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Mediterranean-Style Diet Effect on the Structural Properties of the Erythrocyte Cell Membrane of Hypertensive Patients

The Prevencion con Dieta Mediterranea Study

Francisca Barceló, Javier S. Perona, Jesús Prades, Sérgio S. Funari, Enrique Gomez-Gracia, Manuel Conde, Ramon Estruch, Valentina Ruiz-Gutiérrez

Abstract—A currently ongoing randomized trial has revealed that the Mediterranean diet, rich in virgin olive oil or nuts, reduces systolic blood pressure in high-risk cardiovascular patients. Here, we present a structural substudy to assess the effect of a Mediterranean-style diet supplemented with nuts or virgin olive oil on erythrocyte membrane properties in 36 hypertensive participants after 1 year of intervention. Erythrocyte membrane lipid composition, structural properties of reconstituted erythrocyte membranes, and serum concentrations of inflammatory markers are reported. After the intervention, the membrane cholesterol content decreased, whereas that of phospholipids increased in all of the dietary groups; the diminishing cholesterol:phospholipid ratio could be associated with an increase in the membrane fluidity. Moreover, reconstituted membranes from the nuts and virgin olive oil groups showed a higher propensity to form a nonlamellar inverted hexagonal phase structure that was related to an increase in phosphatidylethanolamine lipid class. These data suggest that the Mediterranean-style diet affects the lipid metabolism that is altered in hypertensive patients, influencing the structural membrane properties. The erythrocyte membrane modulation described provides insight in the structural bases underlying the beneficial effect of a Mediterranean-style diet in hypertensive subjects. **(*Hypertension*. 2009;54:1143-1150.)**

Key Words: Mediterranean diet ■ lipids ■ membrane structure ■ cardiovascular disease ■ hypertension

Cardiovascular disease constitutes the main cause of death in industrialized countries,[1] and hypertension is one of the main modifiable cardiovascular risk factors, especially in the elderly.[2] Healthy diet and lifestyle constitute the first steps in the guidelines for management of hypertension.[3] In this context, the type and amount of dietary lipids influence the lipid composition of cell membranes[4,5] and modulate the interactions with proteins involved in the regulation of blood pressure.[6] Thus, the changes in membrane properties induced by dietary lipids may have important consequences on the blood pressure regulation.

The Mediterranean-style diet (MD) is characterized by a high consumption of virgin olive oil (VOO) and nuts, which are rich natural sources of oleic (18:1; n-9) and α-linolenic (18:3; n-3) acids, respectively. The Prevencion con Dieta Mediterranea (PREDIMED) Study is a large-scale, randomized trial aimed at assessing the effects of a MD enriched with VOO or nuts on primary prevention of cardiovascular disease in patients at high risk for coronary heart disease. The results of the 3-month intervention on the first 772 patients entering the study showed that, compared with a low-fat diet, the MD rich in VOO or nuts reduced systolic blood pressure and serum total cholesterol and triglyceride concentrations and increased serum high-density lipoprotein cholesterol concentration.[7] Although there is evidence indicating that dietary lipids can have a positive effect on cardiovascular risk factors, the mechanisms and effects on the molecular and structural bases underlying the physiological process are largely unknown.

Several studies support the involvement of plasma membrane properties in the modulation of membrane protein activities and cell physiology. The structural properties and function of cell membranes appear to be modified in hypertensive humans and animal models of hypertension.[8–10] Changes in membrane lipid composition of hypertensive subjects have been associated with alterations in the transmembrane fluxes of Na^+ and K^+, including Na^+-Li^+ countertransport, which is a marker of essential hypertension,[10,11]

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and in cell signaling proteins that participate in the control of blood pressure.[12] On the other hand, it has been reported that dietary lipids have an effect on membrane lipid composition and cell signaling proteins.[4,5,12] Considering that changes in the dietary lipid composition yield to variations in the biophysical properties of the plasma membrane, it is likely that cellular functional changes could result from alterations in the structure of the lipid membrane properties influenced by the diet. Thus, the changes in membrane properties induced by dietary lipids may have important consequences on blood pressure regulation.

Dietary habit could play a role as an environmental factor, altering some targeted molecular functions in the cell and, through them, influencing cardiovascular risk factors. In fact, the MD has been associated with changes in membrane structure and function. Consumption of olive oil–rich diets increases the concentration of oleic acid in plasma membrane lipids of different rat and human cells, with beneficial consequences on membrane functionality.[13–16] In contrast, very little is currently known regarding the effects of nuts, another key ingredient of the MD, on membrane lipid composition and structure.

The present study was undertaken to examine the structural basis underlying the effect of the MD on the cardiovascular system, in parallel with the PREDIMED Study that is currently in progress.[7] With this aim, we conducted a structural substudy to assess the effect of the MD supplemented with nuts or VOO on the erythrocyte membrane properties in a group of participants recruited from the parent study after 1 year of intervention. This is the first time that membrane structural analyses are included in an intervention study with an MD.

Materials and Methods

Materials

HEPES was obtained from Sigma Chemical Co. Lipid standards, cholesterol, 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylethanolamine, 1,2-diacyl-*sn*-glycero-3-phosphocholine, 1,2-diacyl-*sn*-glycero-3-phospho-L-serine, *N*-acyl-4-sphingenyl-1-*O*-phosphorylcholine, and lysophosphatidylcholine were purchased from Sigma-Aldrich. Solvents used for lipid extraction and high-performance liquid chromatography–grade solvents were from Romil. The high-performance liquid chromatography column was purchased from Merck.

Methods

Study Design

The PREDIMED Study is a large, parallel-group, multicenter, randomized, controlled, 5-year trial,[7] for which the aim is to assess the effects of the MD on the primary prevention of cardiovascular disease (http://www.predimed.org). Nearly 7500 high-risk participants have been divided into 3 intervention groups, and each group receives a specific diet, one third an MD enriched with VOO, another third an MD enriched with mixed nuts, and the remaining third a low-fat diet. The present study reports the first-year effects of these dietary interventions on the structural membrane properties of the erythrocyte plasma membranes from 36 hypertensive subjects participating in the PREDIMED Study.

Subjects

The first 36 hypertensive participants entering in the PREDIMED Study from 2 nodes (Sevilla and Malaga) were divided into 3 groups and assigned to the following interventions: an MD enriched with VOO (MD+VOO group), an MD enriched with nuts (MD+nuts group), or a low-fat diet (LF group). Each group consisted of 12 subjects to ensure adequate sample size to conduct the X-ray diffraction study, as well as to obtain sufficient statistical significance. Blood pressure was measured, and blood samples were collected from all of the subjects before the dietary intervention (baseline) and after 1 year of intervention with the corresponding diet, as described in Estruch et al.[7] All of the protocols used in this study followed the principles of the Declaration of Helsinki and were approved by the Institutional Committee of Human Research (Hospital Universitario Virgen del Rocío, Sevilla, Spain). All of the procedures followed were in accordance with institutional guidelines, and the subjects gave their informed consent to participate in the study.

Dietary Intervention

Participants in the PREDIMED Study were given a written recommendation for a traditional MD and 3-month allotments of free VOO (1 L/wk) or mixed nuts (30 g/d, as 15.0 g of walnuts, 7.5 g of hazelnuts, and 7.5 g of almonds). A 137-item food-validated frequency questionnaire and a 14-item questionnaire, an extension of a questionnaire designed to assess the degree of adherence to the traditional MD, were used.[7] Please see the online Data Supplement (at http://hyper.ahajournals.org) for detailed information.

Serum Inflammatory Markers

Fasting blood samples were obtained at baseline and after subjects had received the dietary intervention for 1 year, kept at 4°C for during transportation from the hospital to the laboratory (<1 hour) and then stored at −80°C until required for biochemical analyses. Measurements of high-sensitivity C-reactive protein (CRP), interleukin (IL) 6, E-selectin, and P-selectin were taken. Serum concentrations of high-sensitivity CRP were analyzed by particle-enhanced immunonephelometry. Serum IL-6, E-selectin, and P-selectin were measured in duplicate using standard ELISA.

Erythrocyte Model Membrane Preparation

Erythrocyte membranes were isolated as described previously.[16] Briefly, blood samples were collected in heparinized tubes and centrifuged at 1750*g* and 4°C for 10 minutes. The erythrocyte pellets were washed twice with 110 mmol/L of $MgCl_2$. Erythrocyte membranes from the 12 participants of each group were mixed and used to reconstitute model membranes with a lipid composition representative of the 3 (MD+VOO, MD+nuts, and LF) groups of patients. Total lipids of each erythrocyte membrane group were extracted with chloroform:methanol (2:1, vol/vol), as described previously.[17] Multilamellar lipid vesicles, 15% (wt/wt) with total lipid extracts, were prepared in 10 mmol/L of HEPES, 100 mmol/L of NaCl, and 1 mmol/L of EDTA (pH 7.4; HEPES buffer).[12] Lipid mixtures were hydrated, thoroughly homogenized with a pestle-type minihomogenizer (Sigma), and vortexed until a homogeneous mixture was obtained. Then, the suspensions were submitted to 5 temperature cycles (heated up to 70°C and cooled down to 4°C). Samples for X-ray scattering experiments were stored at −80°C under argon and allowed to equilibrate at 4°C for 48 hours before measurements were taken.

Lipid Composition Analyses

Lipid and phospholipid classes were separated by high-performance liquid chromatography in a single chromatogram following a modification of the method by Perona et al.[18] Briefly, triplicates of the lipid extracts were dissolved in chloroform:methanol (2:1, vol/vol), passed through 0.2-μm filters, and subsequently analyzed by liquid chromatography (2695 Alliance, Waters Co.) using a LiChrospher column (250.0×4.6 mm, 5-μm particle size) and an evaporative light-scattering detector (Waters 2420, Waters Co). A ternary gradient of hexane, 2-propanol, and methanol was applied with a flow rate of 0.8 mL/min. Commercially purchased lipid standards were used to identify and quantify the lipid classes. The amounts of cholesterol and phospholipids were quantified using calibration curves from lipid standards. The quantification was based on regression analyses of curves with correlation coefficients >0.999.

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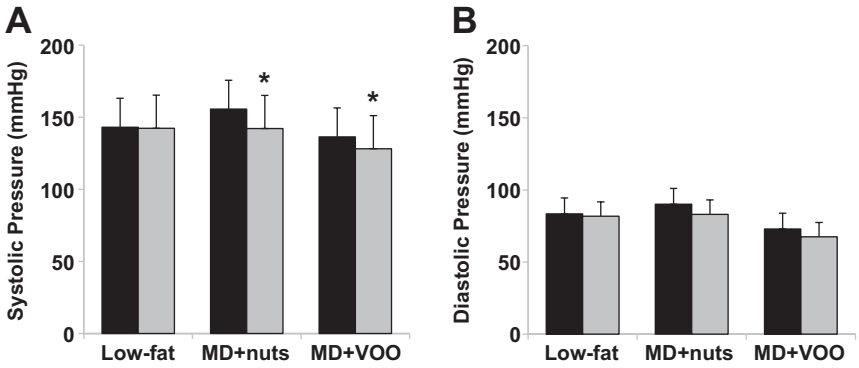

Figure 1. Systolic (A) and diastolic (B) blood pressures of hypertensive subjects assigned to an MD enriched with nuts or VOO or to a low-fat diet at baseline (■) and after a 1-year intervention (▨). *$P<0.05$ vs baseline.

Fatty acid methyl esters were analyzed by gas chromatography using a Hewlett-Packard 5890 series II gas chromatograph equipped with a flame ionization detector (Hewlett-Packard Co) and a Supelcowax 10 capillary silica column (60 m and 0.25 mm ID; Sulpelco Co). Fatty acid methyl esters were identified by comparison of their retention times against those of standards and quantified by internal standardization (tricosanoic methyl ester, 23:0) using peak area integration.[19]

X-Ray Diffraction Studies

Small- and wide-angle synchrotron radiation X-ray scattering analyses were conducted using standard procedures on the Soft Condensed Matter beamline A2 of Hamburger Synchrotronstrahlungslabor at the Deutsches Elektronen Synchrotron. The data collection conditions were as described previously.[12] Samples were heated from 10°C to 70°C. To work in quasiequilibrium conditions, the systems were allowed to equilibrate for 15 minutes at each temperature before measurements. Then, they were kept at the highest temperature for 15 minutes and finally cooled down to the lowest temperature at the same scan rate. Positions of the observed peaks were converted into distances, d, after calibration with the standards rat tendon tail and poly-(ethylene terephthalate) for the small- and wide-angle synchrotron radiation X-ray scattering analysis regions, respectively. Interplanar distances, d_{hkl}, were calculated according to the following equation:

$$(1) \qquad s = l/d_{hkl} = (2 \sin \theta)/\lambda,$$

where s is the scattering vector, 2θ is the scattering angle, λ (0.150 nm) is the X-ray wavelength, and h, k, and l are the Miller indices of the scattering planes.

Statistical Analysis

Data are reported as the mean±SD unless otherwise stated. Variables were examined for normality and skewness (Kolmogorov and Levene tests). We transformed values with a skewed distribution (CRP, IL-6, E-selectin, and P-selectin) to their natural logarithm for analyses. Differences within and between groups were analyzed using the 1-factor ANOVA analysis and the paired t test, when indicated. Values were considered significantly different when $P<0.05$. Analyses were performed using SPSS software version 14.0 (SPSS Inc).

Results

Systolic and Diastolic Pressures

Blood pressure was measured at baseline and after 1 year of intervention in all of the groups. There was a significant reduction of systolic blood pressure in both groups consuming the MD enriched with nuts or VOO ($P<0.05$). In contrast, no changes were found in those following the low-fat diet during the same period time (Figure 1). No changes were observed in weight, body mass index, and energy consumption in any of the experimental groups after the 1-year intervention period (data not shown).

Inflammatory Markers

Figure 2 shows the differences in serum concentrations of CRP, IL-6, E-selectin, and P-selectin at baseline and after 1 year of intervention in the 3 groups. Only the reduction observed after consumption of the MD enriched with VOO in the plasma concentration of CRP ($P<0.01$), IL-6 ($P<0.001$), E-selectin ($P<0.01$), and P-selectin ($P<0.05$) achieved statistical significance.

Erythrocyte Membrane Lipid Composition

The effect on the lipid and fatty acid compositions of erythrocyte membranes from hypertensive patients of an MD enriched in VOO or nuts or a low-fat diet was evaluated. The lipid composition of erythrocyte membranes was modified after 1-year dietary intervention in MD+nuts and MD+VOO groups (Table 1). Triglyceride concentrations were strikingly reduced in both groups, and cholesteryl esters were reduced only in the MD+nuts group. Conversely, the phospholipid concentration was increased, but the difference was only significant after following the MD+nuts diet or the low-fat diet. Significant differences were found in phospholipid classes in all of the groups studied (Table 2). Phosphatidylethanolamine (PE) concentration was increased in those consuming the MD enriched with nuts or VOO but was decreased in erythrocyte membranes of volunteers consuming the low-fat diet. Phosphatidylcholine was reduced in all of the groups after the dietary intervention but only significantly in the MD+nuts and LF groups. Sphingomyelin (SM) was only reduced in patients on the MD+VOO diet. Changes in the content of lysophosphatidylcholine were highly dependent on the diet and was unchanged in the MD+nuts diet, reduced in the MD+VOO diet, and greatly increased in the low-fat diet.

In the fatty acid analysis (Table 3), the concentration of stearic acid increased in the MD+nuts and MD+VOO groups, as did the concentration of palmitic acid in the MD+nuts group compared with the low-fat diet group. The concentration of oleic acid remained unchanged in all of the groups, but that of palmitoleic acid (16:1; n-7) was higher in the MD+nuts and MD+VOO groups and that of vaccenic acid (18:1; n-7) was higher in the MD+VOO group. Polyunsaturated fatty acids were only modified by the MD+nuts diet, by increasing the content of α-linolenic acid (18:3; n-3) and reducing that of

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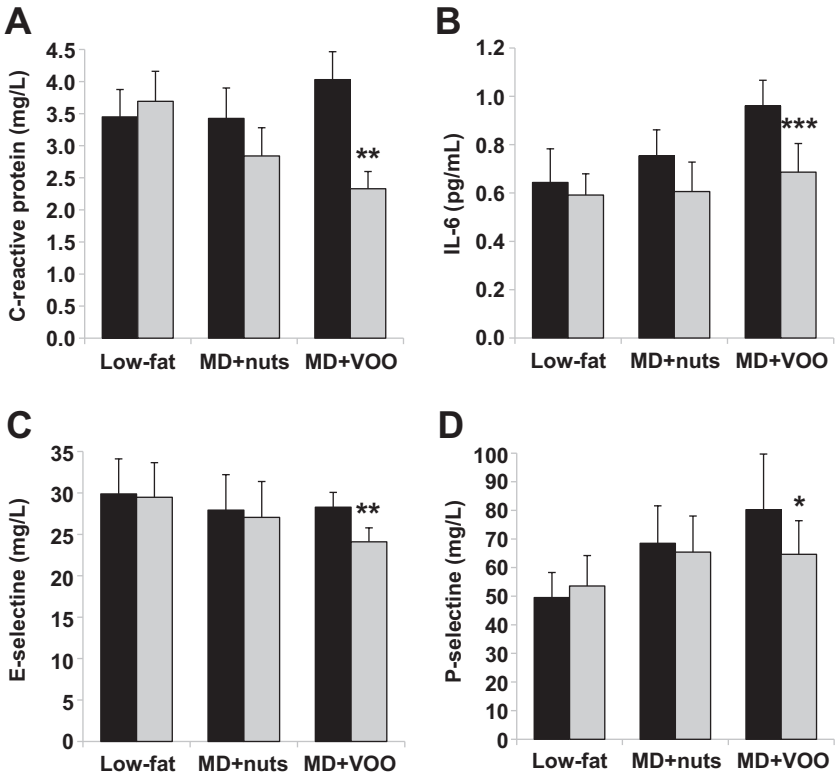

Figure 2. Serum concentrations of CRP (A), IL-6 (B), E-selectin (C), and P-selectin (D) of hypertensive subjects assigned to an MD enriched with nuts or VOO or to a low-fat diet at baseline (■) and after a 1-year intervention (▨). *$P<0.05$; **$P<0.01$; ***$P<0.001$ vs baseline.

linoleic and eicosadienoic (20:2; n-6) acids. In turn, no significant changes were observed in the fatty acid composition of the membranes corresponding with the low-fat diet.

Structural Influence on the Supramolecular Organization of Erythrocyte Model Membranes

For each group of patients (MD+VOO, MD+nuts, and LF), Multilamellar lipid vesicles were prepared with the total lipid extract from the mixture of the erythrocyte membranes from the 12 participants in the basal experiment and after the 1-year dietary intervention. The reconstituted model membranes, with a lipid composition representative of the erythrocyte membrane of the participants assigned to each group, were analyzed by X-ray diffraction (Figure 3 and Table 4). Membranes displayed clear X-ray diffraction patterns composed by a lamellar liquid crystalline phase (L_α), transforming into a nonlamellar inverted hexagonal phase (H_{II}) structure on heating. The thermotropic behavior depended on the membrane group. Control-model membrane samples obtained from the basal experiment (multilamelar lipid vesicles prepared with the erythrocyte membrane total lipid extract from hypertensive patients assigned to low-fat diet [VLF], multilamelar lipid vesicles prepared with the erythrocyte membrane total lipid extract from hypertensive patients assigned to MD+VOO diet, and multilamelar lipid vesicles prepared with the erythrocyte membrane total lipid extract from hypertensive patients assigned to MD+nuts diet [VDF]) showed a lamellar L_α phase with a repeat distance (d) in the range of 7.2 to 7.8 nm at 37°C that developed alone or in coexistence with an H_{II} phase with a $d=\approx 10.0$ nm at 50°C. Since the beginning, the H_{II} phase coexisted with the L_α phase in VLF membranes, and it appeared at ≈25°C or 50°C in VDF or VOO membranes. All of the samples exhibited reversible thermotropic behavior on cooling. We studied the effect of the specific diet by analyzing the model membranes samples (VLF1, VOO1, and VDF1) obtained at a 1-year term

Table 1. Lipid Composition of Erythrocyte Cell Membranes From Hypertensive Patients Assigned to an MD+Nuts, MD+VOO, or LF Diet at Baseline and After a 1-Year Intervention

	LF, mg/100 mg		MD+Nuts, mg/100 mg		MD+VOO, mg/100 mg	
Lipid Class	Baseline	Intervention	Baseline	Intervention	Baseline	Intervention
CE	1.33±0.80	1.18±0.36	3.50±0.73	0.55±0.57†	4.54±1.15	3.70±0.51
TG	0.77±0.52	0.60±0.29	1.12±0.05	0.25±0.12†	1.88±0.50	0.97±0.21†
C	20.22±2.40	16.39±1.10*	18.99±0.10	17.48±0.09*	35.20±5.38	33.13±4.92
PL	77.68±0.71	81.82±0.57*	76.39±1.88	81.73±0.59†	58.38±6.93	62.20±5.17

Data are mean±SD from 3 independent analyses, corresponding with a subgroup of subjects (n=12). CE indicates cholesterol esters; TG, triglycerides; C, cholesterol; PL, phospholipids.

*$P<0.01$ vs baseline.

†$P<0.001$ vs baseline.

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Table 2. Phospholipid Composition of Erythrocyte Cell Membranes From Hypertensive Patients Assigned to an MD+Nuts, MD+VOO, or LF Diet at Baseline and After a 1-Year Intervention

	LF, mg/100 mg		MD+Nuts, mg/100 mg		MD+VOO, mg/100 mg	
PL	Baseline	Intervention	Baseline	Intervention	Baseline	Intervention
PE	25.10±0.81	19.84±1.88‡	21.55±1.95	24.21±1.32†	25.05±3.26	28.35±2.72*
PS	2.44±1.86	3.19±0.67	1.83±0.93	3.54±1.65*	4.25±2.76	6.26±2.30
PC	44.73±1.73	41.13±1.25‡	46.53±1.73	43.11±1.34†	38.13±0.66	37.84±1.66
SM	24.68±1.73	27.25±2.46	28.43±1.77	27.58±2.78	28.74±1.06	24.46±3.23†
LPC	3.06±2.25	8.60±3.78*	1.66±0.23	1.57±0.13	3.83±0.93	3.09±0.21*

Data are mean±SD from 3 independent analyses, corresponding with a subgroup of subjects (n=12). PL indicates phospholipid; PS, phosphatidylserine; PC, phosphatidylcholine; LPC, lysophosphatidylcholine.

*$P<0.05$ vs baseline.

†$P<0.01$ vs baseline.

‡$P<0.001$ vs baseline.

and comparing the respective structural properties in each group. In the MD+nuts group, VDF1 membranes showed a decrease in the structural parameters of the L_α phase (d=7.7 and 7.1 nm at 37°C for VDF and VDF1, respectively) and an increase in the compressibility factor. From 20°C to 25°C and ≤70°C, the L_α phase developed in coexistence with an H_{II} phase (d=10.7 and 10.4 nm at 50°C for VDF and VDF1, respectively) for both groups. However, it is worth noting that VDF membranes displayed a more structured H_{II} phase. In the MD+VOO group, VVOO and VVOO1 membranes showed subtle differences in their thermotropic behavior. The L_α phase developed up to 42°C to 50°C as a sole phase (d=≈7.8 nm) with no compressibility factor and then went into an H_{II} phase with a different diameter (d=≈9.9 and 9.4 nm for VVOO and VVOO1 at 50°C). Indeed, VVOO1 presented a broad diffraction peak at 3.5 nm that developed up to 30°C and was identified as a cholesterol-rich domain organized in the bilayer in coexistence with the L_α phase. In the LF group, VLF and VLF1 (data not shown) membranes did not exhibit significant differences in their thermotropic behavior, and the phases shown coexisted during the temperature range studied with similar structural parameters (L_α phase with d=7.2 nm at 37°C and H_{II} phase with d=9.9 nm at 50°C).

Discussion

The PREDIMED Study is a large-scale, randomized trial designed to assess the effects of the MD enriched in VOO or nuts on cardiovascular outcomes in high-risk, coronary heart disease patients. The results of this study in the first 772 patients after a 3-month intervention showed that, compared with a low-fat diet, the MD rich in VOO or nuts reduced systolic blood pressure and serum total cholesterol and triglyceride concentrations and increased serum high-density lipoprotein cholesterol concentration.[7] In the current study, we have explored molecular and structural bases underlying

Table 3. Fatty Acid Composition of Erythrocyte Cell Membranes From Hypertensive Patients Assigned to an MD+Nuts, MD+VOO, or LF Diet at Baseline and After a 1-Year Intervention

	LF, mg/100 mg		MD+Nuts, mg/100 mg		MD+VOO, mg/100 mg	
Fatty Acid	Baseline	Intervention	Baseline	Intervention	Baseline	Intervention
16:0	20.60±0.74	21.12±1.05	20.18±1.37	23.15±1.64*	21.49±1.20	22.12±1.60
16:1; n-7	1.22±0.53	1.29±0.20	1.24±0.39	0.79±0.16*	4.07±1.47	1.22±0.45‡
18:0	12.02±1.33	11.04±1.77	13.13±3.54	18.10±4.50*	12.42±1.06	15.30±2.25*
18:1; n-9	21.82±3.36	23.50±5.29	21.88±2.05	20.59±2.34	21.28±2.91	21.68±1.84
18:1; n-7	1.51±0.06	1.67±0.18	1.50±0.22	1.35±0.17	1.66±0.18	1.37±0.14†
18:2; n-6	19.37±2.13	17.89±4.23	21.10±3.3	14.91±2.44†	16.15±0.94	15.63±2.35
18:3; n-3	0.77±0.07	0.71±0.10	0.72±0.23	1.32±0.33†	0.68±0.38	0.72±0.27
20:2; n-6	2.06±0.58	1.56±0.44	1.91±0.46	1.50±0.16*	1.62±0.25	1.41±0.27
20:4; n-6	12.13±1.01	11.77±1.21	11.50±2.47	11.12±2.39	11.94±1.33	11.40±1.94
20:5; n-3	0.77±0.23	1.17±0.48	0.93±0.28	1.06±0.32	0.69±0.21	1.28±0.72
22:4; n-6	1.32±0.56	1.60±0.22	ND	ND	2.09±0.56	1.61±0.65
22:5; n-3	1.63±0.83	1.33±0.03	1.26±0.43	1.50±0.45	1.51±0.35	1.32±0.31
22:6; n-3	4.77±1.48	5.35±0.33	4.64±0.81	4.61±1.17	4.40±0.91	4.93±0.68

Data are mean±SD from 3 independent analyses, corresponding with a subgroup of subjects (n=12). ND indicates not detected.

*$P<0.05$ vs baseline.

†$P<0.01$ vs baseline.

‡$P<0.001$ vs baseline.

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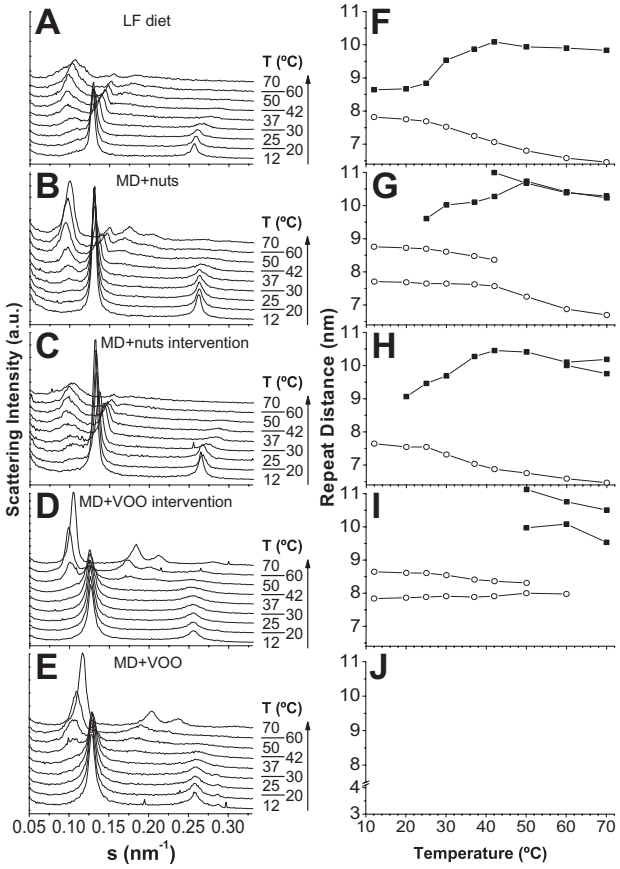

Figure 3. Left, X-ray diffraction sequence of the scattering patterns of model erythrocyte membranes reconstituted with the total lipid extract from the mixture of the erythrocyte membranes from the 12 hypertensive patients assigned to a low-fat diet (LF diet) or to an MD enriched with nuts (MD+nuts and MD+nuts intervention) or VOO (MD+VOO and MD+VOO intervention) at baseline (LF diet, MD+nuts, and MD+VOO) and after a 1-year intervention (MD+nuts intervention and MD+VOO intervention). The sequence of patterns was acquired in quasiequilibrium conditions after equilibrating the sample during 15 minutes at each temperature. Successive diffraction patterns were collected for 20 seconds. The L_α phase was identified by the 2 order reflection peaks on the small-angle synchrotron radiation X-ray scattering analysis and the absence of peaks on the wide-angle synchrotron radiation X-ray scattering analysis region. The H_{II} phase was identified by 3 to 4 higher-order reflection peaks, as indicated. Only the heating sequence is shown from 10°C to 70°C. Right, Dependence of the repeat distance with temperature for the reconstituted membranes. Phases represented are L_α (-○-) and H_{II} (-■-). The broad diffraction peak at 3.5 nm (-▲-) was identified as a cholesterol-rich domain organized in the bilayer. LF diet at a 1-year intervention (data not shown) was similar to the LF diet at baseline.

the effect of an MD supplemented with nuts or VOO and compared it with that of a low-fat diet.

The data presented clearly establish in vivo that dietary lipid management can modulate the structural properties of erythrocyte membranes in addition to a decrease in blood pressure. Membrane lipid composition and structural properties of reconstituted erythrocyte membranes were altered after a 1-year intervention by supplementing an MD diet with nuts or VOO as natural rich sources of α-linolenic or oleic acid, respectively. The main changes in lipid composition were observed in cholesterol and phospholipid concentrations.

Table 4. Structural Properties of Reconstituted Erythrocyte Membrane From Hypertensive Patients Assigned to an MD+Nuts (VDF and VDF1), VOO (VVOO and VVO01), or LF (VLF and VLF1) Diet at Baseline (VDF, VVOO, and VLF) and After a 1-Year Intervention (VDF1 and VVO01)

Sample	*$\Delta T_{L\alpha}$, °C	†$d_{L\alpha}$, nm	†d_{HII}, nm
VLF	10 to 10 (70)	7.25	9.94
VDF	10 to 25 (70)	7.62	10.73
VDF1	10 to 20 (70)	7.03	10.41
VVOO	10 to 50 (60)	7.88	9.97
VVO01	10 to 42 (50)	7.77	9.42

VVOO indicates multilamelar lipid vesicles prepared with the erythrocyte membrane total lipid extract from hypertensive patients assigned to MD+VOO diet.

VLF1 data (not shown) were similar to VLF data.

*Temperature range where the L_α phase was observed is shown in $\Delta T_{L\alpha}$. The parenthesis on the right indicates the temperature limit of the L_α phase in the $L_\alpha+H_{II}$ temperature range coexistence.

†Repeat distance, $d_{L\alpha}$, at 40°C and d_{HII} at 50°C.

Cholesterol content decreased, whereas that of phospholipids increased in all of the groups studied, although the difference was not significant in the MD+VOO group. Previous studies have shown that the hydrophobic core of erythrocyte membranes is less fluid in hypertensive rats and has a high index of a cholesterol:phospholipid ratio.[20] In addition, it has been reported that the increased cholesterol:phospholipid ratio in erythrocyte membranes of hypertensive patients is associated with alterations of the Na^+-Li^+ countertransport activity,[10,11] which can be normalized by short-term VOO intake.[21] In the present study, the changes in the lipid composition that result in a reduction of the cholesterol:phospholipid ratio could be associated with an increase in the membrane fluidity. Although fatty acid composition also contributes to the modulation of membrane fluidity,[22] only minor changes were found in the phospholipid fatty acid profile of the 3 groups of patients analyzed.

Despite the similar increase found in phospholipids in the 3 groups studied, not all of the lipid classes were modified to the same extent. For instance, PE was reduced after following the low-fat diet but increased after both MD diets supplemented with nuts or VOO. Low-membrane PE concentrations have been reported in spontaneously hypertensive rats compared with normotensive animals.[22,23] In this latter strain, VOO consumption led to increased PE content in membranes compared with high-oleic sunflower oil.[9] It is worth noting that, in contrast with VOO, high-oleic sunflower oil was unable to exhibit beneficial effects on blood pressure in hypertensive subjects.[16] Another interesting observation was that the low-fat and MD+VOO diets differed in their effects on SM and lysophosphatidylcholine concentrations in erythrocytes membranes. These two lipid classes were reduced in the group receiving VOO and increased in the group assigned to the low-fat diet. Previous studies suggest that SM content is also increased in spontaneously hypertensive rats.[24] Actually, a hallmark of sphingolipids is that they bring local order to fluid membranes[25] by forming structural microdomains (eg, "lipid rafts") enriched in cholesterol and SM with a high structural order. It is now becoming clear that these lipid

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microdomains play a role in the cell signaling.[26] Some proteins (eg, G proteins) that participate in cell signal transduction and are involved in the physiological process of the control of blood pressure have been associated with lipid rafts.[27] Interestingly, a growing body of data indicates that multiple signal transduction events in the heart occur via plasma membrane receptors located in signaling microdomains.[28]

Changes in the lipid composition because of the diet style were associated with subtle differences in the structural properties of the reconstituted membranes from erythrocytes. Reconstituted membranes from the MD+nuts and MD+VOO groups after the 1-year intervention showed a higher propensity to form nonlamellar H_{II} structures that correlated with an increase in PE lipid class observed in their respective lipid composition. In model membranes, an experimental correlation between an H_{II}-phase propensity and an increase in G-protein localization or protein kinase C activity has been shown,[29,30] demonstrating the influence of the membrane structure on cell signaling proteins that participate in the control of blood pressure. Thus, membrane structural changes induced by the MD diet style may have a cellular functional implication.

On the other hand, when serum inflammatory markers were analyzed, a reduction in CRP, IL-6, E-selectin, and P-selectin concentrations was observed after both MD interventions, although only the differences observed in the MD+VOO group achieved statistical significance. Leukocytes and thrombocytes have been causally related to atherogenesis and vascular thrombosis occlusion. However, more recently, an increased appreciation has been noticed for erythrocyte as a cell involved in atherosclerotic plaque destabilization.[31] Recently, Tziakas et al[32] have shown that IL-8 is increased in the membrane of circulating erythrocyte in patients with acute coronary syndrome. The results of our study also show a possible link between changes in erythrocyte membrane properties and serum inflammatory markers after an MD intervention, especially when this diet is supplemented with VOO.

Perspectives

Cardiovascular disease has a multifactorial etiology. Genetic and environmental factors apparently form the basis for structural membrane properties and function. Considering the in vivo approach of this study, the dietary fat management constitutes an external factor able to reduce the blood pressure and serum inflammatory markers and modulate the structural erythrocyte membrane properties. Adjustment in the lipid composition and structural properties of erythrocyte membranes attributed to MD diets supplemented with nuts or VOO is most probably related to changes in the physicochemical properties of the lipid microenvironment of membrane proteins. The complexity of biological membranes makes it difficult to assign specific changes in membrane structure to membrane-dependent functions (eg, the function of membrane proteins that participate in cell signaling). The alterations in the structural blood cell properties reported could reflect changes in other cell types related to the control of blood pressure and could account for the statistically significant reductions in blood pressure observed in those groups of participants in the PREDIMED Study.

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Disclosures

None.

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MEDITERRANEAN-STYLE DIET EFFECT ON THE STRUCTURAL PROPERTIES OF ERYTHROCYTE CELL MEMBRANE OF HYPERTENSIVE PATIENTS: THE PREDIMED STUDY

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Expanded Materials and Methods

Dietary Intervention

The baseline examination included assessment of standard cardiovascular disease factors, medications and socio-demographic factors. A 137-item food validated frequency questionnaire and a 14-item questionnaire, an extension of a questionnaire designed to assess the degree of adherence to the traditional MD was used (1). On the basis of the baseline 14-item questionnaire each participant was given personalized dietary advice by a dietitian during a 30-minute session. Participants allocated to a low-fat diet were advised to reduce all types of fat and were given written recommendations according to the American Heart Association guidelines. Participants in the MD groups received instructions directed to upscale the 14-item score, including the use of VOO for cooking and dressing, increased consumption of vegetables, nuts, and fish products, consumption of white meat instead of red or processed meat, preparation of home-made sauce by simmering tomato, garlic, onion, and aromatic herbs with VOO to dress vegetables pasta, rice, and other dishes and for alcohol drinkers, to follow a moderate pattern of red wine consumption. No energy restrictions were suggested for any intervention group. Participants in the MD groups were given 3-month allotments of free VOO (1 L/week) or mixed nuts (30 g/day, as 15 g walnuts, 7.5 g hazelnuts and 7.5 g almonds). All participants had free access to their dietitian throughout the study. The fatty acid and minor components composition of the VOO and nuts employed in the study was published elsewhere (1). Biological assessment of the intervention compliance was performed by measuring tyrosol and hydroxytyrosol levels in urine by GC-MS to assess the compliance of the MD rich in VOO group and ♋-linolenic (18:3, n-3) acid in serum by GC as a biomarker of compliance of the MD rich in nuts (1).

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