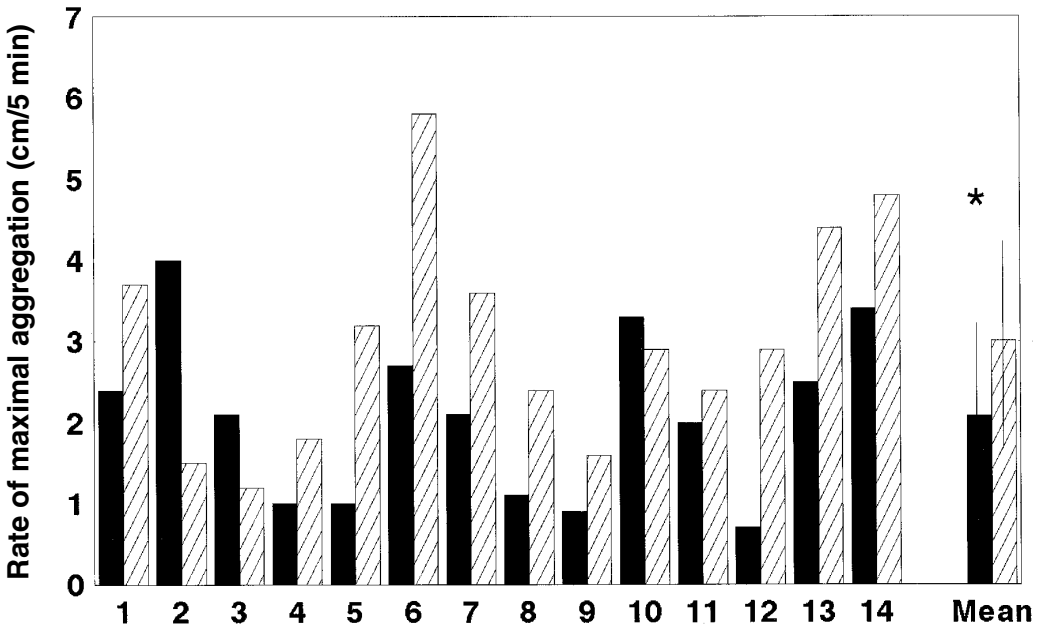

FIG. 2. Rate of maximal aggregation at 5 min (cm/5min) of 14 postmenopausal women consuming extra-virgin olive oil (EVOO, ■) and high-oleic sunflower oil (HOSO, cross-hatched bar). *EVOO vs. HOSO, $P < 0.05$.

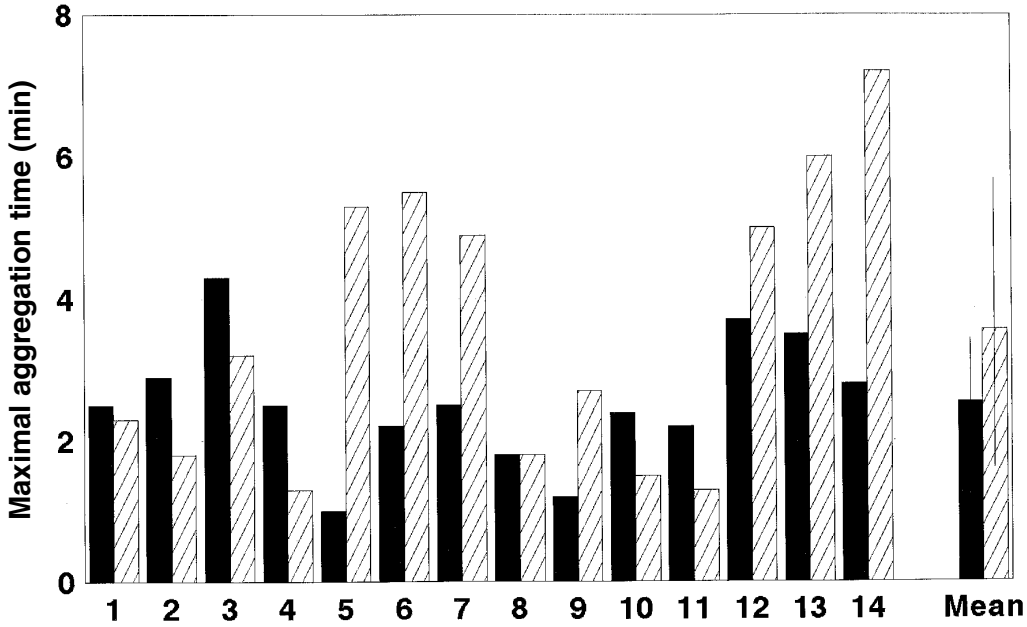

FIG. 3. Maximal aggregation time (min) of 14 postmenopausal women consuming EVOO and HOSO. For abbreviations and key see Figure 2.

tance of plasma and LDL to oxidation have used vitamin E supplementation, either singly (26) or in combination with β-carotene and/or vitamin C (27,28). HOSO contains about 2.6 times more α-tocopherol than EVOO. Nevertheless, it is interesting to consider that vitamin E has no effect on platelet aggregation, even at the concentration 10^{-3} M and for a longer incubation time, as described by Agradi *et al.* (29).

The concentration of polyphenols in extra-virgin olive oil was about 110 mg/kg. Polyphenols are much lower in quantity in HOSO (Table 2), owing to refining processes that destroy a large amount of these compounds.

The great stability of olive oil toward oxidative processes has been attributed to the presence of potent antioxidants of phenolic nature. 2(3,4-Dihydroxyphenyl) ethanol (DHPE), oleuropeine glycoside, and (*p*-hydroxyphenyl) ethanol are the most abundant phenols (30), compounds that are well known for their antioxidant properties. Nevertheless, the biological activities of these compounds are poorly known.

Petroni *et al*. (31) found that the incubation of PRP with DHPE for 10 min before collagen stimulation inhibited, in a dose-dependent manner, the PRP aggregation. Moreover, preincubation of blood with DHPE before collagen stimulation resulted in about a 70% reduction of the TXB_2 level.

The presence of a relatively high hydroxytyrosol content has been demonstrated in extra-virgin olive oil. This compound has also been demonstrated to possess free radical scavenging properties and to be able to quench both the hydroxyl radical and the peroxyl radical antioxidants (32), which would increase resistance to oxidation as determined by the *in vitro* conjugated diene assay. Thus, the lower aggregation after EVOO intake, compared to HOSO intake, would also be attributable to the presence of other natural minor compounds, such as polyphenols.

However, the differences in the content of other minor compounds, such as Δ^5-avenasterol and squalene, in both oils (Table 2) would also have a role in the different aggregation patterns seen after consuming these oils. The effect of different microconstituents of such oils upon aggregation in humans should also be tested in future studies.

Platelets are activated by a variety of agonists: collagen, thrombin, ADP, serotonin, and others. However, LDL particles can also act as platelet agonists. Platelets are able to bind LDL particles to specific receptors in the platelet membrane without internalization. Aviram and Brook (32) found increased ADP-induced aggregation and serotonin release of platelets in patients with type II_a and II_b hyperlipoproteinemia. They also found that ADP-induced aggregation and serotonin release were increased when washed platelets were incubated with isolated very low density lipoproteins and LDL, but decreased in the presence of high-density lipoproteins.

The present study also shows the possible influence of serum cholesterol levels on the two aggregation parameters tested: (i) the rate of aggregation at 5 min and (ii) the time of maximal aggregation When the population was divided into two groups of seven women each according to serum cholesterol levels, less than 220 mg/dL (normocholesterolemics) and more than 220 mg/dL (hypercholesterolemics), the same effect was found. On EVOO diet, platelets tended to aggregate earlier but less intensively than on the HOSO (Table 3). However, when comparisons for the same oil were made, no differences or tendencies were found for the EVOO diet. After consuming HOSO, the platelets of hypercholesterolemic women tended to aggregate earlier and more intensively than those of normocholesterolemics. Once again, these results suggested differences between the oils. Never-

TABLE 3
Platelet Aggregation and Serum Cholesterol Levels[a]

	Extra-virgin olive oil		High-oleic acid sunflower oil	
	<220 mg/dL	≥220 mg/dL	<220 mg/dL	≥220 mg/dL
Rate of aggregation (cm/5 min)	2.0 ± 1.1	2.2 ± 1.2	2.8 ± 1.4	3.2 ± 1.5
Time of maximal aggregation (min)	2.7 ± 1.2	2.6 ± 0.7	3.9 ± 2.3	3.0 ± 1.8

[a]Values are means ± SD.

theless, no significant or relevant effect of the serum cholesterol level upon the level of platelet aggregation was found. More studies must be carried out to understand the differences found between EVOO and HOSO and to assess the role of some minor compounds present in EVOO and other oils on platelet metabolism.

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