Cigarette Smoke Alters Human Vitamin E Requirements¹,²
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ABSTRACT Vitamin E is a lipophilic chain-breaking antioxidant that prevents lipid peroxidation. Although cigarette smoke is a potent source of oxidative stress that depletes vitamin E in vitro, it is unclear whether it has a similar effect in vivo, particularly in humans. Therefore, this review will discuss the role of cigarette smoke on γ-tocopherol (γ-T) nitration, its effect on α-tocopherol (α-T) biokinetics in smokers, and the changes in the synthesis, plasma concentrations, and urinary excretion of the vitamin E metabolite (CEHC; carboxy-ethyl-hydroxy-chroman). Last, the possibility of CEHC as a biomarker of vitamin E status will be assessed as will the question whether smokers have increased dietary requirements of vitamin E. J. Nutr. 135: 671–674, 2005.

Vitamin E consists of 8 lipophilic compounds including 4 tocopherols (T)⁴ (α, β, γ, and δ) and 4 tocotrienols (α, β, γ, and δ) (1). Of these, α- and γ-T are the most important biologically (1). Vitamin E functions primarily as a chain-breaking antioxidant that ceases the propagation of lipid peroxidation due to its ability to scavenge peroxyl radicals, thereby protecting PUFA from oxidation.

Cigarette smoke is a potent exogenous source of oxidative stress in humans because of the inflammatory response it induces and the free radicals present in smoke (2). In fact, a puff of cigarette smoke contains 10¹⁴ and 10¹⁵ free radicals in the tar and gas phases, respectively (3). Moreover, the gas phase also contains high concentrations of nitric oxide, which may slowly undergo oxidation to form nitrogen dioxide (4) or react with superoxide to generate peroxynitrite (5). The in vitro antioxidant ability of vitamin E has led to the hypothesis that cigarette smokers require additional dietary vitamin E.

In vitro investigations have consistently demonstrated that cigarette smoke depletes plasma of vitamin E as well as other antioxidants (6–8). However, the plasma α- and γ-T concentrations of smokers either do not differ (8) or are lower (9) than those found in nonsmokers. Further confusing matters was the observation that, compared with nonsmokers, smokers and passive smokers had higher concentrations of plasma γ-T, but not α-T (10).

Therefore, well-designed human clinical studies evaluating vitamin E utilization under conditions of oxidative stresses such as cigarette smoking are imperative. These studies will help to determine whether a relation between oxidative stress and vitamin E utilization exists in vivo and whether smokers have higher vitamin E requirements. As such, this review is aimed at evaluating the current literature with regard to the influence of cigarette smoking on alterations of vitamin E utilization in humans.

Cigarette Smoking Causes γ-Tocopherol Nitration. Structurally, α- and γ-T differ subtly in that γ-T has a single unsubstituted position on the chromanol head whereas α-T is fully methylated. This enables γ-T nitration to occur, whereas this reaction is impossible for α-T (11). Consequently, γ-T nitration can result in the formation of 5-nitro-γ-T (NGT), likely through a peroxynitrite-mediated reaction (11,12).

Cigarette smoke contains reactive nitrogen oxides and induces an inflammatory response involving neutrophils and macrophages, which may also contribute to the reactive nitrogen species (RNS) pool (13–15). Indeed, cigarette smoke in vitro and in vivo caused NGT formation (8). When human plasma was exposed to cigarette smoke in vitro for 6 h, γ-T concentrations decreased 60% and NGT concentrations increased from 3 to 134 nmol/L, which accounted for ~20% of the initial γ-T content (8). Additionally, plasma samples taken from otherwise healthy nonsmokers and smokers were analyzed for α-, γ- and NGT concentrations. By LC/MS analysis, plasma α- and γ-Ts were not significantly different between groups. However, concentrations of NGT in smokers were roughly double those of nonsmokers. Thus, measuring NGT may be advantageous because plasma α- and γ-T measurements in smokers may not be sensitive enough to serve as an in vivo marker of vitamin E utilization.

That investigation provided the first evidence that smokers have elevated NGT concentrations (8). Because γ-T is quite lipophilic and peroxynitrite often targets lipophilic biomolecules (16), the routine measurement of NGT from biological fluids may enable its use as a biomarker for evaluating RNS-mediated stress in inflammatory diseases and processes as was described in the plasma of coronary heart disease patients (17) and postmortem brains of Alzheimer’s patients (18).

Previously, nitro-tyrosine was used as a marker of RNS-mediated stress (19) and was associated with increased risk for coronary artery disease (20). Importantly, γ-T nitration occurs ~15 times more readily than tyrosine nitration (21). Therefore, NGT may be a better biomarker for RNS-derived inflammatory-related pathologies than the frequently reported measurement of nitro-tyrosine. Future research is warranted in this area.

Cigarette Smoking Increases α-Tocopherol Depletion. The measurement of plasma concentrations or dietary intakes of α- and γ-Ts in smokers and nonsmokers often reveals no significant differences between groups (8,22). Thus, if smok-
ing-induced oxidative stress truly influences vitamin E dietary requirements, then more sensitive techniques are necessary to prove this hypothesis. Therefore, several investigations were conducted to evaluate differences between smokers and non-smokers in vitamin E biokinetics after supplementation with deuterium-labeled α-Ts (23–25).

Bruno et al. (23) evaluated α-T biokinetics in smokers and nonsmokers. On a single occasion, smokers and nonsmokers were provided 50 mg each deuterium-labeled α-T (d1-RRR-α-T) and α-tocopheryl acetate (d6-RRR-α-tocopheryl acetate) after a standard meal. Subsequently, plasma samples were collected at 6, 12, and 27 h and analyzed for labeled and unlabeled α-Ts. No significant differences were observed between plasma concentrations of free and acetate ester forms of α-T, but smokers had lower plasma deuterated α-T concentrations at each of the time points and lower area under the curves (AUCs). Unfortunately, due to study design limitations, it could not be determined whether these differences were due to increased plasma α-T clearance or to decreased α-T absorption.

In an effort to discern these differences, Traber et al. (24) supplemented smokers and nonsmokers with deuterated α-Ts (75 mg each d1-RRR-α-tocopheryl acetate and d6-all rac-α-tocopheryl acetate) for 7 d. Blood samples from fasting subjects were then collected on select days through d 21 postsupplementation to determine α-T fractional disappearance rates among the groups. After 7 d of supplementation (d 0), the percentage of %d1-α-T in plasma did not differ between the groups. The α-T fractional disappearance rates of the groups were not statistically different likely because the investigation was underpowered. However, consistent with the notion of faster disappearance rates, the plasma deuterated α-Ts of smokers were significantly lower at the end of the investigation.

Bruno et al. (25) recently reevaluated α-T biokinetics in smokers and nonsmokers utilizing a similar protocol, but a larger sample size. After 6 d of deuterium-labeled α-Ts supplementation, smokers’ and nonsmokers’ plasma labeled and unlabeled α-Ts did not differ as measured by LC/MS. However, smokers’ plasma α-T fractional disappearance rates (calculated from d 0–17 postsupplementation) were significantly faster and α-T half-lives were ~10 h shorter (79.3 ± 4.1 vs. 88.8 ± 3.8 h in nonsmokers). In addition, urinary excretion of deuterium-labeled and unlabeled α-CEHCs were evaluated as part of this investigation. After 6 d of deuterated α-T supplementation, urinary labeled and unlabeled α-CEHCs did not differ between groups. However, when reevaluated 17 d postsupplementation, smokers had ~one third the excretion of labeled and unlabeled α-CEHCs compared with nonsmokers, corresponding to plasma labeled and unlabeled α-T concentrations that were also significantly lower in smokers. Therefore, because the formation of α-CEHC is produced through the metabolism of α-T via a cytochrome P450 (CYP)-mediated pathway (26), not through a free radical–mediated oxidation pathway (27,28), the most likely explanation for the increased α-T disappearance among smokers was through increased α-T oxidation, consistent with an antioxidant function of α-T.

Further supporting the antioxidant function of α-T was the observation that α-T fractional disappearance rates were inversely correlated with plasma ascorbic acid (AA) concentrations in smokers, but not nonsmokers. Thus, when smokers had low plasma AA levels, their rates of α-T disappearance were faster. These data collectively support the hypotheses that α-T functions as an in vivo antioxidant, that α-CEHC might serve as an in vivo biomarker of α-T status, and that smokers require additional vitamin C as well as vitamin E to cope with the increased oxidative stress.

**Are CEHCs a Biomarker of α-Tocopherol Status?** The study of CEHCs stems from the early work of Chiku et al. (29) who described a δ-T metabolite. Subsequently, Wechter et al. (30) discovered, purified, and chemically synthesized the novel natriuretic factor LLU-α (Loma Linda University Alpha) which was renamed γ-CEHC to reflect that this factor was a γ-T metabolite (31). In 1993 the metabolite of α-T, α-CEHC, was discovered in urine (32). Subsequently, the metabolic pathway was elucidated by the detection of α- and γ-T metabolite intermediates, and the involvement of CYP in T metabolism was described (26,33–37). Collectively, the discovery and regulation of T metabolism has sparked interest in the use of CEHCs as a biomarker to assess vitamin E status (38).

Few studies have investigated the value of plasma or urinary CEHCs as indicators of in vivo vitamin E status. Synthesis of α- and γ-CEHCs occurs in response to supplementation with α- and γ-T, respectively (39). However, urinary recovery of CEHCs represents a small proportion of the administered dose (40). Moreover, metabolism of vitamin E to CEHCs appears to occur in response to excess or unwanted vitamin E forms (40).

For example, the metabolism of γ-T to γ-CEHC seems more active than α-T to α-CEHC because plasma γ-CEHC concentrations are 12 times higher than α-CEHC concentrations despite significantly higher plasma concentrations of α-T compared with γ-T (41). In addition, the use of deuterated α-Ts showed that compared with RRR-α-T, all rac-α-T is preferentially metabolized to α-CEHC (42).

Schulz et al. (43) proposed α-CEHC as a marker of adequate vitamin E intake. They conducted a 5 wk trial in which healthy, nonsmoking, male participants were supplemented incrementally with RRR-α-T (0, 50, 150, 350, 800 mg/d). Subsequently, plasma α-T and urinary α-CEHC were measured. α-CEHC was excreted only after a plasma threshold of ~30–40 μmol/L α-T was achieved, corresponding to a daily intake of 50–150 mg/d RRR-α-T. Thus, they suggested that detection of urinary α-CEHC likely indicated a saturated binding capacity of α-T and could serve as an indicator of optimum vitamin E intake.

More recently, vitamin E status in smokers and nonsmokers was evaluated by analyzing plasma, erythrocytes, lymphocytes, and platelets for α- and γ-Ts and urine for α- and γ-CEHCs (43). Smokers had significantly lower lymphocyte and platelet α-T concentrations than nonsmokers, but no significant differences were observed for plasma or erythrocyte α- and γ-T concentrations. Additionally, urinary α-CEHC did not differ between groups, but smokers had a higher urinary γ-CEHC excretion. Because the groups had similar vitamin E intakes, they speculated that the increased γ-CEHC excretion by smokers may have been due to the induction of CYP enzyme(s) by cigarette smoke. However, it was unclear why urinary α-CEHC was similar between the groups given this explanation.

Previous trials provided limited data concerning the effects of smoking on vitamin E metabolism. Therefore, urinary α-CEHC excretion was investigated in smokers and nonsmokers after 6 d of deuterium-labeled α-tocopheryl acetate dosing (25). As discussed above, 17 d postsupplementation, smokers’ urinary excretion of labeled α-CEHCs was approximately one third that observed in nonsmokers, and plasma labeled α-Ts were also significantly lower in smokers. Thus, it seemed that smokers’ CYP-mediated metabolism of α-T was not as active as that of nonsmokers due to limited substrate availability. In a follow-up analysis of these same participants (44), labeled
and unlabeled α-CEHCs and unlabeled γ-CEHC were measured daily in plasma (44) to ascertain whether substrate availability influences the ability of smokers to produce CEHC or whether smoking increases CEHC synthesis. Despite 6 d of supplementation with labeled α-Ts, plasma labeled α-CEHCs were detectable only from d –5 (~12 h after the first dose) through d 5 regardless of smoking status (Fig. 1). During this time period, neither plasma labeled α-Ts nor dietary vitamin E intake differed between groups. However, the AUCs calculated from d –5 to +5 were ~50% lower among the smokers for d0-, d3-, d6-, and total α-T (44). Furthermore, the slopes of the linear regressions between plasma labeled α-Ts and labeled α-CEHCs were ~50% lower among smokers compared with nonsmokers. The findings of this study showed that smokers had ~50% less plasma α-CEHC (despite similar plasma α-T concentrations), suggesting that decreased α-T metabolism was occurring (Fig. 2); more d0-all rac-α-T was metabolized to α-CEHC than d1-RRR-α-T, regardless of smoking status; nonsmokers’ plasma α-CEHCs peaked at d –2 and quickly declined despite 2 additional days of α-T supplementation, suggesting that alternative pathways may be excreted in excess vitamin E and/or CEHC (40). Therefore, although it appears that α-CEHC concentrations are associated with plasma α-Ts, the plausibility of α-CEHCs as a biomarker for α-T status appears questionable for the following reasons: 1) smokers and nonsmokers’ plasma α-CEHC responses differ for the same plasma α-T concentration, 2) synthetic Ts are preferentially metabolized, and 3) CEHC concentrations and/or production appears to be saturable.

It is currently debated which CYP(s) are involved in vitamin E metabolism. CYP4F2 was reported to metabolize vitamin E (33), but another likely candidate is CYP3A (26,34). Importantly, cigarette smokers have lower CYP3A protein levels than nonsmokers (45) and CYP1A (46) and mouse Cyp2e1 (47) are induced by cigarette smoke. Thus, if CYP3A is involved, it would be expected that smokers would have lower CEHC formation. Alternatively, it is possible that smokers’ tissues were vitamin E depleted at study onset. Therefore, α-T supplementation may have repleted smokers’ tissues; thus α-T was available for the production of α-CEHC. Clearly, the regulatory and molecular mechanisms of vitamin E status require further understanding utilizing more invasive models.

In conclusion, the recent investigations utilizing deuterated Ts demonstrated that cigarette smoking does increase vitamin E requirements in humans. It is clearly preferable to encourage smokers to stop smoking rather than to formulate dietary recommendations that will help them to cope with the magnitude of oxidative stress experienced. Unfortunately, despite increased public awareness of the health risks associated with smoking, the prevalence of smoking in the United States has not declined substantially in the past decade (48). Moreover, only 8% of men and 2.4% of women in the United States, regardless of smoking status, have dietary vitamin E intakes that meet the Estimated Average Requirements (12 mg α-T/d) (49). Thus, it is prudent to suggest that all individuals be encouraged to achieve at least the vitamin E required by nonsmokers (15 mg α-T/d), but it is likely that smokers have even higher requirements. In addition, because smokers’ vitamin E utilization may be related to plasma AA concentrations (25), they should also strive to consume the Recommended Dietary Allowance for AA because the data suggest that higher dietary AA intakes in smokers would decrease vitamin E disappearance.

LITERATURE CITED


FIGURE 1 α-CEHC time course in smokers and nonsmokers. Participants were supplemented (d –6 to +1) with 75 mg each d1-RRR-α-tocopherol acetate and d3-all rac-α-tocopherol acetate. Plasma was measured for labeled (d1-, d2-, and unlabeled (d0-) α-CEHCs from d –5 through d 17. Labeled α-CEHCs were detectable only from d –5 through d 5 regardless of smoking status [adapted from Bruno et al. (44)].

FIGURE 2 Overall effects of cigarette smoking on vitamin E utilization. Cigarette smoke results in oxidative stress marked by increased lipid peroxidation and RNS. Ts can react with peroxyl radicals to halt the lipid peroxidation chain reaction but become oxidized in the process. Ascorbic acid may recycle tocopheroyl radicals and reduce them to Ts. NOx reacts with γ-T to form 5-nitro-γ-T, but the fate of 5-nitro-γ-T is unknown. Last, cigarette smoking does not increase T metabolism to CEHCs. Abbreviations: AA*, ascorbyl radical; γ-T, re-active nitrogen oxides; R*, carbon-centered radical; RRO*, peroxyl radical; ROOH, lipid hydroperoxide; RH, polyunsaturated fatty acid; T, tocopherols; T*, tocopheroyl radical.
metabolized in HepG2 cells by side chain omega-oxidation and consecutive
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