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LIPID ABNORMALITIES IN CHRONIC RENAL FAILURE PATIENTS UNDERGOING HEMODIALYSIS

NELVA T. de GOMEZ DUMM[1], **ANA M. GIAMMONA**[2], **LUIS A. TOUCEDA**[2], **CLEMENTE RAIMONDI**[2]

[1]*Instituto de Investigaciones Bioquímicas de La Plata (INIBIOLP-CONICET-UNLP);* [2]*Laboratorio de Trasplante de Organos, Facultad de Ciencias Médicas, Universidad Nacional de La Plata*

Abstract Lipid abnormalities are common in patients with renal disease, probably contributing to the high incidence of cardiovascular diseases in this population. In this study we determined the plasma and erythrocyte lipid profile in patients with chronic renal failure (CRF) along 30 months under hemodialysis. In the same patients the influence of cuprophane and polysulphone dialysis membranes on the fatty acid pattern of plasma and erythrocytes, before and after dialysis, was also studied. Fluidity in erythrocyte membranes was also assessed by diphenylhexatriene (DPH) fluorescence polarization measurements. Triglyceride levels were increased in the plasma and in erythrocyte membranes of CRF patients compared to healthy subjects. Plasma polyunsaturared fatty acids decreased whereas palmitic and monounsaturated acids increased in CRF patients. No changes were observed in either the fatty acid profile or DPH fluorescence anisotropy of erythrocyte membranes. The lipid composition abnormalities persisted after 18 months and they became more notorious after 30 months. Neither the plasma nor the erythrocyte membrane lipid pattern changed in CRF patients during the dialysis session, regardless of the dialysis membrane used. We conclude that CRF patients under regular hemodialysis evidence a gradual deteriorarion in the fatty acid and triglyceride abnormalities, a finding that might be relevant to the risk of cardiovascular disease in this setting.

Key words: chronic renal failure, dialysis membranes, erythrocytes, hemodialysis, polyunsaturated fatty acids, triglycerides

Resumen ***Trastornos lipídicos en pacientes con insuficiencia renal crónica en hemodiálisis.*** Los trastornos lipídicos observados en pacientes con enfermedad renal probablemente constribuyen al elevado riesgo de enfermedades cardiovasculares que este grupo presenta. En este estudio se determinó el perfil lipídico del plasma y de los eritrocitos en pacientes con insuficiencia renal crónica (IRC) en hemodiálisis regular durante 30 meses. En estos mismos pacientes se estudió también la influencia de membranas de diálisis de cuprofan y polisulfona sobre el perfil de los ácidos grasos del plasma y de los eritrocitos, antes y después de la diálisis. La fluidez de la membrana de los eritrocitos se determinó mediante anisotropía de fluorescencia del difenilhexatrieno. El tenor de triglicéridos del plasma y de las membranas de eritrocitos aumentó en los pacientes con IRC comparados con individuos controles sanos. En esos mismos pacientes disminuyeron los ácidos grasos polinosaturados y aumentaron el ácido palmítico y los ácidos monoinsaturados. No se observaron cambios ni en el perfil de los ácidos grasos ni en la anisotropía de fluorescencia de las membranas de eritrocitos. Las variaciones lipídicas observadas persistieron después de 18 meses de hemodiálisis y se acentuaron al cabo de 30 meses. El perfil lipídico del plasma y de las membranas de eritrocitos de los pacientes con IRC no se modificó como consecuencia de la sesión de diálisis, independientemente del tipo de membrana de diálisis utilizada. Concluimos que los pacientes con IRC en hemodiálisis regular manifiestan un deterioro gradual en la composición de ácidos grasos y de triglicéridos, que podría contribuir a incrementar la morbi-mortalidad cardiovascular.

Palabras clave: insuficiencia renal crónica, membranas de diálisis, eritrocitos, hemodiálisis, acidos grasos polinosaturados, triglicéridos

Patients with severe chronic renal failure (CRF) as well as with the uremic syndrome, and even those treated with hemodialysis, are characterized by a number of biochemical abnormalities including hyperlipidemia. The importance of cardiovascular illnesses as the cause of death in patients with CRF and in patients on dialysis makes it imperative to consider the risk factors involved. Hyperlipidemia has been incriminated as a risk factor of atherosclerotic vascular disease in dialysed patients[1]. Plasma triglycerides, but not cholesterol, are increased in most patients with advanced renal failure. Castelli et

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Postal address: Dr. Nelva T. de Gómez Dumm, Facultad de Ciencias Médicas, Universidad Nacional de La Plata, calles 60 y 120, La Plata. Argentina.
Fax: (54-221) 4258988 e-mail: tacconi@atlas.med.unlp.edu.ar

al.[2] established that a high triglyceride concentration predicts coronary heart disease independently from other known factors. Furthermore, Hahn et al.[3] have found that dialysed patients with cardio vascular disease have a 50 % increase in the triglyceride levels in comparison with unaffected individuals on dialysis.

Essential fatty acids (EFA) are specific polyunsaturated fatty acids (PUFA) for which all animals have an obligatory dietary requirement[4]. EFA play two important roles in the regulation of cell function. The first is structural: phospholipids incorporated into membranes are able to regulate its physico-chemical properties, which in turn influence the conformation, mobility and function of a wide variety of intrinsic and extrinsic membrane-bound proteins[5]. Secondly, EFA of the n-6 and n-3 families, when released from their membrane phospholipid reservoirs become eicosanoids, which are important as autacoids and second messengers in signal transduction of endocrine and other kind of stimuli[6]. EFA deficiency results in biochemical disorders involved in the aetiology of coronary vascular disease[7].

Patients with CRF exhibit plasma fatty acid patterns which are indicative of EFA deficiency. Thus, plasma PUFA are decreased and saturated fatty acid increased[8, 9]. The same changes occur in the lipid bilayer of red blood cell membranes altering their fluidity[8].

The aim of this study was to determine the plasma and erythrocyte lipid profiles at different points in time in CRF patients undergoing regular hemodialysis. The influence of two different hemodialysis membranes, cuprophane or polysulphone, on plasma and red blood cell fatty acid composition was also investigated.

Materials and Methods

Patients: Ten chronic renal failure patients (aged 33.3 ± 3.0 years, both sexes: 6 men, 4 women, body mass index 23.19 ± 4.45) undergoing maintenance hemodialysis were studied. The underlying renal diseases were chronic glomerulonephritis in 6 cases and was unknown in 4 cases. Patients were dialysed with volumetric dialyser machines, bicarbonate buffer based dialysate, blood flow 250 ml/min, dialysate flow 500 ml/min, ktv 1.34 ± 0.12. All patients had been dialysed three times a week for an average of 5 years (range 4-7), each session lasting 4 hours. Cuprophane hollow fiber dialysers (1.7 m^2) and polysuphone hollow fibers dialysers (1.8 m^2), were used.

After 12 hours fasting, blood was drawn from the arteriovenous fistula before the dialysis session. In order to study the effect of time on dialysis on the research variables blood samples were obtained at the beginning and after 18 and 30 months. The influence of cuprophane or polysulphone dialysis membranes on plasma and erythrocyte lipid profiles was studied in 5 uremic patients. Each patient was dialyzed for 6 sessions using the same cuprophane membrane. On the 7th session blood samples were collected at the beginning and at the end of the hemodialysis session. The same procedure was followed with the same patients using a polysulphone membrane.

The control group consisted of 10 age-matched healthy volunteers (4 men, 6 women) who had no history of hematological or renal diseases. In this case, blood samples were drawn from the cubital vein after 12 hours fasting.

Isolation of erythocyte membranes: Blood was collected in test tubes containing an anticoagulant EDTA solution (Wiener Lab., Rosario, Argentina). Whole blood was centrifuged, the plasma was immediately separated and the packed red blood cells were washed four times at 4°C with a buffered solution containing NaCl (140 mM), KCl (5 mM), NaHSO4 (1 mM), Tris buffer (10 mM), pH 7.4. After agitation they were kept at 4°C for 10 min, and centrifuged at 16000 g for 15 min. This last procedure was done twice, leaving a substantially hemoglobin-free pellet of erythrocyte membranes, which was resuspended in a small amount of supernatant and stored (-70°C) until assayed.

Chemical determinations: Plasma cholesterol and triglycerides were determined using commercially-available enzymatic methods (Wiener Lab., Rosario, Argentina). High-density-lipoprotein (HDL) cholesterol was also analyzed enzymatically after precipitation of very-low-density and low-density lipoproteins (LDL) with magnesium-dextran (Wiener Lab., Rosario, Argentina).

Lipid extraction and analysis: Lipids from the plasma and the erythrocyte membranes were extracted with chloroform-methanol (2:1 v/v). An aliquot from the organic phase was methylated and analyzed using a Hewlett-Packard Model 5840-A gas-liquid chromatograph equipped with a flame-ionization detector. Another aliquot from the organic phase was separated to determine phospholipid and neutral lipid content through the flame ionization detector (FID) of an Iatroscan apparatus model TH 10. Lipids were separated on previously activated chromarods type S-III under a double-development system. The first mobile phase was hexane-benzene (70:30 v/v) whereas the second was benzene-chloroform-formic acid (70:25:2 v/v/v). Lipidic species were quantified by comparison with known amounts of pure standards run under the same conditions. The signals from the FID were registered on a Hewlett-Packard model HP-3396 A integrator.

Fluorescence anisotropy measurements: Steady-state fluorescence anisotropy (rs) was measured in erythrocyte membranes in a SLM 4800 C spectrofluorometer as previously described by Garda et al.[10]. The probe used was 1,6-diphenyl-1,3,5 hexatriene (DPH). Excitation wavelength was 360 nm and the emitted light was passed through a sharp cut-off filter (Schott KV 389). Light scattering of blanks represented less than 5% and fluorescence values were corrected accordingly. The phospholipid:probe ratio was maintained at more than 200:1 (mol:mol) in order to minimize possible probe-probe interactions.

Statistical analyses: Results were tested statistically using either the Student t-test compared to the respective control or the one-way analysis of variance (ANOVA) as appropriate.

Results

Plasma cholesterol and triglyceride levels: Figure 1 shows the values for plasma triglycerides in patients at the beginning of the study and at 18 and 30 months. At the beginning of the study, triglyceride values were significantly higher in CRF patients than in controls. After 30 months triglyceride values increased even further and became significant compared to the baseline and to those at 18 months.

Total cholesterol as well as HDL-cholesterol and LDL-cholesterol levels were similar to those of healthy controls and no significant changes were observed over time (data

not shown). Control values were: (average ± SEM) 3.8 ± 0.3, 0.7 ± 0.04 and 2.5 ± 0.3 mmol/L, respectively.

Fatty acid profile of plasma and erythrocyte membranes: The fatty acid composition of plasma is shown in Fig. 2. One of the most important differences observed in CRF patients was the depression in the relative percentages of various polyunsaturated fatty acids of n-6 (linoleic, eicosatrienoic, arachidonic and docosapentaenoic acids) and n-3 (docosahexaenoic acid) series compared to the controls. This behavior persisted after 18 and 30 months of dialysis. Moreover, the statistical significance in some fatty acids increased, whereas in some others it became significant along with time. An increment in the relative percentages of monounsaturated fatty acids was also observed in the study population at baseline, at 18 and 30 months. When compared to healthy controls (average ± SEM: 1.67 ± 0.06), the unsaturation index (calculated as $\Sigma\ n_1x_1/FA$, n_1=number of double bonds in each fatty acid, x_1=moles of each fatty acid, FA=total moles of fatty acids) was significantly decreased in CRF patients (1.40 ± 0.02, P<0.001), and it decreased even further after 30 months (1.35 ± 0.02) (P<0.02 compared to CRF patients after 18 months). A similar behavior was observed when the PUFA/saturated fatty acid ratio was calculated in controls and CRF patients both at baseline and after 30 months (average ± SEM: 2.01 ± 0.1, 1.54 ± 0.1, and 1.34 ± 0.1, respectively) (P<0.01 and P<0.001 compared to the controls).

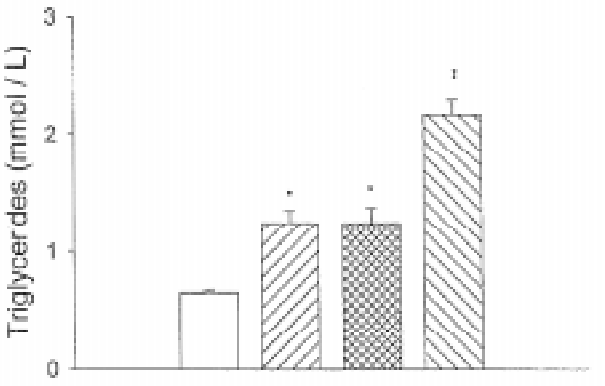

Fig. 1.– Evolution of plasma triglyceride concentration in patients with CRF under regular hemodialysis. Symbols are: healthy controls, CRF at the beginning of the study, CRF after 18 months, CRF after 30 months. Data are the mean ± SEM. Statistical significance at *P<0.001 compared to controls and **P<0.001 compared to CRF patients.

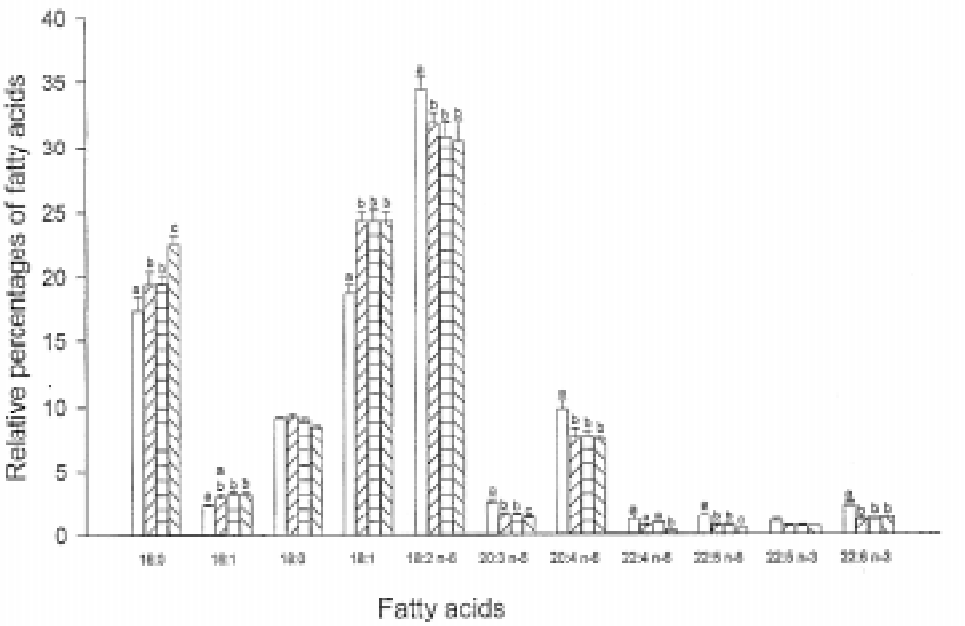

Fig. 2.– Relative percentages of total plasma fatty acids from CRF patients under regular hemodialysis. Symbols are: healthy controls, CRF at the beginning of the study, CRF after 18 months, CRF after 30 months. Fatty acids are identified by: number of carbon atoms in the chain is given first, value following the colon represents number of double bonds (cero means saturated fatty acid); number following n- indicates the position of the last double bond counting the double bond from the terminal methyl group. Data are the mean ± SEM. Values not bearing the same superscript letter are significantly different at P<0.05 or less.

TABLE 1.– *Effect of time on hemodialysis on the lipid composition (mol %) in erythrocyte membranes*

Lipid	Control	Patients on hemodialysis	Patients on hemodialysis after 18 months	Patients on hemodialysis after 30 months
Phospholipids	67.5 ± 0.7	66.0 ± 1.3	67.3 ± 1.4	65.8 ± 0.9
Cholesterol	32.2 ± 0.8	33.3 ± 1.4	31.8 ± 1.3	33.2 ± 0.9
Triglyceride	0.2 ± 0.03	0.4 ± 0.09*	0.5 ± 0.1**	0.6 ± 0.01 t
Free fatty acids	0.1 ± 0.02	0.3 ± 0.05**	0.4 ± 0.04 t	0.4 ± 0.03 t

Data are the mean of 10 determinations ± SEM. Significant difference from control values at * *P<0.05,* ** *P<0.01,* t *P<0.001.*

Relative percentages of fatty acids in red blood cell membranes of patients with CRF, either at the beginning or at the end of the study, were not different from controls (results not shown). Control data were similar to those reported by other authors[11].

Lipid species and steady-state fluorescence anysotropy of DPH in erythrocyte membranes: The amount of different neutral and polar lipids is shown in Table 1. No changes in the relative percentages of phospholipids and cholesterol were observed among the different groups. Triglyceride and free fatty acid distribution in the red blood cells from uremic patients was significantly higher than that in controls, with the values increasing during maintenance hemodialysis.

The mean steady-state fluorescence polarization values of DPH measured in erythrocyte membranes from controls were not different to those obtained in the same membranes of CRF patients, either at the beginning of the study or after 18 months. However, at the end of the study (30 months) a significant increase in rs ($P<0.001$) compared to the previous data (average ± SEM: 0.223 ± 0.003, 0.228 ± 0.002, 0.228 ± 0.005 and 0.235 ± 0.001 from control, CRF patients and CRF patients after 18 and 30 months after baseline, respectively) was observed.

Fatty acid profile of plasma and erythrocyte membranes from CRF patients before and after the dialysis session with cuprophane and polysulphone membranes: Minor changes were observed in the fatty acid pattern of plasma and red blood cells from CRF patients; the samples were compared before and after the dialysis session, regardless of the type of membrane used (data not shown).

Erythrocyte membrane fluorescence anisotropy was unaffected either by cuprophane or by polysulphone membranes during the dialysis session; therefore the results were not shown.

Discussion

Hypertriglyceridemia is the most common plasma lipid abnormality in patients with renal failure, coexisting with cholesterol levels within the normal range. At the beginning of the present study, the hypertriglyceridemia was mild and similar to the one found by Attman and Alaupovic[12] in patients with moderate CRF. In the present study triglyceride levels were also elevated in erythrocyte membranes from CRF patients. At 30 months after baseline determinations, these changes were more pronounced and probably responsible for the increase in fluorescence anisotrophy of DPH, and hence for a decrease in membrane fluidity.

Our study confirms previous findings on lipid disturbances in the plasma fatty acid profile from CRF patients on hemodialysis[8,9,13]. The abnormalities observed in these patients at the beginning persisted and became more marked at the end of the study. Nevertheless, the significant decrease in the unsaturation index observed in plasmatic fatty acids was not evident in the erythrocyte membranes from the same patients as published by other authors[8].

Our findings did not depend on the dialysis membrane used. These results differ from those published by Tanaka et al.[14] who observed a decrease in total cholesterol and in HDL-cholesterol and LDL-cholesterol in patients dialysed using polysulphone or cellulose membranes. The conditions of this study were different from the present report, since the patients were dialysed for 3 months after the blood collection, and additional patients under hemodialysis were selected as controls. We consider our results more valid because we measured the lipid profile at the beginning and at the end of a dialysis session, after the patients had been on dialysis for two weeks with the same membrane. Furthermore, the same patient was studied using both types of membrane. Peuchant et

al.[15] reported a significant increase in cholesterol content, as well as a decrease in the relative percentage of saturated fatty acids, together with an enhancement of PUFA in red blood cells from uremic patients after a dialysis session. This study differs from our observations and also from those described by other authors[8]. The differences might account for the fact that in Peuchant 's work cholesterol levels were higher than in healthy controls before dialysis, while in most of the patients with advanced renal failure cholesterol levels did not change. On the other hand, in the former study the fatty acid composition was not measured in the total lipid of the membrane but in the phospholipid fraction. House et al.[13] found the same amount of plasma triglyceride, cholesterol and lipoproteins in patients hemodialysed with high flux polysulphone membranes for 4 months, when compared to those receiving low flux.

Our results indicate that patients in CRF under regular hemodialysis show important abnormalities of lipid metabolism. The progressive elevation of both triglyceride and fatty acid plasma levels, as well as an increase in erythrocyte membrane triglyceride content, could be relevant to the development of cardiovascular diseases in this population.

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"*The fact is, you have fallen lately, Cecily, into a bad habit of thinking for yourself. You should give it up. It is not quite womanly... Men don't like it*"

"El hecho es, Cecilia, que últimamente has adquirido la mala costumbre de pensar por tí misma. Tendrías que abandonar esta costumbre. No es realmente de mujer... A los hombres no les gusta".

Oscar Wilde (1854-1900)

The Importance of Being Ernest