Eur J Nutr 39 : 183–193 (2000)

ORIGINAL CONTRIBUTION

P. P. Hoppe
G. Krennrich

Bioavailability and potency of natural-source and *all-racemic* α-tocopherol in the human: a dispute

Received: 17 April 2000
Accepted: 24 July 2000

P. P. Hoppe[a] (✉) · G. Krennrich[b]
Nutrition Research Station[a]
Scientific Computing[b]
BASF Aktiengesellschaft
76877 Offenbach, Germany
e-mail: peter-paul.hoppe@basf-ag.de

Summary Alpha-tocopherol occurs in nature as a single stereoisomer (RRR) while synthetic vitamin E is a mixture of eight stereoisomers (all-racemic, all-rac). The presently accepted ratio of biopotency (RRR: all-rac) is 1.36, based on the fetal resorption test in rats. This ratio has been disputed for humans. Clinical endpoint studies in humans are lacking, but plasma responses to RRR- and all-rac were measured in bioavailability studies. In nine studies comparing unlabeled forms, the ratio of plasma parameters (AUC, C_{max} or steady-state concentration) concurred with the accepted ratio of biopotency within accepted bounds of equivalence. Four recent studies with simultaneous application of trideutero-RRR and hexadeutero-all-rac resulted in ratios of up to 2 for plasma, and of ≈ 2.7 and ≈ 3.4 for α-CEHC (a urinary metabolite) and umbilical cord plasma, respectively. Because these results have been widely assumed to reflect the difference in biopotency, this has prompted a proposal to the Food and Nutrition Board, National Academy of Sciences, USA to change the biopotency factor to 2 : 1. We challenge the validity of bioavailability data in lieu of clinical endpoints. Because RRR and all-rac are not chemically identical and differ in plasma and tissue kinetics and metabolism, the ratio of bioavailability parameters does not reflect the ratio of biopotency. This needs to be determined in adequately designed studies using clinical and biochemical endpoints. Until such studies have been performed it does not appear prudent to exchange the presently accepted ratio based on valid bioassays, albeit in a model animal, for another that is based on erroneous conclusions from human studies.

Key words RRR-α-tocopherol – all-racemic α-tocopherol – bioavailability – human

List of abbreviations
RRR: natural-source stereoisomer; all-rac: all-racemic mixture of eight stereoisomers of synthetic α-tocopherol; α-TOH: α-tocopherol; α-TAc: α-tocopheryl acetate; AUC: area under the time-concentration curve; C_{max}: maximum concentration; $T_{1/2\beta}$: terminal elimination rate; LDL: low density lipoprotein.

Background and purpose

Vitamin E is the main physiologic lipid-soluble antioxidant. It is used for prevention and therapy of vitamin E deficiency in premature infants with hemolytic anemia and in low-birthweight infants fed a PUFA-fortified formula [1, 2]. Vitamin E deficiency also results from fat malabsorption in cystic fibrosis patients [3]. Because there is evidence that vitamin E can attenuate oxidative stress, sup-

European Journal of Nutrition, Vol. 39, Number 5 (2000)

plemental vitamin E is used for diseases associated with oxidative stress including the prevention of coronary heart disease, atherosclerosis, diabetes, cataracts, Parkinson's disease, Alzheimer's disease, impaired immune function, and in patients receiving total parenteral nutrition [1].

Vitamin E is the generic term for eight derivatives of 6-chromanol that differ in the site and degree of methylation at the chromanol ring and in the saturation of the side chain, respectively. Of the four tocopherols (α-, β-, γ-, and δ-) with a saturated phytyl side chain and the four respective tocotrienols with an unsaturated isoprenoid side chain, α-tocopherol (αTOH) has the highest vitamin E activity.

αTOH is obtained from several sources. First, directly from foods like vegetable oil where it occurs in association with a variable number of other tocopherols, notably γ-tocopherol, and tocotrienols. In nature, αTOH occurs as a single stereoisomer (Fig. 1) where the three chiral centers have R-configuration (2R, 4'R, 8'R, in short: RRR; formerly d-). Second, via preparations based on extraction of deodorizer destillate from vegetable oils by organic solvents and subsequent methylation and esterification to enhance αTOH yield and stability, respectively. These sources are also known as natural, although in the strict sense, the term applies only to the unaltered αTOH occuring in nature. The correct designation is natural-source vitamin E. Because the latter has RRR configuration it will be designated as natural in this review. Third, from synthetic αTOH produced by present chemical technology. It consists of eight stereoisomers (RRR, RRS, RSS, RSR, SSS, SRR, SSR, and SRS; Fig. 1) in equal proportions and is designated as all-racemic αTOH (all-rac-αTOH, earlier dl-αTOH). Both natural and synthetic vitamin E are mostly used as acetate esters, viz. RRR-αTAc and all-rac-αTAc.

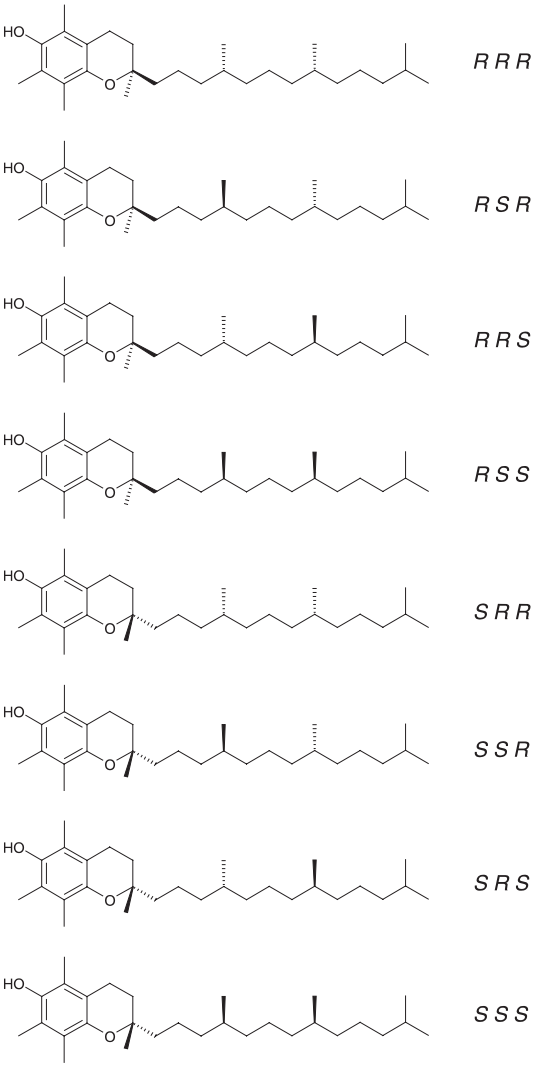

Fig. 1 Stereoisomers of all-racemic α-tocopherol.

The vitamin E activity of α-tocopherol is expressed in International Units (IU), where 1 IU is defined as 1 mg all-rac-αTAc [4]. Relative to all-rac-αTAc, 1 mg of the forms most frequently used has the following potencies: all-rac-α-tocopherol = 1.10 IU, RRR-α-tocopheryl acetate = 1.36 IU, and RRR-α-tocopherol = 1.49 IU. Thus, RRR and all-rac differ by a ratio of 1.36. This ratio was mainly derived from gestation-resorption assays in vitamin-E-depleted rats that determined the relative potency of either source in preventing the resorption (= death) of implanted embryos [5, 6]. Other bioassays were based on curing muscle dystrophy in rats (the plasma pyruvate kinase assay), and on the prevention of encephalomalacia in vitamin-E-deficient chickens. Titrated doses of either vitamin E source were fed and the ratio of potency calculated by dividing the slopes of the dose-response lines. Thus, biopotency was determined by clinical endpoints. It needs to be pointed out that this is the only valid method for assessing the comparative biopotency of substances that are not chemically identical.

For humans, bioassays measuring the potency of vitamin E forms are lacking, and this may be attributed to several reasons. (I) Healthy subjects with a plasma α-tocopherol concentration of ≈25 μmol/L cannot be used. (II) Vitamin E deficiency is so rare – it occurs only as a result of lipid malabsorption or genetic abnormalities in lipoprotein metabolism (1), but not from dietary deficiency [7] – that there are virtually no naturally depleted subjects available for testing. (III) Vitamin E depletion would require more than a year of ingesting a vitamin-E-deficient diet [46] and would be unethical. (IV) Vitamin E deficiency presents as subtle neurological symptoms including loss of sensitivity to vibration that do not lend themselves for measuring a dose-response, unlike fetal resorption, encephalomalacia and myopathy that can be prevented or cured by vitamin E in a dose-dependent way.

Because of the challenges in measuring biopotency of natural and synthetic vitamin E in humans, researchers have resorted to bioavailability studies. These included studies with single and repetitive doses and, more recently,

studies using deuterium-labeled forms. It has been concluded from the studies with labeled forms that the ratio of 1.36 (RRR : all-rac) does not represent the true difference in potency for the human species. Based on these studies, a proposal has recently been submitted to National Academy of Sciences that the ratio be changed to 2 : 1 [47]. The proposal is presently under evaluation by The Panel on Dietary Antioxidants and Related Compounds, Food and Nutrition Board**.** Thus, a critical and comprehensive review of studies in humans is timely.

MEDLINE (National Library of Medicine, Bethesda, MD) and the references in the papers identified were used to search for primary publications addressing in a comparative way the bioavailability of natural-source and synthetic vitamin E. Thirteen studies were identified [8–13, 15, 17–20, 38, 46].

Physiology of tocopherol

Reviews on the physiology and use of vitamin E in humans [1], function and metabolism [7], antioxidant activity, biokinetics and bioavailability [21] and on factors affecting bioavailability including a critical assessment of methodologies [22] have been published. The following is a brief account of the aspects relevant to bioavailability.

The absorption of tocopherols has been reviewed recently [22, 23, 24]. In the small intestine, tocopherols are incorporated into mixed micelles that diffuse across the unstirred water layer and are taken up by enterocytes. Tocopheryl esters are hydrolyzed prior to absorption. The rate of uptake is governed by passive diffusion. In the enterocyte, tocopherols are incorporated into chylomicrons that are secreted into lymph. Intracellular transport within the enterocyte does not appear to rely on a transfer protein. Studies measuring the plasma responses to differently labeled tocopherols have led to the hypothesis that absorption of RRR-αTOH, SRR-αTOH (one of the eight stereoisomers of all-rac-αTOH), and of γ-TOH occurs with roughly equal efficiencies [30]. However, this has not been substantiated by balance trials. Traber et al. concluded that the digestive tract has no detectable role in biodiscrimination between vitamin E forms. Enterohepatic circulation of α-tocopherol is thought to be negligible [7, 26].

In blood, αTOH is transported in plasma lipoproteins [27]. Chylomicrons secreted by the intestines are hydrolyzed by endothelial lipoprotein lipase resulting in transfer of some αTOH into endothelial cells. The resultant chylomicron remnants are recognized by the hepatic remnant receptor and taken up by the liver. In hepatic cells, tocopherols bind to cytosolic α-tocopherol transfer protein (αTTP) that mediates intracellular transfer from the point of entry to the site of VLDL synthesis at the RER/Golgi apparatus. Binding studies in vitro have shown that αTTP has variable affinity for tocopherol homologues and stereoisomers binding RRR-αTOH in preference to β-, γ-, and δ-tocopherols, α-tocotrienol, and SRR-αTOH, respectively [28, 29]. When cynomolgus monkeys were given equimolar doses of RRR- and SRR-αTOH – labeled with different amounts of deuterium – and sacrificed 24 h later, their livers secreted nascent VLDL preferentially enriched (> 80 %) with RRR-αTOH [30]. Patients with a genetic defect in αTTP have very low plasma concentrations and some of them have an impaired ability to discriminate between RRR- and SRR-αTOH. Taken together these findings indicate that the liver plays an important role in the discrimination between tocopherols.

The αTOH plasma pool is turned-over about once a day. Following a single dose, the peak plasma concentration is commonly found at about 12 h [13, 20, 41].

Bioavailability and potency

Bioavailability is defined as "the rate and extent of absorption of a drug". It is determined by analyzing the plasma response. In a single-dose study, the peak plasma concentration (C_{max}), the time to reach C_{max} (t_{max}), and the area under the plasma-concentration time curve (AUC) are determined. The latter is the most reliable parameter because it reflects the entire response over time in contrast to C_{max} which is a one-point measurement and, therefore, inherently more variable. In a multiple-dose study, the most relevant parameter is the concentration in the steady state. In order to reach the steady state, dosing is required for a minimum of five times the plasma half-life ($t_{1/2}$). In human studies with αTOH, $t_{1/2}$ was estimated as 3 days [13] and about 2 days [32], respectively. Hence, dosing should last at least 10–15 days in order to arrive at the steady state.

The bioavailability concept rests on a fundamental law of pharmacology that the magnitude of a drug effect (potency) is a function of its concentration at the site of action. Thus, bioavailability is a surrogate measure for potency. If a test and a reference preparation have the same bioavailability [33] (= are bioequivalent), it can be inferred that they also have the same potency. It is important to note that this inference is valid only if both preparations contain the identical active ingredient.

Criteria for accepting or refuting bioequivalence have been layed down recently as follows [33]. In comparing a test preparation with the standard preparation, the test preparation is regarded as (bio)equivalent when the 90 % confidence interval for the ratio of means (AUC or steady state concentration) falls within 80 % to 125 %. For C_{max}, wider confidence intervals (from 70 % to 134 %) have been suggested [34] because of its variability. These bounds were established in order to allow for the variation in response between subjects and because the clinical effects accruing from dosages within these bounds cannot be distinguished.

Potency is a measure of the biological effects exerted by an active ingredient. In humans, potency of vitamin E

forms has not yet been determined for the reasons outlined above. One assay in humans that may come close to a *functional* assay measures the ability of vitamin E to prevent plasma LDL oxidation *ex vivo* [12, 17].

In publications dealing with vitamin E the terms potency, biopotency and biological activity on the one hand and bioavailability on the other are frequently used interchangeably. This incorrect usage of terminology has resulted in false conclusions as shown below.

Single-dose studies

The earliest published report was based on studies by Horwitt in vitamin-E-depleted subjects given graded doses of all-rac-αTAc and RRR-αTAc [46]. The author hypothesized that all-rac-αTAc may have no more than half the *potency* (sic) of d-αTAc. Because this was essentially a dose-finding study in a few subjects without statistical analysis this suggestion cannot be verified by the data.

In a further study in humans, Horwitt et al. compared various sources (RRR-αTOH, RRR-αTAc, RRR-αTAc containing pectin, all-rac-αTAc, and RRR-α-tocopheryl succinate) in a cross-over study [15]. The preparations were given as a single dose of 800 IU and serum was analyzed at 0, 8, 24, and 48 h following the dose. The percentage increase at 24 h was reported to reflect the differences between preparations. Based on the relative increase at this timepoint, the authors reported that "dTAc was 2.62 times more *potent* than dl-TAc".

In a cross-over study with 24 male volunteers, Yoshikawa et al. [19] gave a single dose of 300 mg each of RRR- and all-rac-αTOH. Plasma responses were measured at 0, 2, 4, 6, 8, 10, 12, 24, 48, and 96 h. Significant differences were found at the timepoints 24 and 48 h. According to the authors "the findings suggest that the absorbability of d-alpha-tocopherol may be higher than that of dl-alpha-tocopherol".

Ferslew et al. compared the kinetics of natural and synthetic αTOH in plasma and erythrocytes following a single dose of 800 mg each [13]. Pharmacokinetic modelling of data showed that k_a and $t_{1/2\alpha}$ was 0.45 h^{-1} and 2.9 h, and β and $t_{1/2\beta}$ was 0.017 h^{-1} and 77 h, respectively, without differences between treatments. T_{max} occurred from 12 to 14 h after ingestion. The AUD_{0-96h} for RRR was 1.5-fold that for all-rac ($p < 0.05$). It was concluded that RRR had a greater bioavailability than all-rac, and that the difference in overall bioavailability was apparently not due to a single pharmacokinetic parameter.

Repetitive-dose studies

In all studies reported, pharmacological doses were used that are typically taken as nutritional supplements or for intervention purposes in patients at high risk [17]. According to Acuff et al. the vitamin E doses most commonly taken in the US are in descending order 400, 800, 200, and 100 IU/d [8]. Such doses result in a measurable plasma response whereas doses near the RDA (≈ 10 mg α-TOH equivalents) do not.

In a controlled, double-blind randomized study in humans, Baker et al. gave 800 IU/d (400 IU twice daily) as RRR- or all-rac-αTAc to two groups of 12 male adults for 28 days [9]. There were no significant differences in plasma αTOH between treatments. The authors concluded that "the results confirm the currently accepted *biopotencies* of 1 IU/mg and 1.36 IU/mg, respectively".

Kiyose et al. supplemented seven women with either 100 mg RRR-αTOH, 100 mg all-rac-αTAc or 300 mg all-rac-αTAc for three separate periods of 28 days, with washout periods of 3 months [16]. The objective of the study was to monitor stereoisomers of αTOH in serum and serum lipoprotein fractions by using a chiral HPLC method that discriminates between the 2R- and 2S isomers. The 2R-isomers resulted in a markedly higher response in plasma lipoproteins than the 2S-isomers.The authors reported that RRR-αTOH had a bioavailability almost three times that of all-rac-α-TAc based on the observation that 100 mg RRR-αTOH (equal to 149 IU) in period 1 resulted in almost the same final serum response as 300 mg all-rac-αTAc (equal to 300 IU) in period 3. However, there was an obvious seasonal effect on serum baseline levels. Objections against the authors' conclusions were raised in a letter to the editor [35], and in their reply, the authors admitted that "the data could not be used to accurately estimate the bioactivity of RRR-αTOH or of all-rac-αTAc" [36].

Winklhofer-Roob et al. carried out a long-term supplementation trial in cystic fibrosis patients to compare RRR-αTOH with two galenic forms of all-rac-αTAc, i. e., a fat-soluble and a water-miscible preparation [18]. Such patients suffer from pancreatic exocrine insufficiency, resulting in malabsorption of fats and fat-soluble vitamins. In the absence of supplementation using 2–10 times the RDA, vitamin E deficiency is invariably present in CF patients with pancreatic achylia [3]. Hence any difference between natural and synthetic vitamin E would be particularly relevant for CF patients. Three randomized parallel groups of ten patients were given 400 IU/day each for 6 weeks. No significant differences in plasma response were found between the preparations. It was concluded that by supplementing with 400 IU/day of any one of the vitamin E sources, plasma values of healthy controls can be achieved.

The susceptibility of plasma low-density lipoproteins (LDL) to Cu-induced oxidation *in vitro* depends on the concentrations of αTOH and other antioxidants. By measuring accumulation of αTOH in LDL and resistance of LDL to oxidation *in vitro,* this test measures bioavailability and also potency. Reaven et al. compared RRR-αTOH and all-rac-αTOH at a dose of 1600 mg/day, given as 800 mg twice daily, in two groups of eight subjects follow-

ing 8-week supplementation [17]. LDL oxidation was determined by measuring formation of conjugated dienes, lipid peroxides, thiobarbituric acid-reactive substances, and by macrophage degradation of LDL exposed to oxidation *in vitro*. From 4 weeks, αTOH in LDL increased significantly and to the same extent in both treatment groups. LDL oxidation also decreased to a similar extent in both groups compared with baseline. The study demonstrated that natural and synthetic α-tocopherol provided equal antioxidant protection. However, the power of detecting differences (test strength) of this test is low because of the low dose-response relationship and high variability of data.

A similar protocol was used by Devaraj et al. in a dose-titration study with RRR-αTOH and all-rac-αTOH [12]. In this randomized, placebo-controlled study, parallel groups of 20 subjects each were given both forms at doses of 100, 200, 400, and 800 IU/d for 8 weeks. Plasma α-tocopherol and Cu-induced LDL oxidation *in vitro* were determined. Plasma αTOH increased in a dose-dependent fashion in both groups without significant differences between the supplements at any dose (Fig. 2). Neither supplement affected the lag time of LDL oxidation at doses below 400 IU/day. At 400 and 800 IU/day both supplements resulted

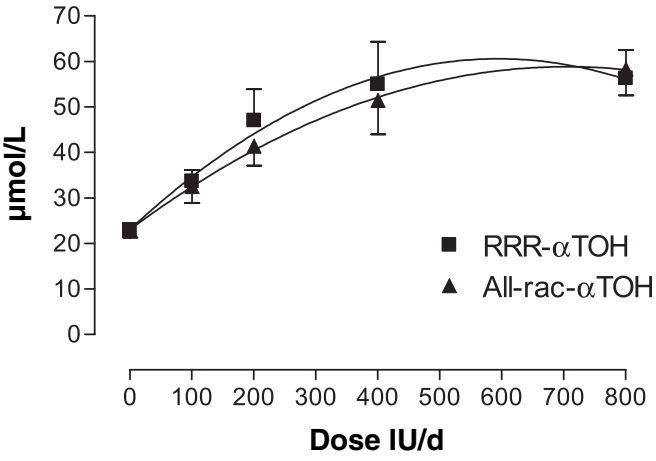

Fig. 2 Plasma responses to RRR-αTOH and all-rac-αTOH supplements in humans. Drawn from data of Devaraj et al. (1997).

Table 1 A complete list of published studies comparing the bioavailability of RRR- and all-rac-α-tocopherol in humans

Authors	Subjects per treatment	Treatments		Duration of dosing (d)	Ratio[a] RRR : all-rac (based on weight)	Parameter
		Dose per day	Preparation			
Horwitt et al. [15]	20	800 IU	RRR-αTAc	single dose		
		800 IU	all-rac-αTAc		2.62	plasma at 24 h
Yoshikawa et al. [19]	24	300 mg	RRR-αTOH	single dose	1.23	C_{max}
		300 mg	all-rac-αTOH		1.33	AUC, plasma
Ferslew et al. [13]	12	800 mg	RRR-αTOH	single dose	*1.52*	AUC, plasma
		800 mg	all-rac-αTOH		*0.98*	C_{max}, plasma
					1.23[b]	AUC, red blood cells
					1.2	C_{max}, red blood cells
Baker et al. [9]	24	800 IU		28	1.36	plasma steady state
		800 IU				
Devaraj et al. [12]	20	100 IU	each dose as	56	1.36	plasma steady state
		200 IU	RRR-αTOH		1.36	protection from LDL
		400 IU	and as			oxidation
		800 IU	all-rac-αTOH			
Kiyose et al. [16]	7	100 mg	RRR-αTOH	3 periods of	[c]	
		100 mg	all-rac-αTAc	28 d		
		300 mg	all-rac-αTAc			
Reaven & Witztum [17]	8	1600 mg	RRR-αTOH	56	< 1.36	LDL αTOH
		1600 mg	all-rac-αTOH			protection from LDL oxidation
Winklhofer-Roob et al. [18]	10 (9)	400 IU	RRR-αTOH	42	1.36	plasma steady state
		400 IU	all-rac-αTAc[d]			
		400 IU	all-rac-αTAc[e]			
Chopra & Bhagavan [11]	12	800 IU	RRR-αTAc	10	1.36	plasma steady state
		800 IU	all-rac-αTAc			

[a] ratios calculated from authors' data are printed in *italics*
[b] calculated from Table 2, Ref. [13]
[c] no valid interpretation possible
[d] as fat-soluble formulation
[e] as water-soluble formulation

in significant prolongation of the lag phase. Thus, the study demonstrated that bioavailability and potency of RRR and all-rac were the same.

A further repetitive dose study comparing, *inter alia*, RRR-αTAc and all-rac-αTAc was published by Chopra & Bhagavan [11]. In this randomized, double-blind study, two parallel groups of healthy volunteers received 800 IU/day, given as 400 IU twice daily, for 10 days. Serum αTOH did not differ significantly between groups at any sampling date from day 0 through 10. The authors concluded that the "data support the currently accepted ratio of 1.36 for the biopotency of RRR- vs. all-rac-αTAc".

An overview of the results is presented in Table 1. It shows that in all studies but one [15] the ratio (RRR : all-rac) of bioavailability parameters was reasonably close to 1.36.

Studies using deuterium labeled preparations

The recent synthesis of stable-isotope labeled αTOH by Burton and collaborators has been a milestone in vitamin E research [37]. d_3-RRR-αTOH was obtained by introducing CD_3 into RRR-γ-tocopherol at carbon 5 of the chroman ring and D_6-all-rac-αTOH was obtained by inserting two CD_3 groups in positions C5 and C7 of the chroman ring. Deuterated forms were used to investigate kinetics in plasma, erythrocytes and tissues [13], tissue accretion, depletion and turnover [39], hepatic VLDL secretion [7, 24, 30], metabolic breakdown to α-CEHC [20], and finally, biodiscrimination between tocopherol homologues and αTOH-stereoisomers [16, 21, 22]. Labeled forms are particularly useful for bioavailability studies. (I) They can be given simultaneously to the same individual, according to the competitive uptake method. (II) Physiologic dosages from about 15 mg may be used. (III) The sensitivity of the HPLC-MS analytical method is high [40]. (IV) Because all covariates, whether related to host (digestive physiology, plasma lipid profile, compliance), diet (e. g., fat) or analysis (recovery) affect both isotopomers in the same way, there are virtually no confounders. (V) Fewer subjects are required than for a conventional study and there is no need for randomization and cross-over.

In the studies reviewed below, a mixture (1 : 1, by wt) of d_3-RRR-αTAc and d_6-all-rac-αTAc from one single source was given simultaneously. Plasma isotopomers were analyzed by GC/MS.

Acuff et al. supplemented healthy adults with the isomeric mixture (300 mg) for 11 consecutive days [8]. Blood was sampled daily during the application period (day 1–11) and thereafter until day 137. Red blood cells and plasma were analyzed for endogenous d_0-αTOH, and for d_3- and d_6-isotopomers. The authors reported a plasma ratio (d_3 : d_6) of 2, based on the AUC over the entire study from day 1–day 137 ($AUC_{0–137d}$). This is challenged because the ratio was not obtained during the steady state. By calculating the AUC for plasma and red blood cells from the authors' data during what appears to be the steady state (day 5–10), ratios of 1.78 and 1.87, respectively, are obtained (Table 2).

Newborn infants have only about one-fifth the plasma αTOH concentration of their mothers possibly indicating low maternal-fetal transfer. Plasma responses to natural and synthetic vitamin E have not yet been compared in mothers and newborns. In a study with pregnant women 5 days prior to delivery, Acuff et al. gave the d_3-RRR- and d_6-all-rac-mixture (1 : 1, by wt) as a total daily dose of 15, 30, 75, 150, or 300 mg until delivery [38]. The responses above baseline in maternal plasma, in plasma lipoprotein fractions, and in umbilical cord plasma were measured on the day of delivery. The d_3 : d_6 ratio in plasma of the mothers averaged 1.86 (± 0.10), ranging from 1.77 to 2.02, and it ranged from 3.35 to 3.55 in cord plasma. The results appear to indicate that the placental-fetal unit, the fetal liver, or both further discriminate between the RRR- and all-rac-forms. However, caution is advocated in interpretation because the ratio was not obtained in the steady state and because of the very low concentrations in the newborns that may limit the analytical accuracy.

Single-dose and repetitive-dose studies

A series of studies measuring plasma and tissue responses to single or repetitive doses of the isomeric mixture (d_3-RRR-αTAc and d_6-all-rac-αTAc) given simultaneously was carried out by Burton et al. [10]. Healthy volunteers, patients awaiting elective surgery [e. g., hernia or gall-bladder disease] and two terminally ill patients were used.

Two groups of five adults consumed 30 mg as a single dose and one month later, 30 mg/day on eight consecutive days, respectively, with the evening meal. The same procedure was followed one month later with a single or eight consecutive 300-mg doses. Plasma d_0, d_3 and d_6-αTOH were analyzed for 5 and > 500 days following the single and eight repetitive doses, respectively.

In the single dose studies plasma concentrations were significantly greater for RRR than for all-rac at all times. The ratio of RRR : all-rac was significantly greater than 1.36, increasing from 1.63 on the first morning after the dose to 1.96 by day 4 (30 mg) and from 1.57 on the first morning after dosing to 1.97 by day 4 (300 mg).

In the study with eight times 30 mg, ingestion of the labeled αTOH resulted in depression of unlabeled αTOH thereby dampening the effect on total (labeled and unlabeled) αTOH. The d_3 : d_6-ratio increased from a mean of 1.48 on the first morning to 1.9 on day 5. In the study with eight times 300 mg the ratio was constant at 1.51 during the application period. On termination of dosing it increased sharply to ≈ 2. The authors concluded that natural vitamin E has roughly twice the availability of synthetic.

Table 2 Studies using simultaneous application of d_3-RRR-αTAc and d_6-all-rac-αTAc in humans

Authors (Ref. No)	Number of subjects per treatment	d3-RRR and d6-all-rac (1 : 1 by wt) mg/day	Duration of dosing (days)	Bioavailability ratio[a] RRR: all-rac (mg basis)	Parameter
Acuff et al. [8]	6	300	11	*1.78*[b]	plasma steady state
				1.87[c]	red blood cells
Acuff et al. [38]	3[d]	15, 30, 75, 150 or 300	5	1.86 ± 0.10	maternal plasma on day 5
				3.42 ± 0.03	umbilical cord plasma
Burton et al. [10]	5	30	single dose	1.67	presumed plasma C_{max}
	5	300	single dose	1.62	presumed plasma C_{max}
	5	100[e]	single dose	*1.65*	presumed plasma C_{max}
	5	30	8	*1.48 ± 0.25*	presumed plasma C_{max}
				1.9 ± 0.1	plasma steady state
	5	300	8	1.51 ± 0.11	plasma steady state
Burton et al. [10]	2[f]	150	3	[1.56 ± 0.02][g]	plasma on day 3
	6[f]	150	8	1.79 ± 0.06	plasma steady state
	5[f]	150	14	1.73 ± 0.10	plasma steady state
	4[f]	150	21	1.74 ± 0.02	plasma steady state
	4[f]	150	29	1.83 ± 0.13	plasma steady state
	1[f]	150	41	*1.77*	plasma steady state
Burton et al. [10]	19[f]	150	19 ± 10	*1.57*	adipose tissue
	8[f]	150	10 ± 5	*1.21*	muscle
	6[f]	150	13 ± 8	*1.54*	skin
	3[f]	150	23 ± 10	*1.38*	vein
	3[f]	150	4 ± 2	*1.5*	nerve
	3[f]	150	9 ± 3	*1.72*	gallbladder
	1[f]	150	7	*0.91*	liver
Burton et al. [10]	1[h]	30	361	1.71 ± 0.27	average from 25 tissues
				2.06	plasma at autopsy
	1[h]	300	615	2.01 ± 0.17	average from 27 tissues
				2.11	plasma at autopsy
Traber et al. [20]	6	300	single dose	1.8	plasma C_{max}
				≈ 2.7	urinary α-CEHC

[a] ratios calculated from authors' data are printed in *italics*
[b] calculated from Table 1 (Ref. 8) for day 5 to 10; authors reported ratio of 2.0
[c] calculated from Table 1 (Ref. 8) for day 5 to 10
[d] pregnant women 5 days from delivery
[e] d_6-RRR and d_3-all-rac (1 : 1)
[f] elective surgery patients
[g] unsuitable for interpretation because of too short duration of dosing
[h] terminally ill patient

Repetitive dose study in elective surgery patients [10]

Supplementation of six groups of healthy adults with 150 mg/day of the isotopic mixture for a mean of 3, 8, 14, 21, 29, or 41 days prior to surgery (see Table 2) resulted in plasma ratios ranging from 1.56 to 1.83. These ratios probably reflected the plasma steady state except for groups 1 and 2 who took supplements for only 3 and 8 days, respectively. In adipose tissue, muscle, skin, vein, nerve, gallbladder, and liver, mean ratios ranged from 0.91 for liver to 1.72 for gallbladder. It is unknown if these ratios reflect the tissue steady state, because equilibration in tissues normally occurs later than in plasma. Therefore, interpretation is open to question.

Long-time application in terminally ill patients

Two critically ill patients were supplemented for ≈ 1 or ≈ 2 years with 30 and 300 mg of the isotopic mixture, respectively [10]. RRR : all-rac ratios were analyzed in a total of 26 tissues taken at autopsy. In the patient given 30 mg/day, tissue ratios ranged from 0.87 (optic nerve) to 2.16 (lumbar spinal cord) averaging 1.71 ± 0.24, and the ratio in plasma was 2.06. In the patient on 300 mg/d, tissue ratios ranged from 1.55 (head of pancreas) to 2.21 (cerebellum) averaging 2.01 ± 0.17, and the ratio in plasma was 2.11.

D_6-RRR- vs. d_3-all-rac

A further group of five subjects were given a single 100-mg dose of the 1 : 1 mixture where the deuterium labeling was reversed [10]. Plasma was analyzed in the morning of day 1 (presumptive C_{max}) and daily until day 5. The mean plasma ratio on days 1–5 was 1.65, 1.82, 1.94, 2.07, and 1.99, respectively (G. Burton, personal communication). The result was the same as for d_3-RRR- and d_6-all-rac indicating that the degree of deuteration does not contribute in any significant way to the observed discrimination between RRR- and all-rac-α-tocopherol.

The authors conclude from these studies that "natural vitamin E has roughly twice the availability of synthetic vitamin E".

Tetramethyl-carboxyethyl-hydroxychroman (α-CEHC) is a metabolite of αTOH that originates from truncation of the phytyl tail of tocopherols and is excreted in urine [31]. It is of interest because it may serve as an indicator of adequate or excess vitamin E supply [31]. Traber et al. investigated if the rate of metabolic breakdown to α-CEHC differs for natural and synthetic vitamin E by giving a single total dose of 300 mg (634 μM) d_3-RRR-αTAc and d_6-all-rac-αTAc (1 : 1, by wt) and monitoring urinary α-d_3-CEHC and α-d_6-CEHC [20]. Urine was collected quantitatively (24 h) prior to and 0, 1, 2, 3, 4, and 8 days after the dose and plasma during the day of dosing, respectively. The plasma $d_3 : d_6$ ratio at C_{max} was 1.7. In urine, unlabeled α-CEHC from diet and body stores increased from 1 μM (day 0) to a maximum of 1.6 μM on day 2 and decreased thereafter. Urinary excretion of d_3-α-CEHC and d_6-α-CEHC was < 0.3 μM/day and < 0.7 μM/day, respectively, accounting for less than 1 molar % of the dose. Thus, it appears that newly ingested tocopherols are metabolized at a markedly lower rate than tocopherols from body stores and plasma.The ratio of total urinary $d_6 : d_3$ α-CEHC excretion was ≈ 2.7. The study indicates that biodiscrimination between natural and synthetic vitamin E is not entirely due to hepatic α-TTP but also to metabolic breakdown to α-CEHC. However, in view of the preference for "old" tocopherols and the minor fraction of the new dose that is metabolized, the value of α-CEHC for assessing bioavailability appears to be limited.

A summary of the $d_3 : d_6$ ratios reported by the authors and calculated from the authors' data is given in Table 2.

Discussion

In the eight conventional studies conducted with unlabeled materials (Table 1) the ratios calculated for varying pharmacokinetic parameters range from 0.98 to 2.62. The latter factor was reported by Horwitt et al. [15] based on the plasma increase after 24 h that was considered "representative and possibly most important". This timepoint is no accepted bioavailability parameter because it is well beyond C_{max}. The authors failed to calculate the $AUC_{0–48h}$ that would have reflected the difference more adequately. The majority of ratios found in these studies is clustered around 1.36. Nonetheless, several studies concluded that the natural-source of vitamin E was more potent than indicated by the factor of 1.36 [13–16, 19].

The studies with deuterated forms indicate higher ratios than the conventional studies (Table 2). This is partly because some authors derived the ratio from timepoints other than layed down by FDA [33] and because bioequivalence was generally refuted on the basis of the null hypothesis. If the FDA-ruling is applied, the ratio for plasma found by Acuff et al. is marginally above the upper bounds of equivalence of 1.70.

In the studies by Burton et al. [10] all but one of the ratios calculated for the presumptive C_{max} in the healthy subjects indicate agreement with the accepted ratio within the bounds of equivalence. The ratio of ≈ 1.8 found in the plasma steady state of elective surgery patients is marginally above the upper bounds. The study with chronic application is based on two subjects only.

The ratio reported for umbilical cord blood is difficult to interpret because the duration of supplementation was short and the concentrations appear to be near the level of determination [38].

The use of urinary α-CEHC to determine bioavailability is an intriguing proposition, because measuring a metabolite in lieu of the parent compound is an acceptable approach. However, because less than 1 % of the oral dose appeared in urine and because tocopherols of dietary and body origin are metabolized in preference to newly ingested preparations, the value of α-CEHC excretion for assessing bioavailability appears to be limited.

Cohn pointed out that the competitive uptake method results in bias against all-rac, because when equal weight doses of RRR and all-rac are given, the total dose contains 50 % d_3-2R, 25 % d_6-2R, and 25 % d_6-2S [42]. Thus, 75 % of the R-form compete with 25 % of the S-form. Because of the higher abundance of the R-form and because it is preferred by αTTP in liver and possibly by metabolism [20], this results in overestimation of the RRR-form and discrimination against all-rac. In contrast, if the two compounds are given separately (in a study with parallel groups), no such discrimination occurs.

In several publications, the *maximum* ratio observed was pointed out [8, 10, 20]. The maximum ratio is no accepted measure of the difference because it is by definition the upper end of the range rather than the mean. Moreover, the maximum ratio was not found at t_{max} or during the steady state but in the elimination phase after withdrawal of supplementation. For these reasons it is of no consequence.

The most serious criticism relates to the interpretation of the results. Although the studies were devised to investigate bioavailability, namely the differential plasma kinetics of RRR and all-rac, the results are often interpreted

as reflecting potency. This was claimed explicitly by several authors of original papers and in their wake, by Natural Source Vitamin E Association. This interpretation is incorrect because potency needs to be derived from bioassays measuring functional parameters. As a substitute method for potency, bioavailability studies may be used only if the two products compared contain the identical active ingredient.

Natural and synthetic vitamin E are not identical. Comparing the plasma response to a single chemical entity (RRR) with a blend of eight entities including RRR (all-rac) is tantamount to comparing apples with fruit (including apples). The question is: which amount of fruit is required to elicit the same biological effect as 1 kg of apples? It is obvious that this cannot be answered by measuring fruit and apple concentrations in any body compartment. Likewise, it is not permissible to determine the potency of RRR relative to all-rac by measuring plasma or tissue concentrations. This criticism also applies to studies using the competitive uptake method. It has undisputed value for comparing plasma kinetics, but is useless for estimating potency.

RRR and all-rac differ in chemical composition and hence, in pharmacokinetics. Ferslew et al. reported that the difference in bioavailability was apparently due to more than one pharmacokinetic parameter [13]. This is also evident from the work of Burton et al. [10] who found that the plasma ratio was around 1.5 during dosing and started to increase immediately after cessation of dosing (Fig. 3, Plot F; Burton et al. [10]) to reach a final value of about 2. This indicates a faster elimination rate of (some enatiomers of) all-rac. Because the ratio varies depending on the timepoint, determination is not unequivocal but arbitrary. In contrast, if two differently labeled preparations containing the identical active ingredient in the same galenic formulation are ingested simultaneously, the ratio is constant across all timepoints and the result is unmistakable.

We have reinvestigated the data from Burton et al. [10] (kindly provided by Dr. M. Traber) and Acuff et al. [8] to make some of the above arguments more explicit. In order to estimate the yet unknown steady-state concentrations from the data we have modelled Burton's 8*30 mg and 8*300 mg data and Acuff's 11*300 mg assay with a biexponential model,

$$0\,c(t) = \begin{cases} a_0 \cdot \left(1 - e^{-a_1 \cdot time}\right) & ; time \le TIME_{MAX} \\ k_0 \cdot e^{-k_e \cdot time} & ; time > TIME_{MAX} \end{cases}$$

where a_0 is the steady-state concentration after infinite dosing time, a_1 the absorption rate (d^{-1}), and k_e the elimination rate (d^{-1}). The biexponential "broken-line" model describes the data appropriately.

Based on the individual estimates for a_0 from the data of Burton et al. [10], a ratio (RRR : all-rac) of 1.99 ± 0.06 (N = 5, ±SEM) is calculated for the 8*30 mg dosing regime while a ratio of 1.51 ± 0.05 (N = 5, ±SEM) is estimated for the 8*300 mg assay ($p < 0.05$). In the same way the ratio calculated from the data of Acuff et al. (11*300 mg) is 1.78 ± 0.04 (N = 6). It is obvious that the ratios differ significantly depending on the dose, indicating lower biodiscrimination at high doses and higher discrimination at low doses. Thus, the ratio varies not only with the timepoint but also with the dose.

Based on the cross-over study of Ferslew et al. [13] that first showed the differences in pharmacokinetics, the studies with labeled materials [10, 40] and our kinetic modelling, respectively, there is compelling evidence that RRR and all-rac differ in plasma and tissue accretion, elimination, and metabolism [22]. In view of the chemical difference, this is not unexpected. It follows that a bioavailability study is no adequate substitute for a potency study and that potency needs to be re-assessed by an appropriate and validated method. The necessity to measure the functionality *in vivo* was also advocated by Acuff [43].

What is the challenge? We need studies aimed at measuring the potency of vitamin E in humans *in vivo* or *ex vivo*. For the reasons given in the chapter on bioavailability and potency, this is a great challenge. Resistance of LDL to oxidation *in vitro* subsequent to loading with vitamin E *in vivo* does not appear promising because pharmacological doses are required for significant enrichment of LDL and prolongation of lag time. It does not appear likely that the sensitivity of this test can be improved such that minor differences in potency between RRR and all-rac can be detected.

Novel biomarkers reflecting vitamin E functions *in vivo* need to be looked for. They may be based on the antioxidant function that modulates the life span of red blood cells and susceptibility to peroxide-induced hemolysis. They may also lie beyond the antioxidant function as pointed out by Brigelius-Flohe and Traber [7] relying on the function in cellular signaling, particularly in smooth muscle cell proliferation and protein kinase C activity [44, 45]. Much needs to be done in this new field, and this aspect cannot be covered here because it would exceed the scope of this review. The most appropriate way of adressing the challenge would be an interaction of experts in the fields of tocopherol function and metabolism, measurement of oxidative stress, bioassay methodology, and related disciplines. This will hopefully result in delineating valid methods for determining the potency of vitamin E forms in humans. In the meantime it appears prudent not to exchange the accepted potency ratio that is based on sound methodology in model animals for another that is based on erroneous inference from studies in humans.*

* During revision of the manuscript the Report on Dietary Reference Intakes has been published [48]. The Panel on Antioxidant Nutrients and Related Compounds concluded that the activity of all-rac-α-TOH relative to RRR-α-TOH is 50%.

References

1. Traber MG, Sies H (1996) Vitamin E in humans: demand and delivery. Annu Rev Nutr 16: 321–47
2. Koletzko B, Desci T, Sawatzki G (1995) Vitamin E status of low birthweight infants fed formula enriched with long-chain polyunsaturated fatty acids. Int J Vitam Nutr Res 65: 101–41
3. Farrell PM (1980) Deficiency states, pharmacological effects, and nutrient requirements. In: Machlin L (ed) Vitamin E. A comprehensive treatise. Marcel Dekker Inc, pp 520–620
4. USP. 21st revision (1985) Rockville, MD: United States Pharmacopoeal Convention Inc
5. Weiser H, Vecchi M (1981) Stereoisomers of α-tocopheryl acetate. I. Characterization of the samples by physicochemical methods and determination of biological activities in the rat resorption-gestation test. Int J Vit Nutr Res 51: 100–113
6. Weiser H, Vecchi M (1982) Stereoisomers of α-tocopheryl acetate. II. Biopotencies of all eight stereoisomers, individually or in mixtures, as determined by rat resorption-gestation tests. Int J Vit Nutr Res 52: 351–370
7. Brigelius-Flohé R, Traber MG (1999) Vitamin E: function and metabolism. FASEB J 13: 1145–1155
8. Acuff RV, Thedford SS, Hidiroglou NN, Papas AM, Odom TA (1994) Relative bioavailability of RRR- and all-rac-alpha-tocopheryl acetate in humans: studies using deuterated compounds. Am J Clin Nutr 60: 397–402
9. Baker H, Handelman GJ, Short S, Machlin LJ, Bhagavan HN, Dratz EA, Frank O (1986) Comparison of plasma α- and γ-tocopherol levels following chronic oral administration of either all-rac-α-tocopheryl or RRR-α-tocopheryl acetate in normal adult male subjects. Am J Clin Nutr 43: 382–387
10. Burton GW, Traber MG, Acuff RV, Walters DN, Kayden H, Hughes L, Ingold KU (1998) Human plasma and tissue alpha-tocopherol concentrations in response to supplementation with deuterated natural and synthetic vitamin E. Am J Clin Nutr 67: 669–684
11. Chopra RK, Bhagavan HN (1999) Relative bioavailabilities of natural and synthetic vitamin E formulations containing mixed tocopherols in human subjects. Int J Vit Nutr Res 69(2): 92–95
12. Devaraj S, Adams-Huet B, Fuller CJ, Jialal I (1997) Dose-response comparison of RRR-alpha-tocopherol and all-racemic alpha-tocopherol on LDL oxidation. Arterioscler Thromb Vasc Biol 17:2273–2279
13. Ferslew KE, Acuff RV, Daigneault EA, Woolley TW, Stanton PE (1993) Pharmacokinetics and bioavailability of the RRR and all racemic stereoisomers of alpha-tocopherol in humans after single oral administration. J Clin Pharmacol 33(1): 84–88
14. Horwitt MK (1980) Relative biological values of d-a-tocopheryl acetate and all-rac-α-tocopheryl acetate in man. Am J Clin Nutr 33: 1856–1860
15. Horwitt MK, Elliott WH, Kanjananggulpan P, Fitch CD (1984) Serum concentrations of α-tocopherol after ingestion of various vitamin E preparations. Am J Clin Nutr 40: 240–245
16. Kiyose C, Muramatsu R, Kameyama Y, Ueda T, Igarashi O (1997) Biodiscrimination of α-tocopherol stereoisomers in humans after oral administration. Am J Clin Nutr 65: 785–789
17. Reaven PD, Witztum JL (1993) Comparison of supplementation of RRR-alpha-tocopherol and racemic alpha-tocopherol in humans: effects on lipid levels and lipoprotein susceptibility to oxidation. Arterioscler Thromb 13: 601–608
18. Winklhofer-Roob BM, van't Hof MA, Shmerling DH (1996) Long-term oral vitamin E supplementation in cystic fibrosis patients: RRR-α-tocopherol compared with all-rac-α-tocopheryl acetate preparations. Am J Clin Nutr 63, pp 722–728
19. Yoshikawa T, Miyagawa H, Takemura T, Oyamada H, Itani K, Tainaka K, Sugino S, Kondo M (1988) Absorption of D- and DL-α-tocopherol in healthy human volunteers. In: Hayaishi O, Niki E, Kondo M, Yoshikawa T (eds) Proc. 4th Biennal Meeting, Society of Free Radical Research, Kyoto, Japan, 9–13 April, pp 295–298
20. Traber MG, Elsner A, Brigelius-Flohé R (1998) Synthetic as compared with natural vitamin E is preferentially excreted as α-CEHC in human urine: studies using deuterated α-tocopheryl acetates. FEBS Letters 437: 145–148
21. Burton GW, Traber MG (1990) Vitamin E: antioxidant activity, biokinetics, and bioavailability. Ann Rev Nutr 10: 357–82
22. Cohn W (1997) Bioavailability of vitamin E. Eur J Clin Nutr 51: S80-S85
23. Cohn W, Gross P, Grun H, Loechleiter F, Muller DP, Zulauf M (1992) Tocopherol transport and absorption. Proc Nutr Soc 51: 179–188
24. Kayden HJ, Traber MG (1993) Absorption, lipoprotein transport and regulation of plasma concentrations of vitamin E in humans. J Lipid Res 34: 343–358
25. Traber MG, Sokol RJ, Kohlschütter A, Yokota T, Muller DPR, Dufour R, Kayden HJ (1993) Impaired discrimination between stereoisomers of α-tocopherol in patients with familial isolated vitamin E deficiency. J Lipid Res 34: 201–210
26. Traber MG, Burton GW, Hughes L, Ingold KU, Hidaka H, Malloy M, Kane J, Hyams J, Kayden HJ (1992) Discrimination between forms of vitamin E by humans with and without genetic abnormalities of lipoprotein metabolism. J Lipid Res 33: 1171–1182
27. Traber MG (1997) Regulation of human plasma vitamin E. Adv Pharmacol 38: 49–63
28. Sato Y, Hagiwara K, Arai H, Inoue K (1991) Purification and characterization of the α-tocopherol transfer protein from rat liver. FEBS Lett 228: 41–45
29. Hosomi A, Arita M, Sato Y, Kiyose C, Ueda T, Igarashi O, Arai H, Inoue K (1997) Affinity for alpha-tocopherol transfer protein as a determinant of the biological activities of vitamin E analogs. FEBS Lett 409: 105–108
30. Traber MG, Rudell LL, Burton GW, Hughes L, Ingold KU, Kayden HJ (1990) Nascent VLDL from liver perfusions of cynomolgus monkeys are preferentially enriched in RRR-compared with SRR-α-tocopherol; studies using deuterated tocopherols. J Lipid Res 31: 687–94
31. Schultz M, Leist M, Petrzika M, Gassmann B, Brigelius-Flohé R (1995) Novel urinary metabolite of α-tocopherol, 2,5,7,8-tetramethyl–2(2'carboxyethyl)–6-hydroxychroman, as an indicator of an adequate vitamin E supply? Am J Clin Nutr 62: 1527S–34S
32. Traber MG, Ramakrishnan R, Kayden HJ (1994) Human plasma vitamin E kinetics demonstrate rapid recycling of plasma RRR-alpha-tocopherol. Proc Natl Acad Sci USA 91: 10005–10008
33. U.S. Department of Health and Human Services, Food and Drug Administration (1997) Guidance for Industry. In vivo bioequivalence studies based on population and individual bioequivalence approaches. Draft guidance, October
34. Rauws AG (1993) Can we fix norms for C_{max} confidence intervals? In: Midha KK, Blume HH (eds) BioInternational. Bioavailability, Bioequivalence and Pharmacokinetics. Scient Publ Medpharm, Stuttgart, pp 133–40
35. Kormann AW, Bachmann H (1998) Biodiscrimination of α-tocopherol stereoisomers in humans after oral administration. Letter to the Editor. Am J Clin Nutr 67: 350–351
36. Kiyose C, Igarashi O (1998) Reply to Kormann AW, Bachmann H. Am J Clin Nutr 67: 351

37. Burton GW, Ingold KU, Cheeseman KH, Slater T F (1990) Application of deuterated α-tocopherols to the biokinetics and bioavailability of vitamin E. Free Rad Res Comms 11: 99–107
38. Acuff RV, Dunworth RG, Webb LW, Lane JR (1998) Transport of deuterium-labeled tocopherols during pregnancy. Am J Clin Nutr 67: 459–464
39. Ingold KU, Burton GW, Foster DO, Hughes E, Lindsay DA, Webb A (1987) Biokinetics of and discrimination between dietary RRR- and SRR-a-tocopherols in the male rat. Lipids 22: 163–172
40. Traber MG, Ingold KU, Burton GW, Kayden HJ (1988) Absorption and transport of deuterium-substituted 2R,4'R,8'R-α-tocopherol in human lipoproteins. Lipids 23: 791–797
41. Cheeseman KH, Holley AE, Kelly FJ, Wasil M, Hughes L, Burton G (1995) Biokinetics in humans of RRR-α-tocopherol: the free phenol, acetate ester, and succinate ester forms of vitamin E. Free Rad Biol Med 19: 591–598
42. Cohn W (1999) Evaluation of vitamin E potency. Letter to the Editor. Am J Clin Nutr 69: 157
43. Acuff RV (1998) Vitamin E. Bioavailability and function of natural and synthetic forms. Am J Nat Med 5: Nov.
44. Azzi A, Aratriu E, Boscoboinik D, Clement S, Ozer NK, Ricciarelli R, Spycher S (1998) Molecular basis of alpha-tocopherol control of smooth muscle cell proliferation. Biofactors 7: 3–14
45. Boscoboinik D, Szewczyk A, Hensey C, Azzi A (1991) Inhibition of cell proliferation by alpha-tocopherol. Role of protein kinase C. J Biol Chem 266: 6188–6194
46. Horwitt MK (1960) Vitamin E and lipid metabolism in man. Am J Clin Nutr 8: 451–461
47. Natural-Source Vitamin E Association (1998) Proposal to change biopotency values for natural vitamin E. October 22
48. Dietary Referene Intakes for vitamin C, vitamin E, selenium and carotenoids. Report of the Panel on Dietary Antioxidants and Related Compounds, Food and Nutrition Board, Institute of Medicine. National Academy Press, Washington, DC, USA, 2000