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ORIGINAL CONTRIBUTION

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Platelet aggregation, thromboxane production and thrombogenic ratio in postmenopausal women consuming high oleic acid-sunflower oil or palmolein

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Summary *Background* Saturated fatty acids exert controversial effects on platelet aggregation and eicosanoid production. *Aim* To investigate the effect of a dietary exchange between palmitic acid and oleic acid on both platelet aggregation and thromboxane B2 (TXB_2) production, and on urine TXB_2, prostacyclin I_2 (PGI_2 as 6-keto-protaglandin $F_1\alpha$), and the thrombogenic ratio (TXB_2/6-keto-protaglandin $F_1\alpha$) in fourteen postmenopausal women. *Experimental design* Women were assigned to two consecutive 28-d dietary periods that were high in cholesterol (~400 mg/d) and fat (~46 %en). In the first period all subjects followed an oleic acid-rich diet prepared with high oleic acid-sunflower oil. This was followed by a second period rich in palmitic acid in the form of palmolein. *Determinations* Nutrient intakes, ADP-platelet aggregation, platelet TXB_2 production, urine TXB_2 and 6-keto-protaglandin $F_1\alpha$ were measured during two dietary periods and the results obtained correlated to serum cholesterol, lipoprotein-cholesterol and peroxides, apolipoproteins and plasma tocopherol. *Results* The palmolein diet led to an increase in the platelet aggregation rate ($p < 0.05$) and in the time for the maximal aggregation rate ($p < 0.02$). No significant differences were observed in platelet TXB_2 production. Palmolein increased urine TXB_2 in pg/mL ($p < 0.05$) and pg/min ($p < 0.01$), whereas the thrombogenic ratio (TXB_2/6-keto-protaglandin $F_1\alpha$) did not change. Most changes were related to oil change, few to serum cholesterol level (< or ≥ 6.2 mmol/L) or age (< or ≥ 65 yr). *Conclusions* Palmolein diet activates platelet aggregation more in normocholesterolemics. Though palmolein increased thromboxane and tended to increase prostacyclin in urine in normo- and hypercholesterolemic women, the thrombogenic ratio did not change. These effects were related to the LDL and HDL concentration increases and to the absence of change in the total cholesterol/HDL-cholesterol ratio found following the dietary intervention.

Key words high oleic acid sunflower oil – palmolein – platelet aggregation – postmenopausal – thromboxane – thrombogenic ratio – urine prostacyclin and thromboxane

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Introduction

Postmenopause is considered to elevate the cardiovascular disease risk in women with an increase in small low density lipoprotein (LDL)-particles, LDL-cholesterol, and a slight decrease in HDL-cholesterol occurring [1]. However, platelet aggregation and eicosanoid production on postmenopausal women have not been extensively studied. Although, saturated fatty acids (SFA) and polyunsaturated fatty acids (PUFA) have been believed to promote and to protect against arterial thrombi formation, respectively, recent data confirm the inconsistent effect of dietary SFA and α-linoleic acid on platelet activation [2, 3]. On the other hand, platelet aggregation was distinctly affected by two oils rich in monounsaturated fatty acids (MUFA) [4]. A promising approach to assess platelet activation *in vivo* is the measurement of thromboxane (TX) metabolites in urine [5] but results on fatty acids other than n-3 PUFA on urinary eicosanoids are scarce and controversial [6–8].

Platelet activation *in vitro* increased in the presence of LDL and very low density lipoproteins (VLDL) while this mechanism was inhibited in the presence of high density lipoproteins (HDL) [8]. However, SFA have been found to increase not only the LDL-cholesterol but also the HDL-cholesterol [9]. Moreover, different studies showed that the presence of minor compounds could change platelet activation [4, 10]. High oleic acid sunflower oil (HOSO) is rich in tocopherols, whereas palmolein is rich in tocotrienols [4]. Both of them may protect against cardiovascular disease because of their antioxidant activity [11] and inhibition of platelet aggregation [12], although others did not report that [13, 14].

Due to the controversial dietary effects of SFA and antioxidants on platelet aggregation and eicosanoid production, the present study is focused on the effect of an exchange of oleic acid and tocopherols (from HOSO) by palmitic acid and tocotrienols (from palmolein) in postmenopausal women. The interaction of dietary intervention and age, and the correlations between the changes in lipoprotein, apolipoproteins (Apo), peroxidation and plasma tocopherol levels on changes in platelet aggregation and eicosanoid production will be also studied. Moreover, because hypercholesterolemia has been related to hyperaggregability and hypercoagulability [4, 15], this study also analyzes the effect of basal plasma cholesterol on platelet aggregation and eicosanoid production in these postmenopausal women.

Methods

Subjects

Fourteen postmenopausal nuns were enrolled in the study. They were part of an enclosed religious community with a regular lifestyle and dietary habits. None of the participants had presented previous cardiovascular, metabolic or systemic disease, or was taking any drug that might affect lipid metabolism or platelet aggregation. All subjects gave their informed consent to be included in the study. The study protocol was approved by the Spanish Comisión Interministerial de Ciencia y Tecnología, a Committee for Human Studies at the University Complutense of Madrid, and was performed in accordance with the Helsinki Declaration. Some characteristics of the population are shown in Table 1.

Experimental

Participants were assigned to two consecutive 28-d experimental periods. In the first period, all subjects consumed a diet rich in oleic acid, HOSO being the only culinary fat. This was followed by a second dietary period rich in palmitic acid from palmolein. In a pilot study we performed in the same community, we used a crossover design [4]. However the logistics involved in food preparation and consumption in the community prevented us from using a crossover design in this study. For example, food preparation at the community kitchen was too difficult and time-consuming because the same food was cooked with two oils. Furthermore, some members of the community complained because

Table 1 Characteristics of the women studied

	Mean ± SD	Range
Age (y)	62.9 ± 11.2	46–75
Height (cm)	153 ± 7	142–164
Weight (kg)	54.3 ± 9.3	41–70
Body mass index (kg/m^2)	23.2 ± 3.4	19.6–29.3
Energy expenditure (kJ)	7548 ±778	5995–9272
Serum cholesterol (mmol/L)	6.41 ± 1.15	4.7–8.8
Serum triglycerides (mmol/L)	0.83 ± 0.19	0.53–1.20
HDL-cholesterol (mmol/L)	1.88 ± 0.40	1.19–2.59
LDL-cholesterol (mmol/L)	3.78 ± 0.8	2.84–5.87
Apolipoprotein AI (g/L)	1.56 ± 0.21	1.25–1.98
Apolipoprotein B (g/L)	1.08 ± 0.21	0.75–1.62
Apolipoprotein AII (g/L)	0.35 ± 0.05	0.28–0.46
Systolic pressure (mmHg)	126.4 ± 16.1	100–160
Diastolic pressure (mmHg)	67.9 ± 12.7	60–100
Serum peroxides (mmol/L)	0.85 ± 0.73	0.14–2.27

Values are mean ± SD, n = 14

they thought that they were consuming a less healthy diet than other members. Therefore, we were concerned about the feasibility of accomplishing a full-size study under those circumstances and we opted for the sequential design. Subjects maintained their normal patterns of activity and did not consume extra food in their meals. The compliance to the experimental diets was total. Moreover, there was no washout between experimental periods because the fact that diet induces stable changes in lipoprotein-lipids after 4 weeks has been described [16] and the average lifespan of circulating platelets is less that 2 weeks [17].

Diets

During the two consecutive 28-d experimental dietary periods, menus and individual ratios were maintained. Experimental diets were assessed for four weeks using the "precise weighing method". The cooked weight of individual portions and table waste were also recorded. Energy and nutrient intakes were calculated using tables of food composition for the raw weights of foodstuffs [18]. The only distinguishing feature of the diets was the oil used: HOSO (Koipe Co., Andújar, Spain), during the first period, and palmolein (AGRA S. A., Bilbao, Spain) during the second period. Both oils were used for cooking (for sautéing, frying and pot-roasting, and the preparation of fish, egg, vegetable and other stews) and for salad dressing. Because palmolein is solid at room temperature, this oil was liquefied by immersing a plastic container with the amount to be used in a water bath at ~30 °C just before use. To ensure constant consumption of palmolein from salads, participants were advised to remove any oil remaining on their dishes using small pieces of bread.

Laboratory analyses

The fatty acid composition was analyzed as previously described [19]. α-tocopherol and tocotrienol were determined by high-performance liquid chromatography on a reverse-phase column [20]. Blood was collected with minimum intrusion after the anthropometric evaluation to avoid stress to the volunteers. In order to avoid damage to the platelets the first 10-mL of blood should not be used for TX analyses [21]. We used them for biochemistry 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.

Serum was separated by low speed centrifugation at 1500 x *g*, at 4 °C for 30 min within 1-h of sampling. LDL and HDL were isolated from serum by 21-h density gradient ultracentrifugation at 272,000 x *g* as previously reported [10]. Total cholesterol was determined in serum samples and in the LDL and HDL fractions using a Technicon RA-500 autoanalyzer (Tarrytown, NY, USA) and standard enzymatic procedures (Boehringer Mannheim, Mannheim, Germany). Quality controls (Precinorm reference 225053, and Precilip reference 781827 (Boehringer Mannheim, Mannheim, Germany) were included in all assays. Serum peroxide and LDL-peroxide concentrations were determined as thiobarbituric acid-reactive substances [22].

The anticoagulated trisodium citrate blood (9:1 V/V) was centrifuged at 200 x *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 the difference between platelet counts on PRP samples from both dietary periods were non-significant. Platelet aggregation was determined using ADP (Chromopag ADP. IZASA, Spain) as an aggregating agent as previously commented on [4]. Data were expressed as the extent or rate of maximal aggregation at 5 min (cm/5 min), the time required for achieving the maximal aggregation rate (min), and the time required for achieving half of the maximal aggregation (min). ADP-stimulated TXA_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 using a silica column [10]. These metabolites were determined using ^{125}I-labelled radio-immunoassay kits purchased from Advanced Magnetics Inc. (Cambridge, MA, USA).

Statistical analyses

The data were compared by a paired Student's *t* test. Repeated measures ANOVA was used to assess the oil effect in women with a total cholesterol level < 6.2 mmol/L (normocholesterolemics) compared with those with a total cholesterol level ≥ 6.2 mmol/L (hypercholesterolemics) and in women aged more or less than 65. Pearson product-moment correlations were performed to assess the influence of the changes in lipoprotein, tocopherol and serum peroxide concentrations on the changes in platelet aggregation and eicosanoid production.

Results

Dietary assessment

The major fatty acid composition of HOSO and palmolein was: palmitic acid, 4.7 % and 40.6 %; stearic acid

4.5 % and 4.2 %; oleic acid, 77.1 % and 40.7 %, and linoleic acid 11.5 % and 12.2 %, respectively. *Trans* fatty acids were present in small amounts in both oils (elaidic acid 0.02 % and 0.16 %, *trans*-linolenic acids 0.07 % and 0.57 %, respectively), while myristic acid and α-linolenic acid were 0.05 % and 0.06 % in HOSO but were 1.2 % and 0.16 % in palmolein, respectively. HOSO and palmolein contained 538 and 96.3 mg/kg oil of α-tocopherol and < 1.0 and 530 mg/kg oil of tocotrienols, respectively. Daily intakes of macronutrients and different fatty acids during basal and experimental diet periods are presented in Table 2. The experimental oils provide ~62 % of the total fat intake in both diets. The diets were normocalorics (adjusted to the energy women requirements) with an adequate energy contribution from protein but rather high from fat and low from carbohydrates (Table 2). It should be noted that the usual diet of the participants in this study did not include meat or meat products but a relatively high amount of eggs and whole milk.

The percentages of linoleic and stearic acids were similar for both dietary periods. However, the palmolein-rich period contained a higher percentage of palmitic acid and a lower percentage of oleic acid. The fats plus oil food group was the main source of oleic and linoleic acids, while milk and dairy products were the main contributors of myristic and α-linolenic acids for the diet periods. During the palmolein diet, SFA and palmitic acid were mainly provided by the fats plus oil food group (data not shown).

Table 2 Daily intake during the high oleic acid sunflower oil dietary period and the palmolein dietary period

	High oleic acid sunflower oil diet	Palmolein diet
Energy (kJ/d)	7328 ± 773.3	7265 ± 781.4
	% of energy	
Protein	11.7 ± 0.4	12.0 ± 0.6
Carbohydrates	42.0 ± 5.8	42.4 ± 3.1
Lipids	46.4 ± 3.0	45.8 ± 3.0
SFA (1)	10.3 ± 1.7	19.6 ± 1.7
MUFA (1)	27.8 ± 1.6	17.5 ± 0.1
PUFA (1)	4.6 ± 0.2	4.2 ± 0.2
Major fatty acids		
Palmitic acid	5.0 ± 0.7	14.4 ± 2.8
Stearic acid	2.8 ± 0.3	2.7 ± 0.3
Oleic acid	27.0 ± 1.6	16.7 ± 1.0
Linoleic acid	4.0 ± 0.2	3.7 ± 0.2
Linolenic acid	0.33 ± 0.04	0.2 ± 0.0
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
n-3/n-6 ratio	0.12 ± 0.01	0.11 ± 0.01
Cholesterol (mg/d)	401.4 ± 33.9	402.7 ± 34.7

Data are mean values ± standard deviations of 14 women. (1): *SFA* saturated fatty acids; *MUFA* monounsaturated fatty acids; *PUFA* polyunsaturated fatty acids

Lipids and lipoprotein-cholesterol, lipid peroxide and plasma tocopherol levels

In previous studies [10, 19], it was stated that the same palmolein diet in respect to the same HOSO diet produced a significant increase in serum TC (7.31 ± 1.19 *vs* 6.21 ± 1.15 mmol/L; $p < 0.001$), LDL-cholesterol (4.33 ± 0.94 *vs* 3.56 ± 0.85 mmol/L; $p < 0.001$) and HDL-cholesterol (2.24 ± 0.36 *vs* 1.95 ± 0.46 mmol/L; $p < 0.05$). However, the total cholesterol/LDL-cholesterol ratio remained unchanged (3.28 ± 0.38 *vs* 3.28 ± 0.61). HDL particles appeared enriched in Apo AII (0.51 ± 0.04 *vs* 0.37 ± 0.05 g/L; $p < 0.001$) but not in Apo AI (1.65 ± 0.23 *vs* 1.67 ± 0.19). The Apo AI/Apo B and the Apo AI/Apo AII ratios decreased significantly (1.41 ± 0.18 *vs* 1.70 ± 0.24; $p < 0.001$ and 3.23 ± 0.58 *vs* 4.64 ± 1.10; $p < 0.001$). Palmolein diet produced a significant decrease in serum peroxides (0.48 ± 0.26 *vs* 0.95 ± 0.70 mmol/L; $p < 0.01$), while the α-tocopherol concentration was not affected (33.5 ± 5.9 *vs* 34.4 mmol/L).

Platelet aggregation

Maximal aggregation rate at 5 min was significantly higher ($p < 0.05$) following the palmolein diet. The time of the maximal aggregation rate increased ($p < 0.02$) (Table 3). The time for half the maximal aggregation also shows significant increases (47.6 %, $p < 0.02$; data not shown). The maximal aggregation rate and the time for the maximal aggregation was significantly affected by the type of oil ($p < 0.05$ and $p < 0.01$, respectively) but not by the serum cholesterol level (Table 3). Oil exchange increased both the maximal aggregation rate and the time for the maximal aggregation ($p < 0.01$ and $p < 0.05$ respectively) only in normocholesterolemic women. The oil exchange tended to increase the maximal aggregation rate more in women over 65 than in their younger counterparts (increase of 1.07 ± 1.16 *vs* 0.34 ± 1.56 cm; not significant). The same was found for the time for the maximal aggregation rate (increase of 2.17 ± 1.54 *vs* 1.07 ± 1.21 min; not significant).

Changes in the maximal aggregation rate did not show any significant correlation with changes in serum lipid or lipoprotein-cholesterol, peroxides levels, plasma tocopherol levels and PRP-TXB_2 (pg/mL). However, change in the time for the maximal aggregation rate appeared inversely correlated ($r = -0.736$, $p < 0.003$) to changes in the urine TXB_2 (pg/mL) concentrations and LDL-Apo B ($r = -0.625$, $p < 0.02$) but positively to the Apo AI/Apo B ratio ($r = 0.670$; $p < 0.01$).

Table 3 Changes in platelet aggregation and thromboxane B2 (PRP-TXB2), urinary TXB2 and prostacyclin I2 (6-keto PGF1α) and the thrombogenic ratio in postmenopausal women due to the dietary intervention

	HOSO All (n = 14)	Palmolein All (n = 14)	Change (%)	HOSO	Palmolein	HOSO	Palmolein	Oil effect	Cholesterol level effect	Interaction
				Normocholesterolemic (n = 8)		Hypercholesterolemic (n = 6)				
Blood Platelet Number (x1000)	221 ± 53.7	239 ± 52.6	7.94	210 ± 47.5	242 ± 45.4	237 ± 61.7	235 ± 65.4	NS	NS	+
PRP platelet Number	281 ± 67.8	304 ± 66.6	8.20	277 ± 60.0	308 ± 57.7	289 ± 78.5	299 ± 82.5	NS	NS	NS
Maximal aggregation (cm/5min)	3.25 ± 1.35	4.02 ± 1.00 *	23.54	2.81 ± 1.38	4.19 ± 1.15*	3.75 ± 1.20	3.81 ± 0.80	*	NS	*
Time for maximal aggregation (min)	3.56 ± 2.02	5.18 ± 1.36*	45.22	3.86 ± 2.31	5.05 ± 1.06**	3.15 ± 1.67	4.67 ± 1.63	**	NS	NS
PRP-TXB_2 (pg/mL)	2109 ± 1478	2351 ± 1182	11.50	2143 ± 1781	2662 ± 1184	2064 ± 1112	1937 ± 1145	NS	NS	NS
PRP-TXB_2 (10^8 platelets pg/mL)	699 ± 369	749 ± 281	7.24	780 ± 415	753 ± 299	589 ± 297	744 ± 283	NS	NS	NS
Urine excretion (mL/24 h)	1075 ± 270	1330 ± 307**	23.77	1128 ± 304	1434 ± 246*	1003 ± 222	1125 ± 260	**	+	NS
Urine TXB_2 (pg/mL)	902 ± 306	1176 ± 433	30.38	951 ± 270	1241 ± 490*	835 ± 364	1088 ± 357	*	NS	NS
Urine TXB_2 (pg/min)	649 ± 213	1065 ± 418**	64.11	650 ± 222	993 ± 438	648 ± 222	1161 ± 408	**	NS	NS
Urine 6-keto $PGF_1\alpha$ (pg/mL)	991 ± 217	1224 ± 247	23.59	1053 ± 256	1275 ± 227	908 ± 126	1157 ± 277	**	NS	NS
Urine 6-keto $PGF_1\alpha$ (pg/min)	720 ± 165	1137 ± 368	57.79 NS	760 ± 163	1079 ± 266	667 ± 166	1213 ± 490	**	NS	NS
Thrombogenic ratio: TXB_2/6 keto $PGF_1\alpha$	0.91 ± 023	1.03 ± 0.30	13.19 NS	0.85 ± 0.19	1.07 ± 0.39	0.98 ± 0.28	0.98 ± 011	NS	NS	NS

High oleic acid sunflower oil (HOSO). Data (mean ± SD) in the same row for all and normocholesterolemic participants bearing one or two asterisks were significantly different (paired Student *t*-test). ** $p < 0.01$, * $p < 0.05$, + $p < 0.1$, *NS* Not significant

Thromboxane B_2 in ADP-stimulated platelet rich plasma

TXB_2 concentration in PRP was not significantly increased after the dietary intervention (Table 3). The type of dietary oil and the cholesterol level also did not have any significant effect on TXB_2 (Table 3). No significant differences were observed in the change induced by the dietary intervention on the PRP-TXB_2 concentration in women over 65 in respect to women under 65 ($+153.2 \pm 437.0$ *vs* -52.0 ± 386.3). Changes in PRP-TXB_2 in pg/mL were inversely correlated ($r = -0.534$, $p < 0.05$) with changes in HDL-cholesterol levels.

Eicosanoids in urine

In the palmolein dietary period, 24-h urinary excretion was significantly higher ($p < 0.01$) (Table 3). The urinary TXB_2 excretion in 24-h in pg/min increased significantly ($p = 0.003$) following the palmolein dietary period in respect to the HOSO. The concentration of urine 6-keto $PGF_1\alpha$ in pg/mL or pg/min did not change significantly because of the dietary intervention. The concentration of TXB_2 and 6-keto $PGF_1\alpha$ in pg/mL or pg/min appeared significantly affected (all, $p < 0.03$) by the oil type but not by the cholesterol level (Table 3).

Urine TXB_2 (in pg/mL) change induced by the dietary intervention was significantly higher in women under 65 than in the older counterparts ($+487.8 \pm 343.0$ *vs*. $+63.1 \pm 302.5$; $p = 0.031$). Similar tendency, although non-significant, was observed for the 6-keto $PGF_1\alpha$ in pg/mL ($+344.4 \pm 167.9$ *vs*. $+122.9 \pm 268.1$). Changes in the 6-keto $PGF_1\alpha$ (pg/mL) appeared inversely and significantly correlated with changes in plasma Apo B ($r = -0.550$, $p = 0.042$), LDL-ApoB ($r = -0.619$, $p = 0.018$) and the Apo A1/Apo B ratio ($r = 0.623$, $p = 0.017$). Changes in the urinary TXB_2 were highly correlated ($r = 0.811$, $p < 0.001$) with changes in the 6-keto $PGF_1\alpha$ (both in pg/min). Changes in the urine TXB_2/6-keto $PGF_1\alpha$ ratio appeared related to changes in total cholesterol ($r = 0.564$; $p = 0.04$) and HDL-cholesterol ($r = 0.628$, $p = 0.016$). The thrombogenic ratio change induced by the dietary intervention was not significantly different in women under 65 than in women over 65 ($+0.204 \pm 0.311$ *vs* $+0.041 \pm 0.27$).

Discussion

This study should be considered a first test in postmenopausal women of the substitution of a relatively high percentage (9–10%) of oleic acid by palmitic acid together with minor compounds present in both oils on the platelet aggregation and platelet and urinary eicosanoid production. Both diets presented the same energy profile and cholesterol content and were rich in MUFA but the energy contribution of palmitic and oleic acids were different while those of other SFA and PUFA were similar. Palmolein presented large amounts of tocotrienols and HOSO of tocopherols.

The increase in the maximal aggregation rate should be firstly related to the higher palmitic acid and lower MUFA contents of the palmolein diet. From earlier reviews concerning the effect of dietary fat type, it can be concluded that SFA promote arterial thrombus formation, whereas unsaturated fatty acids are not thrombogenic and may even be antithrombotic [23]. A significant effect on ADP-induced platelet aggregation was found in 42 healthy subjects [24] and in type IIa and IIb hyperlipoproteinemic patients [8]. These authors also found that ADP-induced aggregation and serotonin release were increased when washed platelets were incubated with isolated VLDL and LDL but decreased in the presence of high HDL [8]. However, in the present study, changes in the platelet maximal aggregation rate did not appear related to changes in LDL-cholesterol or in HDL-cholesterol levels. Furthermore, the increase in the PRP maximal aggregation rate due to the dietary intervention was relevant in the normocholesterolemic population (+49%) but irrelevant (–1.4%) in the hypercholesterolemic one. Disagreement also exists on the effects of tocotrienols on platelet aggregation. Some authors described the reduction of the *in vitro* platelet aggregation by tocotrienols [12] but those results disagree with those found by others [13]. In the present study, changes in PRP maximal aggregation rate did not appear related to serum or LDL-peroxides, tocopherol or tocotrienol levels. In our study, women received about 30 mg of tocotrienols/day during the palmolein diet, an amount that seemed to increase the time for maximal aggregation in normocholesterolemics but not in hypercholesterolemic women in which tocotrienols would not be enough to counterbalance the hyperaggregating effect of SFA. No differences in aggregation velocity between diets rich in palmitic acid and in oleic acid have been described [2]. Tocotrienols accumulate more readily in platelets than in plasma, and at low collagen concentrations tocotrienol supplementation beneficially affected platelet aggregation reactivity [25]. However, it has been observed that the vitamin E has no effect on platelet aggregation, even at the concentration of 10^{-3} μM and for a long incubation time [26].

The measurement of the platelet TXB_2 level *in vitro* assesses the capacity for TXA_2 production under maximal stimulation. The dietary intervention resulted in a non-significant increase of PRP-TXB_2 levels. It has been suggested that TXB_2 production should be produced as a consequence of the increase in LDL particles and this effect can be counterbalanced by the increase in HDL [8, 28]. In fact, changes in HDL-cholesterol were inversely

correlated with changes in PRP-TXB_2 in the present paper.

An optimal balance of the TXA_2/PGI_2 ratio may be important in the prevention of thrombotic conditions. Evidence indicates that dietary fatty acids can alter this balance [6]. Thus, the noted tendency for the urinary excretion of the TXA_2/PGI_2 ratio to increase in normocholesterolemic women following the palmolein diet in respect to the HOSO diet might be critical. In contrast with the present results, others have found a lower TXA_2/PGI_2 after the palmolein period compared to the HOSO period [28].

The increase in TXA_2 has been related to increase in blood pressure [24]. However, an increase of urinary volume due to the higher arterial pressure induced by SFA has also been reported [29]. In the present study the urinary TXB_2 excretion increased significantly after the dietary exchange with no significant modification in 6-keto $PGF_1\alpha$ excretion and the thrombogenic ratio. In contrast to present results, the dietary replacement of palmitic acid by oleic or linoleic acids has been shown to increase the urinary excretion of TXB_2 [7]. Others did not find any differences in 24h 6-keto prostaglandin $F_1\alpha$ excretion between the SFA and MUFA diets [24]. It has been reported that LDL inhibited the PGI_2 synthesis by endothelial cells, while HDL significantly enhanced the transformation of PGH_2 to PGI_2 [30]. Present results suggest that correlations are related to Apo B and the ApoAI/ApoB ratio changes in the case of changes in PGI_2. We are far from knowing the reasons for the discrepancy in results found in normo and hypercholesterolemic women in urinary TXB_2 excretion.

With the exception of urine TXB_2 levels that increased more in younger postmenopausal women following the dietary intervention, age did not seem to exert any relevant effect on the urinary eicosanoid production and in the thrombogenic ratio. Although it has been proposed that the HDL rich in Apo AII are involved in the progression of fatty streaks and thus increase CHD risk [31], present results do not suggest any significant effect of the changes in Apo AII on changes in platelet aggregation and eicosanoid production.

In conclusion, dietary intervention affected the platelet aggregation and urinary TXB_2 production more in normocholesterolemics than in hypercholesterolemics suggesting that the consumption of HOSO should be preferred to that of palmolein. Nevertheless, the thrombogenic ratio was not affected by the dietary intervention. Changes in platelet sensibility and prostacyclin excretion suggest that they were modulated by the changes produced in serum LDL levels while those of the thrombogenic ratio were by the changes in HDL levels. More studies are needed to understand the *in vivo* effect of SFA diets on thrombogenic risk and the relationship between serum cholesterol, serum peroxides, platelet activation and eicosanoid production.

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