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Role of dietary oleic acid from two different sources on fatty acid composition of erythrocyte membrane and blood pressure in healthy subjects

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This study has been undertaken to determine the effect of a diet enriched with olive oil (OO) and high-oleic sunflower oil (HOSO) on fatty acid composition of erythrocyte membrane phospholipids and blood pressure in healthy women. OO and HOSO were used as natural sources of monounsaturated fatty acids (MUFAs) in a random-order sequence over two 4-week periods with a 4-week washout period between both MUFA diets. HOSO diet resulted in significant increases in oleic [(18:1n-9) 8.6%, $P < 0.001$], eicosenoic [(20:1n-9) 33.3%, $P < 0.05$], arachidonic [(20:4n-6) 6.2%, $P < 0.05$], and docosapentaenoic [(22:5n-6) 56.0%, $P < 0.001$] acids, whereas OO diet besides increased the content of stearic acid [(18:0) 13.6%, $P < 0.01$] and long-chain polyunsaturated fatty acids (PUFAs) of the n-3 family (22:5n-3 and 22:6n-3), when compared with the baseline [a diet high in saturated fatty acids (SFAs) and low in MUFAs]. In contrast, there was a significant decrease in linoleic acid [(18:2n-6) 21.8%, $P < 0.001$] for both MUFA diets. Consistent with these data, dietary intake of OO significantly raised total PUFAs (7.2%, $P < 0.05$), the n-3 fatty acids (22.2%, $P < 0.01$) and the PUFAs/SFAs ratio (9.3%, $P < 0.01$), as well as decreased the ratio of cholesterol to phospholipids (26.1%, $P < 0.001$) versus HOSO-based diet. Interestingly, dietary OO, but not HOSO, was able to significantly reduce the systolic (3%, $P < 0.05$) and diastolic (4%, $P < 0.05$) blood pressures. Although both vegetable oils provided a similar content of MUFAs (mainly oleic acid), our findings rather indicate that OO has important benefits to modulate the fatty acid composition of membranes and the mechanisms involved in the regulation of blood pressure in human. (J. Nutr. Biochem. 8:689–695, 1997) © *Elsevier Science Inc. 1997*

Keywords: membrane lipids; fatty acid composition; blood pressure; monounsaturated fatty acids; olive oil; high-oleic sunflower oil; dietary fat

Introduction

Intake of olive oil (OO), as a source of monounsaturated fatty acids (MUFAs), has been one of the proposed strategies for lowering the concentration of plasma cholesterol in humans, particularly when it replaces saturated fatty acids (SFAs).[1,2] It is well established that a positive correlation does exist between elevated plasma total cholesterol or low-density-lipoprotein (LDL) cholesterol concentrations and the prevalence of coronary heart disease, whereas an inverse correlation with high-density-lipoprotein cholesterol concentration.[3,4] Indeed, diets supplemented with OO are reported to have beneficial effects on plasma lipids and lipoprotein profile in healthy subjects[5–7] and hyperlipidemic patients.[8]

It is equally well known that membrane lipids seem to be in equilibrium with plasma lipids,[9] which in turn may perturb bulk lipid fluidity[10] or specific lipid domains[11,12] and important cellular physical and biochemical functions.[13,14] In this regard, the effects of dietary supplemen-

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tation with polyunsaturated fatty acids (PUFAs) of n-3 family (20:5n-3 and 22:6n-3), such as those found in marine fish oils, have been fully investigated and characterized by an increase in membrane n-3/n-6 ratio and a selective attenuation of age-related membrane changes in human erythrocytes.[15] Additionally, meta-analysis of controlled trials indicates a hypotensive effect of n-3 PUFAs.[16,17] It is interesting to note there is also similarity on the value of MUFAs as compared with lipid-lowering capacities of n-3 or n-6 PUFAs.[2,3,18] These findings raise the question whether ingestion of a MUFA-rich diet may modulate fatty acid composition of membranes and blood pressure in healthy subjects. A recent study[19] has demonstrated that OO, when compared with an oleic acid-rich variant of sunflower oil (HOSO), is helpful for normalizing the impaired distribution of membrane cholesterol and reducing elevated activity of Na^+-Li^+ countertransport in erythrocyte of patients with untreated essential hypertension. More importantly, OO (but not HOSO) was able to significantly reduce the blood pressure in hypertensive patients.[20]

The aim of the present study was to determine the effects on erythrocyte membrane fatty acid composition and blood pressure in healthy subjects of a diet rich in OO compared with those of refined HOSO diet, both similar in MUFAs composition. This would help to facilitate more precise recommendations with regard the intake of dietary fats, preventing or delaying the appearance of cardiovascular risk factors.

Methods and materials

Study design

The study was conducted over two 4-week periods, during which each participant followed in a random-order sequence HOSO (a kindly gift from COREYSA, Seville, Spain) or OO (kindly provided by Cooperativa Nuestra Señora de Guadalupe, Córdoba, Spain) diets, with a washout (4-week) period between both MUFA diets. Half of the subjects started with HOSO first. Participants were recruited from a religious communities in Seville (Spain), because of their regular lifestyle and dietary habits. Before the study, the participants recorded their regular dietary intake on four consecutive weeks (baseline), using 24-hr recall and food frequency questionnaires. All dishes were prepared in the same kitchen and planned as 1-week menu for each participant, who was told what food items should be eaten for breakfast, lunch, dinner, and in-between meals. No other food items except water, mineral water, coffee, and tea were allowed to be consumed during the study periods. All participants were asked to record in a diary any event that could affect the outcomes. On admission and during the last days of the treatment periods assessment of biochemical and clinical indices were performed. The washout period that lasted 4-week was enough to ensure re-establishment of initial conditions, i.e., blood pressures (systolic and diastolic) were almost identical at the commencement of each dietary period. The design of the present study was approved by the Institutional Committee on Investigation in Humans (Hospital Universitario Virgen del Rocío, Seville) and all participants gave informed consent.

Subjects

Twelve healthy female volunteers 54 ± 3 years (body mass index, 25.7 ± 3.7 kg/m^2) were enrolled for this study. All of them were postmenopausal to avoid interference related to sexual hormones and hormonal cycle. Criteria for inclusion were that the participants should be reliable and have a regular meal pattern. None of them had received any drug that affected to lipid metabolism. All of them were nonsmokers and no history of alcohol abuse was presented. Throughout the study the participants were free-living. They had a systolic blood pressure (SBP) of < 140 mm Hg and diastolic blood pressure (DBP) of < 90 mm Hg, total plasma cholesterol concentration (TC) of < 5.18 mmol/L, LDL-cholesterol concentration of <3.37 mmol/L, and were in excellent health as defined by clinical evaluation and laboratory tests.

Diets

Diets were based on ordinary food and planned as 1-week menus. The only difference between the diets lay in the edible fats, which were in the form of oils (Virgin olive oil: *Olea europaea*; High-oleic sunflower oil: *Helianthus annuus*) for cooking and for salads and occasionally spread on bread slices. Fatty acid content, nonfatty acid constituents, and triacylglycerol composition of two oils are given in *Tables 1*, *2*, and *3*, respectively. The analysis of fatty acid composition in oils was performed by gas chromatography as described below for fatty acid composition of erythrocyte membrane total lipids. In the nonfatty acid constituents, we fully determined total sterols and squalene by gas chromatography, after extraction of the unsaponifiable fraction of the oils.[21,22] Triacylglycerol composition of oils was determined as described previously.[23] The energy content and the amounts of protein, carbohydrate, cholesterol, and dietary fiber were similar in the diets. Dietary instructions were given by a dietitian before entry into the study. Three duplicate food portions corresponding to each weekday were collected and homogenized to be analyzed for their fat content and other nutrients. Fatty acid content of both MUFA diets (30% fat, 6% SFAs, 21% MUFAs, and 3% PUFAs) was characterized by a lower amount of SFAs (mainly palmitic and stearic acid) and higher amount of oleic acid with regard to baseline (30% fat, 11% SFAs, 16% MUFAs, and 3% PUFAs). The average daily linoleic acid intake was 50, 10, and 5% of energy from PUFAs, at baseline and during the periods on the HOSO and OO diets, respectively. While the average daily n-3 PUFAs intake was 5, 0.5, and 1% of energy from total PUFAs, at baseline and during the periods on the HOSO and OO diets, respectively. The energy consumption (daily caloric intake) of participants was ~8.6 ± 1.4 MJ (~2056 kcal) at baseline, ~8.8 ± 1.1 MJ (~2103 kcal) during the period on the HOSO diet and ~8.7 ± 1.9 MJ (~2094 kcal) during the period on the OO diet. Two investigators were present twice a week in the kitchen during the preparation of the meals and remained blinded along with the subjects to changes in fatty acid composition of erythrocyte membrane phospholipids and blood pressure. The other investigators conducted most laboratory analyses and were blinded to the dietary assignments. Sodium intake was similar on the baseline and MUFA-enriched diets.

Plasma lipid and lipoprotein analyses

Venous blood was obtained after an overnight fast, at the beginning and end of each phase of the study. Plasma samples obtained by centrifugation were individually analyzed fresh. Cholesterol and triacylglycerol concentrations were determined by conventional enzymatic methods.[24,25] High- and low-density lipoproteins were isolated by using a combination of preparative ultracentrifugation[26] and precipitation with polyethylene glycol.[27]

Lipid and fatty acid analyses in erythrocytes

Fasting blood samples were collected in heparinized tubes and the erythrocytes were separated and washed four times with 150 mol/L NaCl at 800 × *g* for 10 min (4°C). Total lipids from erythrocyte

Table 1 Fatty acid composition of high-oleic sunflower oil (HOSO) and olive oil (OO)

	HOSO	OO
	%	
16:0	4.30	11.79
16:1n-7	0.14	0.86
17:0	0.13	0.37
18:0	4.72	2.79
18:1n-9	80.18	79.22
18:2n-6	9.44	3.45
18:3n-3	0.06	0.60
20.0	0.44	0.28
20:1n-9	0.22	0.20
24:0	0.37	0.44

membranes were extracted by the method of Rose and Oklander,[28] using 2,6-di-ter-butyl-*p*-cresol as an antioxidant.

For analysis of fatty acids, samples of erythrocyte membrane lipids were saponified by heating for 5 min with 5 mL of 0.2 mol/L sodium methylate and heated again at 80°C for 5 min with 6% (w/v) H_2SO_4 in anhydrous methanol. The fatty acid methyl esters thus formed were eluted with *n*-hexane and analyzed in a Hewlett-Packard 5890 series II gas chomatograph equipped with flame ionization detector and using a Omegawax 320 fused silica capillary column (30 m × 0.32 mm inner diameter, 0.25 μm film). The initial column temperature was 200°C, which was held for 10 min, then programmed from 200 to 230°C at 2°C/min. The injection and detector temperatures were 250°C and 260°C, respectively. The flow-rate of helium was 2 mL/min, the column head pressure was 250 kPa, and the detector auxiliary flow rate was 25 mL/min. Peak areas were calculated by a Hewlett-Packard 3390A recording integrator. Individual fatty acid methyl esters were identified on isothermal runs by comparison of their retention time against those of standards. Fatty acid methyl esters for which no standard was available were quantified using calibration tables of relative response ratios constructed according to carbon number (using Gas Chromatography-Mass Spectrometry, GC-MS). GC-MS was performed on a Konik KNK-2000 chromatograph interfaced directly to an AEJ MS30/70 VG mass spectrometer, using the electron impact mode. The ion source temperature was maintained at 200°C, multiplier voltage was 4.0 kV, emission current was 100 μA, and electron energy was 70 eV. The data were processed with a VG 11/250 data system.

Blood pressure measurements

Blood pressure measurements were performed at the right brachial artery in seated participants using a mercury-gauge sphygmomanometer. At each visit, three blood pressure readings were recorded and the average was used to determine eligibility. In addition, blood pressure was recorded at the beginning and end of each period of MUFA diet.

Statistical analyses

Data were evaluated by using a two-tailed paired *t*-test. The significance of the differences between the groups were assessed by analysis of variance (ANOVA) with Tukey's post-hoc comparison of the means. The analyses were done with the GraphPAD InStat (GraphPAD Software, San Diego, CA, USA) and CoStat (CoHort Software, Berkeley, CA, USA) statistical packages.

Table 2 Composition in nonfatty acid constituents of high-oleic sunflower oil (HOSO) and olive oil (OO)

	HOSO	OO
Unsaponifiable matter (%)	1.3	1.2
Total sterols (mg/kg)	3300	1400
Campesterol (%)	10.3	4.6
Stigmasterol (%)	13.6	2.5
β-sitosterol (%)	58.1	84.0
Δ-5-avenasterol (%)	2.9	5.9
Δ-7-avenasterol (%)	3.3	2.2
Δ-7-stigmastenol (%)	11.8	0.7
Squalene (mg/kg)	310	4800

Results

All participants responded in a similar manner to the two diets and completed the study according to schedule. Compliance with the diets was estimated to be ~90% from the evaluation of daily food questionnaires and by analysis of the fatty acid composition of the plasma cholesterol ester fraction in each subject.[29] There was a significant increase of oleic acid during MUFA-enriched diets, suggesting good adherence to the diets. Body weight was maintained during both MUFA dietary periods.

Oleic acid (18:1n-9) was the major fatty acid similarly found in OO and HOSO (*Table 1*). However, OO was enriched in palmitic acid (16:0) and α-linolenic acid (18:3n-3), whereas HOSO was enriched in linoleic acid (18:2n-6). Important differences were noted in the amount of nonfatty acid constituents between OO and HOSO (*Table 2*). There was a significant lower content of total sterols in OO than in HOSO, β-sitosterol being the most abundant sterol present in these oils (84% for OO and 58% for HOSO), whereas the content of squalene was significantly greater in OO than in HOSO. With regard to composition in triacylglycerols (TAGs) of oils (*Table 3*), OOO (*sn*-glyce-

Table 3 Average triacylglycerol (TAG) composition of high-oleic sunflower oil (HOSO) and olive oil (OO)

	HOSO	OO
	%	
POP + PLS	0.23	3.12
POS	0.35	1.42
POO	9.63	29.45
PLO + PoOO	2.05	4.33
SOO	10.32	5.27
OOO	63.21	45.82
OLO	6.71	6.54
Others	7.50	4.05

Nomenclature of fatty acids: P, palmitic acid, hexadecanoic acid, 16:0; O, oleic acid, *cis*-9 octadecenoic acid, 18:1n-9; L, linoleic acid, *cis*-9,12 octadecadienoic acid, 18:2n-6; S, stearic acid, octadecanoic acid, 18:0; Po, palmitoleic acid, *cis*-9 hexadecenoic acid, 16:1n-9.
Nomenclature of triacylglycerols: POP, *sn*-glycerol-palmitate-oleate-palmitate; PLS, *sn*-glycerol-palmitate-linoleate-stearate; POS, *sn*-glycerol-palmitate-oleate-stearate; POO, *sn*-glycerol-palmitate-oleate-oleate; PLO, *sn*-glycerol-palmitate-linoleate-oleate; PoOO, *sn*-glycerol-palmitoleate-oleate-oleate; SOO, *sn*-glycerol-stearate-oleate-oleate; OOO, *sn*-glycerol-trioleate; OLO, *sn*-glycerol-oleate-linoleate-oleate.

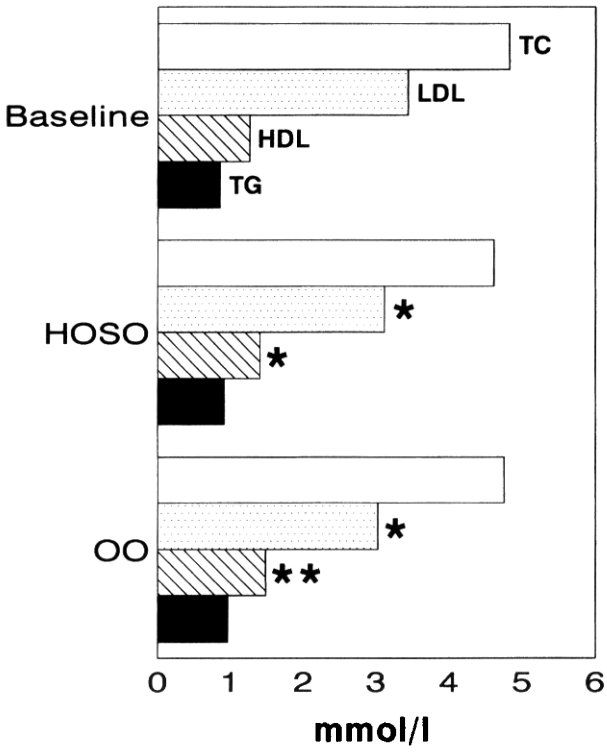

Figure 1 Plasma lipid and lipoprotein concentrations of healthy women at baseline and during the periods on the high-oleic sunflower oil-rich (HOSO) and olive oil-rich (OO) diets. TC, total cholesterol; LDL, low-density lipoprotein cholesterol; HDL, high-density lipoprotein cholesterol; triacylglycerols. Significantly different from baseline diet: $^{*}P < 0.05$, $^{**}P < 0.01$.

rol-trioleate) was the major found in HOSO (63% of the total) and OO (46% of the total). POO (*sn*-glycerol-palmitate-oleate-oleate) was also signified as abundant in OO, making up 30% of the total.

HOSO and OO diets were able to significantly increase plasma HDL-cholesterol (11.1%, $P < 0.05$ and 17.5%, $P < 0.01$, respectively) and to significantly decrease plasma LDL-cholesterol (9.3%, $P < 0.05$ and 11.9%, $P < 0.05$, respectively) (*Figure 1*). However, no significant changes were found for plasma total cholesterol and triacylglycerols.

The fatty acid composition of erythrocyte membrane at the end of the MUFA diets is depicted in *Table 4*. When compared with the baseline, HOSO diet resulted in significant increases in oleic acid (8.6%, $P < 0.001$), eicosenoic acid [(20:1n-9) 33.3%, $P < 0.05$], arachidonic acid (6.2%, $P < 0.05$) and docosapentaenoic acid [(22:5n-6) 56.0%, $P < 0.001$]; in contrast, there was a significant decrease in linoleic acid (21.8%, $P < 0.001$). It tended to increase total MUFAs (7.8%, $P < 0.05$) and the 18:1n-9/18:2n-6 ratio (29.3%, $P < 0.001$), and to decrease total PUFAs (5.8 %, $P < 0.05$), the n-6 fatty acids (6.5%, $P < 0.01$) and the ratio of PUFAs to SFAs (6.5%, $P < 0.01$) of erythrocyte membrane from healthy subjects. OO diet induced a more marked changes, as there was a significant increase in stearic acid (13.6%, $P < 0.01$), oleic acid (7.2%, $P < 0.001$), arachidonic acid (12.9%, $P < 0.001$), docosapentaenoic acids [(22:5n-6) 88.0%, $P < 0.001$; (22:5n-3) 12.9%, $P < 0.05$] and docosahexaenoic acid [(22:6n-3)

Table 4 Fatty acid composition in erythrocyte membrane of healthy women at baseline and during the periods on the high-oleic sunflower oil-rich (HOSO) and olive oil-rich (OO) diets

	Baseline	HOSO	OO
14:0	2.81 ± 0.28	2.86 ± 0.43	0.50 ± 0.12[c,f]
16:0	24.26 ± 0.96	24.87 ± 0.98	23.38 ± 1.41
16:1n-7	0.42 ± 0.22	0.46 ± 0.27	0.41 ± 0.27
18:0	14.81 ± 1.08	14.98 ± 1.01	16.83 ± 1.92[b,e]
18:1n-9	15.92 ± 1.38	17.29 ± 0.98[c]	17.06 ± 1.12[c]
18:1n-7	1.09 ± 0.14	1.07 ± 0.08	1.01 ± 0.11
18:1t	0.18 ± 0.08	0.09 ± 0.05	0.10 ± 0.02
18:2n-6	14.38 ± 0.97	11.25 ± 1.09[c]	11.24 ± 0.89[c]
20:0	0.28 ± 0.05	0.29 ± 0.06	0.18 ± 0.05[b,e]
20:1n-9	0.27 ± 0.08	0.36 ± 0.01[a]	0.28 ± 0.03[d]
20:3n-6	1.43 ± 0.19	1.54 ± 0.25	1.51 ± 0.32
20:4n-6	13.60 ± 0.94	14.44 ± 0.62[a]	15.35 ± 1.43[c,e]
22:0	0.48 ± 0.19	0.57 ± 0.21	0.41 ± 0.15
22:4n-6	3.25 ± 0.48	3.22 ± 0.78	3.27 ± 0.44
22:5n-6	0.50 ± 0.11	0.78 ± 0.13[c]	0.94 ± 0.38[c]
22:5n-3	1.32 ± 0.15	1.26 ± 0.16	1.49 ± 0.20[a,d]
22:6n-3	4.99 ± 0.44	4.91 ± 0.46	6.05 ± 0.54[a,d]
SFAs	42.64 ± 1.40	43.57 ± 0.92	41.30 ± 2.08[d]
MUFAs	17.88 ± 1.16	19.27 ± 0.69[a]	18.85 ± 1.39[a]
PUFAs	39.47 ± 1.37	37.16 ± 1.18[a]	39.85 ± 2.28[d]
n-6	33.16 ± 0.74	30.99 ± 1.32[b]	32.31 ± 1.36
n-3	6.31 ± 0.54	6.17 ± 0.56	7.54 ± 0.98[b,e]
18:1n-9/18:2n-6	1.16 ± 0.12	1.50 ± 0.11[c]	1.48 ± 0.11[c]
PUFAs/SFAs	0.92 ± 0.03	0.86 ± 0.02[b]	0.94 ± 0.09[e]
n-6/n-3	5.21 ± 0.48	5.10 ± 0.50	4.34 ± 0.38[b,e]

Values are expressed as percentages ± SD; $n = 12$.
[a]Significantly different from baseline diet; $P > 0.05$.
[b]Significantly different from baseline diet; $P < 0.01$.
[c]Significantly different from baseline diet; $P < 0.001$.
[d]Significantly different from HOSO diet; $P < 0.05$.
[e]Significantly different from HOSO diet; $P < 0.01$.
[f]Significantly different from HOSO diet; $P < 0.001$.
SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids.

21.2%, $P < 0.05$]; whereas a significant decrease was observed in myristic acid (82.2%, $P < 0.001$), linoleic acid (21.8%, $P < 0.001$) and arachidic acid [(20:0) 35.7%, $P < 0.01$]. Consistent with this was an increase in total MUFAs (5.4%, $P < 0.05$), the n-3 fatty acids (19.5%, $P < 0.01$), and the 18:1n-9/18:2n-6 ratio (27.6%, $P < 0.01$), and a decrease in the ratios of n-6 to n-3 fatty acids (16.7%, $P < 0.01$). When both MUFA diets were compared, significantly higher contents in stearic acid (12.3%, $P < 0.01$), arachidonic acid (6.3%, $P < 0.01$), docosapentaenoic acid [(22:5n-3) 18.3%, $P < 0.05$] and docosahexaenoic acid [(22:6n-3) 23.2%, $P < 0.05$], and significantly lower contents in myristic acid (82.5%, $P < 0.001$), arachidic acid [(20:0) 37.9%, $P < 0.01$] and eicosenoic acid [(20:1n-9) 22.2%, $P < 0.05$], were found after OO diet. Consumption of OO diet also significantly enhanced total PUFAs (7.2%, $P < 0.05$), the n-3 fatty acids (22.2%, $P < 0.01$), and the PUFAs/SFAs ratio (9.3%, $P < 0.01$), as well as decreased total SFAs (5.2%, $P < 0.05$) and the n-6/n-3 ratio (14.9%, $P < 0.01$), versus HOSO-based diet.

The systolic (3%, $P < 0.05$) and diastolic (4%, $P < 0.05$) blood pressures were significantly reduced in healthy subjects after OO diet, but not after HOSO diet (*Figure 2*).

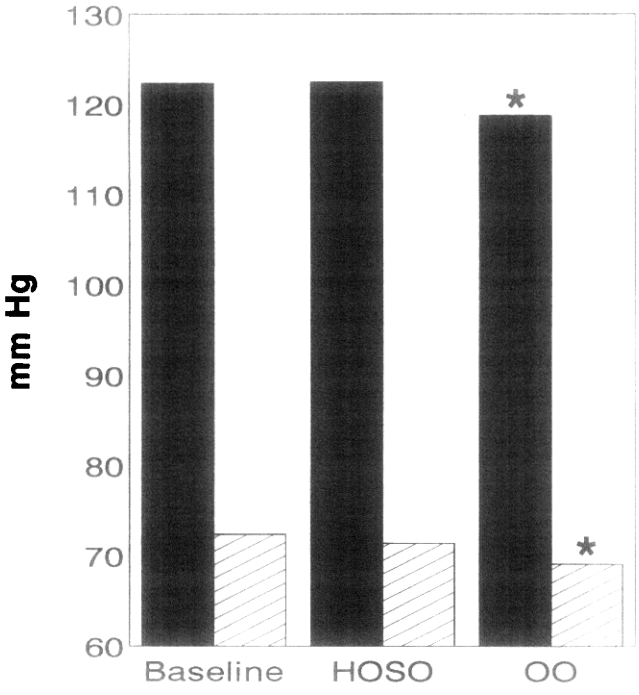

Figure 2 Blood pressure of healthy women at baseline and during the periods on the high-oleic sunflower oil-rich (HOSO) and olive oil-rich (OO) diets. Solid bars, systolic blood pressure (Baseline, 122.3 ± 8.1; HOSO, 122.5 ± 8.7; OO, 118.8 ± 9.6); narrow righ diagonal bars, diastolic blood pressure (Baseline, 72.4 ± 5.3; HOSO, 71.4 ± 5.0; OO, 69.1 ± 5.6). Values are expressed as mm Hg ± SD. Significantly different from baseline and HOSO diets: $^*P < 0.05$.

Discussion

The present study has evaluated the effects of two MUFA diets, one rich in OO and the other one rich in HOSO as major sources of fat, on erythrocyte membrane fatty acid composition and blood pressure of healthy subjects. The diets were based on ordinary food and contained similar amounts of total fats, proteins, carbohydrates, cholesterol, and other nutrients. The only differences between the diets lay in the edible fats.

It has been recently indicated that dietary supplementation with OO or HOSO modified the plasma lipid and lipoprotein profiles in healthy male and female.[7] A beneficial plasma lipid-lowering effect was more evident after OO diet, despite both vegetable oils provided a similar content of MUFAs. Because erythrocyte membrane fatty acids are known to exchange with those in plasma lipids over a relatively short period (<4 weeks),[30] seems to be evident that differences in plasma lipids and lipoproteins may account for differences in erythrocyte membrane fatty acid composition after OO and HOSO diets. Indeed, the present study shows that erythrocyte membrane phospholipids of healthy subjects are increased in oleic acid, which was in accordance with the composition of both MUFA diets. There was also a significant decrease in linoleic acid compensated by an increase in arachidonic acid and docosapentaenoic acid (22:5n-6) after OO and HOSO diets when compared with the baseline. These changes are compatible with an elongation and desaturation of 18:2n-6 to 20:3n-6 and 20:4n-6, and of 20:4n-6 to 22:4n-6 and 22:5 n-6. It was interesting to note that, in balance, the HOSO diet induced a significant decrease in total PUFAs and the content of n-6 fatty acids in the erythrocyte membrane, whereas such parameters did not change but there was an increase in long-chain n-3 fatty acids after OO diet. The shift of competition between n-6 and n-3 fatty acids in favor of n-3 fatty acids is unlikely, because both oils evoked a decrease in linoleic acid intake. This effect of a MUFA-enriched diet in increasing n-3 PUFAs has been also described in platelet membrane phospholipids of healthy subjects[31] and in serum phospholipids of hyperlipidemic patients[32] after dietary low-erucic acid rapeseed oil (canola oil). In addition, PUFAs of the n-3 family were markedly enhanced in phospholipid composition of liver microsomes, erythrocyte membranes, platelets, aorta, cardiac muscle, and brain of rats fed OO, suggesting a positive correlation between OO ingestion and long-chain n-3 fatty acid contents in cell and tissue lipids.[33]

An increase of α-linolenic acid (18:3n-3) in the plasma cholesteryl esters and erythrocyte membrane of mildly hypercholesterolaemic patients has been considered as a biomarker of adherence to MUFA-rich diets,[29] though the present findings rather indicate that 18:3n-3 is elongated and desaturated to 22:5n-3 and 22:6n-3 by dietary OO. In this regard, eicosapentaenoic (20:5n-3) and docosahexaenoic (22:6n-3) acids competitively inhibit the utilization of arachidonic acid by cyclooxygenase pathway and in turn the output of eicosanoids. Therefore, the biologic actions of these chemical mediators may be efficiently modulated by the intake of OO, providing membranes of n-3 PUFAs by upregulating their conversion and/or incorporation. Particularly, in vascular endothelial cells, release of these fatty acids from membrane phospholipids leads to the production of thromboxanes TXA_1 or TXA_3 (less potent vasoconstrictors than TXA_2, which is derived from arachidonic acid) and prostacyclins (powerful vasodilators).[34] It is suggestive of the possibility that OO either may help to modulate the effects of vasoactive substances on vessel tone and/or may participate in vascular structural changes by a rearrangement of the membranes.[13,19,35]

It has been suggested that nonfatty acid constituents at the concentration naturally occurring in OO and HOSO may be important for diet-induced changes in plasma lipid and lipoprotein profiles.[7] Our study shows that both MUFA-rich diets significantly increased the concentration of plasma HDL-cholesterol and decreased the concentration of plasma LDL-cholesterol. This supports the concept that high-MUFA diets result in a lipoprotein profile associated with lower risk for coronary heart disease.[1–6] The composition or distribution of fatty acids in the triacylglycerol (TAG) molecule from oils and their influence on intestinal absorption and metabolic fates appear also of major interest. Indeed, the nutritional and physiologic functions of dietary fats are known to depend on the structure of TAGs.[36,37] Even though a large difference in the composition of dietary TAGs seems to have little effect on postprandial lipemia in healthy subjects,[38] there are no virtually data on different responses after long-term intake of TAGs. The analysis of the oils used along our high-MUFA diets revealed significant differences in the composition of TAGs. The major TAG in OO was OOO (*sn*-glycerol-trioleate) (46% of the

total) and then POO (*sn*-glycerol-palmitate-oleate-oleate) (30% of the total), as we previously found in adipose tissue[39] and very-low-density lipoproteins[40] of healthy subjects consuming presumably OO as the major source of fat. However, HOSO provided mainly of OOO (63% of the total), followed by SOO (*sn*-glycerol-stearate-oleate-oleate) and POO. Whether these differences in nonfatty acid constituents and TAGs composition of OO and HOSO may be responsible of fatty acid composition in erythrocyte membranes is still unknown.

In conclusion, OO but not HOSO may function: (1) as a direct source of MUFAs; and (2) as a mechanism to provide membranes of n-3 PUFAs. Our findings contribute to explain the well known beneficial effects of OO by a possible interactive action of MUFAs (mainly oleic acid) and n-3 long-chain PUFAs as determinants of important parameters (plasma lipid and lipoprotein profiles, membrane fatty acid composition, and blood pressure) associated to the risk of coronary heart disease. The emerging question is now to determine whether oleic acid, n-3 PUFAs, nonfatty acid constituents or TAGs composition, themselves or combinated, is (are) responsible for or equally effective in those biological effects observed after dietary OO.

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