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Review Article

γ-Tocopherol, the major form of vitamin E in the US diet, deserves more attention[1–3]

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ABSTRACT γ-Tocopherol is the major form of vitamin E in many plant seeds and in the US diet, but has drawn little attention compared with α-tocopherol, the predominant form of vitamin E in tissues and the primary form in supplements. However, recent studies indicate that γ-tocopherol may be important to human health and that it possesses unique features that distinguish it from α-tocopherol. γ-Tocopherol appears to be a more effective trap for lipophilic electrophiles than is α-tocopherol. γ-Tocopherol is well absorbed and accumulates to a significant degree in some human tissues; it is metabolized, however, largely to 2,7,8-trimethyl-2-(β-carboxyethyl)-6-hydroxychroman (γ-CEHC), which is mainly excreted in the urine. γ-CEHC, but not the corresponding metabolite derived from α-tocopherol, has natriuretic activity that may be of physiologic importance. Both γ-tocopherol and γ-CEHC, but not α-tocopherol, inhibit cyclooxygenase activity and, thus, possess antiinflammatory properties. Some human and animal studies indicate that plasma concentrations of γ-tocopherol are inversely associated with the incidence of cardiovascular disease and prostate cancer. These distinguishing features of γ-tocopherol and its metabolite suggest that γ-tocopherol may contribute significantly to human health in ways not recognized previously. This possibility should be further evaluated, especially considering that high doses of α-tocopherol deplete plasma and tissue γ-tocopherol, in contrast with supplementation with γ-tocopherol, which increases both. We review current information on the bioavailability, metabolism, chemistry, and nonantioxidant activities of γ-tocopherol and epidemiologic data concerning the relation between γ-tocopherol and cardiovascular disease and cancer. *Am J Clin Nutr* 2001;74:714–22.

KEY WORDS γ-Tocopherol, α-tocopherol, bioavailability, metabolism, electrophile trap, antiinflammatory activity, cardiovascular disease, cancer, review

INTRODUCTION

Oxidative damage is a major contributor to the development of cancer, cardiovascular disease (CVD), and neurodegenerative disorders (1, 2). Antioxidant vitamins defend against oxidative injury and are therefore believed to provide protection against various diseases. α-Tocopherol is quantitatively the major form of vitamin E in humans and animals and has been extensively studied. In contrast, γ-tocopherol—although being the most abundant form of vitamin E in the US diet—has received little attention since the discovery of vitamin E in 1922 and is not included in the current dietary intake recommendations (3). This is mainly because the bioavailability and bioactivity of γ-tocopherol, as assessed in animal studies, are lower than those of α-tocopherol. However, in contrast with the previous assumption that γ-tocopherol is not important because it is not maintained at the same concentrations as is α-tocopherol in the body, recent evidence suggests that γ-tocopherol has properties that may be important to human health and that are not shared by α-tocopherol. The qualities that distinguish γ-tocopherol from α-tocopherol are likely a result of its distinct chemical reactivity, metabolism, and biological activity. In this review we summarize the current knowledge of γ-tocopherol's bioavailability, metabolism, chemistry, nonantioxidant activity, and role in human diseases, with an emphasis on aspects that distinguish it from α-tocopherol.

STRUCTURE OF TOCOPHEROLS AND THEIR MAJOR METABOLITES

Vitamin E occurs in nature in ≥8 structurally related forms, ie, 4 tocopherols (α, β, γ, and δ) and 4 tocotrienols (α, β, γ, and δ) (**Figure 1**), all of which are potent membrane-soluble antioxidants. Tocopherols have a saturated phytyl side chain with 3 chiral centers that are in an *R* configuration (designated as * in Figure 1) at positions 2, 4′, and 8′ in the naturally occurring forms. Tocopherols differ in the number of methyl groups they have at the 5- and 7-positions of the chromanol ring. For instance, γ-tocopherol is unsubstituted at the C-5 position, whereas α-tocopherol is fully substituted in the chromanol ring. It is now clear that all

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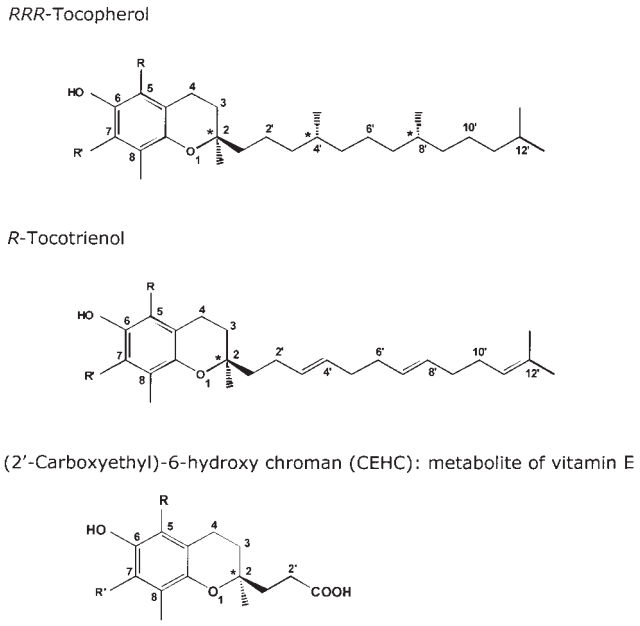

FIGURE 1. Chemical structures of vitamin E and urinary degradation products. *Chiral centers that are in an *R* configuration in tocopherols and tocotrienols and in an *S* configuration in their metabolites.

tocopherols, and possibly all tocotrienols (4, 5), share a similar degradation pathway that involves oxidation of the phytyl chain to the corresponding hydrophilic metabolites without modification of the chromanol ring (6–8). It was estimated, in unsupplemented humans, that 50% of γ-tocopherol is converted to the water-soluble metabolite 2,7,8-trimethyl-2-(β-carboxyethyl)-6-hydroxychroman (γ-CEHC) and is then excreted into the urine (9).

SOURCE, BIOAVAILABILITY, AND BIOACTIVITY

Because humans and animals do not synthesize their own vitamin E, they primarily acquire tocopherols from plants, which are the only species capable of making vitamin E. γ-Tocopherol is often the most prevalent form of vitamin E in plant seeds and in products derived from them (10). Vegetable oils such as corn, soybean, and sesame, and nuts such as walnuts, pecans, and peanuts are rich sources of γ-tocopherol (10). Because of the widespread use of these plant products, γ-tocopherol represents ≈70% of the vitamin E consumed in the typical US diet (10).

In contrast, α-tocopherol is the predominant form of vitamin E in most human and animal tissues, including blood plasma. In rats, α-tocopherol concentrations are generally much higher than those of γ-tocopherol (11, 12) (**Table 1**). In humans, plasma α-tocopherol concentrations are generally 4–10 times higher than those of γ-tocopherol (13). Studies that report γ-tocopherol concentrations in human tissues other than plasma are rare and mostly limited to adipose tissue (17). However, Burton et al (14) reported that γ-tocopherol constitutes as much as 30–50% of the total vitamin E in human skin, muscle, vein, and adipose tissue. Importantly, γ-tocopherol concentrations in these tissues appear to be 20–40-fold greater than those in plasma (14) (Table 1). Furthermore, γ-tocopherol concentrations are substantially higher in human than in rodent tissues. For example, concentrations of γ-tocopherol in human skin and muscle, ie, 180 and 107 nmol/g tissues, respectively, are 20–50-fold higher than those measured in rodents (Table 1) (15, 16). In addition, it is well documented that plasma and tissue γ-tocopherol are suppressed by α-tocopherol supplementation (17, 18). In sharp contrast, γ-tocopherol supplementation leads to a marked increase in both tocopherols (11). The difference in γ-tocopherol concentrations between humans and rodents and α-tocopherol's depression of γ-tocopherol are likely associated with γ-tocopherol's metabolism; this topic will be discussed in the next section of this review.

The biological activity of vitamin E has traditionally been determined with use of the rat fetal resorption assay, in which such activity is defined as the ability of supplemented tocopherol or tocotrienol to prevent embryo death in mothers depleted of vitamin E (19). In this assay, α-tocopherol exhibits the highest biological vitamin E activity, whereas γ-tocopherol exhibits only ≈10–30% of the activity of α-tocopherol (19). This difference in activity, however, appears to be caused by the large difference in retention of α- and γ-tocopherol in rodents, which is reflected by the lower plasma and tissue concentrations of γ-tocopherol than of α-tocopherol, a consequence that can also be explained by their different metabolisms.

ABSORPTION AND METABOLISM OF γ-TOCOPHEROL

The utilization of deuterium-labeled tocopherols (mainly α- and γ-tocopherol) has greatly facilitated our understanding of the

TABLE 1
Concentrations of α- and γ-tocopherol in the plasma and tissues of humans and rodents

	Humans[1]		Rats and mice[2]	
	γ-Tocopherol	α-Tocopherol	γ-Tocopherol	α-Tocopherol
Plasma (μmol/L)	2–7	15–20	1.3–1.7	7.2–13.0
Liver (nmol/g)	—	—	4.5–5.3	30.0–33.4
Adipose (nmol/g)[3]	176 ± 80	440 ± 279	29.5 ± 4.1	79.8 ± 6.9
Muscle (nmol/g)	107	155 ± 163	3.6–5.6	15.1–22.7
Skin (nmol/g)[4]	180 ± 89	127 ± 74	3.0 ± 2.8	8.9 ± 3.0

[1] Data from references 13 and 14.
[2] Data from reference 15 and 16. The animals were fed diets with a ratio of γ- to α-tocopherol of 2:1, 40–60 and 20–30 mg/kg, respectively.
[3] Data for rodents from reference 15.
[4] Data for rodents from reference 16. These mice were fed a diet containing 30 mg α-tocopherol/kg and ≈9 mg γ-tocopherol/kg.

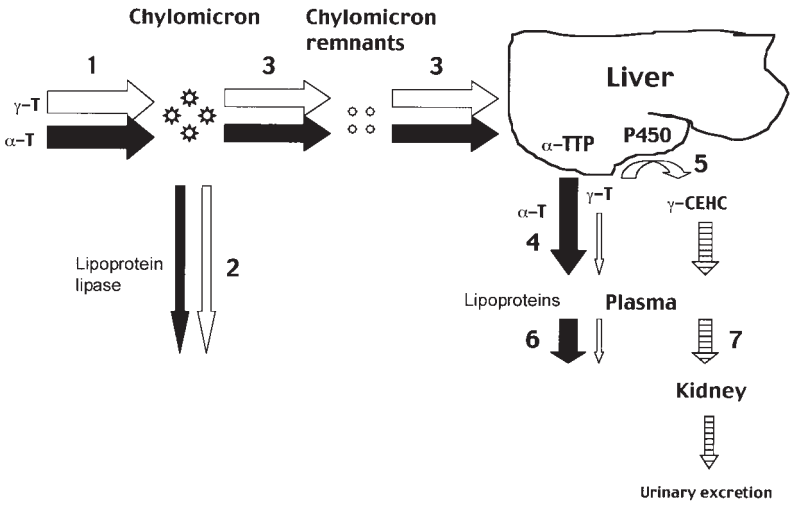

FIGURE 2. Absorption, transport, and metabolism of α-tocopherol (α-T) and γ-tocopherol (γ-T) in peripheral tissues (eg, muscle and adipose). *1*) Both α-T and γ-T are similarly absorbed by the intestine along with dietary fat and are secreted into chylomicron particles. *2*) Some of the chylomicron-bound vitamin E is transported to peripheral tissues with the aid of lipoprotein lipase. *3*) The resulting chylomicron remnants are subsequently taken up by the liver. *4*) In the liver, most of the remaining α-T but only a small fraction of γ-T is reincorporated into nascent VLDLs by α-tocopherol transfer protein (α-TTP). *5*) Substantial amounts of γ-T are probably degraded by a cytochrome P450 3A–mediated reaction to 2,7,8-trimethyl-2-(β-carboxyethyl)-6-hydroxychroman (γ-CEHC). *6*) Plasma vitamin E is further delivered to tissues by LDL and HDL. *7*) γ-CEHC is excreted into urine.

absorption and transport of tocopherols, as documented in an excellent review by Kayden and Traber (20). The recently increasing interest in the study of tocopherol metabolism has led to a rapid expansion of our knowledge in this area. We summarize the current knowledge of the absorption and metabolism of α- and γ-tocopherol in **Figure 2**. Both α- and γ-tocopherol and dietary fat are taken up without preference by the intestine and secreted in chylomicron particles together with triacylglycerol and cholesterol. The nearly identical incorporation of α- and γ-tocopherol in chylomicrons after supplementation with equal amounts of the 2 tocopherols indicates that their absorption is not selective (20, 21). During the subsequent lipoprotein lipase–mediated catabolism of chylomicron particles, some of the chylomicron-bound vitamin E appears to be transported and transferred to peripheral tissues such as muscle, adipose, and brain (22). The resulting chylomicron remnants are subsequently taken up by the liver, where α-tocopherol is preferentially reincorporated into nascent VLDLs by α-tocopherol transfer protein (α-TTP) (21), which enables further distribution of α-tocopherol throughout the body. However, γ-tocopherol appears to be degraded largely to the hydrophilic γ-CEHC (7) by a cytochrome P450–dependent process (23) and is then primarily excreted into urine (9). Catabolism of α-tocopherol by this route appears to be quantitatively much less important than that of γ-tocopherol because the corresponding metabolite of α-tocopherol, α-CEHC, is excreted in large amounts only when the daily intake of α-tocopherol exceeds 150 mg (6) or plasma concentrations of α-tocopherol are above a threshold of 30–40 μmol/L (24). Even then, urinary excretion of α-CEHC is lower than that of γ-CEHC (25, 26).

γ-CEHC was originally discovered by Wechter et al (7) in the pursuit of identifying an endogenous natriuretic factor in human urine. They showed that γ-CEHC possesses natriuretic activity by way of inhibition of the 70 pS potassium channel in the thick ascending limb cells of the kidney, whereas α-CEHC does not exhibit any appreciable activity (7, 27). In a radioisotope tracing study, the same investigators unambiguously established that γ-CEHC is derived from naturally occurring *RRR*-γ-tocopherol. Using X-ray crystallographic analysis, they showed that γ-CEHC has an *S*(+) stereochemistry at the C-2 position, indicating that phytyl-chain oxidation of *RRR*-γ-tocopherol is not accompanied by racemization (28). Although 5′-carboxychroman was subsequently identified as another metabolite in cell culture supernatant fluid and human urine, γ-CEHC appears to be quantitatively far more important (29). Plasma γ-CEHC concentrations are reported to be 50–100 nmol/L in humans (26) and >300 nmol/L in rats (30). In human urine, γ-CEHC exists predominantly as a glucuronide conjugate with concentrations ranging from 4 to 33 μmol/L (9), which increase to >100 μmol/L after supplementation with γ-tocopherol (29).

Recent work by Parker et al (23) strongly suggests that the degradation of tocopherols is a cytochrome P450 3A–dependent process because ketoconazole, a specific inhibitor of this enzyme, markedly reduced accumulation of tocopherol metabolites in the supernatant fluid of cultured hepatocytes supplemented with tocopherols. The codetection of both 3′-(γ-CEHC) and 5′-carboxylate metabolites, products thought to be derived from ω-oxidation followed by β-oxidation of the phytyl side chain, is also consistent with a cytochrome P450–mediated mechanism (6, 29). Parker et al (23) also showed that sesamin, the sesame lignan, inhibited γ-CEHC formation in this system, most likely as a result of the inhibition of cytochrome P450 activity. This observation provides an explanation for the previous finding by Yamashita et al (31, 32) that rats fed a diet containing both γ-tocopherol and sesame seeds or sesame lignans have plasma and liver concentrations of γ-tocopherol comparable with those of α-tocopherol. In the sesame seed– or sesame lignan–treated rats, γ-tocopherol and α-tocopherol similarly inhibited lipid peroxidation, erythrocyte hemolysis, and liver necrosis (32).

In summary, the biological disposition and retention of γ-tocopherol appear to be regulated by a metabolism that is quite different from that of α-tocopherol (Figure 2). Chylomicron-associated

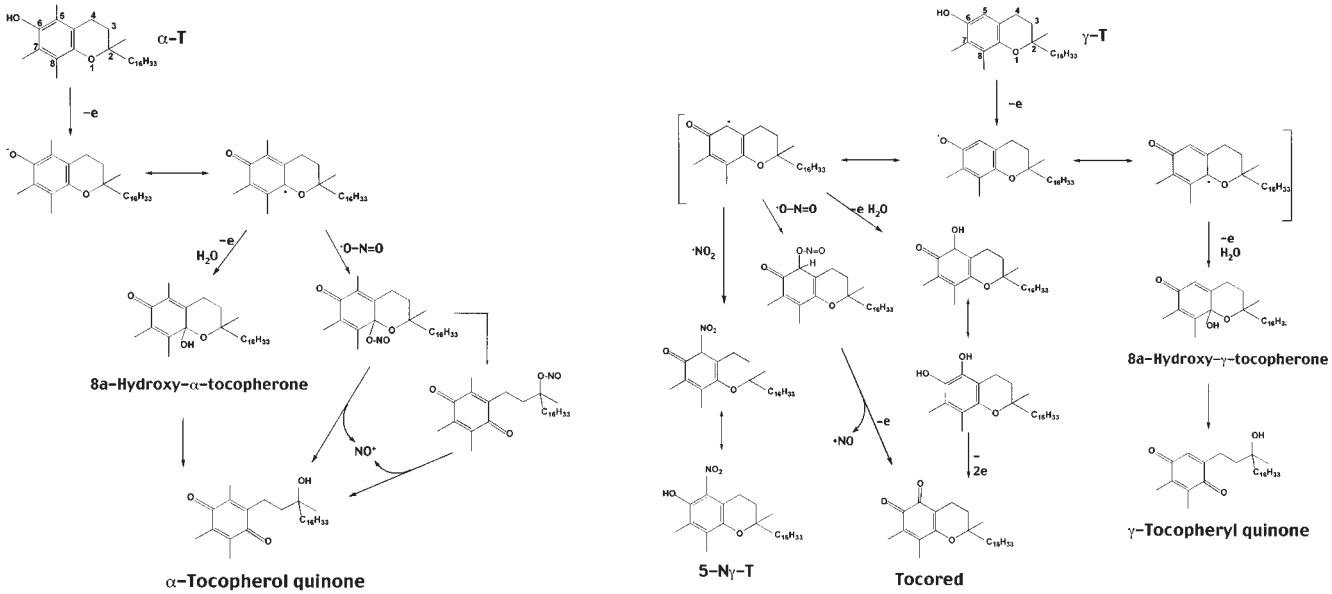

FIGURE 3. Main reactions of α-tocopherol (α-T) and γ-tocopherol (γ-T) with oxygen radicals and nitrogen oxide species. 5-Nγ-T, 5-nitro-γ-tocopherol.

tissue uptake of vitamin E, which occurs before liver metabolism, is possibly important for the accumulation of γ-tocopherol in skin, adipose, and muscle tissue. This could explain the strong correlation in humans between dietary γ-tocopherol uptake and γ-tocopherol concentrations in these tissues (14). However, hepatic catabolism of γ-tocopherol appears to be responsible for the relatively low preservation of γ-tocopherol in plasma and tissues, whereas α-TTP-mediated α-tocopherol transfer plays a key role in the preferential enrichment of α-tocopherol in most tissues. It is possible that α-TTP maintains the α-tocopherol concentration not only by facilitating its reincorporation into nascent VLDLs but also by preventing it from being catabolized (21, 33, 34). This is in contrast with γ-tocopherol, which appears to be largely degraded by cytochrome P450 once it enters the liver. Evidence supporting this possibility includes the findings that both α- and γ-tocopherol are similarly degraded by cytochrome P450–mediated catabolism in cultured hepatocytes (23) and that patients with an α-TTP defect have substantially lower plasma concentrations of α-tocopherol than do individuals with no such defect.

Schuelke et al (24) recently reported that patients with an α-TTP defect have enhanced urinary excretion of α-CEHC despite their having much lower plasma α-tocopherol concentrations than do healthy control subjects. In some of these patients, the reincorporation of *RRR*-α-tocopherol into VLDLs is not preferred to other stereoisomers, such as *SRR*-α-tocopherol (35), in contrast with healthy individuals who preferentially enrich *RRR*-α-tocopherol, presumably by hepatic α-TTP (21). The observation that supplementation of α-tocopherol depletes plasma and tissue γ-tocopherol is likely also rooted in α-TTP's preferential affinity for α-tocopherol. This is likely because an increase in α-tocopherol may further reduce γ-tocopherol's incorporation into VLDLs, which consequently leaves more γ-tocopherol to be degraded by cytochrome P450. On the other hand, γ-tocopherol supplementation may spare α-tocopherol from being degraded, which would explain why γ-tocopherol supplementation results in an increase in α-tocopherol concentrations (11). In addition, cytochrome P450 activity appears to be important in determining plasma and tissue concentrations of γ-tocopherol. The observation that rodents and humans often have substantially different P450 activities (36) may partially explain the finding that rats have lower γ-tocopherol concentrations (12) but higher γ-CEHC concentrations in plasma (30) than do humans (26). This possibility requires further investigation.

Finally, in addition to the urinary excretion of γ-tocopherol as γ-CEHC, biliary excretion may be an alternative route for eliminating excess γ-tocopherol, as proposed earlier (37). This notion is also supported by the fact that the ratio of γ- to α-tocopherol in bile is severalfold higher than that in plasma (31, 37, 38). Excess γ-tocopherol secreted into feces during supplementation may play a role in eliminating fecal mutagens and thus reduce colon cancer (38, 39).

CHEMISTRY OF γ-TOCOPHEROL

The antioxidant activity of tocopherols is rooted in their ability to donate phenolic hydrogens (electrons) to lipid radicals. Because of its lack of one of the electron-donating methyl groups on the chromanol ring, γ-tocopherol is somewhat less potent in donating electrons than is α-tocopherol and is, thus, a slightly less powerful antioxidant (40). Thus, α-tocopherol is generally considered to be more potent than is γ-tocopherol as a chain-breaking antioxidant for inhibiting lipid peroxidation (40). However, the unsubstituted C-5 position of γ-tocopherol appears to make it better able to trap lipophilic electrophiles such as reactive nitrogen oxide species (RNOS). Excess generation of RNOS is associated with chronic inflammation-related diseases such as cancer, CVD, and neurodegenerative disorders (1, 2). RNOS formed during inflammation include peroxynitrite (41), nitrogen dioxide, and nitrogen dioxide–like species generated from myeloperoxidase or superoxide dismutase (SOD)-H_2O_2-NO_2^- (42–44). In pioneering studies, Cooney et al (45, 46) found that γ-tocopherol is superior to α-tocopherol in detoxifying nitrogen dioxide. They showed that reaction of α-tocopherol with nitrogen dioxide leads to the formation of a nitrosating intermediate that, in turn, generates nitroso products. In contrast, they also showed that γ-tocopherol reduces nitrogen dioxide to the less harmful nitric oxide or traps nitrogen dioxide to form 5-nitro-γ-tocopherol (5-Nγ-T), analogous to the nitration of tyro-

sine (**Figure 3**) (45, 46). Subsequently, we (47) and Hoglen et al (48) showed that γ-tocopherol was also nitrated by peroxynitrite and 3-morpholinosydnonimine. Because the chromanol ring of α-tocopherol is fully substituted, this form of vitamin E cannot form a stable nitro adduct (45, 47).

The most important reactions of α- and γ-tocopherol with electron-abstracting oxidants, eg, lipid peroxyl radicals and RNOS, are summarized in Figure 3. In vitro mechanistic studies established that under physiologically relevant conditions, peroxyl radicals or peroxynitrite mainly oxidizes α-tocopherol to 8a-hydroxy-α-tocopherone, which is then hydrolyzed to α-tocopherol quinone (α-TQ) (47, 49). Depending on the nature of the oxidant, oxidation of γ-tocopherol, however, leads to the production of both γ-TQ, the analogue of α-TQ, and of 5-substituted products including tocored and 5-Nγ-T (47, 48). Reaction of γ-tocopherol with the strong electrophile peroxynitrite or SIN-1 primarily generates 5-Nγ-T and tocored (47, 48), whereas γ-TQ is predominant in the reaction with $NO_2^+BF_4^-$ (48), a nitrating agent but also a potent 2-electron oxidant.

We observed that the yield of 5-Nγ-T generated during liposomal peroxidation initiated by peroxynitrite or 3-morpholinosydnonimine was independent of the presence of α-tocopherol, suggesting that γ-tocopherol may complement α-tocopherol in scavenging membrane-soluble RNOS (47). However, this conclusion was later questioned by Goss et al (50), who found that 5-Nγ-T could only be detected after α-tocopherol had been almost completely consumed. Although the reasons for these apparently discrepant findings are not entirely clear, it is likely that they reflect differences in experimental conditions, such as the use of saturated liposomes in some studies (50) and unsaturated liposomes in others (47). Nevertheless, nitration of γ-tocopherol is more extensive than that of tyrosine when LDL is treated with peroxynitrite (47) or when nitration is initiated by SOD-H_2O_2-NO_2^- (42). This is most likely a consequence of a higher reactivity of γ-tocopherol toward electrophilic RNOS (47) and the increased solubility of RNOS in lipid membranes. 5-Nγ-T was therefore proposed as another marker, in addition to 3-nitrotyrosine, for detecting the formation of RNOS (51, 52). Whether nitration of γ-tocopherol is a physiologically relevant process and occurs even in the presence of α-tocopherol can only be determined by in vivo experiments in which adequate analytic methods are used.

Hensley et al (51) recently reported an HPLC method for measuring 5-Nγ-T in which coulometric array detection is used. Using this method, they reported an increase in 5-Nγ-T (both unadjusted and adjusted for γ-tocopherol) in rat astrocytes stimulated with bacterial lipopolysaccharide. We recently developed a highly sensitive HPLC assay with electrochemical detection in which tissue 5-Nγ-T can be measured simultaneously with α-tocopherol, γ-tocopherol, and unesterified cholesterol (S Christen, Q Jiang, MK Shigenaga, BN Ames, unpublished observations, 1998). Using this method, we detected a significant 2-fold increase in γ-tocopherol–adjusted plasma 5-Nγ-T in a rat model of zymosan-induced peritonitis, even in the presence of high plasma α-tocopherol concentrations (53). Surprisingly, the level of γ-tocopherol nitration, even under basal conditions, was in the low percentage range. In contrast, nitration of protein-bound tyrosine generally ranges within parts per million. This may be an indication of the preferred location of RNOS, such as nitrogen dioxide, in lipid environments. Clearly, further studies that consider the metabolism of both γ-tocopherol and nitrated γ-tocopherol (eg, urinary excretion) are needed to clarify the role of γ-tocopherol as an RNOS scavenger in vivo.

The precise location of α- and γ-tocopherol in the lipid environment may be partially responsible for their different reactivities. The lack of a methyl group makes γ-tocopherol relatively less hydrophobic, which may affect its location and interaction with lipids and aqueous-phase components. Although evidence supporting this hypothesis is sparse, the contradictory finding that the protective effect of α- and γ-tocopherol on lipid peroxidation was different in liposomes and LDLs is somewhat supportive. Thus, γ-tocopherol inhibited peroxynitrite- and SIN-1–induced lipid peroxidation in liposomes to a greater degree than did α-tocopherol, whereas α-tocopherol offered better protection in LDL (47). A superior effect of γ-tocopherol was also observed when lipid peroxidation was initiated by peroxynitrite in brain homogenates (54). γ-Tocopherol is predominantly located in the biomembrane of brain homogenates, which have a lipid arrangement similar to that of the liposome model. Differences in the liposomal and LDL particle lipid environments and the arrangement of tocopherol species within these particles could play an important role in the protective effects observed, the understanding of which requires further investigation.

The effect of lipid microenvironment on the chemical reactivity and biological consequence of tocopherols is also evident in tocopherol-mediated lipid peroxidation, a phenomenon that was discovered and studied by Upston et al (55). In this respect, γ-tocopherol has less potent prooxidant (tocopherol-mediated lipid peroxidation) activity than does α-tocopherol because it contains a less active phenolic hydrogen molecule and a relatively more stable phenolic radical (56).

NONANTIOXIDANT ACTIVITY

Besides its well-known chain-breaking antioxidant activity, α-tocopherol at concentrations of 50–100 μmol/L were shown by Tasinato et al (57) to inhibit smooth muscle cell proliferation by inhibiting protein kinase C activity. Although both γ-tocopherol and δ-tocopherol exhibit a similar antiproliferative effect, β-tocopherol does not share this activity (58), indicating that this effect is independent of antioxidant activity. Because smooth muscle cell proliferation plays an important role in the development of atherosclerosis, the potential benefit of vitamin E in preventing CVD may partially stem from its ability to inhibit smooth muscle cell proliferation.

Recently, we found that both γ-tocopherol and γ-CEHC possess antiinflammatory activity (59): γ-tocopherol and γ-CEHC inhibit prostaglandin E_2 synthesis in lipopolysaccharide-stimulated macrophages and in interleukin 1β (IL-1β)–activated epithelial cells at an IC_{50} (ie, the concentration that causes a 50% reduction) of 4–10 μmol γ-tocopherol/L and ≈30 μmol γ-CEHC/L, respectively. In contrast, α-tocopherol has no effect at these concentrations. We further showed that γ-tocopherol and γ-CEHC directly inhibit cyclooxygenase-2 (COX-2) activity in intact cells but do not affect expression of COX-2 protein. Similarly to the antiproliferative effect of α-tocopherol, this antiinflammatory property of γ-tocopherol is yet another effect of vitamin E that is independent of antioxidant activity. Because chronic inflammation contributes to the development of degenerative diseases, the antiinflammatory activity of γ-tocopherol and its major water-soluble metabolite may be important in human disease prevention. Human colon cancer, for example, is associated with an elevated expression of COX-2 and formation of prostaglandin E_2 (60). In addition, frequent use of nonsteroidal antiinflammatory drugs

reduces the incidence of colon cancers (61–63). Interestingly, Cooney et al (45) found that γ-tocopherol is superior to α-tocopherol in inhibiting neoplastic transformation of C3H/10T1/2 cells. The antiinflammatory activity of γ-tocopherol could partially explain this difference in potency.

A recent study by Sjoholm et al (64) found that γ-tocopherol (10 μmol/L), but not α-tocopherol, partially protected insulinoma β cells (RINm5F cells) against IL-1β–induced decreases in cell viability, insulin production, and stimulation of insulin release in response to certain stimuli. These effects were attributed to the superiority of γ-tocopherol in detoxifying RNOS (64). However, it was reported that IL-1β treatment leads to an induction of COX-2 expression and enhancement of PGE_2 release in RINm5F cells (65) and that COX-2 inhibitors protect against the autoimmune destruction of β cells (66). In light of our recent finding that γ-tocopherol inhibits COX-2 activity (59), the abovementioned protective effects of γ-tocopherol may also be partially due to its antiinflammatory activity. In any event, these results suggest that γ-tocopherol may play a role in preventing type 1 diabetes, a devastating complication that affects millions of Americans.

γ-TOCOPHEROL AND CARDIOVASCULAR DISEASE

Potentially beneficial effects of vitamin E on CVD were intensively explored in many intervention and epidemiologic studies (67, 68), which were recently reviewed in the latest *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium and Carotenoids* (3). Most of these studies focused exclusively on α-tocopherol but made no firm conclusions about the protective effects of α-tocopherol supplementation on CVD (67, 68). Although much less is known about γ-tocopherol than about α-tocopherol, much evidence suggests that γ-tocopherol may be important in the defense against CVD. First, several investigations found that plasma γ-tocopherol concentrations are inversely associated with increased morbidity and mortality due to CVD. Ohrvall et al (69) and Kontush et al (70) reported that serum concentrations of γ-tocopherol, but not of α-tocopherol, were lower in CVD patients than in healthy control subjects. In a concomitant cross-sectional study of Swedish and Lithuanian middle-aged men, Kristenson et al (71) found that plasma γ-tocopherol concentrations were twice as high in the Swedish men, but that the Swedish men had a 25% lower incidence of CVD-related mortality. In contrast, this inverse correlation was not observed with α-tocopherol. Second, in a 7-y follow-up study of 34 486 postmenopausal women, Kushi et al (72) concluded that the intake of dietary vitamin E (mainly γ-tocopherol), but not of supplemental vitamin E (mainly α-tocopherol), was significantly inversely associated with increased risk of death by CVD. Recently, these investigators further showed that dietary vitamin E was associated with a reduced incidence of death from stroke in postmenopausal women (73). In contrast, Stampfer et al (74) reported a significantly reduced risk of CVD associated with a high α-tocopherol intake from supplements but not from dietary vitamin E. Although the reasons for this discrepancy are not clear, the overall dietary vitamin E (presumably mainly γ-tocopherol) intake in both studies was much lower than the total intake among supplement users. Finally, it was reported that the regular consumption of nuts, which are an excellent source of γ-tocopherol, lowers the risk of myocardial infarction and death from ischemic heart disease (75).

In addition to the above-cited human studies, several animal studies also provide some evidence that γ-tocopherol might be beneficial against CVD. Saldeen et al (76) investigated the effects of α- and γ-tocopherol supplementation on platelet aggregation and thrombosis in Sprague Dawley rats. They found that γ-tocopherol supplementation led to a more potent decrease in platelet aggregation and delay of arterial thrombogenesis than did α-tocopherol supplementation (76). γ-Tocopherol supplementation also resulted in stronger ex vivo inhibition of superoxide generation, lipid peroxidation, and LDL oxidation. In a follow-up study, this same group reported that γ-tocopherol was significantly more potent than was α-tocopherol in enhancing SOD activity in plasma and arterial tissue and in increasing the arterial protein expression of both manganese SOD and Cu/Zn SOD (77). Furthermore, although both tocopherols increased nitric oxide generation and endothelial nitric oxide synthase activity, only γ-tocopherol supplementation resulted in increased protein expression of this enzyme (77). Because endothelium-derived nitric oxide is a key regulator of vascular homeostasis, up-regulation of endothelial nitric oxide synthase and nitric oxide formation by γ-tocopherol could be important in preventing vascular endothelial dysfunction (78). Together, the abovementioned human and animal studies seem to warrant further investigations into the role of γ-tocopherol in CVD.

γ-TOCOPHEROL, CANCER, AND SMOKING

Recent epidemiologic studies also showed both positive and negative correlations between plasma concentrations of γ-tocopherol and the risk of cancer. Nomura et al (79) showed that serum concentrations of α-carotene, β-carotene, total carotenoids, and γ-tocopherol, but not of α-tocopherol, were significantly lower in patients with upper aerodigestive tract cancers than in control subjects. These investigators observed a statistically nonsignificantly lowered risk of prostate cancer in Japanese American men with relatively high serum γ-tocopherol concentrations in another study (80). Giuliano et al (81) reported that serum concentrations of α- and γ-tocopherol were 24% lower in women with persistently positive papillomavirus infection, which is a high-risk index of cervical cancer. Recently, Helzlsouer et al (82) conducted a nested case-control study to examine the association of α-tocopherol, γ-tocopherol, and selenium with the incidence of prostate cancer. The most striking finding was that men in the highest quintile of plasma γ-tocopherol concentrations had a 5-fold reduction in the risk of prostate cancer compared with those in the lowest quintile. Interestingly, they also found that significant protective effects of high concentrations of selenium and α-tocopherol were only observed when γ-tocopherol concentrations were high. In contrast, higher serum γ-tocopherol concentrations were observed in patients with invasive cervical cancer than in control subjects (83). Zheng et al (84) reported a positive correlation of serum γ-tocopherol and selenium concentrations with the risk of oral and pharyngeal cancer.

Plasma γ-tocopherol concentrations are also affected by smoking, which is associated with the production of RNOS. We recently observed significantly higher plasma γ-tocopherol concentrations in smokers than in nonsmokers matched for dietary antioxidant intake, whereas no difference was found in α-tocopherol concentrations (85). In contrast, a study by Brown (86) showed that plasma γ-tocopherol concentrations were lower in smokers than in nonsmokers, albeit in a small cohort. Interest-

ingly, γ-tocopherol concentrations rapidly increased when long-term smokers ceased to smoke; however, as in the aforementioned study, no significant changes were observed in plasma α-tocopherol concentrations.

Many confounding factors could be responsible for some of these apparent discrepancies. For example, because dietary γ-tocopherol is always associated with high fat intake, which in turn is believed to be connected to many diseases, the high lipid content in foods could impede the beneficial effect of γ-tocopherol. Hence, a match of dietary intake between case and control subjects is mandatory in future studies. In addition, because γ-tocopherol metabolism may be altered under oxidative stress, plasma γ-tocopherol concentrations may not directly reflect its dietary intake. For instance, it was reported that cytochrome P450 activity is inhibited by interleukins and other proinflammatory cytokines (87, 88), which would thus lead to decreased degradation of γ-tocopherol and increased plasma γ-tocopherol concentrations. Other variables such as the type of cancer and the kinetics (or timeline) of the development of specific diseases may also affect the metabolism of γ-tocopherol. It is therefore conceivable that a true association between γ-tocopherol intake and cancer risk may be established only when these factors are further understood and fully considered.

γ-TOCOPHEROL AND AGING

Only a few studies have been conducted to evaluate the relation between γ-tocopherol and aging. Vatassery et al (89) reported that age is associated with a significant decline in the plasma concentration of γ-tocopherol but not of α-tocopherol. However, platelet concentrations of both tocopherols decrease with age. Studies by Lyle et al (90) showed that the sum of serum α- and γ-tocopherol, but neither tocopherol alone, was inversely associated with the incidence of age-related nuclear cataracts. The reasons for these observations and the biological significance of these findings are not known.

SUMMARY AND OUTLOOK

It has been ≈80 y since vitamin E was discovered as an essential element for maintaining reproduction in vertebrates, and yet we are just beginning to understand its physiologic functions and potential benefits in human health. Despite the fact that various forms of vitamin E have been identified, α-tocopherol is the only form that has been extensively studied and is present in most supplements. γ-Tocopherol, being the major form of vitamin E in many plant seeds, is unique in many aspects. Compared with α-tocopherol, γ-tocopherol is a slightly less potent antioxidant with regard to electron-donating propensity but is superior in detoxifying electrophiles such as RNOS, partially because of its ability to form a stable nitro adduct, 5-Nγ-T. γ-Tocopherol is well absorbed and accumulates to a significant degree in some human tissues, but it is also rapidly metabolized to the water-soluble metabolite γ-CEHC. γ-CEHC, but not α-CEHC, exhibits natriuretic activity, which may be physiologically important. In addition, γ-tocopherol and γ-CEHC, in contrast with α-tocopherol, possess antiinflammatory activity. Results from recent epidemiologic studies suggest a potential protective effect of γ-tocopherol against CVD and prostate cancer. These unique properties of γ-tocopherol and its major metabolite raise significant questions about the traditional definition of vitamin E activity, which has been almost exclusively based on the results obtained from the rat fetal resorption assay and which has been used as the primary argument that α-tocopherol is the only important form of vitamin E. We propose that although α-tocopherol is certainly a very important, if not the most important, component of vitamin E, γ-tocopherol may contribute significantly to human health in ways that have not yet been recognized. Because large doses of α-tocopherol are known to deplete plasma and tissue γ-tocopherol, it is our opinion that this possibility should be considered and carefully evaluated.

Controlled intervention studies in humans are required to clearly establish the benefits of γ-tocopherol supplementation (91). Cellular research combined with animal supplementation studies should be valuable in helping to understand the mechanisms behind the biological effects of γ-tocopherol. Potential synergistic effects between γ-tocopherol and other antioxidants should also be explored. These efforts should help to clarify the role of γ-tocopherol in human health.

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