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Re-evaluation of the relative potency of synthetic and natural α-tocopherol: experimental and clinical observations

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Abstract

Nutritionists generally consider *all-rac*-α-tocopherol and *RRR*-α-tocopherol equivalent in vitamin E activity but disagree whether equivalency requires a dosage ratio of 1.36:1 or 2:1. In contrast, we hypothesize that *all-rac*- and *RRR*-α-tocopherols are not equivalent in any dosage ratio. Previous observations that *all-rac*- and *RRR*-α-tocopherols are distributed and eliminated via saturable and stereospecific pathways imply that their relative bioavailability varies with the saturation of these pathways and therefore varies with dosage. Indeed, previous studies observed that the relative bioavailability of *all-rac*- and *RRR*-α-tocopherols varies between tissues as well as with dose, time after dosing, and duration of dosing. This non-constant relative bioavailability predicts non-constant relative activity (i.e., non-parallel dose-concentration curves predict non-parallel dose-effect curves). Non-constant relative bioavailability suggests that a fixed dosage ratio of *all-rac*- and *RRR*-α-tocopherols cannot produce a fixed ratio of effects on all processes in all tissues at all times after all dosages. However, previous studies suggest that *all-rac*- and *RRR*-α-tocopherols have equivalent effects (parallel dose-effect curves) in vitamin E-deficient animals and non-vitamin E-deficient humans. We re-evaluate the data from these animal studies and find non-parallel dose–effect and concentration–effect curves. We discuss pharmacokinetic and pharmacodynamic reasons why previous studies in non-vitamin E-deficient humans did not find non-parallel dose-effect curves for *all-rac*- and *RRR*-α-tocopherols. We note that saturable elimination predicts that *all-rac*- and *RRR*-α-tocopherols might inhibit and/or induce elimination of other compounds (including 30-40% of prescription drugs) eliminated via the same saturable pathways, and stereospecific elimination predicts that *all-rac*- and *RRR*-α-tocopherol have non-parallel dose-effect curves for these interactions.

Keywords: α-Tocopherol; Bioavailability; Drug interactions; Saturability; Stereospecificity; Tocopherol transfer protein

1. Introduction

All-rac- and *RRR*-α-tocopherols are different chemical entities. *All-rac*-α-tocopherol consists of approximately equal proportions of eight stereoisomers (*RRR*, *SRR*, *RRS*, *RSS*, *RSR*, *SSR*, *RSS*, and *SSS*) [1]. *RRR*-α-tocopherol is the only one of these stereoisomers that is naturally present in plants, animals, or humans [2,3]. Differences between stereoisomers in their structural conformation are important because endogenous proteins such as enzymes and receptors usually exist only as one stereoisomer and usually react in a highly stereospecific manner [4]. In fact, the relative potency of stereoisomers of drugs differs for each effect, which may result in differences in therapeutic and adverse effects and in the benefit to risk ratio [5–12]. Stereoisomers of drugs also differ in absorption, bioavailability, protein binding, half-lives, and the rate and extent of metabolism, as well as competing for binding to any enzymes that are saturable [5–7, 13–15]. Thus, pharmacologists consider drug products that consist of different stereoisomers as different drugs rather than different formulations of the same drug [4]. In contrast, nutritionists tend to regard *all-rac*- and *RRR*-α-tocopherols as different formulations of the same nutrient. The United States Pharmacopeia (USP) and the Food and Nutrition Board of the United States Institute of Medicine (FNB-IOM) agree that *all-rac*- and *RRR*-α-tocopherols can produce equivalent vitamin E activity in humans but disagree whether achieving equivalent vitamin E activity requires a 1.36:1 or 2:1 dosage ratio.

However, these dosage ratios are not definitive conclu-

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sions but estimates or hypotheses based on extrapolations. The original estimates of the relative vitamin E activity of natural and synthetic α-tocopherols in humans were extrapolations from their relative vitamin E activity in animals, because researchers discovered symptoms of vitamin E deficiency in animals as early as 1922 but did not discover symptoms of vitamin E deficiency in humans until the 1960s [2,3]. Later researchers continued to rely on extrapolations to estimate relative vitamin E activity in humans because, as discussed below, there are no widely accepted assays of vitamin E activity in humans [16,17]. In 1979, the USP estimated the relative vitamin E activity of *all-rac*- and *RRR*-α-tocopherols in humans by extrapolating from animal studies and from bioavailability studies using unlabeled *all-rac*- and *RRR*-α-tocopherols [16,18]. Others estimate relative vitamin E activity by extrapolating from relative potency for effects other than preventing or treating symptoms of vitamin E deficiency. One such effect is the relative ability of *all-rac*- and *RRR*-α-tocopherols to delay the lag time for oxidation of lipoproteins in humans not deficient in vitamin E [19]. In 2000, the FNB-IOM estimated the relative vitamin E activity of *all-rac*- and *RRR*-α-tocopherols in humans by extrapolating from the relative affinity of tocopherol transfer protein (TTP) for the individual stereoisomers of α-tocopherol [17]. The FNB-IOM noted that the affinity of TTP for the stereoisomers of α-tocopherol [20] is consistent with the 2:1 relative bioavailability of *RRR*- to *all-rac*-α-tocopherol observed in studies using deuterated tocopherols [21–24] but stated that they did not estimate relative biological activity based on relative bioavailability [17,25]. The FNB-IOM suggested that future assays of vitamin E activity in humans might extrapolate from the effects on biomarkers of oxidative stress or inflammation in humans not deficient in vitamin E [17].

In contrast to these extrapolations, we hypothesize that no single dosage ratio of *all-rac*- and *RRR*-α-tocopherols produces equivalent effects on all biological processes or across the entire clinically relevant dosage range. This hypothesis is important because individuals take a variety of dosages of vitamin E supplements for a wide variety of reasons. Supplements at the low end of the dosage range provide the recommended dietary allowance (RDA), which is 15 mg/day for otherwise healthy adults [17]. Nutritionists advise most American adults to take RDA-type supplements to prevent deficiency and to potentially decrease the incidence of chronic diseases of aging [17,26,27]. Many adults who may or may not be otherwise healthy take pharmacological doses typically ranging from 100 IU/day to 800 IU/day in an attempt to optimize health, decrease oxidative stress, prevent disease, or slow the progression of disease. At the highest end of the dosage range, adults with symptomatic vitamin E deficiency receive pharmacological dosages ranging from 800 mg/day to 100 mg/kg/day depending on the cause of deficiency [2,3]. However, neither the USP nor the FNB-IOM restrict the dosage ranges or intended uses for which they apply the estimated equivalent dosage ratios of 1.36:1 or 2:1. Thus, both the USP and FNB-IOM lead the public to believe that these equivalency ratios apply to all dosages, all intended uses, and all biological activities of *all-rac*- and *RRR*-α-tocopherols. Similarly, much of the literature on vitamin E interchangeably uses the terms relative vitamin E activity, relative potency, and relative biological activity. It is important to distinguish assays of vitamin E activity from assays of other effects of *all-rac*- and *RRR*-α-tocopherols.

1.1. Assays of the relative activity of all-rac- and RRR-α-tocopherols in humans

Assays of biologic activity measure effects on clinical endpoints, biomarkers, or surrogate markers of clinical endpoints [4,28]. Plasma or tissue concentrations of tocopherols and their metabolites are measures of bioavailability, not of activity. Biomarkers include anything measurable that changes due to drug administration but do not necessarily correlate with any therapeutic benefit of the drug [28]. A biomarker is a surrogate marker of a clinical endpoint only if changes in the biomarker are sensitive and specific indicators of changes in the clinical endpoint (i.e., only if the dose–effect relationships for changes in the biomarker correlate with the dose–effect relationships for the clinical effect) [28]. Only clinical trials can prove the validity of clinical assays of drug activity and only clinical trials can prove that a biomarker is a valid surrogate marker of a clinical endpoint [28].

Clinical endpoints of vitamin E activity by definition involve only the prevention or resolution of vitamin E deficiency. There are no valid clinical assays of the relative vitamin E activity of *all-rac*- and *RRR*-α-tocopherols (or any other tocopherols or tocotrienols) in humans because no clinical trials show their relative dose–effect relationships in preventing or treating vitamin E deficiency in humans. There are no valid clinical assays of the relative non–vitamin E activity of any tocopherols or tocotrienols in humans because no clinical trials show their relative dose–effect relationships in preventing or treating any nondeficiency disease in humans. Moreover, in the absence of such clinical trials, it is not possible to show correlations between dose–effect relationships for changes in biomarkers and dose–effect relationships for achieving clinical endpoints. Thus, although administration of tocopherols and tocotrienols is associated with changes in numerous biomarkers, it is not possible to determine whether any of these biomarkers are valid surrogate markers of clinical endpoints of the vitamin E activity or the non–vitamin E activity of the tocopherols and tocotrienols in humans. In the absence of valid assays of vitamin E activity, it is important to consider the conditions required to confirm or exclude the hypotheses that a 1.36:1, 2:1, or no dosage ratio of *all-rac*- and *RRR*-α-tocopherols produces equivalent activity.

1.2. Conditions required to confirm or exclude hypotheses

Biological activity is described mathematically by dose versus effect, plasma concentration versus effect, and tissue concentration versus effect curves. Substances with equivalent activity for a specific effect are equally efficacious (i.e., achieve the same maximum clinical effect) but may not be equipotent (i.e., may require a different dosage to achieve the same extent of the same effect). A fixed dosage ratio of different compounds can produce equivalent activity for a specific effect only if their dose–effect curves are parallel. The dose–effect curve for each effect is a composite of dose–concentration and concentration–effect curves for plasma and dose–concentration and concentration–effect curves at the sites of action in tissues. Thus, a fixed dosage ratio of different compounds can produce equivalent activity for a specific effect only if the compounds have parallel dose–effect, dose–concentration, and concentration–effect curves. Similarly, a fixed dosage ratio of different compounds can produce equivalent overall biological activity only if the compounds have parallel dose–effect, dose–concentration, and concentration–effect curves for all effects, in all experimental settings, and over the entire dosage range.

Confirming the USP or FNB-IOM hypotheses therefore requires that every set of dose–effect, concentration–effect, and dose–concentration curves of *all-rac*- and *RRR*-α-tocopherols are parallel in every experimental setting and that each set of parallel curves differ by a ratio of 1.36:1 or 2:1, respectively. In contrast, one can show that no dosage ratio of *all-rac*- and *RRR*-α-tocopherols produces equivalent activity for all effects and all dosages by showing even a single nonparallel dose–effect, dose–concentration, or concentration–effect curve in any situation. We will show nonparallel bioavailability (nonparallel dose–plasma concentration curves and nonparallel dose–tissue concentration curves) and nonequivalent biological activity (nonparallel dose–effect curves and non-parallel concentration-effect curves) in humans and animals.

2. Variable relative bioavailability of *all-rac*- and *RRR*-α-tocopherols

In contrast to recent reviews discussing the ongoing controversy regarding whether *RRR*-α-tocopherol is 1.36-fold or 2-fold more bioavailable than *all-rac*-α-tocopherol [29,30], we focus on observations that their relative bioavailability varies with experimental conditions. Their relative bioavailability varies with time after dosing in humans and animals because *all-rac*- and *RRR*-α-tocopherols are comparably absorbed [2] but differ in the extent of retention [21–24,31–33]. As a result of comparable absorption, the relative bioavailability of labeled *all-rac*- and *RRR*-α-tocopherols is about 1:1 for the first 6 –12 hours after dosing [2]. However, as a result of preferential retention of 2*R* stereoisomers and elimination of 2*S* stereoisomers [31–33], the ratio of retained *RRR*-α-tocopherol to retained *all-rac*-α-tocopherol increases with time after single doses and eventually approximates 2:1 [21–24].

Comparable absorption and differential retention of stereoisomers also causes variations in their relative bioavailability with the duration of dosing. Preferential retention of the 2*R* forms and elimination of the 2*S* forms after every dose results in a progressively greater proportion of retained α-tocopherol being 2*R* forms. For example, in vitamin E–deficient rats receiving *all-rac*-α-tocopheryl acetate, the 2*R* and 2*S* stereoisomers comprised about 75% and 25%, respectively, of plasma α-tocopherol after 8 days and 86% and 14% after 90 days [32]. In humans ingesting multiple doses, several studies show that the ratio of retained *RRR*-α-tocopherol to retained *all-rac*-α-tocopherol increases with duration of dosing and eventually approximates 2:1 [21–24]. One study in humans ingesting daily simultaneous 75 mg doses of deuterated *RRR*- and *all-rac*-α-tocopherols found that the relative bioavailability of *RRR*- and *all-rac*-α-tocopherols did not change with the duration of dosing and approximated 2:1 throughout the study [23]. However, this discrepancy likely arose because humans rapidly eliminate the 2*S* stereoisomers and this study measured α-tocopherol plasma α-tocopherol concentrations about 24 hours after each dose.

The relative availability of synthetic and natural α-tocopherols varies between sites in the body in humans and animals, and the extent of this variation between sites varies with the duration of dosing [21,32,34–37]. For example, in rats fed a diet with an equimolar mixture of deuterated *RRR*- and *SRR*-α-tocopheryl acetate (previously called *dl*-α-tocopheryl acetate), the ratio of deuterated *RRR*- to *SRR*-α-tocopherol after 154 days was 5.3 in the brain, 3.6 in red blood cells, 2.4 in plasma, 1.9 in the heart, and 1.2 in the liver [35]. In rats fed the same diets for 8 days, the ratios were 1.4, 2.0, 1.6, 0.88, and 0.67, respectively [35].

Differences between tissues in the relative availability of stereoisomers might arise from differences in relative uptake and/or relative retention of stereoisomers. Endothelial cells *in vitro* comparably take up all eight stereoisomers of α-tocopherol [38], and other tissues most likely take up all stereoisomers of α-tocopherol in equal proportions because this uptake occurs nonspecifically via the mechanisms of lipid uptake [2]. This suggests that differences between tissues in the relative availability of individual stereoisomers result from differences between tissues in their mechanisms of retaining α-tocopherol. The only known mechanism explaining stereospecific differences between tissues in retention of α-tocopherol is the presence of TTP. TTP has been detected in humans and animals in the liver, brain, and the gravid but not nongravid uterus, and in minute concentrations in the lungs, spleen, and kidneys [39–41]. However, TTP activity cannot be the only mechanism by which tissues differ in the retention of stereoisomers. Acuff *et al*. found that when pregnant humans ingested an equimolar

mixture of deuterated *RRR*- and *all-rac*-α-tocopheryl acetate (d_3-*RRR*- and d_6-*all-rac*-α-tocopheryl acetate) for the last 5–9 days of pregnancy, the ratio of deuterated *RRR*- to *all-rac*-α-tocopherol at delivery was 3.42:1 in fetal blood and 1.86:1 in maternal blood [34]. TTP activity cannot explain the 3.42:1 ratio in fetal blood because enzymes such as TTP that only discriminate between the 2*R* and 2*S* stereoisomers cannot cause greater than 2:1 relative availability of *RRR*- and *all-rac*-α-tocopherols. These observations show that different tissues use different mechanisms to retain different proportions of different stereoisomers of α-tocopherol at different times.

Differences between tissues in the time course of retention of stereoisomers imply that the relative activity of *all-rac*- and *RRR*-α-tocopherols cannot be constant in all tissues at all times, regardless of the ratio of dosages and the units of dosage. For example, consider that all stereoisomers of α-tocopherol are comparably absorbed and are approximately equally potent antioxidants *in vitro* [2,42]. Thus, equal milligram dosages of *all-rac*- and *RRR*-α-tocopherols initially result in approximately equal relative antioxidant activity after *all-rac*- as after *RRR*-α-tocopherol, whereas equal dosages in USP vitamin E units or IU (i.e., 1.36-fold more milligrams of *all-rac*- than *RRR*-α-tocopherol) initially result in approximately 36% greater antioxidant activity after *all-rac*- than after *RRR*-α-tocopherol. However, antioxidant activity eventually becomes greater after *RRR*- than after *all-rac*-α-tocopherol because of greater retention of *RRR*- than *all-rac*-α-tocopherol. If one accepts the FNB-IOM conclusion that *RRR*-α-tocopherol is retained 2.0-fold more than *all-rac*-α-tocopherol, then equal dosages in milligrams eventually result in 50% as much antioxidant activity after *all-rac*- as after *RRR*-α-tocopherol, but equal dosages in USP units eventually result in 68% as much antioxidant activity after *all-rac*- as after *RRR*-α-tocopherol. If one accepts the USP conclusion that *RRR*-α-tocopherol is retained 1.36-fold more than *all-rac*-α-tocopherol, then equal dosages in milligrams eventually result in 74% as much antioxidant activity after *all-rac*- as after *RRR*-α-tocopherol, whereas equal dosages in USP units eventually result in 68% as much antioxidant activity after *all-rac*- as after *RRR*-α-tocopherol. Thus, no dosage ratio produces a constant ratio of antioxidant activity.

Several studies suggest that the relative bioavailability of *all-rac*- and *RRR*-α-tocopherols varies with dosage in humans ingesting single doses and in humans ingesting multiple doses sufficient to achieve steady-state conditions. In α-tocopherol-deficient humans, the ratio of bioavailability of *RRR*- to *all-rac*-α-tocopherols is greater after single doses of less than 30 USP units [43,44]. In healthy nondeficient humans, total plasma α-tocopherol concentrations are about 20% greater after ingestion of a single dose of 100 mg *RRR*-α-tocopheryl acetate than after 100 mg *all-rac*-α-tocopheryl acetate, but total plasma α-tocopherol concentrations are about equal after ingestion of 100 mg *RRR*-α-tocopheryl acetate or 300 mg *all-rac*-α-tocopheryl acetate [33]. As reviewed by Hoppe and Krennrich, multiple-dose studies also show that the relative bioavailability of *all-rac*- and *RRR*-α-tocopherols varies with dose [45].

The above experimental observations show that the relative bioavailability of *all-rac*- and *RRR*-α-tocopherols is not constant, which implies that no fixed dosage ratio of *all-rac*- and *RRR*-α-tocopherols can produce equivalent effects on all processes, at all times, and in all dosages. However, Devaraj *et al*. observed parallel dose–effect, dose–concentration, and concentration–effect curves in healthy humans not deficient in vitamins who were given unlabeled *all-rac*- or *RRR*-α-tocopherols 100, 200, 400, or 800 IU daily for 8 weeks [19]. Indeed, this study showed that a 1:1 dosage ratio in IU (a 1.36:1 ratio in mg) produced a 1:1 ratio of bioavailability (i.e., parallel dose–concentration curves) and a 1:1 ratio of biological activity (i.e., parallel dose–effect curves for prolonging the lag time before oxidation of low-density lipoproteins) [19]. (The reported figures show overlapping curves when the unit for dosage is IU but these curves are parallel rather than comparable when the unit for dosage is milligrams.) We suggest that the discrepancy between this study and our conclusions above arises partly from the stereospecific and saturable kinetics of α-tocopherol discussed in section 3 below and partly from other factors discussed in section 4.

3. Stereospecific and saturable aspects of the kinetics of α-tocopherol

3.1. Stereospecificity and saturability of absorption

Tocopherols and tocotrienols are absorbed nonspecifically and equally well by intestinal mucosal cells via a nonsaturable, non–carrier-mediated passive diffusion process and are then secreted with chylomicrons into the lymph [2]. Absorption is not saturable in humans ingesting labeled *RRR*-α-tocopheryl acetate 15–150 mg [46]. However, absorption appears relatively saturable because humans comparably absorb 50 mg α-tocopherol mixed in a low fat or high fat spread [47] but absorb supplements of 300, 440, 880, or 1320 mg less efficiently with a low-fat than a high-fat meal [48,49]. Humans also absorb 300 mg α-tocopherol more efficiently when they ingest the supplement with dinner compared to ingesting the supplement without food [49]. However, saturable absorption does not affect the relative bioavailability of *all-rac*- and *RRR*-α-tocopherols because absorption occurs via nonspecific processes [2].

3.2. Stereospecificity and saturability of distribution

As reviewed by Traber [2], hepatic TTP directly modulates distribution of α-tocopherol by mediating the preferential recycling of the 2*R* stereoisomers and elimination of the 2*S* stereoisomers. By modulating the availability of the 2*R* stereoisomers for elimination, hepatic TTP thereby in-

directly mediates metabolism and excretion [50]. However, the stereospecificity of TTP is relevant only if TTP is saturable because competition between compounds for binding to enzymes only affects their relative bioavailability if the enzymes are saturable [4]. Consistent with saturability of TTP, urinary α-CEHC concentrations are minimal in normal humans until plasma α-tocopherol exceeds a threshold concentration [51], but no such threshold is detected in TTP-deficient humans [52]. Achieving this threshold plasma concentration apparently requires a dose exceeding 50–150 mg *RRR*-α-tocopherol/day [52]. Consistent with saturability of TTP and/or saturability of other enzymes mediating elimination, increasing dosage of deuterated α-tocopherol from 15 to 150 mg in normal humans causes progressively more rapid disappearance of deuterated α-tocopherol from plasma [46], and administering 335 mg *RRR*-α-tocopherol/day to healthy humans increases plasma α-tocopherol 1.5-to-3-fold but increases plasma α-CEHC 15-to-30 fold [53]. TTP also appears saturable because saturable enzymes are inducible [4], and TTP concentrations are greater in the brains of humans with vitamin E deficiencies or diseases associated with cerebral oxidative stress than in normal human brains [39]. Similarly, in vitro TTP activity is lower in liver tissue from rats fed normal diets than in liver tissue from rats fed vitamin E–deficient diets [54]. Saturability and stereospecificity of TTP imply that *all-rac*- and *RRR*-α-tocopherols differ most in bioavailability at dosages low enough to avoid saturating TTP.

3.3. Stereospecificity and saturability of elimination

Elimination of tocopherols and tocotrienols involves metabolism and excretion. All tocopherols and tocotrienols appear metabolized by a common pathway, resulting in the formation of stable carboxyethyl-hydroxychroman (CEHC) metabolites [53,55–57]. This pathway apparently involves ω-oxidation by cytochrome P450-4F2 (CYP4F2) followed by β-oxidation by CYP3A4 [57–60]. Compared to the 2*R* forms of α-tocopherol, humans appear to preferentially convert tocopherols and tocotrienols that have low affinity for TTP to CEHCs that are eliminated in urine [55,56,61]. In contrast, humans convert less than 3% of *all-rac*- or *RRR*-α-tocopherol to CEHCs that are excreted in urine [61]. However, α-CEHC production is 2.7-fold greater after *all-rac*- than *RRR*-α-tocopherol [61]. Although the bulk of α-CEHC might be excreted in the bile rather than in the urine, this seems unlikely because large proportions of other deuterated tocopherols and tocotrienols are recovered as CEHC metabolites in urine [55,56]. It therefore seems more likely that elimination of α-tocopherol occurs primarily via secretion of unchanged α-tocopherol into bile followed by excretion in feces. Biliary secretion of α-tocopherol in rats appears mediated by the hepatic multidrug resistance P-glycoprotein encoded by the mdr2 gene (Pgp-mdr2) [62]. The stereospecificity of elimination of *all-rac*- and *RRR*-α-tocopherols may simply reflect the stereospecificity of TTP, as TTP apparently modulates the availability of the 2*R* stereoisomers for elimination [50,57], but may also reflect the stereospecificity of metabolic or excretory processes. However, the affinities of CYP3A4, CYP4F2, and Pgp-mdr2 for stereoisomers of α-tocopherol are unknown.

The above observations show that *all-rac*- and *RRR*-α-tocopherols differ in postsystemic elimination, but they may also differ in presystemic elimination. Presystemic elimination occurs after orally ingested substances pass from the intestinal lumen into the enterocytes and before the substances enter the systemic circulation. Postsystemic elimination occurs after substances enter the systemic circulation. Both presystemic and postsystemic elimination involve intestinal metabolism and excretion as well as hepatic metabolism and excretion [63]. The liver likely has a minimal role in presystemic elimination of α-tocopherol because α-tocopherol reaches the systemic circulation via the lymph rather than the portal circulation. In contrast, the intestines likely eliminate α-tocopherol both pre- and postsystemically. The intestinal content of CYP3A4 is 50% that of the liver [63]. P-gp-mdr1 in rats and P-gp-MDR1 in humans mediate secretion of many substrates of CYP3A4 into the intestinal lumen [64]. P-gp-mdr2 mediates secretion of α-tocopherol into the bile in rats [62], and a related ATP-cassette binding protein ABCA1 mediates α-tocopherol efflux from human fibroblasts [65].

Elimination of α-tocopherol is likely saturable because both CYP and P-gp are saturable and another saturable protein (TTP) modulates the availability of α-tocopherol to CYP and P-gp. Although there is no direct evidence of saturable elimination (i.e., plateaus in the rate of α-CEHC production and the rate of biliary secretion of α-tocopherol), one can indirectly show that enzymes of elimination are saturable by showing that one substrate of an enzyme competitively inhibits elimination of another substrate or by showing that the enzymes are inducible [4].

3.4. Competitive inhibition of distribution and elimination

Competitive inhibition of elimination can explain interactions seen between stereoisomers of α-tocopherol. The relative bioavailability of *RRR*- to *all-rac*-α-tocopherol is greater when they are given together rather than separately [45,66]. *All-rac*-α-tocopheryl acetate is 31% more active in preventing fetal resorption in rats than expected based on the sum of activities of all stereoisomers [67]. Similarly, 2-*ambo*-α-tocopheryl acetate (50% *RRR* and 50% *SRR*-α-tocopheryl acetate) and 4′-*ambo*-8′-*ambo*-α-tocopheryl acetate (25% of each of *RRR*, *RSR*, *RRS*, and *RSS*) are 15% more active than expected [67]. These observations suggest that other stereoisomers inhibit the elimination of *RRR*-α-tocopherol. If so, greater doses of other stereoisomers result in greater sparing of the *RRR*-component of *all-rac*-α-tocopherol, which suggests that *all-rac*- and *RRR*-α-tocopherols differ less in bioavailability and bioactivity at higher dosages. Moreover, because humans and animals compara-

bly retain the *RRR*, *RSR*, *RRS*, and *RSS* stereoisomers of *all-rac*-α-tocopherol, these stereoisomers compete for binding to TTP and other saturable enzymes and receptors.

Competitive inhibition of binding to TTP and competitive inhibition of binding to enzymes of elimination can explain interactions observed between α-tocopherol and other compounds. Plasma and tissue concentrations of γ-tocopherol decrease in humans supplemented with α-tocopherol [68]. This is consistent with greater affinity of TTP for α- than for γ-tocopherol [20] and suggests that TTP is saturable. In contrast, tissue concentrations of α-tocopherol are greater in vitamin E-deficient rats fed diets containing both α- and γ-tocopherol than in rats fed α-tocopherol alone [69,70]. Moreover, in rats fed diets containing a constant amount of α-tocopherol and varying amounts of γ-tocopherol, tissue α-tocopherol concentrations increase progressively more as the ratio of γ- to α-tocopherol in the diet is progressively increased [69]. These observations suggest that elimination of α- and γ-tocopherols involves the same saturable pathways and that γ-tocopherol inhibits the elimination of α-tocopherol. γ-Tocopherol might inhibit the elimination of α-tocopherol by CYP4F2 (which has greater activity for γ- than α-tocopherol [59]) or by CYP3A4 (but the relative activity of CYP3A4 for α- and γ-tocopherol is unknown). The metabolism of tocopherols by CYP3A4 appears saturable because ketoconazole (an inhibitor of CYP3A4) inhibits metabolism of α- and γ-tocopherol in rat primary hepatocytes and inhibits metabolism of γ- and δ-tocopherols in human HepG2/3A cells [58]. The metabolism of tocopherols by CYP4F2 appears saturable because the sesame seed lignan sesamin inhibits the tocopherol-omega-hydroxylase activity of CYP4F2 in rat or human liver microsomes [59] and inhibits γ-tocopherol metabolism *in vitro* by HepG2/3A cells [58]. Consistent with inhibition of tocopherol metabolism, sesamin enhances vitamin E activity in rats fed a low α-tocopherol diet [71], synergistically acts with γ-tocopherol in producing vitamin E activity in rats [72], and increases γ-tocopherol levels *in vivo* in rats [73]. Consistent with saturability of P-gp, α-tocopherol polyethylene glycol 1000 succinate (TPGS), a water-soluble formulation of *RRR*-α-tocopherol, inhibits P-gp activity in rats *in vivo* [74] and inhibits human MDR1 activity *in vitro* [75]. However, Wang *et al*. found that α-tocopherol does not affect P-gp function *in vitro* [76].

3.5. Induction of elimination of α-tocopherol

Several observations show induction of elimination of *all-rac*- and *RRR*-α-tocopherols. HepG2 human hepatoma cells release α-CEHC metabolites of *all-rac*-α-tocopherol *in vitro* only after 10 days of exposure to *all-rac*-α-tocopherol [60]. In contrast, HepG2 cells release minimal or no α-CEHC metabolites of *RRR*-α-tocopherol even after pretreatment with *RRR*-α-tocopherol [60,77]. Thus, *all-rac*-α-tocopherol but not *RRR*-α-tocopherol induces its own metabolism. However, HepG2 *in vitro* release 4-fold more α-CEHC metabolites of *RRR*-α-tocopherol after pretreatment with rifampicin, which induces CYP3A4 [60].

Induction of CYP3A4 involves binding of compounds to the pregnane X receptor, also known as the steroid X receptor (PXR/SXR), thereby causing increased transcription of CYP3A4 [78]. Compounds that induce CYP3A4 activity generally also induce P-gp activity because the PXR/SXR receptor coordinately regulates expression of both CYP3A4 and P-gp [79]. Tocopherols and tocotrienols bind to the PXR/SXR [78,80] and thereby likely induce CYP3A4 and P-gp activity. However, tocopherols and tocotrienols might be less potent inducers of P-gp than other compounds that bind to the PXR/SXR receptor, because reactive oxygen species upregulate P-gp expression and antioxidants attenuate this upregulation of P-gp expression [81,82]. Binding of tocopherols and tocotrienols to the PXR/SXR receptor suggests that tocopherols and tocotrienols might induce elimination of other compounds eliminated via these saturable pathways. For example, induction of elimination of simvastatin, which is a substrate of human CYP3A4 and P-gp [83], might explain why the activity of simvastatin and niacin was less in patients receiving simvastatin, niacin, beta-carotene, ascorbic acid, α-tocopherol, and selenium compared to patients receiving simvastatin and niacin alone [84].

4. Discrepancies between studies showing nonconstant relative bioavailability and human studies suggesting constant relative bioavailability and bioactivity

4.1. Pharmacokinetic considerations

For compounds characterized by saturable kinetics, bioavailability varies with the size of each dose, the number of doses, and the duration of dosing [4]. Moreover, only when enzymes are saturable does their relative affinity for stereoisomers determine the relative bioavailability of the stereoisomers [4]. If distribution and elimination of α-tocopherol were not saturable, then both the absolute and relative bioavailability of *all-rac*- and *RRR*-α-tocopherols would be constant. If distribution and elimination of α-tocopherol were saturable but not stereospecific, the absolute bioavailability of *all-rac*- and *RRR*-α-tocopherols would vary with the saturation of those pathways but their relative bioavailability would be constant. However, the combination of saturable and stereospecific distribution and elimination implies that both the absolute and relative bioavailability of *all-rac*- and *RRR*-α-tocopherols change with the saturation of those pathways. This suggests that the body handles *all-rac*- and *RRR*-α-tocopherols most similarly when processes of distribution and elimination are saturated (e.g., when subjects who are not deficient in vitamin E are given pharmacologic doses of vitamin E). This at least partially explains the observations by Devaraj et al. that pharmacological dosages of all-rac- and *RRR*-α-tocopherols produce

parallel dose–concentration curves in humans without vitamin E deficiency [19].

In contrast, saturable and stereospecific distribution and elimination suggest that the body handles *all-rac*- and *RRR*-α-tocopherols most differently when distribution and elimination are least saturated (e.g., in vitamin E–deficient subjects receiving low dosages). Indeed, experimental observations confirm that *all-rac*- and *RRR*-α-tocopherols have nonparallel dose–concentration curves in vitamin E–deficient humans. Changes in plasma α-tocopherol concentrations correlate linearly with changes in dosage in vitamin E–deficient humans given very low doses (5–17 mg/day) of *RRR*-α-tocopherol but do not correlate linearly with changes in dosage in vitamin E–deficient humans receiving greater than 17 mg/day of *RRR*-α-tocopherol or any dosage of *all-rac*-α-tocopherol [43,44]. Dose–concentration curves of *all-rac*- and *RRR*-α-tocopherols are not parallel in vitamin E–deficient humans because these curves are linear for *RRR*-α-tocopherol at low doses and nonlinear for *all-rac*-α-tocopherol at all doses. Consistent with nonparallel dose–concentration curves, the only studies comparing dose–effect relationships of *all-rac*- and *RRR*-α-tocopherols in vitamin E–deficient humans noted nonparallel dose–effect curves in preventing hemolysis [43,44,77]. However, the FNB-IOM notes that these human studies do not provide enough data to accurately assess the relative activity of *all-rac*- and *RRR*-α-tocopherols [17].

Experimental observations in humans not deficient in vitamin E also suggest that changes in relative bioavailability with dosage might occur in studies giving low dosages of *all-rac*- and *RRR*-α-tocopherols. In humans without vitamin E deficiency, a linear correlation between dose and bioavailability has been shown only in subjects receiving low doses of *RRR*-α-tocopherol (15–150 mg/day) [46]. However, this linear change in bioavailability with dose has been shown only by linear changes in the area under the curve (AUC) of plasma concentration versus time with increasing dose [46]. Plasma concentrations do not change linearly with dose in humans without vitamin E deficiency who were receiving *RRR*-α-tocopherol 15–150 mg/day [46], 75 mg/day [86], 100–800 IU/day [87], 400–800 IU/day [88], or 100 IU/kg/day [2,80] or *all-rac*-α-tocopherol 100–800 IU/day [19] or 440–1320 IU/day [48]. Thus, studies may not observe changes in bioavailability with dose if they measure changes in plasma concentration with dose rather than changes in AUC with dose. Moreover, no studies comparing dose–concentration or dose–effect curves of *all-rac*- and *RRR*-α-tocopherols in humans not deficient in vitamin E have given doses of less than 100 IU/day. Thus, although the stereospecificity and saturability of distribution and elimination predict *all-rac*- and *RRR*-α-tocopherols have nonparallel dose–concentration or dose–AUC curves, no experiments in humans not deficient in vitamin E have adequately tested this prediction because none gave sufficiently low dosages and determined dose–AUC curves as well as dose–concentration curves.

Another reason why experiments in humans not deficient in vitamin E have not shown nonparallel dose–concentration or dose–effect curves for *all-rac*- and *RRR*-α-tocopherols is that newly administered labeled α-tocopherol rapidly displaces "old" unlabeled α-tocopherol in plasma and erythrocytes rather than proportionately increasing total plasma α-tocopherol concentrations [21–23,46]. That is, changes in plasma labeled α-tocopherol concentrations are proportionately greater than changes in total circulating α-tocopherol [21–23,46]. Indeed, this displacement process occurs even when total plasma and erythrocyte *RRR*-α-tocopherol concentrations do not change significantly [21,24,46]. This shows that the body responds to administered α-tocopherol by changing how it handles endogenous α-tocopherol. Moreover, newly administered deuterated α-tocopherol comprises a greater proportion of total plasma α-tocopherol in humans given deuterated *RRR*-α-tocopherol than in humans given deuterated *all-rac*-α-tocopherol even when the total plasma α-tocopherol concentrations are comparable [21,46]. This shows that the relative bioavailability of *all-rac*- and *RRR*-α-tocopherols varies with time after dosing even when the total bioavailability of labeled plus unlabeled α-tocopherol does not vary with time after dosing [21,46]. That is, in humans not deficient in vitamin E, dose–concentration curves of newly administered *all-rac*- and *RRR*-α-tocopherols are not parallel yet dose–concentration curves of total plasma α-tocopherol are parallel. Thus, in humans without vitamin E deficiency, differences in the relative bioavailability of newly administered *all-rac*- and *RRR*-α-tocopherols do not cause differences in their relative activity because the effects of newly administered α-tocopherol depends not on the bioavailability of the newly administered α-tocopherol but on the bioavailability of total (new plus previously present) α-tocopherol.

The rapidity of the displacement of endogenous α-tocopherol by newly administered α-tocopherol suggests that no dosage ratio of *all-rac*- and *RRR*-α-tocopherols results in equivalent activity. After normal humans ingest 15 mg of deuterated *all-rac*-α-tocopherol plus 15 mg of deuterated *RRR*-α-tocopherol daily for eight days, labeled α-tocopherol comprises 11% and 33% of total plasma α-tocopherol one day and eight days after the first dose, respectively [21]. After normal humans ingest 150 mg of deuterated *all-rac*-α-tocopherol plus 150 mg of deuterated *RRR*-α-tocopherol daily for eight days, labeled α-tocopherol comprises 55% and 80% of total plasma α-tocopherol one day and eight days after the first dose, respectively [21]. The high proportion of labeled relative to total plasma α-tocopherol suggests that the newly administered tocopherols compete for access to saturable enzymes. These observations of competition for saturable enzymes support the argument by Cohn that competitive dosing studies are valid only if the studies administer trace dosages of competing tocopherols [66]. Accordingly, Cohn suggests that competitive dosing studies using more than trace dosages of *all-rac*- and *RRR*-α-tocopherols underestimate the relative bioavailability of *all-rac*-

α-tocopherol because TTP is stereoselective and saturable [66]. However, we suggest that if differences in dosing protocols between studies cause inconsistencies in the observed relative bioavailability of *all-rac*- and *RRR*-α-tocopherols, then these inconsistencies support the hypothesis that no dosage ratio of *all-rac*- and *RRR*-α-tocopherols results in equivalent activity.

4.2. Pharmacodynamic considerations

The above discussion highlights pharmacokinetic advantages of giving low dosages of *all-rac*- and *RRR*-α-tocopherols and comparing their activity in vitamin E–deficient subjects, but these methods are also important for pharmacodynamic reasons. Studies in vitamin E–deficient subjects are necessary to determine relative vitamin E activity because vitamin activity refers only to prevention or treatment of deficiency symptoms. As discussed above, the only studies comparing dose–effect relationships of *all-rac*- and *RRR*-α-tocopherols in vitamin E–deficient humans noted marked differences in their dose–effect curves in preventing hemolysis as well as in their dose–concentration curves [43,44,85]. However, the FNB-IOM notes that these human studies do not provide enough data to accurately assess the relative activity of *all-rac*- and *RRR*-α-tocopherols [17]. We discuss dose–effect studies in vitamin E–deficient animals below. The size of the dose is relevant because different effects might be evident at different dosages. One might detect vitamin E activity most easily at low dosages but may have difficulty distinguishing vitamin E and non–vitamin E activities at higher dosages. Some effects appear evident only at higher dosages. For example, dosages less than 400 IU/day of *all-rac*- or *RRR*-α-tocopherols do not significantly increase the lag time before oxidation of low-density lipoproteins [19].

The effects of dosage likely vary with the specificity of the mechanisms responsible for those effects. Increasing dosage of *all-rac*- or *RRR*-α-tocopherol likely increases the extent of nonspecific effects relative to the extent of the specific effects because nonspecific effects are less likely than specific effects to involve saturable mechanisms. Increasing dosage may have different effects on the relative antioxidant and nonantioxidant activity of *all-rac*- and *RRR*-α-tocopherols because their antioxidant effects appear derived nonspecifically from their phenolic structure [2,42], but their non-antioxidant effects apparently involve specific interactions with molecules that mediate specific processes [89]. Non-antioxidant processes involving α-tocopherol that appear saturable include those mediated by tocopherol transfer protein, cytochrome P450 isoenzymes, and P-glycoproteins. Increasing dosage likely causes decreasing differences in the relative effects of *all-rac*- and *RRR*-α-tocopherols on saturable non-antioxidant processes as these processes become saturated. In contrast, their antioxidant effects do not appear saturable, although α-tocopherol has pro-oxidant effects under some experimental conditions [90]. Thus, increasing dosage likely does not change the relative antioxidant activity of *all-rac*- and *RRR*-α-tocopherols except to the extent that increasing dosage changes their relative bioavailability, because all of the stereoisomers of α-tocopherol have similar antioxidant potency *in vitro*. These considerations are relevant because most studies measure antioxidant effects on intravascular processes (e.g., erythrocyte hemolysis or lipoprotein oxidation) rather than non-antioxidant effects on extravascular processes. However, ratios of intravascular antioxidant activity may not correlate with ratios of extravascular antioxidant activity or with ratios of intravascular or extravascular non-antioxidant activity.

4.3. How might studies in humans not deficient in vitamin E show nonconstant relative bioavailability and biological activity for all-rac- and RRR-α-tocopherols?

One might expect to observe non-parallel dose–effect curves most easily by studying effects that are evident at physiologic rather than pharmacologic dosages because saturable and stereospecific kinetics imply that non-parallel dose concentration curves are most evident after low dosages of *all-rac*- and *RRR*-α-tocopherols. However, in humans not deficient in vitamin E, even low dosages of new α-tocopherol displace endogenous circulating α-tocopherol rather than proportionately increasing total plasma α-tocopherol, so dose–effect curves will likely remain parallel for those effects determined by total plasma α-tocopherol concentration and for effects mediated by antioxidant mechanisms or other nonspecific mechanisms. Thus, if *all-rac*- and *RRR*-α-tocopherols actually have nonparallel dose–effect curves at any dosages, demonstrating these nonparallel curves may require assessing effects mediated by highly specific nonantioxidant mechanisms.

The above discussion suggests that *all-rac*- and *RRR*-α-tocopherols have similar effects for many of the purposes for which humans without vitamin E deficiencies take pharmacological strength supplements. However, we hypothesize that clinically significant non-parallel dose-effect curves exist for the interactions of *all-rac*- and *RRR*-α-tocopherols with other compounds metabolized by CYP3A4 or excreted by P-gp. Saturable elimination predicts that *all-rac*- and *RRR*-α-tocopherol interact with other compounds eliminated via the same saturable pathways, and stereospecific elimination predicts that *all-rac*- and *RRR*-α-tocopherol interact differently with these compounds. If *all-rac*- and *RRR*-α-tocopherols in fact interact differently with other compounds, these differences might be clinically quite significant, as discussed in the conclusion. We do not propose methods for studying drug interactions because these studies are beyond the scope of this article and are standard practice during preclinical phases of new drug development and during the continuous post-marketing surveillance of drug safety.

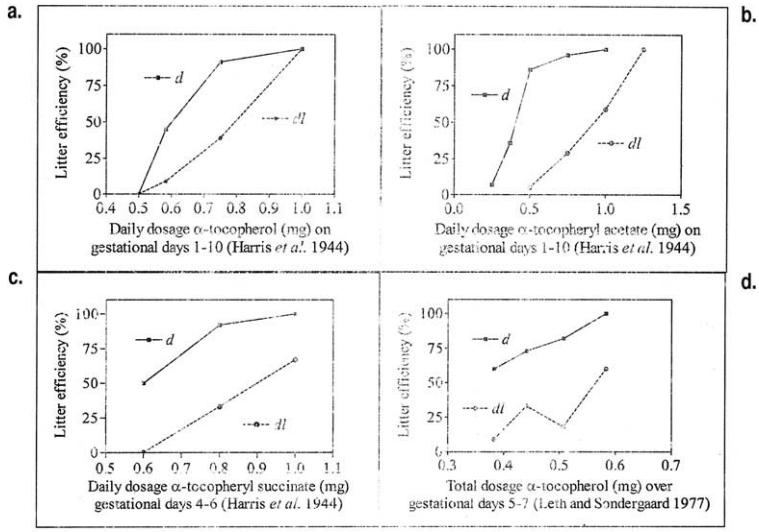

Fig. 1. Dose–effect curves for *d*- and *dl*-α-tocopherols in the rat fetal resorption assay.

5. Discrepancies between studies showing nonconstant relative bioavailability and studies suggesting constant relative activity in vitamin E–deficient animals

One might question the clinical relevance of our conclusion that no fixed dosage ratio of *all-rac*- and *RRR*-α-tocopherols can produce mathematically equivalent biological effects because many studies in vitamin E–deficient animals report that a 1.36:1 dosage ratio produces a constant 1:1 ratio of vitamin E activity. However, we reach different conclusions by reevaluating the data in those studies that present sufficient data to compare the dose-effect curves of synthetic and natural α-tocopherol. This re-evaluation excludes studies that did not administer sufficient dosages to obtain dose–effect curves [91], administered different dosages of natural and synthetic α-tocopherols [92], did not report the effects of each dose [93–97], or reported rejecting any data showing nonlinear or nonparallel dose–effect curves [98]. This re-evaluation includes older studies in which synthetic and natural α-tocopherols are designated *dl*- and *d*-α-tocopherols, respectively, and recent studies in which synthetic and natural α-tocopherols are designated *all-rac* and *RRR*-α-tocopherols, respectively. *dl*-α-Tocopherol is a racemic (equimolar) mixture of one pair of diastereoisomers (*RRR*- and *SRR*-α-tocopherols, previously called *d*- and *l*-α-tocopherols, respectively), whereas *all-rac*-α-tocopherol is a mixture of almost equal proportions of four pairs of diastereoisomers (*RRR* and *SRR*, *RRS* and *RSS*, *RSR* and *SSR*, and *RSS* and *SSS*) [1,17].

5.1. Dose–effect curves of synthetic and natural α-tocopherols

Gestation–fetal resorption assays in vitamin E–deficient female rats are the most widely accepted assays of vitamin E activity in animals. Typical fetal resorption studies fed female rats a diet deficient in all tocopherols and tocotrienols for at least three months to induce vitamin E deficiency and then allowed these females to mate with normal nondeficient males. The female rats were then given supplemental tocopherols, tocotrienols, or synthetic antioxidants on gestational days 1–10 [95], days 4–6 [91], days 4–8 [97], days 5–7 [99], or days 5–14 [1,67,92]. Between days 16 and 20 of the 20-day gestational period, researchers determined the number of live fetuses, dead fetuses, and fetal implantation sites. Positive responses were defined as one or more living fetuses [93,97,99], one or more living fetuses in rats with at least four implantation sites [1,67,92], or two or more living fetuses in rats with at least four implantation sites [91]. Most assays reported responses in terms of litter efficiency (LE, the percentage of mated females with positive responses) and/or median fertility dose (MFD, the dose of each compound that produced a litter efficiency of 50%).

Comparing the MFD of different tocopherols is analogous to comparing the dose of different drugs that achieves 50% of the maximum effect (ED_{50}), but the ED_{50} or MFD accurately describe relative potency only when dose–response curves are parallel. The USP emphasized the rat fetal resorption assays reported by Harris and Ludwig in 1949

Table 1
Effect of selenium on the bioavailability of tritiated *d*- and *l*-α-tocopheryl acetate in chicks [103]

Dose of selenium (mg)	Beta counts/min/mL serum 48 h after feeding tritiated α-tocopheryl acetate		Ratio of retained *d* to retained *l*
	d-α-TA	*l*-α-TA	
0	9146	2260	4.0
0.5	13224	2934	4.4
1	18396	3910	4.7

Table 2
The effect of selenium on the efficacy of *d*- and *l*-α-tocopheryl acetate in the chick curative muscular dystrophy assay [103]

Dosage *d*-αTA mg/kg diet	Dosage *l*-αTA mg/kg diet	Muscular dystrophy score		
		Dietary selenium 0.0 ppm	Dietary selenium 0.1 ppm	Dietary selenium 1.0 ppm
0	0	4	3.5	2.2
2.5		3.4	1.8	0.7
10		1.4	0	0
	10	3.1	2.8	1.5
	40	0.4	0	0

[94,96], but these studies reported the MFD without reporting the responses to each individual dosage, so we cannot determine if they observed parallel dose–effect curves. However, Harris *et al*. reported the MFD and the responses to each individual dosage [100]; Figs. 1a–1c show dose–effect curves drawn from that data. The dose–effect curves for *dl*- and *d*-α-tocopherols are not parallel in Figs. 1a and 1b and are mostly parallel in Fig. 1c. Figure 1d shows nonparallel dose–effect curves for *dl*- and *d*-α-tocopherols drawn from the data of Leth and Sondergaard [101].

Dose–effect curves for *d*- and *dl*-α-tocopherol appear parallel in the chick curative muscular dystrophy assay (data not shown) [102]. However, administering selenium with α-tocopherol increases the retention of *d*-α-tocopherol relatively more than that of *l*-α-tocopherol (Table 1) and enhances the effectiveness of *d*-α-tocopherol relatively more than that of *l*-α-tocopherol (most notable for the dosage of 10 mg α-tocopherol/kg diet in Table 2) [103]. Tables 1 and 2 suggest that adding selenium caused proportionately greater changes in the relative activity of *d*- and *1*-α-tocopherol than in their relative bioavailability. Different effects of selenium on the availability and activity of *d*- and *l*-α-tocopherols imply different effects on *d*- and *dl*-α-tocopherols. Thus, some dose–effect curves of *d*- and *dl*-α-tocopherol are not parallel in the chick curative muscular dystrophy assay.

In contrast to observations that some dose–effect curves for *dl*- and *d*-α-tocopherols are not parallel in rats and chicks, Weiser and Vecchi reported approximately parallel dose–effect curves for *all-rac*- and *RRR*-α-tocopherols in rat fetal resorption assays [1,67,92,104]. Figures 2a and 2b show data from two of these four reports [1, 67]. Nonparallel dose–effect curves for *dl*- and *d*-α-tocopherols and parallel dose–effect curves for *all-rac*- and *RRR*-α-tocopherols are consistent with the fact that different stereoisomers comprise *dl*- and *all-rac*-α-tocopherols, and are consistent with observations that differences in the potency of *dl*- and *all-rac*-α-tocopherols in the rat fetal resorption assay almost reach statistical significance [104]. Nonparallel dose-effect curves for *dl*- and *d*-α-tocopherols in rats and chicks are significant despite apparently parallel dose-effect curves for *all-rac*- and *RRR*-α-tocopherols, because all dose–effect curves are parallel for all effects and for all sites of action when drugs differ in potency but have the same mechanisms of action [4]. The next section discusses studies of the relative potency of individual stereoisomers.

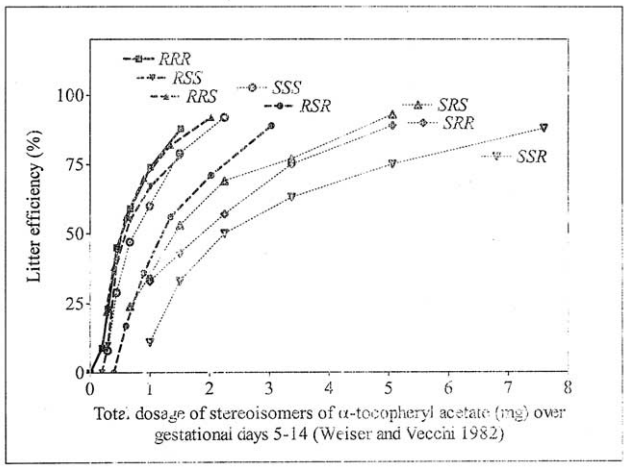

Fig. 3. Dose–effect curves for each individual stereoisomer of α-tocopheryl acetate drawn from data reported by Weiser and Vecchi [67]. This report by Weiser and Vecchi includes data from 11 experiments. Rats received *RRR*-α-tocopheryl acetate in 10 of these 11 experiments; this figure shows average data from all 10. Rats received each of the other seven stereoisomers in only one of the 11 experiments. Figure 2a shows data from the only one of the 11 experiments that compared *all-rac*- and *RRR*-α-tocopheryl acetates.

5.2. Dose–effect curves of the stereoisomers of α-tocopherol

Figure 3 shows dose–effect curves for each stereoisomer of α-tocopherol in the rat fetal resorption assay, using data reported by Weiser and Vecchi [67]. In contrast to the theory that the 2*R* or 2*S* conformation determines the activ-

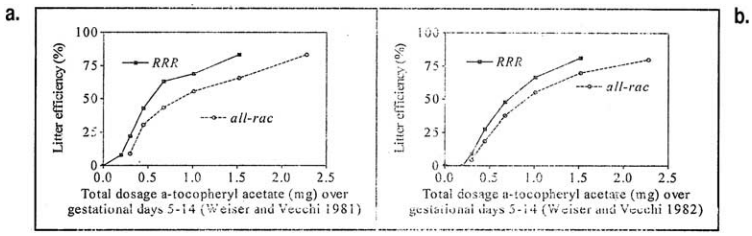

Fig. 2. Dose–effect curves for *RRR*- and *all-rac*-α-tocopherols in the rat fetal resorption assay.

Table 3
Relative potency of 2*R* and 2*S* stereoisomers of each pair of diastereoisomers of α-tocopheryl acetate [67]

Relative potency of each stereoisomer		Ratio of relative potency of 2*R* to 2*S* diastereoisomers	
RRR	100	*RRR/SRR*	3.3
SRR	31		
RRS	90	*RRS/SRS*	2.4
SRS	37		
RSS	73	*RSS/SSS*	1.2
SSS	60		
RSR	57	*RSR/SSR*	2.7
SSR	21		

Table 4
Relative potency and relative bioavailability of the stereoisomers of α-tocopheryl acetate [32,67]

Stereoisomer	Relative potency	Relative bioavailability
RRR	100	100
RRS	90	99
RSS	73	101
SSS	60	24
RSR	57	96
SRS	37	51
SRR	31	32
SSR	21	25

ity of each stereoisomer, these investigators observed marked differences in activity between the *RSR* and *RRR* stereoisomers and marked differences in activity between all four of the 2*S* stereoisomers. Indeed, Fig. 3 shows that *RSR*-α-tocopheryl acetate is markedly less potent than *SSS*-α-tocopheryl acetate. Similarly, Table 3 shows the relative potency reported by Weiser and Vecchi for each stereoisomer compared to the potency of *RRR*-α-tocopheryl acetate [67], and shows almost a 3-fold variation in the relative potency of the 2*R* and 2*S* forms of each pair of diastereoisomers. These observations show that the 2*R* or 2*S* conformation is not the only determinant of the relative activity of the stereoisomers, although the 2*R* or 2*S* conformation is the primary determinant of the relative affinity of the stereoisomers for TTP and the relative bioavailability of the stereoisomers. If different structural characteristics determine the relative bioavailability and relative biological activity of the stereoisomers, then the relative potency of the stereoisomers should not parallel their relative bioavailability. Experimental observations discussed below support this conclusion. Figure 3 is also important because not only are the dose–effect curves not parallel for the 2*R* and 2*S* stereoisomers, but not all of the 2*R* curves are parallel and none of the 2*S* curves are parallel. This suggests that the stereoisomers differ in mechanisms of action because drugs that act by the same mechanisms have parallel dose-effect curves.

5.3. Relative potency does not parallel relative bioavailability

Comparing bioavailability and potency (i.e., assessing concentration–effect relationships) is important because for substances such as α-tocopherol that are characterized by nonlinear kinetics, activity is more accurately shown by concentration–effect relationships than by dose–effect relationships [4,105,106]. No studies show the actual concentration–effect relationships of each stereoisomer, but we indirectly compare the relative bioavailability and the relative potency of the stereoisomers by combining data from the only study showing the relative bioavailability of each stereoisomer in any species [32] and the only study showing the relative potency of each stereoisomer in any species [67].

Table 4 compares the relative bioavailability of each stereoisomer in vitamin E-deficient rats, as observed by Weiser *et al*. [32], to the relative potency of each stereoisomer in preventing fetal resorption in vitamin E-deficient rats, as observed by Weiser and Vecchi [67]. Weiser *et al*. assessed relative bioavailability after administering each stereoisomer for 8, 32, 64, and 90 days [32] but Table 4 shows the relative bioavailability of stereoisomers in plasma at 8 days because Weiser and Vecchi administered the stereoisomers for 10 days in the fetal resorption study [67]. The relative bioavailability of stereoisomers varies with the duration of dosing [32], but this does not materially affect the comparison of these studies. Weiser *et al*. defined relative bioavailability as the concentration of each stereoisomer as a percentage of the total α-tocopherol concentration [32]. After eight days of dosing, the proportion of total plasma α-tocopherol comprised by each stereoisomer was *RRR* 19.0%, *RRS* 18.9%, *RSR* 18.3%, *RSS* 19.1%, *SSR* 4.7%, *SSS* 4.5%, *SRS* 9.6%, and *SRR* 6.0% [32]. However, Table 4 shows relative bioavailability as the proportion of plasma α-tocopherol comprised by each stereoisomer divided by the proportion of plasma α-tocopherol comprised by *RRR*-α-tocopherol. Showing relative bioavailability as a percentage of the bioavailability of *RRR*-α-tocopherol facilitates comparing these studies because Weiser and Vecchi reported the relative potency of the stereoisomers as a percentage of the potency of *RRR*-α-tocopherol, as shown in Table 4 [67].

One might question the validity of this indirect comparison of the relative concentration-effect relationships of the stereoisomers because the dose-concentration study administered *all-rac*-α-tocopheryl acetate 0.82 mg/day for 90 days [32] and the dose-effect study administered individual stereoisomers 0.2 to 7.5 mg/day for 10 days [67]. Indeed, this indirect comparison requires assuming that the *relative bioavailability* of the stereoisomers (not the *actual bioavailability* of each stereoisomer) is constant despite these differences in dosage. There are two possible ways in which making this assumption and indirect comparison can confirm our hypothesis. First, if one argues that the indirect

comparison is not valid because the assumption of constant relative bioavailability is not valid over this dosage range, then that confirms our hypothesis that no dosage ratio of *all-rac*- and *RRR*-α-tocopherols results in a constant ratio of bioavailability or biologic activity. Second, if this assumption is valid over this dosage range and if the relative potency of the stereoisomers does not correlate with their relative bioavailability, then the observation of non-parallel concentration-effect curves confirms our hypothesis.

As shown in Table 4, the observations by Weiser *et al*. of the relative bioavailability of the stereoisomers in vitamin E-deficient rats [32] do not parallel the observations by Weiser and Vecchi of the relative potency of the stereoisomers in preventing fetal resorption in vitamin E-deficient rats [67]. Notably, *SSS*-α-tocopherol is the most un-natural stereoisomer (having the *S* rather than the natural *R* configuration at each chiral center) and is 24% as bioavailable but 60% as potent as *RRR*-α-tocopherol. Indeed, *SSS*-α-tocopherol is only 25% as bioavailable as *RSR*-α-tocopherol yet is 5% more potent. Moreover, both the *RSR* and the *RSS* stereoisomers have about 30-40% lower relative potency than relative bioavilability. The combined observations that the stereoisomers differ in availability in plasma and that their relative availability in plasma does not parallel their relative potency demonstrate that the stereoisomers do not have parallel plasma concentration versus effect curves.

Non-parallel plasma concentration versus effect curves suggest that the stereoisomers differ in their tissue concentration versus effect curves and/or the relative availability of the stereoisomers in tissues does not parallel their relative availability in plasma. However, their relative availability in tissues can differ from their relative availability in plasma only if the stereoisomers differ in affinity for biologic compounds other than TTP and only if the relative affinity of these unknown compounds for the stereoisomers differs from the relative affinity of TTP for the stereoisomers. This is consistent with the observations noted in section 2 that the relative availability of the stereoisomers varies markedly between tissues. This is inconsistent with the conclusion of the FNB-IOM that the relative affinity of the stereoisomers for TTP is the sole determinant of the relative bioavailability and the relative potency of the stereoisomers. Nonparallel plasma concentration-effect curves show that no single dosage ratio can produce equivalent effects and suggest that differences in potency do not result only from differences in bioavailability. This suggests that the stereoisomers differ in their mechanisms of action as well as in their availability at sites of action.

5.4. Implications regarding mechanisms of action

The only known differences in mechanisms of action of *all-rac*- and *RRR*-α-tocopherols are in their affinity for TTP. However, three observations in animals suggest that *all-rac*- and *RRR*-α-tocopherols also differ in other mechanisms of action. First, reports that any dose–effect curves for *all-rac*- and *RRR*-α-tocopherol, *d*- and *dl*-α-tocopherol, and the stereoisomers are not parallel suggest differences in mechanisms of action because dose–effect curves are always parallel for drugs that have the same mechanisms of action [4]. Second, the almost 3-fold variation in the relative potency of the 2*R* and 2*S* forms of each pair of diastereoisomers is not consistent with observations that the 2*R* or 2*S* conformation is the primary determinant of affinity of the stereoisomers for TTP and the relative bioavailability of the stereoisomers. This inconsistency suggests that the relative bioavailability and relative biological activity of the stereoisomers depend on different structural characteristics, which implies that the stereoisomers act by different mechanisms, and therefore implies that *all-rac* and *RRR*-α-tocopherols act by different mechanisms. Third, nonparallel relationships between relative potency and relative bioavailability of the stereoisomers (i.e., nonparallel concentration versus effect curves) show that differences in potency do not result only from differences in bioavailability. Nonparallel concentration–effect relationships also suggest the stereoisomers differ in mechanisms of action because concentration–effect curves are parallel for drugs that have the same mechanisms of action [4]. Thus, observations in rats suggest that the stereoisomers differ in mechanisms of action, and observations in rats and chicks suggest that *all-rac*- and *RRR*-α-tocopherols differ in mechanisms of action. These observations suggest that *all-rac*- and *RRR*-α-tocopherols also differ in mechanisms of action in humans, despite the fact that vitamin E deficiency in humans does not cause fetal resorption or muscular dystrophy. Indeed, one cannot reasonably expect *all-rac*- and *RRR*-α-tocopherols to have the same relative potency and relative efficacy for every clinical effect because one cannot expect the relative affinity of the eight stereoisomers of α-tocopherol to be the same for every enzyme and receptor.

6. Conclusions and clinical implications

A single dosage ratio of *all-rac*- and *RRR*-α-tocopherols can produce equivalent biological effects only if every set of their dose–effect, dose–concentration, and concentration–effect curves are parallel in every experimental setting. In contrast, one can confirm the hypothesis that *all-rac*- and *RRR*-α-tocopherols do not have equivalent activity in any dosage ratio by showing a single nonparallel dose–effect, concentration–effect, or dose–concentration curve in any situation. We have shown a number of such nonparallel curves. The relative bioavailability of *all-rac*- and *RRR*-α-tocopherols is not constant in humans or animals because their relative concentrations vary between tissues and vary with time after dosing, duration of dosing, and the amount of each dose. Their relative bioavailability cannot be constant because their distribution and elimination involve pro-

cesses that are saturable as well as stereospecific; their relative bioavailability must change with the saturation of those processes. Thus, no fixed dosage ratio of *all-rac*- and *RRR*-α-tocopherols can produce a constant ratio of bioavailability for all dosages, which implies that no dosage ratio can produce a constant ratio of effects in any process. We also showed in vitamin E–deficient animals that the stereoisomers of α-tocopherol have nonparallel dose–effect curves and nonparallel relationships between relative bioavailability and relative potency. This shows that no fixed dosage ratio produces equivalent effects and suggests that the stereoisomers act by different mechanisms.

However, the clinical relevance of the conclusion that *all-rac*- and *RRR*-α-tocopherols do not have mathematically equivalent effects appears questionable because there is no evidence of clinically significant differences between *all-rac*- and *RRR*-α-tocopherols in benefits or risks in humans or animals. We hypothesize that a clinically significant difference between *all-rac*- and *RRR*-α-tocopherols is their interactions with prescription drugs. Saturable distribution and elimination predict that *all-rac*- and *RRR*-α-tocopherols interact with drugs eliminated by the same saturable pathways, and stereospecific elimination predicts that *all-rac*- and *RRR*-α-tocopherols interact differently with drugs. Although we are not aware of any reports proving clinically significant drug interactions with either *all-rac*- or *RRR*-α-tocopherol, we suggest that the question is not whether these interactions occur but whether these interactions are clinically significant.

Clinically significant drug interactions mediated by CYP3A4 and P-gp are common because CYP3A4 and P-gp are saturable, CYP3A4 metabolizes almost 30–40% of drugs and xenobiotic compounds, and CYP3A4 and P-gp overlap in substrate specificity [4,64,107,108]. The saturability of CYP3A4 and P-gp suggests that *all-rac*- and *RRR*-α-tocopherols might inhibit these enzymes. Inhibition of CYP3A4 activity or inhibition of P-gp activity in excretory tissues (intestines, liver, and kidneys) can increase plasma and tissue concentrations of drugs eliminated by CYP3A4 or P-gp. Thus, individuals who regularly take drugs eliminated by CYP3A4 and P-gp and suddenly begin taking pharmacologic dosages of α-tocopherol might have side effects caused by increased drug bioavailability. α-Tocopherol likely also induces increased activity of CYP3A4 and P-gp because tocopherols and tocotrienols activate the pregnane X receptor that coordinately regulates expression of both CYP3A4 and P-gp [78–80]. However, coordinate induction of CYP3A4 and P-gp causes different changes in bioavailability of different drugs because drugs vary in the relative proportion of their elimination mediated by CYP3A4 or P-gp [79].

Thus, long-term intake of α-tocopherol might inhibit and/or induce CYP3A4 and P-gp, and determining the short-term and long-term effects of *all-rac*- and *RRR*-α-tocopherols on the bioavailability and bioactivity of other drugs requires studying these interactions for each drug in each clinical setting. If the net long-term effect is increased activity of CYP3A4 and P-gp, this limits drug bioavailability in all tissues by increasing elimination (imcreasing metabolism by CYP3A4 in the liver and intestines, increasing drug excretion by the P-gp mdr1 into the intestinal lumen and urine, and increasing drug excretion by the P-gp mdr2 into the bile). This is clinically significant because the risk of severe adverse drug interactions may be greatest when patients abruptly stop taking the compounds that had induced more rapid elimination of other drugs, because decreased induction of elimination markedly increases drug bioavailability [4]. Moreover, whereas changes in CYP3A4 activity or changes in the P-gp activity in excretory tissues (liver, kidney, intestines) proportionately change drug concentrations in plasma and in most tissues, changes in P-gp activity in non-excretory tissues (brain, placenta, and testes) can change drug concentrations in those tissues in the absence of changes in plasma drug concentrations. For example, inhibiting P-gp increases drug transport across the blood-brain barrier [109–112] and placenta [113,114]. Thus, a compound that inhibits and induces CYP3A4 and P-gp activity might increase or decrease drug concentrations in the brain or fetus, depending on the relative dose-effect curves for inhibition and induction of CYP3A4, P-gp in excretory tissues, and P-gp in non-excretory tissues.

It is not possible to predict the relative concentration-effect curves and dose-effect curves of *all-rac*- and *RRR*-α-tocopherols for their interactions with any given drug because *all-rac*- and *RRR*-α-tocopherols are different chemical entities. In theory, *all-rac*-α-tocopherol might be more likely than *RRR*-α-tocopherol to inhibit or induce the elimination of drugs because TTP limits the availability of *RRR*-α-tocopherol to the pathways of elimination or because *all-rac*-α-tocopherol includes eight different stereoisomers that might cause interactions. However, in theory, *all-rac*-α-tocopherol might be less likely to inhibit or induce the elimination of drugs because for any given dosage, the concentrations of each stereoisomer in patients taking *all-rac*-α-tocopherol are lower than the concentrations of *RRR*-α-tocopherol in patients taking *RRR*-α-tocopherol. Moreover, for any given drug interaction, the relative concentration-effect curves for each stereoisomer might be such that interactions require higher or lower dosages of *all-rac*- than *RRR*-α-tocopherols. Drug interactions also occur by mechanisms other than inhibition or induction of elimination, and one cannot assume that *all-rac*- and *RRR*-α-tocopherols have the same relative potencies for any other possible drug interactions. Thus, the relative safety of *all-rac*- and *RRR*-α-tocopherols likely varies according to the clinical situation and other drugs taken concomitantly. Pending further research on interactions between drugs and α-tocopherol, we advise anyone taking prescription drugs to consult physicians before changing their dosage or type of α-tocopherol.

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