

# Effects of Vitamins C and E on Oxidative Stress Markers and Endothelial Function in Patients with Systemic Lupus Erythematosus: A Double Blind, Placebo Controlled Pilot Study

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**ABSTRACT.** *Objective.* Patients with systemic lupus erythematosus (SLE) experience excess morbidity and mortality due to coronary artery disease (CAD) that cannot be fully explained by the classical CAD risk factors. Among emerging CAD risk factors, oxidative stress is currently being emphasized. We evaluated the effects of longterm antioxidant vitamins on markers of oxidative stress and antioxidant defense and endothelial function in 39 patients with SLE.

*Methods.* Patients were randomized to receive either placebo or vitamins (500 mg vitamin C and 800 IU vitamin E daily) for 12 weeks. Markers of oxidative stress included malondialdehyde (MDA) and allantoin. Antioxidants measured included erythrocyte superoxide dismutase and glutathione peroxidase, plasma total antioxidant power (as FRAP value), and ascorbic acid and vitamin E concentrations. Endothelial function was assessed by flow-mediated dilatation (FMD) of the brachial artery and plasma concentration of von Willebrand factor (vWF) and plasminogen activator inhibitor type 1 (PAI-1). Primary outcome of the study included the change in lipid peroxidation as revealed by MDA levels. Secondary outcomes included changes in allantoin and antioxidant levels and change in endothelial function.

*Results.* After treatment, plasma ascorbic acid and  $\alpha$ -tocopherol concentrations were significantly ( $p < 0.05$ ) increased only in the vitamin-treated group, associated with a significant decrease ( $p < 0.05$ ) in plasma MDA. Other oxidative stress markers and antioxidant levels remained unchanged in both groups. FMD and vWF and PAI-1 levels remained unchanged in both groups.

*Conclusion.* Combined administration of vitamins C and E was associated with decreased lipid peroxidation, but did not affect endothelial function in patients with SLE after 3 months of therapy. (J Rheumatol 2005;32:275–82)

## Key Indexing Terms:

SYSTEMIC LUPUS ERYTHEMATOSUS  
ENDOTHELIAL FUNCTION

OXIDATIVE STRESS      ANTIOXIDANTS  
CONTROLLED TRIAL

Systemic lupus erythematosus (SLE) is a chronic, systemic, inflammatory disorder characterized by a wide range of

autoantibody production. Patients with SLE have an increased risk of cardiovascular diseases (CVD), particularly myocardial infarction. The risks of hospitalization resulting from acute myocardial infarction and stroke in young female North American patients were roughly 8 and 2 times higher, respectively, than age matched controls even after adjustment for multiple conventional risk factors, suggesting that other factors associated with lupus may predispose to these events<sup>1</sup>.

The endothelium plays an integral role in the regulation of vascular tone, fibrinolysis, and thrombosis, and is intimately involved in the development of atherosclerosis. Endothelial dysfunction and lipid peroxidation are key events in the initiation, progression, and rupture of atherosclerotic plaque<sup>2</sup>. Lipid peroxidation results from increased oxidative stress, and there is accumulating evidence that this also accounts for endothelial dysfunction<sup>3</sup>. The inflammatory action of SLE implies that a state of oxidative stress exists in this disease. Studies have shown that oxidative stress

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indices including lipid peroxidation in plasma and red blood cells and superoxide and hydrogen peroxide generation by peripheral leukocytes are significantly higher in SLE patients compared with healthy controls<sup>4-6</sup>, and this has been reported to be a risk factor for CVD in patients with SLE<sup>4,6</sup>. Oxidant byproducts such as superoxide anion and hydrogen peroxide are produced in the body as a consequence of normal aerobic metabolism. These molecules are highly reactive with other biologic molecules, and are referred to as reactive oxygen species (ROS). Under normal physiologic conditions, the production of oxygen free radicals and peroxides is balanced by an efficient system of antioxidants, which are molecules capable of "scavenging" ROS, thereby preventing oxidative damage<sup>7</sup>. Naturally-occurring antioxidants include enzymatic antioxidants, such as superoxide dismutase (SOD), which plays an important role in the conversion of ROS to oxygen and water, and glutathione peroxidase (GPX), which reduces all organic lipid peroxides. Nonenzymatic antioxidants are also important in scavenging free radicals, including the lipid-soluble antioxidant vitamin E and the water-soluble antioxidants vitamin C and glutathione. Oxidative stress is defined as the tissue damage resulting from an imbalance between an excessive generation of oxidant compounds and insufficient antioxidant defense mechanisms<sup>8</sup>. In patients with SLE, oxidative stress resulted not only from excess generation of oxidant compounds, but was also associated with lower antioxidant enzyme levels<sup>9</sup>.

Endothelium-derived nitric oxide (EDNO) is a pivotal molecule in the regulation of vascular tone and homeostasis<sup>10</sup>. Endothelial vasodilator dysfunction has been observed in patients with coronary artery disease (CAD) or subjects with coronary risk factors, including patients with SLE<sup>11,12</sup>. Most of these conditions are associated with increased oxidative stress, particularly increased production of superoxide radicals, which can inactivate EDNO<sup>13</sup>. In addition, oxidized low density lipoprotein (OxLDL) has been shown to inhibit the synthesis of EDNO or attenuate its biological activity<sup>14</sup>. Vitamin C can decrease the levels of superoxide radicals and OxLDL<sup>15</sup>, both of which react with and inactivate NO in high concentration. Vitamin E decreases ROS and apoptosis in endothelial cells induced by OxLDL<sup>16</sup>, and preserves NO by scavenging ROS and inhibiting OxLDL formation. The combination of vitamins C and E has been shown to protect endothelial cells from the cytotoxic effects of OxLDL<sup>17</sup>. Data suggest that supplementation with vitamin C or E alone can improve endothelial dysfunction in some conditions associated with increased oxidative stress<sup>18-20</sup>, but not in other conditions<sup>21-24</sup>. The controversy may be related to inadequate dosage, although the plasma vitamin concentrations had risen several-fold, but previous trials seldom assessed the efficacy of antioxidant supplementation by measuring markers of lipid peroxidation.

Moreover, when vitamin E works as an antioxidant, it is oxidized to a potentially harmful radical, which needs to be reduced back to  $\alpha$ -tocopherol by vitamin C<sup>25</sup>, hence the use of vitamins C and E in combination was thought to have a synergistic action. Whether the combination of vitamins C and E is effective as an antioxidant in SLE patients who are in a chronic state of increased oxidative stress is of great interest, since this may be a potential therapeutic intervention to reduce cardiovascular morbidity and mortality.

Estimates of different types of endothelial dysfunction may be obtained indirectly by measuring endothelial-dependent vasodilatation and high levels of endothelial-derived regulatory proteins including von Willebrand factor (vWF) and plasminogen activator inhibitor type 1 (PAI-1). Our study was undertaken to test the hypothesis that a combination of vitamins C and E could reduce lipid peroxidation in a group of Chinese patients with SLE. We also examined the effect of a combination of vitamins C and E on other oxidative stress markers, as well as the effect on the endothelial function in patients with SLE.

## MATERIALS AND METHODS

Patients were recruited from the Rheumatology Clinic of the Prince of Wales Hospital, Hong Kong. All patients fulfilled the 1997 American College of Rheumatology revised criteria for the classification of SLE<sup>26</sup>. All eligible women who were 18 years of age or older were invited to participate in this randomized, double blind, placebo controlled trial regardless of their history of cardiovascular events. Patients who were taking vitamin C or E or other drugs with antioxidant properties [except angiotensin-converting enzyme inhibitors (ACEI) and statins] were required to stop the medications for 3 months before participating. Patients were randomized into 2 study arms to receive either the vitamin combination or a matched placebo for a 12 week period. The vitamin combination contained 500 mg of vitamin C (Natrol, Chatsworth, CA, USA) and 800 IU of vitamin E (D-alpha tocopheryl succinate; Twin Laboratories, Ronkonkoma, NY, USA). The clinical assessment, flow-mediated dilatation (FMD), and patients' biochemical measures were evaluated at baseline and at 12 weeks. Compliance was assessed by tablet counting, and subjects with less than 70% compliance were excluded from analyses. Patients received their usual medications throughout the study. If there was a flare of SLE requiring increase in immunosuppressive agents, that patient was excluded from the analyses, since changes in disease activity and immunosuppressant affect oxidative stress markers and endothelial function; evaluation for adverse events was carried out in these patients. Each participant provided an authorization for release of medical information, and pertinent hospital and outpatient records were reviewed for any subject reporting a prior cardiovascular event. The Ethics Committee of the Chinese University of Hong Kong approved this study, and all women provided written informed consent.

Forty consecutive female patients with stable SLE whose last disease flare was  $\geq 3$  months in the past were recruited. One patient had a disease flare at recruitment and was excluded from the study; thus a total of 39 patients participated.

*Traditional cardiovascular risk factors.* The clinic visit included anthropometric measurements (height and weight), blood pressure, and a fasting blood draw. Blood samples were used to measure total cholesterol, high density lipoprotein cholesterol, triglycerides, urate, and glucose with standardized laboratory procedures. Hypertension status was defined as systolic blood pressure  $\geq 140$  mm Hg or diastolic blood pressure  $\geq 90$  mm Hg or the use of antihypertensive agents. Information was also collected on age,

smoking habits, family history of CVD (i.e., myocardial infarction in a first-degree relative < 60 years of age), menopause status (follicle-stimulating hormone levels were obtained when menopausal status was uncertain), estrogen replacement, and diabetes.

**SLE activity and complications.** The SLE Disease Activity Index (SLEDAI) and the Systemic Lupus International Collaborating Clinics/American College of Rheumatology (SLICC/ACR) damage index were used to indicate disease activity and damage, respectively. Laboratory studies included complete blood count, renal and liver function tests, lupus anticoagulant (LAC) test (partial thromboplastin time or Russell's viper venom time with mix), C3, C4, and antibody tests for anticardiolipin (aCL; IgG > 15 GPL units; Euroimmun, Sonnenberg, Germany), antiphosphorylcholine antibodies (Dynatech Laboratories, Alexandria, VA, USA), and anti-native DNA (*Criethidia luciliae*). Proteinuria and creatinine clearance were estimated from a 24 hour urine collection. The current and cumulative steroid dosage and duration taking steroid were obtained from chart review. Concomitant use of hydroxychloroquine and immunosuppressants was noted.

**Inflammatory markers.** C-reactive protein (CRP) was measured by ELISA and fibrinogen levels by a modified clot-rate assay.

**Endothelial-derived regulatory proteins.** vWF and PAI-1 were measured by ELISA using commercial kits (Diagnostica Stago, Freres Chausson, France).

**Markers of oxidative stress and antioxidants.** Markers of oxidative stress included malondialdehyde (MDA), a commonly used index of lipid peroxidation, and allantoin, the nonenzymatic oxidation product of uric acid. MDA, uric acid, and allantoin were measured using high performance liquid chromatography (HPLC) techniques<sup>27,28</sup>. Antioxidants measured included erythrocyte SOD, GPX, plasma total antioxidant power (as the FRAP value), ascorbic acid, and vitamin E levels (total and lipid standardized). SOD and GPX were measured using commercial kits (Randox, Antrim, Northern Ireland). Plasma FRAP and ascorbic acid were measured by a modification of the FRAP assay (Benzie IFF and Strain JJ, US patent) as described<sup>29</sup>. Vitamin E as  $\alpha$ -tocopherol was measured by HPLC technique<sup>30</sup>.

**Flow-mediated dilatation.** All examinations were performed using a high resolution 5–12 MHz linear array transducer (ATL 5000, Advanced Technology Laboratories, Bothell, WA, USA). Ultrasound examination was performed by a single examiner blinded to clinical details and the stage of the experiment. The subject rested supine for 15 min before the first measurement. Baseline arterial diameter and arterial flow velocity were measured by a pulsed Doppler signal at a 60° insonation angle to the vessel, with gate length 1.5 mm positioned in the center of the brachial artery. Four cardiac cycles were analyzed for each examination and the measurements were averaged (baseline examination). Then a pneumatic tourniquet placed around the mid-arm was inflated to a pressure of at least 250 mm Hg for 5 min. A second measurement of arterial diameter was taken 1 min before and 90 s after cuff deflation, as well as a flow velocity recording for the first 15 s after cuff release (reactive hyperemia examination). Reactive hyperemia was calculated as the maximum flow recorded in the first 15 s after deflation divided by the flow during the resting (baseline) examination. The patient then rested for 15 min and a further resting examination was undertaken (15-min rest examination). Sublingual glyceryl trinitrate (GTN) spray (400  $\mu$ g) was then administered, and after 4 min a fourth examination was performed (GTN examination). The arterial diameter was measured using ultrasonic calipers, from the anterior to the posterior “m” line at end diastole, incident with the R-wave on the electrocardiogram. Arterial diameters in examinations after reactive hyperemia, 15-min rest, and GTN were expressed as percentages of the baseline examination. Flow was calculated from Doppler flow velocity and arterial diameter; since velocity was taken from the center of the artery absolute values may be overestimated, but relative values before and after cuff inflation are accurate. The coefficient of variation of the measurements was determined by making 5 repeated measurements in 5 subjects over a period of 10 min.

Using analysis of variance, the between-measurement of variance was found to be 0.02 mm with confidence limits of 0.24 mm.

**Randomization.** The method of concealed random allocation was used. Simple randomization was conducted by a computer-generated random list from the Chinese University of Hong Kong School of Pharmacy. The project coordinator and other investigators were blind to the randomization and the group assignments. The code was broken at the end of the clinical trial.

**Outcomes.** Primary outcomes of the study included the change in MDA concentrations. Secondary outcomes included changes in allantoin and antioxidant levels and changes in endothelial function.

**Statistical analyses.** Results are expressed as mean  $\pm$  SD for normally distributed data; non-normally distributed data are expressed as median (interquartile range, IQR). Differences between the 2 groups at baseline were assessed by Student t test for normally distributed data. Mann-Whitney U test was used for non-normally distributed data. Chi-square test or Fisher's exact test was used for categorical variables where appropriate. The changes observed before and after vitamin or placebo supplements were assessed using paired t tests for normally distributed data and Wilcoxon signed-rank test for non-normally distributed data. A p value < 0.05 was considered statistically significant. All tests were 2-tailed. Changes in brachial artery diameter were expressed as percentage change in diameter of the artery from baseline obtained just before increasing flow (FMD) or GTN. SPSS for Windows v. 10.0 (SPSS Inc., Chicago, IL, USA) was used for analyses.

## RESULTS

**Demographic, SLE, and cardiovascular characteristics.** Thirty-nine female patients with SLE were recruited. The mean age was  $46 \pm 9$  years and the mean body mass index (BMI) was  $22.2 \pm 3.1$ . The mean disease duration was  $14 \pm 8$  years. One patient had preexisting CAD. Seventeen (44%) were postmenopausal and one patient was undergoing hormone replacement therapy. Two patients (5%) were current smokers. Six (15%) patients had family history of CAD. Twenty-five (64%) patients had preexisting hypertension and were taking antihypertensive agents including calcium channel blockers (n = 12), ACEI (n = 17), diuretics (n = 8), and  $\beta$ -blockers (n = 11). Four (10%) patients had hyperlipidemia and were given statins. Sixteen (41%) patients were taking hydroxychloroquine. Seven patients (18%) were taking vitamin D supplements for prophylaxis of steroid-induced osteoporosis. No patient was taking vitamin C or E or drugs with antioxidant properties. Twenty-nine (74%) patients were currently taking prednisolone, with a mean daily dose of  $6.4 \pm 4.4$  mg. Fifteen (38.5%) patients were currently taking immunosuppressants, including azathioprine (n = 12), cyclophosphamide (n = 2), and cyclosporin A (n = 1).

At the time of the first assessment, the majority of patients had only mild to moderate disease activity, with a median SLEDAI score of 4 (IQR 2–8). Twenty-four (62%) patients had nephritis, with a median serum creatinine level of 83 (IQR 73–107)  $\mu$ mol/l and proteinuria of 0.5 (IQR 0.1–1.3) g/day. Thirteen (33%) had elevated LDL ( $\geq 3.4$  mmol/l). Two patients were positive for LAC. aCL and antiphosphorylcholine antibodies were negative in all patients.

Twenty patients were randomized to receive antioxidants

and 19 were randomized to receive matching placebo. Table 1 presents the demographic, SLE-specific, and cardiovascular variables for the 2 groups. All 39 SLE patients completed the study, with an overall compliance of 95% by pill counts. No adverse event was reported. The baseline demographic and clinical profile was similar between the 2 groups, except the number of patients currently taking prednisolone was higher in the placebo group. The CAD risk factor profile was also similar between the 2 groups. CRP levels were similar between the 2 groups, but the fibrinogen level was significantly higher in the placebo group, suggesting a higher degree of inflammation. There was no significant change in disease activity, biochemical markers of inflammation, CAD risk factors, or treatment during the whole study period for the 2 groups of patients (data not shown).

*Effects on oxidative stress and antioxidant markers.* Table 2

summarizes the oxidative stress and antioxidant status of the 2 groups. At baseline, MDA levels were similar between the 2 groups; however, the allantoin level was significantly higher in the placebo group, suggesting a tendency of higher oxidative stress level. The overall antioxidant power and other antioxidant levels were similar between the 2 groups. After 12 weeks of vitamin supplements, the antioxidant vitamin C and E levels were significantly increased in the vitamin group, and there were significantly lower plasma levels of MDA in the vitamin group (Figure 1), whereas allantoin, FRAP, and other antioxidant levels remained unchanged. No significant change in the vitamin levels, oxidative stress markers, and antioxidant levels was observed in the placebo group.

*Effects on endothelial function: Flow-mediated vasodilatation.* Under basal conditions, the brachial artery diameters of the 2 groups were similar (Table 3). After vitamin supplementation, no significant change in either endothelium-

Table 1. Baseline demographic, clinical, and cardiovascular characteristics in the vitamin supplement and placebo groups. Data are expressed as mean  $\pm$  SD, percentage, or median (interquartile range).

	Vitamin Group, n = 20	Placebo Group, n = 19
Demographic and clinical profile		
Age, yrs	44 $\pm$ 6	48 $\pm$ 11
Disease duration, yrs	14 $\pm$ 8	13 $\pm$ 8
SLEDAI	4 (2–8)	4 (4–9)
SLICC	1 (1–2)	2 (1–4)
Creatinine, $\mu$ mol/l	77 (69–102)	92 (80–107)
Anti-dsDNA, %	65	63
Lupus anticoagulant, %	10	0
Anticardiolipin antibodies, %	0	0
C3, g/l	0.88 $\pm$ 0.34	0.77 $\pm$ 0.28
C4, g/l	0.17 $\pm$ 0.13	0.17 $\pm$ 0.09
Current medications, %		
Prednisolone	55**	95
Median current dose, mg/day	3.8 (3.8–5.0)	5.0 (5.0–5.0)
Median cumulative dose, g	16.5 (8.2–38.0)	29.6 (21.4–48.0)
Hydroxychloroquine	55	26
Immunosuppressives	20	53
CAD risk factors		
Smokers, %	5	5
BMI, kg/m <sup>2</sup>	22 $\pm$ 3	22 $\pm$ 3
Postmenopausal, %	40	47
CAD, %	5	0
Hypertension, %	60	84
Antihypertensive medications, %	50	79
Systolic blood pressure, mmHg	136 $\pm$ 21	135 $\pm$ 25
Diastolic blood pressure, mmHg	78 $\pm$ 11	78 $\pm$ 12
Glucose, mmol/l	4.5 $\pm$ 0.5	4.4 $\pm$ 0.4
Total cholesterol, mmol/l	5.4 $\pm$ 1.2	5.7 $\pm$ 1.3
Total triglycerides, mmol/l	1.9 $\pm$ 2.3	1.6 $\pm$ 0.7
HDL cholesterol, mmol/l	1.6 $\pm$ 0.5	1.8 $\pm$ 0.6
LDL cholesterol, mmol/l	3.2 $\pm$ 1.1	3.2 $\pm$ 0.9
Inflammatory markers		
C-reactive protein, mg/l	3.0 (3.0–4.1)	3.0 (3.0–9.9)
Fibrinogen, g/l	4.3 $\pm$ 0.9*	5.2 $\pm$ 1.2

\* p = 0.008 by Student's t test; \*\* p = 0.008 by Fisher's exact test comparing vitamin supplement and placebo groups.

Table 2. Effects of antioxidant vitamin supplements on biomarkers of oxidative stress and antioxidant status. Data are expressed in mean  $\pm$  SD, except for allantoin, expressed as median (interquartile range).

	Vitamin Group, n = 20	Placebo Group, n = 19
Oxidative stress markers		
MDA, $\mu\text{mol/l}$ , entry	1.52 $\pm$ 0.27	1.57 $\pm$ 0.25
MDA, $\mu\text{mol/l}$ , week 12	1.42 $\pm$ 0.29*	1.50 $\pm$ 0.32
Allantoin, $\mu\text{mol/l}$ , entry	13.5 (11.0–16.5)	23.0 (14.0–32.0)***
Allantoin, $\mu\text{mol/l}$ , week 12	14.0 (9.3–16.8)	21.0 (12.0–52.0)
Antioxidants		
Ascorbic acid, $\mu\text{mol/l}$ , entry	65.2 $\pm$ 15.5	53.6 $\pm$ 19.2
Ascorbic acid, $\mu\text{mol/l}$ , week 12	80.2 $\pm$ 24.3*	62.9 $\pm$ 23.8
Lipid standardized vitamin E, $\mu\text{mol/mmol TC} + \text{TG}$ , entry	5.23 $\pm$ 1.61	4.94 $\pm$ 1.65
Lipid standardized vitamin E, $\mu\text{mol/mmol TC} + \text{TG}$ , week 12	7.89 $\pm$ 2.16**	5.15 $\pm$ 1.67
Superoxide dismutase, U/g Hb, entry	1415 $\pm$ 216	1339 $\pm$ 232
Superoxide dismutase, U/g Hb, week 12	1452 $\pm$ 247	1333 $\pm$ 253
Glutathione peroxidase, U/g Hb, entry	62.7 $\pm$ 16.6	72.5 $\pm$ 14.0
Glutathione peroxidase, U/g Hb, week 12	68.2 $\pm$ 20.9	74.2 $\pm$ 16.6
FRAP, $\mu\text{mol/l}$ , entry	1233 $\pm$ 252	1279 $\pm$ 352
FRAP, $\mu\text{mol/l}$ , week 12	1238 $\pm$ 269	1199 $\pm$ 239

TC: total cholesterol, TG: total triglyceride, Hb: hemoglobin. \*  $p < 0.05$ , \*\*  $p < 0.005$ , comparing before and after vitamin supplement for 12 weeks by paired  $t$  tests; \*\*\*  $p < 0.005$  by Mann-Whitney  $U$  test comparing placebo and vitamin groups at baseline.

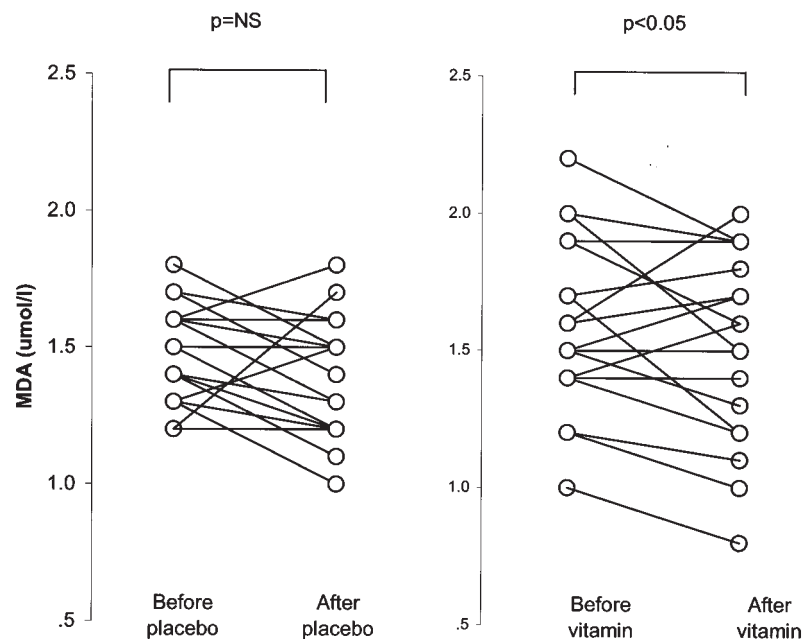


Figure 1. Changes in plasma MDA level in the vitamin supplement group (right) and the placebo group (left) ( $p < 0.05$  comparing before and after vitamin supplement).

dependent flow-mediated vasodilatation (FMD) or endothelium-independent vasodilatation [by glyceryl trinitrate-mediated dilatation (GMD)] was observed. FMD and GMD were also unchanged in the placebo group.

**Endothelial-derived regulatory proteins.** At baseline, the vWF level was significantly higher in the placebo group

(Table 3). There was no significant change in the vWF levels after vitamin supplements. There was a tendency for reduction in the vWF level in the placebo group ( $p > 0.05$ ).

The levels of PAI-1 were similar for the 2 groups at baseline, and remained unchanged after vitamin supplements or placebo (Table 3).

Table 3. Effects of antioxidant vitamins on endothelial function and endothelial-derived regulatory proteins. Values are expressed as median (interquartile range).

	Vitamin Group, n = 20	Placebo Group, n = 19
Basal brachial artery diameter, mm	3.38 (3.20–3.75)	3.55 (3.42–3.90)
FMD, %, entry	9.00 (7.41–10.67)	9.33 (7.27–11.11)
FMD, %, week 12	8.70 (5.76–9.64)	8.82 (5.88–11.54)
GMD, %, entry	18.8 (14.8–21.9)	18.8 (15.5–23.9)
GMD, %, week 12	18.3 (14.7–25.0)	20.6 (17.6–26.5)
vWF, %, entry	9.73 (8.23–16.16)	17.68 (11.74–22.52)*
vWF, %, week 12	12.54 (7.77–16.20)	11.38 (8.91–17.64)
PAI-1, ng/ml, entry	17.63 (11.87–45.85)	23.63 (15.40–47.60)
PAI-1, ng/ml, week 12	25.67 (10.12–54.17)	21.40 (10.62–51.03)

FMD: flow-mediated dilatation; GMD: glyceryl trinitrate-mediated dilatation; vWF: von Willebrand factor; PAI-1: plasminogen activator inhibitor -1. \*  $p = 0.017$  by Mann-Whitney U test comparing the vitamin supplement and placebo groups at baseline.

## DISCUSSION

This is the first randomized controlled trial on antioxidant vitamins in patients with SLE. Our results showed that daily supplementation of 500 mg vitamin C and 800 IU vitamin E for 12 weeks elevate plasma vitamin C and E levels by 23% and 51%, respectively, and may decrease lipid peroxidation, as indicated by the modest decrease in MDA concentrations.

We conducted this trial based on previous findings showing that oxidative stress indices are significantly higher in SLE patients compared with healthy controls<sup>5,6</sup>. We did not include a group of controls in this study for comparison; however, from our unpublished data using the same laboratory methods on 20 healthy female subjects (mean age  $49 \pm 6.5$  yrs), the median allantoin level was 5.5 (IQR 0.80–11.7)  $\mu\text{mol/l}$  and mean MDA level was  $0.72 \pm 0.19$   $\mu\text{mol/l}$ . These were substantially lower than levels in the SLE patients, whose allantoin and MDA levels were 17.0 (13.0–29.0) and  $1.54 \pm 0.26$   $\mu\text{mol/l}$ , respectively, supporting the notion that oxidative stress indices were increased in our group of patients with SLE. In view of the much higher baseline level of MDA in lupus patients compared to healthy controls, the modest reduction in MDA level and the lack of changes in other oxidative stress markers suggest that antioxidant supplementation is likely to be ineffective apart from the small reduction in lipid peroxidation.

There were several baseline differences between the 2 groups due to the small sample size, resulting in higher allantoin, vWF, and fibrinogen levels, and a higher number of patients taking prednisolone in the placebo group. Ames, *et al* demonstrated that SLE patients undergoing steroid treatment had a lower oxidative stress level<sup>5</sup>. In our study, although the number of patients taking prednisolone was higher in the placebo group, the MDA levels were similar between the 2 groups at baseline. Further, the dosage of prednisolone remained unchanged during the study, thus the change in MDA levels after vitamin supplementation should not have been confounded by the baseline prednisolone dosage.

In terms of the choice of lipid peroxidation marker, some reports suggest that F2 isoprostanes may be a more specific index of lipid peroxidation<sup>31</sup>. Their measurement is not without problems, however, and is expensive<sup>31</sup>. We did not have the resources to measure F2 isoprostanes. The MDA method we used involved inhibition of “new” (artefactual) peroxidation by addition of butylated hydroxytoluene to the reaction mixture, optimization of reaction conditions, organic (butanol) extraction, and HPLC separation of the TBA-MDA adduct. Each of these steps increases the specificity of the test in terms of lipid peroxidation. We believe, then, that the MDA method we used is acceptably specific and sensitive for our purposes.

Oxidative stress is thought to play an important role in atherosclerotic vascular disease<sup>2</sup>. Supplementation with antioxidants is therefore an attractive potential therapy to prevent late CVD complications. Despite increases in vitamin C and E levels and decreased lipid peroxidation, the endothelial function of the SLE patients did not change after antioxidant supplements. The reported effects of vitamin C supplements on FMD are mixed. In patients with heart failure or CAD, oral vitamin C (0.5 to 4 g daily) supplement for one month resulted in significant improvement in FMD<sup>18–20</sup>. In other conditions, however, vitamin C (1 to 1.5 g daily) supplement did not have a sustained effect on endothelial function in smokers<sup>21</sup> or in patients with type II diabetes<sup>22</sup>.

Clinical studies have shown that  $\alpha$ -tocopherol increases endothelium-dependent vasodilatation in individuals with coronary risk factors<sup>32–34</sup>. Two of these studies also showed a decrease in markers of lipid oxidation<sup>33,34</sup>. Other studies found no effect of vitamin E supplementation on endothelial dysfunction<sup>23,24</sup> or on lipid peroxidation<sup>23,35</sup>.

Combinations of antioxidants may be of benefit due to the possible synergistic interaction between vitamins C and E. However, of the few studies in which patients with coronary risk factors received vitamin C and E supplements<sup>36–38</sup>, only one<sup>36</sup> found improvement in FMD despite a reduction

of the susceptibility of LDL to oxidation. Antioxidant therapy (vitamin C 1 g and vitamin E 800 IU daily) improved FMD in subjects with type 1 but not type 2 diabetes<sup>38</sup>. Nonetheless, similar combinations (vitamin C 0.5–1 g and vitamin E 400–800 IU daily) have been shown to improve FMD in children and adolescents with endothelial dysfunction due to hereditary hypercholesterolemia without affecting the biomarkers of oxidative stress<sup>37,39</sup>. In smokers, vitamin C (2 g/day) with vitamin E (400–800 IU/day) has been shown to improve FMD, but vitamin C alone had no effect<sup>40</sup>. It is notable that the baseline FMD and vitamin C and E concentrations were within the accepted normal ranges in our subjects<sup>41,42</sup>. It is possible that vitamin C and E supplement is only effective in improving endothelial function when baseline FMD or plasma vitamin C and E concentrations are low; the baseline antioxidant status of our subjects was good. We cannot exclude whether increasing the vitamin C to 1 g daily would improve endothelial function, although this effect has been reported at doses of 500 mg/day<sup>20</sup>. Indeed, higher oral doses of vitamin C are unlikely to have been more effective, as bioavailability is maximal at a dose of 1 g/day; at higher doses the extra ascorbate appears in the urine<sup>43</sup>. It is unlikely that treatment for a longer period would further increase intracellular concentrations of vitamin C substantially, since at daily doses of 100–200 mg plasma concentrations plateau after 3 weeks, indicating saturation of cells with ascorbic acid<sup>43</sup>. However, it is possible that longer treatment would have led to some clinically discernible effect on endothelial function. In addition, our study showed no effect of antioxidant supplement on the endothelial-derived regulatory proteins including vWF and PAI-1. Plasma vWF is a key mediator of platelet aggregation and adhesion. Although platelets contain some vWF, virtually all plasma vWF is derived from the endothelium<sup>44</sup>, and vWF is thought to be a good marker for endothelial injury<sup>45</sup>. PAI-1 is a fast-acting inhibitor of plasminogen activation. It is produced by the vascular endothelium but is also present in platelets, adipocytes, smooth muscle cells, and monocytes, and is considered an important regulatory element in fibrinolysis that is intimately linked to the risk of thrombosis<sup>46</sup>. Suppression of fibrinolysis due to high plasma concentrations of PAI-1 is associated with the development of myocardial infarction<sup>47</sup>. Results from recent studies using antioxidant supplements on these markers are controversial. Chronic vitamin E supplements decreased plasma PAI-1 concentrations in renal transplant recipients and diabetics<sup>48,49</sup>. In smokers, low doses of antioxidant were not effective in reducing vWF<sup>50</sup> and PAI-1<sup>51</sup>. PAI-1 and vWF were decreased only in those who received high-dose vitamin C and E<sup>40</sup> in studies in which the vitamin C dosage was 4-fold higher than in our study.

The vitamin E and C supplements were both safe. Adherence to treatment and bioavailability of the supplements were good, based on the significant increases in plas-

ma vitamin levels. The strengths of our study include the randomized, placebo controlled design and investigation of biomarkers for oxidative stress and antioxidant defense. As this is a proof of principle study, a limitation would be the small sample size, which may explain the variability of baseline characteristics between the groups. However, based on our data (baseline MDA level  $1.52 \pm 0.28 \mu\text{mol/l}$ ), a sample size of 20 per study arm would achieve 80% power to detect a difference of 0.2 between the group means with standard deviation of 0.28 at a significance level (alpha) of 0.05 using a one-sided z test (PASS 2000; NCSS, Kaysville, UT, USA).

In summary, combined administration of vitamin C (500 mg/day) and vitamin E (800 IU/day) was associated with a modest reduction in lipid peroxidation, but did not affect other oxidative stress markers or endothelial function, in patients with SLE after 3 months' therapy. The clinical significance of the reduction in lipid peroxidation requires clarification in future studies.

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