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Mediterranean wild plants reduce postprandial platelet aggregation in patients with metabolic syndrome

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ARTICLE INFO

Article history:
Received 23 November 2010
Accepted 13 July 2011

ABSTRACT

Postprandial platelet hyperactivity and aggregation play a crucial role in the pathogenesis of metabolic syndrome. The purpose of the present study was to evaluate the effect of boiled wild plants consumption on the postprandial platelet aggregation in metabolic syndrome patients. Patients consumed 5 meals in a random order (ie, 4 wild plant meals, namely, *Reichardia picroides* [RP], *Cynara cardunculus*, *Urospermum picroides* [UP], and *Chrysanthemum coronarium*, and a control meal, which contained no wild plants). Several biochemical indices as well as platelet activating factor (PAF)– and adenosine diphosphate–induced ex vivo platelet aggregation were measured postprandially. Moreover, the ability of plants extract to inhibit rabbit platelet aggregation was tested in vitro. The consumption of RP and UP meals significantly reduced ex vivo adenosine diphosphate–induced postprandial platelet aggregation compared with the control meal. The consumption of UP meals significantly reduced the ex vivo PAF-induced platelet aggregation postprandially. Both UP and RP extracts significantly inhibited PAF-induced rabbit platelet aggregation in vitro. Wild plants consumption reduced postprandial platelet hyperaggregability of metabolic syndrome patients, which may account for their healthy effects.

1. Introduction

The *metabolic syndrome* (MS) is defined as a cluster of metabolic abnormalities, namely, insulin resistance and hyperinsulinemia, dyslipidemia with elevated triacylglycerols and/or low high-density lipoprotein cholesterol (HDL-C) in plasma, hypertension, and obesity [1,2]. More recently, the prothrombotic and proinflammatory states have been recognized as components of the MS [3]. A prothrombotic condition may arise when the balance between the 3 main parts of hemostasis, namely, coagulation system, fibrinolysis, and platelet function, becomes disrupted. Metabolic syndrome patients exhibit a pattern of coagulation factors that promote thrombosis or retard thrombolysis [4] as well as higher platelet activity [5-7]. Platelets activation is thought to play a crucial role in the initiation and development of atherosclerotic lesions [8,9] and diabetes [10]. In parallel, MS is connected with inflammation [11] and cardiovascular events [12].

Platelet activating factor (PAF) is a potent mediator of inflammation and platelet aggregation [13]. In addition, PAF is produced during low-density lipoprotein (LDL) oxidation [14]

Authors' contributions: EF: wrote the manuscript and performed the biochemical analysis; PD: performed the blood handling and partially the biochemical analysis; TN: performed the biochemical analysis and drafted the manuscript; EP: performed the anthropometric measurements and partially performed the biochemical analysis, DBP: performed the statistical analysis; SA: designed the study and critically reviewed the manuscript.

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0026-0495/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2011.07.006

and constitutes a juxtacrine signaling molecule at the surfaces of activated human platelets that are critical in cell-cell interactions in inflammatory and thrombotic responses [15]. Moreover, the main enzyme responsible for PAF degradation and inactivation, lipoprotein-associated phospholipase A_2 (Lp-PLA_2), also known as *serum PAF-acetylhydrolase*, is differentiated across levels and topology of adiposity [16].

Apart from the elevated fasting glucose and lipids levels, postprandial hyperglycemia and hypertriglyceridemia are also considered as cardiovascular risk factors [17]. This is of particular importance given the long duration of postprandial state in the daily life. Both postprandial hyperglycemia/hyperinsulinemia and hyperlipidemia affect the hemostatic system including platelet activation [18,19].

Although the etiology of MS has not yet been fully elucidated, complex interactions between genetic, metabolic, and environmental factors including diet are thought to be involved. The Adult Treatment Panel III recommendations for MS patients are consistent with the general dietary recommendations [20]. Recently, the scientific advisory committee of the American Heart Association has stated that a Mediterranean-style diet has favorable effects against the progression of cardiovascular diseases [21]. The existence of PAF antagonists in Mediterranean diet may provide an explanatory mechanism for this atheroprotective effect [15]. Indeed, previous results indicate that the reduction of PAF-induced rabbit platelet aggregation is associated with decreased thickness of atherosclerotic lesions compared with cholesterol-fed rabbits [22]. Traditionally, over the Mediterranean basin, a diversity of plants is consumed, often gathered from the wild. However, little is known about the postprandial effects of wild plants [23]; and especially, there are no data concerning their effect on postprandial platelet aggregability.

The wild plants *Reichardia picroides* (RP), *Cynara cardunculus* (artichokes) (CY), *Urospermum picroides* (UP), and *Chrysanthemum coronarium* (CC) belong to the Asteraceae family and are widely consumed by the Greek people. The extracts from the above plants have revealed important in vitro biological activity concerning antioxidant, anti-inflammatory, antiproliferating, and antidiabetic activities as well as their ability to inhibit specific enzymes [24,25].

The aim of the present study was to examine the effect of boiled wild plants, which are widely consumed by the Greek people and other Mediterranean populations, on postprandial platelet aggregation, mainly against PAF, in patients with MS. Because the postprandial condition is recognized as a procoagulant state, any reduction in postprandial platelet aggregation would imply a reduced risk of thrombosis and related conditions.

2. Methods

2.1. Materials

All solvents were of analytical grade and supplied by Merck (Darmstadt, Germany). Semisynthetic PAF, adenosine diphosphate (ADP), and bovine serum albumin (BSA) free of fatty acids were obtained from Sigma (St Louis, MO).

2.2. Wild plants

The wild plants were collected in Crete during April. The plants were immediately delivered to Harokopio University where the seed heads were cleaned up. The edible green parts of each plant (ie, the heart of *Cynara* and the leaves of the rest of the plants) were weighted, separated into equal portions of 200 g, scalded for 5 minutes, and stored at approximately –20°C until the day of the experiment. This is a common way in which Greeks preserve the wild plants for later consumption. The MS volunteers consumed 450 g of RP, UP, and CC (water replenishment) or, in the case of CY, 100 g of boiled plant. The nutritional data have been calculated for boiled plants.

2.3. Extraction of total lipids

Two hundred grams of each plant was boiled, and total lipids were extracted according to the method of Bligh and Dyer [26]. The extracted total lipids were stored under air nitrogen at –20°C until used. For the biological assay, an amount of each extract was dissolved in 2.5 mg of BSA per milliliter of saline.

2.4. In vitro washed rabbit platelets aggregation

The effect of lipid extracts on platelet activity was tested on washed rabbit platelets according to the method of Demopoulos et al [27]. Platelet activating factor was dissolved in 2.5 mg of BSA per milliliter of saline. The platelet aggregation induced by PAF (final concentration, 0.6×10^{-9} mol/L), namely, PAF-induced aggregation, was measured in washed rabbit platelets before (considered as 0% inhibition) and after the addition of various concentrations of the examined sample. Consequently, the plot of percentage inhibition (ranging from 20% to 80%) vs different concentrations of the sample was linear. From this curve, the concentration of the sample that inhibited 50% PAF-induced aggregation was calculated. This value was defined as the IC_{50}, namely, inhibitory concentration producing 50% inhibition. The IC_{50} values are expressed as micrograms of lipid extracts required for 50% inhibition against PAF.

2.5. Subjects

A group of volunteers from the region of Athens diagnosed with MS was recruited (n = 24). The diagnosis of MS was made according to the Adult Treatment Panel III criteria [20] by physicians at the Endocrinology Department of the General Hospital of Athens and the Diabetology Department of the Nikea General Hospital of Athens. The exclusion criteria are described elsewhere [23]. The purpose and procedures of the study were explained to each subject, and all participants gave written informed consent.

2.6. The meals

Each plant meal consisted of a boiled green plant (RP, CY, UP, or CC), 3 slices of bread, 60 mL of refined oil, and 7.5 mL of canned lemon juice. The control meal included all the above except the plant. All meals were freshly prepared on the day of the intervention. The nutritional composition of the wild plants and control meals is shown in Table 1. The calculation

of the nutrient content of the meals was based on the following databases: the US Department of Agriculture database for the white, commercially prepared bread; canned lemon juice; and wild plants except artichoke (taking boiled radish as reference plant because no data exist for RP, UP, and CC) [28]; the food database developed at the University of Crete for the wild artichoke [29]; and the food database of Trichopoulou for the analysis of olive oil [30].

2.7. Study design

All subjects (n = 24) were asked to consume the following 5 meals: RP, CY, UP, CC, and a control meal, which contained no wild plants. The order of meals consumed was random for each individual. The randomization was performed using a research randomizer (Copyright 1997-2011 by Geoffrey C Urbaniak and Scott Plous). The consumption of all meals was spaced 2 weeks for each subject. Of the prespecified number of participants, 15 completed the study by consuming all 5 meals, whereas the rest of the volunteers consumed 3 or 4 meals. Thus, RP was consumed by n = 18 participants, CY was consumed by n = 19 participants, UP was consumed by n = 18 participants, CC was consumed by n = 17 participants, whereas all participants consumed the control meal. At least a week before the experiment, the volunteers were given detailed advice about following a diet low in dietary fiber, vegetables, green plants, and generally antioxidant and phenolic compounds. Special attention was given not to drink coffee, tea, or alcohol (washout period); and they were advised to keep their usual physical activity. After a 12-hour fast, the volunteers visited Harokopio University. Before the administration of the meal, a cannula with an Intima 20-gauge intravenous catheter (Becton Dickinson, Rutherford, NJ) was inserted into the brachial vein; and after a 15-minute rest, a fasting blood sample was drawn by registered personnel. Subjects consumed the entire meal within 15 minutes, and the time that subjects finished consuming the meal was considered to be time 0 for subsequent measurements. Blood was collected again at 0.5, 1, 2, 3, and 4 hours. The volunteers completed a detailed questionnaire concerning the demographic characteristics, medical history, smoking, dietary habits, and physical activity. Subjects remained supine for the duration of the study and were allowed to drink only mineral water.

The Bioethics Committee of Harokopio University approved the aforementioned protocol. Moreover, all procedures were performed according to the Declaration of Helsinki (revised in 1983).

2.8. Blood sampling and handling

All blood samples were collected from the brachial vein of the volunteers. Venous blood samples for the determination of serum glucose, insulin, triacylglycerols, total cholesterol, LDL cholesterol (LDL-C), HDL-C, apolipoprotein (apo) A-I, apo B, and homocysteine were drawn into evacuated glass tubes (BD Vacutainer Systems; Becton Dickinson, Plymouth, United Kingdom). Serum was collected after 30 minutes by centrifugation at 1200*g* for 10 minutes at 10°C. For the determination of fibrinogen, 1.8 mL blood was drawn into evacuated BD citrated Vacutainers and immediately centrifuged at 800*g* for 10 minutes at 4°C ;and the supernatant plasma was collected. Plasma or serum was immediately aliquoted and stored at approximately –80°C.

2.9. Anthropometric measurements

Anthropometry (ie, weight, height, and waist and hip circumference) was carried out on each visit for MS subjects according to standard procedures published elsewhere [23]. A physician measured the blood pressure of volunteers after at least 20 minutes of seated rest and before the meal consumption. For each subject, 3 measurements were taken at 1-minute intervals; and the average of the last 2 was used. Body mass index (BMI) was calculated as weight (in kilograms) divided by height (in meters) squared.

2.10. Ex vivo human platelet-rich plasma aggregation

Blood (10 mL) was collected at baseline and postprandially (2, 3, and 4 hours) from the brachial vein in polyethylene

Table 1 – Nutritional composition of the control and plants meals consumed by MS patients

Nutritional composition	Control meal				Plant meal (control + plant)		Artichoke meal (control + CY)	
	Refined olive oil	White bread	Lemon juice	Total meal	Boiled plant [a]	Total meal	Boiled artichoke [b]	Total meal
Weight (g)	56.8	75.0	7.50	139.4	450	589.3	100	239.4
Carbohydrate (g)	0	40.8	0.49	41.29	15.9	57.19	9.3	50.5
Fiber (g)	0	1.9	0	1.9	7.38	9.28	5.4	7.3
Protein (g)	0	6.75	0.03	6.78	4.05	10.83	3.20	9.98
Fats (g)	56.7	3.00	0.02	59.72	0.9	60.62	0.16	59.88
SFA (g)	8.10	0.43	0.003	8.54	NA	8.533	0.037	8.47
MUFA (g)	41.70	0.597	0.00	42.30	NA	42.30	0.01	42.30
PUFA (g)	4.20	1.57	0.01	5.77	NA	5.77	0.07	5.84
Water (g)	0	22.8	6.93	29.73	427.5	457.2	81.9	111.63

SFA indicates saturated fat; MUFA, monounsaturated fat; PUFA, polyunsaturated fat.

[a] *Reichardia picroides*, UP, and CC.

[b] *Cynara cardunculus*, artichoke.

tubes containing 0.065 mol/L citric acid and 0.085 mol/L trisodium citrate as anticoagulant (blood to anticoagulant ratio of 9:1). Platelet-rich plasma (PRP) was obtained by centrifugation at 173*g* for 8 minutes at 24°C. The PRP was transferred to polypropylene tubes at room temperature for the biological assay. Platelet-poor plasma was obtained by further centrifugation of the residue at 1.120*g* for 20 minutes at 24°C. The density of platelets in the PRP was adjusted to 500 000 cells per microliter by adding platelet-poor plasma from the same donor.

The aggregation induced by various concentrations of PAF or ADP was measured in human PRP. The maximum reversible or the least not reversible aggregation was evaluated for PAF and ADP to assess 100% aggregation. The plot of percentage aggregation (ranging from 20% to 80%) vs different concentrations of aggregatory agent (PAF or ADP) is linear. From this curve, the concentration of each aggregatory agent that induces 50% of maximum aggregation was calculated. This value was defined as the EC_{50}, namely, equivalent concentration for 50% aggregation.

2.11. Biochemical measurements

Serum glucose, triacylglycerols, total cholesterol, LDL-C, and HDL-C were determined enzymatically with an ACE biochemical analyzer (Schiapparelli Biosystems, Fairfield, NJ) using reagents from Alfa Wassermann (Woerden, the Netherlands). Serum insulin was determined by an immunoenzymometric assay on an AIA 600 II enzyme immunoassay system (Tosoh, Tokyo, Japan). Serum apo A-1 and apo B were determined using an immunoturbidimetric assay, where they formed a precipitate with a specific antiserum (sheep antiserum for apo A and rabbit antiserum for apo B), which was determined turbidimetrically at 340 nm by using the Cobas Integra 700 biochemical analyzer (Roche Diagnostics, Mannheim, Germany). Serum homocysteine concentrations were measured by high-performance liquid chromatography with fluorometric detection. For the fibrinogen levels evaluation, citrated plasma was immediately loaded on a Coatron 2 analyser (Biotechnology, Athens, Greece); and fibrinogen was measured using Teclot Fib (W. Kaolin, TECO Medical). Homeostasis model assessment of insulin resistance was calculated using the following formula: fasting insulin (microunits per milliliter) × fasting glucose (millimoles per liter)/22.5.

The Lp-PLA_2 activity in serum was determined by the trichloroacetic acid precipitation method using (^{3}H) PAF as the substrate. Briefly, 2 μL of serum was incubated with 4 nmol of (^{3}H) PAF (20 Bq/nmol) for 15 minutes at 37°C in a final volume of 200 μL of 100 mmol/L Tris/HCl buffer (pH 7.2) containing 1 mmol/L EGTA. The reaction was terminated by the addition of 2 μL BSA 100 μg/μL and cold trichloroacetic acid (10% final concentration). The samples were then placed in an ice bath for 30 minutes and subsequently centrifuged for 2 minutes. The (^{3}H)-acetate released into the aqueous phase was measured on a liquid scintillation counter. All assays were performed in duplicate. The enzyme activity was expressed as nanomoles of PAF degraded per minute per milliliter of serum.

2.12. Statistical analysis

Normally distributed continuous variables are presented as mean ± standard deviation; normality was evaluated using the Kolmogorov-Smirnov criterion. Categorical variables are presented as frequencies. Differences in the mean values of the baseline characteristics of the subjects before the consumption of the control or plant meal (within each intervention group) were evaluated by a paired *t* test. Differences in the mean values of subjects' baseline characteristics between the different intervention groups were assessed by analysis of variance (ANOVA) after checking for homoscedasticity using the Levene test. Associations between categorical variables were evaluated using the χ^2 test. Generalized estimating equations (GEEs) were fitted to compare the postprandial changes of the biochemical parameters studied between the intervention groups, as well as across time points (baseline, 1 hour, 2 hours, and 4 hours), using as covariates age (in years), sex, BMI (in kilograms per square meter), alcohol intake (in grams per day), current smoking (yes/no), and baseline triacylglycerols values (in milligrams per deciliter). Intervention groups were entered with dummy variables encoding. Because normality was achieved for the dependent variables, the normal distribution was used for fitting GEE, with the identity as the link function. The unstructured formation of the correlation matrix was used after comparing various scenarios using the corresponding quasilikelihood under the independence criterion for model's goodness-of-fit. First-order interactions between intervention groups and time points were also evaluated; and marginal means were calculated for each group, at each time point, as well as at intervention group by time point. Moreover, post hoc analysis for comparing mean values of the studied characteristics between the intervention groups, across time points as well as between different time points, was applied using the Bonferroni correction rule to adjust for the inflation of type I error. Results from GEE are presented as *b*-coefficients and the corresponding standard errors. The intention-to-treat analysis was the approach used for the analysis of the data. The SPSS 18.0 for Windows (Chicago, IL) was used for all the analysis.

3. Results

3.1. In vitro activity of plant extracts against platelet aggregation

The extracted total lipids from each boiled plant were tested for their ability either to induce platelet aggregation or to inhibit PAF-induced washed rabbit platelet aggregation. None of them induced platelet aggregation, whereas all the extracts inhibited PAF-induced platelet aggregation as shown in Fig. 1. The RP and UP extracts revealed a more potent inhibitory activity against PAF-induced platelet aggregation (lower IC_{50} values) compared with the other plant extracts ($P = .05$).

3.2. Baseline characteristics of the participants

The baseline profile of the participants in the studied groups is shown in Tables 2 and 3. Elevated levels of glucose and

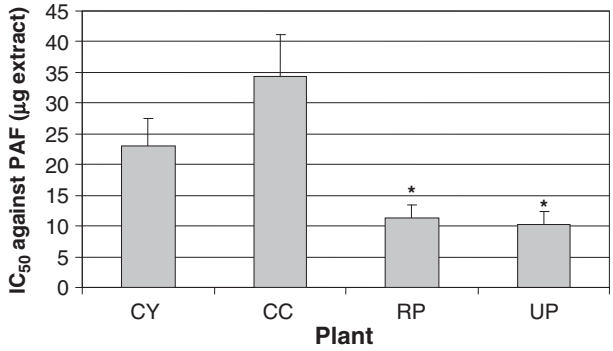

Fig. 1 – IC_{50} values of boiled plants lipid extracts against PAF-induced rabbit platelet aggregation. Data are expressed as mean ± standard deviation. *Statistical difference in relation to CY and CC ($P < .05$).

insulin; abnormally high levels of total cholesterol, LDL-C, triacylglycerols, and fibrinogen; and low levels of HDL-C were observed, indicating a general dysregulation of the metabolic system of these volunteers, as expected. All the baseline demographic, lifestyle, anthropometric (Table 2), and biochemical (Table 3) characteristics did not differ between the intervention groups. Moreover, no significant day-to-day variation between the control and the specific plant meal in fasting levels of the measured biochemical parameters was observed within each intervention group (Table 3).

3.3. *Effect of control meal on postprandial levels of biochemical indices*

Compared with baseline values, glucose and insulin were significantly increased at 0.5, 1, and 2 hours after the consumption of the control meal (glucose: $P < .001$, $P < .001$, $P = .005$, insulin: $P < .001$, $P < .001$, $P = .027$, respectively). Triacylglycerols levels were also increased at 1 hour after the meal consumption and progressively increased up to 4 hours as compared with baseline values (all Ps $< .001$). A decrease was observed in homocysteine levels at 2 ($P < .001$) and 3 hours ($P = .007$) after the consumption of the control meal, whereas the homocysteine values at 4 hours were similar to the baseline levels. Postprandial levels of fibrinogen did not differ at any time point after the consumption of the control meal as compared with baseline values (all Ps $> .05$).

3.4. *Effect of control meal on postprandial ex vivo platelet aggregation*

All patients showed the same response to the control meal in terms of ex vivo platelet aggregation. A time-dependent increase in ADP-induced PRP aggregation (lower EC_{50}) was observed. In particular, lower EC_{50} were observed at 3 ($P = .06$) and 4 hours ($P = .04$) after the control meal consumption as compared with baseline values. In addition, PAF-induced PRP aggregation showed a borderline reduction of EC_{50} values at 2 hours after the control meal consumption as compared with baseline values ($P = .11$) (Figs. 2 and 3).

3.5. *Effect of plant meals on postprandial levels of biochemical factors*

After adjusting for age, sex, BMI, and smoking, no significant differences in glucose levels were observed after the consumption of all plants meal as compared with the control meal (all Ps $> .2$). However, compared with the control group, a significant difference of insulin levels in the RP group ($b \pm$ SE: -7.43 ± 2.94, $P = .01$) and the UP group ($b \pm$ SE: -5.10 ± 2.02, $P = .01$) was observed after adjusting for age, sex, BMI, and smoking. Moreover, a significant difference was also observed in triacylglycerols levels in the RP group ($b \pm$ SE: 20.8 ± 4.65, $P < .001$), in fibrinogen levels in the UP group ($b \pm$ SE: -26.0 ± 9.35, $P = .005$), and in homocysteine levels in the CY group ($b \pm$ SE: 0.49 ± 0.46, $P = .007$) as compared with the control group.

3.6. *Effect of plant meals on postprandial ex vivo ADP-induced platelet aggregation*

The EC_{50} values of the participants' PRP against ADP-induced platelet aggregation are presented in Fig. 2. As regards the RP intervention group, EC_{50} values against ADP-induced platelet

Table 2 – Demographic, lifestyle, and anthropometric characteristics of volunteers at baseline and according to the intervention group

	RP	UP	CC	CY	P
No. of subjects (n)	18	18	17	19	–
Male/female (n)	6/12	6/12	6/11	5/14	.96
Age (y)	58.6 ± 11.3	58.6 ± 11.3	57.7 ± 12.3	56.0 ± 12.0	.86
Smoking (yes)	10	6	6	10	.55
BMI (kg/m^2)	34.0 ± 4.9	33.9 ± 4.9	33.9 ± 5.1	34.7 ± 5.4	.80
Waist circumference (cm)	104 ± 16	101.9 ± 15.9	102.9 ± 16.6	104.2 ± 14.9	.90
Hip circumference (cm)	113 ± 10	111.9 ± 9.4	112.1 ± 10.6	114.6 ± 10.9	.37
Waist to hip ratio	0.982 ± 0.266	0.907 ± 0.089	0.988 ± 0.282	0.908 ± 0.009	.58
Systolic BP (mm Hg)	135 ± 18	136 ± 16	137 ± 19	138 ± 15	.98
Diastolic BP (mm Hg)	80.6 ± 7.9	81.8 ± 6.3	79 ± 8	82 ± 7	.94

Data are expressed as mean ± standard deviation. Differences in the baseline characteristics of subjects between intervention groups were assessed by ANOVA. Differences in the baseline characteristics of subjects between intervention groups for nonparametric variables (ie, sex and smoking status) were assessed by Kruskal-Wallis nonparametric test. BP indicates blood pressure.

Table 3 – Biochemical characteristics of volunteers with MS at baseline and according to the intervention group

	RP		UP		CC		CY	
	Control meal	Plant meal	Control meal	Plant meal	Control meal	Plant meal	Control meal	Plant meal
Glucose (mg/dL)	135.6 ± 52.8	135.7 ± 46.7	137.6 ± 51.9	136.7 ± 47.1	140.5 ± 51.9	139.7 ± 49.3	137.5 ± 50.3	132.1 ± 46.9
Insulin (μU/mL)	9.6 ± 4.2	10.6 ± 4.6	10.2 ± 4.7	9.2 ± 3.5	10.2 ± 4.7	9.4 ± 2.4	12.1 ± 6.5	11.1 ± 4.8
HOMA-IR	3.23 ± 2.03	3.42 ± 2.01	3.46 ± 2.13	2.94 ± 1.17	3.58 ± 2.19	3.17 ± 1.22	4.0 ± 2.3	3.5 ± 1.4
Fibrinogen (mg/dL)	352.8 ± 111.7	341.3 ± 77.7	359.1 ± 110.9	344.2 ± 75.4	338.4 ± 63.7	332.7 ± 66.9	381.6 ± 114.9	360.2 ± 76.4
Total cholesterol (mg/dL)	234.9 ± 67.2	221.8 ± 36.2	219.4 ± 47.5	205.2 ± 42.9	219.9 ± 46.0	206.5 ± 37.4	227.8 ± 41.5	224.9 ± 37.5
LDL-C (mg/dL)	158.0 ± 35.4	158.5 ± 31.5	150.9 ± 35.9	146.5 ± 33.2	153.9 ± 33.3	150.9 ± 28.6	155.1 ± 29.3	161.1 ± 35.5
HDL-C (mg/dL)	42.8 ± 3.0	42.2 ± 4.9	42.4 ± 4.0	41.8 ± 4.8	42.5 ± 4.11	42.9 ± 4.6	42.5 ± 4.1	42.8 ± 3.6
Triglycerides (mg/dL)	188.1 ± 51.6	184.8 ± 41.6	190.7 ± 53.9	196.6 ± 48.0	193.5 ± 53.1	177.9 ± 40.3	187.4 ± 53.1	193.2 ± 58.3
Apo A (mg/dL)	140.4 ± 20.7	140.4 ± 21.9	139.7 ± 20.3	140.2 ± 20.8	139.9 ± 21.4	141.0 ± 20.5	142.3 ± 17.0	139.4 ± 14.2
Apo B (mg/dL)	97.0 ± 20.3	96.0 ± 20.6	94.3 ± 21.5	94.7 ± 24.5	95.6 ± 20.4	93.5 ± 20.9	96.1 ± 17.6	98.8 ± 17.6
Homocysteine (μmol/L)	15.15 ± 2.63	14.73 ± 2.59	15.29 ± 3.82	15.14 ± 4.04	15.66 ± 3.28	15.19 ± 3.25	13.62 ± 3.09	12.58 ± 2.61

Data are expressed as mean ± standard deviation. Differences in the baseline characteristics of subjects before the consumption of the control and plant meal (within each group) were assessed by a paired *t* test. Differences in the subjects' baseline characteristics between different groups were assessed by ANOVA. No significant differences in baseline biochemical characteristics between the control and plant meals, within each group, were observed (*P*s > .05). No significant differences in baseline biochemical characteristics between the intervention groups as regards the consumption of the control or plant meal were observed (*P*s > .05). HOMA-IR indicates homeostasis model assessment of insulin resistance.

aggregation were higher at 2 hours compared with baseline ($P = .003$); as regards the UP intervention group, EC_{50} values were higher at 2 ($P = .01$) and 4 hours ($P = .03$) compared with baseline; as regards the CY group, EC_{50} values were lower at 2 hours compared with baseline ($P = .01$); and in the CC group, EC_{50} values were higher at 2 ($P < .001$) and 3 hours ($P = .006$) compared with baseline values. No other significant differences were observed as regards the aforementioned intervention groups.

Unadjusted analysis revealed an effect of intervention group on EC_{50} values against ADP-induced aggregation, an effect of time ($P = .002$), as well as a significant interaction between intervention by time points ($P < .001$), suggesting an overall different pattern of EC_{50} values between intervention groups as compared with the control group across time (Fig. 2). In particular, a difference of EC_{50} values against ADP-induced platelet aggregation was observed only between the UP intervention and the control group ($b \pm SE$: 0.142 ± 0.06, $P = .02$). When the analysis was adjusted for age, BMI, alcohol intake, smoking, and baseline triacylglycerols, EC_{50} values of the UP intervention group were still higher as compared with the control ($b \pm SE$: 0.118 ± 0.05, $P = .03$); also, the RP intervention group had higher EC_{50} as compared with the control ($b \pm SE$: 0.065 ± 0.03, $P = .05$). Afterward, the analysis was further adjusted for sex; and the aforementioned results slightly lost their significance (for UP vs control, $b \pm SE$: 0.109 ± 0.06, $P = .07$; for RP vs control, $b \pm SE$: 0.055 ± 0.03, $P = .07$). No significant differences on EC_{50} values against ADP-induced platelet aggregation between intervention groups were found (all $P > .2$).

3.7. *Effect of plant meals on postprandial ex vivo PAF-induced platelet aggregation*

The EC_{50} values of the participants' PRP against PAF-induced aggregation are presented in Fig. 3. It was observed that, for the RP intervention group, EC_{50} values were higher at 2 hours as compared with baseline ($P = .02$); regarding the UP group, EC_{50} values were higher at 2 ($P < .001$) and 3 hours ($P = .01$) compared with baseline values, whereas regarding the CC group, EC_{50} values were higher at 3 hours as compared with baseline ($P = .03$). No other significant differences on EC_{50} values against PAF-induced aggregation were observed as regards the aforementioned intervention groups.

Unadjusted analysis revealed a significant effect of intervention groups on EC_{50} values against PAF-induced aggregation, an effect of time ($P = .002$), as well as an interaction between intervention groups by time points ($P < .001$), suggesting an overall different pattern of EC_{50} values between intervention and control group across time (Fig. 3). Specifically, a significant difference of EC_{50} values against PAF-induced platelet aggregation only between the UP intervention and the control group ($b \pm SE$: 7.66 ± 3.09, $P = .01$) was observed. Afterward, the analysis was adjusted for age, sex, BMI, alcohol intake, smoking, and baseline triacylglycerols levels and revealed that EC_{50} values of the UP intervention group were slightly higher as compared with the control group ($b \pm SE$: 6.57 ± 3.9, $P = .07$). The consumption of RP, CY, and CC plant meals did not alter EC50 values against PAF-induced aggregation as compared with the control meal (all *P*s > .50). Moreover, the CC intervention group had lower EC_{50} values as compared with the UP intervention group ($b \pm SE$: −7.8 ± 2.8, $P = .07$). No other differences on EC_{50} values against PAF-induced aggregation between intervention groups were found (all *P*s > .20). Moreover, no effect of sex was observed as regards the aforementioned results.

In addition, Lp-PLA_2, the main enzyme for PAF degradation, is present in PRP because it is bound to lipoproteins and mainly to LDL particles. To examine if the observed reduction in platelet sensitivity in response to PAF was due to a possible enzymatic reduction of its levels, the activity of its Lp-PLA_2 was also measured. No statistical difference was observed in Lp-PLA_2 after the consumption of both control and plant

meals, indicating that this enzyme is not involved in the observed EC_{50} PAF reductions. Baseline premeal values of the enzyme were not statistically different between the groups (data not shown).

4. Discussion

The present work aimed to evaluate whether the consumption of various plant meals may alter the postprandial platelet aggregability of individuals with MS. To the best of our knowledge, this is the first time that the postprandial effects of edible boiled plants on platelet aggregability were studied. The main finding was that the consumption of plant meals by MS patients could reduce postprandial platelet sensitivity to PAF and/or ADP. Moreover, the ex vivo protective effects of plant consumption were almost in accordance with their extracts' ability to reduce the in vitro platelet aggregation in response to PAF. It is also noteworthy that previous studies on patients with type 2 diabetes mellitus showed that a 1-month diet consisting of several traditional foods, capable of inhibiting platelet aggregation ex vivo, reduced platelet sensitivity of this group compared with the control group, indicating accordance between in vitro and in vivo results [31].

To test the effects of plant consumption on MS patients, a traditional meal with boiled plants accompanied with olive oil, lemon juice, and bread was provided, which represents a common way that Greek people consume the boiled plants [32]. Refined olive oil was used instead of virgin olive oil because the latter is a rich source of polyphenols and other micronutrients that may affect the postprandial actions of wild plants, which also have a high content of similar micronutrients. The selection of the specific plants was based on their previously observed potent in vitro antioxidant, anti-inflammatory, antiproliferating, and antidiabetic activities [24].

This study expands the already reported list of the in vitro biological activities of these plants because boiled plant extracts were used. It should be mentioned that, during cooking, several ingredients of the plant can decompose or be extracted by the water. The tested extracts inhibited the in vitro PAF-induced washed rabbit platelet aggregation, which is of great importance because PAF, apart from its role in platelet aggregation, also plays a central role in the inflammatory process. More specifically, the RP and the UP extracts were the most potent ones followed by the CY and CC extracts. This finding indicates the possible existence of compounds capable of reducing platelet aggregation against PAF, which has been documented for other Mediterranean foods [33].

The postprandial state is recognized as procoagulant; and studies have investigated the effects of several aggregatory agents such as ADP, thrombin, and collagen in this state. However, there are no data about the postprandial sensitivity of platelets to PAF as aggregating agent, which is also a potent lipid mediator [15]. In this study, one of the most common agents, ADP, was selected for comparison. In the postprandial state, LDL particles are subject to oxidative or glycemic modification, become more atherogenic, and are enriched

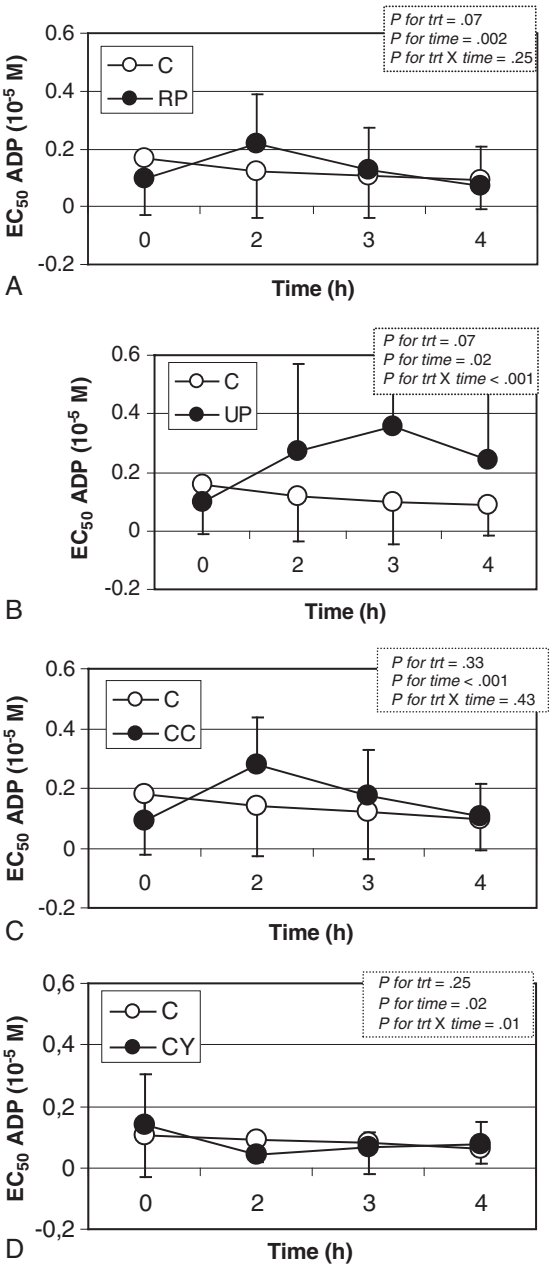

Fig. 2 – EC_{50} values of MS participants' PRP against ADP-induced aggregation after the consumption of plant and control meals. A, The EC_{50} values of MS participants' PRP against ADP-induced aggregation after the consumption of the RP and the control (C) meal. B, The EC_{50} values of MS participants' PRP against ADP-induced aggregation after the consumption of the UP and C meal. C, The EC_{50} values of MS participants' PRP against ADP-induced aggregation after the consumption of the CC and C meal. D, The EC_{50} values of MS participants' PRP against ADP-induced aggregation after the consumption of the CY and C meal. Data are expressed as mean ± standard deviation. Generalized estimating equation was used to assess the effect of group, time, and group by time interaction on postprandial values using age, sex, BMI, alcohol intake, smoking, and baseline triacylglycerols levels as covariates. *P* values for the main effect of the treatment group (trt), time, and group by time interaction are presented.

with PAF [14] and oxidized phospholipids, namely, PAF-like lipids [34]. These lipids exhibit similar biological activities to PAF, including platelet aggregation [35,36]. Therefore, the possible existence of PAF antagonist in plants could have a dual role by reducing platelet sensitivity against PAF and blocking PAF inflammatory actions.

The control meal used in the present study consisted of refined olive oil, bread, and lemon juice, which could be characterized as a meal with medium to high fat and carbohydrate. As expected, it increased postprandial glucose, insulin, and triacylglycerol levels. In addition, an increase in platelet sensitivity to ADP was observed at 3 and 4 hours after the control meal; a borderline increase in platelet sensitivity to PAF was also observed (at 2 hours). Previous results are conflicting concerning the effect of postprandial lipemia on ADP- induced platelet aggregation [37,38], whereas no data exist for PAF. Several mechanisms could be responsible for these observations. Hyperglycemia, hyperinsulinemia, as well as hypertriglyceridemia may lead to (*a*) impaired calcium homeostasis, (*b*) inhibition of the platelet membrane Na^+/K^+-adenosine triphosphatase activity, (*c*) activation of protein kinase C, (*d*) decreased production of nitric oxide, (*e*) increased formation of reactive oxygen species, and (*f*) nonenzymatic glycation of platelet membrane glycoproteins [19,39]. These actions are capable of inducing platelet shape change as well as increasing platelet aggregation to agonists.

The effect of plant meals consumption on platelet aggregation seems to be plant and agonist specific. All plant interventions altered the EC_{50} values against ADP-induced platelet aggregation across time compared with their baseline values. However, the treatment-specific analysis revealed that only the RP and UP interventions improved ADP platelet sensitivity as compared with the control group. Concerning PAF-induced platelet aggregation, all plant interventions changed the EC_{50} across time compared with their baseline values. However, the treatment-specific analysis revealed that only the UP intervention led to a borderline reduction of PAF platelet sensitivity as compared with the control intervention.

The differentiation of plant action against postprandial platelet aggregation cannot be explained by their macronutrient content, which is similar. In addition, their effects cannot be attributed to postprandial glucose changes because no alterations were observed compared with the control meal. The RP and UP meals reduced postprandial insulin levels compared with control meal. Insulin is known to inhibit platelet aggregation against ADP [40]; therefore, it is unlikely that the effect of plant on platelet sensitivity may be attributed to the differences in insulin responses. In addition, published results have shown that extracts from these plant species do not differ in their antioxidant capacity in vitro [24]. A more plausible explanation may lie in the different microconstituents, which may act as PAF and/or ADP inhibitors and therefore contribute to the observed variation in their antiaggregatory action. Concerning the artichoke plant, previous studies have shown the existence of bioactive phenolic compounds that may inhibit

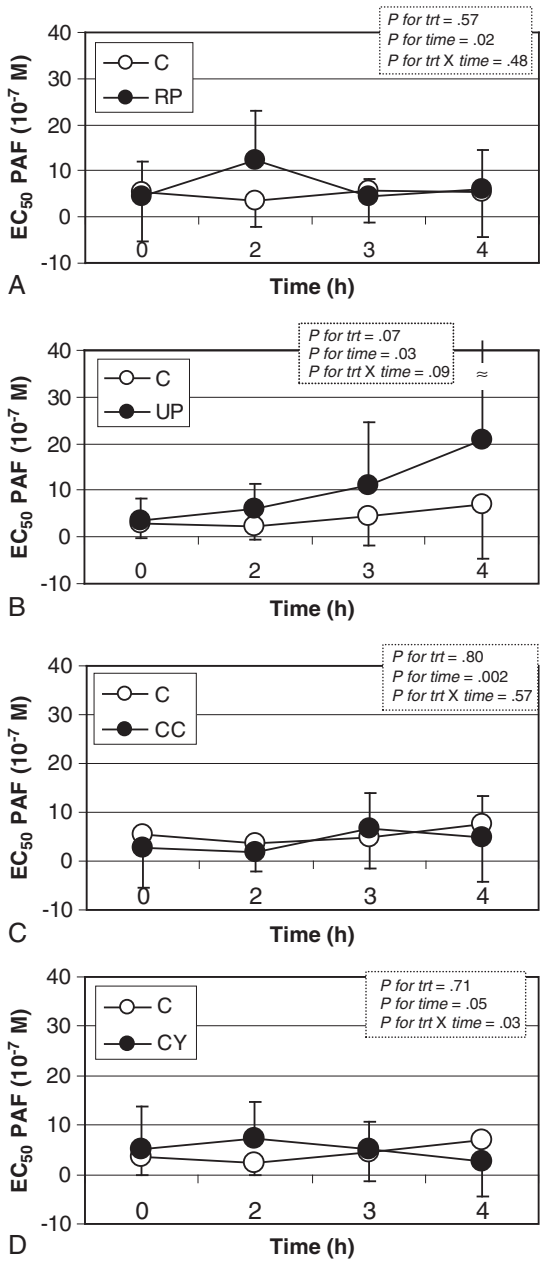

Fig. 3 – EC_{50} values of MS participants' PRP against PAF-induced aggregation after the consumption of plant and control meals. A, The EC_{50} values of MS participants' PRP against PAF-induced aggregation after the consumption of the RP and C meal. B, The EC_{50} values of MS participants' PRP against PAF-induced aggregation after the consumption of the UP and C meal. C, The EC_{50} values of MS participants' PRP against PAF-induced aggregation after the consumption of the CC and C meal. D, The EC_{50} values of MS participants' PRP against PAF-induced aggregation after the consumption of the CY and C meal. Data are expressed as mean ± standard deviation. Generalized estimating equation was used to assess the effect of group, time, and group by time interaction on postprandial values using age, sex, BMI, alcohol intake, smoking, and baseline triacylglycerols levels as covariates. *P* values for the main effect of the treatment group (trt), time, and group by time interaction are presented.

platelet aggregation through nitric oxide and prostacyclin production [41], whereas scarce data so far exist about the bioactive compounds of the other plants that may affect platelet aggregation [42].

In conclusion, the results from this study suggest that the consumption of specific boiled plants could improve platelet sensitivity of MS patients, which implies a reduced risk of thrombosis and related conditions in this high-risk group. The different pattern observed promotes the idea that the plant actions may be attributed to their microconstituent content. However, further studies are needed to determine the beneficial effects of long-term consumption of wild plants against platelet sensitivity as well as to identify their protective mechanisms.

Funding

This work was supported by the European Commission grant EC/QLK1-CT-2001-00173.

Acknowledgment

We wish to thank Zacharias Kypriotakis for the identification and collection of the wild artichoke plants and Margaret Christea for her excellent technical support.

Conflict of Interest

No conflict of interest or any financial disclosure exists.

REFERENCES

[1] Reaven GM. Role of insulin resistance in human disease. Diabetes 1980;37:1595-607.
[2] Grundy SM. Metabolic syndrome: a multiplex cardiovascular risk factor. J Clin Endocrinol Metab 2007;92:399-404.
[3] Marsland AL, McCaffery JM, Muldoon MF, Manuck SB, Systemic inflammation and the metabolic syndrome among middle-aged community volunteers. Metabolism: clinical and experimental;59:1801-8.
[4] Trost S, Pratley R, Sobel B. Impaired fibrinolysis and risk for cardiovascular disease in the metabolic syndrome and type 2 diabetes. Curr Diab Rep 2006;6:47-54.
[5] Serebruany VL, Malinin A, Ong S, Atar D. Patients with metabolic syndrome exhibit higher platelet activity than those with conventional risk factors for vascular disease. J Thromb Thrombolysis 2008;25:207-13.
[6] Anfossi G, Russo I, Massucco P, et al. Impaired synthesis and action of antiaggregating cyclic nucleotides in platelets from obese subjects: possible role in platelet hyperactivation in obesity. Eur J Clin Invest 2004;34:482-9.
[7] Arteaga RB, Chirinos JA, Soriano AO, et al. Endothelial microparticles and platelet and leukocyte activation in patients with the metabolic syndrome. Am J Cardiol 2006;98: 70-4.
[8] Lindemann S, Kramer B, Daub K, Stellos K, Gawaz M. Molecular pathways used by platelets to initiate and accelerate atherogenesis. Curr Opin Lipidol 2007;18: 566-73.
[9] Totani L, Evangelista V. Platelet-leukocyte interactions in cardiovascular disease and beyond. Arterioscler Thromb Vasc Biol 2010;30:2357-61.
[10] Colwell JA, Nair RM, Halushka PV, Rogers C, Whetsell A, Sagel J. Platelet adhesion and aggregation in diabetes mellitus. Metabolism 1979;28:394-400.
[11] Martinez MA, Puig JG, Mora M, et al. Metabolic syndrome: prevalence, associated factors, and C-reactive protein: the MADRIC (MADrid RIesgo Cardiovascular) Study. Metabolism 2008;57:1232-40.
[12] Gupta A, Gupta V. Metabolic syndrome: what are the risks for humans? Biosci Trends 2010;4:204-12.
[13] Antonopoulou S, Nomikos T, Karantonis HC, Fragopoulou E, Demopoulos CA. PAF, a potent lipid mediator. In: Tselepis AD, editor. Bioactive phospholipids. Role in inflammation and atherosclerosis; 2009. p. 85-134.
[14] Liapikos TA, Antonopoulou S, Karabina SP, Tsoukatos DC, Demopoulos CA, Tselepis AD. Platelet-activating factor formation during oxidative modification of low-density lipoprotein when PAF-acetylhydrolase has been inactivated. Biochim Biophys Acta 1994;1212:353-60.
[15] Demopoulos CA, Karantonis HC, Antonopoulou S. Platelet activating factor a molecular link between atherosclerosis theories. Eur J Lipid Sci Technol 2003;105: 705-16.
[16] Detopoulou P, Nomikos T, Fragopoulou E, et al. Lipoprotein-associated phospholipase A2 (Lp-PLA2) activity, platelet-activating factor acetylhydrolase (PAF-AH) in leukocytes and body composition in healthy adults. Lipids Health Dis 2009;8:19.
[17] Roche HM, Gibney MJ. The impact of postprandial lipemia in accelerating atherothrombosis. J Cardiovasc Risk 2000;7: 317-24.
[18] Silveira A. Postprandial triglycerides and blood coagulation. Exp Clin Endocrinol Diabetes 2001;109:S527-32.
[19] Duttaroy AK. Postprandial activation of hemostatic factors: role of dietary fatty acids. Prostaglandins Leukot Essent Fatty Acids 2005;72:381-91.
[20] Executive summary of the third report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). JAMA 2001;285:2486-97.
[21] Krauss RM, Eckel RH, Howard B, et al. AHA dietary guidelines: revision 2000: a statement for healthcare professionals from the Nutrition Committee of the American Heart Association. Circulation 2000;102:2284-99.
[22] Karantonis HC, Antonopoulou S, Perrea DN, et al. In vivo antiatherogenic properties of olive oil and its constituent lipid classes in hyperlipidemic rabbits. Nutr Metab Cardiovasc Dis 2006;16:174-85.
[23] Nomikos T, Detopoulou P, Fragopoulou E, Pliakis E, Antonopoulou S. Boiled wild artichoke reduces postprandial glycemic and insulinemic responses in normal subjects but has no effect on metabolic syndrome patients. Nutr Res 2007;27:741-9.
[24] The Local Food-Nutraceuticals Consortium. Understanding local Mediterranean diets: a multidisciplinary pharmacological and ethnobotanical approach. Pharmacol Res 2005;52: 353-66.
[25] Kammoun M, Koubaa I, Ben Ali Y, et al. Inhibition of pro-inflammatory secreted phospholipase A2 by extracts from Cynara cardunculus L. Appl Biochem Biotechnol 2010;162:662-70.
[26] Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. Can J Biochem Physiol 1959;37:911-7.
[27] Demopoulos CA, Pinckard RN, Hanahan DJ. Platelet-activating factor. Evidence for 1-O-alkyl-2-acetyl-sn-

glyceryl-3-phosphorylcholine as the active component (a new class of lipid chemical mediators). J Biol Chem 1979;254: 9355-8.
[28] US Department of Agriculture. USDA national nutrient database for standard reference release 23. Accessed at: http://www.nal.usda.gov/fnic/foodcomp on 14 November 2010.
[29] Department of Social Medicine of the University of Crete. Composition table of Greek foods. Accessed at: http://nutrition.med.uoc.gr/GreekTables/Main/main.htm. on 14 November 2010.
[30] Trichopoulou A. Composition tables of food and Greek dishes. Athens: Parisianos SA; 2004.
[31] Antonopoulou S, Fragopoulou E, Karantonis HC, et al. Effect of traditional Greek Mediterranean meals on platelet aggregation in normal subjects and in patients with type 2 diabetes mellitus. J Med Food 2006;9:356-62.
[32] Kafatos A, Verhagen H, Moschandreas J, Apostolaki I, Van Westerop JJ. Mediterranean diet of Crete: foods and nutrient content. J Am Diet Assoc 2000;100:1487-93.
[33] Nomikos T, Fragopoulou E, Antonopoulou S. Food ingredients and lipid mediators. Curr Nutr Food Sci 2007;3:255-76.
[34] Marathe GK, Davies SS, Harrison KA, et al. Inflammatory platelet-activating factor–like phospholipids in oxidized low density lipoproteins are fragmented alkyl phosphatidylcholines. J Biol Chem 1999;274:28395-404.
[35] Watson AD, Leitinger N, Navab M, et al. Structural identification by mass spectrometry of oxidized phospholipids in minimally oxidized low density lipoprotein that induce monocyte/endothelial interactions and evidence for their presence in vivo. J Biol Chem 1997;272: 13597-607.
[36] Tanaka T, Iimori M, Tsukatani H, Tokumura A. Platelet-aggregating effects of platelet-activating factor–like phospholipids formed by oxidation of phosphatidylcholines containing an sn-2-polyunsaturated fatty acyl group. Biochim Biophys Acta 1994;1210:202-8.
[37] Nimpf J, Malle E, Leopold B, Wurm H, Kostner GM. Postprandial hyperlipemia inhibits platelet aggregation without affecting prostanoid metabolism. Prostaglandins Leukot Essent Fatty Acids 1989;37:7-13.
[38] Freese R, Mutanen M. Postprandial changes in platelet function and coagulation factors after high-fat meals with different fatty acid compositions. Eur J Clin Nutr 1995;49: 658-64.
[39] Ferroni P, Basili S, Falco A, Davi G. Platelet activation in type 2 diabetes mellitus. J Thromb Haemost 2004;2: 1282-91.
[40] Trovati M, Anfossi G. Insulin, insulin resistance and platelet function: similarities with insulin effects on cultured vascular smooth muscle cells. Diabetologia 1998;41:609-22.
[41] Grande S, Bogani P, de Saizieu A, Schueler G, Galli C, Visioli F. Vasomodulating potential of Mediterranean wild plant extracts. J Agric Food Chem 2004;52:5021-6.
[42] Lai JP, Lim YH, Su J, Shen HM, Ong CN. Identification and characterization of major flavonoids and caffeoylquinic acids in three Compositae plants by LC/DAD-APCI/MS. J Chromatogr B Analyt Technol Biomed Life Sci 2007;848: 215-25.