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Plasma Concentrations of Vitamins E and A, and Effects of Vitamin E Supplementation on Oxidative Stress and Immune Status in Korean Non-Insulin Dependent Diabetic Patients*

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ABSTRACT

Plasma concentrations of Vitamins E and A were measured in 15 non-insulin dependent Korean female subjects and 15 age-matched normal subjects using reversed-phase high-performance liquid chromatography. No differences were found in plasma Vitamin E concentrations between the 2 groups. Plasma Vitamin A concentrations were higher in subjects with non-insulin dependent diabetes mellitus (NIDDM). The effects were evaluated of 4 weeks of daily supplementation of 400 mg Vitamin E on plasma levels of these two vitamins. In addition, the effects were observed for Vitamin E supplementation on oxidative stress and immune-related compound productions in non-insulin dependent diabetic patients and control subjects. After treatment with Vitamin E, plasma Vitamin E concentrations were significantly elevated in both groups. Basal plasma thiobarbituric acid reactive substances (TBARS) were identical, and a decreased level of TBARS caused by Vitamin E was observed only in the diabetic group (0.02739 ± 0.0024 versus 0.01814 ± 0.0008 nmols malondialdehyde equivalents/dl plasma ; $p<0.05$). The basal and after-treatment levels of immunoglobulins A, G, M were identical in control and diabetic groups, indicating that Vitamin E did not appear to alter gross humoral responses in this study. However, elevation of Complement 3 (C_3) was noticed due to Vitamin E supplementation, revealing a possible effect of Vitamin E on one aspect of humoral immunity. Furthermore, an increase in Prostaglandin E_2 (PGE_2) levels in diabetic patients was normalized by Vitamin E supplementation. This suggests indirectly that the depressed cell-mediated response due to elevated PGE_2 could be normalized. For the definitive antioxidant intake recommendations for prevention and treatment of adverse effects of non-insulin dependent diabetes, evidence from intervention trials like this study should be collected. The present data suggests that Vitamin E may exert some protective effects against oxidative damage and might have beneficial effects of partial immuno-stimulation.

KEY WORDS : VE supplementation · NIDDM subjects · oxidative stress · immune status.

INTRODUCTION

It is generally accepted that free radicals are produced in the body as by-products of normal metabolism and also as a result of exposure to environmental agents, and can lead to damage to cellular components.[1] In organisms, free radicals are known to be neutralized to a certain degree by antioxidant enzyme systems and nutrient-derived antioxidant molecules such as vitamin E, vitamin C, etc. In the body, oxidative stress may occur as a result of increased free radical generation or decreased levels of antioxidants and/or impaired regeneration of reduced forms of antioxidants.[2][3] The evidence for increased oxidative stress in diabetes includes observations of exaggerated free radical activity, decreased antioxidant plasma concentrations in both diabetic subjects and animal models of diabetes, and reports of increased plasma lipid peroxides in this metabolic disorder.[4-13]

α-Tocopherol is recognized as one of the most important lipid-soluble antioxidants in tissues, red cells, and plasma.[14] α-Tocopherol and other forms of vitamin E are chain-breaking antioxidants that prevent oxidation by trapping peroxyl free radicals, thereby providing first-line protection against lipid peroxidation.[15-17] There are several reports confirming the protective role of Vitamin E against oxidative stress in diabetic subjects.[18-22] It has also been shown that the onset of diabetes in experimental animals can be delayed by feeding supplements rich in free radical scavengers, including Vitamin E.[23-25] There is growing interest in the beneficial effects of Vitamin E on prevention and complications of diabetes. This has been stimulated by observations such as epidemiological findings that there is an inverse relationship between plasma Vitamin E concentration and risk for NIDDM in 944 Finnish males over a 4 year period of study.[26] Therefore, human data

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that is linked to determination of the optimum amount of Vitamin E in various pathological conditions seems necessary at present. Despite this need, the effect of α-Tocopherol supplementation on the antioxidant status of Korean diabetic subjects has not been investigated.

Although Vitamin A has been studied less than Vitamin E, Vitamin A is also known to protect lipid membranes against free radical-mediated damage.[27][28] Before clarifying the effect of Vitamin E supplementation, it seems logical to evaluate the circulating concentrations of Vitamin A and Vitamin E in NIDDM and control subjects.

In addition to the major role on oxidative stress, Vitamin E has been recognized as one of the nutrients that influence the immune system. Vitamin E is found in high concentrations in the membranes of immune cells, because they are at high risk for oxidative damage.[29][30] It became evident from several animal and human studies that Vitamin E deficiency is strongly associated with inadequate immune responses.[31-33] It is presumed that Vitamin E might have protective effects on immune status in illnesses with high oxidative stress.[34] However, the effects of Vitamin E supplementation on immune status in NIDDM have not been investigated previously. This study was undertaken to investigate the oxidative state and immune status in diabetes and the effect of treatment with antioxidant α-Tocopherol on these parameters in order to evaluate the protective effect of Vitamin E over oxidative stress and immune functions. The result of this study could give some background information for the determination of the optimum intake of Vitamin E in abnormal metabolic states.

METHODS AND MATERIALS

1. Subjects and study design

Female NIDDM patients were recruited from the outpatient clinic in Seoul ,and age, weight-matched normal subjects were selected from the healthy population. Diabetic patients were between 51 and 60 years of age with an average duration of illness 5 years, and they were without any serious complications of diabetic symptoms. Neither diabetic or control subjects were using insulin, vitamins, minerals, or any food supplements. Experimental subjects had non-insulin dependent diabetes mellitus and were treated with oral blood glucose lowering agents with no other medication. All of the subjects were non-smokers. Participants underwent a brief physical examination, and then their blood was taken after an overnight 10-12 hour fast. They received DL-α-tocopherol acetate (Yuhan Pharmaceutical Co.) 400 mg per day for 4 weeks. After 4 weeks, the blood sampling procedure was repeated. While they were taking Vitamin E, they were checked periodically for their supplement consumption. They were advised to follow the same dietary patterns and daily activities as before the intervention trials and were asked to report the results.

2. Collection of blood samples and biochemical analysis

After an overnight fast, blood was collected in heparinized tubes. Plasma was separated from cells by centrifugation. Plasma samples were then stored at −70℃ until analysis. Plasma Vitamin E and Vitamin A (retinol) were analyzed with the High Pressure Liquid Chromatography system (Shimadzu SCL-10A system, Japan) according to Official method of Food Analysis.[35] The HPLC conditions for vitamin analysis are as follows(Table 1).

The 200 μl of plasma was combined with 1.2 ml of chloroform/methanol (2 : 1,v/v), vortexed, centrifuged for 10 min at 1,000 rpm, and a chloroform/methanol layer was taken. The organic solvent layer was evaporated to dryness under nitrogen and reconstituted with 100 μl mobile phase solution, and 20 μl of sample was used for the HP LC analysis.

Plasma glucose was measured using a glucose oxidase-peroxidase assay according to the reported method.[36]

TBARS in plasma were determined with the thiobarbituric acid reagent. The assay was started by mixing plasma with 1/12 N sulfuric acid and 10% phosphotungstic acid for 5 min, and after centrifugation at 3,000 rpm for 10 min, precipitants were treated once more with the agents. The resulting precipitants were mixed with H_2O and thiobarbituric acid reagent and sealed, and then the samples were heated for 60 min in a boiling water bath, cooled and extracted with n-butanol, and absorbance was determined at 534. The concentrations of TBARS were expr-

Table 1. Chromatographic condition for HPLC

Condition	α-tocopherol	Retinol
Column	Cap cell pack 18 (shimadzu)	Cap cell pack 18 (shimadzu)
Mobile phase	Acetonitrile/Methanol (75 : 25,v/v)	Acetonitrile/Methanol (75 : 25,v/v)
Detector	Spectrofluoromertric (Em 285nm, E×325mn)	Spectrofluoromertric (Em 340nm, E×460mn)
Flow rate	2ml/min	1ml/min

essed as malondialdehyde equivalents (nmol) per 100 ml plasma.

Plasma Immunoglobulin A, G, M (IgA, IgG, IgM) and C_3 were measured according to the method of rate nephelometry.[37] Plasma Prostaglandin E_2 (PGE_2) levels were determined by enzyme-linked immunoabsorbant assay (EL ISA kit, Amersham, United Kingdom).

3. Statistical analysis

Statistical analysis was basically performed by using the Statistical Analysis System (SAS).[38] Data was expressed as the mean with standard error, and statistically significant differences between subgroup means were evaluated primarily by Student's t-test.

RESULTS AND DISCUSSION

The mean age and anthropometric characteristics of the study subjects are shown in Table 2. The mean age and anthropometric measurements were comparable between NIDDM subjects and control subjects. Fasting plasma glucose was significantly higher in diabetic subjects than in normal control subjects. Table 3 shows the result of plasma Vitamin E and A concentrations analysis before and after Vitamin E supplementation. Before supplementing with Vitamin E, both vitamin levels appeared to be within normal ranges.[39] However, subjects with NIDDM had higher serum Vitamin A concentrations than normal subjects, whereas Vitamin E concentrations were similar in both of the groups. Previous investigations[40-42] have shown that individuals with NIDDM have higher serum Vitamin A concentrations than normal subjects. Presently, the reason for elevated Vitamin A in NIDDM is unclear. It has been suggested that high insulin levels in insulin-resistant animals lead to increased circulating Vitamin A concentrations by depleting hepatic stores of Vitamin A.[43] The Vitamin A increase in NIDDM subjects has shown to occur independently from insulin resistance and the lipid status of NIDDM subjects in other study.[41] The result of supplementing Vitamin E for 4 weeks shows that the average plasma Vitamin E concentrations almost dou-bled after feeding 400 mg of Vitamin E. The plasma Vita-min E levels after supplementation are somewhat lower than in reported supplementation studies on diabetic and nondiabetic subjects, despite the duration and supplementation levels were similar.[44-46]

Fig. 1 shows plasma concentrations of TBARS, which represent products of fatty acid peroxidation products. No differences in basal levels of TBARS were found between NIDDM and control groups. Most of the previous studies[44][47] on plasma Vitamin E concentrations in diabetic subjects showed an increased TBARS value compared to control. The absence of elevated TBARS in diabetic subjects shown in this study may indicate that these subjects are under the same degree of oxidative stress as control subjects. The reason for this observation awaits further studies. However, the differences in the characteristics of diabetic subjects, such as the degree of diabetes, sample size, or dietary intake might be possible reasons. After supplementing with 400 mg of Vitamin E for 4 weeks,

Table 2. Characteristics of Korean normal and NIDDM subjects

	Normal (n=15)	NIDDM (n=15)
Age (year)	54.8±1.6[1]	54.8± 1.6
eight (cm)	152.0±1.8	153.9± 3.4
Weight (kg)	58.8±3.7	61.0± 3.8
BMI (kg/m^2)	24.5±1.3	25.7± 1.2
Fasting blood glucose (g/dl)	82.5±3.3	146.7±18.8[#2]

1)Mean±SE
2)# : Significantly different between normal and NIDDM groups by Student t-test, $p<0.05$

Table 3. Plasma concentrations of Vitamin E and Vitamin A in Korean normal and NIDDM subjects before and after supplementing 400mg of α-Tocopherol for 4 weeks

		Normal (n=15)	NIDDM (n=15)	Total
Vitamin E	Before	1182.1±110.6[1]	1200.8±107.1	1187.4± 83.3
(μg/dl)	After	2748.0±256.8[**2]	2104.3±345.9[*]	2565.2±213.8[**]
Vitamin A	Before	81.1± 6.1	134.2± 11.1[#3]	98.0± 7.6
(μg/dl)	After	64.2± 3.8[*]	126.6± 15.0[#]	82.0± 8.0

1) Mean±SE
2) * : Significantly different at $p<0.05$ by Student t-test between before and after. ** : $p<0.001$
3) # : Significantly different at $p<0.05$ by Student t-test between normal and NIDDM

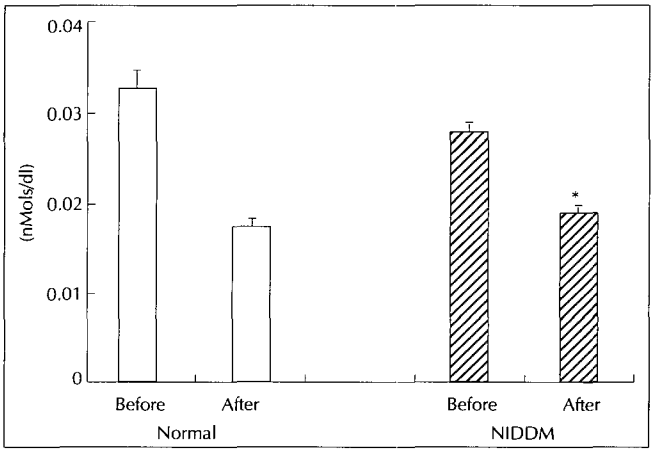

Fig. 1. Plasma TBARS concentrations in Korean normal and NIDDM subjects before and after supplementing 400 mg of α-Tocopherol for 4 weeks.
* : Significantly different at $p<0.05$ by student t-test between before and after.

the mean values of TBARS lowered in both of the groups. A statistically significant decrease was observed only in NIDDM subjects. Distinctive decreases in the concentration of TBARS after Vitamin E treatment are common observations, especially in diabetic patients[44)48)] and diabetic rats.[49)] No significant changes occurred in TBARS concentrations after Vitamin E treatment in normal subjects with wider individual variations in TBARS after Vitamin E treatment (data not shown). Intervention trials to supplement Vitamin E to Korean NIDDM patients, either in a small scale or a large scale, have not been performed before. In this study, determination of effects of Vitamin E on oxidative status was attempted, but studies on the beneficial effects of Vitamin E treatment on insulin sensitivity and other complications in lipid metabolism seems to be greatly needed to provide basis for the appropriate level or safe dosage of Vitamin E intake in Korean diabetics.

Figs. 2, 3 and 4 represent plasma immunoglobulin A, G, and M concentration before and after Vitamin E treatment. Other studies of Immunoglobulins usually show that NIDDM patients had significantly elevated IgA or IgM levels.[50)51)] In contrast, these subjects did not have increased levels of these immunoglobulins compared to normal subjects. Significant effects were not noted in the levels of immunoglobulins after the Vitamin E treatment. This result can not be reconfirmed by other data, since the effects of Vitamin E supplementation on humoral im-

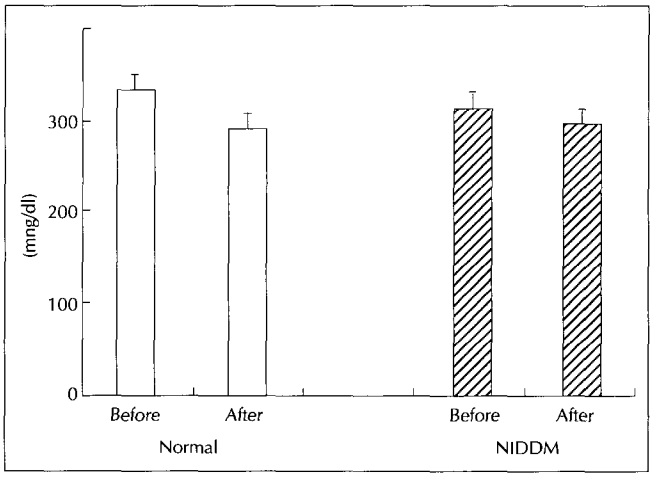

Fig. 2. Plasma Immunoglobulin A concentration in Korean normal and NIDDM subjects before and after supplementing 400 mg of α-Tocopherol for 4 weeks.

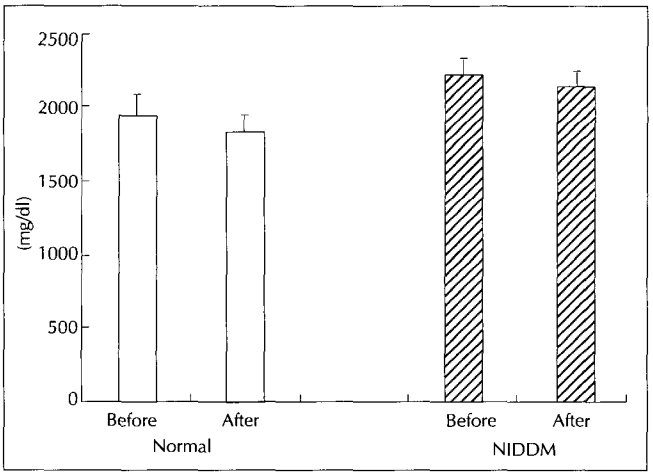

Fig. 3. Plasma Immunoglobulin G concentrations in Korean normal and NIDDM subjects before and after supplementing 400 mg of α-Tocopherol for 4 weeks.

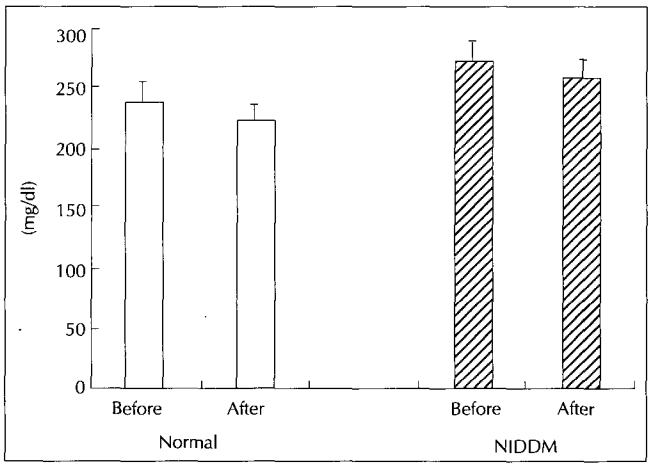

Fig. 4. Plasma Immunoglobulin M concentrations in Korean normal and NIDDM subjects before and after supplementing 400 mg of α-Tocopherol for 4 weeks.

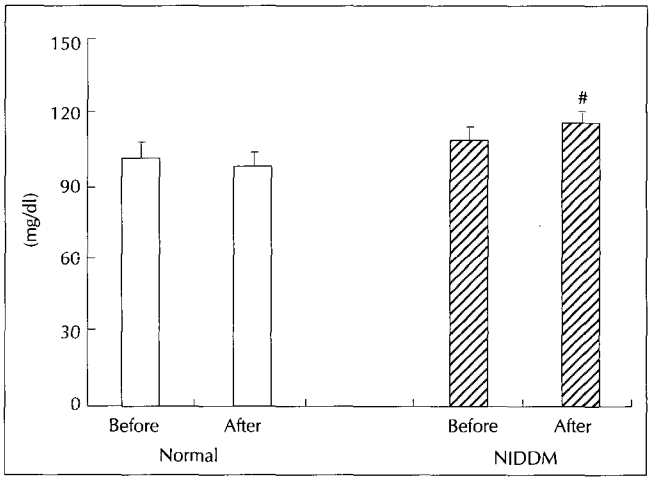

Fig. 5. Plasma complement C_3 concentrations in Korean normal and NIDDM subjects before and after supplementing 400 mg of α-Tocopherol for 4 weeks.
* : Significantly different at $p<0.05$ by student t-test between normal and NIDDM.

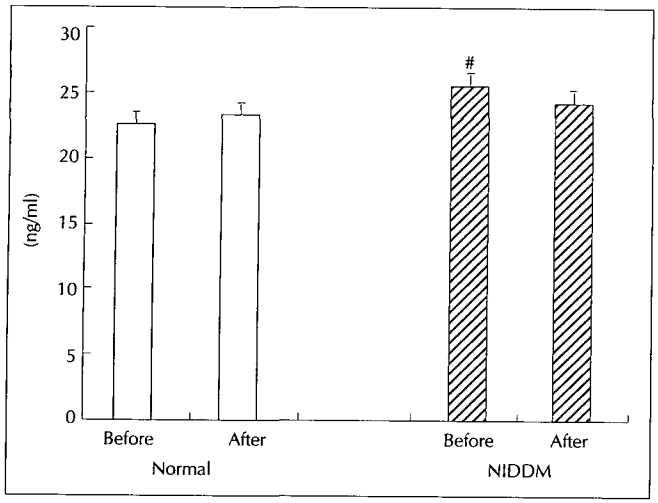

Fig. 6. Plasma prostaglandin E_2 concentrations in Korean normal and NIDDM subjects before and after supplementing 400 mg of α-Tocopherol for 4 weeks.
: Significantly different at $p<0.05$ by student t-test between normal and NIDDM.

munity in diabetes have not yet been investigated with either Korean or non-Korean subjects. The demonstration of the effectiveness of Vitamin E supplementation on humoral responses was limited with normal elderly subjects.[52] Besides the effects or immunoglobulin levels, the effect of Vitamin E supplementation on C_3 concentration was also observed (Fig. 5). Feeding 400 mg of Vitamin E for 4 weeks significantly elevated the level of C_3 only in diabetic subjects. The previous study of changes in C_3 levels by Vitamin E treatment in normal elderly subjects showed no significant effects.[53]

To assess the effect of Vitamin E supplementation on lymphocyte proliferation indirectly, plasma prostaglandin E_2 (PGE_2) concentrations were determined. Diabetic subjects showed significantly elevated PGE_2 and this disappeared after supplementing with Vitamin E (Fig. 6). PGE_2 is known to have an inhibitory effect on lymphocyte proliferation and thereby depressing immune responses, specially cell-mediated responses.[54] The elevation of PGE_2 in diabetic subjects before Vitamin E supplementation might indicate the possibility for a depressed state of cell-mediated immunity in NIDDM subjects. The supportive evidence shows that enhancements of immune responses through decreased PGE_2 production in humans[55] and in animals,[56][57] occurred with concurrent increases in cyclooxygenase activities. However, to confirm the relation between PGE_2 levels and cell-mediated immunity, the further studies are needed.

The data from the present study shows a lowering of the elevated peroxidation of lipids in diabetic patients. Vitamin E supplementation in diabetic patients appeared to be beneficial in some aspects of immune status such as stimulation of C_3 production and lowering of elevated PGE_2, which were not observed in normal control subjects.

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