How an Increased Intake of Alpha-Tocopherol Can Suppress the Bioavailability of Gamma-Tocopherol

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α-Tocopherol is the only form of vitamin E in vitamin supplements, whereas γ-tocopherol is the predominant form of vitamin E in the US diet. γ-Tocopherol has beneficial properties as an anti-inflammatory and possibly anti-atherogenic and anticancer agent. Excess α-tocopherol taken in supplements causes a reduction of γ-tocopherol concentration in plasma. The biochemical mechanism of this effect, which is important to human nutrition, has recently been elucidated.

Key words: α-tocopherol; α-tocopherol transfer protein; antioxidant; anti-inflammatory; α-carboxyethyl-hydroxychroman (αCEHC); γ-carboxyethyl-hydroxychroman (γCEHC); γ-tocopherol; vitamin E

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Vitamin E was discovered by Evans and Bishop in 1922 as a vitamin necessary for reproduction in rats. Its function as an antioxidant was first described by Cummins and Mattill in 1931. Since then, its antioxidant function as a free radical scavenger protecting the organism against oxidative damage has been firmly established.

Vitamin E occurs in plant sources in eight different analogs. α-Tocopherol (αT) (Figure 1) is the predominant form of the vitamin found in mammalian plasma and tissues. It is the only form present in vitamin supplements. Another form of vitamin E, γ-tocopherol (γT), is the principal vitamin E form in the US diet, being about 2.5 times as abundant in food as αT. Its source is principally vegetable oils, and it is less active as an antioxidant than αT. It has one fewer methyl group on the benzene ring than the αT form (Figure 1).

αT and γT are absorbed equally well from the intestine, and are carried to the liver by chylomicrons.

αT is released into the circulation from the liver in combination with a carrier protein, α-tocopherol transfer protein (αTTP), where it is incorporated into very low density lipoprotein (VLDL) for delivery to tissues. In animals lacking αTTP, such as in transgenic mice (Ttpa−/− mice), high dietary doses of αT can overcome the lack of the carrier protein, with the vitamin being delivered directly into the bloodstream. In humans suffering from ataxia caused by a genetic defect resulting in a lack of αTTP, high dietary αT can overcome the disease. γT can also combine with αTTP, but only to a small extent (9%). Therefore, αTTP is the regulator of the supply of αT but not γT to the tissues.

That portion of vitamin E not transported out of the liver is metabolized by ω- and β-oxidation through the action of cytochrome P450. The oxidation products are then excreted in urine as glucuronides. αT is metabolized to 2,5,7,8-tetramethyl-2-(2′-carboxyethyl)-6-hydroxychroman (αCEHC) and γT to 2,7,8-trimethyl-2-(2′-carboxyethyl)-6-hydroxychroman (γCEHC) (Figure 1). γCEHC has physiological activity as a natriuretic agent.

In view of the much larger amount of γT than αT in the human diet, the question arose, does γT have a function in the animal organism other than as a weak antioxidant? Though the level of γT in human plasma is low (αT, 32 μM; γT, 1.9 μM), the two forms are present in comparable concentrations in human muscle (αT, 155 nmol/g; γT, 107 nmol/g) and human skin (αT, 127 nmol/g; γT, 180 nmol/g).

In 2001, Jiang et al. published an important review entitled “γ-Tocopherol, the major form of vitamin E in the U.S. diet, deserves more attention.” More attention was then paid to γT, and a number of functions of this form of vitamin E were revealed. γT, in contrast to αT, can act as an anti-inflammatory agent by virtue of a dose-dependent reduction of the synthesis of the mediator of inflammation, prostaglandin E2, through inhibition of the enzyme cyclooxygenase 2 (COX 2). Due to an unsubstituted position on its benzene ring (Figure 1), γT, in contrast to αT, can react with reactive nitrogen species such as peroxynitrite. Peroxynitrite is formed in macrophages from reactive oxygen and the
stable nitric oxide free radical during inflammation, and can damage protein, lipids, and DNA. γT readily mops up peroxynitrite by forming 5-nitro-γT.15,16 Reactive nitrogen species are especially abundant in cigarette smoke, and smokers have twice the level of 5-nitro-γT in their plasma compared with nonsmokers.17 The anti-inflammatory action may also explain the influence of γT in lowering the incidence of coronary heart disease, shown by both animal and clinical data.18,19 The most striking result was obtained in a 7-year study of 34,486 women20 that revealed a significant inverse relationship between γT intake (dietary vitamin E, mostly γT) and the risk of cardiovascular disease. This was not the case when vitamin E was consumed from supplements (mostly αT). Other studies21,22 confirmed this result by showing a strongly significant inverse relationship between serum γT and the risk of coronary heart disease.

In an important prospective, 7-year case-control study of the association of prediagnostic blood levels of γT among 10,458 males, Huang et al.23 found γT concentrations strongly and inversely associated with subsequent risk of prostate cancer. The blood levels of γT among the 142 men with prostate cancer were lower than in the 284 matched controls (P = 0.0001).

In light of the beneficial effects of γT and the great expansion of the use of dietary supplements, an important consideration for human nutrition has been the influence of αT from supplements on the availability to the organism of γT from the diet. The early work of Handelman et al.24 in a survey of 86 elderly adults showed an inverse correlation of plasma αT compared with γT (P < 0.001) (Figure 2). After supplementation of a group of middle-aged volunteers with 400 IU of αT three times daily for 2 months, plasma αT increased to 200%–400% and γT decreased to 30%–50% of initial levels. In later work by Handelman’s group,25 tocopherols in the adipose tissue of 10 male human subjects were assayed. After a 1-year supplementation with αT (800 mg/d), followed by another year without supplementation, γT had declined by about 50%, whereas αT had remained unchanged. Similarly, Traber and Kayden26 found that 24 hours after a single dose of 1000 mg αT given to six human subjects, plasma αT increased, whereas γT declined precipitously. A most convincing recent study by Huang and Appel27 demonstrated that supplementing the diet of 184 adult nonsmokers with 296 mg/d of α-tocopheryl acetate for 2 months reduced the serum γT level by 58%. Clearly, the α form of the vitamin in the organism can somehow suppress the γ form.

The questions then arose, is the reduction of γT in plasma and adipose tissue the result of competition in the...
course of absorption? Does αT suppress the release of γT from the liver into the circulation, either directly or by competition for access to the transfer protein αTTP (even though only a small fraction of γT can bind to αTTP)? Does an increase in αT in liver lead to a greater metabolic breakdown and excretion of γT, perhaps in competition for a metabolic enzyme? Do high doses of αT increase an enzyme that preferentially breaks down γT?

Some answers to these questions have been found. As to absorption, Traber et al. found that in human subjects, αT and γT labeled with deuterium were absorbed from the intestine equally well. More recently, however, evidence has suggested that, at least in mice, absorption of tocopherols from the intestine is mediated by passive diffusion, the rest was transported by the SR-BI receptor-transporter from the apical to the basolateral side of the enterocyte in mice. SR-BI antibody blocked 80% of uptake. By means of intestinal cells (Caco-2TC-7) in culture, competitive inhibition of the absorption of tocopherols was observed. The authors suggest that this competitive effect was probably caused by competitive binding to the SR-BI receptor-transporter.

As to the question of the release into the circulation and metabolic breakdown of the tocopherols, two recent reports by Traber’s group provided important answers.

Previous work by the authors had established that αT is transferred from the liver to the plasma by αTTP. Nevertheless, in αTTP-knockout mice, plasma αT levels could be maintained, if high amounts of αT were provided in the diet, by a direct transfer of αT from the liver to the plasma. The authors found very low levels, both of αT and γT, in plasma of Ttpa mice fed a normal diet (αT in the diet, 31 mg/kg; γT, 8.8 mg/kg). Feeding a diet high in γT, low in αT (αT in the diet, 10.2 mg/kg; γT, 550 mg/kg) to Ttpa mice was expected to result in high plasma levels of γT by direct transfer; however, as was the case with αT, this did not occur. Even liver γT concentrations in those mice were low. Indeed, liver γT levels did not increase in proportion to increases in diet. Clearly, γT must be rapidly metabolized to γCEHC. Interestingly, when Ttpa mice were given a high-γT diet, their plasma γT concentration was higher than in the corresponding Ttpa mice, showing that, despite the low fraction of γT transferred by αTTP, this transfer protein can carry significant amounts of γT into the circulation.

The authors identified and determined the P450 enzyme in liver metabolizing the tocopherols as CYP3a. The concentration of CYP3a was correlated with liver αT, but not γT, levels (Figure 3). The levels of the metabolite of γT, γCEHC, in liver was correlated with the concentrations of both αT and γT (Figure 4). The authors suggested that αT influences γT metabolism, in that an increase in dietary αT increases the hepatic enzyme metabolizing not only αT, but also γT, leading to the excretion of both metabolites in urine. Whereas all remaining αT is taken to the plasma by αTTP, all of the γT is metabolized and excreted. In other words, increased αT, by causing an increase in CYP3a, stimulates the metabolic breakdown of γT. This mechanism explains the decline in plasma and adipose γT in humans whose diets contain high levels of αT.

The metabolic kinetics of αT and γT in humans was investigated by Traber’s group. The authors administered a 1:1 molar mixture of 50 mg deuterium-labeled α- and γ-tocopheryl acetate (d-αT; d-γT) to five women and nine men. Deuterium in metabolites was determined by liquid chromatography/mass spectrometry. Plasma samples were collected at different times up to 72 hours; urine samples were collected throughout. In plasma, d-γT peaked earlier (8.6 ± 2.6 h) than d-αT (11.1 ± 1.4 h), at one-third the concentration of d-αT. Areas under the curves were 6 times greater for d-αT than for d-γT. Half-lives were calculated from fractional disappearance rates from plasma. Plasma αT had a half-life about four times that of γT (57 ± 19 h vs. 13 ± 4 h). Women had a greater fractional disappearance

![Figure 3. Relationship of hepatic α- and γ-tocopherol concentrations with constitutive hepatic CYP3a concentrations. Hepatic α- (A) and γ-tocopherol (B) concentrations (nmol/g liver) were correlated with hepatic Cyp3a concentrations (in nanomoles of CYP3A2 cross-reactive protein per milligram of liver homogenate protein) in male mice (●) (P < 0.0002) and in all mice (○ and ●) (P < 0.0001). Used with permission from Traber MG, Siddens LK, Leonard SW, et al. αTocopherol modulates Cyp3a expression, increases γ-carboxyethyl-hydroxychroman (αCEHC) production, and limits tissue γ-tocopherol accumulation in mice fed high γ-tocopherol diets. Free Radical Biol Med. 2005;38:773–785.](image-url)
rate of γT than men; the rates for αT were the same for women and men.

γT was metabolized rapidly to γCEHC, which appeared in plasma within a few hours of tocopherol administration. Women excreted four times as much of the γT metabolite than men did. No aCEHC was found in plasma. The deuterium enrichment in γT and γCEHC was closely matched at all times, showing rapid equilibriums between the γT and the γCEHC pools. The much shorter half-life of γT is clearly caused by the more rapid and greater extent of metabolism of γT than αT, confirming the conclusions reached from their work with mice. The authors suggested that “the relatively lesser conversion of αT to αCEHC was not dependent upon competition between αT and γT . . . rather, the differences in metabolism seem to be the result of preferential metabolism of non-αT forms.”

In conclusion, it appears that the reduction in plasma γT during enhanced intake of αT can be explained by the more rapid metabolism of γT occurring when αT intake is increased. Recent experiments with mice have suggested that competition for an intestinal transporter protein may also play a part in the reduction of γT by large doses of αT.

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