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Eicosanoid production, thrombogenic ratio, and serum and LDL peroxides in normo- and hypercholesterolaemic post-menopausal women consuming two oleic acid-rich diets with different content of minor components

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The present paper compares the effects of two monounsaturated oils, extra virgin olive oil (EVOO) and high-oleic acid sunflower oil (HOSO), on serum and LDL peroxides, eicosanoid production and the thrombogenic ratio (thromboxane (TX) B_2:6-keto-prostaglandin $F_{1\alpha}$) in fourteen non-obese post-menopausal women. The subjects, mean age 63 (SD 11) years, were assigned to two consecutive oleic acid-rich 28 d dietary periods. EVOO and HOSO represented 62 % of the total lipid intake and were used as the only culinary fat during the first and second dietary periods respectively. Serum peroxides, plasma α-tocopherol and TXB_2 levels in stimulated platelet-rich plasma (PRP-TXB_2) were significantly higher ($P < 0{\cdot}01$, $P < 0{\cdot}001$, and $P < 0{\cdot}05$ respectively) after the HOSO diet than after the EVOO diet. The relationship between the serum cholesterol level (<6·21 mmol/l or ≥6·21 mmol/l) and the type of dietary oil on eicosanoids, peroxides and α-tocopherol were evaluated by two-way ANOVA. Dietary oil significantly affected ($P < 0{\cdot}05$) the PRP-TXB_2 level, whereas serum and LDL peroxides were significantly affected ($P < 0{\cdot}001$ and $P < 0{\cdot}01$ respectively) by the serum cholesterol level. The plasma α-tocopherol level was significantly affected by the serum cholesterol level and the type of dietary oil (both $P < 0{\cdot}001$). No significant relationships were found between serum cholesterol levels, serum peroxide or LDL peroxide levels, plasma α-tocopherol concentrations or α-tocopherol intakes with eicosanoid production or the thrombogenic ratio due to dietary changes. However, in spite of their higher α-tocopherol levels, hypercholesterolaemic subjects showed increased peroxidation in serum and LDL in comparison with normocholesterolaemic subjects on the HOSO diet in comparison with the EVOO diet. These findings suggest that differences in the type of minor compounds, as well as in the concentration of linoleic acid, in both these monounsaturated oils may play an important role in modulating eicosanoid production and lipoprotein peroxidation when they constitute a large proportion of the diet of post-menopausal women.

Monounsaturated oils: Eicosanoid production: Lipoprotein peroxidation: α-Tocopherol

In the UK, CHD is responsible for 23 % of all female mortality (Office of Population Censuses and Surveys, 1993). Protective effects of oestrogen may be important in the prevention of CHD, and consequently the risk of CHD increases in post-menopausal women due to decreased oestrogen synthesis (Williams, 1997).

The lower incidence of CHD in Mediterranean populations may be due to the high proportion of dietary monounsaturated fatty acids (MUFA), in relation to saturated fatty acids, consumed by the population of these countries (Keys *et al.* 1986). Although the traditional source of dietary MUFA in Mediterranean countries has

Abbreviations: EVOO, extra virgin olive oil; HOSO, high-oleic acid sunflower oil; MUFA, monounsaturated fatty acids; PG, prostaglandin; PRP, platelet-rich plasma; TX, thromboxane.

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been olive oil, other sources of oils with similar fatty acid compositions but with different amounts and types of plant sterols, polyphenols, vitamins and other substances are available (Trichopoulou & Lagiou, 1997).

Dietary fatty acid composition may have an effect on the development of thrombosis and atherosclerosis (Kwon *et al.* 1991; Knapp, 1997). Saturated fatty acids promote the formation of arterial thrombi; linoleic acid has been defined as anti-thrombotic, but oleic acid and other MUFA probably have little effect on arterial thrombosis (Kwon *et al.* 1991). However, controversial effects of linoleic acid on eicosanoid synthesis, which appeared to be dose-dependent, have been described (Galli, 1992).

On the other hand, oxidised LDL have been found in human atherosclerotic lesions (Ylä-Hertuala *et al.* 1989). According to Holvoet & Collen (1994) these particles and autoimmune antibodies against oxidised LDL may enhance the progression of these lesions by: (1) increasing platelet adhesion and aggregation, which may stimulate macrophage foam-cell generation and smooth-muscle cell proliferation; (2) triggering thrombosis; (3) impairing vasodilatation, which results in increased shear stress. Moreover, it has also been demonstrated that oxidised LDL decrease the secretion of endothelium-derived relaxing factor, resulting in enhanced platelet adhesion (Chin *et al.* 1992).

Hypercholesterolaemic patients show hyperaggregability and hypercoagulability (Miller, 1993; Sánchez-Muniz *et al.* 1998). The LDL of patients with CHD are more susceptible to oxidation, possibly as a result of a decreased amount of endogenous antioxidants (Holvoet & Collen, 1994).

The purposes of the present study were: (1) to compare the effects of diet on eicosanoid production in post-menopausal women on a high-fat high-MUFA diet, containing either extra virgin olive oil (EVOO) or high-oleic acid sunflower oil (HOSO); (2) to assess whether the effects of the diets on thrombogenesis also depend on serum cholesterol levels; (3) to study the possible relationship between eicosanoid production and serum and LDL peroxides.

Material and methods

Subjects

Nuns from an enclosed convent were studied. The subjects were chosen because of their regular lifestyle, age and dietary habits. Fifteen women were initially enrolled in the study, but one was later excluded because she was premenopausal. The mean age of the fourteen subjects who finished the study was 63 (SD 11) years. The mean body weight of the subjects was 54·3 (SD 9·3) kg and the mean BMI was 23·2 (SD 3·4) kg/m^2. No subject reported any previous cardiovascular, metabolic or systemic disease or took any drug that might affect lipid metabolism or platelet aggregation. The study protocol was approved by the Spanish Comisión Interministerial de Ciencia y Tecnología, and performed in accordance with the Helsinki Declaration.

Experimental design

Subjects were assigned to two consecutive 28 d periods when they received diets rich in EVOO and HOSO. There was no washout period between experimental diets because according to Keys *et al.* (1965) diet induces stable changes in lipoprotein-lipids after 4 weeks and the average lifespan of circulating platelets is less than 2 weeks (Paulus & Aster, 1985).

Diets

The diet of the community was assessed using the precise weighing method (Marr, 1971). All ingredients used in the preparation of dishes, as well as the inedible waste, were weighed. The cooked weights of individual portions and table waste were also recorded. Energy and nutrient intakes were calculated using food composition tables for the raw weights of foodstuffs (Moreiras *et al.* 1996). Two dietitians were present every day in the community's kitchen during the preparation of meals. A 14 d menu cycle was used.

Body weight was measured twice weekly and energy intake adjusted as needed to maintain the initial weight. To avoid radical dietary changes that would stress participants, subjects were assigned to two consecutive 28 d dietary periods, in which menus and individual ratios were kept constant with respect to the regular diet. The regular diet contained, as culinary fat, a homogenous mix (1:1, v/v) of olive oil and refined sunflower oil. The single distinguishing feature of the experimental diets was the oleic acid-rich oil used. In the first period all subjects consumed a diet rich in EVOO (Comunal Olivarera Mora, Toledo, Spain). This period was followed by a second period rich in HOSO (Koipe Co., Andújar, Spain). Both these oils were used raw as well as for cooking. A high percentage of the oils (about 45) was consumed raw in salad dressing, while the rest was used for sautéing, frying and pot-roasting, and in the preparation of fish, eggs, vegetables and stews.

The percentage of oleic acid (18:1n-9) in EVOO and HOSO was similar; however, EVOO contained more palmitic acid (16:0) and less linoleic acid (18:2n-6) than HOSO (Table 1). Total polyphenol, squalene and Δ^5-avenasterol concentrations were 4, 9·2, and 2·7 times higher respectively in the EVOO, while total sterols, β-sitosterol

Table 1. Composition of extra virgin olive oil (EVOO) and high-oleic acid sunflower oil (HOSO)

	EVOO	HOSO
Major fatty acids (mg/100 mg total fatty acids)		
Palmitic acid	9·6	4·5
Oleic acid	74·0	73·5
Linoleic acid	7·1	15·0
SFA	14·1	9·1
MUFA	74·7	75·0
PUFA	11·2	15·0
Minor components (mg/kg oil)		
α-Tocopherol	192·7	538·0
Total polyphenols	108·0	25·0
Total sterols	1457	3941
Campesterol	45·3	340·5
β-Sitosterol	1217	2101
Δ^5-Avenasterol	117·7	66·6
Squalene	4600	500

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

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and α-tocopherol levels were 2·7, 1·7 and 2·6 times higher respectively in the HOSO. MUFA contribute about 28 % energy in both diets, but linoleic acid was 45 % lower in the EVOO diet than in the HOSO diet.

Laboratory analyses

The fatty acid composition of the oils was analysed using a Hewlett-Packard 5890 Series II gas chromatograph (Palo Alto, CA, USA). Reverse-phase HPLC (Hess *et al.* 1991) was used for α-tocopherol determination, and total polyphenols were determined using the method of Folin-Ciocalteau (Singleton & Rossi, 1965). Other minor components were determined in the non-saponifiable fraction of the oils by GC (Las Comunidades Europeas, 1991).

After overnight fasting (12 h), blood samples were collected by venepuncture. Blood was collected with minimum intrusion after the anthropometric evaluation to avoid stress to the volunteers. A vacuum system was not used, although it allows quicker blood extraction, because it can cause platelet damage and greater blood vessel stress. According to Subbiah *et al.* (1991) to avoid damage to platelets the first 10 ml blood should be not used for thromboxane analysis. Thus, we used the first 10 ml for biochemical analyses and the remainder of the sample for eicosanoid analyses. Moreover, because endothelial cells can be stressed by venepuncture, potentially affecting the blood determination of prostacyclin, we determined this eicosanoid only in the urine.

Total cholesterol in serum was determined using a Technicon RA-500 AutoAnalyzer (Tarrytown, NY, USA) by the enzymic esterase–cholesterol oxidase method of Boehringer Mannheim Gmbtt, Mannheim, Germany.

Lipoproteins were isolated density-gradient ultracentrifugation for 22 h using the salt gradient composition suggested by Terpstra *et al.* (1981) followed by removal of the salt. However, an ultracentrifugation temperature of 8°C was used instead of 20°C to minimise thermal damage to lipoproteins.

Serum peroxide and LDL peroxide concentrations were determined as thiobarbituric acid-reactive substances according to the method of Yagi (1993).

The platelet-rich plasma (PRP) was separated from anticoagulated blood, and ADP-stimulated thromboxane (TX) A_2 synthesis was measured in PRP. At the end of each dietary period 24-h urine samples were collected. TXB_2, a stable metabolite of TXA_2, was extracted from PRP and urine samples, while 6-keto-prostaglandin (PG) $F_{1\alpha}$, a stable metabolite of prostacyclin I_2 (PGI_2) was extracted from urine by the procedures of Powell (1980). These metabolites were determined using ^{125}I-labelled radioimmunoassay kits purchased from Advanced Magnetics Inc. (Cambridge, MA, USA).

Statistical analyses

The paired Student's *t* test was used to assess the effects of both oils on serum and LDL peroxidation, plasma α-tocopherol and PRP-TXB_2 production, and 24 h urinary TXB_2 and 6-keto-$PGF_{1\alpha}$ excretion. Two-way ANOVA was used to assess the effects of each oil in women with a total cholesterol level <6·21 mmol/l compared with those with a total cholesterol level of ≥6·21 mmol/l on these variables.

Table 2. Daily intake of post-menstrual women during the extra virgin olive oil (EVOO) dietary period and the high-oleic acid sunflower oil (HOSO) dietary period†

(Mean values and standard deviations for fourteen women. Values are expressed as a percentage of the total energy intake, except where stated)

	EVOO diet		HOSO diet	
	Mean	SD	Mean	SD
Energy (kJ/d)	7347	775	7347	775
Protein	11·7	0·4	11·7	0·4
Carbohydrates	42·1	3·0	42·1	3·0
Lipids	46·2	3·0	46·2	3·0
SFA	11·8	1·7	10·3*	1·7
MUFA	28·5	1·7	27·8	1·6
PUFA	2·8	0·1	4·6*	0·2
Major fatty acids				
Palmitic acid	6·6	0·7	5·0*	0·7
Oleic acid	27·5	1·6	27·0	1·6
Linoleic acid	2·2	0·1	4·0*	0·2
Linolenic acid	0·37	0·04	0·32	0·04
Eicosapentaenoic acid	0·05	0·01	0·05	0·01
Docosapentaenoic acid	0·02	0·00	0·02	0·00
Docosahexaenoic acid	0·09	0·01	0·09	0·01
Cholesterol (mg/d)	401·4	33·9	401·4	33·9
Fibre (g/d)	16·5	2·1	16·5	2·1

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.
Mean values were significantly different from those for EVOO diet: *$P < 0·05$.
† For details of subjects, diets and procedures, see p. 42 and Table 1.

Results

Dietary assessment

Food intake was the same throughout both dietary periods (Table 2). Fats and oils contributed two-thirds of the total lipids in both experimental periods, and the experimental oils provided approximately 62 % of the total fat intake in each case. The energy contribution of palmitic and linoleic acids, however, as well as the amount of α-tocopherol consumed, was significantly different ($P < 0·05$ in each case) between the two dietary periods. The average daily fibre intake was 16·5 g, while that of cholesterol was approximately 400 mg.

Serum peroxides, LDL peroxides and plasma α-tocopherol

Table 3 shows that the women displayed higher total lipid peroxide concentrations ($P < 0·01$) following the HOSO diet than after the EVOO diet. No differences were found for LDL peroxides. Statistical analysis (two-way ANOVA) showed a highly significant effect of the blood cholesterol level on serum peroxides and LDL peroxides ($P < 0·001$ in both cases), whereas the type of dietary oil did not significantly affect those variables. No significant interaction between the cholesterol level and the dietary oil was found (Table 4).

At the end of the HOSO period α-tocopherol levels were significantly higher ($P < 0·001$) than those at the

Table 3. Concentration of thromboxane (TX) B_2 in platelet-rich plasma (PRP) and urine, 6-keto-prostaglandin (PG) $F_{1\alpha}$ in urine, thrombogenic ratio (TXB_2 : 6-keto-$PGF_{1\alpha}$) in urine and lipid peroxidation in serum of post-menopausal women consuming extra virgin olive oil (EVOO) and high-oleic acid sunflower oil (HOSO)†

(Mean values and standard deviations for fourteen women)

	EVOO diet		HOSO diet	
	Mean	SD	Mean	SD
Platelets (/mm³) in PRP	298 214	43 773	280 857	67 837
TXB_2 in PRP (pg/10^8 platelets)	584·3	356·3	698·5*	369·4
Urinary volume (litres)	1·19	0·29	1·07	0·27
TXB_2 in urine: pg/ml	871·0	383·7	901·6	306·1
pg/min	666·1	241·8	648·5	213·3
6-keto-$PGF_{1\alpha}$ in urine: pg/ml	1044·8	333·2	990·7	216·7
pg/min	816·1	150·6	720·4	164·8
TXB_2 : 6-keto-$PGF_{1\alpha}$ in urine	0·82	0·26	0·91	0·23
Serum total lipid peroxides‡ (nmol/ml)	0·87	0·73	0·95 **	0·70
LDL peroxides‡ (nmol/ml)	0·28	0·21	0·29	0·22
α-Tocopherol (mmol/l)	28·6	6·4	34·4 ***	5·4

Mean values were significantly different from those for EVOO diet: *$P < 0·05$, **$P < 0·01$, ***$P < 0·01$.
† For details of subjects, diets and procedures, see p. 42 and Table 1.
‡ Determined as thiobarbituric acid-reactive substances, according to the method of Yagi (1993).

end of the EVOO period for all subjects (Table 3). Statistical analysis (two-way ANOVA) showed a highly significant effect of both the serum cholesterol level and the type of dietary oil on α-tocopherol levels ($P < 0·001$ in both cases). However, there was no significant interaction between serum cholesterol level and type of dietary oil (Table 4).

Thromboxane B_2 in ADP-stimulated platelet-rich plasma

TXB_2 concentration in PRP was significantly lower ($P < 0·05$) in the women after consuming the EVOO diet than after the HOSO diet (Table 3). Statistical analysis (two-way ANOVA) showed that the type of dietary oil had a significant effect ($P < 0·05$) on TXB_2 concentration in PRP (Table 4).

Eicosanoids in urine and the thrombogenic ratio

The urinary excretion of TXB_2 and 6-keto-$PGF_{1\alpha}$ (expressed as pg/ml and pg/min) and the thrombogenic ratio TXB_2:6-keto-$PGF_{1\alpha}$ are shown in Table 3. These were no significant differences between the types of dietary oil for any of these variables. Statistical analysis (two-way ANOVA) showed no significant effect of the serum cholesterol level or the type of dietary oil was found (Table 4).

Discussion

Dietary assessment

The concentrations of polyphenols, total and individual sterols, and squalene were rather different in EVOO and HOSO. Both diets presented the same energy profile and

Table 4. Serum cholesterol level and concentration of thromboxane (TX) B_2 in platelet-rich plasma (PRP) and urine, 6-keto-prostaglandin (PG) $F_{1\alpha}$ in urine, thrombogenic ratio (TXB_2 : 6-keto-$PGF_{1\alpha}$) in urine and lipid peroxidation in serum of normocholesterolaemic (<6·21 mmol/l) or hypercholesterolaemic (≥6·21 mmol/l) post-menopausal women consuming diets rich in extra virgin olive oil (EVOO) or high-oleic acid sunflower oil (HOSO)*

(Mean values, and standard deviations for seven women per group)

	EVOO diet				HOSO diet				Statistical significance (two-way ANOVA) of:		
Serum cholesterol (mmol/l)...	<6·21		≥6·21		<6·21		≥6·21		Effect of oil type	Effect of serum cholesterol level	Interaction
	Mean	SD	Mean	SD	Mean	SD	Mean	SD			
TXB_2 in PRP (pg/10^8 platelets)	592·8	393·6	572·9	336·5	755·6	428·9	622·4	291·6	$P < 0·05$	NS	NS
TXB_2 in urine (pg/min)	714·4	255·8	601·6	222·4	676·9	225·6	620·0	214·1	NS	NS	NS
6-keto-$PGF_{1\alpha}$ in urine (pg/min)	792·3	173·4	847·9	121·6	737·4	171·1	667·3	151·6	NS	NS	NS
TXB_2 : 6-keto-$PGF_{1\alpha}$ in urine	0·91	0·26	0·70	0·23	0·87	0·18	0·94	0·28	NS	NS	NS
Serum total lipid peroxides† (nmol/ml)	0·39	0·26	1·50	0·68	0·50	0·24	1·54	0·68	NS	$P < 0·001$	NS
LDL peroxides† (nmol/ml)	0·17	0·07	0·42	0·25	0·17	0·08	0·45	0·25	NS	$P < 0·01$	NS
α-Tocopherol (mmol/l)	24·8	5·4	33·6	3·5	31·4	4·8	38·5	3·3	$P < 0·001$	$P < 0·001$	NS

* For details of subjects, diets and procedures, see p. 42 and Table 1.
† Determined as thiobarbituric acid-reactive substances, according to the method of Yagi (1993).

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cholesterol content, and were very rich in MUFA but the energy contributions of palmitic and linoleic acids were lower and higher respectively in the EVOO diet than in the HOSO diet. It has been reported that minor dietary components have potential effects on lipoprotein peroxidation (Chimi *et al.* 1991; Montedoro *et al.* 1992), and on platelet aggregation and eicosanoid production (Petroni *et al.* 1994; Sánchez-Muniz *et al.* 1998). This factor was taken into account when considering both the fatty acid profile of the diets and the levels of minor components in the oils.

Serum peroxide, LDL peroxide and α-tocopherol levels

A large percentage of women (71) had increased serum lipid peroxide levels after the HOSO dietary period compared with the levels found at the end of the EVOO dietary period. These results seem paradoxical considering both the higher α-tocopherol content of the HOSO diet and the higher α-tocopherol levels in plasma at the end of the HOSO dietary period.

Δ^5-Avenasterol and other sterols are potent antioxidants, while squalene has a moderate antioxidant effect (Boskou, 1999). Moreover, antioxidant properties have been shown in some polyphenols present in EVOO (e.g. 2-(3,4-dihydroxyphenyl) ethanol, oleuropeine glycoside, *p*-hydroxyphenyl ethanol, tyrosol; Montedoro *et al.* 1992). The lower content of such minor components, together with the higher linoleic acid level in HOSO, would account for the significantly higher serum levels of TBARS found in the women after the HOSO diet compared with that observed after the EVOO diet. However, although in the present study no data are available on tocopherol or antioxidant distribution in lipoproteins; the results show that pro-oxidant–antioxidant mechanisms resulted in similar LDL peroxidation levels following both dietary periods.

According to our data, a high proportion of peroxides is associated with lipoproteins other than LDL (e.g. VLDL, lipoprotein(a) and HDL). This finding is in agreement with the findings of Szczeklik & Gryglewski (1980) and Hagihara *et al.* (1984) relating to TBARS in human lipoproteins. Solà *et al.* (1997) studied the influence of dietary fat on the oxidation of HDL particles. These authors found that a diet rich in oleic acid (18:1) produced HDL_3 that contain more 18:1 and TBARS than the HDL_3 produced following a diet rich in linoleic acid (18:2). Moreover, TBARS in those particles correlated negatively with the 18:1:18:2 value and positively with the 18:1 content of phospholipids.

Higher serum and LDL peroxide levels were found in hypercholesterolaemic women following both diets. This finding suggests a higher susceptibility of LDL to oxidation. According to Holvoet & Collen (1994) the higher susceptibility of LDL to oxidation in patients with CHD would be a result of fatty acid composition of the LDL particles as well as reduced endogenous antioxidants such as vitamin E. However, hypercholesterolaemic women had higher plasma vitamin E levels, which would prevent the increase in peroxidation. With the aim of finding a possible explanation, serum total lipid peroxides:α-tocopherol was calculated (data not shown). Normocholesterolaemic subjects had a ratio of 0·016 after both diets, while for hypercholesterolaemic subjects the ratio was 0·045 and 0·040 after EVOO and HOSO diets respectively, suggesting that hypercholesterolaemic subjects are more susceptible to peroxidation than normocholesterolaemic subjects.

Thromboxane B_2 level in ADP-stimulated platelet-rich plasma, and excretion of urinary eicosanoids

Platelet aggregation is modulated by the production of TXA_2 and PGI_2. An optimal balance of TXA_2–PGI_2 may be important in prevention of thrombotic conditions. Evidence indicates that dietary fatty acids can alter this balance (Knapp, 1997).

On stimulation platelets produce TXA_2. Thus, the measurement of the platelet TXB_2 level *in vitro* assesses the capacity for TXA_2 production under maximum stimulation. On the other hand, measurement of TXB_2 in urine is considered to be an acceptable and non-invasive estimate of *in vivo* production (Mensink *et al.* 1999). Thus, the level of the stable metabolite of PGI_2 (6-keto-$PGF_{1\alpha}$) was also determined to assess the effect of the diets on TXA_2:PGI_2.

About 80 % of the women showed lower PRP-TXB_2 levels after the EVOO diet than after the HOSO diet. The effect of polyunsaturated fatty acids on eicosanoid synthesis is controversial and highly dose dependent. According to Galli (1992), at levels between 0 and 5–6 % total energy intake linoleic acid enhances the platelet formation of eicosanoids derived from arachidonic acid. The energy contribution of linoleic acid in both experimental diets was <5 %, but the linoleic acid content was about twice as high in the HOSO diet as that in the EVOO diet, explaining why TXB_2 levels were higher at the end of the HOSO dietary period.

Small differences in other fatty acids between diets would also cause variations in PRP-TXA_2 synthesis. The higher *n*-6:*n*-3 fatty acids or the higher linoleic acid:α-linolenic acid in the HOSO diet may partially explain the greater PRP-TXB_2 level following this diet. Experimental and clinical studies have shown that there is competition between linoleic and α-linolenic acids in relation to the Δ^6-desaturase enzyme system (Knapp, 1997).

Differences in the concentrations of minor components of the two oils may also account for some of the differences observed in TXB_2 levels in stimulated PRP after the two dietary periods. According to Petroni *et al.* (1994) the incubation of PRP with 2-(3,4-dihydroxyphenyl) ethanol before collagen stimulation, resulted in about a 70 % reduction in the TXB_2 level. In agreement with the present results, in a previous study using the same subjects lower platelet aggregation was found after an EVOO diet than after a HOSO diet (Sánchez-Muniz *et al.* 1998).

Urinary TXB_2 or PGI_2 levels were not affected by the either the type of dietary oil or the serum cholesterol level. Urinary TXB_2 measurement provides time-integrated information about *in vivo* TXA_2 biosynthesis (Mensink *et al.* 1999). However, although a considerable proportion of urinary TXA_2 is derived from TXA_2 synthesised by platelets (FitzGerald *et al.* 1987), the urinary concentrations

of TXA_2 may be confounded by the eicosanoid production of the kidney. This factor may explain the different results for PRP-TXB_2 and urinary TXB_2 levels found after the two dietary periods. However, the synthesis of PGI_2 by the aortas of animals eating a diet with more than 40 g linoleic acid/kg is lower than or identical to that of animals eating a diet with less than 40 g linoleic acid/kg (Szczeklik *et al.* 1985), explaining the absence of significance found for PGI_2 between the two dietary periods in the current study.

In conclusion, although EVOO and HOSO are defined as monounsaturated oils, they show differences in the content of other fatty acids (e.g. palmitic and linoleic acids) and in minor components that account for the different results seen in serum peroxide and plasma α-tocopherol levels and PRP-TXB_2 production. Further studies are necessary in order to understand the different physiological effects induced by the ingestion of EVOO and HOSO, and to assess the role of some minor components present in these oils.

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