Vitamin E bioavailability from fortified breakfast cereal is greater than that from encapsulated supplements[1–3]

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ABSTRACT

Background: Conflicting results from vitamin E intervention studies suggest supplemental vitamin E malabsorption.

Objective: We compared vitamin E bioavailability from a supplement with that from a fortified breakfast cereal.

Design: Vitamin E bioavailability was evaluated by using deuterium-labeled *all-rac*-α-tocopherol in three 4-d trials (2 wk apart). Five fasting subjects sequentially consumed the following (with 236 mL fat-free milk): 400 IU d_9-α-tocopheryl acetate (400-IU capsule), 41 g ready-to-eat wheat cereal containing 30 IU d_9-α-tocopheryl acetate (30-IU cereal), and 45 g cereal containing 400 IU d_9-α-tocopheryl acetate (400-IU cereal). Five months later (trial 4), they consumed a 400-IU capsule with 41 g vitamin E–free cereal. Blood was obtained up to 72 h after the start of each trial.

Results: The mean (±SD) vitamin E bioavailabilities of the 30-IU cereal and the 400-IU cereal were 6 ± 2 and 26 ± 8 times, respectively, the vitamin E bioavailability of the 400-IU capsule. The areas under the 0–72-h d_9-α-tocopherol curves for the 400-IU capsule, the 30-IU cereal, and the 400-IU cereal were 30 ± 7, 153 ± 43, and 765 ± 164 μmol · h/L (all trial comparisons, $P < 0.0001$). In trial 4, 3 subjects barely responded and 2 subjects had areas under the curve that were similar to their 400-IU cereal responses.

Conclusion: The low bioavailability of vitamin E from the 400-IU capsule and the variability observed when the capsule was consumed with cereal suggest that encapsulated vitamin E is poorly absorbed when consumed with a low-fat meal and that bioavailability can be enhanced by food fortification with vitamin E. *Am J Clin Nutr* 2004;79:86–92.

KEY WORDS Tocopherol, low-fat meal, clinical trial, mass spectrometry, vitamin E bioavailability, supplements

INTRODUCTION

Controversies have surrounded vitamin E since its discovery in 1922 (1) and the subsequent description of forms other than α-tocopherol that had some vitamin E biological activity (2). Currently, only α-tocopherol has been shown to reverse human vitamin E deficiency symptoms, and α-tocopherol is the only form of vitamin E that meets the year 2000 vitamin E recommended dietary allowance (3).

In addition to the prevention of deficiency symptoms, the potential of antioxidants, especially vitamin E, to decrease the risk of chronic disease has been a popular topic in the nutrition field. Nonetheless, many Americans do not consume vitamin E–adequate diets (3). Vitamin E has been touted for decades as "heart protective," but credible scientific evidence has been lacking. In the 1990s, epidemiologic evidence (4, 5) and a relatively small (2002 subjects), randomized, placebo-controlled intervention study gave credence to the concept that vitamin E supplements could decrease heart attack risk (6). Subsequently, larger vitamin E intervention trials failed to show cardiovascular-protective effects (7, 8). Moreover, dietary, but not supplemental, vitamin E has been reported to be associated with beneficial outcomes in heart disease (9), cancer (10), and Alzheimer disease (11).

The lack of consistency in the outcomes of vitamin E supplement studies prompted us to consider the hypothesis that the bioavailability of supplemental vitamin E is highly dependent on the way in which the supplement is consumed. It is well known that fat malabsorption syndromes (eg, cholestatic liver disease) and genetic abnormalities in lipoprotein synthesis (eg, abetalipoproteinemia) or in the α-tocopherol transfer protein (eg, ataxia with vitamin E deficiency) result in vitamin E malabsorption or abnormally low plasma transport (12). Indeed, supplemental vitamin E bioavailability is highly influenced by prandial status (13). Despite the requirement for normal fat digestion and absorption, it is generally assumed that vitamin E malabsorption does not occur in healthy humans. However, the amount of dietary fat needed for optimal vitamin E absorption is unknown.

The possibility of vitamin E malabsorption from supplements led us to devise a trial to compare supplements with vitamin E–enriched foods. Vitamin E–fortified, ready-to-eat breakfast cereals are a major food source of α-tocopherol in the American diet (14). Therefore, using stable-isotope-labeled

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TABLE 1
Subject characteristics[1]

	Value
Weight (kg)	79.4 ± 10.0
Height (cm)	173.7 ± 9.1
BMI (kg/m^2)	26 ± 3
Age (y)	32 ± 7
Total cholesterol (mmol/L)	
Baseline[2]	4.09 ± 0.45
All times[3]	4.11 ± 0.59
Triacylglycerol (mmol/L)	
Baseline[2]	1.09 ± 0.46
All times[3]	1.11 ± 0.51

[1] $\bar{x}$ ± SD. $n = 3$ women and 2 men.
[2] Averaged for the 4 trials.
[3] Combined average measured at all time points during the 4 trials.

TABLE 2
Blood chemistry and hematologic values of the subjects at screening[1]

	Value[2]	Reference range
Sodium (mmol/L)	140 ± 1	135–145
Potassium (mmol/L)	4.5 ± 0.4	3.5–5.1
Chloride (mmol/L)	103 ± 2	100–111
Bicarbonate (mmol/L)	24.6 ± 1.5	22–30
Glucose (mmol/L)	5.6 ± 0.4	3.9–5.8
Serum urea nitrogen (mmol/L)	4.6 ± 0.7	2.1–6.8
Creatinine (μmol/L)	79.6 ± 8.84	35.4–97.2
Uric acid (μmol/L)	321 ± 65	143–420
Calcium (mmol/L)	2.27 ± 0.10	2.10–2.55
Phosphorus (mmol/L)	1.25 ± 0.06	0.87–1.45
Total protein (g/L)	72 ± 4	64–83
Albumin (g/L)	42 ± 5	34–50
SGOT (U/L)	18 ± 3	0–31
LDH (U/L)	157 ± 38	94–250
SGPT (U/L)	14 ± 4	0–31
Alkaline phosphatase (U/L)	71 ± 14	39–117
γ-GT (U/L)	16 ± 8	7–33
Total bilirubin (μmol/L)	10 ± 7	2–18
Globulin (g/L)	30 ± 2	23–35
Lipid profile		
Triacylglycerols (mmol/L)	1.00 ± 0.56	<2.26
Cholesterol (mmol/L)		
Total	4.42 ± 0.34	<5.20
HDL	1.45 ± 0.28	≥0.91
VLDL	0.47 ± 0.29	<1.03
LDL	2.48 ± 0.41	<3.36
Blood count		
WBC (×10^3/μL)	6.7 ± 1.4	4.0–10.8
RBC (×10^6/μL)	4.9 ± 0.4	4.3–5.6
Hemoglobin (g/L)	140 ± 13	130–168
Hematocrit (%)	42 ± 4	38–49
MCV (fL)	86 ± 1	81–93

[1] SGOT, serum glutamic oxaloacetic transaminase; LDH, lactic dehydrogenase; SGPT, serum glutamic pyruvic transaminase; γ-GT, γ-glutamyltransferase; WBC, white blood cells; RBC, red blood cells; MCV, mean corpuscular volume.
[2] $\bar{x}$ ± SD.

α-tocopherol, we tested whether encapsulated vitamin E consumed with fat-free milk was as effective in raising plasma α-tocopherol concentrations as was vitamin E–fortified, wheat-based cereal eaten with fat-free milk. The doses used were equivalent to the US recommended dietary allowance for vitamin E (30 IU) or those in a typical vitamin E supplement (400 IU). The form of vitamin E used was synthetic (*all-rac*-α-tocopheryl acetate) because this is the form that is routinely used to fortify breakfast cereals. To estimate vitamin E bioavailability, plasma labeled and unlabeled α-tocopherol concentrations were measured, and areas under the curves (AUCs) for deuterated tocopherol were approximated.

SUBJECTS AND METHODS

Subjects

This study was approved by the Institutional Review Board at Oregon State University and was reviewed by the staff at Bell Institute of Health and Nutrition, General Mills, Inc. Five active, healthy, nonsmoking adults (3 women and 2 men) who were not currently taking vitamin or antioxidant supplements and were not allergic to wheat were recruited to participate. Each subject signed an informed consent statement before the study.

The subjects' characteristics and blood chemistry and hematologic values at screening are shown in **Tables 1** and **2**, respectively. The mean BMI was on the high end of the normal range (15); otherwise, all the laboratory measures were within the normal ranges for Good Samaritan Hospital's clinical laboratory (Corvallis, OR).

Deuterium-labeled tocopherol and cereal enrichment

Deuterium-labeled α-tocopheryl acetate [2-CH_3-5,7,8-$(CD_3)_3$-tetramethyl-2*RS*-(4'*RS*,8'*RS*,12-trimethyltridecyl)-6-chromanyl acetate] (d_9-*all-rac*-α-tocopheryl acetate) was synthesized by Isotec Inc (Miamisburg, OH). The α-tocopherol deuterium distribution, which was determined by liquid chromatography–mass spectrometry, was 88.4% d_9, 11.0% d_8, and 0.6% d_7. Note that 1 IU is equivalent to 1 mg *all-rac*-α-tocopheryl acetate or 0.45 mg 2*R*-α-tocopherol [2*R*-(4'*RS*,8'*RS*,12 trimethyltridecyl)-6-chromanol], as defined by the Food and Nutrition Board (Table 6.1) for the 2000 vitamin E dietary reference intakes (3). The 400-IU capsules did not contain any carrier or diluents.

Unlabeled α-tocopherol (used as a standard) and *RRR*-α-5,7-$(CD_3)_2$-tocopheryl acetate (d_6-α-tocopheryl acetate, used as an internal standard) were gifts from James Clark (Cognis Nutrition and Health, LaGrange, IL).

Ready-to-eat wheat-based cereal without added vitamin E, as well as cereal fortified with labeled vitamin E, was prepared at General Mills, Inc, by using standard industrial fortification practices. To fortify the cereal with d_9-*all-rac*-α-tocopheryl acetate, the labeled vitamin E was added to a vitamin E–free vitamin emulsion. The emulsion contained a preemulsified, vitamin E–free, fat-soluble vitamin formulation (Hoffman-LaRoche, Nutley, NJ); a water-soluble vitamin formulation (Hoffman-LaRoche); polysorbate-80; mono- and diglycerides; gum arabic; and butylated hydroxytoluene as a preservative. The emulsion was evenly applied to the cereal with a fine-tipped syringe and air-dried at 12.8 °C for 30 min. The cereal serving sizes were determined on the basis of the experimentally determined concentrations of labeled vitamin E in the cereal (*see* below).

TABLE 3
Nutrient composition of the meals consumed

	Breakfast[1]	Lunch[2]
Energy (kcal)	250	550
Carbohydrates (g)	49	93
Protein (g)	14	20
Total fat (g)	1	10
Fat (% of energy)	4	16
Fat (% by wt)	2	8

[1] Breakfast (trials 2, 3, and 4) consisted of cereal and 236 mL fat-free milk; during trial 1, only fat-free milk was consumed for breakfast.

[2] Lunch for all trials consisted of a 15.2-cm-long turkey submarine sandwich with lettuce, tomato, and mustard; 236 mL orange juice; and 30 g wheat chips.

Study design

The study, which was conducted at the Linus Pauling Institute, Oregon State University, Corvallis, consisted of four 4-d trials and used a sequential feeding design. Each trial began with "breakfast" at 0700 on day 1, when subjects who had fasted for ≥10–12 h consumed vitamin E, either encapsulated or in a fortified cereal, along with 236 mL fat-free milk (Safeway, Pleasanton, CA). Blood was collected at 0, 3, 6, 9, 12, 24, 36, 48, and 72 h after the initial breakfast in each trial. In trial 1, the subjects consumed a capsule containing 400 IU d_9-α-tocopheryl acetate (400-IU capsule). Two weeks later (trial 2), the same subjects consumed 41 g cereal containing 30 IU d_9-α-tocopheryl acetate (30-IU cereal). Two weeks after trial 2 (trial 3), the same subjects consumed 45 g cereal containing 400 IU d_9-α-tocopheryl acetate (400-IU cereal).

Five months after trial 3 (trial 4), the same subjects consumed a capsule containing 400 IU d_9-α-tocopheryl acetate along with 41 g cereal without added vitamin E (400-IU capsule with cereal). The delay between trials 3 and 4 was used to allow the high plasma d_9-α-tocopherol concentrations resulting from the 400-IU cereal to return to baseline concentrations. During trials 1 and 2, the baseline plasma sample (before eating breakfast) contained no detectable d_9-α-tocopherol, but during trial 3 the baseline sample contained d_9-α-tocopherol (0.29 ± 0.16 μmol/L) that was subtracted from the subsequent samples. The delay between trials 3 and 4 did, in fact, allow the high plasma d_9-α-tocopherol concentrations to return to baseline concentrations—no d_9-α-tocopherol was detected in the baseline sample for trial 4.

Lunch was controlled on the first day of each of the 4 trials and was consumed between 1130 and 1230. Lunch consisted of a turkey sandwich with lettuce and tomato (no mayonnaise), 236 mL orange juice (Minute Maid; Coca-Cola Company, Houston), and 30 g wheat chips (Sun Chips; Frito-Lay, Dallas) (**Table 3**). Other meals were consumed ad libitum.

Measurement of plasma vitamin E and lipids

Blood was drawn from the antecubital vein (alternating between arms for the various time points) into evacuated tubes containing 0.05 mL 15% (wt:vol) EDTA (Becton Dickinson, Franklin Lakes, NJ), and the plasma was promptly separated by centrifugation at 4 °C for 15 min at 500 × *g* (model TJ-6; Beckman Coulter, Palo Alto, CA) and stored at −80 °C until analyzed. Plasma was extracted as described (16) and resuspended in methanol:ethanol (1:1, vol:vol). The plasma extracts were analyzed by using liquid chromatography–mass spectrometry [Waters 2690 Separations Module (Waters, Milford, MA) and Micromass ZQ 2000 single-quadrupole mass spectrometer (Micromass, Manchester, England) with Micromass MASSLYNX NT version 3.4 software] with a modification of a method described previously (17). Briefly, tocopherols were separated by using a Symmetry LC-18 column (4.6 × 150 mm, 5 μm; Waters) with a mobile phase of methanol (flow rate of 1 mL/min), a run time of 8 min, and mass spectrometric detection with atmospheric pressure chemical ionization in negative ionization mode. The analysis parameters were set as follows: current in corona discharge electrode, 15.0 μA; temperature of atmospheric pressure chemical ionization probe, 450 °C; flow rate of heater gas (nitrogen) for atmospheric pressure chemical ionization, 350 L/h; pressure of nebulizer gas (nitrogen), 0.55 MPa (80 psi); flow rate of cone gas (nitrogen), 25 L/h; cone voltage, −40 V; dwell time, 0.20 s. Mass-to-charge ratios were obtained for d_0-, d_6-, and d_9-α-tocopherols as follows: d_0-α-tocopherol, 429.3; d_6-α-tocopherol, 435.3; d_9-α-tocopherol, 438.3.

Plasma d_0- and d_9-α-tocopherol concentrations were quantitated by normalizing the respective peak areas to the area of the internal standard (d_6-α-tocopherol). Then the peak areas were estimated by using external calibration curves. Plasma triacylglycerol and total cholesterol concentrations were measured by using standard assays (Sigma Aldrich, St Louis).

The cereal without vitamin E was analyzed by HPLC with amperometric detection (16) and was found to contain 0.1 mg unlabeled α-tocopherol/g cereal. The cereal with added d_9-α-tocopheryl acetate was analyzed by liquid chromatography–mass spectrometry after saponification and extraction as described above.

Mathematical and statistical analysis

Data are expressed as means ± SDs. The AUCs for the plasma d_9-α-tocopherol concentration were calculated by using the trapezoidal rule for the time points from 0 to 72 h; the slopes and *y* intercepts of the logarithmically transformed d_9-α-tocopherol concentrations were estimated by using the linest function of EXCEL (Microsoft, Seattle). The maximum d_9-α-tocopherol concentration (C_{max}) and the time at which the maximum concentration was reached (t_{max}) were estimated by visually inspecting the data. Total vitamin E concentrations were calculated by summing the labeled and unlabeled plasma α-tocopherol concentrations for each subject at each time point. The plasma total α-tocopherol C_{max} values were estimated from the concentrations in the first 24 h after isotope administration. The percentage increase above baseline was calculated by dividing the difference between the maximum and baseline total α-tocopherol concentrations by the baseline concentration and multiplying by 100.

Statistical analyses of logarithmically transformed unlabeled α-tocopherol and d_9-α-tocopherol concentrations and of lipid-standardized vitamin E concentrations for trials 1, 2, and 3 were performed by using repeated-measures analysis of variance (STATVIEW, version 4; SAS Institute Inc, Cary, NC) with Bonferroni-Dunn correction for post hoc comparisons. Results were considered to be statistically significant at the 95% confidence level ($P < 0.05$). The variance of the results

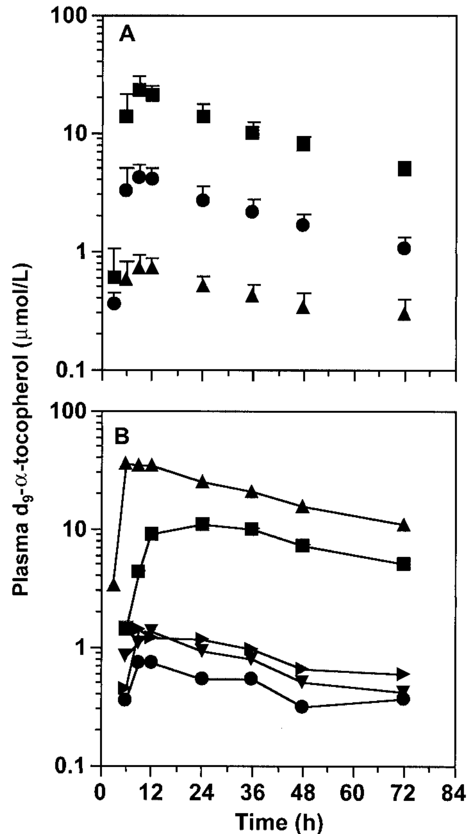


FIGURE 1. A: Mean (+SD) plasma d_9-α-tocopherol concentrations in 5 subjects who sequentially consumed 400 IU d_9-α-tocopheryl acetate (400-IU capsule, ▲; trial 1); 41 g ready-to-eat wheat cereal containing 30 IU d_9-α-tocopheryl acetate (30-IU cereal, ●; trial 2); and 45 g cereal containing 400 IU d_9-α-tocopheryl acetate (400-IU cereal, ■; trial 3). The maximum d_9-α-tocopherol concentration differed significantly between trials (all comparisons, $P < 0.005$). B: Plasma d_9-α-tocopherol concentrations in 5 individual subjects (subject 1, ▼; subject 2, ▶; subject 3, ▲; subject 4, ●; subject 5, ■) who consumed the 400-IU capsule with cereal (trial 4).

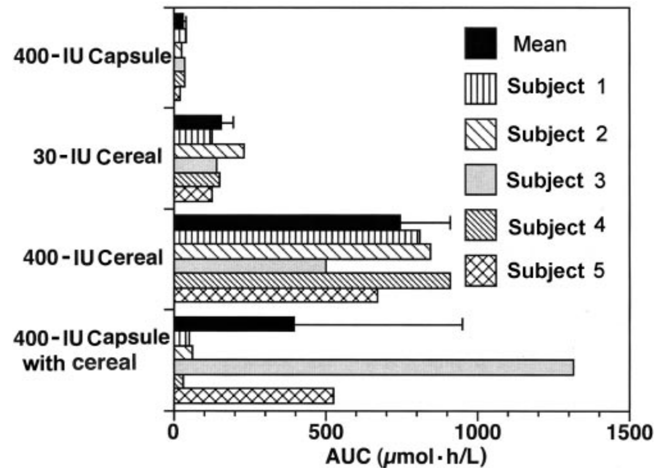


FIGURE 2. Mean (+SD) and individual areas under the curve (AUCs) for plasma d_9-α-tocopherol concentrations for each trial shown in Figure 1. In pairwise-comparisons, the AUCs for the first 3 trials (ie, 400-IU capsule, 30-IU cereal, and 400-IU cereal) differed significantly from one another ($P < 0.0001$). [Trial 4 (ie, 400-IU capsule with cereal) was not evaluated.]

obtained in trial 4 precluded statistical evaluation; therefore, values for individual subjects are shown instead.

The low-fat breakfasts generated little within-subject variation in plasma cholesterol and triacylglycerol concentrations (Table 1). Although lipid-standardized (cholesterol plus triacylglycerols) unlabeled α-tocopherol and d_9-α-tocopherol concentrations were calculated and statistical analysis was performed, similar findings were obtained whether or not the data were adjusted for plasma lipid concentrations. Therefore, only unadjusted unlabeled α-tocopherol and d_9-α-tocopherol concentrations are presented.

RESULTS

Plasma deuterated α-tocopherol concentrations

In response to the breakfast containing d_9-α-tocopheryl acetate, plasma d_9-α-tocopherol concentrations peaked at similar times in all the trials. The values for t_{max} (in h) after the breakfast were as follows: trial 1, 10 ± 2; trial 2, 8 ± 1; trial 3, 10 ± 1; trial 4, 12 ± 7. The overall t_{max} was 10 ± 4 h (**Figure 1**A). The variability in t_{max} during trial 4 was due to a difference between subject 5 and the other subjects in the time at which the plasma d_9-α-tocopherol concentration peaked (24 h in subject 5 compared with 6-12 h in the other subjects). Note that in the 3 preceding trials, the concentration in subject 5 peaked consistently between 9 and 12 h.

Plasma d_9-α-tocopherol concentrations were dependent on both dose and route of administration. C_{max} values observed in response to the 400-IU capsule (0.7 ± 0.2 μmol/L) were significantly lower than those observed after consumption of the 30-IU cereal (3.6 ± 1.9; $P < 0.0001$) or the 400-IU cereal (23.1 ± 7.5; $P < 0.0001$).

When the 400-IU capsule was consumed with cereal (trial 4), a wide range of plasma d_9-α-tocopherol concentrations were observed (Figure 1B). In 3 of the subjects, plasma d_9-α-tocopherol concentrations peaked between 0.7 and 1.4 μmol/L, similar to the C_{max} values observed in response to the 400-IU capsule alone, whereas in the remaining 2 subjects, plasma d_9-α-tocopherol concentrations peaked at higher values (10.8 and 35.6 μmol/L, respectively). The variability in trials 1–3 (assessed as the CV of the d_9-α-tocopherol concentrations at each time point) averaged 33 ± 10%, whereas the variability in trial 4 was 162 ± 35%. The 2 subjects with the greatest responses were women—1 had the highest triacylglycerol concentrations (average over all trials: 1.69 ± 0.67 mmol/L), and 1 had the lowest (0.78 ± 0.11 mmol/L). However, as shown in Table 2, all the subjects had cholesterol and triacylglycerol concentrations that were within the respective normal ranges. There were no significant differences between baseline lipid (cholesterol plus triacylglycerols) concentrations and concentrations at other time points within or between trials.

AUCs for plasma d_9-α-tocopherol were also calculated for each subject in each trial (**Figure 2**). Bioavailability can be estimated from the ratios of the AUCs derived from the various doses (18). Vitamin E bioavailability after consumption of the 30-IU cereal was 6 ± 2 times that after consumption of the 400-IU capsule, and vitamin E bioavailability after consumption of the 400-IU cereal was 26 ± 8 times that after consumption of the 400-IU capsule. The AUCs in trials 1–3 differed significantly from one another ($P < 0.0001$). In trial 4, the

TABLE 4
y Intercepts, rates of disappearance, and half-lives for d_9-α-tocopherol by treatment[1]

	y Intercept	Rate of disappearance[2]	Half-life
	μmol/L	$\mu mol \cdot L^{-1} \cdot h^{-1}$	*h*
400-IU Capsule	1.1 ± 0.2	0.028 ± 0.013	29 ± 14
30-IU Cereal	4.5 ± 0.8[3,4]	0.025 ± 0.007	29 ± 8
400-IU Cereal	27.5 ± 9.4[3]	0.028 ± 0.006	26 ± 7
400-IU Capsule with cereal	12.9 ± 18.2	0.020 ± 0.005	36 ± 9

[1] $\bar{x}$ ± SD. The variance in *y* intercepts for the 400-IU capsule with cereal was so large that these data were not included in the statistical analysis.
[2] After the peak concentration was reached.
[3] Significantly different from the 400-IU capsule, $P < 0.0001$ (Bonferroni-Dunn post hoc test).
[4] Significantly different from the 400-IU cereal, $P < 0.0001$ (Bonferroni-Dunn post hoc test).

AUCs varied widely: 3 subjects hardly responded, and 2 subjects had a response similar to that observed after consumption of the 400-IU cereal (Figure 2).

Similar to the AUCs, the estimated maximum plasma d_9-α-tocopherol concentrations (given as the *y* intercept; **Table 4**) had the following order: 400-IU capsule < 30-IU cereal < 400-IU cereal. However, there were no significant differences between the trials in disappearance rates. These data suggest that the higher AUCs (and thus the greater bioavailability) observed with the cereals than with the supplement were a result of higher absorption of vitamin E, which led to higher maximum d_9-α-tocopherol concentrations.

Plasma total α-tocopherol increase

Only minor changes in plasma total α-tocopherol concentration (sum of d_9- and d_0-α-tocopherol concentrations) were observed after consumption of either the 400-IU capsule or the 30-IU cereal. The percentage increases in plasma total α-tocopherol concentration from the baseline value to C_{max} were 14 ± 15%, 28 ± 11%, and 99 ± 39% in response to the 400-IU capsule, the 30-IU cereal, and the 400-IU cereal, respectively (**Figure 3**). The response to the 400-IU cereal was significantly greater than the response to either of the first 2 treatments ($P < 0.001$). After consumption of the 400-IU capsule, the plasma total α-tocopherol t_{max} value was 5.4 ± 2.5 h, which was significantly lower than the t_{max} values (range: 8.4–10.2 h) for any of the other trials ($P < 0.02$). In response to the 400-IU capsule with cereal (trial 4), plasma total α-tocopherol concentrations increased only in those subjects in whom plasma d_9-α-tocopherol concentrations increased.

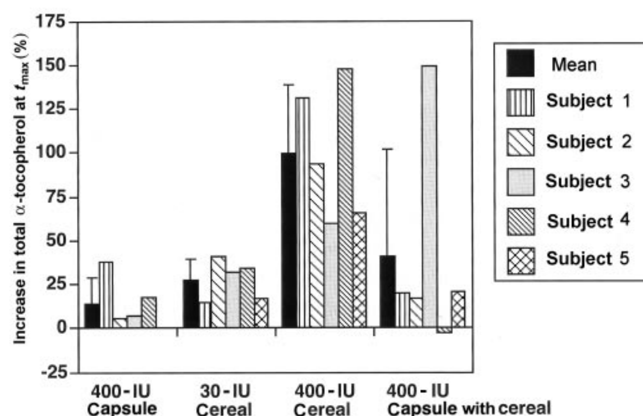


FIGURE 3. Mean (+SD) and individual percentage increases in plasma total α-tocopherol at the time at which the maximum total α-tocopherol concentration was reached (t_{max}) during the 4 trials shown in Figure 1. The percentage increase in total α-tocopherol after consumption of the 400-IU cereal was significantly greater than that after consumption of the 400-IU capsule or the 30-IU cereal ($P < 0.001$). [Trial 4 (ie, the 400-IU capsule with cereal) was not evaluated.]

DISCUSSION

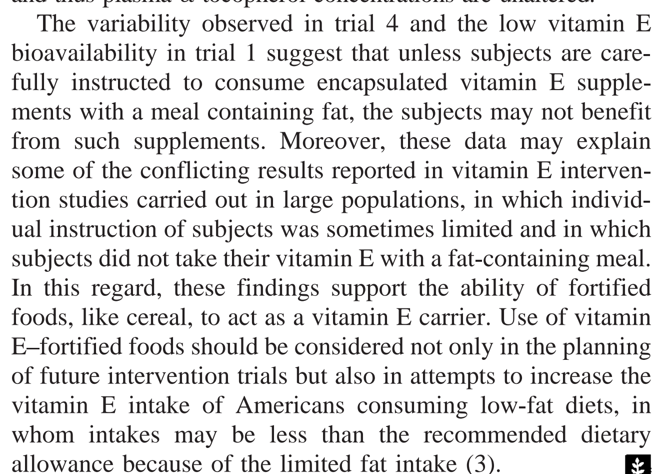
The bioavailability of vitamin E (400 IU d_9-α-tocopheryl acetate) from a fortified breakfast cereal was ≈25-fold that from a supplement when both the cereal and the supplement were consumed with fat-free milk. Indeed, the 30-IU cereal had greater vitamin E bioavailability than did the 400-IU capsule: the 30-IU cereal, which was approximately one-tenth of the dose of the 400-IU capsule, resulted in a maximum plasma d_9-α-tocopherol concentration that was ≈5-fold that observed with the 400-IU capsule. (Note that these comparisons do not take into account differences in administered dose.) When the 400-IU capsule was consumed with cereal and milk (trial 4), plasma d_9-α-tocopherol concentrations increased in only 2 of the 5 subjects. The variability observed in trial 4 has also been observed in other studies using deuterated vitamin E. In a study of 30 healthy subjects, Roxborough et al (19) reported a broad range of plasma responses to deuterated vitamin E: AUCs varied from 12.9 to 493 μmol · h/L ($\bar{x}$ ± SD: 220 ± 143 μmol · h/L). In the present study, the AUCs for the 400-IU capsule, the 30-IU cereal, and the 400-IU cereal were 30 ± 7, 153 ± 43, and 765 ± 164 μmol · h/L, respectively, with the greatest variability occurring after consumption of the 400-IU capsule with cereal (394 ± 554 μmol · h/L, trial 4). The protocol for our trial 4 (*see* Subjects and Methods) was similar to that used by Roxborough et al (19) (gelatin capsule containing 75 mg d_6-*RRR*-α-tocopheryl acetate consumed with 125 mL fat-free milk, 2 slices of buttered toast, and 125 mL tea or coffee; blood samples were taken up to 51 h). Thus, the absorption of deuterated vitamin E given as a capsule with a low-fat meal is apparently quite variable. Similarly, in a study of vitamin E kinetics in smokers and nonsmokers, we (20) reported that although some of the subjects had a maximal response after one capsule, plasma concentrations reached a plateau in all the subjects only after the third dose of deuterium-labeled vitamin E was administered.

The findings of the present study have important public health implications. When encapsulated vitamin E supplements

are consumed with fat-free milk, α-tocopherol absorption is minimal at best. The fat-free milk consumed with the breakfasts contained only 0.5% fat by wt (21), which may have contributed to the limited vitamin E absorption from the 400-IU capsule. This result was expected because vitamin E absorption requires biliary and pancreatic secretions, as well as chylomicron synthesis (12). However, the breakfast that contained <5% fat (consisting of vitamin E–fortified cereal plus fat-free milk) unexpectedly increased vitamin E bioavailability. Hydrolysis of α-tocopheryl acetate and absorption of α-tocopherol were probably aided by the fine dispersal of vitamin E on the surface of the cereal flakes, in contrast to the capsule, in which the vitamin E was concentrated in a globule. These findings are significant because fortified breakfast cereals are a major source of vitamin E in the American diet (14). It should be emphasized that the 30 IU in the breakfast cereal did not increase total α-tocopherol concentrations, and this result is consistent with the findings of Hayes et al (22), who used unlabeled vitamin E and found that plasma α-tocopherol concentrations did not differ whether 30 IU vitamin E was provided in capsules or as a fine emulsion in milk. Importantly, Hayes et al found that the vitamin E bioavailability, as estimated from plasma concentrations, of α-tocopheryl acetate (100–200 mg/d) provided as a microdispersion in milk was double that of the same dose provided in capsules or orange juice. Again, these results emphasize that the physical properties involved in how vitamin E is presented to the intestinal absorptive surfaces have great bearing on its bioavailability.

The variability in vitamin E bioavailability observed in trial 4 is not a result of subjects being "nonresponders"; the same subjects participated in all the trials and showed consistent results in the first 3 trials (*see* Figure 3). Only in the last trial, in which the 400-IU capsule was consumed with a low-fat breakfast that consisted of fat-free milk and cereal, were large variations in plasma d_9-α-tocopherol concentrations observed. Wide variability in response to deuterium-labeled vitamin E supplements has been suggested to be a result of large differences in biological response, because subjects had similar responses at different times when the same protocol was used (19). From the results of our study, such large variation is apparently observed in response to encapsulated vitamin E supplements only, not to fortified foods (or at least not to vitamin E–fortified cereal). Vitamin E bioavailability was previously reported to be higher when subjects consumed vitamin E capsules containing Aqua-Biosorb (polysorbate 80, ethanol, propylene glycol 10; RP Scherer Pty Ltd, Victoria, Australia) than when they consumed capsules containing vitamin E in soybean oil (23), which suggests that the key step in vitamin E bioavailability is delivery of emulsified vitamin E to enterocytes. The critical role of bile acids in vitamin E absorption (24) also suggests that the key step in vitamin E absorption is entry into enterocytes, which is followed by packaging into chylomicrons (25). In a study by Borel et al (26), vitamin E absorption and secretion into chylomicrons occurred between 3 and 5 h after administration of a vitamin E dose in an emulsion containing 57% fat; there were no differences in vitamin E absorption between small and large fat-particle sizes. However, vitamin E absorption and triacylglycerol absorption appeared to be temporally separated (26); thus, secretion into chylomicrons can be accomplished if the vitamin E is absorbed into enterocytes. To control for fat intake from lunch in the present study, we ensured that all the subjects in every trial consumed the same lunch after administration of the deuterated vitamin E at breakfast. We believe that the differences observed in vitamin E bioavailability in our study were not related to fat intake.

Finally, plasma total (sum of labeled and unlabeled) α-tocopherol concentrations increased markedly only in response to the 400-IU cereal and in 2 subjects in trial 4. In dose-response studies using deuterium-labeled vitamin E, we (27) previously found that newly absorbed α-tocopherol replaced, rather than added to, circulating α-tocopherol. Plasma α-tocopherol concentrations are regulated by the α-tocopherol transfer protein (α-TTP) (28). α-TTP probably salvages hepatic α-tocopherol, thereby preventing its excretion, because patients with defective α-TTP become deficient in vitamin E (29) as a result of a rapid loss of plasma α-tocopherol (30). In healthy subjects, large vitamin E doses apparently exceed the regulatory function of α-TTP and increase plasma α-tocopherol concentrations. However, plasma total α-tocopherol concentrations increase maximally only 2-3-fold (31); the mechanism for this limitation is unknown but may involve increased metabolism (32). In the present study, only the deuterated α-tocopherol absorbed from the 400-IU cereal (or from the capsule in 2 subjects in trial 4) appears to have exceeded the α-TTP regulatory capacity, thus resulting in increased plasma total α-tocopherol concentrations. The major advantage of using stable-isotope-labeled vitamin E in the present study is that the newly absorbed α-tocopherol and the nuances in response to dietary α-tocopherol concentrations can be detected. These findings also provide an explanation for observations that plasma α-tocopherol concentrations are not correlated with dietary vitamin E, but only with vitamin E supplement intakes (33–35). That is, the amount of newly absorbed α-tocopherol from dietary sources is insufficient to exceed the capacity of α-TTP, and thus plasma α-tocopherol concentrations are unaltered.



The variability observed in trial 4 and the low vitamin E bioavailability in trial 1 suggest that unless subjects are carefully instructed to consume encapsulated vitamin E supplements with a meal containing fat, the subjects may not benefit from such supplements. Moreover, these data may explain some of the conflicting results reported in vitamin E intervention studies carried out in large populations, in which individual instruction of subjects was sometimes limited and in which subjects did not take their vitamin E with a fat-containing meal. In this regard, these findings support the ability of fortified foods, like cereal, to act as a vitamin E carrier. Use of vitamin E–fortified foods should be considered not only in the planning of future intervention trials but also in attempts to increase the vitamin E intake of Americans consuming low-fat diets, in whom intakes may be less than the recommended dietary allowance because of the limited fat intake (3).

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