Bioavailability of Vitamin E as Function of Food Intake in Healthy Subjects
Effects on Plasma Peroxide–Scavenging Activity and Cholesterol-Oxidation Products

L. Iuliano, F. Micheletta, M. Maranghi, G. Frati, U. Diczfalusy, F. Violi

Abstract—Clinical trials with vitamin E have yielded contrasting results. In these trials, the amount of vitamin E given was different, and the compliance was not assessed in all studies. In addition, the modality of intake, ie, in relation to food, was not specified in any trial. Vitamin E is lipophilic, and its absorption is expected to be increased by food. We studied the bioavailability of vitamin E in relation to food intake and the effect on the lipid peroxide–scavenging activity of plasma and on 7β-hydroxycholesterol and 7-ketocholesterol (oxysterols) as markers of oxidant stress. Twenty healthy Italian subjects were randomly assigned to take vitamin E at 300 mg/d on an empty stomach (group A) or during dinner (group B) for 15 days. Plasma vitamin E markedly increased in group B (84%) compared with group A (29%). The lipid peroxide–scavenging activity of plasma increased significantly in group B (14%, \( P < 0.005 \)) but did not change in group A. All subjects showed very low levels of plasma oxysterols, which were not affected by vitamin E supplementation in either group. This study shows that plasma concentration of vitamin E and plasma antioxidant activity in response to oral supplementation are markedly affected by food intake. Healthy Italian subjects show very low levels of cholesterol oxidation products; these low levels are possibly related to the Mediterranean diet. To obtain maximal absorption, vitamin E must be given at meals. These data should be taken into account in clinical trials with vitamin E. (Arterioscler Thromb Vasc Biol. 2001;21:e34-e37.)

Key Words: atherosclerosis ■ antioxidants ■ oxidant stress ■ oxysterols ■ vitamin E

The most effective vitamin E isomer, α-tocopherol, is a naturally occurring lipophilic chain–breaking antioxidant that circulates in the blood bound to β-lipoproteins. It is present in cell membranes, in which it exerts a potent defense against lipid peroxidation. Lipid peroxidation is one of the hallmarks of oxidant stress in living organisms. It is considered to play an important role in the pathophysiology of several conditions, including cardiovascular disease. A large body of evidence has been gathered in support of the hypothesis that free radical–mediated oxidative processes, particularly the oxidation of LDL, play a key role in atherogenesis.

The effectiveness of vitamin E in preventing atherosclerotic processes is supported by antioxidant chemistry, by in vitro and ex vivo studies in cell systems and animal models, and by in vivo studies in animals.

Although initial studies in humans provided encouraging results, clinical trials with vitamin E supplementation showed contrasting findings. The effect of vitamin E supplementation in reducing the incidence of nonfatal myocardial infarction shown by the Cambridge Heart Antioxidant Study (CHAOS) was not confirmed by subsequent clinical trials. The 2 largest trials using vitamin E in patients with coronary artery disease, the Heart Outcomes Prevention Evaluation (HOPE) study and the Gruppo Italiano per lo Studio della Streptochinasi nell’Infarto Miocardico (GISSI)-Prevenzione trial, did not find a significant reduction of cardiovascular events. Nevertheless, in the Secondary Prevention With Antioxidants of Cardiovascular Disease in Endstage Renal Disease (SPACE) trial, vitamin E administration was able to reduce vascular events in hemodialysis patients, who are at high risk of cardiovascular complications. The cause of these conflicting results is not clear, and several factors have been suggested to play a role. These include compliance, the design of trials, the dosage regime used (ranging from 50 mg to \( \sim 1600 \) mg [800 IU] per day), and the source of vitamin E linked to the unlike bioavailability of natural and synthetic form of vitamin E. Compliance has also been questioned because not all studies estimated vitamin E plasma levels in placebo and target groups.

Vitamin E is lipid soluble, and food intake could potentely influence its bioavailability. Accordingly, we evaluated the
bioavailability of synthetic vitamin E in relation to food intake in healthy subjects. In addition, we studied the effect of supplementation on the lipid peroxide–scavenging activity of plasma (LISAP) to assess whether vitamin E supplementation affects another plasma marker, which can be used in large population studies. Finally, plasma oxysterols were also assessed as markers of oxidant stress in vivo.

Methods
Twenty healthy Italian volunteers from the hospital medical staff were randomly assigned to take 300 mg/d vitamin E (Dl-tocopheryl acetate) at 11:00 AM, which was 3 hours before lunch (group A), or at 8:00 PM during dinner (group B) for 15 days. Subjects of group A were invited to take only coffee at 7:00 AM. All participants followed a Mediterranean diet, with an average intake of 2200 calories (55% carbohydrates, 10% saturated fatty acids, 10% monounsaturated fatty acids, 10% polyunsaturated fatty acids, and 15% proteins); these subjects did not use any supplements containing vitamin E, vitamin C, carotenoids, or iron for at least 30 days before the study. The subjects’ body weights did not change in the month before the study. All subjects gave informed consent to participate in the study. Blood samples were taken from patients who had fasted for at least 12 hours, between 8:00 PM and 9:00 AM. Plasma levels of vitamin E were assessed at baseline and at 7 and 15 days after the initiation of supplementation. In each patient, LISAP, oxysterols, cholesterol, triglycerides, and urate were evaluated at baseline and at 15 days after the initiation of supplementation. In each patient, LISAP, oxysterols, cholesterol, triglycerides, and urate were evaluated at baseline and at the end of treatment period.

Plasma levels of \( \alpha \)-tocopherol were analyzed by high-performance liquid chromatography, as described,\(^6\) with the use of 50 µL plasma, \( \alpha \)-tocopherol acetate as an internal standard, and extraction with ethyl acetate; levels were expressed as milligrams vitamin E per gram plasma lipids (cholesterol plus triglycerides).\(^10\) Plasma samples for oxysterol analysis were frozen and transported on dry ice to Sweden for analysis. The samples were kept at \(-70^\circ\)C until analysis. Plasma oxysterols were measured by mass spectrometry with the use of an isotope dilution method as previously described.\(^11\)

LISAP, measured by the inhibition method of peroxyl radical–mediated dipyridamole fluorescence decay,\(^12\) was expressed in trolox equivalents.

Randomization was achieved by using the table of random numbers according to Polock.\(^13\)

Mean±SD values were calculated for all measured parameters. The Friedman 2-way ANOVA was used to determine the significance of difference in each parameter between the 2 groups and between data collected at different times. A difference was considered statistically significant at \( P<0.05 \).

Results
Twenty healthy subjects participated in the present study. Ten subjects in group B took vitamin E at 8:00 pm during dinner. Ten subjects in group A took vitamin E at 11:00 AM on an empty stomach. The baseline characteristics of the population study are described in the Table. There were no differences in age, sex, or smoking habit between subjects in group A and group B. Plasma total cholesterol, triglycerides, uric acid, vitamin E, LISAP, and oxysterols were not statistically different in the 2 groups. No changes in body weight and blood lipids were observed in either group during the follow up.

Plasma vitamin E levels rose during treatment in groups A and B (Figure 1). In group A, the baseline level of vitamin E (6.10±1.00 mg/g lipids) increased by 34% after 7 days (8.2±1.7 mg/g lipids, \( P=0.011 \)) but the increase was slightly less (29%) after 15 days (7.87±0.9 mg/lipids, \( P=0.011 \)). The plasma vitamin E levels of 3 subjects in group A were unchanged during the treatment period.

All subjects in group B showed an increase in vitamin E plasma levels after supplementation. Baseline vitamin E in group B rose from 5.61±1.22 to 9.61±2.4 mg/g lipids (71%, \( P=0.002 \)) after 7 days and further increased to 10.33±1.6 mg/g lipids (84%, \( P=0.0015 \)) after 15 days. Plasma vitamin E, which was not different between the 2 groups at baseline (Table), was significantly higher (31.2%, \( P=0.0015 \)) in group B than in group A at the end of the treatment period.

LISAP increased by 14% (\( P=0.005 \)) in group B subjects at the end of supplementation period but did not change in group A subjects (Figure 2). At baseline LISAP was not correlated with plasma vitamin E concentration in either group but was highly correlated with uric acid (\( r=0.88, P<0.005 \)). At the end of treatment, LISAP was highly correlated with plasma vitamin E (for group A, \( r=0.72 \) and \( P<0.02 \); for group B, \( r=0.81 \) and \( P<0.005 \)). Baseline values of oxysterols were 2.37±0.91 and 3.58±1.69 ng/mL for 7β-hydroxycholesterol and 7-ketocholesterol, respectively. Baseline 7β-hydroxycholesterol was correlated with 7-ketocholesterol (\( r=0.77, P<0.02 \)) and total cholesterol (\( r=0.57, P<0.05 \)). On the other hand, 7β-hydroxycholesterol was negatively correlated with LISAP (\( r=-0.59, P<0.05 \)). Nor were oxysterols correlated with plasma vitamin E. After 15 days of supplementation, oxysterols showed a trend in reduction in both groups, which did not reach statistical significance. Thus, 7β-hydroxycholesterol and 7-ketocholesterol were reduced in group A by \( \approx 24\% \).
and 19%, respectively, and in group B by ≈18% and 16%, respectively (data not shown).

Discussion

In the subjects of group B, who took the antioxidant with meals, plasma vitamin E concentration at the end of supplementation period was significantly higher than that in the subjects of group A, who took the antioxidant on an empty stomach. After the initial increase, in group A subjects, vitamin E tended to decline in the second week of supplementation. In comparison, group B showed a constant increase in plasma vitamin E throughout the study. The plasma vitamin E concentration in all group B subjects was increased, whereas 30% of the subjects in group A were unaffected by supplementation. LISAP activity increased in group B subjects and remained unchanged in group A subjects. It is likely that vitamin E concentration in plasma must increase to a threshold point to affect LISAP activity. Because LISAP activity is the result of the maximal ability of plasma to scavenge peroxyl radicals, it is dependent on the concentration of several antioxidants dissolved in plasma. It is largely affected by uric acid, which is an efficient peroxyl radical scavenger, and is present in plasma at submillimolar concentration. The bimolecular rate constant for reaction of urate with peroxyl radical is 5.8 to \(9 \times 10^2\) (mol/L)\(^{-1}\) s\(^{-1}\), comparable to that of vitamin E, which is 5.8 to 13.5\(\times 10^2\) (mol/L)\(^{-1}\) s\(^{-1}\). However, urate concentration in plasma is 1 order of magnitude higher than that of vitamin E. The participation of urate in LISAP activity questions the relevance of urate in the antioxidant defense system. Urate is widely recognized as an efficient scavenger of oxygen radicals and is suggested to be an important physiological antioxidant against oxidative injuries that may have replaced (during the course of evolution) some of the antioxidant functions of ascorbic acid. In contrast, urate has been suggested to be a risk factor for heart disease through still unknown mechanisms.

An efficient vitamin E absorption is necessary to induce an efficient increase in its plasma concentration and, in turn, in LISAP activity. LISAP activity as a surrogate of the plasma antioxidant defense system has the potential advantage of a simple and rapid test to assess the effectiveness of antioxidant supplementation in vivo and could be easily used in clinical settings with enhanced oxidant stress.

Vitamin E supplementation did not affect the levels of oxysterols in the present study. Oxysterols can be produced by free radical–catalyzed oxidation of cholesterol and are candidate markers of oxidant stress in vivo. Oxysterols are associated with an increased risk of atherosclerosis, and the levels seem to be modulated by vitamin E. Subjects in the present study had very low plasma oxysterol levels compared with values reported previously in the literature. The low levels of plasma oxysterols in the present investigation are close to the detection limit (0.5 ng/mL) and could represent a background arising from artifactual generation during processing rather than the actual in vivo production. In agreement, it has recently been reported that vitamin E supplementation has no significant effect on the urinary excretion of isoprostanes and 4-hydroxynonenal in healthy persons.

Interestingly, the values of \(7\beta\)-hydroxycholesterol for the healthy Italian volunteers (2.37±0.9 ng/mL) participating in the study were lower than the values for Lithuanians (12±5 ng/mL), who are at high risk of cardiovascular disease. This may in part reflect the effect of diet, ie, the Mediterranean diet, which is well known to have an effect on cardiovascular disease. In this context, the recruitment of populations with different dietary habits in clinical trials, such as the HOPE study, has been proposed as the source of error. In conclusion, the present study shows that bioavailability of vitamin E is highly influenced by prandial status. In agreement, a previous study showed that optimal uptake of vitamin E require a limited amount of fat in the meal. The results of the present study imply that clinical trials with vitamin E that lack adequate information on its plasma levels should be closely scrutinized. Furthermore, it suggests that vitamin E consumption after food intake is useful to increase its bioavailability and eventually its antioxidant capacity.

References

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