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Research Article

Factor VII polymorphisms influence the plasma response to diets with different fat content, in a healthy Caucasian population

Rafael Ángel Fernández de la Puebla, Pablo Pérez-Martínez, José Carmona, José López-Miranda Carmen Marín, Juan Antonio Paniagua, Francisco Fuentes, and Francisco Pérez-Jiménez

Reina Sofia University Hospital, Lipids and Atherosclerosis Research Unit, University of Cordoba, Ciber Fisiopatologia Obesidad y Nutricion, Instituto Salud Carlos III, Cordoba, Spain

To determine the influence of the factor VII gene polymorphisms, R353Q and 5'F7, on factor VII Ag plasma levels after the consumption of diets with different fat contents. Methods: 59 healthy individuals (42 RR, 16 RQ, 1 QQ at the R353Q and 46 A1A1, 13 A1A2 at the 5'F7) consumed 3 diets during 4-weeks each: a Saturated diet (SAT) enriched in saturated fatty acid (SFA) (38% fat, 20% SFA), followed by a carbohydrate (CHO)-rich diet (30% fat, 55% CHO) or a Mediterranean diet (MEDIT) enriched in monounsaturated fatty acid (MUFA) (38% fat, 22% MUFA) following a randomized crossover design. Plasma lipids and FVII Ag plasma levels were determined at the end of each dietary period. Results: After a SAT diet, RR homozygotes had greater concentrations of FVII Ag compared with MEDIT and CHO diets than did carriers of the minority Q allele (82.76 ± 1.3 *vs*. 75.02 ± 2.4, p = 0.001). The 5'F7 polymorphism behaved in a similar fashion (A1A1 81.98 ± 1.4 *vs.* A1A2 75.37 ± 2.4, p = 0.026). Conclusions: Our data show that carriers of the RR and/or A1A1 genotype present higher FVII Ag levels after the consumption of a SAT diet compared with the MEDIT and CHO rich diets.

Keywords: Factor VII polymorphism / Gene – diet interactions / Mediterranean diet / Nutrigenetics / Olive oil

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1 Introduction

Factor VII coagulant activity [1, 2] and plasma activated FVII levels [3] are directly associated with the risk of developing coronary heart disease, irrespective of other factors such as systolic arterial pressure, triglycerides, obesity, or apolipoprotein (apo) A-1 levels. On the other hand, the presence of the minority alleles of the R353Q [4] and 5F7 [5] polymorphisms at factor VII gene is associated with a lower risk of myocardial infarction. This may be explained by the lower plasma concentrations of this factor in carriers of these minority alleles [6]. Consequently, factor VII plasma levels, as well as its gene polymorphisms, might play an important role in the pathogenesis of atherosclerosis.

It has been reported that several polymorphisms in the FVII gene are associated with plasma levels of FVII (–401G/T, –402G/A, 5′F7A1/A2, IVS7, and R353Q). The substitution of glutamine for arginine at position 353 in the catalytic domain (R353Q) and a 10-bp insertion in the promoter region (5′F7) may be responsible for one third of the variations in plasma FVII levels. Bernardi *et al.* showed that genetic variation associated with the 5′F7, IVS7, and 353Arg-Gln polymorphisms contributes about 26, 17, and 30% of the variance of FVIIc and 23, 13, and 23% of that of FVIIAg, respectively, in a group of females and males from a rural area [6]. Lam *et al.* found that the R353Q polymorphism accounted for 12% of the concentration variability of factor VII in diabetic patients, and only 1% in nondiabetic subjects [7]. Another author observed that the influence of these polymorphisms was more marked in men than in women [8].

Dietary fat also influences postprandial plasma factor VII concentrations [9], given that the intake of saturated

Correspondence: Dr. Pablo Perez-Martinez, Unidad de Lipidos y Arteriosclerosis, Hospital Universitario Reina Sofía, Avda. Menendez Pidal, s/n. 14004, Cordoba, Spain
E-mail: pablopermar@yahoo.es
Fax: +34-957218229

Abbreviations: Apo, apolipoprotein; **CHO,** carbohydrate; **MEDIT,** Mediterranean; **SAT,** saturated; **SFA,** saturated fatty acid, **SSPG,** steady-state plasma glucose; **SSPI,** steady-state plasma insulin; **TC,** total cholesterol

fatty acids (SFAs) increases its plasma levels and activates factor VII [10]. In previous studies, we have reported that the Mediterranean (MEDIT) diet reduces activated factor VII plasma levels in healthy men (data not published); a fact that might be related to its rich use of olive oil [11]. The interaction between the polymorphisms of factor VII, chiefly the R353Q, and postprandial activation of FVII by different dietary fats has been studied in different populations. Mennen *et al.* [12] reported that elderly women with the RR genotype had the highest concentrations of activated factor VII after a fatty breakfast. In men with ischemic cardiopathy, carriers of the minority Q allele showed lower concentrations of activated factor VII in response to fat intake [13], although other authors have not confirmed this finding [14].

Individual differences in the response of activated factor VII levels to diet may be related to the R353Q and 5′F7 polymorphisms of this coagulation factor in each person. However, precisely how the interaction between diet and FVII genetics affects FVII Ag levels is not well understood. Consequently, our objective was to study the influence of factor VII gene polymorphisms on its factor VIIAg plasma levels in healthy normolipemic subjects.

2 Material and methods

2.1 Subjects and diets

Fifty-nine healthy normolipemic (total plasma cholesterol levels lower than 5.3 mmol/L) subjects 30 males (21 RR and 9 RQ), (22 A1A1 and 8 A1A2), and 29 females (21 RR and 8 RQ), (24 A1A1 and 5 A1A2), attending the University of Cordoba, volunteered to participate in the study. All had a comprehensive medical history, physical examination, and clinical chemistry analyses before enrollment. Subjects were below 30 years of age (mean age 22 ± 0.4 SD), with no evidence of any chronic illness (such as hepatic, renal, thyroid, or cardiac dysfunction), or unusual high levels of physical activity.

Dietary information, including alcohol consumption, was collected over seven consecutive days. Individual energy requirements were calculated by taking into consideration subjects' weight and physical activity. Subjects were encouraged to maintain their 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 a saturated fat (SAT) enriched diet, with 15% protein, 47% carbohydrate (CHO) and 38% fat (20% SAT, 12% MUFA and 6% PUFA) (Fig. 1). After this period, 30 subjects received a MEDIT-enriched diet for 28 days in a randomized, crossover design. The diet contained 15% protein, 47% CHOs and 38% fat (<10% SFA, 6% PUFA, 22% MUFA). The MEDIT enriched diet was followed by a high CHO diet for 28 days containing 15% protein, 55% CHOs and <30% fat (<10% SFA, 6% PUFA, 12% MUFA). The other 29 subjects received the CHO diet before the MEDIT diet. Volunteers were randomly assigned to this sequence of diets. Cholesterol content remained constant (under 300 mg/day) during the three periods. Eighty percent of the MEDIT diet was provided by virgin olive oil, which was used for cooking, salad dressing, and as a spread. CHO intake of the CHO diet was based on the consumption of biscuits, jam, and bread. Butter and palm oil were used during the SAT dietary period. The composition of the experimental diet was calculated using the United States Department of Agriculture (USDA) food tables [15] or the Spanish food composition tables for local foodstuffs [16]. Fourteen menus, prepared with regular solid foods, were rotated during the experimental period. We used palm oil and butter as a source of SAT fat. Lunch and dinner were consumed in the hospital dining room, whereas breakfast and an afternoon coffee break were taken in the medical school cafeteria. A dietician supervised all meals. Duplicate samples from each menu were collected, homogenized, and stored at −80°C. Protein, fat, and CHO content of the diet were analyzed using standard methods (Table 1). The Human Investigation Review Committee approved this study at the Reina Sofia University Hospital.

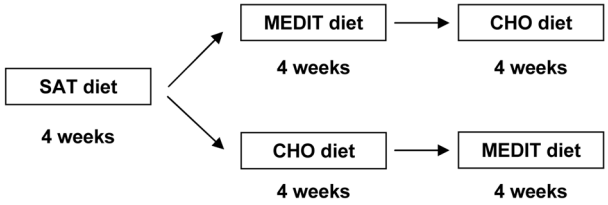

Figure 1. Intervention study design. Subjects consumed three diets during four-weeks each following a randomized crossover design.

2.2 Blood sampling and biochemical determinations

Venous blood samples for glucose, lipid, and lipoprotein analysis were collected in EDTA-containing tubes from subjects after a 12-h overnight fast. Cholesterol and triglycerides were assayed by enzymatic procedures [17, 18]. HDL cholesterol was measured by analyzing the supernatant obtained after precipitation of a plasma aliquot with extra sulfate-Mg^{2+} [19]. LDL cholesterol level was calculated from the total cholesterol (TC), triglycerides, and HDL-cholesterol values using the Friedewald formula [20]. Apo A-I (apo A-I) and apo B were determined by immunoturbidimetry [21]. Glucose levels were determined by enzymatic tests and insulin levels by radioimmunoanalysis [22]. Plasma FVII Ag concentrations were determined using an

Table 1. Daily intake during each dietary intervention period

	SAT		CHO		MEDIT	
	Calculated	Analyzed	Calculated	Analyzed	Calculated	Analyzed
Energy (MJ)	10.2	10.8 ± 1.1	10.2	10.6 ± 1.0	10.2	10.8 ± 1.5
Proteins (%)	15.0	18.1 ± 2.5	15.0	17.6 ± 1.5	15.0	17.5 ± 2.0
Fats (%)						
SFA	20.0	22.6 ± 4.1	10.0	9.2 ± 3.5	10.0	9.2 ± 4.2
MUFA	12.0	10.1 ± 2.9	12.0	13.5 ± 1.2	22.0	24.4 ± 2.2
PUFA	6.0	5.0 ± 1.5	6.0	5.2 ± 2.0	6.0	4.8 ± 1.1
CHOs (%)	47.0	44 ± 8.3	57.0	54.5 ± 8.6	47.0	44.1 ± 7.8
Cholesterol (mg/day)	285	272	285	275	285	277
Fiber (g/day)	25	25.9 ± 7	25	26.1 ± 6.1	25	24.9 ± 8.2

Means ± SD. The different nutrients are expressed as a percentage of energy intake. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

ELISA kit (Asserachrom® VII:Ag, Diagnostica Stago) with a detection limit of 0.5%. We determined peripheral sensitivity to insulin at the end of each intervention period using a modified version of the insulin suppression test described by Reaven and *coworkers* [23]. Unesterified free fatty acid levels were analyzed by an enzymatic colorimetric assay (Boehringer Mannheim) as described by Shimizu *et al.* [24]. To reduce interassay variation, plasma for biochemical determinations was stored at −80°C and analyzed at the end of the study in duplicate.

2.3 Determination of the genotypes

DNA was extracted from 10 mL of peripheral venous blood in EDTA (1 mg/mL) containing tubes by the salting-out method. The 5′F7 polymorphism was determined by PCR amplification of a fragment of 214 or 224 bp of the FVII gene using 1 μg of genomic DNA, 200 μmol/L of nucleotides, 1.5 mmol/L $MgCl_2$, 2.5 U of Taq polymerase (Biotaq, Bioline) and 0.2 μmol of each "primer" (5′F7-1, 5′-GGCCTGGTCTGGAGGCTCTCTTC-3′and 5′F7-2, 5′-GAGCGGACGGTTTTGTTGCCAGCG-3′) in a final volume of 50 μL. DNA was denatured at 95°C for 2 min followed by 29 cycles of denaturation at 95°C for 30 s, "annealing" at 55°C for 30 s, and extension at 72°C for 1 min. Ten microliters of the PCR amplification products were digested with Sty-I restriction enzyme in a total volume of 35 μL. The R353Q polymorphism was determined by PCR amplification of a fragment of 239-bp of the FVII gene using 1 μg of genomic DNA, 200 μmol/L of nucleotides, 1.5 mmol/L $MgCl_2$, 2.5 U of Taq polymerase (Biotaq, Bioline) and 0.2 μmol of each primer (RQ-1, 5′-GCAGCAAGGACTCCTGCAAG-3′ y RQ-2, 5′-CCACAGGCCAGGGCTGCTGG-3′) [25] in a final volume of 50 μL. DNA was denatured at 95°C for 3 min followed by 34 cycles of denaturation at 95°C for 1 min, annealing at 65°C for 30 s, and extension at 72°C for 1 min and a final cycle at 72°C for 5 min. Ten microliters of the PCR amplification products were digested with MspI restriction enzyme in a total volume of 35 μL. The products obtained were submitted to 8% nondenaturing polyacrylamide electrophoresis at 150 V for 45 min followed by silver staining.

2.4 Glucose suppression test

A modified insulin suppression test was carried out on all the subjects at the end of the dietary period [26]. The study began at 8:00 am, after 12 h of fasting. A continuous infusion of somatostatin (214 nmol/h), insulin (180 pmol/m^2/m) and glucose (13.2 pmol/m^2/m^1) were infused in the same vein. Somatostatin was used to inhibit endogenous insulin secretion. Blood was sampled every 30 min for the first 2.5 h, by which time steady-state plasma glucose (SSPG) and steady-state plasma insulin (SSPI) concentrations were achieved. Blood was then sampled at 10-min intervals for the last 30 min (at minutes 150, 160, 170, and 180) for measurement of plasma glucose and insulin concentrations. These four values determined the SSPG and SSPI concentrations. Since SSPI concentrations were similar in all subjects, SSPG concentrations provided a measure of the ability of insulin to promote the disposal of infused glucose. Subjects with high SSPG are relatively more insulin resistant than others with lowers SSPG.

2.5 Statistical analyses

ANOVA for repeated measures was used to analyze the differences among several study groups. When statistically significant effects were demonstrated, Tukey's post-hoc test was used to identify differences between groups. The Kolmogorov – Smirnov one-sample test was used to test the normality of the distribution. Variables that were not normally distributed were log-transformed before their analysis. A value of $p < 0.05$ was considered significant. All data are presented in the text and tables as means ± SE. In order to determine which variables influence the response of FVII Ag levels to diet, we carried out two multiple linear regression analyses. As dependent variables we used two new

Table 2. Basal characteristics according to R353Q and 5′F7 polymorphisms

	R353Q			5′F7		
	RR (n = 42)	RQ (n = 17)	p	A1A1 (n = 46)	A1A2 (n = 13)	p
Gender (m/f)	21/21	9/8	NS	22/24	8/5	NS
BMI (kg/m^2)	21.7 ± 0.4	21.1 ± 0.9	0.498	21.5 ± 0.4	21.5 ± 1.1	0.998
TC (mmol/L)	4.18 ± 0.09	4.20 ± 0.1	0.895	4.19 ± 0.08	4.18 ± 0.1	0.864
LDL-C (mmol/L)	2.62 ± 0.07	2.53 ± 0.07	0.570	2.63 ± 0.07	2.47 ± 0.1	0.357
HDL-C (mmol/L)	1.21 ± 0.03	1.34 ± 0.07	0.111	1.22 ± 0.03	1.35 ± 0.08	0.130
TG (mmol/L)	0.74 ± 0.07	0.70 ± 0.06	0.629	0.73 ± 0.04	0.59 ± 0.08	0.842
Apo A-I (g/L)	1.49 ± 0.02	1.48 ± 0.03	0.850	1.50 ± 0.02	1.45 ± 0.03	0.339
Apo B (g/L)	0.73 ± 0.02	0.72 ± 0.03	0.748	0.79 ± 0.01	0.71 ± 0.03	0.621
Glucose (mmol/L)	6.98 ± 0.5	7.44 ± 0.9	0.662	6.95 ± 0.5	7.68 ± 0.2	0.519

Mean ± standard error. TC, total cholesterol; LDL-C, LDL cholesterol; HDL-C, HDL cholesterol; Apo A-1, apolipoprotein A-1; Apo B, apolipoprotein B; TG, triglycerides.

variables that resulted from comparing FVII Ag concentrations after the SAT diet, with those obtained after the other two dietary periods. These two variables reflect the theoretic effect that could arise from the replacement of a SAT-rich diet with the hypolipemic diets (MEDIT and CHO-rich diets). As independent variables we included those which have already been (or could be) related with FVII Ag concentrations. These are gender, the 5′F7 polymorphism, the R353Q polymorphism, basal LDL-C, basal HDL-C, and TG [27, 28]. For statistical analysis we used the SPSS 11.0 package (SPSS, Chicago).

3 Results

We found no significant differences in basal characteristics between carriers of the different genotypes of either of the two polymorphisms studied (Table 2).

In Table 3 we present concentrations of FVII Ag, lipids, and plasma lipoproteins after each dietary period. The isocaloric replacement of the SAT diet with the CHO or the MEDIT diets determined a reduction in the levels of Apo A-1, ApoB, TC, HDL-C, and LDL-C. The replacement of the CHO diet with a MEDIT was associated with a marked increase in Apo A-1 ($p < 0.001$), and in HDL-C ($p < 0.001$) levels. We found no statistically significant differences in the levels of FVII Ag, ApoB, TC, LDL-C or triglycerides after the MEDIT and CHO diets.

Among the volunteers we found 42 homozygotes for arginine at position 353 (71%-RR), 16 heterozygotes (27%-RQ), and a single homozygote for glutamine (2%-QQ) Therefore, R allele frequency was 0.85 and Q allele frequency was 0.15. These values reflect figures previously described in the literature. The statistical analysis compares two groups (RR and RQ/QQ) that are based on the presence or absence of the Q allele. For the 5′F7 polymorphism, we found 46 A1A1 homozygotes (78%), and 13 A1A2 heterozygotes (22%). There were no A2A2 homozygotes among the volunteers. Therefore, the frequency of the A1 allele

Table 3. Concentrations of FVIIAg, lipids, and apoproteins at the end of each dietary period

	SAT	CHO	MEDIT	p value
FVII Ag (%)	80.5 ± 1.2	79.0 ± 1.4	78.8 ± 1.3	0.188
TC (mmol/L)	4.18 ± 0.07	3.62 ± 0.07*	3.69 ± 0.07*	0.001
LDL-C (mmol/L)	2.58 ± 0.07	2.14 ± 0.07*	2.17 ± 0.07*	0.001
HDL-C(mmol/L)	1.24 ± 0.02	1.13 ± 0.02*	1.18 ± 0.02*†	0.001
TG (mmol/L)	0.73 ± 0.04	0.77 ± 0.03	0.76 ± 0.04	0.437
Apo A-1 (g/L)	1.49 ± 0.02	1.39 ± 0.03*	1.43 ± 0.02*†	0.001
Apo B (g/L)	0.73 ± 0.02	0.66 ± 0.02*	0.66 ± 0.02*	0.001

Mean ± standard error. ANOVA for repeated measures.
* $p < 0.05$ *versus* saturated-
† $p < 0.05$ *versus* CHO. Mean ± standard error. TC, total cholesterol; LDL-C, LDL cholesterol; HDL-C, HDL cholesterol; Apo A-1, apolipoprotein A-1; Apo B, apolipoprotein B; TG, triglycerides.

was 0.89 and the frequency of the A2 allele was 0.11. These values are slightly lower than those described in the literature with Spanish populations (0.167). We found no significant differences between the groups in the basal characteristics of the population. The genotypes did not influence TC, LDL-C, HDL-C, TG, Apo A, and Apo B plasma concentrations.

The effect of the two polymorphisms on diet-induced changes in the factor VII Ag plasma levels is displayed in Table 4. For the R353Q polymorphism, we noted an interaction between genotype and diet in the FVII Ag concentration. Thus, after the intake of SAT diet, RR homozygotes presented higher concentrations of FVII Ag than the Q allele carriers ($p = 0.001$). Conversely, we observed no significant differences between the MEDIT diet and the CHO diet in both groups of volunteers.

Results obtained with the 5′F7 polymorphism were in the same way to those found with the R353Q polymorphism. We noted a genotype effect after the SAT diet, with higher concentrations of FVII Ag in the A1A1 homozygotes than in the A1A2 heterozygotes ($p < 0.026$). In addition, no dif-

Table 4. Influence of diet on FVII Ag levels (%) according to R353Q and 5′F7 polymorphisms

	R353Q		5′F7	
	RR ($n = 42$)	RQ/QQ ($n = 17$)	A1A1 ($n = 46$)	A1A2 ($n = 13$)
SAT	82.76 ± 1.3	75.02 ± 2.4	81.98 ± 1.4	75.37 ± 2.4
CHO	78.61 ± 1.4	80.08 ± 3.4	79.06 ± 1.5	78.95 ± 3.2
MEDIT	80.01 ± 1.5	75.79 ± 2.6	79.82 ± 1.5	75.13 ± 1.9
p	Genotype effect	0.117	Genotype effect	0.112
	Diet effect	0.837	Diet effect	0.771
	Gene-diet interaction	0.001	Gene-diet interaction	0.026

ANOVA for repeated measures. Mean ± standard error.

Table 5. Multiple regression analysis

Dependent variable	Independent variable	Multiple *r*	r^2	*p* value
FVIIag SAT-MEDIT	Basal HDL-C	0.276		0.043
	Mean glucose	0.253		0.041
	Basal TG	−0.272		0.036
	R353Q	0.220		0.071
Summary of the model		0.548	0.300	0.001
FVIIag SAT-CHO	Basal TG	−0.205		0.088
	Mean glucose	0.164		0.170
	R353Q	0.471		<0.001
Summary of the model		0.542	0.294	<0.001

FVII Ag values have been used as a dependent variable after replacement of the SAT diet with the MEDIT diet, and the SAT with the CHO diet. R353Q, polymorphism of the factor VII gene.

ferences were found between the two groups after the intake of the MEDIT and CHO-rich diets.

A multiple linear regression analysis was performed using the changes in FVII Ag caused by the switch from the SAT to the MEDIT diet as the dependent variable. We observed that such changes are determined by mean glucose during the stabilization period of the insulin sensitivity test, basal HDL-C, basal TG, and the R353Q polymorphism. The resulting model accounts for 30% of the variability in the changes in FVII Ag concentration. Likewise, the changes in FVII Ag that were caused by replacing the SAT diet with the CHO diet are determined by the R353Q polymorphism (Table 5).

4 Discussion

Our data show that carriers of the RR and/or A1A1 genotype present higher FVII Ag levels after the consumption of a SAT diet compared with the MEDIT and CHO-rich diets, than did individuals carrying the Q and/or A2 allele.

Several studies have shown that dietary fat has an impact on coagulant FVII concentrations. Shifting from a SAT diet to a CHO-rich diet generally reduces FVIIc. There are a few research papers that have examined the link between the consumption of different types of dietary fat and FVII Ag levels. Marckmann *et al.* [29], in a controlled crossover study, reported a 13% reduction in FVII Ag to be associated with the consumption of a low-fat diet in young volunteers. However, these results were not confirmed in an older population [30]. The same author did not find this effect when changes were made from saturated to monounsaturated fat. We did not find variations in FVII Ag concentrations between the different dietary periods. However, we did observe different responses in the different allele carriers of the R353Q and 5′F7 polymorphisms. Compared to carriers of the minority (RQ or QQ) allele, FVII Ag levels were higher in homozygotes for the majority RR allele. This occurred only after the consumption of the SAT diet. We obtained the same results for the 5′F7 polymorphism.

In the elderly, the postprandial response of activated factor VII and coagulant FVII to fat intake was more marked in those with the RR genotype [31]. Similar results were observed for factor VII coagulant activity in a group of post-myocardial infarction men [13]. In contrast, FVIIc increased in middle-aged males after a high fat diet, irrespective of the R353Q genotype. In a transversal study, the inverse association between coagulant FVII and fiber was more marked in subjects with the RR genotype [31]. In another large transversal study with elderly women, the magnitude of the association between FVII activity and serum triglycerides varied according to this polymorphism [32].

Niskanen *et al.* [33] conducted a previous intervention study, with 28 middle-aged persons with CHO intolerance, which was similar to this study. A reduction in FVIIc concentrations was only observed in RR homozygotes for the R353Q polymorphism with the CHO diet. However, in our study we observed the same reduction in both the CHO diet and the MEDIT diet. The multiple regression model showed that insulin sensitivity, measured by the insulin suppression test with somatostatin, was a determining factor in FVII Ag modifications when switching from a SAT diet to a MEDIT or CHO-rich diet. This coincides with the correlation found between FVII concentrations and insulin levels [34].

The R353Q polymorphism leads to the replacement of the aminoacid arginine with glutamine at position 353 of the protein, which is in very close proximity to the catalytic region. This induces a change in plasma levels FVII due to a reduction in protein secretion in the liver, modification of its stability in plasma, alteration in synthesis autoregulation due to protein activity, or the linkage disequilibrium between this polymorphism, the 5′F7 and the other promoter polymorphisms. The 5′F7 polymorphism is a decanucleotide insertion at position −323 of the FVII gene promoter. At the same time, linkage sequences of transcription

factors such as C/EBPalpha, C/EBPbeta, or IL-6 [35], similar to nuclear transcription factor kappa-B (NFkappa-B), are involved in inflammatory processes. We believe that, due to its antioxidant and anti-inflammatory effect [35, 36], the MEDIT diet may reduce the activity of one or several of these proinflammatory transcription factors (*i. e.*, NFkappa-B) [37] and consequently decrease FVII synthesis. Future studies may consider potential modifying factors such as age, ethnicity, metabolic syndrome, dyslipemia, and others. In addition, our results should be replicated in larger populations.

In conclusion, the consumption of a MEDIT or a CHO diet is associated with lower concentrations of FVII Ag in healthy subjects that were homozygotes for the majority allele of both polymorphisms studied, which accounted for 70% of the population. Given the importance of FVII Ag in thrombus formation on atheromatous plaque, the reduction of FVII Ag in the majority of alleles, through the consumption of MEDIT or CHO-rich diets, would seem to present further evidence for the significance of these diets in cardiovascular protection.

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