Research paper

Enhanced oxidative stress with a gradient between plasma and muscle interstitial fluid in patients with end stage renal failure on hemodialysis

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ABSTRACT

OBJECTIVE: The concentration of 8-iso-prostaglandin-$F_{2\alpha}$ (8-iso-$PGF_{2\alpha}$) in biological fluids has been considered as the most reliable biochemical index of the lipid peroxidation and oxidative stress in patients with several pathological conditions including end stage renal failure. However, there is no reference regarding the influence of Hemodialysis (HD) on the values of 8-iso-$PGF_{2\alpha}$ in the muscle Interstitial Fluid (IF) of patients with end stage renal failure. The aim of our study was to determine 8-iso-$PGF_{2\alpha}$ concentration in the IF during hemodialysis and the gradient between plasma and IF in patients with end stage renal failure. DESIGN: In this study, two microdialysis probes were inserted into the vastus lateralis muscle of the right leg of six male patients with end stage renal failure who were on hemodialysis, and in six healthy males (controls). The samples of IF (12 dialysate fluids) were collected after an equilibration of 30 min: a) during the 1st hour preceding hemodialysis (group CRF_0), b) during the 1st, 2nd, 3rd and 4th hour while on hemodialysis (groups CRF_1, CRF_2, CRF_3 and CRF_4) and c) during the 1st hour following hemodialysis (group CRF_5). At the end of the above periods and simultaneously, blood samples were drawn from the arteriovenous fistula. In the controls, the IF samples (twelve dialysate fluids) were collected during a period of one hour and the blood samples at the end of this period. The levels of 8-iso-$PGF_{2\alpha}$ were measured with an enzyme-immunoassay method. Statistical evaluation was carried out with the statistical program NCSS 2000 and the ANOVA test. RESULTS: Plasma and IF levels of 8-iso-$PGF_{2\alpha}$ in the patients were significantly higher than in controls at base line. During hemodialysis, the 8-iso-$PGF_{2\alpha}$ rose progressively both in plasma and IF but remained higher in plasma than in IF. CONCLUSIONS: Lipid peroxidation is higher in patients on hemodialysis than in controls but it is lower in the IF compared to plasma. The mechanism for this gradient is speculative.

Key words: Hemodialysis, Lipid peroxidation, Microdialysis, Oxidative stress 8-iso-$PGF_{2\alpha}$, Renal failure, 15-F_{2t}-isoprostane

INTRODUCTION

8-iso-prostaglandin-$F_{2\alpha}$ (8-iso-$PGF_{2\alpha}$) is a characteristic F_2-isoprostane, which is produced non-enzymatically in human cells via a free radical-catalysed lipid peroxidation mechanism of arachidonic acid, independent of the cyclooxygenase pathway. 8-iso-prostaglandin-$F_{2\alpha}$ or 8-iso-$PGF_{2\alpha}$ is the old nomenclature, while the new one proposed by Taber is 15-F_{2t}-isoprostane or 15-F_{2t}-IsoP. In this paper, however, the old nomenclature will be used.

There are 64 diastereoisomers isoprostanes but 8-iso-$PGF_{2\alpha}$ is the most biologically active. It is present in humans in two distinct forms: esterified in LDL phospholipids and as free acid. The ratio of these two forms is approximately 2:1. 8-iso-$PGF_{2\alpha}$ has been shown to be a potent vasoconstrictor and a potent mediator of hepatorenal syndrome and atherosclerosis. It has been also postulated to act as a pathophysiological mediator, able to modify the fluidity and integrity of cell membranes.[1-4]

The plasma levels of 8-iso-$PGF_{2\alpha}$ have been considered the most reliable biochemical index of lipid peroxidation and oxidative stress level in patients with several pathological conditions, including end stage renal failure.[5-12] However, there is no reference in the literature regarding the effect of hemodialysis on the production and accumulation of 8-iso-$PGF_{2\alpha}$ in the Interstitial muscle's Fluid (IF) of hemodialysed patients, nor are there data on the simultaneous changes of the levels of 8-iso-$PGF_{2\alpha}$ in the plasma and IF during hemodialysis.

During the last ten years, a microdialysis technique has been applied in different tissues such as the brain, the bones, the connective tissue, the adipose tissue and the skeletal muscles.[13-16] This technique is based on the principle of diffusion of substances with molecular weight below 20,000 Da (like 8-iso-$PGF_{2\alpha}$) through a semi-permeable membrane (microdialysis probe). The dialysate fluid contains no enzymes or proteins, hence this technique allows the isolation, purification and quantitative measurements of these compounds in the interstitial space of various tissues.[17,18]

The eicosanoid hormones (derivatives of arachidonic acid, prostaglandins, prostacyclins, thromboxanes and F_2-isoprostanes) have low molecular weight (<5.000 Da) and can thus be isolated from the IF of the muscle with the use of this microdialysis technique. After the isolation of these locally produced hormones, it is easy to measure their concentration in the IF via a sensitive immunoassay method such as Radioimmunoassay (RIA) or Enzyme Immunoassay (EIA).[17-19]

In a previous study using the microdialysis technique, we demonstrated the existence of 8-iso-$PGF_{2\alpha}$ in the IF of the skeletal muscles of healthy individuals.[18]

In the present study, we determined the values of 8-iso-$PGF_{2\alpha}$ as an index of lipid peroxidation in plasma and IF thoughout hemodialysis, also looking for a possible gradient between plasma and IF in patients with end stage renal failure.

SUBJECTS AND METHODOLOGY

The study included two groups: a) six stabilized male patients with end stage renal failure who were on hemodialysis (non smokers, aged 49 to 55 years) and b) six healthy individuals, non smokers, of the same age range, who served as controls (Table 1). All patients had well-functioning forearm arteriovenous fistula. The respective diagnoses were diabetic nephropathy, nephrosclerosis and polycystic kidney disease; their time on dialysis ranged from 15 to 35 months (Table 1). The filters used were fresinius polysulfone capillary dialysis with 1.6 m^2 effective surface area.

Two microdialysis probes (length 30 mm, inner diameter 0.52 mm, permeable by substances with a molecular mass less than 20,000 Da) were inserted with a CMA 60 microdialysis catheter (CMA medical, Stockholm, Sweden) into the vastus lateralis of the quadriceps femoris muscle group of the right leg. Before the insertion of the catheters, local anesthesia was achieved with 1 ml of lidocoine (10 mg/ml) administered above the muscle facia. After insertion, the microdialysis probes were attached to a perfusion pump (CMA model 102) and perfused at a rate of 0.3 μl/min with ringer acetate solution. The samples (12 dialysate fluids) were collected after an equilibration of 30 min: a) during one hour preceding hemodialysis (group CRF_0), b) during the 1st, 2nd, 3rd

Table 1. Descriptive data of the patients (age, gender, cause of hemodialysis (HD), time on HD, smoking).

Patients	Age (years)	Gender	Cause of HD	Time on HD (months)	Smoking
1.	49	♂	Diabetic nephropathy	19	NO
2.	51	♂	nephrosclerosis	15	NO
3.	55	♂	Diabetic nephropathy	28	NO
4.	53	♂	nephrosclerosis	22	NO
5.	52	♂	Polycystic kidney disease	25	NO
6.	54	♂	Diabetic nephropathy	35	NO
mean±SD	52.33±2.16			24.00±4.62	

and 4th hour while on hemodialysis (groups CRF_1, CRF_2, CRF_3 and CRF_4) and c) during the 1st hour following hemodialysis (group CRF_5).

As the volume in the sampling system from the distal end of the catheter to the microvials was 2.9 μl, the time required for the microdialysate to reach the microvials from the interstitial tissue was approximately 10 min. This sampling delay was taken into account when the dialysate fluids were collected. In order to eliminate the effect of local inflammation and tissue disturbances, the catheters were inserted 30 min before the collection of the dialysate fluid began (before hemodialysis). In order to avoid bleeding complications due to heparinization, the catheter was inserted 90 min before the beginning of hemodialysis.

A blood sample was drawn at the end of each IF sampling from the arteriovenous fistula (in sealed ice-cold microcentrifuge tubes) using EDTA (3 mg/ml) as anticoagulant and indomethacin (10 μg/ml) as prostaglandin synthetase inhibitor. In the controls, the samples (12 dialysate fluids) were collected during a period of one hour and the blood samples at the end of this period. After the centrifugation (at $4°$ C) of the blood samples at about 1500 g, plasma samples and dialysate fluid samples were stored at $-70°$ C until determination of 8-iso-$PGF_{2α}$ (about a month). In order to measure the levels of 8-iso-$PGF_{2α}$ in the interstitial fluid of the vastus lateralis muscle and in the plasma samples after purification in a C-18 SPE cartridge (Catalog No. 400020), we employed a sensitive and reliable enzymo-immunoassay method using kits of Cayman chemical company www.caymanchem.com (catalogue No 516351). The above method can

measure the free (non-esterified) or the total (free and esterified) 8-iso-$PGF_{2α}$. In this study the free form was measured.[18]

The performance characteristics of this immunoassay method were as follows:

a) Precision: The intra and inter assay coefficient of variation (CV%) was 5.1% and 7.3%, respectively.

b) Sensitivity: The sensitivity of the assay was 5 pg/ml.

c) Specificity: The 8-iso-$PGF_{2α}$ antiserum used in this assay, binds to: 8-iso-$PGF_{2α}$ 100.00%, 8-iso-$PGF_{3α}$ 20.55%, 2.3-dinor-8-iso-$PGF_{2α}$=4.0% 8-iso-PGE_2 1.84%, 2,3-dinor-8-iso-$PGF_{1α}$ 1.7%, 8-iso-PGE_1 1.56%, $PGF_{1α}$ 0.71%, $PGF_{3α}$ 0.66%, PGE_1 0.39%, PGD_2 0.16%, 6-keto-$PGF_{1α}$ 0.14%, $PGF_{2α}$ 0.14%, 2,3 dinor-6-keto$PGF_α$ 0.09%, 8-iso-$PGF_{1β}$ 0.08%, TXB_2 0.08%, 11-$(OH)_2$-TXB_2 0.07% and other prostaglandine metabolites <0.1%.

d) Recovery: The average % recovery of this assay was 109% (range 98-119%).

e) The microdialysis probe recovery was evaluated at almost 100% in experimental conditions because the perfusion pump perfused at an equally very slow rate of 0.3 μl/min before, during and after hemodialysis. The low flow rate of 0.3 μl/min was used to allow almost complete equilibration with the interstitial fluid and to avoid draining of the extracellular space. This almost 100% recovery with a flow rate of 0.3 μl/min was determined in our previous study.[18]

The statistical evaluation was carried out with

the statistical program NCSS 2000 and the ANOVA test and, specifically, with the randomized complete block design where blocks were the subjects and treatment the repeated measures. Bonferroni correction was used to adjust for multiple comparisons between groups.

Informed written consent was obtained from the subjects included in this study and the study was approved by the Ethics Committee of the School of Medicine, Aristotle University of Thessaloniki, Greece.

RESULTS

The levels of $8\text{-iso-PGF}_{2\alpha}$ in the plasma and IF of the patients as well as their statistical evaluation are shown in Tables 2 and 3 and in Figure 1.

As shown in Table 2 and in Figure 1, the plasma levels of $8\text{-iso-PGF}_{2\alpha}$ in the patients prior to hemodialysis initiation were higher (330.8 ± 42.2 pg/ml) than in controls (25.6 ± 5.1 pg/ml) ($p<0.001$). During hemodialysis, the plasma levels of $8\text{-iso-PGF}_{2\alpha}$ in the patients gradually increased and reached a maximum value at the end of the third hour of the hemodialysis, was almost stabilized at the end of the fourth hour and decreased significantly at the first hour following hemodialysis. Specifically, the plasma levels of $8\text{-iso-PGF}_{2\alpha}$ were as follows: a) one hour after hemodialysis initiation, they were higher (533.6 ± 55.2 pg/ml) than

those prior to hemodialysis initiation ($p=0.006$), b) at the second hour of hemodialysis, the values were 584.2 ± 57.8 pg/ml, significantly different than during the first hour ($p=0.049$), c) at the third hour of hemodialysis, they reached the level of 664.2 ± 73.1 pg/ml and were higher than the second hour of hemodialysis ($p=0.039$). At the fourth hour on hemodialysis, the values were stabilized at 648.0 ± 60.3 pg/ml without a significant difference from the levels of the third hour of hemodialysis ($p=0.386$). At the first hour after hemodialysis, the values decreased (549.0 ± 64.4 pg/ml) and were lower than the levels at the fourth hour of hemodialysis ($p=0.033$).

As shown in Table 3 and in Figure 1, the IF levels of $8\text{-iso-PGF}_{2\alpha}$ of hemodialysed patients before the beginning of hemodialysis were significantly higher (150.1 ± 22.2 pg/ml) than the levels in the controls (24.1 ± 4.1 pg/ml) with a p value <0.001. During hemodialysis, the IF levels of $8\text{-iso-PGF}_{2\alpha}$ gradually increased and reached a maximum value at the end of the third hour of the hemodialysis, were almost stabilized at the end of the fourth hour and decreased significantly at one hour following hemodialysis. Specifically, the IF levels of $8\text{-iso-PGF}_{2\alpha}$ were as follows: a) at one hour on hemodialysis, the values were higher (271.9 ± 31.4 pg/ml) than those before the start of hemodialysis ($p=0.006$), b) at the second hour on hemodialysis, they rose further and were higher (299.7 ± 32.6 pg/ml) than the levels during
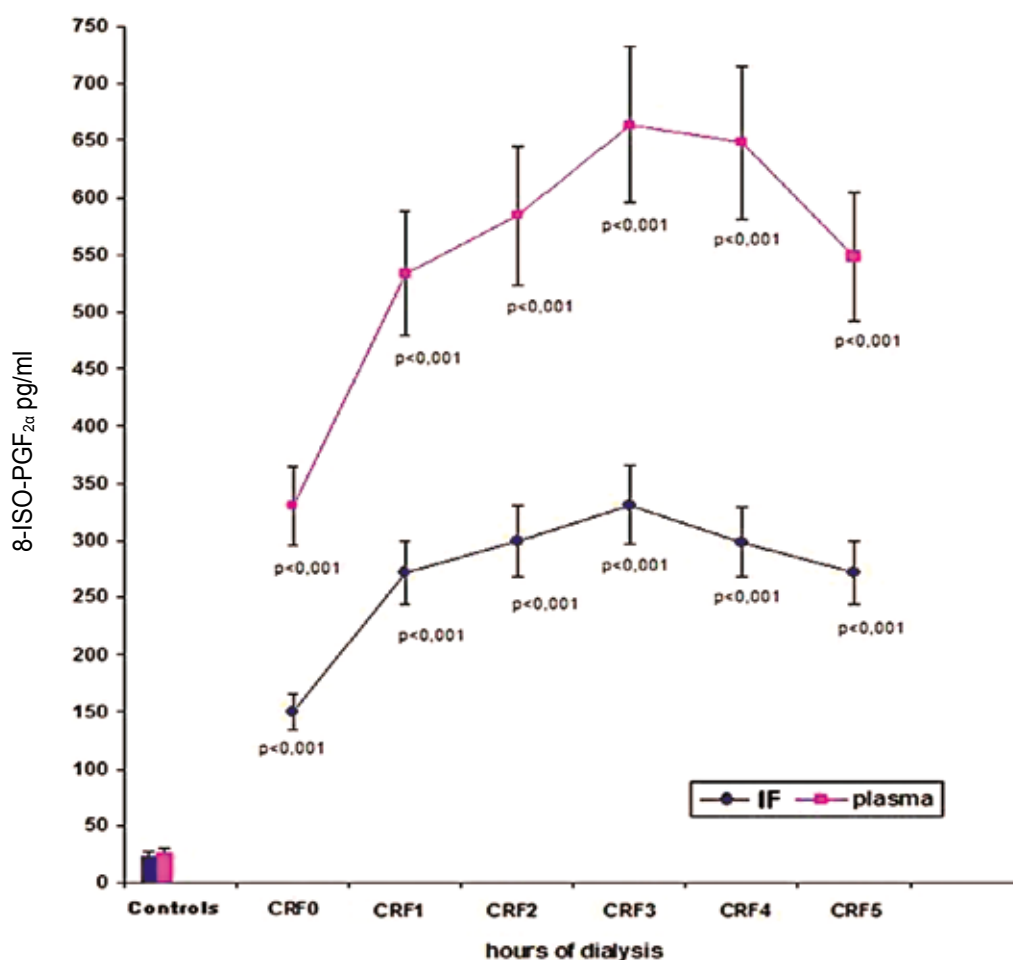
Table 2. Plasma $8\text{-iso-PGF}_{2\alpha}$ (mean$\pm$SD) in Controls (C) and in patients prior to (CRF_0), and during hemodialysis (CRF_1, CRF_2, CRF_3, CRF_4 and CRF_5) and their statistical evaluation.

			$8\text{-iso-PGF}_{2\alpha}$ pg/ml				
	C	CRF_0	CRF_1	CRF_2	CRF_3	CRF_4	CRF_5
	25.6	330.4	533.6	584.2	664.2	648.0	549.0
	±5.1	±42.2	±55.2	±57.8	±73.1	±60.3	±64.4
1.		$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$
2.			$p=0.006$	$p=0.004$	$p=0.001$	$p=0.003$	$p=0.005$
3.				$p=0.049$	$p=0.015$	$p=0.025$	$p=0.982$
4.					$p=0.039$	$p=0.053$	$p=0.508$
5.						$p=0.386$	$p=0.023$
6.							$p=0.033$

Comparison of the mean values of $8\text{-iso-PGF}_{2\alpha}$ of the groups:
1. C versus CRF_0, CRF_1, CRF_2, CRF_3, CRF_4 and CRF_5
2. CRF_0 versus CRF_1, CRF_2, CRF_3, CRF_4 and CRF_5
3. CRF_1 versus CRF_2, CRF_3, CRF_4 and CRF_5
4. CRF_2 versus CRF_3, CRF_4 and CRF_5
5. CRF_3 versus CRF_4 and CRF_5
6. CRF_4 versus CRF_5
Designation of CRF_1 to CRF_5 are given in the section on subjects and methods.

Figure 1. Values of 8-iso-PGF$_{2\alpha}$ (mean ± SD) in the plasma (■) and in the Interstitial Fluid (IF) (●) of the muscles in patients with chronic renal failure are higher than in controls. The values of 8-iso-PGF$_{2\alpha}$ (mean ± SD) in plasma and IF rise during hemodialysis with a significant gradient between plasma and IF prior to (CRF$_0$) and during hemodialysis (CRF$_1$-CRF$_5$). Controls: Columns ● IF, ■ plasma.

Table 3. Interstitial fluid 8-iso-PGF$_{2\alpha}$ (mean±SD) in controls (C) and in patients prior to (CRF$_0$), and during hemodialysis (CRF$_1$, CRF$_2$, CRF$_3$, CRF$_4$ and CRF$_5$) and their statistical evaluation.

	C	CRF$_0$	CRF$_1$	CRF$_2$	CRF$_3$	CRF$_4$	CRF$_5$
	21.1	150.1	271.9	299.7	330.8	298.9	272.0
	±4.1	±22.2	±31.4	±32.6	±33.6	±34.9	±31.8
1.		p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001
2.			p=0.006	p=0.003	p=0.001	p=0.004	p=0.005
3.				p=0.048	p=0.022	p=0.049	p=0.734
4.					p=0.035	p=0.999	p=0.048
5.						p=0.238	p=0.024
6.							p=0.049

Comparison of the mean values of 8-iso-PGF$_{2\alpha}$ of the groups:
1. C versus CRF$_0$, CRF$_1$, CRF$_2$, CRF$_3$, CRF$_4$ and CRF$_5$ 4. CRF$_2$ versus CRF$_3$, CRF$_4$ and CRF$_5$
2. CRF$_0$ versus CRF$_1$, CRF$_2$, CRF$_3$, CRF$_4$ and CRF$_5$ 5. CRF$_3$ versus CRF$_4$ and CRF$_5$
3. CRF$_1$ versus CRF$_2$, CRF$_3$, CRF$_4$ and CRF$_5$ 6. CRF$_4$ versus CRF$_5$
Designation of CRF$_1$ to CRF$_5$ are given in the section on subjects and methods.

the first hour (p=0.048), c) at the third hour on hemodialysis, they reached their maximum levels (330.8±33.6 pg/ml) and were higher than the levels during the second hour of hemodialysis (p=0.035), d) at the fourth hour on hemodialysis, the values were stabilized at 298.9±34.9 pg/ml without a statistically significant difference from the levels of the third hour (p=0.999), e) at one hour after hemodialysis, the values decreased (272.0±31.8 pg/ml) significantly and were lower compared to those at the fourth hour of hemodialysis (p=0.049).

DISCUSSION

The concentration of 8-iso-prostaglandin-$F_{2\alpha}$ (8-iso-$PGF_{2\alpha}$) in biological fluids has been considered as the most reliable biochemical index of lipid peroxidation in patients with several pathological conditions including end stage renal failure.

Using an enzyme-immunoassay method which has good precision, sensitivity and specificity,[18,22,25,26] we showed that the plasma and IF levels of 8-iso-$PGF_{2\alpha}$ of patients with end stage renal failure prior to hemodialysis initiation were significantly higher than the base line levels in controls. During hemodialysis, the plasma and IF levels of 8-iso-$PGF_{2\alpha}$ gradually increased to reach a peak at the third hour.

A gradient between plasma and IF with regard to the 8-iso-$PGF_{2\alpha}$ values was observed throughout the study period, the values in plasma being higher than in the IF space. The increased plasma levels of 8-iso-$PGF_{2\alpha}$ found in this study in patients with end stage renal failure before, during and after hemodialysis are in agreement with the findings of certain researchers who used either the classical gas Chromatography/ Mass Spectrometry Method (GC/MS)[10,19-21] or the new enzymo-immunoassay method that we used[18,22] for 8-iso-PGF2α determination. However, Ferraro et al[23] and Kim et al[24] found no difference in the 8-iso-$PGF_{2\alpha}$ plasma levels between normal subjects and hemodialysis patients before the hemodialysis session. Nevertheless, Kim et al found much higher values (6-fold) in patients after hemodialysis compared to normal subjects. We should note that the values found by Kim et al in normal subjects were much higher (960 pg/ml) than the respective values in healthy individuals reported by other researchers

(~150 pg/ml) and also found in our study (25.6±5.1 pg/ml). These differences may be attributed to environmental and dietery factors[25-30] or possibly to the fact that the normal subjects studied by Kim et al were active or passive smokers. It is known that smokers have 7-fold higher levels of 8-iso-$PGF_{2\alpha}$ in their plasma than non-smokers.[19,28] Similarly, Ferraro et al[23] found no difference in 8-iso-$PGF_{2\alpha}$ plasma levels (246 pg/ml) between patients before hemodialysis and normal subjects (252 pg/ml). This difference may also be due to inappropriate selection of their normal subjects. It should be mentioned that small differences in the values obtained are possibly due to different dietetic and environmental exposure of the patients[26-31] in the various studies.

In our study, the plasma levels of 8-iso-$PGF_{2\alpha}$ were significantly higher than the respective levels in the IF prior to and at all phases of the hemodialysis (p<0.001). The plasma levels of 8-iso-$PGF_{2\alpha}$ before the hemodialysis initiation were 16-fold higher than those in controls, while in the IF they were 10-fold higher. IF levels of 8-iso-$PGF_{2\alpha}$ prior to hemodialysis initiation found in this study confirmed the findings of a previous study of ours,[32] whereas the gradual increase of 8-iso-$PGF_{2\alpha}$ in the IF during hemodialysis, disclosed in the present study, has not previously been reported. It must be underlined that, compared to plasma levels, the IF levels of 8-iso-PGF_{2a} more accurately reflect the status of oxidative stress in the organism, since enzymes in plasma can break the 8-iso-PGF_{2a} molecule down into its derivatives, a reaction which is not expected in the IF.[17,18]

The higher levels of 8-iso-PGF_{2a} in plasma compared to those in the IF could be attributed to: a) an increased oxitative stress derived from a direct contact of blood elements with the surface of the hemodialysis filter connections, and b) to the much higher peroxidation of the arachidionic acid in the plasma by the free radicals compared to the oxidation of the arachidionic acid of the phospholipids of tissue cellular membranes.[33,34]

The high 8-iso-PGF_{2a} levels in the biological fluids in patients with Chronic Renal Failure (CRF) might be attributed to the increased oxidative stress and the increased lipid peroxidation in these patients, especially those in the last stages of the disease. The

increased oxidative stress and the increased lipid peroxidation of these patients must be attributed to the increased production of free radicals (oxygen and nitrogen mainly) and to the loss of the antioxidants during hemodialysis.[11,12,31,32]

The increased oxidative stress and the increased lipid peroxidation of the patients with CRF on hemodialysis are probably due to: a) the uremic environment which causes the increased production of free radicals from polymorphonuclear leucocytes,[35,36] b) the loss of hydrosoluble antioxidizing-reducing substances of low molecular weight during hemodialysis,[37,38] c) the bio-incompatibility of filter membranes of the artificial kidney that are used during hemodialysis,[33,34] d) the contamination of the hematocatharsis solution by endotoxins and pyrogens, which activate the macrophages and produce larger quantities of free radicals.[33,34] The contact of the blood with the plastic tubes during hemodialysis activates the polymorphonuclear leucocytes, resulting in an even higher production of free radicals. The afore-mentioned interpretations are reinforced by the fact that in the present study, 8-iso-PGF$_{2\alpha}$ levels were more than double in the blood plasma of the patients during hemodialysis than in the muscle IF.[33,34]

Moreover, all patients undergoing hemodialysis are exposed to various environmental as well as nutritional factors, which exert a significant effect, though at a different level from that experienced by patients suffering from CRF but not on hemodialysis.[26-30] Thus, recent literature data indicate that, in general, the oxidative stress and the resultant lipid peroxidation are increased further by: a) smoking (active or passive),[28] and b) exposure to increased environmental (O$_3$)[25] and Ultraviolet radiation (UV).[27] On the other hand, individuals who consume large quantities of vitamin C and/or vitamin E, drink large quantities of black or green tea or consume olive oil have lower oxidative stress and lower lipid peroxidation.[29-31]

In summary, in patients with end stage renal failure: a) lipid peroxidation is increased, as compared to that of normal individuals, b) lipid peroxidation rises further and in parallel in plasma and in IF during hemodialysis, c) lipid peroxidation in the IF space is lower than in the plasma prior to and during hemodialysis. The mechanism for this gradient remains speculative.

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