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Effects of Vitamin E on Fasting and Postprandial Oxidative Stress, Inflammatory Markers, Glucose Status, Insulin Resistance, Blood Pressure and Pulse Rate in Type-2 Diabetic Patients: A Randomized Clinical Trial

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Abstract

Background: Atherosclerosis is one of the prevalent complications in diabetic patients. Increased free radical levels in diabetes activate stress-sensitive signaling pathway, resulting in this outcome. This study examines the effect of short-term supplementation of vitamin E on different biochemical markers in type 2 diabetic patients to prevent from atherosclerosis.
Materials and Methods: In this single-blind placebo controlled trial, 30 type 2 diabetic patients were randomly divided into two groups of study to receive vitamin E (400IU) or identical placebo capsules daily for 6 weeks. Serum level of lipoproteins, glucose, insulin, malondialdehyde (MDA), interleukin-6 (IL-6), high-sensitivity C-reactive protein (hs-CRP), pulse rate and blood pressure were measured in fasting and postprandial (after a fatty meal) states before and after six weeks of supplementation.**Results:** There was not any significant difference in fasting and postprandial lipid profile (Triglyceride, HDL-, LDL- and total Cholesterol), glucose, insulin and HOMA-IR after six weeks of intervention between the two groups. However, results of our study showed a significant decrease in fasting and postprandial MDA levels and postprandial pulse rate and a significant increase in fasting IL-6 in vitamin E group compared to the controls after supplementation. There were no significant differences between the groups in other markers.**Conclusion:** This study suggests that short term supplementation of vitamin E can reduce oxidative stress in fasting and postprandial states in type 2 diabetic patients and may prevent diabetic complications; in addition, increment of IL-6 after supplementation may play a role in attenuating Type 2 diabetes by anti-inflammatory effects.**[GMJ. 2015;4(3):67-74]**

Keywords: Vitamin E; Diabetes; Postprandial Period; Oxidative Stress; Inflammation; Atherosclerosis

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Introduction

Type 2 diabetes is an established risk factor for atherosclerosis. Different complications of diabetes such as hyperlipidemia, hypertention, hyperglycemia, inflammation and disturbed endothelial function are involved in initiation of and progression to atherosclerosis [1-3]. Increased oxidative stress may be the underlying mechanism of this process [4]. Different studies have demonstrated decreased serum concentrations of α-tocopherol and ascorbate against increased levels of free radicals in diabetic patients; these are contributing factors for oxidative stress [5].

Evidence shows that postprandial state, i.e. the period that comprises and follows a meal, can affect oxidative stress more strongly than fasting state [6]. Postprandial hyperglycemia and lipidemia are two predictors of cardiovascular disease (CVD); both of these may have direct toxic effects on the vascular endothelium mediated by increased oxidative stress [7]. Increased oxidative stress in postprandial state is associated with excessive production of free radicals just as reactive oxygen species (ROS) [8].

These radicals activate several biochemical pathways, which are associated with insulin resistance, enhanced expression of cytokines, decreased concentration of nitric oxide (NO) as a vasodilator and increased peroxidation of lipids through stress sensitive signaling pathway by activating protein kinase-C (PKC), NF-kB and activator protein-1 (AP-1) [9, 10]. Depletion of antioxidants in diabetic patients with increased production of free radicals in these patients showed that with repletion of antioxidant reserves, secondary complication of diabetes such as atherosclerosis may be prevented.

Vitamin E (-tocopherol) is one of the most readily available dietary antioxidants. This fat soluble antioxidant is transported in plasma lipoproteins and precipitated into the membrane and fat storage sites [11]. Vitamin E scavenges peroxyl radicals as a chain-breaking antioxidant and protects from poly-unsaturated fatty acids (PUFA) in membrane phospholipids and in plasma lipoproteins [12]. Lipid radicals react with vitamin E 1,000 times more rapidly than they do with PUFA. Vitamin E donates hydrogen to lipid radicals and produces an antioxidant radical which is neutralized with ascorbic acid and converts back to alpha-tocopherol [11].

Although theoretical debates support supplementation of vitamin E in diabetic patients, the question which still remains is whether this is really effective. The aim of this study is to evaluate the effects of vitamin E supplementation on different markers such as blood glucose, insulin, lipid profile, Malondialdehyde (MDA) as lipid peroxidation marker, inflammatory markers, pulse rate and blood pressure in fasting and postprandial states in diabetic patients to show that whether this supplementation can have beneficial effects on modulating underlying risk factors in order to decrease or prevent cardiovascular disease in this group.

Materials and Methods

Participants

Thirty patients (8 men and 22 women) with type 2 diabetes mellitus participated in this randomized single blind placebo controlled clinical trial from Diabetes Association of Shiraz, Iran which is affiliated to Shiraz University of Medical Sciences after screening of 408 diabetic patients for eligibility.

Patients were included in the study with the following inclusion criteria: diabetes' duration 1-15 years and a fasting blood glucose (FBG) ≥126 mg/dl or diagnosed with type 2 diabetes by a co-advisor endocrinologist. Those patients, who had the history of smoking, alcohol abuse, clinical evidence of overt vascular disease and acute or chronic inflammatory diseases were excluded.

The included patients should not have been treated with lipid lowering drugs, hormone replacement therapy, insulin, diuretics, β-blockers, aspirin and supplemental vitamins.

All the subjects were fully informed of the purpose and procedures of the trial and were free to leave the trial at any time. A written informed consent was obtained from all participants. The research protocol was approved by the Ethics Committee on Human Experimentation of Shiraz University of Medical Scienc-

es (NO: 88-4600) and this study was registered to Iranian Registry of Clinical Trials (IRCT) with the ID of IRCT138904073236N1.

Diabetic participants were divided into two groups of study by block randomization with fixed block size of four. The treatment group received vitamin E (400IU/per day) and the control group received placebo (acetate cellulose) for six weeks. The supplement and placebo capsules looked identical and were especially prepared for this study by General Nutrition Center (GNC) in the USA. We asked the participants to avoid any changes in their oral hypoglycemic drugs whenever possible.

Treatment Protocol

This study began at 07:00 a.m. after a 12-hour overnight fasting. 5 milliliters of blood was drawn for serum total, LDL and HDL-cholesterol, triglyceride, malondialdehyde (MDA), hs-CRP, interleukin-6 (IL-6), glucose and insulin measurements. Pulse rate, systolic and diastolic blood pressures were also measured in fasting state. Each subject was then given a breakfast that contained 80 grams fat (including a piece of cake, a small cheese sandwich and 200 ml high fat milk); afterwards they were told to take a rest in a chair and watch a television without having any food, tea and or coffee. Two hours after finishing the test meal, biochemical markers, pulse rate, systolic and diastolic blood pressures were measured repeatedly (postprandial state).

At this point, the patients in the treatment group received vitamin E (400 IU/day) and the control group received identical placebo for six weeks. At the end of the sixth week, the baseline procedure was repeated and fasting and 2-hour postprandial biochemical markers, pulse rate and systolic and diastolic blood pressures were measured.

Lipid profiles and blood glucose were measured by biosystemA-25 autoanalyser. Plasma MDA concentration was measured by determining the levels of TBARS (Thiobarbituric acid reactive substances) as a measure of lipid peroxidation by using spectophotometric assay. Serum concentration of Insulin and hs-CRP were measured using a commercially available ELISA kit (biosource kit for insulin and Immuno-Biological Laboratories kit for hs-CRP). We used RIA method for measurement of IL-6 (Biosource kit). Moreover, insulin resistance was measured by homeostasis model assessment (HOMA-IR) based on this equation: [glucose (mmol/L) × insulin (μIU/mL)] / 22.5 [13].

Pulse rate detection and blood pressure measurement were conducted by the same one for each patient before and after intervention.

Data Analysis

Data analysis was carried out using Mann-Whitney U-test to compare mean differences between both groups in SPSS software version 16. Basic data were expressed as mean±standard deviation, fasting and postprandial biochemical parameters before and after the intervention expressed as median (inter-quartile range). It was considered significant if P value was lower than 0.05.

Results

There was no significant difference between the vitamin E and placebo groups at baseline in demographic characteristics such as sex, age, duration of diabetes, body mass index (BMI), daily dose of drugs and biochemical parameters. Three patients were excluded from statistical analysis because they interrupted the trial treatment (Table1).

As shown in Tables 2 and 3, there wasn't any significant difference in fasting and postprandial lipid profile (Triglyceride, HDL-, LDL- and total Cholesterol), glucose, insulin and HOMA-IR after six weeks of intervention between the two groups.

Significant decreases in fasting and postprandial serum MDA levels (0.033 and <0.001, respectively) in the treatment group were recorded compared to the placebo group (Table 4). No significant differences were shown in fasting and postprandial inflammatory markers except fasting IL-6 (p=0.008) (Table 4).

Table 5 shows the fasting and postprandial systolic/diastolic blood pressure and pulse rates in the two groups. Following 6 weeks of supplementation, there was a significant decrease in postprandial pulse rate in the treatment group compared to the placebo group. There were no significant changes in fasting and postprandial systolic/diastolic blood pressure and fasting pulse rate.

Table1. Patient Characteristics and Biochemical Profiles

Parameters	Groups		P-value
	Vitamin E*	Placebo*	
N (female/male)	14 (11/3)	13 (9/4)	0.847
Age (years)	48±6.28	46.61±7.58	0.905
Duration of diabetes(years)	5.14±3.82	4.92±4.78	0.841
Body mass index (kg/m²)	29.22±6.62	28.81±4.04	0.258
Metformin (g/day)	1±0.85	0.73±0.75	0.135
Glibenclamide (mg/day)	7.5±8.49	9.61±8.02	0.814
Glu (mg/dl)	157.78±50.21	138±39.92	0.629
TG (mg/dl)	168.78±66.77	147.15±37.38	0.944
Chol (mg/dl)	208.64±29.3	200.15±17.54	0.354
LDL-C (mg/dl)	142.47±25.33	133.32±15.01	0.747
HDL-C (mg/dl)	32.41±9.83	37.4±5.6	0.116

*Data expressed as mean±SD except N (number of participants).

Table2. Lipid Profile in Fasting and Postprandial States before and after Supplementation

Parameters	Groups				P-value†
	Vitamin E Median(IQR*)		Placebo Median(IQR)		
	Baseline	After 6 weeks	Baseline	After 6 weeks	
Before meal(fasting)					
TG (mg/dl)	170.5(104.25)	162(61.75)	152(69)	138(42)	0.685
Chol (mg/dl)	211.5(33.75)	211.5(26.5)	204(24)	198(44)	0.519
LDL-C (mg/dl)	136.85(38.88)	142.9(30.57)	138.3(30)	126.8(40.2)	0.43
HDL-C (mg/dl)	30.1(14.72)	29.7(13.6)	38.5(7.4)	36.9(14.3)	0.55
2 h after meal					
TG (mg/dl)	263(150.25)	220(87)	227(80)	233(131)	0.756
Chol (mg/dl)	211(49)	203.5(39.25)	202(32)	209(42)	0.867
LDL-C (mg/dl)	117.6(35.32)	126.9(48.75)	122.2(28.3)	111.7(36.2)	0.145
HDL-C (mg/dl)	33.5(13.4)	28.1(14.62)	37.5(6.4)	36.7(12.9)	0.116

* The interquartile range (IQR) is the distance between the 75th percentile and the 25th percentile.
† indicates difference between two groups

Table3. Glycemic Properties in Fasting and Postprandial States before and after Supplementation

Parameters	Groups				P-value
	Vitamin E Median(IQR)		Placebo Median(IQR)		
	Baseline	After 6 weeks	Baseline	After 6 weeks	
Before meal(fasting)					
Glucose(mg/dl)	147(91.5)	138.5(56.5)	132(57)	116(57)	0.519
Insulin (µIU/ml)	9.8(7.25)	10(4.6)	10.2(10.5)	12.9(7.7)	0.325
HOMA index	3.49(2.73)	3.5(1.95)	3.52(3.07)	3.58(3.81)	0.519
2 h after meal					
Glucose(mg/dl)	177.5(107.5)	180(87.75)	177(60)	185(74)	0.905
Insulin (µIU/ml)	30.45(36.18)	41.75(27.83)	44.4(70.9)	36.8(131.8)	0.867
HOMA index	16.16(13.16)	19.17(15.68)	24.43(26.3)	15.59(27.46)	0.905

Table4. Inflammatory and Oxidative Stress Markers in Fasting and Postprandial States before and after Supplementation

Parameters	Groups				P-value
	Vitamin E Median(IQR)		Placebo Median(IQR)		
	Baseline	After 6 weeks	Baseline	After 6 weeks	
Before meal(fasting)					
hs-CRP(µg/ml)	2.3(10.98)	3.6(5.32)	3.6(7.9)	2.9(9.1)	0.085
IL-6(pg/ml)	3.8(4.5)	19.2(15.4)	4.8(7.6)	10.4(3.6)	0.008*
MDA(µmol/L)	8.87(4.9)	6.72(3.26)	5.3(3.75)	6.75(3.75)	0.033*
2 h after meal					
hs-CRP(µg/ml)	2.05(7.3)	3(7.53)	3.6(8.1)	3.7(10.5)	0.56
IL-6(pg/ml)	4.8(7.5)	4(4.4)	3.6(7.6)	5.6(7.6)	0.432
MDA(µmol/L)	9.25(4.65)	6.42(3.19)	4.5(1.35)	6.7(4.1)	<0.001*

* P≤0.05 be significant.

Table5. Blood Pressure and Pulse Rate in Fasting and Postprandial States before and after Supplementation

Parameters	Groups				P-value†
	Vitamin E Median(IQR*)		Placebo Median(IQR)		
	Baseline	After 6 weeks	Baseline	After 6 weeks	
Before meal(fasting)					
Systolic BP‡	125(15.25)	119(28.25)	132(15)	132(19)	0.375
Diastolic BP	78(13)	79(20.25)	87(13)	85(7)	0.325
Pulse rate	76.5(16)	80(11.75)	79(27)	86(15)	0.202
2 h after meal					
Systolic BP	126.5(22)	119(29.25)	135(17)	129(14)	0.616
Diastolic BP	79(16.25)	73(16.75)	85(15)	84(8)	0.094
Pulse rate	93(12.25)	83.31(16.75)	89(18)	97(11)	0.007*

***IQR:** The interquartile range
†P≤0.05 be significant
‡ **BP:** blood pressure.

Discussion

This study explored the hypothesis that vitamin E supplementation might improve fasting and postprandial oxidative stress, lipid profile, glucose metabolism, hypertension, pulse rate and inflammation in type 2 diabetic patients. The results show that supplementation with 400IU vitamin E per day for 6 weeks decreases MDA levels in fasting and postprandial states significantly. We also observed a significant decrease in postprandial pulse rate and a significant increase in fasting IL-6 in vitamin E group compared to the controls, but there were no significant effects on other biochemical parameters in fasting or two hours after eating fatty meal. These findings are consistent with the results obtained by Mazloom *et al*. in a 6 week placebo-controlled supplementation trial with vitamin C (1000mg/d) as an antioxidant in type 2 diabetic patients [14]. Also, the levels of total cholesterol, LDL-c, HDL-c and TG did not vary significantly after two months of administering 1200IU vitamin E to diabetic patients [15].

Esposito *et al*. demonstrated that consumption of antioxidants (vitamin C, 184mg, vitamin E, 19mg and ß-caroten, 15mg) with a high fat meal compared to a high fat meal without antioxidant did not significantly change the glucose, HDL-c and blood pressure after meals [16]. Likewise, LU *et al* in their investigation indicated that treating type two diabetic patients with 1 gram vitamin C three times per day for two weeks could not change IL-6, hs-CRP and oxidant form of LDL significantly [4]. In contrast, Lawrence *et al*. demonstrated that consumption of 800IU vitamin E and 2g vitamin C with oral glucose loading in healthy subjects cannot affect MDA levels and other oxidative stress markers [17]. Two studies showed that supplementation of type 2 diabetic patients with 800IU/per day and 1200IU/per day vitamin E for one and three months respectively decreased inflammatory markers significantly [18, 19].

Increased production of free radicals concordant with reduced antioxidant defense system in diabetic patients shows the importance of antioxidant supplementation in this group [20, 21].

Diabetes is associated with lipid peroxidations increasing production of free radicals such as peroxyl and superoxide anions and thereby increasing reactive oxygen species (ROS) and reactive nitrogen species (RNS) levels in the body that cause oxidative stress condition [6, 22].This situation has been reported to be involved in pathogenesis of diabetic complications [23]. The increase of serum glucose, lipid profile (mainly TG) and insulin levels in diabetic patients are more rapid than healthy adults after eating meal (postprandial state) [24]; this state provides more substrates to produce more free radicals and is associated with higher risk of cardiovascular disease compared to fasting state.

In this regard, antioxidant consumption such as vitamin E can be effective. Vitamin E is a chain breaking antioxidant protecting the lipid phase of cells from oxidative chain reactions [24, 25] and is an important lipid-soluble antioxidant in human plasma. The antioxidant effect of tocopherols is mainly due to their ability to donate hydrogens from the phenolic ring of the molecule to lipid radicals [26]. A tocopheroxyl-radical is then formed that can be reduced back to tocopherol. This property leads to a decrease in lipid peroxidation markers like MDA, as occurred in this study.

Elevated circulating levels of free fatty acids liberated from adipose tissue or diets and hyperglycemia in diabetes increase free radicals which can activate stress sensitive signaling pathways that result in pro-inflammatory gene expression, elevated levels of endothelin-1 and angiotensin-2 and decreased levels of prostacyclin and nitric oxide (NO) [27]. These changes increase the blood pressure and pulse rate which can be intensified with the increase of free radicals such as that after a meal. In our study, we showed that consumption of vitamin E with a fatty meal thwart free radicals and decrease pulse rate postprandially.

Some studies suggest that IL-6 may exert anti-inflammatory effects by decreasing circulatory level of tumor necrosis factor α (TNF α) and stimulating the production of anti-inflammatory cytokines. Furthermore, accumulating evidence shows a link between IL-6 and AMPK (AMP-activating protein kinase). AMPK activation stimulates fatty acid oxida-

tion and increases glucose uptake. IL-6 was shown to increase AMPK activation in both skeletal muscle and adipose tissue.
Thus, this trend shows that IL-6 might have a protective role in defending type-2 diabetes [28]. So in our study the increase in fasting IL-6 after supplementation might have beneficial effects in these patients. Further studies are needed to find the exact molecular mechanism of vitamin E supplementation in this regard.

Conclusion

In conclusion, our study shows that short term supplementation of vitamin E is safe and effective in decreasing oxidative stress and pulse rate in type 2 diabetic patients. This supplementation can be considered as alternative or additive treatments for diabetic patients to prevent chronic complications. Further studies are recommended to evaluate their long term efficacy and safety, different dose actions and their effect in longer hours postprandially.

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Conflicts of Interest

The authors declare that there is no conflict of interest.

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