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Influence of Dietary Vitamin E on Susceptibility to Ozone Exposure

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It is widely reported that vitamin E intake may markedly reduce the occurrence of ozone-induced respiratory tract damage (Chow 1983; Chow and Tappel 1972; Dungworth et al. 1975; Goldstein et al. 1969). Considerably less data are available concerning whether vitamin E nutritional status may affect the occurrence of ozone-induced systemic alterations such as the blood cell changes (Chow and Kaneko 1979; Menzel et al. 1975; Goldstein and Balchum 1967; Chow et al. 1981). Nevertheless, the data that are available suggest a certain degree of protection. The present study is designed to extend this previous research by evaluating the extent to which vitamin E supplementation in humans over a four week period would affect the *in vitro* erythrocyte susceptibility to hydrogen peroxide (H_2O_2) which Goldstein (1973) reported is formed in whole blood following ozone exposure.

MATERIALS AND METHODS

Twelve healthy male subjects, aged 23-27, were recruited for this study. Each subject was randomly assigned to either a control group or a vitamin E group. The control group was given a standard fructose placebo. The vitamin E group was given a total of 600 mg of α-tocopherol daily. The vitamin E was taken in tablet form in a divided dose twice daily: in the morning and at night. No attempt was made to control individual diets, except for the vitamin supplementation administered during the study.

Prior to the initiation of vitamin supplementation, 30 ml of blood were drawn in heparinized tubes via venipuncture. This blood was then incubated with three levels of H_2O_2 for one half hour. Control tubes containing blood without H_2O_2 were also incubated. The level of H_2O_2 was

determined during preliminary research to yield methemoglobin (METHB) increases of approximately 2-3% for a "low" stress level (i.e., level #1), 5-8% for a "moderate" stress level (i.e., level #2), and 15-20% for a "high" stress level (i.e., level #3). Tested stressor levels also reduced glutathione (GSH) levels by approximately 5-20 mg% in a dose-dependent fashion. Reduced GSH, METHB, plasma vitamin E and hematocrit levels were then measured in the blood.

Immediately following the collection of the baseline data, the subjects started the vitamin regimen. After two weeks of vitamin/placebo treatment, 30 ml of blood were drawn from each subject with the same blood and urine analyses being performed. The subjects continued their respective vitamin/pacebo regimens for an additional two weeks, following which 30 ml of blood were again drawn and the same blood chemistry tests were done. Each subject acted as his own control, thus providing both internal and external controls.

The hydrogen peroxide was generated from a glucose:glucose oxidase enzyme system. The methodology was adapted from Barker et al. (1973) for use on whole blood.

β- D(+) Glucose was purchased from Sigma Scientific Products and a 2 molar solution was made using distilled water. Type II glucose oxidase, with an activity of 17,800 units/gm, was also purchased. A stock solution of glucose oxidase was made containing 1780 units/ml of distilled water. The final concentration recommended by Barker et al. (1973) of 41.6 millimolar of glucose/ml and 1.5 units of glucose oxidase/ml were utilized in this study. The final amounts incubated with the blood for the three respective dosage levels were 1.2 units of activity/1 ml whole blood, 2.4 units and 3.6 units.

The blood samples were evaluated using biochemical assays for METHB formation, GSH level and plasma vitamin E. Methemoglobin and GSH parameters were chosen for study because under oxidative stress to red blood cells, hemoglobin is oxidized to METHB and GSH to GSSG. Therefore, both parameters are considered good indicators of oxidant stress. A standard hematological test for hematocrit (Hct) was also performed, as a Hct value was necessary for the mathematical calculation of GSH. All tests were performed on whole blood. A qualitative vitamin C test was conducted on a urine sample provided by each subject. Each assay or test is outlined below.

Due to time constraints on the testing days, the vitamin E assay was performed after the completion of the actual experiment. However, on the test days, 3-4 ml of the subject's blood was centrifuged for ten minutes, and 1 ml of plasma was withdrawn. This plasma was then frozen at -20°C until the assay could be performed. Plasma vitamin E was determined using the procedure outlined by Hansen and Warwick (1966). Only free vitamin E was performed, as free vitamin E is highly positively correlated to total vitamin E (Calabrese unpub. obser.). METHB was measured according to the method of Brown (1973) using potassium ferricyanide and potassium cyanide as reagents and measuring changes in optical density at 630 nm. A colorimetric reaction employing 5,5'-dithiobis nitrobenzoic acid (DTNB) was used to measure the amount of GSH in blood according to Prins and Loos (1969) at 412 nm.

A Repeated Measures Analysis of Variance (RMANOVA) was applied to the data, using a BMDP statistical package (University Computing Facility, University of California, Los Angeles). The dependent variables were percent METHB and mg GSH per 100 ml red blood cell. The independent variables were hydrogen peroxide (H_2O_2) levels and vitamin E levels.

Prior to the RMANOVA, the data were adjusted by subtracting each subject's baseline values from their two and four week values, yielding a variable labelled "D" (defined as difference between values). Then, the subject's two and four week control values (referring to blood without stressor) were subtracted from their two and four week "D" values, yielding an "AD" variable (defined as adjusted). These adjustments were done to incorporate each subject's internal controls. The RMANOVA was then applied to the adjusted variables.

Following the RMANOVA, the means for the vitamin group, at each stressor level, were compared to the means for the placebo group using Duncan's Multiple Range Test with significance level set at 0.05.

RESULTS AND DISCUSSION

The vitamin E treatment was found to significantly reduce ($P < 0.05$) METHB formation at both the second (-7.7%) and fourth (-5.4%) weeks, respectively at the highest stressor level (Table 1). Vitamin E treatment did not have any statistically significant effect on METHB levels at the lower two treatment stressor levels.

The vitamin E treatment had no statistically significant ($P > 0.05$) effect on the GSH levels at any level of oxidant stress (Table 1).

Table 1. The Effects of Vitamin E on H_2O_2-Induced Changes in Erythrocyte Methemoglobin and Glutathione Levels

Glutathione

Relative Change from Placebo in mg/100 ml Red Blood Cell

Vitamin E Level	Hydrogen Peroxide 1	2	3
2 week	-1.1	-5.1	-1.8
4 week	+1.7	-2.0	+2.0

Methemoglobin

Relative Change from Placebo in Percent

Vitamin E Level	Hydrogen Peroxide 1	2	3
2 week	+1.7	-1.7	-7.7*
4 week	+2.0	+1.1	-5.4*

*significant difference ($P < 0.05$) from placebo controls

Numerous reports exist which indicate that vitamin E intake markedly affects the pulmonary toxicity of ozone in animal models (Chow 1983; Chow and Tappel 1972; Dungworth et al. 1975; Goldstein et al. 1975). Comparable findings, although less numerous, reveal that vitamin E nutritional supplementation likewise reduces the magnitude of ozone induced red blood cell alterations in animal models (Chow and Kaneko 1979; Menzel et al. 1975; Goldstein and Balchum 1967; Chow et al. 1981). However, there is a general lack of validation concerning the extent to which these findings in animal models may be predictive of what possibly occurs in humans. In 1975, Menzel et al. reported that supplementation of human volunteers with up to 200 mg of α-tocopherol per day for two weeks, markedly reduced the toxicity of a proposed ozone intermediate, methyl oleate ozonide (MOO). More specifically, they reported that the vitamin E supplementation reduced the ozonide induced formation

of Heinz bodies over a range of Heinz body formation (i.e., 26.9 -91.9%). No evaluation of a possible protective effect at lower levels of MOO stress was made.

The present studies are partially consistent with the findings of Menzel et al. (1975), in that they reveal that at the highest level of H_2O_2 induced stress (i.e. up to 16 - 18% METHB formation), volunteers taking 600 mg vitamin E per day showed a 5.4 to 7.7% decrease ($p < 0.05$) in METHB values in comparable in vitro testing to the Menzel at al. (1975) study. However, no protective effects were seen at lower levels of H_2O_2 induced stress for METHB. No significant ($p > 0.05$) vitamin E induced protective effects as indicated by increased GSH values were found at any conditions involving H_2O_2. Furthermore, no protective effect ($p > 0.05$) was observed with respect to another proposed toxic ozone intermediate, methyl oleate hydroperoxide, (MOHP) (Victor 1983). In fact, the vitamin E treatment appeared to enhance slightly the oxidative stress of MOHP, although these changes were statistically insignificant ($p > 0.05$). At the present, no adequate explanation exists to elucidate the divergent pattern of vitamin E induced protective effects, which is protecting against H_2O_2 and MOO (Menzel et al. 1975) induced changed, but not against MOHP induced changes.

These findings suggest that vitamin E supplementation may play a role in protecting against ozone induced toxicity in red blood cells, at appreciable levels of oxidant stress. In contrast to the higher levels of stress, no protection from vitamin treatments occurred at lower, more realistic levels of stress.

There is considerable debate over whether people living in high ozone areas, such as the Los Angeles county basin, should supplement their diets with vitamin E (Lee et al. 1983). The present study certainly does not resolve this issue. For even though vitamin E supplementation reduced H_2O_2 induced changes in METHB at high stressor levels, the data suggest that it does not have any effect at low levels of stress. Clearly, further research is needed in this area.

Finally, it must be emphasized that while H_2O_2 is generated in whole blood by ozone (Goldstein 1973), it is highly uncertain as to what the range of possible toxic ozone intermediates may be as well as what serum concentrations these possible intermediates may correspond to in terms of the inhalation of

ambient ozone.

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