ELSEVIER

Journal of Nutritional Biochemistry 13 (2002) 653–663

THE JOURNAL OF Nutritional Biochemistry

Vitamin C and E supplements improve the impaired antioxidant status and decrease plasma lipid peroxides in hemodialysis patients☆

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Received 3 January 2002; received in revised form 19 April 2002; accepted 6 May 2002

Abstract

This study investigated the supplementation with vitamin C or/and E on the antioxidant system in hemodialysis patients. Thirty-eight hemodialysis patients (27 males and 11 females) with the average of 60 years old were divided into four groups: placebo (400 mg starch/time), vitamin C (400 mg/time)-, vitamin E (400 mg d,l- α-tocopheryl acetate/time)-, and vitamin C (400 mg/time) + E (400 mg d,l-α-tocopheryl acetate/time)-supplemented groups for 6-week supplementation. The patients orally received three capsules of placebo or antioxidant(s) three times a week after finishing hemodialysis. Thirty-six healthy subjects (22 males and 14 females) with the average of 58 years old were recruited as the control group. Hemodialysis patients significantly decreased plasma vitamin C by 32%, erythrocyte glutathione by 26%, and plasma total antioxidant status by 9%, but increased plasma lipid peroxide levels by 102% compared with the control group at the baseline. The levels of plasma vitamin C and total antioxidant status significantly decreased by 24% and 18%, respectively, from the post-dialysate compared with those from the pre-dialysate. At week 6, vitamin C + E-supplemented group significantly increased plasma vitamin C and E, erythrocyte glutathione, and plasma antioxidant status, and inhibited plasma lipid peroxides compared with placebo group. Additionally, vitamin C + E-supplemented group had higher plasma vitamin C, vitamin E, and total antioxidant status, and lower plasma lipid peroxides than placebo group even at least 2 weeks after the termination of the supplements. Therefore, antioxidant vitamin supplements could improve antioxidant status and decrease lipid peroxides of hemodialysis patients.

Keywords: Vitamin C; Vitamin E; Hemodialysis; Antioxidant status; Lipid peroxidation

1. Introduction

A profound imbalance between oxidants and antioxidants as well as the abnormality of plasma lipid profile have been observed in chronic renal failure (CRF) patients [1–6]. Previous studies reported that dialysis patients had an impaired antioxidant system, including antioxidant status [7–10], antioxidant enzyme activities [11–17], and reduced antioxidant defense against lipid peroxidation [4,5,14–18]. Due to hemobioincompatibality of the dialysis system, the formation of free oxygen radical species and trace amounts of endotoxins were induced during the inflammatory state in hemodialysis patients [6]. Both the abnormalities in the antioxidant defense system and the increased oxidative stress may increase their susceptibility to lipid peroxidation in low density lipoprotein (LDL), which may lead to the subsequent development of atherosclerotic cardiovascular disease in hemodialysis patients [5–7].

The concentrations of plasma antioxidants changed in hemodialysis patients. Plasma retinol significantly elevated in hemodialysis patients [8,9], but absolute plasma concentrations of α- or β-carotene did not differ between CRF patients with or without hemodialysis and the control [8]. Plasma α-tocopherol concentration in hemodialysis patients did not differ from the control group [7–10]. Additionally, plasma α-tocopherol concentration was not affected during a single session of hemodialysis [7–10], and no α-tocopherol was detected in the dialysate [10]. However, the hemodialysis process acutely lowered plasma antioxidant status, and plasma total ascorbate, thiol, and uric acid levels, which can be lost in the dialysate due to their water-soluble property [7–10]. During a 3-h hemodialysis session, the mean

☆ Supported by National Science Council of Taiwan, R.O.C. (grant NSC 89–2320-B-038–036 and NSC 89–2320-B-038–050).

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0955-2863/02/$ – see front matter
PII: S0955-2863(02)00209-7

decrease in total vitamin C was 40% [10]. Additionally, the ascorbyl free radical/vitamin C ratio elevated after the hemodialysis session [7]. Some studies have demonstrated that vitamin supplements are required to meet the specialized needs of renal failure or to improve the redox status in hemodialysis patients [5,6,19]. Vitamin E supplementation via oral administration or bound on dialyzer membrane improved the redox status by increasing antioxidant levels and lowering oxidative damage in hemodialysis patients [5,20–23]. However, it is unclear for the effect of combined antioxidant vitamin supplements on the antioxidant system during and after supplementation in a manner of time course. Of potential antioxidants in the foods, vitamin C and E are the principal dietary antioxidants to protect from reactive oxygen species (ROS) damage. Due to the conflicting findings for the effect of β-carotene on ROS-associated diseases and elevated plasma retinol level in hemodialysis patients, β-carotene supplement is not considered in this study. Therefore, the proposes of the study was to investigate the effect of oral vitamin C or/and vitamin E supplements on the antioxidant defense system, and to follow up the effects after the termination of the supplements in hemodialysis patients. The antioxidant capacity was evaluated by blood antioxidant levels, total antioxidant status, and lipid peroxidation.

2. Materials and methods

2.1. Subjects

Thirty-eight patients (27 males and 11 females) of the average 60 years old (27~85 years old) with chronic renal failure and receiving regular hemodialysis for at least 3 months (3~261 months) were recruited from the center of hemodialysis at Taipei Medical University Hospital. All patients regularly received 3–5 h hemodialysis carried on cellulose or poly methyl methacrylate membrane three times weekly. Thirty-six healthy subjects (22 males and 14 females) of the average 58 years old (28~81 years old) were recruited as the control group. The subjects were selected by interviewing their motivation, commitment to completing the experiment, dietary habits (the intake amount and frequency of alcohol, coffee, and tea), physical activity (the type and frequency of exercise), smoking habit (the amount, frequency, and duration), and medical history, as well as by laboratory examination of their blood pressure, plasma lipids, blood glucose, and general health conditions by the investigators and physicians. The exclusion criteria for hemodialysis patients included those with acute illness, severe diabetes (blood glucose >8.06 mmol/L *ante cibum* and >11.11 mmol/L *post cibum*), severe hypertension (blood pressure >150/95 mmHg during hemodialysis), hepatic, respiratory, or chronic inflammatory diseases. The exclusion criteria for all subjects were obesity, malnutrition, drastic body weight changes (> 2.5 kg in last 1 month), hormone replacement therapy, heavy smoking, vegetarian diet, drug abuse, the habit of alcohol, coffee or tea drinking (> 3 cups/day) in last 3 months, or dietary supplementation of high-dosage (> 200% Recommended Dietary Allowances) vitamins, minerals, antioxidants, herbs, or fish oil in last 3 months. All qualified subjects were asked for attending this study, and the informed consent was obtained prior to the beginning of the study. To prevent the potential risk for their illness, hemodialysis patients were allowed to take their regular medication without any antioxidant effect but decreasing the dosage to the minimum. If the medication has an antioxidant effect (such as probucol), it was replaced two weeks before the supplementation by the physicians. During the experimental period, the patients were followed up for their health conditions routinely by the physicians, and for their obedience and complaint irregularly by the investigators. If any serious illness or complaint occurs, the patient was permitted dropping out at any time. During the experimental period, five hemodialysis patients withdrew. One in placebo group was dead, one and two in vitamin C- and vitamin E-supplemented groups, respectively, were poor health conditions, and one in vitamin C + E-supplemented group transferred to other hospital. Other than those, the patients completed the experiment without any complaint. All subjects were allowed to maintain their physical activity and regular life style. The study was performed in accordance with the regulations of the ethics committee of Taipei Medical University Hospital.

2.2. Dietary intake

To evaluate the effect on the antioxidant system caused by antioxidant supplements rather than by dietary intake, the dietary intake was assessed in all subjects. The subjects were asked to record their daily intake once a week for at least three times. The investigators then interviewed the subjects for their dietary record one day after. Energy and nutrient intake was calculated by food composition analysis software developed by Institute of Biomedical Sciences, Academia Sinica.

2.3. Antioxidant supplements

It was designed as a double-blind control study. According to age, sex ratio, the degree of the diseases, the medical treatment, and the duration of receiving hemodialysis therapy, the patients were divided into the following four groups: placebo (400 mg starch/time), vitamin C (400 mg L-ascorbate/time)-, vitamin E (400 mg d,l- α-tocopheryl acetate/time)-, and vitamin C (400 mg L-ascorbate/time) + E (400 mg d,l- α-tocopheryl acetate/time)-supplemented groups for 6 weeks. To achieve the double-blind study, all supplements were filled into the same-looking hard capsules without any flavor, and given to hemodialysis patients by the nurses rather than by the investigators. Due to the limitation of the volume of the hard capsule, the amount of

the supplement per time was evenly filled into three hard capsules produced by China Chemical & Pharmaceutical Co., Ltd. (GMP #99–203791–00, Taipei, Taiwan, R.O.C.). Considering the loss of the supplements during hemodialysis, the patients were orally given three capsules of the supplement after the end of hemodialysis under the supervision of the nurse three times a week to ensure totally oral intake and to increase their compliance. After 6 weeks, the supplements were terminated and the antioxidant status of hemodialysis patients was followed up for another 4 weeks.

2.4. Biochemical analyses

Pre- and post-dialysis venous blood samples (6 mL) were drawn at week 0, 3, 6, 8, and 10. Blood samples were collected in EDTA-containing tubes for lipid peroxidation assay or heparinized tubes for other analyses, and centrifuged at 2,500 × *g* at 4°C for 10 min to separate cells and plasma. The erythrocyte pellet was washed three times by 0.9% NaCl, and centrifuged for 5 min at 2,500 × *g* after each wash. The pellet was prepared freshly for glutathione analysis.

Vitamin C was analyzed by reverse phase HPLC [24]. The plasma (0.5 mL) was mixed with an equal volume of cold 10% (wt/vol) metaphosphoric acid (MPA), and frozen stored until analysis. Prior to apply to HPLC, samples were centrifuged at 10,000 × *g*, 4°C, for 10 min. The supernatant (50 μL) was run through C_{18} column (RP-18E, 4 × 250 mm, 5μm, Merck Taiwan Ltd., Taipei, Taiwan, R.O.C.). The mobile phase of HPLC system (Jasco PU-980 Intelligent HPLC Pump, Jasco UV-975 Intelligent UV/VIS Detector, Jasco AS-950 Intelligent Sampler, Jasco Cor., Tokyo, Japan) was 5 mM 1-pentane sulfuric acid sodium salt (pH 3.1), and the flow rate was 0.8 mL/min. Vitamin C was detected at UV 254 nm. Different concentrations of L-ascorbate in 10% MPA were used as vitamin C standards. The retention time for L-ascorbate was 4.5 min.

Vitamin E was analyzed by reverse phase HPLC as described previously [25]. The plasma (0.5 ml) was mixed with 2 mL of 1% pyrogallol in absolute alcohol, 0.1 mL of 12 M HCl, and 6 mL of n-hexane containing 0.125% butylated hydroxytoluene (BHT) to extract vitamin E. The n-hexane layer containing vitamin E was evaporated under nitrogen, and the residue was solubilized in 0.1 mL methanol, and was run through C_{18} column (RP-18E, 4 × 250 mm, 5μm). The mobile phase of HPLC system was 100% methanol, and the flow rate was 1 mL/min. Vitamin E was detected at UV 292 nm. Different concentrations of α-tocopherol in absolute alcohol containing 0.1% BHT were used as vitamin E standards. The retention time for α-tocopherol was 5.5 min. Vitamin C and E concentrations were analyzed by SISC-LAB (32) chromatographic analysis software (Scientific Information Service Cor., Taipei, Taiwan, R.O.C.).

The concentrations of reduced glutathione were measured spectrophotometrically at 400 nm using a commercial kit (Calbiochem 354102, Calbiochem-Novabiochem Cor., La Jolla, CA) [26]. The erythrocyte pellet (50 μL) was resuspended in 200 μL of 6% MPA at 4°C followed by centrifugation at 3,000 × *g* for 15 min. The supernatant (100 μL) was diluted with 800 μL of assay buffer (200 mM potassium phosphate, pH 7.8/0.2 mM diethylene-triaminepentaacetic acid/0.025% LUBROL). The diluted supernatant was added 50 μl of the chromogenic reagent (12 mM solution in 0.2 M HCl) followed by mixed with 50 μL of 30% NaOH at room temperature. After 10-min reaction in the dark, glutathione levels were determined at 400 nm.

Plasma total antioxidant status was determined by evaluation of the ability of the antioxidants in the samples to inhibit the oxidation of ABTS® (2,2′-azino-di-[3-ethylbenzthiazoline sulfonate]) to $ABTS^{\bullet +}$ by metmyoglobin (a peroxidase) using a commercial kit (Randox NX 2332, Randox Laboratories Ltd., Antrim, UK). The amount of $ABTS^{\bullet +}$ produced can be monitored at 600 nm [27]. The standard (1.65 mmol/L 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and plasma (20 μL) was mixed with 1 mL chromogen (5 μM metmyoglobin and 500 μM ABTS® in 66 mM phosphate buffer saline, pH 7.4), and the initial absorbance was read at 600 nm at 37°C. After the incubation with 250 μM H_2O_2 for 3 min, the final absorbance was read at 600 nm at 37°C.

The concentration of lipid peroxides in the plasma was assessed colorimetrically at 586 nm using a commercial kit (Calbiochem 437634, Calbiochem-Novabiochem Cor., La Jolla, CA) [28]. The plasma (200 μl) was mixed with 650 μl of Reagent 1 (7.7 mM N-methyl-2-phenylindole in 75% acetonitrile and 25% methanol) and 150 μL of Reagent 2 (15.4 M methanesulfonic acid) at 45°C for 40 min. The levels of malondialdehyde (MDA) and 4-hydroxy-2(E)-nonenal (4-HNE), the end products derived from peroxidation of polyunsaturated fatty acids and related esters, were measured at 586 nm.

2.5. Statistical analysis

All data were analyzed by Statistical Analysis System (SAS) software (v6.12, SAS Institute Inc., Cary, NC). The differences of sex ratio and the numbers of hemodialysis patients with cardiovascular disease, diabetes mellitus, or erythropoietin therapy were analyzed by chi square. The mean differences between healthy subjects and hemodialysis patients were analyzed by Student's *t* test, and those between the pre- and post-dialysis in hemodialysis patients were analyzed by paired *t* test. General linear model was used to analyze the differences among four supplemented groups at different time points. Fisher's least significant difference test was used to make *post-hoc* comparisons if the treatment effect was demonstrated. Statistical significance is assigned at the 0.05 level.

Table 1
Clinical data of the control group and the four subgroups of hemodialysis patients at the baseline[1]

	Control (n = 36)	Hemodialysis			
		Placebo (n = 8)	Vitamin C (n = 10)	Vitamin E (n = 10)	Vitamin C + E (n = 10)
Sex	M22, F14	M6, F2	M7, F3	M7, F3	M7, F3
Cardiovascular disease, n	—	7	8	8	6
Diabetes mellitus, n	—	3	2	3	2
Erythopoietin therapy, n	—	7	10	7	6
Age, year	58 ± 19	66 ± 12	57 ± 14	62 ± 8	58 ± 17
Body mass index, kg/m^2	23.7 ± 3.1	23.6 ± 3.2	19.7 ± 2.4	22.9 ± 2.7	23.8 ± 2.5
Duration of hemodialysis therapy, month	—	61 ± 55	61 ± 77	57 ± 67	57 ± 78
Plasma albumin, g/L	35–55[2]	37 ± 5	37 ± 3	39 ± 3	37 ± 4
Plasma total cholesterol, mmol/L	3.36–5.69[2]	3.54 ± 0.77	4.21 ± 0.83	3.96 ± 0.90	4.20 ± 0.89
Plasma triglycerides, mmol/L	0.34–1.95[2]	2.47 ± 2.85	2.13 ± 1.08	3.02 ± 2.79	2.69 ± 1.80

[1] Values are mean ± SD ($P > 0.05$).
[2] Normal range values in laboratory data.

3. Results

3.1. Baseline data and dietary intake

Clinical data of the control and hemodialysis subjects at the baseline were shown in Table 1. Sex ratio (male:female = 61%:39% vs 71%:29%), age (58 ± 19 vs 60 ± 13 years), and body mass index (23.7 ± 3.1 vs 22.4 ± 2.7) were not different between the control and hemodialysis groups. The numbers of hemodialysis patients with cardiovascular disease, diabetes mellitus, or recombinant human erythropoietin therapy, the averages for duration of hemodialysis therapy (59 months), plasma albumin (39 g/L), total cholesterol (4.00 mmol/L), and triglyceride levels (2.58 mmol/L) were not significantly different among four hemodialysis groups. The averages of plasma albumin and total cholesterol concentrations in hemodialysis patients were in the normal ranges. However, the average of plasma triglyceride concentration was higher than the normal range in all hemodialysis groups.

The dietary intake of the control and hemodialysis subjects were shown in Table 2. Due to no significant difference among dietary assessments from different days and among hemodialysis groups, the daily average intake of the control and hemodialysis groups was used. The daily dietary intake for energy, carbohydrate, protein, lipid, saturated fatty acids (SFA), unsaturated fatty acids (UFA), vitamin C, vitamin E, and crude fiber did not significantly differ between the control and hemodialysis groups. The intake for carbohydrate, protein, and lipid in the control and hemodialysis groups was 48.8%, 18.6%, and 32.6%, as well as 53.6%, 14.5%, and 31.9% energy, respectively. The UFA/SFA ratio in the control and hemodialysis groups was 2.9 and 2.2, respectively. The average vitamin C intake in the control and hemodialysis groups was beyond Dietary Reference Intakes (DRI) [29]. Whereas the average vitamin E intake in both groups was less than DRI.

3.2. Plasma vitamin C and E concentrations

Plasma vitamin C concentrations in hemodiaytic patients significantly lowered by 32% ($P < 0.05$) than those in the control group at the baseline (Table 3). Plasma vitamin C levels also significantly lowered by 24% ($P < 0.05$) from the post-dialysis compared with those from the pre-dialysis of hemodialysis patients during 10 weeks. Vitamin C + E-supplemented group had lower plasma vitamin C concentration ($P < 0.05$) than other hemodialysis groups at week 0. During the supplemented period, plasma vitamin C levels significantly increased by 33% and 63% ($P < 0.05$) in vitamin C-supplemented group, and by 35% and 72% ($P < 0.05$) in vitamin C + E-supplemented group at week 3 and 6 compared with the respective baselines. Both vitamin C- and vitamin C + E-supplemented groups had higher plasma

Table 2
The dietary intake of the control and hemodialysis subjects[1]

	Control (n = 36)	Hemodialysis (n = 38)
Energy, kcal	2028 ± 432	1935 ± 647
kJ	8484 ± 1805	8097 ± 2707
Carbohydrate, g	247.9 ± 98.7	259.5 ± 75.8
% energy	48.8	53.6
Protein, g	94.4 ± 31.7	70.3 ± 23.5
% energy	18.6	14.5
Lipid, g	73.5 ± 29.3	68.8 ± 11.4
% energy	32.6	31.9
Saturated fatty acids, g	18.9 ± 10.4	21.1 ± 12.4
% energy	8.3	9.8
% lipid	25.7	30.6
Unsaturated fatty acids, g	53.5 ± 20.2	46.7 ± 17.5
% energy	23.7	21.7
% lipid	72.8	67.8
Vitamin C, mg	126.1 ± 92.6	98.6 ± 74.9
Vitamin E, mg α-TE	9.75 ± 4.54	8.04 ± 3.71
Crude fiber, g	3.64 ± 1.75	2.83 ± 2.09

[1] Values are mean ± SD ($P > 0.05$).
α-TE: α-tocoherol equivalent.

Table 3
Plasma vitamin C concentrations of the control and hemodialysis subjects before, during, and after antioxidant vitamin supplements[1]

	Plasma vitamin C concentration, μmol/L (%)[2]				
	Week 0	Week 3	Week 6	Week 8	Week 10
Control	17.0 ± 3.1 (n = 36)				
Hemodialysis	11.6 ± 3.1[3] (n = 38)				
Placebo	(n = 8)	(n = 8)	(n = 8)	(n = 7)	(n = 7)
Pre-dialysis	13.5 ± 3.1$^{b\#}$	12.7 ± 3.3$^{\#}$ (94)^{a}	12.0 ± 3.0$^{\#}$ (89)^{a}	10.5 ± 3.1$^{\#}$ (78)^{a}	11.7 ± 3.3$^{\#}$ (87)^{a}
Post-dialysis	10.3 ± 3.2¶	9.5 ± 2.8¶	9.2 ± 2.5¶	8.0 ± 2.9	8.8 ± 2.5¶
Vitamin C	(n = 10)	(n = 10)	(n = 10)	(n = 9)	(n = 9)
Pre-dialysis	10.7 ± 3.1$^{ab\#}$	14.2 ± 2.5*$^{\#}$ (133)^{b}	17.4 ± 4.0* (163)^{b}	15.9 ± 2.8 (149)^{c}	12.2 ± 2.6$^{\dagger\#}$ (114)^{b}
Post-dialysis	8.1 ± 2.4¶	10.7 ± 3.2¶	13.1 ± 3.6¶	12.1 ± 2.8¶	9.3 ± 2.4¶
Vitamin E	(n = 10)	(n = 9)	(n = 8)	(n = 8)	(n = 8)
Pre-dialysis	12.5 ± 3.2$^{b\#}$	13.2 ± 3.6$^{\#}$ (106)^{a}	13.5 ± 3.9 (108)^{a}	13.4 ± 4.1 (107)^{b}	13.2 ± 3.7$^{\#}$ (106)^{ab}
Post-dialysis	9.2 ± 3.2¶	9.9 ± 3.3	10.2 ± 3.2¶	10.2 ± 3.9	10.1 ± 3.0¶
Vitamin C + E	(n = 10)	(n = 10)	(n = 9)	(n = 9)	(n = 9)
Pre-dialysis	9.6 ± 2.9$^{a\#}$	13.0 ± 3.0*$^{\#}$ (135)^{b}	16.5 ± 3.2* (172)^{b}	15.9 ± 4.0 (166)^{c}	13.9 ± 3.2 (145)^{c}
Post-dialysis	7.1 ± 2.5	9.7 ± 2.4¶	12.5 ± 3.5¶	12.1 ± 3.1¶	10.6 ± 3.0¶

[1] Values are mean ± SD.
[2] Values are the percentage of the respective group at week 0 from the pre-dialysis in hemodialysis patients.
[3] Values from the pre-dialysis of all hemodialysis patients at week 0 vs the control group, $P < 0.05$.
Values of hemodialysis patients in each column not sharing the same letter significantly differ ($P < 0.05$) by Fisher's least significant difference test.
* Week 3, 6 (during the supplemented period) vs week 0 (baseline), $P < 0.05$.
† Week 8, 10 (the terminated period without supplementation) vs week 6 (the end of the supplemented period), $P < 0.05$.
$^{\#}$ The hemodialysis group vs the control group, $P < 0.05$.
¶ Post-dialysis vs pre-dialysis, $P < 0.05$.

vitamin C levels ($P < 0.05$) than placebo and vitamin E groups. After the termination of the supplements, plasma vitamin C levels remained the same in vitamin C- and vitamin C + E-supplemented groups for another 2 and 4 weeks, respectively. At week 8, plasma vitamin C levels significantly increased ($P < 0.05$) in vitamin C-, vitamin E-, and vitamin C + E-supplemented groups compared with placebo group. At week 10, plasma vitamin C levels in vitamin C- and vitamin C + E-supplemented groups were higher ($P < 0.05$) compared with placebo group, but only in vitamin C + E-supplemented group was not significantly different from those of the control group.

Plasma vitamin E levels were not different between the control and hemodialysis groups, between the post- and pre-dialysis, and among the hemodialysis groups at week 0 (Table 4). Plasma vitamin E concentrations significantly elevated by 35% and 70% ($P < 0.05$) in vitamin E-supplemented group, and by 36% and 73% ($P < 0.05$) in vitamin C + E-supplemented group at week 3 and 6 compared with the respective baselines. During the supplemented period, both vitamin E- and vitamin C + E-supplemented groups had higher plasma vitamin E levels ($P < 0.05$) than placebo and vitamin C groups. At week 6, plasma vitamin E levels were higher ($P < 0.05$) in vitamin E- and vitamin C + E-supplemented groups than those in the control group. Placebo and vitamin C did not affect plasma vitamin E levels during 10 weeks. After the termination of the supplements, plasma vitamin E concentrations remained higher ($P < 0.05$) in vitamin E- and vitamin C + E-supplemented groups compared with placebo and vitamin C-supplemented groups. At week 10, plasma E levels in vitamin E-supplemented group were still higher ($P < 0.05$) than those in the control group.

3.3. Erythrocyte glutathione concentration

Hemodialysis patients significantly lowered erythrocyte glutathione levels by 26% ($P < 0.05$) compared with the control group (Table 5). Except for lower erythrocyte glutathione in placebo group at week 10 from the post-dialysis, erythrocyte glutathione concentrations in the pre- and post-dialysate did not significantly differ (Table 5). Erythrocyte glutathione levels increased with both the supplemented duration and antioxidant vitamin supplements ($P < 0.05$). At week 3 and 6, all antioxidant supplemented groups had higher erythrocyte glutathione levels ($P < 0.05$) than the respective baselines and placebo group. After the termination of the supplements, erythrocyte glutathione levels in vitamin C- and vitamin E-supplemented groups were not significantly different from those at week 6 and from those in the control group. Additionally, erythrocyte glutathione levels were not significantly different among the hemodialysis groups at week 8 and 10.

3.4. Plasma total antioxidant status

Except for placebo group, hemodialysis patients had significantly decreased plasma total antioxidant status by 9% ($P < 0.05$) compared with the control group at the baseline (Table 6). Plasma total antioxidant status significantly impaired by 18% ($P < 0.05$) from the post-dialysis compared with that from the pre-dialysis throughout 10 weeks. At the

Table 4
Plasma vitamin E concentrations of the control and hemodialysis subjects before, during, and after antioxidant vitamin supplements[1]

	Plasma vitamin E concentration, μmol/L (%)[2]				
	Week 0	Week 3	Week 6	Week 8	Week 10
Control	12.9 ± 3.4 (n = 36)				
Hemodialysis	11.0 ± 3.6 (n = 38)				
Placebo	(n = 8)	(n = 8)	(n = 8)	(n = 7)	(n = 7)
Pre-dialysis	12.0 ± 3.0[a]	11.7 ± 3.1 (98)[a]	12.4 ± 3.3 (103)[a]	11.8 ± 2.9 (98)[a]	12.7 ± 2.8 (106)[a]
Post-dialysis	11.4 ± 3.6	10.7 ± 3.1	11.8 ± 3.2	10.9 ± 3.4	11.5 ± 3.0
Vitamin C	(n = 10)	(n = 10)	(n = 10)	(n = 9)	(n = 9)
Pre-dialysis	11.0 ± 3.8[a]	12.0 ± 2.6 (109)[a]	12.3 ± 3.2 (112)[a]	10.9 ± 3.8 (99)[a]	11.1 ± 2.9 (101)[a]
Post-dialysis	11.2 ± 3.8	11.1 ± 3.2	11.7 ± 3.0	10.2 ± 3.8	9.8 ± 3.4
Vitamin E	(n = 10)	(n = 9)	(n = 8)	(n = 8)	(n = 8)
Pre-dialysis	11.3 ± 4.1[a]	15.3 ± 3.9* (135)[b]	19.2 ± 4.1*[#] (170)[b]	17.8 ± 3.3[#] (158)[b]	16.5 ± 4.6[#] (146)[b]
Post-dialysis	9.9 ± 3.5	15.4 ± 3.4	18.6 ± 3.4	16.7 ± 3.5	15.3 ± 3.5
Vitamin C + E	(n = 10)	(n = 10)	(n = 9)	(n = 9)	(n = 9)
Pre-dialysis	9.8 ± 3.3[a]	13.3 ± 3.2* (136)[b]	17.0 ± 4.1*[#] (173)[b]	16.8 ± 3.7[#] (171)[b]	15.9 ± 3.6 (162)[b]
Post-dialysis	10.2 ± 4.0	12.9 ± 2.8	17.6 ± 3.9	16.6 ± 4.1	14.7 ± 3.8

[1] Values are mean ± SD.
[2] Values are the percentage of the respective group at week 0 from the pre-dialysis in hemodialysis patients.
Values of hemodialysis patients in each column not sharing the same letter significantly differ ($P < 0.05$) by Fisher's least significant difference test.
* Week 3, 6 (during the supplemented period) vs week 0 (baseline), $P < 0.05$.
[#] The hemodialysis group vs the control group, $P < 0.05$.

baseline, plasma total antioxidant status was below the normal range (1.30~1.77 mmol/L) from the pre-dialysis in eleven (29%) patients, and from the post-dialysis in all patients (100%). Total antioxidant status significantly enhanced with the duration of antioxidant vitamin supplements ($P < 0.05$). Vitamin C-, vitamin E-, and vitamin C + E-supplemented groups significantly elevated plasma total antioxidant status by 9%, 26%, and 24% ($P < 0.05$) at week 3, and by 21%, 33%, and 33% ($P < 0.05$) at week 6 compared with the respective baselines. Vitamin E- and vitamin C + E-supplemented groups had greater plasma total antioxidant status ($P < 0.05$) than the control, placebo, and vitamin C-supplemented groups at week 3. At week 6, all antioxidant vitamin supplements significantly elevated

Table 5
Erythrocyte glutathione concentrations of the control and hemodialysis subjects before, during, and after antioxidant vitamin supplements[1]

	Erythrocyte glutathione concentration, μmol/L (%)[2]				
	Week 0	Week 3	Week 6	Week 8	Week 10
Control	309 ± 40 (n = 36)				
Hemodialysis	228 ± 44[3] (n = 38)				
Placebo	(n = 8)	(n = 8)	(n = 8)	(n = 7)	(n = 7)
Pre-dialysis	236 ± 39[a#]	242 ± 33[#] (103)[a]	249 ± 43[#] (106)[a]	242 ± 21[#] (103)[a]	249 ± 20[#] (106)[a]
Post-dialysis	205 ± 36	212 ± 32	213 ± 38	218 ± 28	213 ± 23[¶]
Vitamin C	(n = 10)	(n = 10)	(n = 10)	(n = 9)	(n = 9)
Pre-dialysis	231 ± 54[a#]	289 ± 58* (125)[b]	304 ± 41* (131)[b]	260 ± 57 (113)[a]	256 ± 57 (111)[a]
Post-dialysis	210 ± 44	265 ± 45	279 ± 37	231 ± 52	233 ± 54
Vitamin E	(n = 10)	(n = 9)	(n = 8)	(n = 8)	(n = 8)
Pre-dialysis	234 ± 36[a#]	300 ± 50* (128)[b]	310 ± 57* (132)[b]	270 ± 46 (115)[a]	262 ± 48 (112)[a]
Post-dialysis	199 ± 38	262 ± 48	271 ± 50	234 ± 42	230 ± 45
Vitamin C + E	(n = 10)	(n = 10)	(n = 9)	(n = 9)	(n = 9)
Pre-dialysis	210 ± 48[a#]	273 ± 39* (130)[b]	294 ± 56* (140)[b]	241 ± 37[†#] (115)[a]	238 ± 38[†#] (113)[a]
Post-dialysis	184 ± 44	244 ± 39	267 ± 51	217 ± 33	214 ± 33

[1] Values are mean ± SD.
[2] Values are the percentage of the respective group at week 0 from the pre-dialysis in hemodialysis patients.
[3] Values from the pre-dialysis of all hemodialysis patients at week 0 vs the control group, $P < 0.05$.
Values of hemodialysis patients in each column not sharing the same letter significantly differ ($P < 0.05$) by Fisher's least significant difference test.
* Week 3, 6 (during the supplemented period) vs week 0 (baseline), $P < 0.05$.
[†] Week 8, 10 (the terminated period without supplementation) vs week 6 (the end of the supplemented period), $P < 0.05$.
[#] The hemodialysis group vs the control group, $P < 0.05$.
[¶] Post-dialysis vs pre-dialysis, $P < 0.05$.

Table 6
Plasma total antioxidant status of the control and hemodialysis subjects before, during, and after antioxidant vitamin supplements[1]

	Plasma total antioxidant status, mmol/L (%)[2]				
	Week 0	Week 3	Week 6	Week 8	Week 10
Control	1.46 ± 0.09 (n = 36)				
Hemodialysis	1.33 ± 0.14[3] (n = 38)				
Placebo	(n = 8)	(n = 8)	(n = 8)	(n = 7)	(n = 7)
Pre-dialysis	1.40 ± 0.21[a]	1.36 ± 0.16 (97)[a]	1.42 ± 0.18 (101)[a]	1.28 ± 0.16[#] (91)[a]	1.36 ± 0.17 (97)[a]
Post-dialysis	1.10 ± 0.24[¶]	1.04 ± 0.06[¶]	1.14 ± 0.10[¶]	1.09 ± 0.24	1.14 ± 0.21[¶]
Vitamin C	(n = 10)	(n = 10)	(n = 10)	(n = 9)	(n = 9)
Pre-dialysis	1.34 ± 0.09[a#]	1.46 ± 0.18* (109)[a]	1.62 ± 0.19*[#] (121)[b]	1.39 ± 0.23 (104)[ab]	1.33 ± 0.27[†] (99)[a]
Post-dialysis	1.08 ± 0.10[¶]	1.12 ± 0.17[¶]	1.33 ± 0.15[¶]	1.10 ± 0.17[¶]	106 ± 0.25[¶]
Vitamin E	(n = 10)	(n = 9)	(n = 8)	(n = 8)	(n = 8)
Pre-dialysis	1.26 ± 0.16[a#]	1.59 ± 0.14*[#] (126)[b]	1.68 ± 0.15*[#] (133)[b]	1.47 ± 0.13 (117)[bc]	1.37 ± 0.12 (109)[a]
Post-dialysis	1.17 ± 0.07[¶]	1.28 ± 0.12[¶]	1.47 ± 0.18[¶]	1.22 ± 0.16[¶]	1.24 ± 0.13
vitamin C + E	(n = 10)	(n = 10)	(n = 9)	(n = 9)	(n = 9)
Pre-dialysis	1.35 ± 0.11[a#]	1.67 ± 0.30*[#] (124)[b]	1.80 ± 0.25*[#] (133)[b]	1.67 ± 0.26[#] (124)[c]	1.52 ± 0.14[†#] (113)[a]
Post-dialysis	1.08 ± 0.14[¶]	1.32 ± 0.09[¶]	1.48 ± 0.34	1.40 ± 0.22[¶]	1.23 ± 0.27[¶]

[1] Values are mean ± SD.
[2] Values are the percentage of the respective group at week 0 from the pre-dialysis in hemodialysis patients.
[3] Values from the pre-dialysis of all hemodialysis patients at week 0 vs the control group, $P < 0.05$.
Values of hemodialysis patients in each column not sharing the same letter significantly differ ($P < 0.05$) by Fisher's least significant difference test.
* Week 3, 6 (during the supplemented period) vs week 0 (baseline), $P < 0.05$.
[†] Week 8, 10 (the terminated period without supplementation) vs week 6 (the end of the supplemented period), $P < 0.05$.
[#] The hemodialysis group vs the control group, $P < 0.05$.
[¶] Post-dialysis vs pre-dialysis, $P < 0.05$.

plasma total antioxidant status ($P < 0.05$) compared with the control and placebo groups. However, plasma total antioxidant status was not different among antioxidant vitamin supplemented groups. After the termination of the supplements, vitamin E- and vitamin C + E-supplemented groups still had greater plasma total antioxidant status ($P < 0.05$) than placebo group at week 8. At week 10, only vitamin C + E-supplemented group had greater plasma total antioxidant status ($P < 0.05$) than the control group. However, plasma total antioxidant status was not significantly different among the hemodialysis groups.

3.5. Plasma lipid peroxidation

Hemodialysis patients had significantly increased plasma lipid peroxidation by 102% ($P < 0.05$) compared with the control group at the baseline (Table 7). However, plasma lipid peroxide levels were not significantly different between the post- and pre-dialysis during 10 weeks (Table 7). At week 3, vitamin E-supplemented group significantly lowered plasma lipid peroxides ($P < 0.05$) compared with placebo and vitamin C-supplemented groups. Plasma lipid peroxide levels significantly decreased by 29%, 31%, and 47% ($P < 0.05$) in vitamin C-, vitamin E-, and vitamin C + E-supplemented groups at week 6 compared with those in placebo group and those in the respective baselines. Only in vitamin E- and vitamin C + E-supplemented groups plasma lipid peroxide levels were not significantly different from those of the control group from week 6 to week 10. Plasma lipid peroxide levels were still lower in antioxidant vitamin supplemented groups than placebo group at week 8. At week 10, placebo and vitamin C-supplemented groups significantly increased plasma lipid peroxide levels by 54% and 124% ($P < 0.05$) compared with the control group. Additionally, vitamin E- and vitamin C + E-supplemented groups had lower plasma lipid peroxides ($P < 0.05$) than placebo and vitamin C-supplemented groups.

4. Discussion

Hemodialysis patients in the study had normal plasma albumin and total cholesterol levels, suggesting that the patients were not malnutrition. It is also supported by the results of dietary intake, energy and macronutrients intake in hemodialysis patients was not different from the control group. Although protein, vitamin C, and crude fiber intake tended to be slightly lower in hemodialysis patients, there was not significantly different from the control group. Energy, macronutrients, crude fiber, vitamin C and vitamin E intake was very similar between hemodialysis and control groups. Therefore, the difference observed in this study was totally due to the supplementation rather than dietary intake.

Oral dosage of vitamins each time in this study was 4- and 5-fold of DRI for vitamin C in males (90 mg) and females (75 mg), and 18-fold of DRI for vitamin E (15 mg α-tocopherol), respectively [29]. Because a certain part of oxalate in the urine derived from metabolized vitamin C, high intake of vitamin C was susceptible to cause secondary

Table 7
Plasma lipid peroxide concentrations of the control and hemodialysis subjects before, during, and after antioxidant vitamin supplements[1]

	Plasma malondialdehyde + 4-hydroxy-2(E)-nonenal concentration, μmol/L (%)[2]				
	Week 0	Week 3	Week 6	Week 8	Week 10
Control	23.6 ± 9.8 (n = 36)				
Hemodialysis	47.7 ± 21.4[3] (n = 38)				
Placebo	(n = 8)	(n = 8)	(n = 8)	(n = 7)	(n = 7)
Pre-dialysis	37.7 ± 19.8[a#]	40.0 ± 19.7[#] (106)[b]	42.4 ± 22.2[#] (112)[c]	44.0 ± 26.8[#] (117)[c]	36.3 ± 24.6[#] (96)[b]
Post-dialysis	38.4 ± 20.2	42.6 ± 20.2	41.2 ± 23.7	44.4 ± 23.6	38.0 ± 24.5
Vitamin C	(n = 10)	(n = 10)	(n = 10)	(n = 9)	(n = 9)
Pre-dialysis	56.3 ± 20.0[a#]	52.9 ± 16.4[#] (94)[b]	40.0 ± 11.1*[#] (71)[b]	45.3 ± 18.9[#] (80)[b]	52.9 ± 19.8[†#] (94)[b]
Post-dialysis	56.1 ± 19.7	54.3 ± 19.0	41.6 ± 16.8	43.2 ± 20.1	59.5 ± 20.2
Vitamin E	(n = 10)	(n = 9)	(n = 8)	(n = 8)	(n = 8)
Pre-dialysis	43.5 ± 17.3[a#]	36.0 ± 15.4[#] (83)[a]	30.2 ± 12.4* (69)[ab]	22.7 ± 17.1 (52)[a]	25.5 ± 19.1 (59)[a]
Post-dialysis	44.7 ± 16.6	36.0 ± 13.2	28.1 ± 11.7	24.2 ± 19.4	24.1 ± 16.7
Vitamin C + E	(n = 10)	(n = 10)	(n = 9)	(n = 9)	(n = 9)
Pre-dialysis	53.1 ± 28.4[a#]	42.7 ± 20.9[#] (80)[a]	28.0 ± 16.1* (53)[a]	25.3 ± 19.4 (48)[a]	32.3 ± 21.6 (61)[a]
Post-dialysis	52.4 ± 26.0	40.2 ± 21.7	29.4 ± 15.2	25.7 ± 22.8	33.2 ± 20.1

[1] Values are mean ± SD.
[2] Values are the percentage of the respective group at week 0 from the pre-dialysis in hemodialysis patients.
[3] Values from the pre-dialysis of all hemodialysis patients at week 0 vs the control group, $P < 0.05$.
Values of hemodialysis patients in each column not sharing the same letter significantly differ ($P < 0.05$) by Fisher's least significant difference test.
* Week 3, 6 (during the supplemented period) vs week 0 (baseline), $P < 0.05$.
[†] Week 8, 10 (the terminated period without supplementation) vs week 6 (the end of the supplemented period), $P < 0.05$.
[#] The hemodialysis group vs the control group, $P < 0.05$.
[¶] Post-dialysis vs pre-dialysis, $P < 0.05$.

hyperoxalemia and oxalosis in hemodialysis patients. Costello et al. [30] reported that increased plasma vitamin C status was not correlated with plasma oxalate level. However, Ono [31] found that there was a strong correlation between plasma vitamin C and oxalate levels ($r = 0.755$, $P < 0.01$). Vitamin C at a dose of 100 mg/day for 1 month had no significant effect on oxalate metabolism, including plasma concentration, metabolic pool size, tissue accumulation rate, production rate, and dialysis clearance in hemodialysis patients [32]. However, plasma oxalate levels changed from 50.4 ± 8.2 μmol/L to 34.1 ± 1.4, 33.3 ± 3.7, and 25.7 ± 3.9 μmol/L, respectively, in hemodialysis patients with daily oral vitamin C intake from 500 mg for more than 6 months to 100, 50, and 0 mg for 4 weeks [31]. Similarly, oral administration of vitamin C at a dose of 500 mg/day for 3 weeks, plasma oxalate rose form 30.3 ± 3.5 to 48.4 ± 6.1 μmol/L in hemodialysis patients [33]. Oxalate levels in pre- and post-dialysate significantly increased following an increase of vitamin C dosage from 100 to 500 mg/day [34]. It is suggested that hemodialysis patients who have a defect in vitamin C or oxalate metabolism may restrict daily vitamin C intake below 500 mg. Whereas vitamin C bioavailability in healthy males was 80%, 72%, and 63%, respectively, for oral administration of pure vitamin C at 100, 200, and 500 mg as a single dose [35,36]. In our study, oral intake vitamin C at 400 mg/time three times a week should not be risk for oxalosis or calcium oxalate kidney stones in hemodialysis patients, although we did not measure plasma oxalate.

Our study showed that plasma vitamin C levels significantly decreased by 24% during hemodialysis. Similar to a previous finding [10], the mean decrease in plasma total vitamin C was 40% during a 3-h hemodialysis session. Additionally, plasma vitamin C levels significantly decreased by 32% in hemodialysis patients compared with the control group at the baseline. Although dietary intake of vitamin C in hemodialysis patients was similar to the control group, lower plasma vitamin C levels probably resulted from the loss during hemodialysis because of its water-soluble property, poor absorption of vitamin C in the gastrointestinal tract, or/and the utilization for the defense of free radicals in hemodialysis patients. After 6-week supplementation, vitamin C- and vitamin C + E-supplemented groups had significantly greater plasma vitamin C levels than the baselines and placebo group. Additionally, plasma vitamin C concentrations were not different among vitamin C-, vitamin C + E-supplemented, and the control groups at week 6, indicating vitamin C supplement alone or with vitamin E could elevate plasma vitamin C levels to the normal concentration in hemodialysis patients. After the termination of the supplements, plasma vitamin C levels maintained at the normal levels at least for another 2 weeks in vitamin C- and vitamin C + E-supplemented groups, which was supported by the half-life data of plasma vitamin C in previous studies [37,38]. Hornig [37] demonstrated that the overall half-life of plasma vitamin C was 10–20 days for daily supplements of 30–180 mg, and dependent upon plasma vitamin C levels. Blanchard [38] also showed that the half-life of vitamin C was inversely related to entry vitamin C levels in the plasma below 85 μmol/L, whereas above this concentration half-life was approximately constant and averaged 14.2 days [38]. In contrast with plasma

vitamin C levels, plasma vitamin E concentrations were not different between the pre- and post-dialysis, as well as the hemodialysis and the control groups. Previous studies also found that plasma α-tocopherol concentrations unchanged during hemodialysis [7–10]. However, Pastor et al. [39] showed erythrocyte vitamin E levels were lower in hemodialysis patients than in the control group. Plasma vitamin E levels significantly increased in vitamin E- and vitamin C + E-supplemented groups at week 6. Similar to our results, both plasma and RBC vitamin E concentrations significantly increased in hemodialysis patients receiving daily oral supplement of vitamin E at 600 mg for 30 days [20]. Additionally, serum vitamin E significantly increased in hemodialysis patients with oral vitamin E at a dose of 300 [21] or 800 mg/day [5] for 8 or 3 weeks, respectively. Peuchant et al. [18] also found that increased erythrocyte vitamin E level was observed in CRF patients on the restricted protein diet (0.3 g/day) supplemented with iron and multivitamins, including 7.5 mg vitamin A, 1000 IU vitamin D_3, 5 mg α-tocopheryl acetate, 75 mg vitamin C, and vitamin B complex (B_1, B_2, B_6, and B_{12}) daily for 6 months compared with pre-diet results. Our data indicated that the increased effect of plasma vitamin E lasted for another 4 weeks during the termination of the supplements probably due to its water-insoluble property.

Erythrocyte glutathione concentrations significantly decreased in hemodialysis patients compared with the control group, indicating hemodialysis patients may have impaired glutathione-related antioxidant enzyme system. The activities of plasma and erythrocyte glutathione peroxidase (GSH-Px) significantly decreased in hemodialysis patients compared with the healthy subjects [13]. Erythrocyte or polymorphonuclear leukocyte GSH-Px activities were also lower in the patients after hemodialysis than the control group [11–13,17]. Plasma GSH-Px activity significantly elevated during hemodialysis [13]. All antioxidant vitamin supplemented groups significantly increased erythrocyte glutathione levels to the normal level at week 6, suggesting that antioxidant vitamin supplementation may reserve the consumption of glutathione for the protection from free radical damage. After the termination of the supplements, erythrocyte glutathione concentrations were not significantly different among the hemodialysis groups. Both vitamin C- and vitamin E-supplemented groups had similar erythrocyte glutathione levels as the control group from week 3 to week 10.

Plasma total antioxidant status significantly decreased by 9% in hemodialysis patients compared with the control group at the baseline, and impaired by 18% during hemodialysis. It is indicated that plasma water-soluble antioxidants may be lost during hemodialysis, which results in impaired plasma antioxidant status in hemodialysis patients. After 6-week supplementation, all antioxidant vitamin supplements significantly enhanced plasma total antioxidant status, which could be accompanied by the increases in plasma vitamin C, vitamin E, and erythrocyte glutathione levels. Although plasma total antioxidant status was lower at the baseline in hemodialysis patients, vitamin C + E supplementation could improve plasma total antioxidant status even greater than the control group throughout the supplementation and termination periods.

In consistent with the results of total antioxidant status, the data showed that plasma lipid peroxide levels significantly increased by 102% in hemodialysis patients compared with the control group. Similar to our results, previous studies indicated that lipid peroxidation products in plasma [4,5,10,16] and RBC [17,18] increased in hemodialysis patients compared with the healthy subjects. It is suggested that oxidative damage occurs during hemodialysis. At week 6, all antioxidant vitamin supplemented groups significantly decreased plasma lipid peroxide levels compared with the respective baselines and placebo group. A previous study demonstrated that significantly reduced erythrocyte lipid peroxidation was found in CRF patients on the restricted protein diet (0.3 g/day) supplemented with iron and vitamin E-containing multivitamins [18]. Supplementation with vitamin E at 300 mg/day for 1 month significantly inhibited both plasma and erythrocyte lipid peroxidation in hemodialysis patients [22]. Additionally, daily oral administration of vitamin E at 600 mg for 2 weeks resulted in a significant decrease of LDL lipid peroxidation in hemodialysis patients [23]. However, Galli et al. [5] found that oral supplementation with 800 mg/day vitamin E for 3 weeks did not significantly affect plasma lipid peroxide-thiobarbituric acid reactants in hemodialysis patients. After the termination of the supplements, vitamin E- and vitamin C + E-supplemented groups still had similar plasma lipid peroxide levels as the control group. However, plasma lipid peroxide levels were not different between vitamin E- and vitamin C + E-supplemented groups. Whereas placebo and vitamin C-supplemented groups had higher plasma lipid peroxide levels than the control group throughout 10 weeks. The data suggest that the inhibition of plasma lipid peroxidation in hemodialysis patients may be primarily contributed by the antioxidant action of vitamin E.

In conclusion, vitamin C (400 mg L-ascorbate/time) + E (400 mg d,l-α-tocopheryl acetate/time) supplements three times a week for 6 weeks not only effectively corrected the decreased plasma vitamin C, erythrocyte glutathione and plasma total antioxidant status at least to the normal levels, but also inhibited the increased plasma lipid peroxidation to the normal level in hemodialysis patients. Additionally, vitamin C + E supplements had the overall longer lasting period for maintaining antioxidant status after the termination of the supplements. For clinical application, it is feasible and inexpensive that oral administration of combined vitamin C (400 mg) and E (400 mg) after finishing hemodialysis session is helpful to prevent the development of cardiovascular disease by improving antioxidant status and decreasing oxidative damage in hemodialysis patients.

Acknowledgments

We thank the nurses for assisting oral administration of the supplements in the center of hemodialysis at Taipei Medical University Hospital.

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