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Vitamin E-Coated Dialyzer and Antioxidant Defense Parameters: Three-Month Study

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Atherosclerotic cardiovascular disease is the most frequent cause of death in patients with end-stage renal disease who have undergone dialysis treatment. Oxidative stress, increased lipid peroxidation, and impaired function of antioxidant systems may contribute to the accelerated development of atherosclerosis in chronic renal failure patients during renal replacement therapy. The aim of this study was to investigate the influence of a vitamin E-coated dialyzer on antioxidant defense parameters in hemodialysis (HD) patients. In 14 HD patients, hemodialysis was performed using a vitamin E-coated dialyzer (Terumo CL-E15NL; Terumo Corporation, Tokyo, Japan) during a 3-month study. In these patients, erythrocyte (ER) antioxidant enzymes, superoxide dismutase (SOD), glutathione peroxidase (GPX), glutathione reductase (GR) and catalase (CAT), plasma total antioxidant capacity (TAC), RBC glutathione (GSH), plasma malondialdehyde (MDA), plasma, and RBC vitamin E were investigated. Each parameter was measured at the beginning of the study, after the 1st, 2nd, and 3rd month of the study, and 10 weeks after the interruption of the use of vitamin E-coated dialyzer. All HD patients were treated by erythropoietin (EPO) and received vitamin C 50 mg/d, pyridoxine 20 mg/d, and folic acid 5 mg/wk during the entire study. The 3-month treatment with the vitamin E-coated dialyzer led to a significant decrease of plasma MDA level (from 2.85 ± 0.44 to $2.25 \pm 0.37 \mu\text{mol/L}$) and to an increase of plasma TAC, RBC, GSH, and the vitamin E levels both in plasma (from 25.9 ± 2.8 to $33.6 \pm 3.8 \mu\text{mol/L}$) and in the RBCs (from 6.7 ± 0.8 to $7.4 \pm 0.7 \mu\text{mol/L}$) by 30% and 10.5%, respectively. Ten-week interruption of the use of the vitamin E-coated dialyzer led to near initial values of MDA ($2.90 \pm 0.28 \mu\text{mol/L}$), plasma ($28.6 \pm 3.5 \mu\text{mol/L}$), and RBC ($6.9 \pm 0.7 \mu\text{mol/L}$) vitamin E and of other investigated parameters. Statistical analysis of results was performed by conventional methods and analysis of variance. The findings of the current study confirm the beneficial effect of the vitamin E-coated dialyzer against oxidative stress in HD patients.

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Cardiovascular disease is a major cause of mortality in patients receiving hemodialysis (HD) or continuous ambulatory peritoneal dialysis (CAPD) because of chronic renal failure. Increased lipid peroxidation and depletion of antioxi-

dants may contribute to increased risk for atherosclerosis and damage of other tissues and organs in these patients.¹ Oxidative stress was more severe in HD than CAPD patients, and was exacerbated by HD.^{2,3}

Plasma and erythrocyte antioxidant defense mechanisms, enzymatic (superoxide dismutase [SOD], glutathione peroxidase [GPX], glutathione reductase [GR], and catalase [CAT]),⁴⁻⁸ and nonenzymatic (erythrocyte [ER] glutathione [GSH], vitamin E),^{6,9-12} are suppressed in chronic renal fail-

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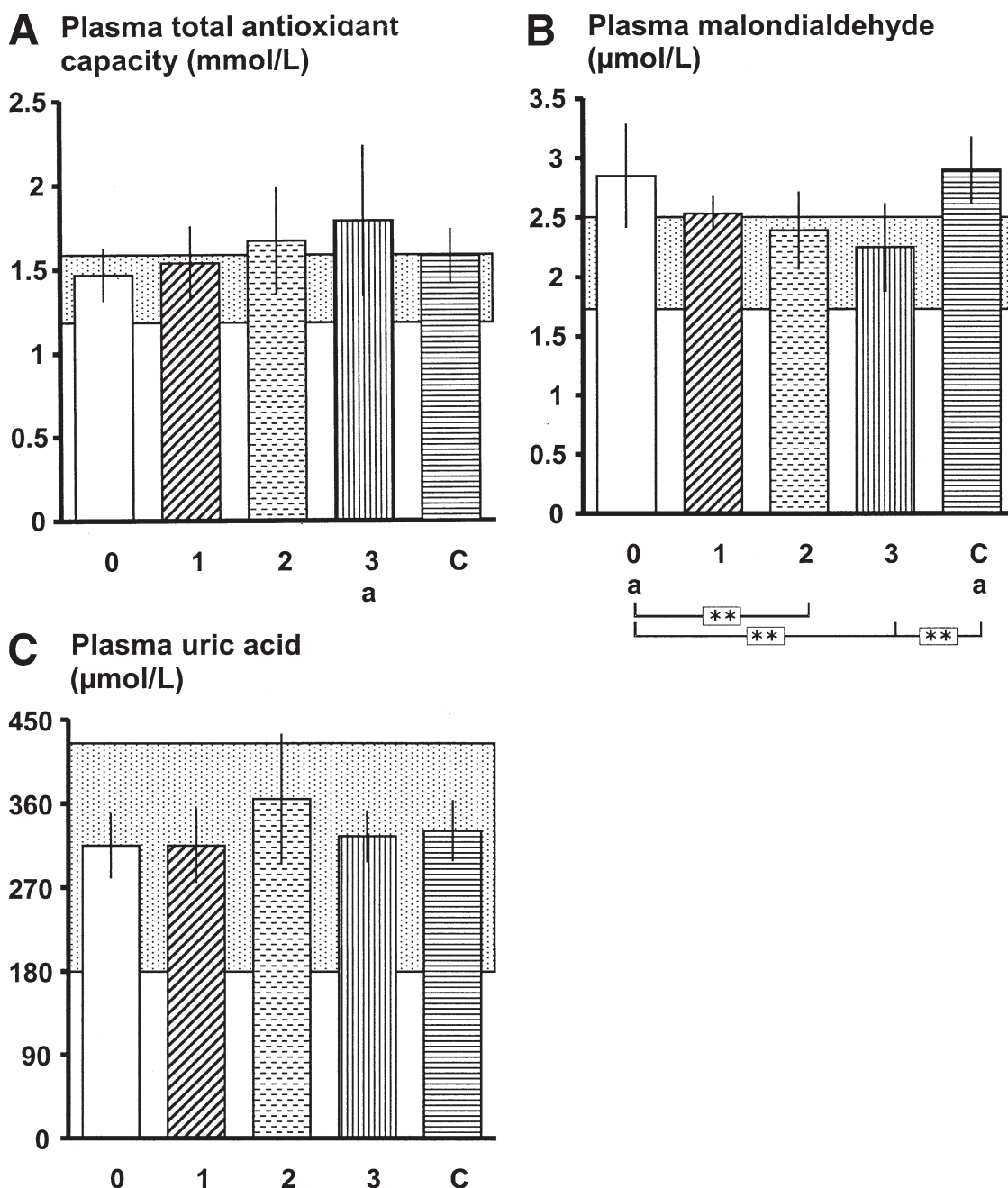


Figure 1 Influence of the vitamin E-coated dialyzer on (A) plasma total antioxidant capacity, (B) MDA, and (C) uric acid. 0, 1, 2, 3 correspond to month of the study. C, 10 weeks after study end. a, $P < .05$ versus normal range. * $P < .05$. ** $P < .01$ between months of the study. ▨, normal range.

ure, HD, and CAPD patients. It is proposed that reduced antioxidant defense mechanisms in erythrocytes is one of the important factors leading to peroxidation in the membrane lipid structure of the erythrocytes and thereby to hemolysis and anemia in these patients.⁴

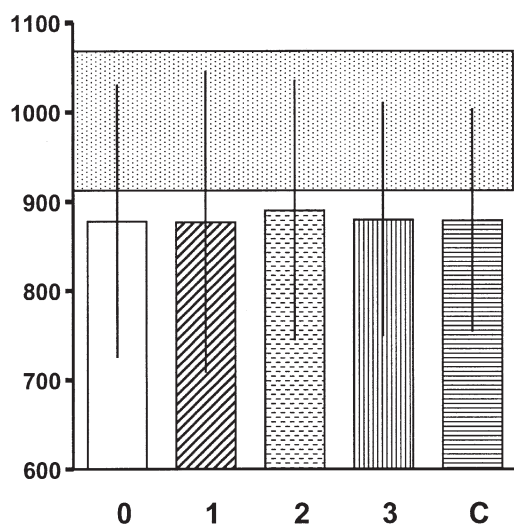
Malondialdehyde (MDA) levels as a product of lipid peroxidation were increased in uremic patients,^{1,3,7,13-18} indicating accelerated lipid peroxidation is a consequence of multiple pathogenetic factors.¹⁵ MDA is a short-chain aldehyde, the end product of unsaturated fatty acid oxidation. The unsaturated fatty acids are protected against oxidation by vita-

min E- and GSH-related systems. There is evidence that vitamin E is the most important antioxidant agent.¹⁴

The major cellular antioxidant that protects protein thiols and inhibits cellular damage caused by oxygen free radicals is GSH. Patients undergoing dialysis have low levels of blood GSH, which may lead to increased susceptibility of oxidative stress.^{6,9-12}

Antioxidants such as GSH, vitamin E, and vitamin C, and enzymatic antioxidants such as GSH-related enzymes, have been described as inadequate to cover the demand for protection against the oxidant insult of plasma lipoproteins and

A Erythrocyte superoxide dismutase (U/gHb)



B Erythrocyte catalase (U/gHb)

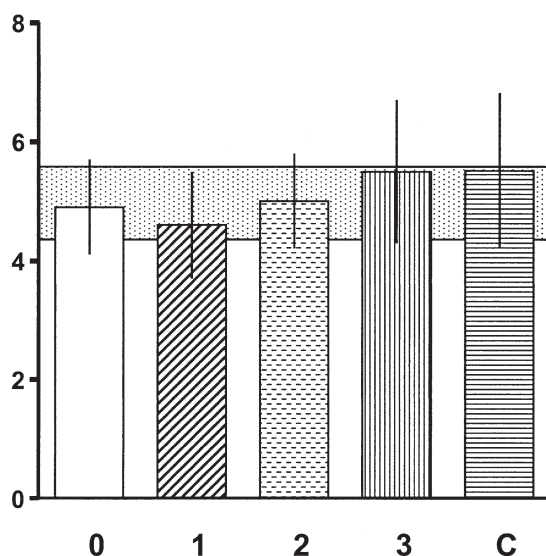


Figure 2 Influence of the vitamin E-coated dialyzer on (A) erythrocyte SOD and (B) erythrocyte CAT. 0, 1, 2, 3 correspond to month of the study. C, 10 weeks after study end. ■, normal range.

cell membranes. Lower antioxidant protection may act to shorten the ER lifespan and impair erythropoietin (EPO) function.¹⁶ At the same time, optimized correction of renal anemia (ie, by EPO supplementation) has been shown to interfere with reactive oxygen molecule production. It still is unclear whether EPO can favor or reduce oxidant stress in HD patients.¹⁶ Some investigators described a positive effect of EPO, that is, a significant reduction of oxidative stress and therefore in the reduction of organ tissue damage.^{7,15,16,19}

One of the new antioxidant-based dialysis strategies is a vitamin E-coated cellulose membrane dialyzer. According to

the recent data, the use of a vitamin E-coated dialyzer in HD patients during long-term study led to a decrease of the low-density lipoprotein MDA and oxidized low-density lipoprotein in comparison with conventional membranes.²⁰

However, there are discrepancies about serum vitamin E levels in uremic patients on HD and CAPD. In HD patients serum vitamin E is in the normal range^{12,16,21} or lower^{12,19,22} compared with healthy subjects, and ER tocopherol is decreased in HD patients.^{12,14,19,23} Erythrocyte tocopherol deficiency in HD patients could be caused by an insufficiency of vitamin E transfer from plasma high-density lipoprotein fractions to erythrocytes because it is known that vitamin E is transferred from serum to erythrocytes by high-density lipoprotein.²⁴ Some other causes could influence it too, such as a greater antioxidant consumption in these patients.²³

The aim of the study was to investigate the influence of the vitamin E-coated cellulose membrane dialyzer on markers of oxidative stress and antioxidant defense parameters in HD patients during the 3-month study. The present study is the continuation of the previous short-term study (3 wk) in which we compared the influence of vitamin E-coated dialyzers with conventional dialyzers and with peroral supplementation of vitamin E on MDA and antioxidant defense parameters in HD patients.²¹

Patients and Methods

In the study we investigated 14 HD patients (7 women, 7 men; mean age, 43 ± 5 y). All patients underwent regular bicarbonate HD treatment 3 times for 4 h/wk. Six patients suffered from chronic glomerulonephritis, 5 patients suffered from tubulointerstitial nephritis, 2 patients suffered from diabetic nephropathy, and 1 patient suffered from vascular nephrosclerosis. The Kt/V value was $1.49 \pm .18$. The patients were treated by EPO, the value of the hematocrit and hemoglobin concentration was in the range of .29 to .39 and 93.5 to 22.8 g/L, respectively, and they were supplemented with pyridoxine (20 mg/d), vitamin C (50 mg/d), and folic acid (5 mg/wk).

The study lasted 3 months and regular hemodialysis was performed using a vitamin E-coated dialyzer (Terumo Clirans CL-E15NL; Terumo Corporation [currently Asahi Corporation], Tokyo, Japan). All investigated parameters were determined at the beginning of the study and after the first, second, and third month of the study. In addition, the same parameters were determined 10 weeks after interruption of the use the dialyzer with vitamin E.

Plasma total antioxidant capacity (TAC) was determined by colorimetric method (Randox Laboratories, Crumlin, United Kingdom).²⁵ Erythrocyte antioxidant enzymes (SOD, GPX, and GR) were determined by spectrophotometric methods using RANSOD, RANSEL, and GR kits (Randox Laboratories),²⁶⁻²⁷ on a Cobas Mira automatic analyzer (Roche, Basel, Switzerland) and ER CAT by the spectrophotometric ultraviolet method.²⁸ Erythrocyte GSH level was determined by the spectrophotometric method with

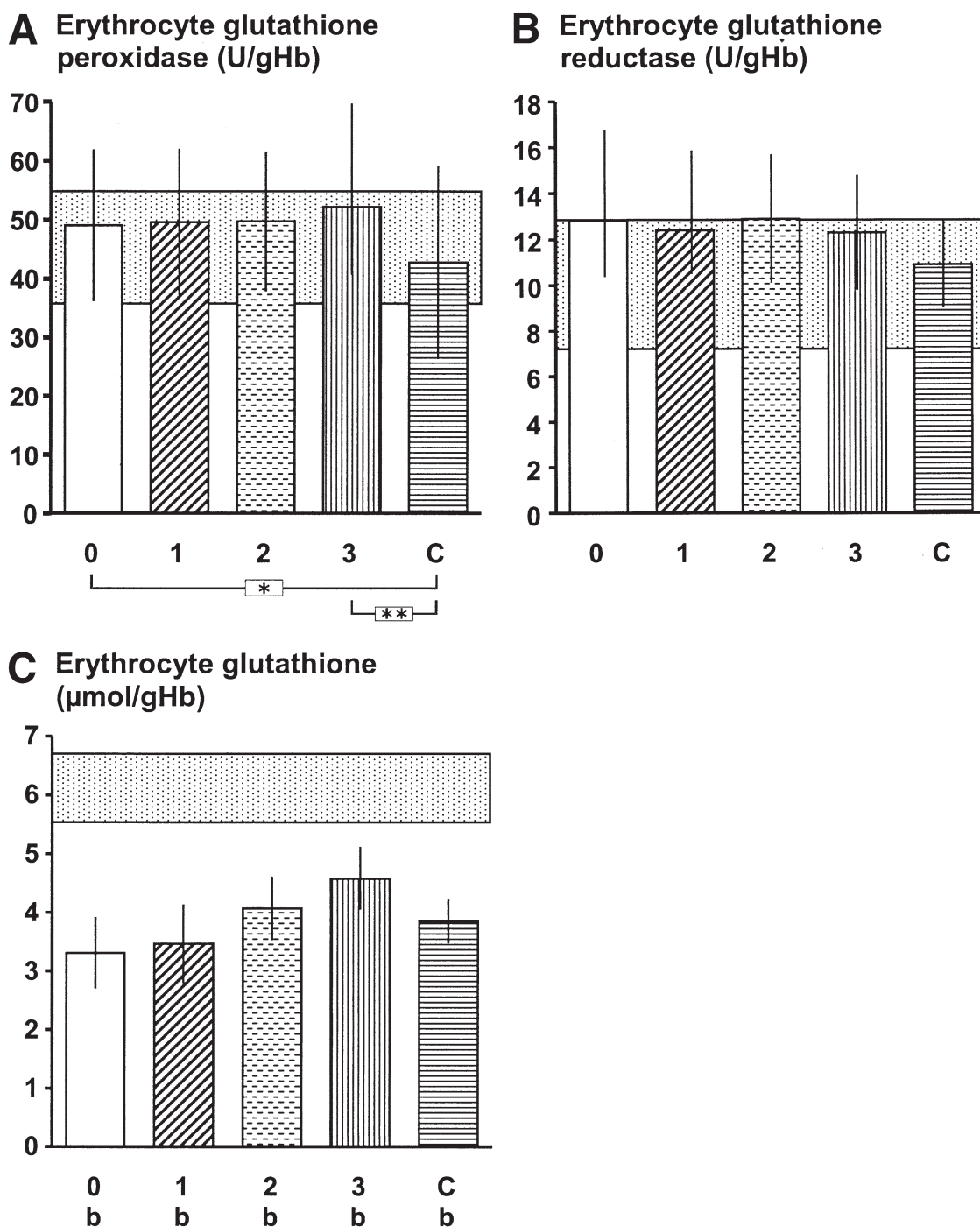


Figure 3 Influence of the vitamin E-coated dialyzer on (A) erythrocyte GPX, (B) erythrocyte GR, and (C) erythrocyte glutathione. 0, 1, 2, 3 correspond to month of the study. C, 10 weeks after study end. b, $P < .01$ versus normal range. * $P < .05$. ** $P < .01$ between months of the study. □, normal range.

5,5-dithio-bis(2-nitro)benzoic acid.²⁹ Plasma MDA level as the thiobarbituric acid reactive substance was determined by the spectrofluorometric method.³⁰ Plasma and erythrocyte vitamin E levels were determined by the modified spectrofluorometric method.^{31,32} Plasma uric acid and lipid parameters were determined by standard biochemical methods. Statistical analysis of results was performed by conventional methods and analysis of variance.

Results

Plasma TAC was in the normal range at the beginning of the study and 3-month use of the vitamin E-coated dialyzer led to a nonsignificant increase of plasma TAC. The value of plasma TAC at the end of the study was increased significantly in comparison with the normal range. At the beginning of the study, plasma MDA level was increased significantly in

HD patients and after 3 months of vitamin E-coated dialyzer use a significant decrease in value was found. Ten-week interruption of the use of the vitamin E-coated dialyzer led to a nonsignificant decrease of plasma TAC and to the significant increase of plasma MDA level near initial values. Plasma uric acid was in the normal range during the whole study (Fig. 1).

The activity of RBC SOD was decreased in comparison with the control group and activity of ER CAT was in the normal range during the whole study. No influence of vitamin E dialyzer on these parameters was found (Fig. 2). Activities of ER GPX and RBC GR in HD patients were in the normal range and the vitamin E-coated dialyzer did not influence these parameters. Erythrocyte GSH level in investigated patients was very low in comparison with the control group and the use of the vitamin E-coated dialyzer led to a nonsignificant increase of its value (Fig. 3). Concentration of plasma vitamin E was in the normal range but ER vitamin E was decreased significantly in HD patients. The use of the vitamin E-coated dialyzer led to the significant increase of plasma vitamin E (30%) and to the increase of ER vitamin E (10.5%) within normal range. Ten-week interruption of the use of the vitamin E-coated dialyzer led to the decrease of plasma and ER vitamin E near initial values (Fig. 4). In HD patients direct relationships between ER and plasma vitamin E at the beginning of the study ($r = .58, P < .05$), at the end of the study ($r = .61, P < .05$), and 10 weeks after the end of the study ($r = .62, P < .05$) were found.

Serum lipids (total cholesterol, triglyceride, high-density and low-density lipoprotein cholesterol) were in the normal range during the whole study. No influence of the vitamin E-coated dialyzer on these parameters was found (Table 1). In addition, direct relationships between plasma vitamin E and serum total cholesterol at the beginning ($r = .79, P < .01$), at the end of the study ($r = .58, P < .05$) and 10 weeks after the end of the study ($r = .65, P < .05$) were found.

Discussion

In the present study we investigated the influence of the vitamin E-coated dialyzer on the markers of oxidative stress and antioxidant defense mechanisms in HD patients over 3 months. The concentration of plasma MDA as a marker of lipid peroxidation in our HD patients was increased in comparison with the normal range. Three-month treatment with the vitamin E-coated dialyzer led to a decrease of plasma MDA levels.^{16,18,20,21}

Jackson et al¹ described increased plasma TAC in HD patients, but we are able to confirm these results only after 3-month use of the vitamin E-coated dialyzer. In HD patients the activity of ER SOD was low throughout the whole study. Our observation was in agreement with the data of some investigators.^{4,5,7,21} These results show in HD patients the existence of an oxidizing stress, mainly intracellular, which could be caused, in part, by a decrease in SOD activity.⁵ On the other hand, we observed normal levels of ER GPX and CAT activities in contrast to the results of other investigators, who observed significantly low levels of GPX^{4-6,8} and

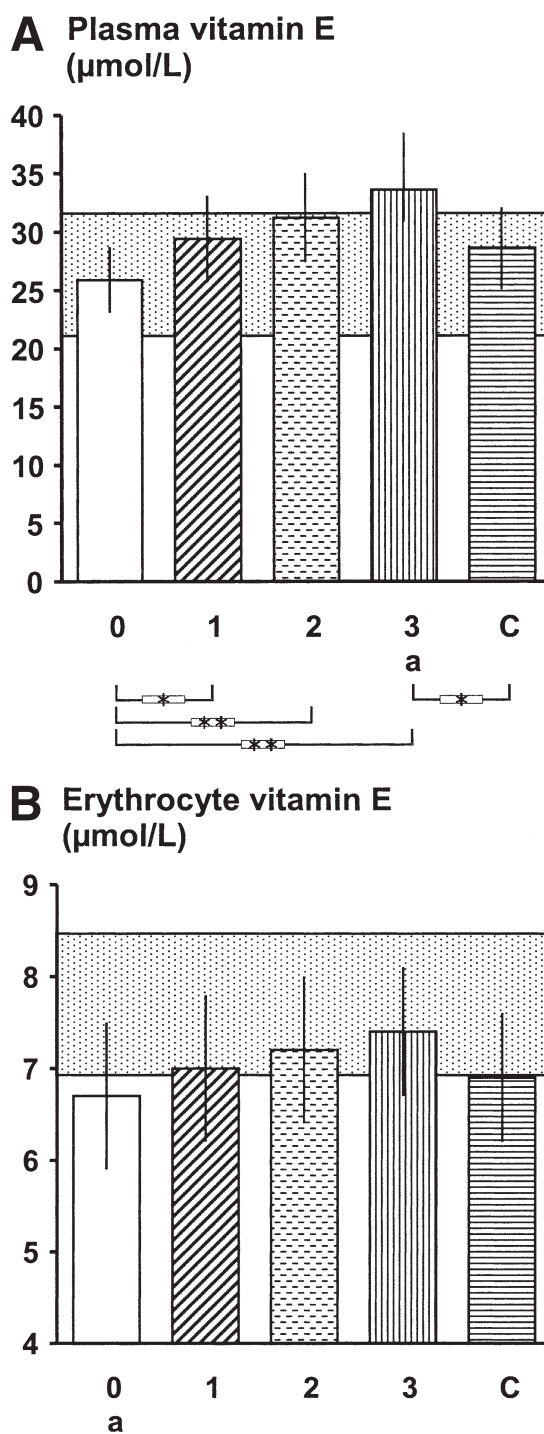


Figure 4 Influence of the vitamin E-coated dialyzer on (A) plasma vitamin E and (B) erythrocyte vitamin E. 0, 1, 2, 3 correspond to month of the study. C, 10 weeks after study end. a, $P < .05$ versus normal range. * $P < .05$. ** $P < .01$ between months of the study. ■, normal range.

CAT.^{4,7,8} On the contrary, in CAPD patients we found decreased activity of ER GPX.³ In agreement with the data of Ceballos-Picot et al,⁶ we found the activity of ER GR to be in the normal range. We did not observe significant changes of ER enzyme activities during the 3-month use of the vitamin E-coated dialyzer.

Table 1 Serum Lipids During 3-Month Study Using the Dialyzer With Vitamin E in Haemodialyzed Patients

Month of the Study	Total Cholesterol Level (mmol/L)	Triglyceride Level (mmol/L)	HDL Cholesterol Level (mmol/L)	LDL Cholesterol Level (mmol/L)
0	4.7 ± 0.7	1.59 ± 0.5	1.17 ± 0.3	3.03 ± 0.5
1	4.3 ± 0.7	1.58 ± 0.6	1.21 ± 0.3	2.74 ± 0.5
2	4.7 ± 1.0	1.55 ± 0.6	1.04 ± 0.2	2.76 ± 0.5
3	4.9 ± 0.7	1.69 ± 0.7	1.27 ± 0.4	2.95 ± 0.5
C*	5.1 ± 0.9	1.64 ± 0.6	1.38 ± 0.4	3.23 ± 0.7
Normal range	4.4–6.2	0–2	1.16–1.68	1.50–4.10

*Ten weeks after the end of the study.

Levels of RBC GSH, which is a major cellular antioxidant, were very low in HD patients in the present study.^{6,9-12} The 3-month use of the vitamin E-coated dialyzer led to the gradual nonsignificant increase of ER GSH, but not to normalization of its value. Our finding is similar to the data of Galli et al.¹² Moberly et al¹¹ administered to their HD patients 2-oxothiazolidine-4-carboxylic acid, which resulted in a significant increase in whole-blood GSH.

The erythrocyte vitamin E level was decreased in uremic patients on chronic hemodialysis^{12,14,19,23} in contrast to the results of Paul et al,⁵ who found the RBC vitamin E level to be in the normal range. According to the literature, long-term treatment by EPO in chronic renal failure patients led to the increase of ER vitamin E level to the normal range.¹⁹ We were not able to confirm that finding because our HD patients were treated simultaneously with EPO, their hematocrit values and hemoglobin concentrations reached target values and despite that the ER vitamin E level was decreased significantly. Normalization of ER vitamin E values were observed only after treatment with the vitamin E-coated dialyzer. EPO treatment in chronic renal failure patients leads to improvement of anemia, influences the RBC lifespan in circulation,³³ decreases lipid peroxidation in blood, and increases antioxidant protection.^{7,15,16} According to the results of the present study, the treatment by EPO is inadequate only for the normalization of ER vitamin E levels, sufficient correction was achieved by simultaneous use of the vitamin E-coated dialyzer.

Ten-week interruption of the use of the vitamin E-coated dialyzer led to a significant increase of plasma MDA and to a decrease of plasma TAC, ER GSH, plasma, and RBC vitamin E to near the initial values. This evidence suggests the beneficial effect of the vitamin E-coated dialyzer against oxidative stress in HD patients or its positive influence, at least partially, to reduce HD-related oxidative stress.^{12,13,16,18,20}

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