EPIDEMIOLOGY AND PREVENTION

Effect of the Mediterranean Diet on Fasting Concentrations of Activated Factor VII in Healthy Persons

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Introduction and objectives. Many clinical and epidemiologic studies suggest that activated factor VII may be involved in the pathogenesis of coronary heart disease. Our objective was to determine the effect of a Mediterranean diet on plasma levels of activated factor VII in comparison to a low-fat diet and a diet rich in saturated fat.

Patients and method. The study population comprised 16 healthy normolipemic men who consumed 3 different diets in consecutive 28-day periods. The first diet was rich in saturated fat (38% calories as fat, 20% saturated fat), the second was a low-fat, high-carbohydrate diet (28% calories as fat, 10% saturated fat), and the third was enriched in monounsaturated fatty acids (38% calories as fat, 22% monounsaturated fat). At the end of each period, plasma concentrations of total cholesterol, HDL cholesterol, LDL cholesterol, total triglycerides, apolipoprotein A-I, apolipoprotein B, and glucose were measured. Activated factor VII was determined with a coagulation assay.

Results. The diet rich in saturated fat was associated with a significant increase in total cholesterol, LDL cholesterol, apolipoprotein AI, and apolipoprotein B in comparison to the other 2 diets. There were no significant differences between the carbohydrate-rich diet and the Mediterranean diet in any of the lipid parameters. The Mediterranean diet decreased plasma levels of factor VIIa in comparison to the diet rich in saturated fat (34.6±15.3 mU/mL vs 101.5±19.2 mU/mL; P<.05).

Conclusions. In comparison to the diet rich in saturated fat or the high-carbohydrates diet, the Mediterranean diet decreased plasma concentrations of activated factor VII in healthy young men. This phenomenon may constitute another protective mechanism of the Mediterranean diet in reducing cardiovascular risk.

Key words: Mediterranean diet. Activated factor VII. Cardiovascular risk.

Efecto de la dieta mediterránea en los valores plasmáticos de factor VII activado en personas sanas

Introducción y objetivos. Numerosos estudios clínicos y epidemiológicos sugieren que el factor VII activado puede estar implicado en la patogenia de la enfermedad coronaria. Nuestro objetivo es determinar el efecto de una dieta típica mediterránea en los valores plasmáticos de dicho parámetro, cuando se compara con una dieta pobre en grasa y con una dieta rica en grasa saturada.

Pacientes y métodos. Dieciséis varones sanos normolipémicos recibieron 3 dietas, durante 4 semanas cada una. La primera era rica en grasa saturada (38% grasa, 20% saturada), la segunda rica en hidratos de carbono y pobre en grasa (28% grasa, 10% saturada) y, finalmente, una dieta mediterránea (38% grasa, 22% de grasa monoinsaturada). Al final de cada período se determinaron las concentraciones plasmáticas de colesterol total, colesterol unido a lipoproteínas de baja (cLDL) y alta (cHDL) densidad, triglicéridos totales, apolipoproteína A-1, apolipoproteína B y glucosa. El factor VII activado se midió mediante un ensayo de coagulación.

Resultados. La dieta rica en grasa saturada se asoció con un incremento significativo de los valores de colesterol total, cLDL, apolipoproteína A-1 y apolipoproteína B, en comparación con las otras 2 dietas. No hubo diferencias significativas entre la dieta rica en hidratos de carbono y la mediterránea para cualquiera de los parámetros lipídicos examinados. El paso de una dieta rica en grasa saturada a una dieta mediterránea produjo un descenso en los valores de FVIIa (101,5 ± 19,2 frente a 34,6 ± 15,3 mU/ml; p < 0,05).

Conclusiones. La dieta mediterránea, cuando se compara con la dieta rica en grasa saturada o la rica en hidratos de carbono, disminuye las concentraciones plasmáticas del factor VII activado en varones sanos. Este fenómeno podría constituir otro mecanismo protector de la dieta mediterránea en la reducción del riesgo cardiovascular.

Palabras clave: Dieta mediterránea. Factor VII activado. Enfermedad cardiovascular.

ABBREVIATIONS

HDL: high-density lipoprotein.
LDL: low-density lipoprotein.
FVII: factor VII.
FVIIc: factor VII clotting.
FVIIa: activated factor VII.

INTRODUCTION

Factor VII (FVII) is a vitamin K-dependent plasma glycoprotein which has an important role in initiating the tissue-factor induced coagulation cascade (the extrinsic pathway of blood clotting).[1] Several clinical studies have suggested that factor VII clotting (FVIIc) activity in middle-aged persons is directly associated with the risk for cardiovascular disease.[2,3] Moreover, the values of activated factor VII (FVIIa) are also related with cardiovascular risk, independently of other factors, such as systolic blood pressure, triglycerides, obesity, or levels of apolipoprotein A-l.[4] Thus, FVII may have an important role in the pathogenesis of arteriosclerosis.

Although several different techniques are available to measure FVII, they are not all comparable, which may explain the inconsistent results of different studies. The most commonly used assay is FVIIc, which measures total concentrations of both FVIIc and FVIIa. Since the function of the activated form is to initiate the coagulation chain, measurement of FVIIa would appear to be the most interesting method of studying FVII. To date, specific assays to measure FVIIa in plasma, which is a better predictor of risk than either FVII or FVIIc, have shown that healthy subjects have significantly low levels.[5]

Recent dietary intervention studies have shown that various plasma components related to the thrombotic process may be affected by the fatty acid composition of the diet.[6] An example is the demonstration that a diet enriched in saturated fatty acids raises plasma levels of FVII and induces activation of FVIIc during the postprandial period.[7-9] Earlier studies have shown that diets poor in fats and rich in carbohydrates have a reduced effect on FVIIc. However, the influence of this type of diet on FVIIa has not yet been studied. Accordingly, we examined the effects on FVIIa concentrations of 3 types of diet: a diet rich in saturated fats, a diet rich in carbohydrates, and a Mediterranean diet.

PATIENTS AND METHODS

Study Population and Diets

The study population comprised 16 healthy normolipidemic men from the University of Cordoba. Prior to the study, a clinical history was taken and a laboratory analysis was performed for the participants, all of whom were younger than 30 years of age (mean age, 20.8±2.1), had plasma concentrations of total cholesterol <5.2 mmol/L, no evidence of any chronic liver, kidney, heart or thyroid disorder, and none of them had a regularly high level of physical exercise. The participants had no family history of cardiovascular disease and had not taken any medication or dietary or vitamin supplements for 6 months prior to the study. The dietary information, which included alcohol consumption, was collected for 7 consecutive days. The individual energy requirements were calculated taking into consideration each person's physical activity. The participants were encouraged to continue their usual lifestyle and level of physical exercise and were asked to write down in a diary any event which might alter this pattern, such as stress, changes in smoking habits, and the consumption of alcohol or meals not included in the experimental design.

All the participants consumed 3 different diets over consecutive 28-day periods. In order to maintain a stable weight, the diets were isocaloric in relation to the participants' previous usual energy intake. The first diet consisted of a stabilization diet rich in saturated fat (SAT diet), containing 15% of the energy in the form of proteins, 47% as carbohydrates and 38% as fat (20% saturated fat, 12% monounsaturated fat, and 6% polyunsaturated fat). The second diet was rich in carbohydrates (CH diet) and consisted of 15% proteins, 57% carbohydrates, and 28% fat (<10% saturated fat, 12% monounsaturated fat, and 6% polyunsaturated fat). Finally, the third diet was a Mediterranean diet (MED diet), which was rich in olive oil, with 15% proteins, 47% carbohydrates, and 38% total fat (10% saturated fat, 22% monounsaturated fat, and 6% polyunsaturated fat). There was no washout period between diets. The mean intake of cholesterol during the 3 dietary periods was 115 mg/1000 kilocalories. The study was approved by the Clinical Research Ethics Committee of the Hospital Universitario Reina Sofía, Cordoba, Spain.

The composition of the experimental diets was calculated from the food tables of the United States Department of Agriculture (USDA)[10] or the Spanish food composition tables corresponding to local foods.[11] The experimental design of the study involved 20 previously established rotating menus, each of which used natural foods calibrated to provide the established proportions for each of the dietary periods. The monounsaturated fat in the Mediterranean diet was derived from the virgin olive oil used to cook the meals, dress the salads and put on toast. Lunch and dinner were always provided by and eaten in the dining room of the Hospital Universitario Reina Sofía, under supervision by us and a team dietician. Breakfast and tea were served according to our instructions in the cafete-

rias of the University Faculties of Medicine and Science. Breakfast and tea consisted of white coffee and biscuits with marmalade during the period of the carbohydrate rich diet. The biscuits and marmalade were replaced by toast with olive oil or margarine on it for the Mediterranean and western diets, respectively.

Two samples of each menu were collected, homogenized, and stored at –80ºC. The protein, fat and carbohydrate contents of the diets were analyzed by standard techniques. The dietary follow-up of the fatty acids in the low-density lipoprotein (LDL) cholesterol esters was collected at the end of each dietary period.[12]

Biochemical Measurements

At the end of each of the dietary intervention periods, and after a 12-hour fast, 15 mL of venous blood were drawn in EDTA tubes. The venous blood to measure the plasma concentrations of FVIIa was placed in tubes containing a 1:9 ratio of 3.8% sodium citrate. Plasma with a low platelet concentration was obtained by centrifugation at 3000 g for 1 hour at room temperature. Enzymatic methods were used to measure the plasma concentrations of total cholesterol and triglycerides.[13,14] The high-density lipoprotein (HDL) cholesterol was measured by precipitation with phosphotungstic acid.[15] The LDL cholesterol was calculated from the Friedewald equation.[16] The concentrations of apolipoprotein A-1 and apolipoprotein B were measured by immunoturbidimetry.[17] The plasma concentrations of FVIIa were measured by a coagulation assay using a soluble recombinant tissue factor which possesses activity as a cofactor for FVIIa but fails to maintain the activation of FVII.[5] Measurement of FVIIa was undertaken with a commercial device (Staclot VIIa-rTF, Diagnostica Stago, France). To reduce the interassay variation the samples were stored at –80ºC and analyzed in triplicate at the end of the study period.

Statistical Analysis

Statistical analysis was carried out using the computer software program SPSS (version 7.5, in Spanish, Inc., United States, 1997). The effect of the various diets on the study variables was measured by analysis of variance (ANOVA) for repeated measurements. If the differences were significant, a *post hoc* analysis was undertaken with the Tukey test to identify differences between the groups. Because the differences in the plasma concentrations of FVIIa between the 3 diets did not follow a normal distribution they were analyzed by Friedman ANOVA and the Wilcoxon test (which corresponds to paired data). Values were considered significant if the *P* was less than .05. Data are presented as the mean ± standard deviation (SD).

RESULTS

Table 1 shows the age, the body mass index, and the baseline values of plasma lipids and apolipoprotein levels in the study subjects. The dietary composition was analyzed in duplicate portions of meals and was in agreement with that given in the corresponding food composition tables (Table 2). The plasma concentrations of lipids and apolipoproteins at the end of each dietary intervention period are shown in Table 3. The SAT diet had higher levels of total cholesterol, LDL cholesterol, apolipoprotein A-1, and apolipoprotein B than the CH diet ($P<.05$) and the MED diet ($P<.001$). No significant differences were detected for any of the lipid parameters studied between the CH and the MED diets. Consumption of the MED diet was associated with a significant reduction in plasma concentrations of FVIIa in comparison with the CH diet ($P<.05$) and the SAT diet ($P<.05$) (Table 3).

DISCUSSION

The results of this study showed that eating a Mediterranean type diet, rich in olive oil, lowered the levels of FVIIa in a group of healthy volunteers, in comparison with a diet rich in saturated fatty acids and another rich in carbohydrates. There was a positive correlation between the reduction in FVIIa levels and the concen-

TABLE 1. **Baseline Characteristics (Mean ±SD) of the Men (n=16) Who Participated in the Study**[*]

Age, years	20.8±2.1
BMI	23.6±1.9
Triglycerides, mmol/L	0.9±0.4
Total cholesterol, mmol/L	3.9±0.6
HDL cholesterol, mmol/L	1.2±0.3
LDL cholesterol, mmol/L	2.3±0.6
Apo A-1, g/L	1.10±0.2
Apo B, g/L	0.56±0.1

*Apo indicates apolipoprotein; BMI, body mass index.

TABLE 2. **Daily Mean Intake (mean ±SD) During the 3 Dietary Intervention Periods**[*]

	SAT Diet	CH Diet	MED Diet
Protein, % energy	17.9±2.5	17.3±1.5	17.5±2.0
Fat, % energy	38.1±2.9	28.1±2.8	38.6±3.5
Saturated	23.1±4.1	9.0±3.5	9.1±4.2
Monounsaturated	10.0±2.9	13.12±1.2	24.7±2.2
Polyunsaturated	5.0±1.5	6.0±2.4	4.8±1.1
Carbohydrates, % energy	44.0±8.3	54.6±8.6	43.9±7.8
Cholesterol, mg/1000 kcal	112±39	114±48	115±42
Energy, MJ	10.2±1.1	10.3±1.0	10.5±1.5

*CH indicates carbohydrate-rich diet; MED, diet rich in monounsaturated fats; SAT, diet rich in saturated fats.

TABLE 3. **Plasma Concentrations of Lipids and Apolipoproteins (mmol/L) at the End of Each Dietary Intervention Period**[*]

	SAT diet	CH diet	MED diet
Cholesterol, mmol/L	4.35±0.6†,‡	3.62±0.4	3.79±0.5
Triglycerides, mmol/L	0.91±0.3	0.85±0.4	0.80±0.3
HDL cholesterol, mmol/L	1.60±0.2	1.16±0.3	1.24±0.3
LDL cholesterol, mmol/L	2.67±0.5†,‡	2.06±0.4	2.18±0.5
Apo A-1, g/L	1.20±0.1†,‡	1.08±0.2	1.12±0.2
Apo B, g/L	0.58±0.1†,‡	0.46±0.1	0.50±0.1
FVIIa, mU/mL	101.5±19.2	67.8±11.5	34.6±15.3†,$

*Apo indicates apolipoprotein; CH, carbohydrate-rich diet; BMI, body mass index; MED, diet rich in monounsaturated fats; SAT, diet rich in saturated fats.
†Significantly different from the CH diet ($P<.004$).
‡Significantly different from the MED diet ($P<.0004$).
$Significantly different from the SAT diet ($P<.002$).

trations of total cholesterol, LDL cholesterol, and apolipoprotein B. This association is interesting, because both the Northwick Park Heart Study[1] and the PRO-CAM[2] study found an association between fatal events from ischemic heart disease and the levels of FVIIc. Moreover, our study showed that the concentrations of HDL cholesterol were not significantly altered during the 3 dietary intervention study periods, although there was a biological trend to higher levels during the SAT diet, which may not be unusual if we consider that during this latter diet the levels of total cholesterol increased, which means an increase in both LDL cholesterol and HDL cholesterol.

The content of total fats in the diet is known to influence the concentrations of FVII. In general, replacement of a fat-rich diet by a diet low in fat results in a reduction in FVIIc.[18] In our case both the CH diet and the MED diet reduced FVIIc levels in comparison with the SAT diet, although the lowest concentrations were seen with the MED diet. Several studies have investigated the effect of the different fatty acids. Some found no differences when comparing saturated fat with monounsaturated or polyunsaturated fats, either in FVIIc, or in FVIIAg.[19-21] The difference between our results and those of previous studies[21] may be because the previous studies analyzed hyperlipidemic patients and this metabolism disorder may cause the response to be different from that seen in our participants, who were all healthy and normolipidemic. To this extent, substitution of diets rich in saturated fatty acids for diets rich in monounsaturated fatty acids in young healthy persons reduces the postprandial activation of FVII.[22,23] Likewise, comparison of different oils showed a decrease in FVIIc after consumption of olive oil,[24] a fact which supports our results because the MED diet was especially rich in olive oil. Persons with hypertriglyceridemia who consume a diet rich in monounsaturated or n-3 polyunsaturated fatty acids experience a reduction in FVIIa in comparison with consumption of a low-fat, carbohydrate-rich diet,[25] which partly coincides with our results. Another study,[26] however, showed that three types of diets rich in saturated fatty acids resulted in a lesser increase in FVIIa than the diet rich in oleic acid. The discordance between the results of these different studies may be due to differences in study design or because other nutrients with an additional effect were supplied together with the monounsaturated fat.

Regarding the mechanism by which the MED diet reduced FVIIa, Hoffman et al[27] found a positive association between various vitamin K-dependent clotting factors and plasma concentrations of total cholesterol and LDL cholesterol, which would suggest a possible association between cholesterol metabolism and the vitamin K-dependent clotting factors produced in the liver.

Because the three diets were given sequentially not randomly, the possibility cannot be excluded of the presence of a time or lag phenomenon depending on the order of consumption of the diets. Nevertheless, exhaustive control of the protocol and strict periodic follow-up of the participants mean that the results of this study are unlikely to be due to this phenomenon.

In conclusion, we found that a group of healthy normolipidemic men who changed from a diet rich in saturated fatty acids to a diet rich in carbohydrates and especially to a Mediterranean type diet based on olive oil experienced a reduction in FVIIa. This factor is involved in the formation of thrombi on atheromatous plaques. Moreover, in persons with hypercholesterolemia, FVIIa is associated with different inflammatory parameters, such as C reactive protein and the interleukin-6 soluble receptor, which are related to cardiovascular events.[28] Given the involvement of FVIIa in the thrombogenic process and its association with inflammatory parameters, the reduction of this factor with a Mediterranean diet is one more argument in favor of this diet for cardiovascular protection.

ACKNOWLEDGEMENTS

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