

ORIGINAL COMMUNICATION

Mediterranean diet, but not red wine, is associated with beneficial changes in primary haemostasis

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Objective: (1) To compare the effect of an alcohol-free Mediterranean-type diet (MD) and a high-fat diet (HFD) on variables of primary haemostasis (bleeding time, plasma von Willebrand factor and platelet aggregation/secretion). (2) To test whether red wine supplementation modified these variables, independently of the diet.

Design, subjects and intervention: Controlled prospective intervention study. Two groups, each consisting of 21 healthy male university students (22 ± 3.4 y), received either MD or HFD during 90 days. Between days 30 and 60, both diets were supplemented with 240 ml/day of red wine. Baseline (T0) and T30, T60 and T90-day samples were drawn. Bleeding time was measured before (day 30) and after (day 60) wine supplementation. No drop out from the study was experienced.

Setting: University campus and outpatient nutrition clinic.

Results: All baseline (day 0) variables did not differ significantly between study groups. On day 30, individuals on MD had significantly higher levels of plasma β -carotene, folate, ascorbate, and eicosapentaenoic acid in plasma lipid fractions, than those on HFD. Total plasma cholesterol, HDL and LDL did not change significantly in either study group at any time point. After 30 days on each diet, individuals on MD had longer bleeding time (BT) than those on HFD (7.6 ± 2.8 vs 5.8 ± 1.7 min; $P = 0.017$). BT did not change significantly after 1 month of wine supplementation (7.1 ± 2.0 vs 5.5 ± 2.0 min, respectively). Plasma von Willebrand factor (vWF:Ag) on day 0 was 89 ± 40 and $111 \pm 70\%$ in MD and HFD groups, respectively ($P = 0.21$). These values did not change significantly at 30, 60 or 90 days. MD intake was associated with an increase in platelet serotonin secretion ($P = 0.02$) and a marginal increase in platelet aggregation after stimulation with epinephrine ($P = 0.07$). Wine intake resulted in a marginal decrease in platelet ¹⁴C-5-HT secretion with 4 μ M ADP ($P = 0.07$). However, both platelet aggregation and secretion were consistently increased when using collagen as agonist (1 and 2 μ g/ml, $P = 0.01$).

Conclusion: The longer BT in individuals on MD, obtained independently of red wine, denotes less interaction of platelets with the vascular wall, which could be beneficial from the point of view of cardiovascular (CV) risk. This effect is not explained by changes in the measured haemostatic determinants of BT (plasma vWF, ex vivo platelet function), and might be attributed to other as yet unknown vascular factors. Moderate consumption of red wine results in a significant increase in ex vivo platelet aggregation and secretion after stimulation with collagen. This observation contradicts previous reports, although further studies are required to elucidate the influence of this finding on CV risk.

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of data and in preparing the manuscript. AC and OC participated in the clinical control of participants, diet design and daily supervision of the diet. PS, CM, OP and BM provided administrative, technical and logistic support and completed the experimental work. GM was responsible for statistical analysis.

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Introduction

Intervention and epidemiological studies show that certain dietary habits and light-to-moderate alcohol consumption provide cardiovascular (CV) protection. These effects were recently summarized, indicating that foods of animal origin were directly correlated with coronary heart disease mortality, whereas vegetable-food groups, fish and alcohol were inversely correlated (Menotti *et al*, 1999). Populations consuming a diet rich in vegetables, olive oil, fish and wine (Mediterranean diet, MD) had the lowest mortality among the seven countries included in that study. The benefits of this diet can apparently be transferred to populations of other ethnic origins (Kouris-Blazos *et al*, 1999) and have also been reproduced in secondary prevention trials (de Lorgeril *et al*, 1999).

Alcoholic beverages, mostly wine, are a constitutive component of the MD. Wine itself appears to add an independent CV benefit to this diet (Grønbaek *et al*, 2000). However, despite reports indicating that light-to-moderate alcohol consumption reduces both CV mortality (Thun *et al*, 1997) and the risk of stroke (Berger *et al*, 1999), wine drinking also has been related to the intake of healthier diets (Tjønneland *et al*, 1999) and other advantageous lifestyle characteristics (Wannamethee & Shaper, 1999), to which CV benefits are attributed. Thus, the health benefits of phenolic acids and polyphenols contained in red wine may be indiscernible in populations that ingest large amounts of fruit and vegetables, whereas these benefits could be distinctly apparent in populations with relatively low intakes of these kinds of foods (de Lorgeril *et al*, 1999). In this regard, the proposal that wine is a major determinant of the benefits derived from MD (Renaud & de Lorgeril, 1992) still needs to be demonstrated.

Lower rates of CV disease and mortality in Southern European countries can be only partially explained by differences in fat intake or blood lipid profiles (Langer *et al*, 1992). As suggested in several previous reports (Bijnen *et al*, 1996; Roche *et al*, 1998; Mezzano *et al*, 1999; Rimm *et al*, 1999), diet-and/or wine-induced changes in haemostatic cardiovascular risk factors (HCVRF) may contribute to this protection. We recently showed that young volunteers consuming MD had lower levels of plasma procoagulant HCVRF (fibrinogen, factor VIIc and factor VIIIc) than paired controls consuming a high-fat diet (HFD). Supplementation of these diets with red wine resulted in further reduction of plasma fibrinogen and factor VIIc and increases in both tissue plasminogen activator antigen (tPA Ag) and plasminogen activator inhibitor antigen (PAI-1 Ag; Mezzano *et al*, 2001). These results indicate that the effects of MD and a moderate red wine intake on CV risk markers are independent and complementary.

Platelet-vascular wall interactions (primary haemostasis) play a key role in arterial thrombotic events. This explains the increasing use of platelet inhibitors to prevent or treat these conditions. In this context, some of the beneficial effects of MD and wine consumption could be mediated by

a reduction in platelet reactivity. Several studies report that diets enriched with fish, α -linolenic acid and spices, abundant in the case of MD, include substances that reduce or could reduce platelet function (Vognild *et al*, 1998; Allman *et al*, 1995; Janssen *et al*, 1998; Ackerman *et al*, 2001; Srivastava *et al*, 1995). Similarly, other reports indicate that the intake of moderate amounts of ethanol or nonalcoholic components of wine, for 2 or more weeks, is associated with a decrease in *ex vivo* platelet function (Pikkar *et al*, 1987; Pellegrini *et al*, 1996; Pace-Asciak *et al*, 1996; Freedman *et al*, 2001).

The aims of the current study were two-fold: firstly, to compare the effects of two alcohol-free diets, a Mediterranean-type and a high-fat Western-type diet (HFD) on primary haemostasis variables (cutaneous bleeding time (BT), plasma concentration of von Willebrand factor antigen and platelet aggregation and secretion *ex vivo*); and secondly, to assess changes in these variables after supplementing each diet with a moderate amount of red wine.

Subjects and methods

Subjects and study design

The subjects and design of this intervention study have been previously described in greater detail (Mezzano *et al*, 2001). Forty-two healthy undergraduate or graduate university male students, aged 22 ± 3.4 y, gave their informed consent to participate in the study. Incorporation criteria included: absence of clinical disease and obesity, serum lipids and glucose within the normal range, normal blood pressure and absent or mild alcohol drinking habits. All the participants were physically active, non-smokers and received no medication or vitamin supplementation. The experimental protocol was approved by the university Medical Ethics Committee, prior to its initiation. All the participants were interviewed and the average of three 24 h dietary recalls (two on weekdays and one on weekends) were used to estimate basal daily consumption of different nutrients and alcoholic beverages.

For 90 days, 21 volunteers received a Mediterranean-type diet and the remaining 21 a high-fat Western-type diet, as previously detailed (Mezzano *et al*, 2001). From day 31 through day 60, both diets were isoenergetically supplemented with 240 ml/day of red wine (cabernet sauvignon), providing 23.2 g of ethanol/day. To avoid a drug-induced inhibition of platelet function, volunteers were instructed to abstain from aspirin and non-steroidal anti-inflammatory drugs. No other alcoholic beverages were allowed during the 3-month study period. Thirty students were randomly allocated to either the MD or HFD program. The remaining 12 were allowed to select the diet according to their preferences, in order to preserve their compliance with the study protocol. Measurements of haemostatic and selected nutritional variables were performed on fasting blood samples drawn around 8:00 on baseline (day 0) and days 30, 60 and 90, along with clinical assessments of each participant. Forearm

BT was performed on two occasions: after one month of dietary treatment (day 30), and after one month of dietary supplementation with red wine (day 60). Volunteers declared that they would comply with the recommendations made, and none left the study prior to completion.

Diets

Diets prepared by a catering company were supervised daily by one of the authors. They were delivered at the campus at midday or at home at night and at weekends, in personalized boxes. Specific indications for breakfast and snacks were provided to all the volunteers in concordance with each diet. Both diets were designed to provide the same energy intake, with a daily average of 8168 kJ/day (2565 kcal/day). Proteins supplied 17.6% of total energy in each diet. Fats supplied 27.3 and 39.9% of energy for MD and HFD, respectively. Mean contents of saturated, monounsaturated (MUFA), polyunsaturated (PUFA) and ω -3 fatty acids provided by MD and HFD were 22.7 vs 35.8, 37.9 vs 35.5, 9.6 vs 32.0 and 1.64 vs 0.96 g/day, respectively. Individuals on MD consumed an average of 32 ml/day of olive oil. Fruit and vegetable consumption were on average 675 g/day in MD and 246 g/day in HFD. White meat, fish and legumes were the main source of proteins in MD, whereas HFD was rich in red meat and low in fish. Diet composition was calculated employing the Food Processor II computer program (Esha Research, Salem, OR). Lean weight was calculated by bio-electrical impedance analysis.

Laboratory methods

Analytical procedures for measurements of serum albumin, blood haemoglobin, total plasma cholesterol, triacylglycerides, plasma β -carotene, α -tocopherol, lycopene, ascorbate, serum vitamin B₁₂ and folate, as well as the percentage of eicosapentaenoic acid in plasma fatty acids, have been described in our previous study (Mezzano *et al*, 2001). The forearm BT test was performed using a commercial device (Simplat II, Organon Teknika, Belgium). Plasma von Willibrand factor was measured by sandwich-type ELISA, using a capture monoclonal antibody (vW1, kindly provided by Dr Robert R Montgomery, Milwaukee, WI, USA) and a peroxidase-conjugated rabbit antibody for detection (Dako Corp., USA). Platelet aggregation and ¹⁴C-5-HT (serotonin) secretion in platelet-rich plasma (2×10^5 platelets/ μ l) were performed as previously described (Mezzano *et al*, 1996). Low concentrations of ADP (4 μ M), collagen (1 μ g/ml) and epinephrine (8 μ M), known to elicit primary and secondary waves of aggregation in most healthy subjects, were employed. Higher concentrations of ADP (8 μ M), collagen (2 μ g/ml) and sodium arachidonate (1 mM) were selected in order to elicit full aggregation.

Statistical analyses

The two-tailed Mann-Whitney test was used to compare the means of different variables between MD and HFD groups at baseline. Proportions of O and non-O blood types between groups on MD and HFD were compared using the two-tailed Fisher's exact test. A linear mixed effects model (repeated measure Anova) was used to compare both diets and the effect of wine on haemostatic factors during the study period at 30, 60 and 90 days. To correct for small group differences, explained by subject variability, and to adjust for a lack of absolute randomization, the values at 30, 60 and 90 days were controlled by the baseline measurement, which was used in the model as a covariate. Anova-type F-statistics were calculated to test the effect of diet (MD vs HFD) across the study time frame (30, 60 and 90 days), using initial values as controlling factors. The effect of wine, combining the two dietary groups, was evaluated by comparing the response of all subjects at 60 days vs average values at 30 and 90 days, using F-statistics based on within-subject source of variability. Moreover, to control a longer than expected wine effect, the response at 60 days vs values at 30 days was compared. Finally, the effect of wine was also tested for each dietary group independently, despite loss of statistical power. Normal distribution was checked on the residuals of all variables considered in this work. For cases displaying a digression from the normality assumption, a similar analysis was performed using rank statistics instead of raw data, as a nonparametric alternative. The proc MIXED of the SAS statistical program was used for these purposes.

Results

Analysis of dietary recalls before the start of the intervention study disclosed no significant mean differences in intake of several nutrients between MD and HFD groups (data not shown). Alcohol consumption, mostly beer and spirits, averaged less than 1.5 drinks/week in both study groups. Anthropometric, haematological and nutritional measurements at baseline (day 0) were outlined in a previous report (Mezzano *et al*, 2001). In brief, no significant differences between individuals assigned to MD or HFD treatments were detected for age, body weight, body mass index, lean weight, serum albumin, blood haemoglobin, total plasma cholesterol, triacylglycerides, plasma β -carotene, α -tocopherol, lycopene, ascorbate, serum vitamin B₁₂ and folate, as well as percentage of eicosapentaenoic acid in plasma fatty acids.

Despite the lack of absolute randomization, no significant differences in blood platelet count and platelet aggregation/secretion with all agonists (Table 1), as well as plasma vWF: Ag concentrations (Figure 1), were observed at baseline (T0) between groups on MD and HFD.

After 30 days of intervention, individuals on MD had significantly higher levels of plasma β -carotene (0.49 ± 0.19 vs 0.23 ± 0.14 μ mol/l; $P < 0.0001$), ascorbate (55.9 ± 11.2 vs 29.7 ± 8.9 μ mol/l; $P < 0.0001$), folate (14.2 ± 4.3 vs 10.5 ± 2.6 nmol/l; $P = 0.0019$) and eicosapentaenoic acid in

Table 1 Effects of Mediterranean-type and high-fat diets, with (T60) and without (T30 and T90) red wine, on blood platelet count, platelet aggregation (PA) and platelet secretion (PS), measured for various agonists in healthy subjects

Variable		T0 ^a	T30	T60	T90	P ^b diet	P ^c wine
Platelet count ($\times 10^3/\mu\text{l}$)	MD	236 \pm 42	235 \pm 36	228 \pm 45	224 \pm 41	0.78	0.37
	HFD	221 \pm 49	224 \pm 60	212 \pm 51	215 \pm 63		
PA 4 μM ADP (%)	MD	38 \pm 18 (7–72)	52 \pm 21 (15–81)	47 \pm 17 (15–73)	50 \pm 22 (12–100)	0.82	0.51
	HFD	34 \pm 21 (8–71)	44 \pm 21 (15–80)	44 \pm 20 (19–100)	52 \pm 24 (10–84)		
PS 4 μM ADP (%)	MD	14 \pm 20 (0–59)	19 \pm 18 (0–61)	14 \pm 15 (0–56)	14 \pm 15 (0–46)	0.34	0.07
	HFD	8 \pm 11 (0–35)	12 \pm 14 (0–47)	8 \pm 13 (0–52)	13 \pm 14 (0–41)		
PA 8 μM ADP (%)	MD	55 \pm 17 (20–76)	66 \pm 14 (40–85)	60 \pm 15 (30–89)	65 \pm 12 (40–88)	0.68	0.17
	HFD	50 \pm 22 (14–80)	60 \pm 17 (22–85)	60 \pm 15 (32–87)	69 \pm 14 (35–87)		
PS 8 μM ADP (%)	MD	23 \pm 23 (0–94)	23 \pm 17 (0–58)	20 \pm 16 (0–61)	20 \pm 15 (0–44)	0.86	0.31
	HFD	16 \pm 16 (0–48)	20 \pm 16 (0–47)	18 \pm 14 (0–47)	22 \pm 17 (0–53)		
PA collagen 1 $\mu\text{g}/\text{ml}$ (%)	MD	39 \pm 28 (0–81)	53 \pm 29 (0–82)	58 \pm 25 (0–87)	60 \pm 21 (4–82)	0.98	0.13
	HFD	37 \pm 33 (0–81)	42 \pm 27 (0–75)	62 \pm 18 (7–78)	65 \pm 22 (2–90)		
PS collagen 1 $\mu\text{g}/\text{ml}$ (%)	MD	25 \pm 16 (0–54)	26 \pm 16 (3–57)	30 \pm 17 (0–62)	25 \pm 14 (4–51)	0.85	0.003
	HFD	19 \pm 15 (0–43)	21 \pm 15 (0–57)	32 \pm 18 (0–74)	24 \pm 11 (4–42)		
PA collagen 2 $\mu\text{g}/\text{ml}$ (%)	MD	61 \pm 23 (0–88)	67 \pm 21 (0–86)	72 \pm 18 (16–100)	68 \pm 20 (8–95)	0.77	0.013
	HFD	57 \pm 28 (0–82)	61 \pm 22 (0–85)	75 \pm 7 (58–85)	72 \pm 10 (46–88)		
PS collagen 2 $\mu\text{g}/\text{ml}$ (%)	MD	40 \pm 22 (0–100)	35 \pm 16 (4–65)	42 \pm 12 (6–59)	31 \pm 15 (0–58)	0.90	0.005
	HFD	33 \pm 17 (4–70)	33 \pm 15 (3–51)	44 \pm 21 (0–100)	33 \pm 12 (12–47)		
PA epinephrine 8 μM (%)	MD	23 \pm 19 (1–64)	42 \pm 30 (1–82)	36 \pm 23 (5–78)	36 \pm 24 (5–80)	0.07	0.50
	HFD	26 \pm 23 (2–77)	27 \pm 27 (0–86)	28 \pm 19 (8–76)	36 \pm 27 (2–86)		
PS epinephrine 8 μM (%)	MD	11 \pm 22 (0–72)	26 \pm 26 (0–78)	23 \pm 21 (0–53)	20 \pm 20 (0–58)	0.02	0.47
	HFD	10 \pm 17 (0–57)	11 \pm 17 (0–52)	9 \pm 17 (0–55)	15 \pm 17 (0–53)		
PA arachidonate 1 mM (%)	MD	68 \pm 23 (1–85)	72 \pm 25 (0–91)	67 \pm 26 (0–94)	67 \pm 26 (2–86)	0.84	0.26
	HFD	63 \pm 32 (0–93)	69 \pm 24 (0–86)	64 \pm 26 (0–87)	68 \pm 24 (0–90)		
PS arachidonate 1 mM (%)	MD	38 \pm 22 (0–100)	35 \pm 18 (3–74)	32 \pm 20 (0–65)	25 \pm 11 (0–50)	0.87	0.90
	HFD	30 \pm 18 (0–52)	36 \pm 21 (0–95)	30 \pm 16 (7–79)	27 \pm 13 (0–43)		

Data represent mean \pm s.d. and (range) of observed values.

^aComparison of values at T0 for each variable revealed no significant differences between MD and HFD (two-tailed unpaired Student's *t*-test or Mann–Whitney test).

^bThe effect of diet was evaluated by repeated measure ANOVA, controlled using initial values and comparing both dietary groups at 30, 60 and 90 days.

^cThe effect of wine was evaluated by combining both groups (MD and HFD) and comparing their response at both T60 vs (T30 + T90)/2 and T60 vs T30 *P*-value shown is the least significant of both calculations.

the plasma lipid fraction (1.5 ± 0.7 vs $0.85\% \pm 0.3$; $P = 0.001$), than those on HFD. Dietary and wine interventions did not

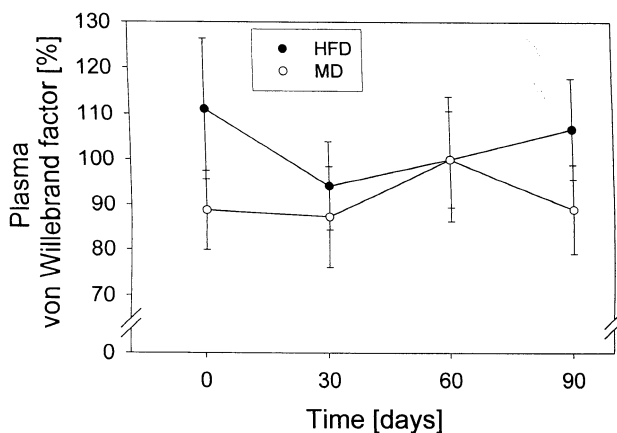


Figure 1 Comparative effect of Mediterranean diet (MD) and high-fat diet (HFD) and the effect of red wine on plasma concentrations of von Willebrand factor. Volunteers received red wine from days 30 to 60. No significant difference was observed between MD and HFD groups at baseline (T0). The differences between diets at different times and after wine intake were not statistically significant. Values represent mean \pm SE.

result in significant changes, at any time point, in total plasma cholesterol in either study group, as reported previously (Mezzano *et al*, 2001).

Effect of diets on primary haemostasis

After 1 month on each diet, mean BT for the MD group was significantly longer than that for HFD subjects (7.6 ± 2.7 vs 5.8 ± 1.7 min; $P = 0.017$, (Figure 2). The BT reference range in our laboratory is 4.5–9.5 min. As shown in Figure 1, MD and HFD intake for 1 month did not result in significant changes in plasma vWF:Ag concentrations. This variable is partially determined by ABC blood type. A higher, though not statistically significant proportion of individuals of blood type O belonged to the HFD group (11/21) as compared with the MD group (5/21); ($P = 0.11$).

The effects of diet on platelet aggregation and ¹⁴C-serotonin secretion are shown in Table 1. Platelet responses to ADP, collagen and arachidonate were not significantly different between MD and HFD groups. Intake of MD was associated with a slightly increased platelet secretion after stimulation with epinephrine.

Effect of red wine on primary haemostasis

The effect of wine was evaluated by combining the results of both dietary groups and comparing the response of all subjects at both T60 vs T30 and T60 vs (T30 + T90)/2, and the results are presented in Table 1. In both cases, red wine consumption was associated with increased platelet ^{14}C -5-HT secretion after stimulation with collagen (1 and 2 $\mu\text{g}/\text{ml}$), and increased platelet aggregation at the higher collagen concentration. This effect is graphically depicted in Figure 3. A slight, though not statistically significant, decrease in platelet secretion with the low concentration of ADP (4 μM) was also observed. Red wine intake was not associated with significant changes in platelet aggregation/secretion after stimulation with either sodium arachidonate or epinephrine (Table 1). No significant effects for red wine treatment were observed on BT (Figure 2), plasma vWF:Ag concentration (Figure 1) and blood platelet count (Table 1).

Discussion

Diets and primary haemostasis

One month of MD had no significant effects on *ex vivo* platelet aggregation/secretion with most agonists. The marginal increase in platelet reactivity to epinephrine in the MD group is probably not significant from a physiological point of view, considering that, in this study, it was associated with longer, not shorter, BT. Previous intervention studies have shown that distinctive components of MD reduce platelet function, and this effect has been proposed as a mechanism contributing to the CV benefit of this diet. In fact, fish oils (Goodnight *et al*, 1981; Agren *et al*, 1997; Vognilid *et al*, 1998), α -linolenic acid (Renaud & Nordøy, 1983; Allman *et al*, 1995; Freese *et al*, 1994) and various spices (Janssen *et al*, 1998; Ackerman *et al*, 2001; Srivastava *et al*, 1995) induce a

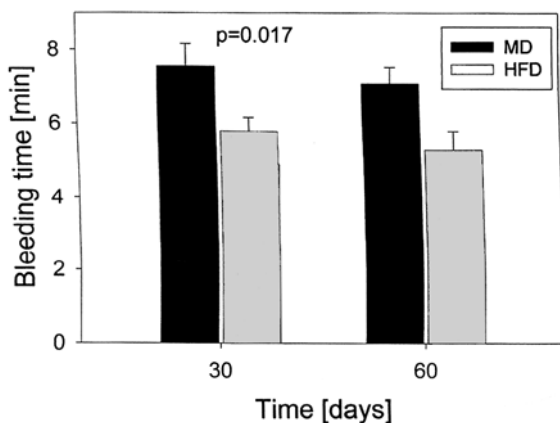


Figure 2 Comparative effect of Mediterranean diet (MD) and high-fat diet (HFD) and the effect of red wine on bleeding time. Values were recorded after 1 month on each diet (day 30) and after 1 month of supplementation of both diets with red wine (day 60). *P*-value reflects the difference in BT between diets. Red wine intake was not associated with significant changes in bleeding time. Bars represent mean \pm SD.

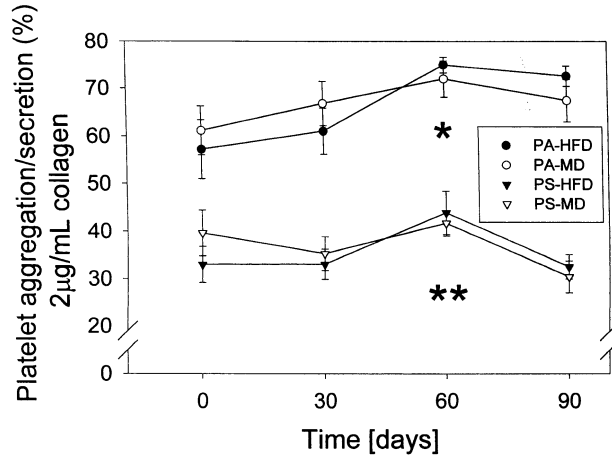


Figure 3 Platelet aggregation (PA) and secretion (PS) with 2 $\mu\text{g}/\text{ml}$ collagen at baseline (T0), after 30 days on MD or HFD (T30), after supplementation of each diet with red wine (T60), and 30 days after withdrawal of wine, while maintaining the same diet (T90). Data express mean \pm 1 S.E. As the effect of wine was similar in MD and HFD individuals, the statistical analysis was performed by combining both groups and comparing their response at both T60 vs (T30 + T90)/2 and T60 vs T30. For PA, *P* = 0.013 and 0.0017 with each statistical approach, respectively. **Corresponding values for PS were *P* = 0.0002 and 0.005.

decrease of platelet function. The apparent discrepancy of our results with those findings is probably explained by the lower content of each one of these substances in the complete MD, as opposed to the amounts used for single components of MD in selective intervention studies. Accordingly, our findings stand against the notion that the beneficial CV effects of MD are mediated through by a decrease in platelet reactivity.

We also found that plasma vWF, considered a HCVRF (Folsom *et al*, 1999; Smith *et al*, 2000), did not change significantly with MD. This result suggests that the CV benefit of MD would not be mediated by a decrease in plasma vWF levels. Intake of a Mediterranean-type diet, rich in fats and MUFA (38 and 22% of total energy, respectively), has been reported to result in a decrease of plasma vWF (Pérez-Jiménez *et al*, 1999). A lower fat content of the diet in our study (27.3% of total energy as fat intake) might explain this discrepancy.

Forearm cutaneous BT reflects the interaction of platelets with the vessel wall. The time required to stop bleeding from a superficial skin incision is modulated mainly by platelet function (including plasma von Willebrand factor function), and by factors derived from the vascular wall, principally the endothelium. We found that BT was significantly longer in the MD group than in HFD subjects, even though all values were within the normal range. This observation is consistent with the protective effect of MD against CV disease, based on the fact that drugs used in the prevention of arterial thrombotic disease, like aspirin, induce a similar effect on BT. It is unlikely that abundance of fish or ω -3 fatty acids in the diet explains the observed effect (Dyerberg & Bang, 1979; Good-

night *et al*, 1981), because fish intake in our MD group was lower than that used in previous specific intervention studies, and it did not result in significant changes in platelet aggregation/secretion.

Nitric oxide (NO) and prostacyclin (PGI₂) produced by endothelial cells reduce platelet–vessel wall interactions and both induce prolongation of BT (Simon *et al*, 1995; Ubatuba *et al*, 1979; Gerrard *et al*, 1992). In this context, it is possible that the modulation of NO on platelet–endothelium interactions could account for the longer BT presently observed for individuals on MD. This explanation would be concordant with the results of a recent report showing that hypercholesterolaemic men fed with MD had an increase in endothelium-dependent vasodilation, mainly induced by NO (Fuentes *et al*, 2001). Taken together, these observations reinforce the hypothesis that the effect of MD on BT could be mediated by endothelium-derived products.

Red wine and primary haemostasis

Red wine supplementation of MD and HFD did not modify significantly the BT and plasma vWF concentrations. These results confirm those of a previous intervention study, using a similar experimental design (Pellegrini *et al*, 1996). *Ex vivo* platelet function studies were performed in fasting samples drawn 10–12 h after the last drink, in the absence of ethanol. In this setting, no significant changes in aggregation and 5-HT secretion in platelets stimulated with ADP, epinephrine and arachidonic acid were detected after one month of wine intake. However, a highly significant increase in the aggregation and secretion of platelets exposed to low and high collagen concentrations was observed. This finding contests the widespread notion, summarized in recent reviews, that red wine inhibits platelet function (Wollin & Jones, 2001; Booyse & Parks, 2001). In fact, the undisputed CV benefits of moderate red wine consumption parallel the wide consensus that wine effects are, at least in part, induced by inhibiting platelet reactivity. This notion is weakened by the fact that published studies assessing the effect of wine on platelets are highly heterogeneous and that the inhibition observed with a single stimulus is often not reproduced with other agonists. Moreover, a distinct increase in *ex vivo* platelet function has been observed with some agonists in association with wine intake, and an additional increase in platelet reactivity, referred to as the rebound effect, has been noted after alcohol withdrawal (Renaud & Ruf, 1996). In this regard, our finding of increased platelet aggregation/secretion with collagen closely resembles a rebound effect, given that it arises several hours after the last drink, with no alcohol remaining in the blood. This situation best reflects the physiological status of a moderate wine drinker.

It is difficult to compare platelet function results obtained in this study with those published previously, due to differences in experimental design, species and subjects of study, volume and type of wine (red or white), ethanol and non-alcoholic components of wine, interference with diets con-

sumed by the participants, modality of consumption (acute or chronic), time elapsed between last drink and platelet function study, difficulties in laboratory standardization, and type and concentration of platelet agonists tested. This heterogeneity has precluded the incorporation of platelet aggregation tests in meta-analysis to quantitate the effect of alcohol on CV risk (Rimm *et al*, 1999). Our study did not assess the acute effect of wine intake on platelet function, and therefore our findings are not contrary to the inhibition of platelets observed in acute intervention studies. It has also been reported that the type of diet can influence the effect of wine, whereby the inhibition of platelets appears more significant in subjects with a diet rich in saturated fats (Renaud *et al*, 1992). Again, we did not observe such differences in platelet function between MD and HFD individuals.

In conclusion, the longer BT associated with MD, found here to be independent of red wine treatment, is a signal of decreased platelet–vascular wall interactions, which could partially account for the benefits of this diet on CV risk. This effect is not explained by changes in known haemostatic determinants of BT (plasma vWF, platelet function), and might be attributed to other vascular factors that, as yet, remain unknown. Moderate consumption of red wine, independent of diet, resulted in significant increases in platelet aggregation and secretion after stimulation with collagen, with no effect on BT. The mechanisms underlying the specificity for this agonist are unknown, but our findings indicate that future studies directed to analyse the effect of red wine on platelet collagen receptors or signal transduction pathways (Watson *et al*, 2000) are needed. The biological significance of our observations remains to be determined. The participation of platelets is crucial in arterial thrombotic events, but *ex vivo* platelet aggregation tests do not predict risk for future CV events (Elwood *et al*, 1998, 2001). So, the increased wine-related platelet aggregation/secretion reactions detected here do not denote an increased risk of CV events. Nonetheless, they suggest that the CV benefits of red wine may be best explained as a consequence of its effects on plasma lipids and other haemostatic CV risk factors.

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