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Review Article

Importance of tocopherols beyond α-tocopherol: evidence from animal and human studies

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Abstract

Although the tocopherol content in food has been shown to be inversely associated with mortality from cardiovascular disease, dietary supplementation with α-tocopherol alone has a modest protective effect. The lack of natural tocopherols such as γ- and δ-tocopherol in most vitamin E preparations may be a limiting factor for promoting health. Although α-tocopherol and γ-tocopherol are 2 principle tocopherols in vegetable oils, the latter is in greater abundance in the edible oils processed in the United States. In contrast to α-tocopherol, γ-tocopherol has biologic activity that potentially protects against chronic diseases such as inflammation. Evidence indicates that the mixed tocopherols found in native vegetable oils afford additive and synergistic activities that support their broader beneficial biologic functions. Both γ- and δ-tocopherol may be necessary for preventing lipid peroxidation and in counteracting the prooxidant effect of α-tocopherol. Moreover, all tocopherols except β-tocopherol inhibit smooth muscle proliferation. In our research, a preparation of mixed tocopherols, containing γ-, δ-, and α-tocopherol (5:2:1), has been shown to have better antioxidant and anti-inflammatory actions than α-tocopherol alone. This mixture did not have any adverse effects in a limited number of preliminary clinical investigations. Thus, among the tocopherols, α-tocopherol is not the only important isomer for human health. Based on the evidence in this review, further research and additional clinical studies should be conducted on mixed tocopherol preparations.

Keywords: Tocopherols, α-Tocopherol; β-Tocopherol; δ-Tocopherol; γ-Tocopherol; Cardiovascular disease

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1. Introduction

There are 4 different tocopherols (vitamin E), α-, β-, γ-, and δ-tocopherols, with minor structural differences (Fig. 1). All occur naturally as a mixture in plant-based foods. The tocopherol content in food is inversely associated with mortality from cardiovascular disease [1,2]; however, α-tocopherol alone has been found to have no effect on cardiovascular events and death in some investigations [2-8]. A survey performed in Sweden on all scientific reports on vitamin E published from 1989 to 1996 [9] concluded that when taken with food, vitamin E can prevent heart disease, but there was no evidence that vitamin E in the form of a supplement (α-tocopherol) has a preventive effect based on clinical studies. Therefore, it is surprising that

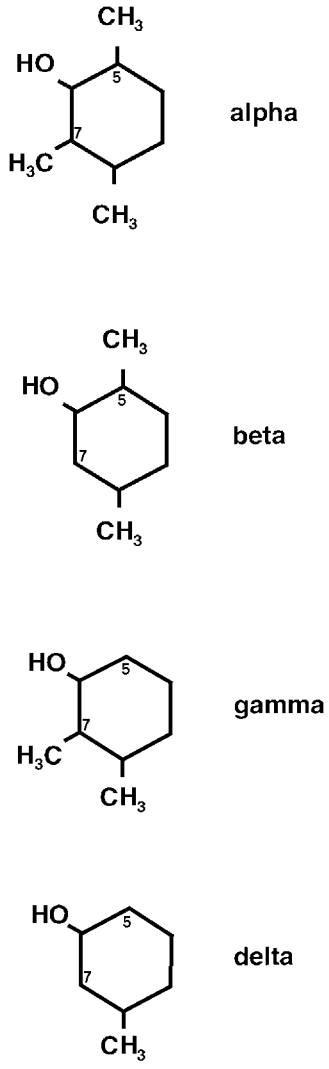

Fig. 1. The chroman ring of the tocopherols. The number of methyl groups at the positions 7 and 5 of the ring differs between different tocopherols.

only α-tocopherol and not the other natural tocopherols, especially γ- and δ-tocopherol, is on the "positive list" in the Food Supplements Directive of the European Union.

Pure "natural" α-tocopherol is produced from tocopherol-containing extracts from a variety of oilseed crops. After processing of soybean oil by a chemical methylation, the conversion of non–α-tocopherols to α-tocopherol occurs. Pure α-tocopherol is therefore not strictly natural, in contrast to extracts of mixed tocopherols from vegetable oils. It is of great interest as presented in this review that not only α-tocopherol, but also the non–α-tocopherols, should be regarded as valuable food components and supplements. This review describes the beneficial effects of natural tocopherols, with a focus on non–α-tocopherols.

2. History

In 1922, researchers at the University of California discovered that vitamin E was essential for reproduction in rats. In 1936, vitamin E was isolated from wheat germ, and its structure was elucidated shortly thereafter. The word *tocopherol* is from the Greek words *tokos*, meaning offspring, and *pherein*, meaning "to bear and bring forth." Pregnant rats deficient in this vitamin were found to suffer spontaneous abortion.

3. Tocopherols in food and antioxidant capacity

Of the tocopherols, γ-tocopherol and α-tocopherol are the 2 most common found in food, γ-tocopherol being the predominant tocopherol in US food. For example, the tocopherol content in corn oil and soybean oil are 77% and 70% γ-tocopherol, 2% and 23% δ-tocopherol, and 14% and 7% of α-tocopherol, respectively.

Vegetable oils, the source of tocopherols for animals and humans, contain polyunsaturated fatty acids which are protected from oxidative damage by antioxidants, for example, tocopherols. These oils contain not only α-tocopherol, but also other tocopherols, especially γ- and δ- tocopherol. α-Tocopherol alone can have a prooxidant effect as it contains a more active phenolic hydrogen molecule and a less stable phenolic radical [10]. α-Tocopherol may work more efficiently with other tocopherols such as γ- and δ-tocopherol to control its prooxidant activity. Food supplements containing polyunsaturated fatty acids should therefore also contain not only α-tocopherol, but also γ- and δ-tocopherol to inhibit lipid peroxidation in the oil and in the consumer [11,12]. Lipid peroxidation has been described in humans, for example, after intake of fish oils containing α-tocopherol alone and no other tocopherols [13-16].

α-Tocopherol may, in theory, be a more potent chain-breaking antioxidant than the other tocopherols because these latter tocopherols lack 1 or 2 of the electron-donating methyl groups of the chroman ring (Fig. 1). However, the unsubstituted C-5 position of γ-tocopherol makes this tocopherol more active to trap reactive nitrogen oxide species, such as peroxynitrite, nitrogen oxide, and nitrogen-like species. It has been shown that γ-tocopherol is superior to α-tocopherol in detoxifying nitrogen dioxide [17,18]. γ-Tocopherol levels are low in smokers, improve after cessation of smoking, and are low in patients with age-related nuclear cataracts. Another reason for the weakness of

α-tocopherol compared with γ- and δ-tocopherol as antioxidants in certain biologic systems is that α-tocopherol has no metal chelating power [19].

Our interest in the tocopherols originated years ago during studies of the stability of different oils containing polyunsaturated fatty acids. We observed that α-tocopherol sometimes could be only a very weak antioxidant and could even behave as a prooxidant. Both δ- and γ-tocopherol had a much stronger antioxidant effect, compared with the α form. When we combined different tocopherols, the antioxidant effect became even stronger. We then performed studies in human erythrocytes, in which we induced lipid peroxidation by adding hydrogen peroxide [20]. Working with different mixtures of α-, β-, δ-, and γ-tocopherol into the lipid layers of the cell membrane before exposure to hydrogen peroxide resulted in a mixture of γ-, δ-, and α-tocopherol with the ratio of 5:2:1 that had a much better antioxidant effect than α- tocopherol alone. This mixture is similar to that found in nature. The concentration of tocopherols was found to be important, and at high concentrations, no antioxidant effect was observed [20]. Interestingly, the total uptake of tocopherol in cell membranes was much higher after incubation of the cells with the mixed tocopherol preparation than when the same concentration of α-tocopherol alone was used (Fig. 2) [20]. Lipid peroxidation resulted in a decrease in polyunsaturated fatty acids in cell membranes, and this decrease was inhibited by the mixed tocopherol preparation.

4. Anti-inflammatory effects

γ-Tocopherol possesses anti-inflammatory activity [21]. It reduces prostaglandin E_2 synthesis, whereas α-tocopherol has no effect and γ-tocopherol inhibits cyclooxygenase-2

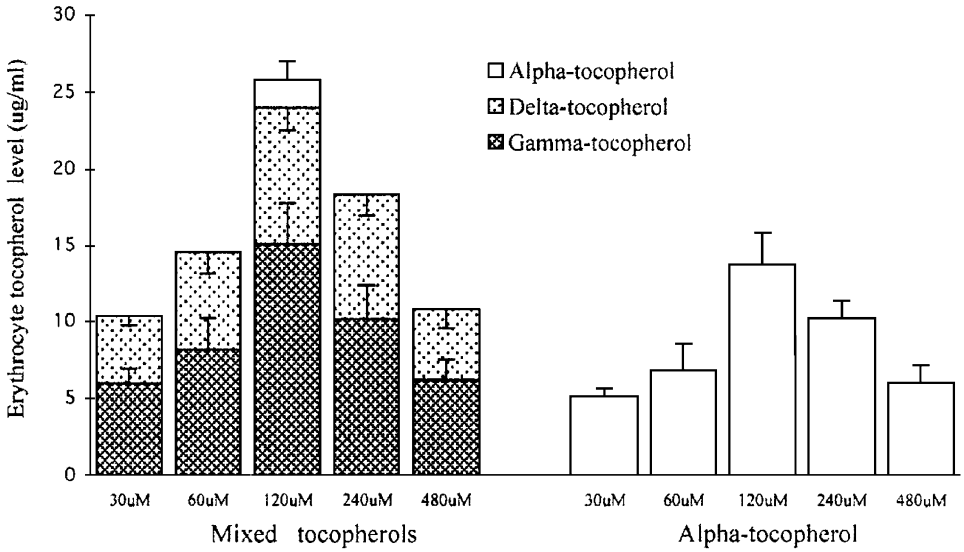

Fig. 2. Uptake of different tocopherols in human erythrocytes after incubation with a mixed tocopherol preparation (Cardi-E) containing α-, δ-, and γ-tocopherol (1:2:5) or α-tocopherol alone. The uptake of tocopherol was much higher after incubation with the mixed tocopherol preparation [20]. Data are presented as mean ± SE.

activity. It has been suggested that this anti-inflammatory effect of γ-tocopherol might prevent the incidence of colon cancer [22] because human colon cancer is associated with increased expression of cyclooxygenase-2 and formation of prostaglandin E_2. Jiang and Ames [23] recently demonstrated that it is γ-tocopherol, not α-tocopherol, that can attenuate proinflammatory eicosanoid production and inflammatory damage by reducing synthesis of prostaglandin E_2 and inhibiting the formation of leukotriene B_4 and tumor necrosis factor α.

5. Antidiabetic effect

One report suggests that γ-tocopherol, but not α-tocopherol, protects pancreatic beta cells against an inflammation-induced decrease in cell viability and insulin production [24]. Another report indicates that γ-, δ-, and α-tocopherol, but not β-tocopherol intake, is inversely related to the risk of type 2 diabetes [25]. These data suggest that γ-tocopherol may play a role in reducing risk of diabetes.

6. Attenuation of vascular smooth muscle proliferation

Vascular smooth muscle cell proliferation is a major mechanism underlying certain diseases, such as atherosclerosis and hypertension. This type of cellular proliferation can be inhibited by α-tocopherol. Protein kinase C (PKC) plays a major role in this inhibition [26]. Although α-tocopherol inhibits PKC activation in cells, β-tocopherol lacks this effect and may abolish the inhibition of smooth muscle cell proliferation and PKC activity caused by α-tocopherol. Thus, β-tocopherol acts as an antagonist in this system, in contrast to the other tocopherols. We have shown that a mixture of α-, δ-, and γ-tocopherol has the same positive effect as α-tocopherol on PKC activity in both platelets and leukocytes in human subjects [27,28]. Our data would suggest that α-, δ-, and γ-tocopherol, but not β-tocopherol, should be included in mixtures of tocopherols used for the prevention of atherosclerosis and hypertension.

7. Prevention of prostate cancer

In a study on more than 20 000 men, patients who developed prostate cancer had significantly lower blood levels of γ-tocopherol than men who did not develop this type of cancer [29,30]. Thus, the higher the blood level of γ-tocopherol, the lower the risk of prostate cancer. Men with the highest levels were 80% less likely to develop prostate cancer, compared with men with the lowest levels. γ-Tocopherol has some capacity to inhibit the growth of prostate cancer cells to a greater degree than α-tocopherol [31,32]. Moreover, both γ-tocopherol and δ-tocopherol were found to be superior to α-tocopherol in inhibiting neoplastic transformation of certain cells [17,33].

8. Alzheimer disease

Oxidative stress is believed to be an important contributing factor in the pathogenesis of Alzheimer disease. A significant increase in the lipid nitration product 5-nitro-γ-tocopherol

has been observed in affected regions of the brain from patients with this disease [34]. γ-Tocopherol, but not α- tocopherol, was found to inhibit nitrative stress in the brain. The results suggest that γ-tocopherol, but not α-tocopherol, may protect the brain against reactive nitrogen species.

9. Effects of tocopherol metabolites

A carboxyethylhydroxychroman derivative of γ-tocopherol has been shown to be a natriuretic factor that promotes sodium excretion and contributes to the regulation of the extracellular fluid volume [35]. This derivative may protect the cardiovascular system by lowering blood pressure [36].

10. Animal and clinical investigations

We compared the effects of α-tocopherol alone with those of a γ-tocopherol–rich preparation of mixed tocopherols in several experimental and clinical investigations. All human and animal studies were approved by the regional ethics committees. We found that the mixed tocopherol preparation had much more favorable effects than α-tocopherol alone on constitutive nitric oxide (NO) synthase (ecNOS) and superoxide dismutase (SOD) activity, as well as protein expression in rats and humans [28,37] (Figs. 3 and 4).

The mixed tocopherol preparation was more effective than α-tocopherol alone in decreasing platelet aggregation and inhibiting thrombus formation in a rat model [37]. Recently, Hensley et al [36], using an identical thrombosis model, found that thrombosis was

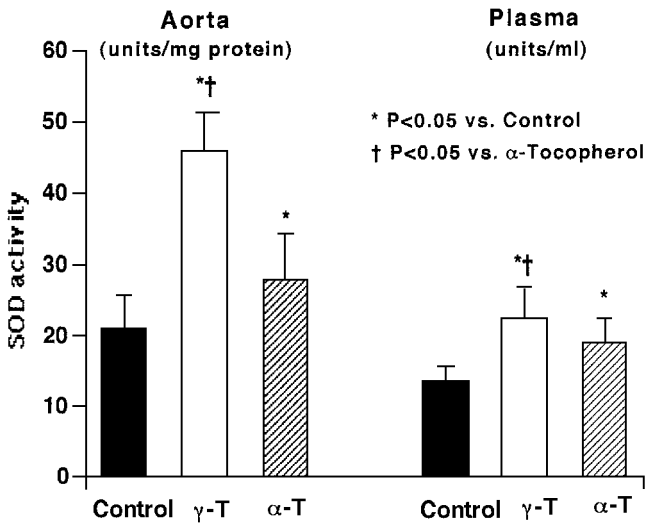

Fig. 3. Superoxide dismutase activity in aortic homogenates and plasma from rats fed different diets. Feeding rats with the γ-tocopherol–rich mixed tocopherol preparation increased SOD activity in supernatants of aortic tissue homogenates and plasma compared with α-tocopherol alone [37]. Data are presented as mean ± SD ($P < .05$, ANOVA, and Fisher exact test).

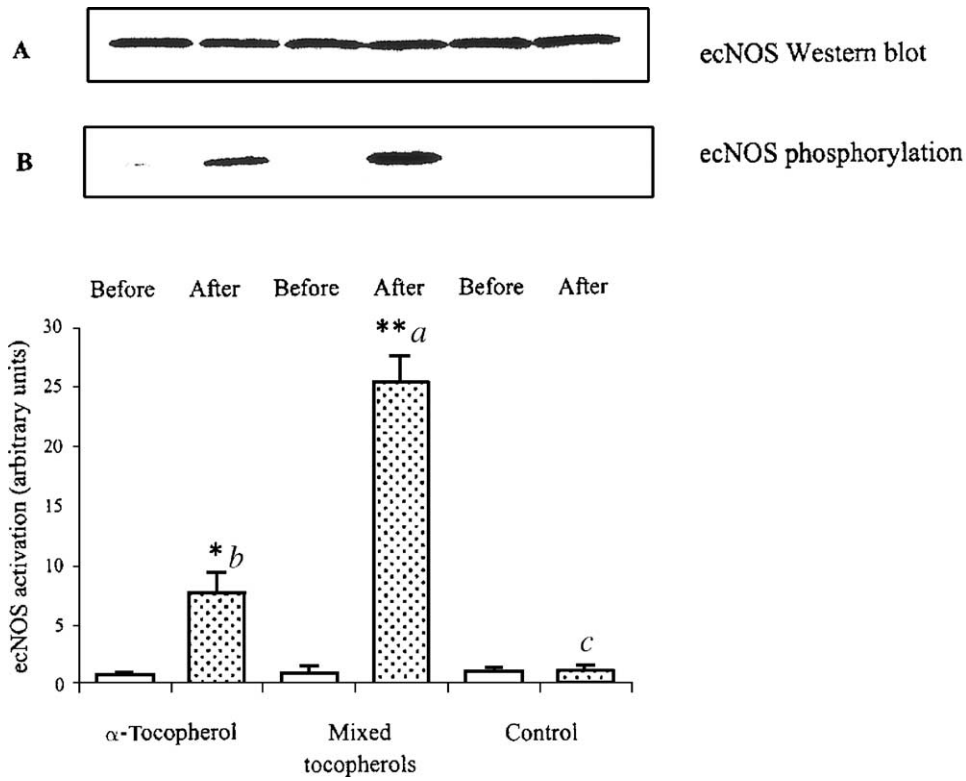

Fig. 4. Endothelial ecNOS protein content (A) and activation (phosphorylation) (B) in platelets before and after supplementation for 8 weeks with α-tocopherol or the mixed tocopherol preparation in human subjects. Supplementation with the mixed tocopherol preparation increased ecNOS activation compared with α-tocopherol alone [28]. For 8 weeks of data, means with different letters are significantly different ($P < .01$). *$P < .01$; **$P < .001$ compared with means before supplementation, ANOVA, and Tukey test.

associated with appearance of 5-NO2-γ-tocopherol in the circulating plasma, the first data demonstrating formation of nitrated tocopherol products during a thrombotic event. In our study, the mixed tocopherol preparation was also more effective in decreasing arterial superoxide anion generation and lipid peroxidation. [37]. Low-density lipoprotein (LDL) oxidation decreased to a greater extent after administration of mixed tocopherols, and endogenous SOD activity in plasma and arterial tissue increased more after supplementation with mixed tocopherols. In addition, protein expression of manganese SOD and copper/zinc SOD increased in arterial tissue. Only the mixed tocopherol preparation increased constitutive nitric oxide synthase (cNOS) protein expression [38]. In another experiment, human platelets were incubated with α-, δ-, and γ-tocopherol or a combination of the 3 (1:2:5). The 3 forms of tocopherol appeared at least to partly attenuate platelet aggregation through a decrease in free radical generation and an increase in platelet cNOS activity. Interestingly, the 3 tocopherols had a synergistic platelet inhibitory effect [39].

When we compared the effect of the mixed tocopherol preparation with that of α-tocopherol after 8 weeks of supplementation in human subjects, we found that the mixed tocopherol

preparation, but not α-tocopherol alone, inhibited adenosine diphosphate–induced platelet aggregation. In addition, the release of NO from platelets and ecNOS activation were higher after supplementation with the mixed tocopherol preparation (Fig. 4), which may be one mechanism for the effect of this preparation [28]. The mixed tocopherol preparation increased the SOD protein level in platelets, suggesting that the combination treatment of tocopherols up-regulate intrinsic SOD expression at a protein level. This process may be another important mechanism underlying the effect of the mixed tocopherol preparation on platelet aggregation. In addition, there was an increase in ecNOS activation in the leukocytes, which was much greater after supplementation with mixed tocopherols than with α-tocopherol alone [27], which may be important for the cardiovascular actions of the mixture. Nitric oxide is a key determinant in the development of atherosclerosis, and inhibition of NO synthesis increases the leukocyte-endothelial interaction [40]. Although the results of these clinical investigations are very promising, there is a need for additional clinical studies with mixed tocopherol preparations.

11. Cellular studies

We have further investigated the mechanism underlying the effect of γ-tocopherol in experiments in which we studied the role of γ-tocopherol in oxidized (ox)-LDL-induced nuclear factor-kB activation and apoptosis in human coronary artery endothelial cells. Treatment of the cells with γ-tocopherol attenuated the ox-LDL–induced activation of NF-kB and also reduced ox-LDL–induced apoptosis [41]. The attenuation of ox-LDL–induced NF-kB activation and apoptosis found in our studies may constitute a unique mechanism for the beneficial effect of the γ-tocopherol–rich mixed preparation in coronary artery disease (CAD).

In other experiments [42], we compared the effect of the mixed tocopherol preparation with that of α-tocopherol alone on SOD activity and inducible nitric oxide synthase (iNOS) expression in cultured myocytes exposed to hypoxia-reoxygenation (H-R). H-R resulted in

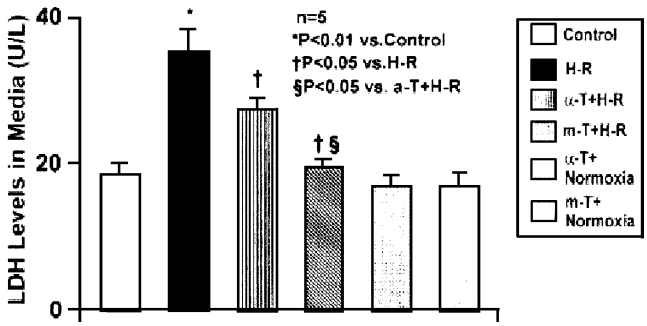

Fig. 5. LDH release in the media of myocytes. Hypoxia-reoxygenation caused an increase in LDH release. Preincubation of myocytes with either α-tocopherol alone or the mixed tocopherol preparation before hypoxia markedly attenuated the increase in LDH release. The mixed tocopherol preparation was more effective than α-tocopherol alone in reducing myocyte injury [42]. Data are presented as mean ± SD ($P < .05$, ANOVA and Student t test).

myocyte injury (as observed by lactate dehydrogenase [LDH] release) (Fig. 5), a decrease in SOD activity, and an up-regulation of iNOS expression and activity. The mixed tocopherol preparation was superior to α-tocopherol alone in terms of myocyte protection from the adverse effects of H-R (Fig. 5).

12. Plasma tocopherol levels and cardiovascular disease

Supplementation with α-tocopherol can decrease γ- and δ-tocopherol levels in the blood and tissues, as shown by our group and others [38,43,44] (Fig. 6). This may be an unfavorable effect because γ-tocopherol has properties which are not shared with α-tocopherol, and it is γ-tocopherol, not α-tocopherol that is expressed in reduced amounts in the plasma in patients with CAD [45,46]. Subjects with high plasma levels of γ-tocopherol had a 70% lower risk for myocardial infarction than those with low levels [47]. It has been shown that the chief explanation for the difference in mortality from CAD between Swedish and Lithuanian subjects is the plasma level of γ-tocopherol [48].

13. Adverse effects of mixed tocopherols

The tocopherol preparation Cardi-E, which contains a mixture of different natural tocopherols, mainly γ-, but also δ-, and α-tocopherol (5:2:1), has been used in several clinical

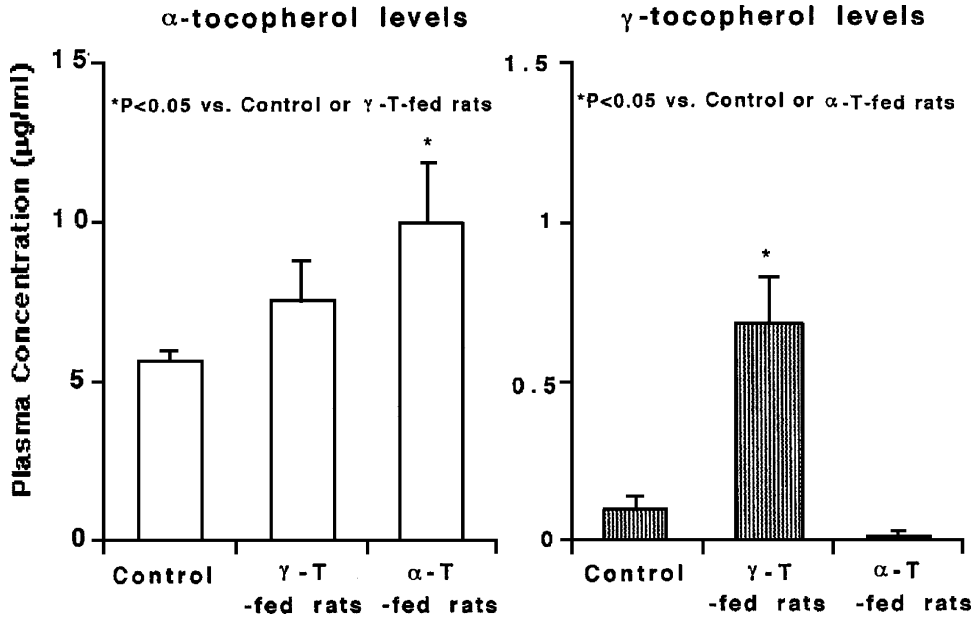

Fig. 6. Plasma levels of α- and γ-tocopherol in rats fed different diets. Plasma α-tocopherol levels increased markedly in the α-tocopherol–fed rats, and γ-tocopherol levels decreased as compared with control rats. The plasma γ-tocopherol levels were markedly elevated in rats fed the γ-tocopherol–rich mixed tocopherol preparation, and α-tocopherol levels did not differ from those in control animals [37]. Data are presented as mean ± SD ($P < .05$, ANOVA, and Fisher exact test).

investigations for up to 6 years, and no adverse effects have been reported [27,28,48]. Thus, at this time, the mixed tocopherol preparation appears to be safe.

14. Reasons why non–α-tocopherols have been considered to be much less potent than α-tocopherol

One reason why non–α-tocopherols such as γ- and δ- tocopherol have been considered to be less important than α- tocopherol is their low plasma concentrations compared with α-tocopherol. However, not only plasma concentrations, but also tissue concentrations, are important for the effect. Thus far, few investigations have focused on tissue concentrations of non–α-tocopherols, but one important such study was recently published [49]. This study found that γ-tocopherol represents 31% of the vitamin E stored in human adipose tissue, 38% in muscle tissue, and 53% in the skin. According to the authors, the unexpectedly high tissue concentrations of γ-tocopherol that were found spur reevaluation of the potential importance of this previously underestimated tocopherol.

The explanation for the high tissue vs plasma concentrations of γ-tocopherol is likely due to the lipoprotein lipase–mediated catabolism of chylomicron particles, in which part of the chylomicron-bound vitamin E is transported to the peripheral tissues [22] and, thus, escapes the liver. The chylomicron remnants are delivered to liver, and α-tocopherol is preferentially incorporated into very low-density lipoprotein by the α-tocopherols transfer protein, whereas γ-tocopherol is primarily degraded by a cytochrome P_{450}–dependent process and is then excreted into the urine. As mentioned earlier, this degradation product of γ-tocopherol has been shown to have natriuretic activity [35,36,50], which might be important in the prevention of hypertension and congestive heart failure.

The traditional expression of tocopherol activity in international units is based on the rat fetal resorption assay, where the activity is defined as the ability to prevent embryonic death in pregnant dams depleted of vitamin E. Based on this assay, α-tocopherol has been considered, for example, to be 10 times more potent than γ-tocopherol. This bioassay, although relevant to 1 biologic end point, is not valid for all the biologic activities of different tocopherol compounds. Hence, γ-tocopherol fails to substitute for α-tocopherol, and may have little value in the potential health of the human. Moreover, the γ-tocopherol levels in rat tissues are much lower (20-50 times) than in the human [51]. Hensley et al [36] also state that there is no a priori reason to expect that tocopherol efficacy as a fertility agent will correlate with other biologic activities.

15. Conclusions

A preparation of mixed tocopherols, containing γ-, δ-, and α-tocopherol (5:2:1), has been shown to have better antioxidant and anti-inflammatory effects than α-tocopherol alone in animal models and in a limited number of preliminary clinical investigations. This preparation was found to have no adverse effects. Thus, other tocopherols besides α-tocopherol have biologic importance. Further clinical studies with mixed tocopherol are needed to understand their role in human health and disease prevention.

Acknowledgment

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