Middle-East Journal of Scientific Research 10 (5): 577-580, 2011
ISSN 1990-9233

Effects of Antioxidant Vitamins (C, E, Beta-Carotene) Supplementation on Serum 8-iso-Prostaglandin PGF2α in Male Subjects with Risk Factors for Cardiovascular Diseases

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Abstract: Numerous studies have shown that subjects with high levels of serum8-iso-Prostaglandin F2α(8-iso PGF2α) has an increased risk of dying of coronary heart disease or stroke.The aimed of this study was to determine the effects of antioxidant vitamins E, C, beta-carotene and combined (E, C and beta-carotene) supplementation on serum 8-iso PGF2αin male subjects with risk factors. One hundred eight one (181) male subjects were recruited in this study. The mean values for 8-iso-PGF2α at the baseline were shown for C group (66.04 ± 21.86 pg/ml), E group (67.97 ± 16.62 pg/ml), B group (68.15 ± 19.15 pg/ml), combined group (67.29 ± 21.64 pg/ml) and P group (64.14 ± 18.97 pg/ml) and after 12 weeks of intervention, the values were 60.82 ± 9.43 pg/ml, 55.98 ± 13.20 pg/ml, 56.17 ± 10.43pg/ml, 57.20 ± 8.84 pg/ml, 62.18 ± 15.78 pg/ml, respectively. The percent changes in the concentration of 8-iso-PG F2α through supplementations of vitamin C, E, beta-carotene and combined group were -7.92, -17.62, -17.56 and -14.97, respectively. The current study showed that vitamin C (500mg) had no effect on serum 8-iso-PGF2α levels in the subjects however, there were significant reduction in the 8-iso-PGF2αconcentration with vitamin E (400IU), beta-carotene (15mg) and the combination of vitamins (E, C and beta-carotene) intakes.

Key words: 8-iso-Prostaglandin F2α · Vitamin E · Vitamin C · Beta-carotene · Biomarkers · Cardiovascular

INTRODUCTION

Oxidative Stress is implicated in most human diseases [1, 2]. Antioxidants may decrease the oxidative damage and its alleged harmful effects [3]. Many people are taking antioxidant supplements, believing to improve their health and prevent diseases. Whether antioxidant supplements are beneficial or harmful is uncertain [4, 5]. Many primary or secondary prevention trials of antioxidant supplements have been conducted to prevent several diseases [6]. Numerous epidemiologic studies have supported the hypothesis that antioxidants could be used as an inexpensive means of prevention [7-9] and possibly treatment of cardiovascular diseases [10]. Many studies have shown that subjects with high levels of urinary 8-iso PGF2α had an 80% increased risk of dying of coronary heart disease or stroke. However, this founded to support involvement of oxidative stress [11] in the pathophysiology of cardiovascular disease [12]. F2-Isoprostanes, derived from non-enzymatic free-radical-mediated peroxidation of arachidonic acid, are arguably the best established biomarker of oxidative stress. The results showed significantly increased in atherosclerotic plaques compared with healthy non-atherosclerotic vascular tissue; systemic F2-isoprostanes have also been found to increase following angioplasty [13]. Studies have shown that F2-isoprostanes are reliable biomarkers of lipid peroxidation and could therefore, be used as potential indicators of oxidative stress in diverse conditions [14].

It has now been suggested that measurement of F2-isoprostanes is the most reliable approach to assess oxidative stress status *in vivo*, providing an important tool to explore the role of oxidative stress in the pathogenesis of human disease. In addition, products of the isoprostane pathway have been found to exert potent biological actions and therefore may be pathophysiologic mediators of disease [15].

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MATERIALS AND METHODS

The research design of this study was a single-blinded randomized trial that was aimed at determining the effects of antioxidant vitamins (E, C and beta-carotene) supplementation on prostaglandin F2α in male subjects with risk factors for cardiovascular diseases.181 subjects were recruited through advertisement in local newspapers and announcement via radio in and around the study region of Bushehr, Iran. The criteria for participation were: (1) age≥40 years, (2) elevated risk of cardiovascular disease, defined as the presence of one to three of the following six known conditions: hypertension, dyslipidemia, type 2 diabetes, obesity, family history of premature CVD and smoking, (3) not taking any vitamin and mineral supplements, in the last 3 months before the intervention period. The vitamin E group took vitamin E (400 IU), vitamin C group took vitamin C (500 mg), beta-carotene group took beta-carotene (15 mg) and combined group took (E+ C+ beta-carotene) respectively, by oral supplementation. Subjects were asked to come for the blood sample collection after 12 hours of taking their last meal. Fasting venous blood samples were taken for measurement of plasma concentrations of 8-iso-PGF2α and serum concentrations of vitamin E, C, beta-carotene, FBS, TG, T-Chol, HDL and LDL cholesterol. The analysis of the fasting blood samples were carried out at the Persian Gulf Health Research Center on the same day of blood collection, using a Selectra 2 Autoanalyzer (Vita Scientific, Spankeren, The Netherlands).

The 8-iso-Prostaglandin F²αconcentration in serum was analyzed with an 8-isoprostane enzyme immunoassay kit (kit no. 516351; Cayman, Ann Arbor, MI, USA) according to the manufacturer's instructions. The Statistical Package for the Social Sciences (SPSS windows version 15) was used for statistical analysis. Nonparametric statistics such as Wilcoxon was employed. For variable that met normality of distribution, Analysis of Variance (ANOVA), Independent t-test and paired sample t-test were utilized to identify mean difference between and within group. Statistical significance has been assigned at level of $p< 0.05$.

RESULTS

Table 1. Shows the mean±SD of the lipid profile for the treatment and control groups at the baseline and after intervention. The mean values at the baseline in vitamin C group were 86.17±24.62 mg/dl for FBS, 206.65±131.38 mg/dl for TG, 218.65±36.31 mg/dl for TC, 45.65±7.95 mg/dl for HDL-C, 132.05±28.32 mg/dl for LDL-C and in vitamin E group were 4.29±39.73 mg/dl for FBS, 241.24±159.66 mg/dl for TG, 208.75±41.31 mg/dl for TC, 40.18±11.39 mg/dl for HDL-C, 124.08±45.59 mg/dl for LDL-C and in beta-carotene group were 97.02±45.57 mg/dl for

Table 1: Distribution of number and percentage (%) of the participants according to the guideline of American Diabetes Association ADA and ATP III Classification of LDL, Total and HDL Cholesterol (mg/dl).

	C Group (n=35)		E Group (n=37)		B Group (n=34)		COM Group (n=40)		P Group (n=35)	
	Before	After	Before	After	Before	After	Before	After	Before	After
FBS (mg/dl)										
<110 n (%)	27(77.1)	28(80)	31(83.8)	29(78.4)	26(76.5)	26(76.5)	29(72.5)	30(75)	32(91.4)	31(88.6)
110-125 n (%)	6(17.1)	5(14.3)	2(5.4)	2(5.4)	1(2.9)	2(5.9)	3(7.5)	4(10)	1(2.9)	2(5.7)
=126 n (%)	2(5.7)	2(5.7)	4(10.8)	6(16.2)	7(26.6)	6(17.6)	8(20.0)	6(15)	2(5.7)	2(5.7)
TG (mg/dl)										
<150 n (%)	16(45.7)	20(57.1)	11 (29.7)	11(29.3)	13 (38.2)	9(26.5)	12 (30	11(29)	16 (45.7)	18(51.4)
150-199 n (%)	3 (8.6)	6(17.1)	12 (32.4)	9(24.3)	5 (14.7)	13(38.2)	10 (25)	5(12.5)	8 (22.9)	6(17.1)
=200 n (%)	16 (45.7)	9(25.7)	14 (37.8)	17(45.9)	16 (47.1)	12(35.2)	18 (45)	23(57.5)	11 (31.4)	11(31.4)
TC (mg/dl)										
<200 n (%)	9(25.7)	14(40)	18(48.6)	16(43.2)	13 (38.2)	9(26.5)	12 30.0	12(30)	16 (45.7)	18(51.4)
200-239 n (%)	16(45.7)	11(13.4)	10(27)	11(29.7)	5 (14.7	13(38.2)	10 25.0	5(12.5)	8 (22.9)	6(17 (1)
=240 n (%)	10(28.6)	10(28.6)	9(24.3)	10(27)	16 (47.1)	12(35.2)	18 45.0	23(57.5)	11 (31.4)	11(31.4)
LDL-C (mg/dl)										
<130 n (%)	18(51.4)	20(57.1)	22(59.5)	21(56.8)	6(76.5)	26(76.5)	27(67.5)	25(62.5)	22(74.3)	28(80)
130-159 n (%)	12(34.3)	8(22.9)	8(21.6)	8(21.6)	6(17.6)	6(17.6)	9(22.5)	12(30)	3(8.6)	7(20)
=160 n (%)	5(14.3)	7(20)	7(18.9)	8(22.2)	2(5.9)	2(5.9)	4(10)	3(7.5)	6(17.1)	2(11)
HDL-C (mg/dl)										
<40 n (%)	13(37.5)	9(25.7)	23(62.2)	17(45.9)	17(50)	14(41.2)	23(57.5)	19(47.5)	16(45.7)	13(37.1)
40-59 n (%)	20(57.1)	21(60)	13(35.1)	19(51.4)	14(41.2)	17(50)	12(30)	19(47.5)	18(51.4)	21(60)
=60 n (%)	2(5.7)	5(41.3)	1(2.7)	1(2.7)	3(8.8)	2(5.9)	5(12.5)	2(50)	1(2.9)	1(2.6)

•Note: TC, total cholesterol; TG, triglycerides; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol

Table 2: Changes in the 8-iso-PGF2a (pg/ml) in serum at the baseline and after the intervention (Mean ± SD and Mean d ± SE d)

	8-iso-PGF2a (pg/ml)				
	C group	E group	B group	COM group	P group
Before	66.04± 21.86	67.97±16.62	68.15±19.15	67.29± 21.64	64.14±18.97
After	60.82±9.43	55.98 ±13.20	56.17± 10.43	57.20±8.84	62.18±15.78
Mean d ±SE d (within groups	-5.22±3.64	-11.98±2.07	-11.97±3.07	*0.014	-1.96±1.21
Changing Percent	-7.92	-17.62	-17.56	-14.97	-3.50
P value	0.422	0.000*	0.002*	0.000*	0.092

•P-value <0.05 is significantly different.
•Test non-parametric, Wilcoxon, was used to compare the pre-post tests.

FBS, 220.67±123.28 mg/dl for TG, 196.85±32.59 mg/dl for TC, 41.32±9.73 mg/dl for HDL-C and 110.23±29.05 mg/dl for LDL-C . The mean values at the baseline in the combined (C+E+beta-carotene) group were as follows: 96.85±37.55 mg/dl for FBS (), 233.75±161.96 mg/dl for TG, 209.30±31.92 mg/dl for TC, 42.02±11.78 mg/dl for HDL-C and 118.50±31.36 mg/dl for LDL-C. The mean values at the baseline in the placebo group were: 83.08±20.543 mg/dl for FBS, 176.68±91.26 mg/dl for TG, 199.11±36.48 mg/dl for TC, 40.41±8.57 mg/dl for HDL-C and160.45±24.69 mg/dl for LDL-C .It is important to note that the paired t-test carried out showed no significant difference ($p>0.05$) in the values of the FBS, lipid profile and antioxidant vitamins (C, E, beta-carotene) both at the baseline and after the intervention.

The mean values for 8-iso-PGF2α at the baseline were shown for C group (66.04 ± 21.86 pg/ml), E group (67.97 ± 16.62 pg/ml), B group (68.15 ± 19.15 pg/ml), combined group (67.29 ± 21.64 pg/ml) and P group (64.14 ± 18.97 pg/ml) and after 12 weeks of intervention, the values were 60.82 ± 9.43 pg/ml, 55.98 ± 13.20 pg/ml, 56.17 ±10.43pg/ml, 57.20 ± 8.84 pg/ml, 62.18 ± 15.78 pg/ml, respectively (Table 2). However, applying Wilcoxon indicated that there was a significant difference found for the mean 8-iso-PGF2αbetween E, B and combined groups($p<0.05$) before and after the intervention.

DISCUSSION

The present result were similarly shown by several other studies revealing a reduction in the markers of F-prostaglandin with vitamin C and E supplementation [16]. Marangon*et al.* [17] has shown that supplementation of healthy adults with 400 IU/day α-tocopherol for 8 weeks resulted in lower levels of urinary F2-isoprostanes. Intake of ascorbic acid has been shown to be negatively associated with PGF2a formation [18].

In 1996, Reilly *et al.* [19]established that the supplementation among heavy smokers with 2000mg vitamin C/day for duration of 5 days had significantly decreased the urinary excretion of 8-epi-PGF2a by 29 %. A Study showed that vitamin C (1.5 g daily) intake for 3 weeks produced no significant reduction in oxidative stress,, as assessed by plasma concentrations of the isoprostane 8-epi-PGF2a [20].

A similar significant depression of 8-epi-PGF2α excretion (mean, 23%) was observed when the smokers were treated with vitamin E 800 U/d in combination with vitamin C [19]. A-tocopherol (500 mg) supplementation resulted in reduced plasma F2-isoprostanes concentration by 22.9 % [21].

Several studies have illustrated a negative relationship between F2-IsoPs and fruit and vegetable or mixed carotenoids intakes. Another study has shown a decrease in urinary F2-IsoPs with high dietary intakes of fruit and vegetables in human [22]. Similarly, many cross-sectional studies have documented significant inverse associations between plasma F2-IsoPs and plasma beta-carotene [23].

However another study, Mayne *et al.* [24] found that 8-iso-prostaglandin F2α concentrations decreased after the consumption of supplements containing beta-carotene alone, or in combination with other antioxidant vitamins, such as vitamin E and vitamin C, whereas beta-carotene alone did not yield any significant reduction.

The current study showed that vitamin C (500mg) had no effect on serum 8-iso-PGF2α levels in the subjects however, there were significant reduction in the 8-iso-PGF2a concentration with vitamin E (400IU), beta-carotene (15mg) and the combination of vitamins (E, C and beta-carotene) intakes. The percent changes in the concentration of 8-iso-PG F2α through supplementations of vitamin C, E, beta-carotene and combined group were -7.92, -17.62, -17.56 and -14.97, respectively.

In conclusion, the findings from this study demonstrating that supplementation of antioxidant vitamins decreased harmful biomarker for cardiovascular diseases such as 8-iso-PGF2α. This imply that people at risk for heart disease should be encouraged to use supplementation of antioxidant vitamins for reducing cardiovascular diseases.

ACKNOWLEDGMENT

Our special appreciation goes to SOVEH Company for their cooperation and all individuals who have helped us in one way or another, in making this study a success.

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