# BENEFICIAL EFFECTS OF VITAMIN C AND VITAMIN E ON BLOOD PRESSURE IN HYPERANDROGENIC WOMEN

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***Abstract-*** ***Hyperandrogenism affects 2-6% of all women. Hypertension is one of disturbances which is related to androgen excess. Higher intakes of vitamin C and vitamin E have been associated with lower blood pressure. To investigate the effect of these vitamin supplementation on blood pressure in hyperandrogenic women, a randomized, double-blind, placebo-controlled study was designed on 56 women 18-45 years old. Women were randomly allocated to one of four treatment groups: spironolactone plus vitamin C and vitamin E (SCE), spironolactone plus vitamins placebo (SP), vitamin C plus vitamin E (CE), vitamins placebo (P). The treatment consisted of oral administration of 100 mg spironolactone, 1000 mg vitamin C and 900 mg vitamin E (alpha-tocopherol acetate) daily for about 3 months. Results indicate that compared to pretreatment, vitamins supplementation significantly lowered systolic (119.1±12.6 vs. 112.6±15.4 mmHg) and mean blood pressure (97.4±11.5 vs. 92±12.1 mmHg) in SCE group (p<0.05), diastolic (87.3±12 vs. 80±12 mmHg) and mean blood pressure (96.9±12.7 vs. 89.9±12.8 mmHg) in CE group (p< 0.05). Blood levels of vitamin C and alpha-tocopherol increased in all SCE and CE subjects. In conclusion, vitamin C and vitamin E supplementation reduced blood pressure in hyperandrogenic women who are at risk of hypertension.***
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***Key Words:*** ***Vitamin C, vitamin E, blood pressure, androgen excess***

## INTRODUCTION

Hyperandrogenism is present in about 2-6% of all women (1). The majority of these women have hirsutism and or polycystic ovary syndrome (PCOS) (2). Some of the hyperandrogen related disturbances are dyslipidemia, diabetes, hypertension, insulin resistance, android obesity and cardiovascular disease (3,4). Hyperandrogenic women have significantly higher systolic (SBP) and diastolic (DBP) blood pressure as compared to euandrogenic women (5). Higher intake or plasma levels of vitamin C and E have been reported to be associated with lower blood pressure (BP) (6-11.). The results of some small size clinical trials showed that supplementation of vitamin C and E may decrease BP both in hypertensive and normotensive subjects (12-14). There is some evidence that spironolactone, as an antiandrogen drug, may increase serum levels of insulin (15). This effect is important especially in obese and or hyperinsulinemic subjects (15). There have been no previous studies assessing the effect of antioxidant supplements on BP in hyperandrogenic women. Therefore, this study was designed to examine the effects of spironolactone adminstration with or without supplementation of vitamin C and E on blood levels of these vitamins and BP in hyperandrogenic women.

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## MATERIALS AND METHODS

### Patients

The study was approved by Iranian Ministry of Health and Medical Education's Ethics Committee for Medical Science Research and written informed consent was obtained from all subjects.This study was carried out between June 2000 and June 2001. Sixty three 18-45 years old women with high levels of testosterone (≥ 2.9 nmol/L) and/or androstene-dione (≥ 3.2 pg/ml) and/or hirsutism (Ferriman and Gallwey scores≥ 8) (16) in a reproductive endocrinology outpatient clinic were enrolled in this randomized, double-blind, placebo-controlled study. Estimation of sample size was based on systolic blood pressure criteria. The α and β levels were considered 0.05 and 0.1 respectively. The majority of selected subjects (n=59) had biochemical evidences of androgen excess. Fifty six subjects completed the study. None of subjects received any kind of vitamin supplement, hormonal drugs, antiandrogen therapy or other drugs known to affect endocrine function or

BP up to one month prior of taking part in the study and none of them had thyroid disorders, hyperprolactinemia and diabetes. Only one of them was smoking 6 cigarettes per day. The subjects were randomized using block design to 1 of 4 treatment groups: 1) spironolactone plus vitamin C and vitamin E (SCE); 2) spironolactone plus vitamins placebo (SP); 3) vitamin C plus vitamin E (CE); 4) vitamins placebo (P). The treatment consisted of spironolactone in two doses of 50 mg, vitamin C capsule in two doses of 500 mg and vitamin E capsule (alpha-tocopherol acetate) in two doses of 450 mg daily for about 3 months. The vitamins supplements were provided by Daru Pakhsh Company. Patients were followed each every other week by phone. Compliance was assessed by counting the remaining of vitamin/placebo capsules and spironolactone tablets.

### BP and blood vitamins measurements

SBP and DBP were measured from the right arm in the sitting position after 10 min of rest using a mercury sphygmomanometer and by the same investigator. The mean blood pressure (MBP) was calculated using the following formula ( 17):

(MBP)= DBP +1/3 (SBP-DBP)

Fasting blood samples were taken on the 20±2 days of menstrual cycle after BP measurements. Ascorbic acid was measured in whole blood with the method of Lowry (18). For measurement of serum alpha-tocopherol 200 µl methanol and 200 µl ethanol were added to 200 µl serum. Thereafter, 1 ml hexane with 0.1% butylated hydroxytoluene (BHT) was added and the sample was shaken. The two phases were separated and the supernatant was analysed by high-performance liquid chromatography (HPLc) using a Bondapack $NH_2$ 30 cm×3.9 mm column and UV-Vis detection ($\lambda$=298 nm) (19).

### Statistical analysis

Data were analysed using SPSS for Windows [SPSS INC, 1993]. The means of SBP, DBP, MBP, whole blood ascorbic acid and serum alpha-tocopherol levels of each subject at baseline and after 3 months of treatment were compared using paired t test. Comparison among the 4 groups was performed with one-way analysis of variance (ANOVA).

## RESULTS

Before treatment, the marital and infertility status, mean age, BMI, WHR, whole blood vitamin C, serum alpha-tocopherol, androgens levels (Table 1) SBP, DBP and MBP (Table 2) were similar among all groups. After treatment, BMI had small but significant increase in CE group as compared to the pretreatment value ($p< 0.03$). In addition, the mean whole blood vitamin C and serum alpha-tocopherol levels increased significantly in the SCE and CE groups as compared to the pretreatment values ($p< 0.002$ and $p< 0.001$ respectively). Table 2 shows that SBP and MBP were significantly decreased in SCE group as compared to the baseline levels ($p< 0.03$ and $p< 0.02$ respectively). In addition, DBP was also decreased, but not significantly ($p< 0.07$) in this group. Vitamin C and vitamin E supplementation also reduced DBP and MBP in CE group as compared to the baseline levels ($p< 0.01$ and $p< 0.02$ respectively). DBP in SCE group and SBP in SP group decreased nonsignificantly after supplementation as compared to the baseline levels ($P< 0.06$) (Table 2). The mean of serum androgens, SBP, DBP and MBP were not significantly different among four groups after intervention. Mean changes of SBP, DBP and MBP have been shown in figure 1. SCE and CE groups had higher changes than SP and P groups.

## DISCUSSION

Hyperandrogenic women are at risk of dyslipidemia, diabetes, hypertension, insulin resistance, android obesity and cardiovascular disease (3,4). Inverse correlation has been shown between serum vitamin C and E levels with obesity (11,20), hypertension (11) and cardiovascular disease (21). The antioxidant status of hyperandrogenic women has not been compared to euandrogenic women. Our study is the first one that investigated the effect of antioxidant supplements on BP in these women who are at risk of high BP. The result of this study demonstrated a beneficial effect of vitamin C and vitamin E supplementation on BP in hyperandrogenic women. Our findings are consistent with other studies which showed beneficial effects of vitamin C and alpha-tocopherol supplementation on reducing BP in hypertensive and normotensive subjects (13,14,22). However, some other studies did not find any significant reduction in BP after supplementation with these vitamins (23-25). Some of these studies did not have control group (24) and some of them used lower dose of alpha-tocopherol (300 mg) (23). One study obtained significant reduction in SBP, but not in DBP, with 1 g ascorbic acid daily supplementation for 6 weeks (14). Spironolactone has also some effects on BP. In one study, daily administration of 200 mg spironolactone for 9 months reduced significantly SBP (15). However, in our study daily intake of 100 mg spironolactone without antioxidants did not show any significant effects on BP. Since SBP was decreased significantly in SCE group and not in CE group, it

might indicate that spironolactone with its diuretic effect (26) together with vitamins reduced SBP.

**Table 1.** Characteristics of subjects in the 4 groups

| | | SCE (n= 14) | SP (n= 15) | CE (n= 15) | P **(n= 12)** |
|---|---|---|---|---|---|
| **Marital status** | | | | | |
| | **single(%)** | **4(28.6)** | **3(20)** | **4(26.7)** | 4(33.3) |
| | **married(%)** | **10(71.4)** | **12(80)** | **11 (73.3)** | 8(66.7) |
| **Infertility** | | | | | |
| | **yes (%)** | **6(66.7)** | **8(66.7)** | **5(45.4)** | 6(75) |
| | **no (%)** | **3 (33.3)** | **4(33.3)** | **6(54.6)** | 2(25) |
| **Age (y)** | | **24.6±4.5*** | **27.3±5.6** | **26.1±5** | 25.1±5.7 |
| **Body Mass Index (BMI) (kg/m$^2$)** | | | | | |
| | **before** | **26.6±5** | **27.6±3.7** | **27.4±5.6** [a] | 24.9±3.8 |
| | **after** | **26.8±5** | **27.6±3.6** | **27.8±5.9** | 25.2±3.8 |
| **Waist/hip ratio** | | | | | |
| | **before** | **0.8±0.07** | **0.8±0.08** | **0.8±0.06** | 0.8±0.07 |
| | **after** | **0.8±0.07** | **0.8±0.08** | **0.8±0.05** | 0.8±0.06 |
| **Whole blood vitamin C (mg/dl)** | | | | | |
| | **before** | **1.5±0.5** [b] | **1.4±0.4** | **1.3±0.4** [c] | 1.5±0.4 |
| | **after** | **2.4±0.7** [d] | **1.8±0.8** | **2±0.6** | 1.5±0.6 |
| **Serum alpha-tocopherol (µg/ml)** | | | | | |
| | **before** | **10.5±3.7** [c] | **9.4±2.7** [+] | **10.3±3.6**[++,c] | 10.7±4.1[+++] |
| | **after** | **23±11.3** [e] | **9.9±4** | **22.4±10.6** | 12.6±7.6 |
| **Testosterone (nmol/l)** | | | | | |
| | **before** | **2.7±1.4** | **2.4±1.3**[++] | **2.7±1.3** | 2.9±1.6 |
| | **after** | **2.2±1.2** | **2.3±1.0**[++] | **2.4±1.1** | 2.5±1.4 |
| **Androstenedione (µg/ml)** | | | | | |
| | **before** | **2.0±0.9** | **2.2±1.0** | **2.2±0.9** | 2.4±1.3[+++] |
| | **after** | **1.9±0.9** | **2.1±1.0** | **2.1±1.1** | 1.9±0.9[+++] |
| **Dehydroepiandrosterone (DHEAS) (µg/dl)** | | | | | |
| | **before** | **220.3±160.4** | **209.8±181.8** | **231.4±128.3** | 296.5±240.3 |
| | after | 176.9±97.5 | 186.8±145.2 | 205.6±87.8 | 290.9±217.1 |

SCE: Spironolactone + Vitamin C + Vitamin E; SP: Spironolactone + Placebo; CE: Vitamin C + Vitamin E; P: Vitamins Placebo.

* values are mean±SD

+) n= 12 , ++)n= 14, +++) n= 11

Statistical significance between before and after treatment (paired student's t test): a) $p<0.03$, b) $p<0.002$, c) $p<0.001$

Statistical significance among 4 groups (ANOVA): d) $p< 0.03$, e ) $p< 0.001$

**Table 2.** Blood systolic, diastolic, mean pressure, glucose and insulin before and after 3 months of vitamin C and E supplementation in hyperandrogenic women

| | SCE (n=14) | SP (n= 15) | CE (n=15) | P (n=12) |
|---|---|---|---|---|
| **Systolic pressure (mmHg)** | | | | |
| before | **11 9.1±12.6**[*b] | **114.6±11.9** | **116±16** | 115.3±13.4 |
| after | **112.6±15.4** | **110.2±12.8** | **110.3±15.6** | 111.8±16.8 |
| **Diastolic pressure (mmHg)** | | | | |
| before | **86.6±12.1** | **84.5±12.2** | **87.3±12**[d] | 85.8±10.8 |
| after | **81.7±11.1** | **81.5±10.7** | **80±12** | 81.9±11.6 |
| **Mean blood pressure (mmHg)** | | | | |
| before | **97.4,11.5** [c] | **94.6±11.8** | **96.9±12.7** [c] | 95.7±11.2 |
| after | **92±12.1** | **91.1±11** | **89.9±12.8** | 91.9±13.1 |
| **Glucose (mg/dl)** | | | | |
| before | **91.9±10.5** | **88.2±6.4** [e] | **91.1±6.9** | 91.9±11.4 |
| after | **90.7±10.4** | **94.8±12** | **94.5±10** | 93.1±14.1 |
| **Insulin (ulU/ml)** | | | | |
| before | **12.7±5.8** | **9.6±4.4**[f] | **13.0±5.8** | 10.1±3.8 |
| after | **11.5±7.1** | **12.6±6.4** | **13.0±5.4** | 10.9±3.7 |
| **Glucose/insulin ratio** | | | | |
| before | **8.8±4.2** | **10.8±4.2** [a] | **8.0±2.5** | 10.1±3.2 |
| **after** | 9.7±3.7 | 9.2±4.4 | 8.6±3.7 | 9.4±3.3 |

SCE: Spironolactone + Vitamin C + Vitamin E; SP: Spironolactone + Placebo; CE: Vitamin C + Vitamin E; P: Vitamins Placebo

* values are mean±SD

Statistical significance between before and after treatment (paired t test): a) p<0.03, b) p<0.02, c) p<0.01

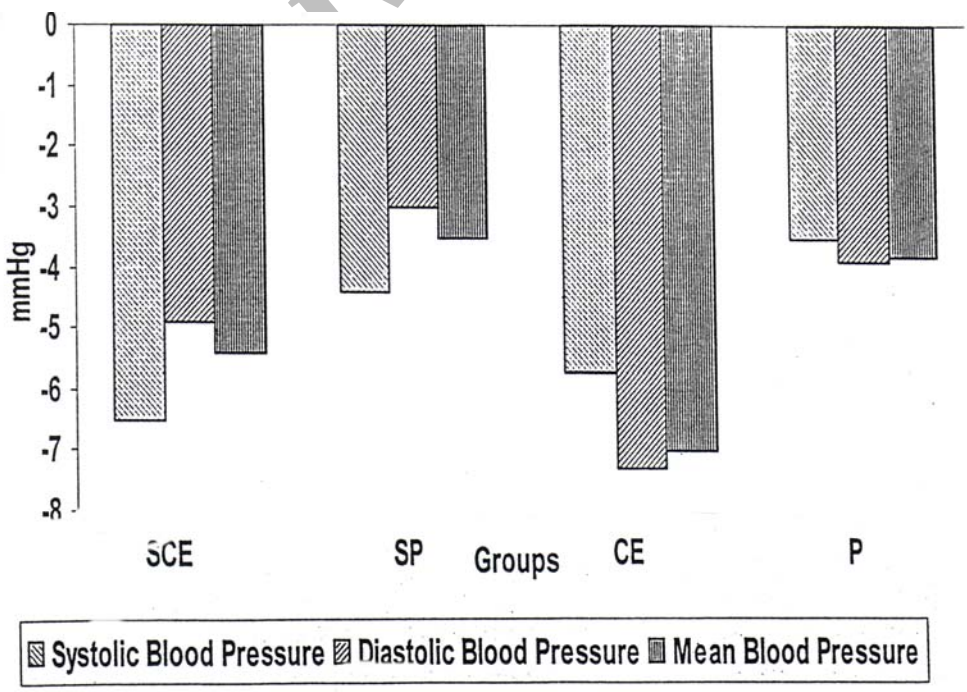


**Fig. 1.** Mean changes of blood pressures in vitamins or placebo 13 supplemented hyperandrogenic women

However, after treatment small but significant increase of BMI in CE group might be the other reason for nonsignificant decrease of SBP in this group. It has been shown that antioxidants supplements reduce BP by removal of superoxide anion and enhancing of nitric oxide availability (13). In addition, ascorbic acid potentiates nitric oxide synthesis via a chemical stabilization of tetrahydrobiopterin which is nitric oxide synthase cofactor (27). Whether these antioxidant vitamins reduce other risk factors (dyslipidemia, insulin resistance, etc.) in hyperandrogenic women need further investigation. In conclusion, ascorbic acid and alpha-tocopherol supplementation in mega doses reduced BP in hyperandrogenic women and hence, may prevent the elevation of BP later in life of these women. These findings need to be confirmed in large studies.

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