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Circulating levels of endothelial function are modulated by dietary monounsaturated fat

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Abstract

Background: For the most part, the benefits of monounsaturated-rich diets (MUFA-diet) have been related to their action on plasma lipid levels. However other non-lipidic effects could also be involved in their protective effects. One of these involves the decrease in plasma levels of plasminogen activator inhibitor type 1 (PAI-1), the main inhibitor of fibrinolysis. Given that the PAI-1 is of endothelial origin, one hypothesis is that the MUFA-diet could protect against CHD by modulating some endothelial components. **Methods and results:** Healthy male subjects ($n = 25$) received three different consecutive diets, each lasting 28 days: a low fat NCEP-I-diet, with 28% calories as fat, 10% saturated fat (SAT), 12% monounsaturated (MUFA) and 6% polyunsaturated (PUFA); a MUFA-diet, with 38% calories as fat, 10% SAT, 22% MUFA and 6% PUFA; and a SAT rich-diet (SAT-diet), with 38% calories as fat, 20% SAT, 12% MUFA and 6% PUFA. After each dietary period, the plasma lipid profile was determined, including total cholesterol, HDL cholesterol, LDL cholesterol, total triglyceride, apo A1, apo B plasma levels and conjugated diene formation, after incubation of LDL particles with Cu 5 μ M/l. Endothelial products measured in plasma were von Willebrand factor (vWF), E-selectin, Thrombomodulin and Tissue Factor Pathway Inhibitor (TFPI) levels. We observed a decrease in vWF, PAI-1 and TFPI plasma levels and an increase in lag time of conjugated diene formation after the MUFA-diet. There was a positive correlation between the decreases in TFPI and vWF and the changes in total cholesterol, LDL-C, apo B plasma levels. The decrease in TFPI was negatively correlated with the increase in lag time of conjugated diene formation. PAI-1 plasma levels were positively correlated with total cholesterol, LDL-C and triglycerides and negatively correlated with HDL-C. **Conclusions:** Consumption of a Mediterranean-type MUFA-diet produces a decrease in plasma levels of vWF, TFPI and PAI-1 plasma levels in young healthy males. Given that these substances are of endothelial origin, one could suggest that the MUFA of the diet has a beneficial effect on endothelial function resulting in protective changes against thrombogenesis. © 1999 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Endothelial function; Monounsaturated-rich diet; Plasminogen activator inhibitor type 1; Conjugated diene formation; Plasma lipid profiles

1. Introduction

The role of the endothelium far exceeds that of a passive barrier between the blood and subendothelial cells. It plays a unique and important role in the

interface between blood and tissues. It is involved in numerous homeostatic mechanisms, such as the maintenance of a non-thrombotic surface, the metabolism of lipoproteins, participation in the regulation of vascular tone and a role in the immune response [1]. Endothelial cells produce a large number of substances involved in adhesion and transendothelial migration of circulating leucocytes into the vascular wall, as well as coagulation and fibrinolysis, all of which are involved in atherosclerosis.

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rotic development [2]. In vitro studies have demonstrated that the rolling of monocytes on endothelial cells is mediated by adhesion molecules, one of them, E-selectin, being a product of endothelial origin [3]. Recently, it has been shown that in vivo expression of E-selectin by the aortic endothelium is increased in atherosclerosis in hyperlipemic diabetic rabbits [4]. Another important factor is von Willebrand factor (vWF), a coagulation component. It is generally assumed that its function involves interaction with components of the subendothelium on the one hand and also interaction with platelet membrane receptors. In this case, vWF could participate in arterial thrombosis, binding to platelet membrane glycoproteins in both adhesion and aggregation, leading to thrombus formation in high shear stress rates [5]. Also, vWF has been considered as a specific endothelial product and a possible indicator of endothelial cell damage [6]. In contrast to this thrombotic function, the endothelial cells also show an active antithrombotic behaviour. One example of this is given by Tissue Factor Pathway Inhibitor (TFPI), an activated factor X dependent inhibitor of tissue-factor induced coagulation [7]. Another antithrombotic system is the natural anticoagulant mechanism dependent on the protein C-thrombomodulin mechanism. When thrombin binds to thrombomodulin, several coagulation reactions, including fibrin generation and platelet activation, are inhibited [8].

High intake of saturated fat (SAT) has been associated with an increased incidence of CHD, whereas high intake of monounsaturated fat (MUFA) has been associated with a protective effect [9,10]. One of the mechanisms by which dietary fats can modify CHD risk is by their effect on plasma LDL and HDL cholesterol levels [11]. However, these effects do not appear to explain the magnitude of CHD protection observed in individuals living in countries with a high intake of MUFA. An additional mechanism may involve the oxidative modification of LDL particles. This process plays an important role in the initiation and progression of atherosclerosis and previous studies have shown that LDL particles obtained after a MUFA-diet have a longer lag time than particles obtained after a polyunsaturated enriched diet [12]. Furthermore, oxidative modified LDL particles could also affect endothelial cell function [13] and other non-lipid related mechanisms may also be affected by dietary fat [14]. Recently [15], we have shown that consumption of diets rich in MUFAs decreases PAI-1 plasma activity, the main physiological inhibitor of fibrinolysis, originated in hepatocytes and endothelial cells [16]. Moreover, these diets have been reported to decrease vWF in NIDDM patients [17]. These data might suggest that dietary MUFAs could modulate endothelial function. The main objective of this study was to determine the effect of a MUFA-diet, typical of Mediterranean countries,

compared to an NCEP-I diet, on different soluble factors originating from endothelial cells.

2. Methods

2.1. Subjects and diets

Twenty-five healthy normolipemic (total cholesterol < 5.1 mmol/l) male students attending the University of Cordoba volunteered to participate in the study. All had a comprehensive medical history, physical examination and clinical chemistry analysis before enrolment. Subjects under 30 years of age with total cholesterol plasma levels lower than 5.1 mmol/l on their usual diets, with no evidence of any chronic illness (such as hepatic, renal, thyroid, or cardiac dysfunction), or unusually high levels of physical activity were selected. None of the subjects had a family history of CHD and none had received medication or vitamin supplements in the 6 months prior to the study. Dietary information, including alcohol consumption, was collected over 7 consecutive days. Individual energy requirements were calculated by taking into consideration each subject's physical activity. Baseline mean body-mass index (BMI) was calculated as kg/m^2 (mean \pm S.D.) and remained constant during the experimental period. Subjects were encouraged to maintain regular physical activity and life-style and were asked to record in a diary any event that could affect the outcome of the study, such as stress, change in smoking habits, and alcohol consumption or foods not included in the experimental design.

The study design included an initial 28-day period during which all subjects consumed an NCEP-I-diet [18] containing 15% of energy as protein, 57% as carbohydrate, and 28% as fat (10% SAT, 12% MUFA, and 6% PUFA). The second diet lasted 28 days, and all subjects consumed a MUFA-diet, with 15% of energy as protein, 47% as carbohydrate, and 38% as fat (10% SAT, 22% MUFA, and 6% PUFA). The third dietary period also lasted for 28 days and all subjects consumed a SAT-diet, with 15% of energy as protein, 47% as carbohydrate and 38% as fat (20% SAT, 12% MUFA and 6% PUFA). Dietary cholesterol was maintained constant in our experimental design and the mean intake was 115 mg/1000 kcal over the three periods. This study was approved by the Human Investigation Review Committee of the Reina Sofia University Hospital.

The composition of the experimental diets was calculated using the USDA food tables or the Spanish food composition tables for local foodstuffs. Fourteen menus, prepared with regular solid foods, were rotated during the experimental period. We used virgin olive oil for cooking and salad dressing during the high MUFA

period and palm oil and butter for the SAT-diet. During the NCEP-I diet, some olive oil or palm oil was replaced by biscuits, bread and jam. Lunch and dinner were prepared in the hospital kitchen and consumed in the dining hall. Breakfast and an afternoon snack were prepared by each individual with the recommended food products according to our instructions. Duplicate samples from each menu were collected, homogenized, and stored at -80°C . Protein, fat and carbohydrate content of the diet were analyzed using standard methods. Dietary follow-up was verified by analyzing fatty acids in LDL-cholesterol (LDL-C) esters at the end of each dietary period [19].

2.2. Blood sampling and biochemical determinations

Venous blood for lipid and lipoprotein analysis was collected in EDTA-containing tubes, from subjects after a 12-h overnight fast, at three timepoints at the end of each dietary period. Venous blood for endothelial products was pooled in tubes containing 3.8% sodium citrate in a 1:9 ratio. Platelet-poor plasma was obtained by centrifugation at 4°C , at $3000 \times g$, within 1 h of venipuncture. Cholesterol and triglycerides were assayed by enzymatic procedures [20,21]. HDL-cholesterol (HDL-C) was measured after precipitation of apo B-containing lipoproteins with phosphotungstic acid [22]. LDL-C level was calculated from the total cholesterol, triglycerides, and HDL-C values using the Friedewald formula [23]. Apo A1 and apo B concentrations were determined by turbidimetric methods [24]. To reduce interassay variation, plasma and lipoprotein fractions were stored at -80°C and analyzed at the end of the study in triplicate.

Plasma levels of vWF were determined by ELISA (Asserachrom vWF, Boehringer Mannheim, Mannheim, Germany) [25]. Plasma levels of soluble E-selectin were determined by ELISA (Human soluble E-selectin, R&D Systems, Minneapolis, MN) [26]. Levels of plasma thrombomodulin were determined by ELISA (Imubind Thrombomodulin, American Diagnostica, Greenwich, CT) [27]. Finally, TFPI levels were also determined by ELISA (Imubind Total TFPI, American Diagnostica, Greenwich, CT) [28]. PAI-1 activity was quantified by a chromogenic assay for determination of PAI-1 activity using Spectrolyse/fibrin PAI Kit (Biopool, Umea, Sweden) [29]. Vitamin E content of LDL particles was measured by high-performance liquid chromatography according to a modification of methodology described by Kaplan et al. [30]. The intraassay coefficient of variation for von Willebrand Factor was 2.12%, for TFPI, 2.05%, E-selectin, 1.87%, thrombomodulin, 2.35% and PAI-1 was 2.57%.

2.3. LDL oxidation

The formation of conjugated dienes was measured by incubating 100 μg LDL protein with 5 $\mu\text{M/l}$ CuSO_4 in 1.0 ml PBS medium. The absorbance at 234 nm was measured continuously in a spectrophotometer as previously described [31]. Results are expressed as the duration of lag time before propagation of the oxidation reaction determined by the absolute increase in absorbance above the initial value.

2.4. Statistical analysis

Statistical analysis was carried out using the CSS statistical package (Statsoft, Tulsa, OK). ANOVA for repeated measures was used to analyze the differences in plasma lipid levels and endothelial products between dietary phases. When statistically significant effects were demonstrated, the Tukey's post-hoc test was used to identify between group differences. Correlation analyses were performed with Spearman's rank correlation. A value of $P < 0.05$ was considered significant. All data are presented in the text and tables as means \pm S.D.

3. Results

The age, BMI and basal plasma lipid levels and apolipoprotein levels of the 25 subjects who participated in the study are presented in Table 1. Dietary composition was analyzed in duplicate. The values obtained (Table 2) were similar to those calculated from the tables. Analysis of the fatty acid composition of the LDL-C esters for each of the diet phases (Table 3) demonstrated a significant increase in palmitic acid during the dietary period rich in saturated fats compared to the other two dietary periods and an increase in oleic acid composition during the dietary period rich in monounsaturated fats suggesting good adherence of the subjects to the experimental diets.

Plasma levels of thrombomodulin and E-selectin did not significantly change after any of the experimental

Table 1
Baseline characteristics (mean \pm S.D.) of the 25 subjects that completed all diet phases^a

Age (years)	20.6 \pm 2.1
BMI (kg/m^2)	23.8 \pm 2.6
Triglycerides (mmol/l)	0.9 \pm 0.4
Total cholesterol (mmol/l)	3.9 \pm 0.7
HDL-cholesterol (mmol/l)	1.2 \pm 0.3
LDL-cholesterol (mmol/l)	2.3 \pm 0.7
Apo A1 (g/l)	1.1 \pm 0.2
Apo B (g/l)	0.6 \pm 0.1

^a Apo, apolipoprotein.

Table 2

Mean daily intake (mean \pm S.D.) during each experimental diet period^a

	NCEP-I diet	MUFA diet	SAT diet
Protein (% of energy intake)	17.6 \pm 1.8	17.5 \pm 2	18.1 \pm 1.9
Fat (% of energy intake)	27.9 \pm 2.6	38.4 \pm 3.1	37.7 \pm 2.9
Saturated	9.2 \pm 3.8	9.2 \pm 4	22.6 \pm 4.3
Monounsaturated	13.5 \pm 1.6	24.4 \pm 2.7	10.1 \pm 2.9
Polyunsaturated	5.2 \pm 1.2	4.8 \pm 1.3	5 \pm 1.1
Carbohydrates (% of energy intake)	54.5 \pm 8.6	44.1 \pm 7.6	44.2 \pm 7.8
Cholesterol (mg/1000 kcal)	113 \pm 45	117 \pm 39	112 \pm 37
α -Tocopherol (mg)	12.2 \pm 2.2	12.4 \pm 2.3	12.1 \pm 3.0
Energy (MJ)	10.1 \pm 1	10.2 \pm 1.2	10.3 \pm 1.3

^a NCEP-I, National Cholesterol Education Program; MUFA, monounsaturated-diet; SAT, saturated-diet.

dietary periods (Table 4). Consumption of the MUFA-diet brought about significant decreases in the plasma levels of vWF, PAI-1 and TFPI in comparison with the NCEP-I-diet ($P < 0.001$) and the diet rich in saturated fats ($P < 0.001$) (Table 4Fig. 1). In addition, PAI-1 plasma levels during the NCEP-I diet were significantly lower than at the end of the diet rich in saturated fats.

Lipid and lipoprotein levels at the end of each dietary period are presented in Table 5. There were no significant differences between the NCEP-I-diet and the MUFA-diet in any of the lipid parameters. As expected, consumption of a diet rich in saturated fats led to a significant increase in total cholesterol ($P < 0.01$), LDL-cholesterol ($P < 0.01$), apo A1 ($P < 0.01$) and apo B ($P < 0.01$) compared to the other two dietary periods.

The study of the production of conjugated dienes by the LDL revealed a significant increase in lag time after the MUFA-diet compared to the NCEP-I-diet ($P <$

Table 3

Fatty acid composition (mean \pm S.D.) of LDL-cholesterol esters during each diet phase^a

Fatty acid	NCEP-I diet	MUFA diet	SAT diet
16:0	19 \pm 3.9	15.1 \pm 0.4	27 \pm 1.4 ^{†,‡}
16:1	2.3 \pm 0.3	1.9 \pm 0.2	2.2 \pm 0.9
18:0	2.4 \pm 0.8	2.5 \pm 0.4	2.8 \pm 1.1
18:1	38.5 \pm 9	49.7 \pm 4.7 [§]	45.5 \pm 4.4
18:2	33.6 \pm 16	26.4 \pm 4.8	20.2 \pm 3.6

^a NCEP-I, National Cholesterol Education Program; MUFA, monounsaturated; SAT, saturated.

[†] Significantly different from NCEP-I-diet ($P < 0.004$).

[‡] Significantly different from MUFA-diet ($P < 0.0004$).

[§] Significantly different from NCEP-I-diet ($P < 0.03$).

Table 4

Plasma substances (mean \pm S.D.) of endothelial origin at the end of each dietary period^a

	NCEP-I diet	MUFA diet	SAT diet	P -value [†]
von Willebrand Factor (%)	81.3 \pm 11.9	71.8 \pm 13.1 ^{‡,§}	78.6 \pm 13.3	0.00001
TFPI (ng/ml)	8.1 \pm 1.6	6.5 \pm 1 ^{‡,§}	7.8 \pm 1.5	0.000002
Thrombomodulin (ng/ml)	3.1 \pm 1.1	3.6 \pm 2.1	3.4 \pm 1.4	0.6872
E-Selectine (ng/ml)	28.7 \pm 11.5	29.7 \pm 14.1	30.6 \pm 10.7	0.6021
PAI-1 (mU/ml)	3.54 \pm 2.35 [§]	1.49 \pm 1.14 ^{‡,§}	5.51 \pm 2.89	0.0001

^a NCEP-I, National Cholesterol Education Program; MUFA, monounsaturated fat; SAT, saturated.

[†] Probability values determined by ANOVA.

[‡] Significantly different from NCEP-I-diet ($P < 0.001$).

[§] Significantly different from SAT-diet ($P < 0.001$).

0.01) and SAT-diet (Table 5). However, there were no differences in the α -tocopherol levels in LDL particles and LDL-C/apo B ratio between the three different dietary periods (Table 5).

Positive correlations were found between changes in vWF and TFPI levels and also with changes in total cholesterol, LDL-C and apolipoprotein B (Table 6Fig. 2). The lag time of conjugated diene formation, after LDL oxidation, was negatively correlated with a decrease in plasma levels of TFPI (Table 6). There was no correlation between lag time, LDL-C/apo B ratio and LDL- α -tocopherol levels. PAI-1 plasma levels were positively correlated with total cholesterol, LDL-C and triglycerides and negatively correlated with HDL-C.

4. Discussion

The present study shows for the first time that after consumption of a MUFA-diet by healthy young men for 28 days, both vWF plasma activity and total TFPI plasma concentration were significantly lower compared with levels recorded after the periods in which the same subjects consumed a low-fat carbohydrate-rich diet or a high SAT-diet, suggesting that a MUFA-diet might beneficially affect endothelial function. We also confirmed our previous results which found a decrease in PAI-1 plasma levels after consumption of a high MUFA diet.

Our data suggest that the decrease observed in vWF and TFPI plasma levels could be explained by the change in MUFA concentration in the MUFA-diet,

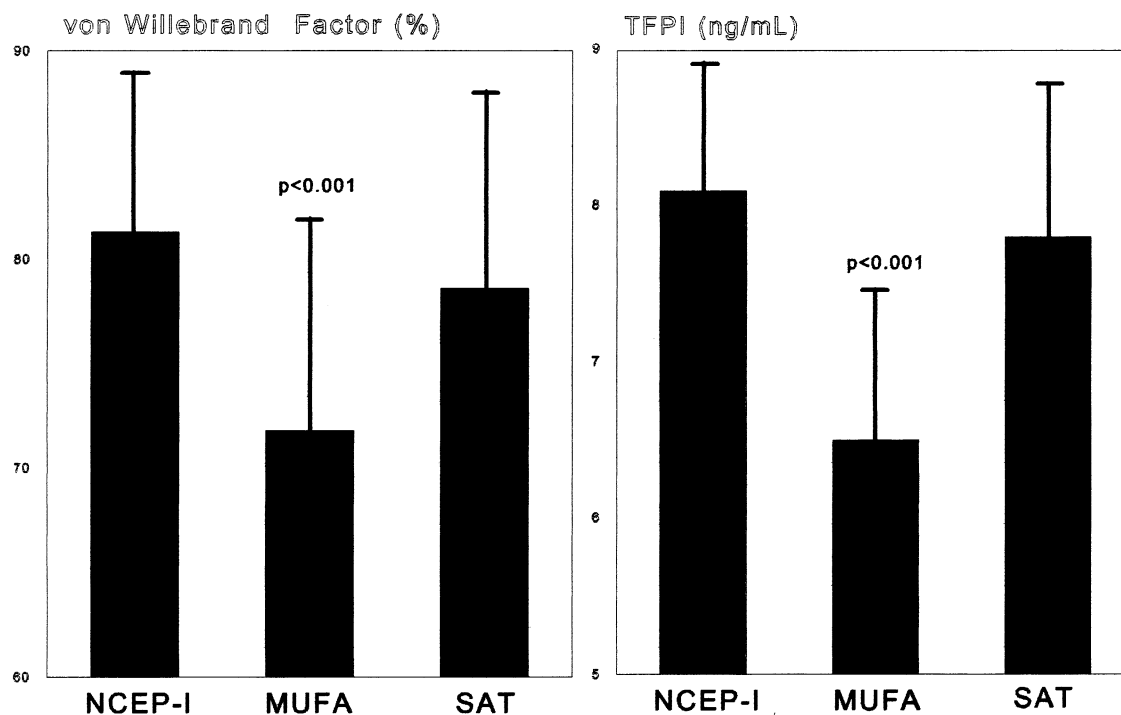


Fig. 1. Plasma von Willebrand Factor activity (A) and Tissue Factor Pathway Inhibitor levels (B) (mean \pm S.D.) at the end of each dietary period. NCEP-I, National Cholesterol Education Program; MUFA, monounsaturated-diet; SAT, saturated-diet. $P < 0.001$ as compared with NCEP-I diet and SAT diet.

since the principal change introduced during this dietary period was the concentration of this nutrient. Since an increase in the amount of MUFA is linked to a concomitant reduction in the carbohydrate content, a reduction in carbohydrate could be in part responsible for the effect on vWF and TFPI. However, replacement of carbohydrate by SAT was not associated with any change in the values of these parameters. It is also possible that, in addition to the fatty acid-induced effects, other minor components present in the olive oil used during the MUFA period could contribute to the changes associated with consumption of the MUFA-diet, although LDL vitamin E plasma levels were no different between the three dietary periods.

The study design was sequential and non-randomized. It is, therefore, possible that order of diet and period effects could influence the findings. However, to minimize the carryover effects, subjects were encouraged to maintain regular physical activity and life-style and there were no differences in any event that could affect the outcome of the study, such as stress, change in smoking habits, consumption of alcohol and foods not included in the experimental design between the three dietary periods. In addition, lunch and dinner were prepared in the hospital kitchen and consumed by all subjects in the dining hall, and the dietary follow-up was verified by analyzing fatty acids in LDL-cholesterol esters at the end of each dietary period.

Since vWF and TFPI are products derived principally from endothelium our results suggest that a MUFA-diet could perhaps have an effect on endothelial cell function. These findings are also consistent with the decreased PAI-1 plasma levels produced in part in the endothelium. The possibility of a modification in the biology of the endothelial cells by the diet has been suggested previously. A lipid lowering low fat diet [6]

Table 5

Plasma lipid, lipoproteins and apolipoproteins concentrations (mean \pm S.D.) at the end of each dietary period^a

	NCEP-I diet	MUFA diet	SAT diet
Cholesterol (mmol/l)	3.7 \pm 0.5	3.7 \pm 0.5	4.3 \pm 0.6 ^{†,‡}
Triglycerides (mmol/l)	0.9 \pm 0.3	0.9 \pm 0.3	1 \pm 0.3
HDL-c (mmol/l)	1.1 \pm 0.3	1.2 \pm 0.3	1.2 \pm 0.2
LDL-c (mmol/l)	2.1 \pm 0.5	2.1 \pm 0.5	2.6 \pm 0.6 ^{†,‡}
Apo A1 (g/l)	1.1 \pm 0.1	1.1 \pm 0.2	1.2 \pm 0.1 [†]
Apo B (g/l)	0.5 \pm 0.1	0.5 \pm 0.1	0.6 \pm 0.1 ^{†,‡}
Lag time (min)	41.4 \pm 31.3	61.1 \pm 45.7 ^{†,‡}	47.4 \pm 42.2
LDL- α -tocopherol (μ g/ml)	36.3 \pm 30	31.5 \pm 19	30.7 \pm 28
LDL-C/apo B ratio	4.51 \pm 0.56	4.38 \pm 0.58	4.58 \pm 0.62

^a NCEP-I, National Cholesterol Education Program; MUFA, monounsaturated fat; SAT, saturated fat; Apo, apolipoprotein.

[†] Significantly different from NCEP-I diet ($P < 0.01$).

[‡] Significantly different from SAT diet ($P < 0.01$).

Table 6
Correlation between percent changes of plasma levels of von Willebrand Factor and Tissue Factor Pathway Inhibitor and with percent changes of different parameters, after different dietary periods (SAT-diet to MUFA-diet, SAT-diet to NCEP-I-diet and MUFA-diet to NCEP-I-diet)^a

	vWF		TFPI		PAI	
	<i>R</i>	<i>P</i> -value [†]	<i>R</i>	<i>P</i> -value [†]	<i>R</i>	<i>P</i> -value [†]
Total cholesterol	0.33	0.003	0.36	0.001	0.41	0.006
Triglycerides	0.14	0.23	0.07	0.52	0.61	0.001
HDL-cholesterol	0.08	0.47	-0.16	0.15	-0.30	0.044
LDL-cholesterol	0.24	0.03	0.39	0.005	0.44	0.002
Apo A1	0.17	0.14	-0.05	0.66	-0.12	0.48
Apo B	0.24	0.03	0.28	0.01	0.24	0.08
Lag time	0.18	0.11	-0.26	0.02	-0.24	0.11
vWF	-	-	0.51	0.00002	0.17	0.26
TFPI	0.51	0.00002	-	-	0.12	0.44
PAI	0.17	0.26	0.12	0.44	-	-

^a NCEP-I, National Cholesterol Education Program; MUFA, monounsaturated fat; SAT, saturated fat; Apo, apolipoprotein; vWF, von Willebrand factor; TFPI, Tissue Factor Pathway Inhibitor.

[†] Spearman correlation test.

and a MUFA-diet [17] brought about a decrease of vWF in groups of patients with coronary heart disease or non-insulin dependent diabetes mellitus, usually suffering from high plasma levels of vWF. However, in the MUFA intervention study diabetic patients received a very high fat diet (50% of total calories as fat), with a high content in MUFA (30% of total calories). Unlike previous studies our population consisted of healthy normolipemic young subjects. Our data, therefore, provide new and relevant information because the decrease in endothelial parameters was observed in a group of subjects with normal vWF plasma levels. Our results may, therefore, be more easily extrapolated to the general population than earlier observations. Furthermore, we used a diet of high palatability whereas the study mentioned previously was performed with a very high fat diet, with a MUFA content higher than that usually present in the Mediterranean diet. Hence, the beneficial effect of MUFA in the diet can be gained by consumption of a diet that is easily adopted and accepted by the population, since this is the same as that normally consumed in Mediterranean countries.

The mechanisms involved in the MUFA-induced effect on endothelial function are not known. TFPI is a lipoprotein-associated coagulation inhibitor and 95% of TFPI in normal plasma is carried by lipoproteins [7]. A previous study has shown that TFPI in the plasma of crab-eating monkeys increases markedly in response to a high-cholesterol diet [32]. On the other hand, vWF plasma levels have also been shown to be correlated with plasma total cholesterol. These findings could suggest that the dietary effect is induced by modifying the cholesterol metabolism [6], since the nutrients in the diet are important determinants of plasma cholesterol, and hyperlipidemia is able to induce prelesional modifications of the endothelium and alter vascular tone and

reactivity [13]. In agreement with this hypothesis we found, in the group as a whole, that percent changes in vWF and TFPI, between the different dietary periods, correlated with percent changes in total cholesterol, LDL cholesterol and apo B plasma levels. However, it is interesting to note that vWF and TFPI plasma levels only decreased with the Mediterranean diet but not with the NCEP-I-diet, despite the fact that both diets induced similar changes in lipid plasma levels. This finding could support the hypothesis that the MUFA-diet has a specific effect, independent of the influence on cholesterol metabolism. Another mechanism which could affect endothelial function is the oxidative modification of the LDL [13]. It has been demonstrated that oxidized LDL can modify the endothelium, and the addition of antioxidants, such as β -carotene and α -tocopherol in rabbits fed a diet rich in cholesterol, can restore the endothelium dependent relaxation [33]. In support of this hypothesis, we found that the Mediterranean type diet increased the resistance to LDL oxidation and this increase was correlated with the decrease in TFPI with the dietary intervention. All this suggests that oxidation and damage could occur in healthy subjects. Another possibility is that the MUFA-diet modifies endothelial function by changing the phospholipid concentration of the arterial wall as shown previously [34]. It is noteworthy that other soluble substances of endothelial origin such as thrombomodulin and E-selectin are not modified with this diet. A similar observation has been shown in pharmacologically treated patients with dyslipidemia in which E-selectin but none of the other soluble cell adhesion molecules was shown to significantly decrease. It has been suggested that this may be due to differences in mechanisms of gene regulation [35]. Future studies should focus on elucidating this mechanism.

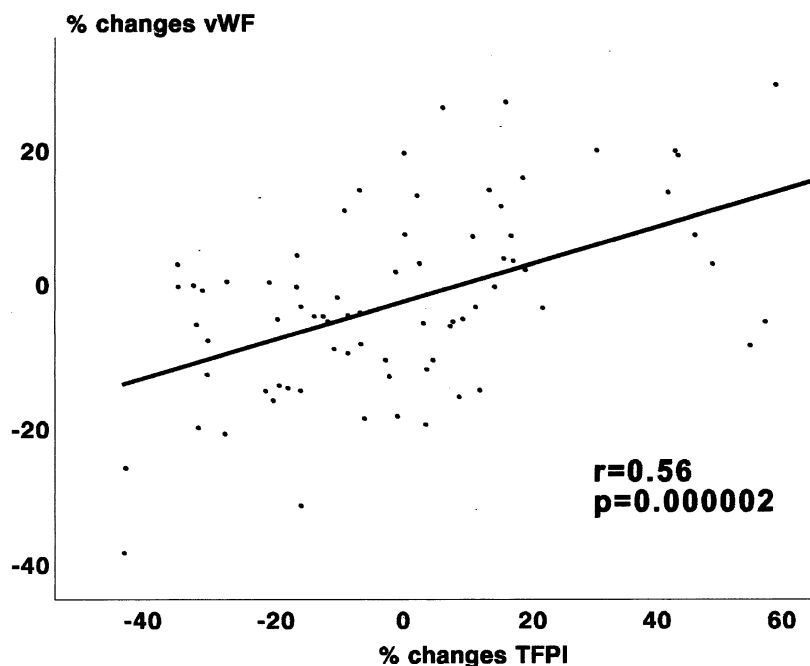


Fig. 2. Correlation between percent change of plasma levels in von Willebrand Factor and Tissue Factor Pathway Inhibitor with the different dietary periods (SAT-diet to MUFA-diet, SAT-diet to NCEP-I-diet and MUFA-diet to NCEP-I-diet). NCEP-I, National Cholesterol Education Program; MUFA, monounsaturated-diet; SAT, saturated diet.

The appearance of thrombotic phenomena on an unstable plaque leads to a sudden increase of stenosis with or without angina, or acute occlusion with myocardial infarction, unstable angina, or ischaemic sudden death. The European Concerted Action on Thrombosis and Disabilities Angina Pectoris Study [36] constitutes a major advance in the establishment of haemostatic variables as cardiovascular risk factors. It showed that raised fibrinogen, t-plasminogen activator (t-PA) and vWF were strong predictors of the risk of myocardial infarction or sudden death in patients with angina pectoris. On the other hand, Tissue Factor (TF) is the predominant initiator of the coagulation cascade and its regulation is a critical aspect of endothelial cell haemostatic function [37]. Expression of TF by endothelium monocytes or other cells is also thought to play a role in the pathogenesis of atherosclerosis [38]. The main role of TFPI seems to be to inhibit small amounts of TF, which is probably essential for maintaining a normal haemostatic balance [7] and it has been speculated that TFPI on endothelial cells *in vivo*, plays a role in the regulation of thrombosis [39]. The decrease in plasmatic TFPI, as previously suggested [32] could reflect an increase of this protease in the endothelial surface which would have a regulatory effect on thrombogenesis. Therefore, the decrease in vWF, PAI-1 and TFPI would be interpreted as a change in the protective effect against thrombogenesis.

In conclusion, the changes induced by the MUFA-diet, both on vWF and TFPI levels as well as those

previously described and now confirmed on PAI-1 [15] could be involved in the lower incidence of CHD in populations that have a high oleic acid consumption, such as in the Mediterranean area, implying that replacement of SATs in the diet by MUFAs could be preferable to their replacement by carbohydrates.

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