Human vitamin E requirements assessed with the use of apples fortified with deuterium-labeled α-tocopheryl acetate

Richard S Bruno, Scott W Leonard, Su-il Park, Yanyun Zhao, and Maret G Traber

ABSTRACT

Background: Little is known about factors that modulate dietary α-tocopherol bioavailability.

Objectives: The study aimed to assess the efficacy of vitamin E–fortified apples as a low-fat vitamin E delivery system, the influence of fat on vitamin E absorption, and human vitamin E requirements by using plasma α-tocopherol kinetics at a dosage of α-tocopherol found in food.

Design: Apples fortified with deuterium-labeled α-tocopheryl acetate were consumed by 5 participants at a breakfast containing 0%, 6%, or 21% kcal from fat in 3 sequential trials. The trials were separated by a 2-wk washout period. Blood samples were obtained up to 72 h, and plasma was analyzed for labeled and unlabeled α-tocopherol.

Results: Compared with observations in the 0% fat trial, the maximum observed plasma δ6-α-tocopherol concentrations (Cmax) and the areas under the curve increased 2- and 3-fold during the 6% and 21% fat trials, respectively. The mean (±SD) estimated percentage δ6-α-tocopherol absorbed increased from 10 ± 4% during the 0% fat trial to 20 ± 3% and 33 ± 5% during the 6% and 21% fat trials, respectively. The mean time to Cmax (9 ± 2 h), fractional disappearance rates (0.022 ± 0.003 pools/d), and half-lives (32 ± 4 h) did not differ significantly between the trials. With the use of fractional disappearance rates and baseline plasma α-tocopherol concentrations, the estimated daily plasma α-tocopherol efflux was 13–14 mg.

The estimated rate of α-tocopherol delivery to tissues was 5 mg/d.

Conclusions: Given an estimated 33% absorption, the amount of dietary vitamin E needed daily to replace irreversible losses is only 8% of men and 2% of women in the United States had vitamin E intakes from food (3) that met the 2000 Estimated Average Requirement of 12 mg α-tocopherol/d (5). Low-fat foods fortified with vitamin E might be a solution that allows consumers to eat more α-tocopherol–dense foods.

Fresh-cut fruit and vegetables can be fortified with micronutrients with the use of vacuum impregnation without otherwise changing the physiochemical food properties. This is a technique used by the food industry to fortify the functional composition of high-porosity foods. We have used vacuum impregnation to incorporate micronutrients, such as α-tocopherol (6) or zinc and calcium (7), into low-fat, low-energy foods.

Although vacuum impregnation of fresh apples with vitamin E incorporates substantial amounts of α-tocopherol, little is known about α-tocopherol bioavailability from this low-fat matrix. In most foods, vitamin E is associated with fats and oils (8). Intestinal α-tocopherol absorption is dependent on the same processes that enable fat digestion, uptake into the enterocyte, and secretion in chylomicrons (9). Therefore, we hypothesized that α-tocopherol bioavailability from vitamin E–fortified apples could be significantly enhanced by the simultaneous ingestion of dietary fat. To test this hypothesis, deuterium-labeled vitamin E–fortified apples, which were designed so that a serving contained approximately the vitamin E daily value (30 IU) (5), were consumed with a breakfast that contained increasing amounts of fat followed by a controlled lunch in 3 sequential trials. Importantly, because the amount of vitamin E consumed was in the range for dietary intakes, we also estimated human vitamin E requirements based on α-tocopherol turnover kinetics.

SUBJECTS AND METHODS

Materials

HPLC-grade methanol was obtained from Fisher (Fair Lawn, NJ). Ascorbic acid, butylated hydroxy toluene, and potassium hydroxide were obtained from Sigma-Aldrich (St Louis, MO).

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Vitamin E standards, including unlabeled (d0) RRR-α-tocopherol, labeled (d6) RRR-α-tocopheryl acetate (d6-α-tocopherol acetate), and dγ-γ-tocopherol, were gifted from James Clark of Cognis Nutrition and Health, LaGrange, IL. The isotopic purity of the d6-α-tocopheryl acetate was determined to be 89% by liquid chromatography–mass spectrometry (LC-MS); the remainder was dγ-α-tocopherol acetate. dγ-all rac-α-Tocopheryl acetate, which was used as an internal standard, was provided by Carolyn Good of General Mills (Minneapolis, MN) and was synthesized by Isotec Inc (Miamisburg, OH).

Study participants and protocol

The protocol for the present study was approved by the Institutional Review Board for the Protection of Human Subjects of the Oregon State University, and all participants provided written consent. Healthy, nonsmoking, normolipidemic volunteers (n = 5; 3 men and 2 women) were selected on the basis of age (range: 18–35 y; ± SD: 26.6 ± 6.2 y), no nutritional supplement use (>6 mo), and exercise status (<5 h/wk of aerobic activity). To verify the participants’ health status, a serum chemistry profile was conducted at the Good Samaritan Regional Medical Center in Corvallis, OR. All participants had body mass indexes (BMI; ± SD: 23.0 ± 2.0 kg/m2) and serum chemistries (not shown) within normal limits.

The participants sequentially completed each of the 3 trials, which were separated by a 2-wk washout period. During each trial, the participants consumed a serving of apples (≈80 g) that was fortified with 22 mg d6-α-tocopherol (see below for vacuum impregnation and deuterated vitamin E quantitation). The participants ingested the fortified apples alone (0 g fat; 0% kcal from fat) during trial 1, with a low-fat breakfast (2.4 g fat; 6% kcal from fat) consisting of a bagel with 30 g low-fat cream cheese during trial 2, or with a regular-fat breakfast (11.0 g fat; 21% kcal from fat) consisting of a bagel with 30 g regular-fat cream cheese during trial 2 (see Table 1 for complete dietary details). Lunch was controlled on the first day of each trial and was consumed between 1130 and 1230. Lunch (1300 kcal, 53 g fat, 36% kcal from fat) consisted of a turkey sandwich with lettuce and tomato (no mayonnaise), 236 mL orange juice (Minute Maid; Coca-Cola Company, Houston, TX), and 30 g chips (Sun Chips; Frito-Lay, Dallas, TX). Other food (ie, snacks after 1500, dinner, etc) was consumed ad libitum. To estimate additional nutrient intakes until 0700 the following day, all participants completed a food record. The protocol for the present study was approved by the Institutional Review Board for the Protection of Human Subjects of the Oregon State University, and all participants provided written consent.

Vacuum impregnation of apples with vitamin E

To emulsify and stabilize the labeled α-tocopheryl acetate in the water-based vacuum impregnation solution, a mixture (1:1: by wt) of 0.8 g d6-α-tocopheryl acetate, 0.8 g acetylated monoglyceride (Grindsted Acetem 50–00 pk; Danisco, New Century, KS), and 0.24 g polysorbate 80 (Integra, Renton, WA) was heated to 70 °C followed by the addition of 200 g 70 °C 20% high-fructose corn syrup solution (Western Family Foods Inc, Portland, OR). The solution was then homogenized (Polytron PT 10–35; Kinematica AG, Littau, Switzerland) for 90 s at 10 000 rpm.

Fujif apples (Washington State, fall 2004 crop) were washed with distilled water, and cylindrical apple chunks (15 mm height × 15 mm diameter pieces, without the skin) were obtained with a sterile stainless steel tubular cork borer and a knife. The apple cylinders were cut in the axial direction. The pieces were immediately immersed into distilled water to avoid contact with air.

The goal was to enrich the apples with ≈50 mg vitamin E/100 g apples. For treatment, freshly prepared apple pieces (≈50 g) were immersed into 150 mL vacuum impregnation solution and placed in a chamber that was connected to a vacuum pump (Model 0211-P204; Gast Mfg Corp, Benton Harbor, MI). Vacuum pressure (100 mm Hg) was applied at room temperature for 15 min, then atmospheric pressure was restored for 30 min. The apples were removed from the solution, left to drain at room temperature for 15 min, then packed in 8” hinged clear shallow containers (Gourmet Classics DV 800S; Barrett Parkway, St Louis, MO)...

### Table 1

Dietary intakes during the first day of each trial

<table>
<thead>
<tr>
<th></th>
<th>Trial 1 (0% fat)</th>
<th>Trial 2 (6% fat)</th>
<th>Trial 3 (21% fat)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Energy (kcal)</strong></td>
<td>2258 ± 4612</td>
<td>2743 ± 582</td>
<td>2768 ± 235</td>
</tr>
<tr>
<td><strong>Carbohydrate (g)</strong></td>
<td>269 ± 548a</td>
<td>321 ± 488b</td>
<td>346 ± 288b</td>
</tr>
<tr>
<td><strong>Protein (g)</strong></td>
<td>84 ± 19a</td>
<td>126 ± 31b</td>
<td>112 ± 19b</td>
</tr>
<tr>
<td><strong>Fat (g)</strong></td>
<td>94 ± 23</td>
<td>108 ± 39</td>
<td>106 ± 12</td>
</tr>
<tr>
<td><strong>Carbohydrate (% of kcal)</strong></td>
<td>48 ± 2</td>
<td>48 ± 6</td>
<td>50 ± 4</td>
</tr>
<tr>
<td><strong>Protein (% of kcal)</strong></td>
<td>15 ± 3</td>
<td>19 ± 4</td>
<td>16 ± 2</td>
</tr>
<tr>
<td><strong>Fat (% of kcal)</strong></td>
<td>38 ± 3</td>
<td>35 ± 6</td>
<td>34 ± 2</td>
</tr>
<tr>
<td><strong>d6-α-Tocopherol (mg)</strong></td>
<td>8 ± 5</td>
<td>7 ± 3</td>
<td>6 ± 1</td>
</tr>
<tr>
<td><strong>dγ-α-Tocopherol (mg)</strong></td>
<td>0.3</td>
<td>0.5</td>
<td>0.5</td>
</tr>
</tbody>
</table>

*The participants (n = 5) completed a 24-h food record. Nutrient values within rows with different superscript letters are significantly different, P < 0.05.

* ± SD (all such values).

* x (all such values).
Louis, MO). The packed samples were stored at 2 °C and 88% relative humidity until consumed within 48 h.

Apple pieces were pooled from various enrichment sessions and mixed thoroughly, and then aliquots were obtained for analysis. The \( \alpha \)-tocopherol enrichment of the apple pieces was measured with the use of LC-MS (11). The exact weight of apples that was necessary for delivery of 22 mg \((i \pm SD: 21.89 \pm 0.34 \text{ mg})\) \( \alpha \)-tocopherol per serving was calculated on the basis of the apple vitamin E concentration of each batch of apples for each trial.

Mathematical and statistical analysis

Areas under the curve (AUCs) of plasma \( \alpha \)-tocopherol concentrations were calculated for each person for each trial with the trapezoidal rule. Time to maximal concentration \((T_{\text{max}})\) and maximal concentrations \((C_{\text{max}})\) were identified by visual inspection of the data. \( \alpha \)-Tocopherol fractional disappearance rates (FDRs) and half-lives were calculated from the plasma \( \alpha \)-tocopherol concentrations, as previously described (13).

Statistical comparisons between the treatments were performed with GRAPHPAD PRISM (version 4; GraphPad Software, San Diego, CA). The statistical significance of treatment effects on AUC, \( T_{\text{max}} \), and \( C_{\text{max}} \) was evaluated by a one-way analysis of variance with repeated measures followed by Tukey’s post hoc test when a significant main effect \((P < 0.05)\) was observed. Data are reported as means ± SDs throughout the text; means ± SEs are shown in the figures.

The \( C_{\text{max}} \) increment that was dependent on dietary fat was estimated by calculating the linear relation between the plasma \( \alpha \)-tocopherol \( C_{\text{max}} \) and fat ingested for each participant, then the slopes and intercepts were averaged. The amount absorbed was estimated by multiplying the \( C_{\text{max}} \) with the plasma volume, which converted the value from \( \mu \text{mol to mg} \). The fractional absorption was estimated from the amount absorbed divided by the dose administered \((22 \text{ mg} \alpha \)-tocopherol).

No significant differences in plasma total cholesterol or triglycerides concentrations were observed between dietary treatments. Because it was suggested that plasma tocopherol concentrations should be adjusted for lipid concentrations (14), we performed the \( \alpha \)-tocopherol kinetic analyses with and without adjustment for circulating lipid concentrations. After adjustment for lipids, we nonetheless observed no significant differences in the results and, therefore, have presented the \( \alpha \)-tocopherol concentrations and analyses without adjustment for circulating lipids.

RESULTS

Dietary fat intakes

The participants consumed vitamin E–enriched apples at breakfasts that varied in amounts of fat, followed by a lunch that contained a constant amount of fat \((1300 \text{ kcal, } 53 \text{ g fat, } 36\% \text{ kcal from fat})\). During the first 24 h of each trial, the participants’ daily fat intakes did not differ significantly, although there was some variability in the carbohydrate and protein intake between trials largely due to the differences in breakfast composition (Table 1).

Plasma unlabeled and deuterium-labeled \( \alpha \)-tocopherols

Compared with the 0% fat trial, plasma \( \alpha \)-tocopherol \( C_{\text{max}} \) doubled and tripled during the 6% and 21% fat trials, respectively (Figure 1, Table 2). The increasing breakfast fat also increased the mean (±SD) maximum percentage plasma \( \alpha \)-tocopherol absorbed from the total (labeled plus unlabeled) \( \alpha \)-tocopherol consumed from 8 ± 3% during the 0% fat trial to 14 ± 2% and 22 ± 6% for the 6% and 21% fat trials, respectively.

Mean (±SD) plasma \( \alpha \)-tocopherol \( T_{\text{max}} \) \((9 \pm 2 \text{ h})\) did not significantly differ between the 3 trials, nor did either FDR \((i \pm SD\) for all trials: 0.52 ± 0.07 pools/d) or the corresponding half-lives \((i \pm SD\) for all trials: 32 ± 4 h). Additionally, FDRs were not significantly correlated with fat intake. The AUC is often used as a measure of bioavailability. Compared with the 0% dietary fat trial, the plasma \( \alpha \)-tocopherol AUC \((0–72 \text{ h})\) doubled in the 6% fat trial and tripled in the 21% fat trial, increasing from a mean (±SD) 68 ± 42 \(\mu \text{mol/L} \cdot \text{h} \) during the 0% fat trial to 124 ± 29 and 209 ± 57 \(\mu \text{mol/L} \cdot \text{h} \) for the 6% and 21% fat trials, respectively \((P < 0.05\) between each of the trials). Given the lack of change in \( T_{\text{max}} \) and FDR between the trials, the increase in bioavailability with increased fat intake resulted from an increase in vitamin E absorption.

Vitamin E absorption was estimated from each participant’s plasma \( \alpha \)-tocopherol \( C_{\text{max}} \). The mean (±SD) percentage \( \alpha \)-tocopherol absorbed increased from 10 ± 4% in the absence of fat to 20 ± 2.5% during the 6% fat trial and 33 ± 5% with the 21% fat trial (Table 2). These data show that \( \alpha \)-tocopherol absorption was enhanced by the simultaneous consumption of increasing amounts of dietary fat.

Importantly, \( \alpha \)-tocopherol was absorbed from the apples in the absence of fat. The increase in \( C_{\text{max}} \) that was dependent on dietary fat was also estimated (Figure 2). In the absence of fat, the average (±SD) \( C_{\text{max}} \) was 2.0 ± 0.8 \(\mu \text{mol/L} \), which is equivalent to 2.7 ± 1.0 mg \( \alpha \)-tocopherol absorbed. With each gram of fat consumed, \( C_{\text{max}} \) increased by 0.33 \(\mu \text{mol/L} \), which is equivalent to an increase of 0.43 mg \( \alpha \)-tocopherol absorbed. The data estimated from the disappearance curves were similar to the \( C_{\text{max}} \) actually measured for the participants (Figure 1).

At baseline \((t = 0 \text{ h})\), plasma unlabeled \( \alpha \)-tocopherol concentrations were not significantly different between trials 1, 2, and 3 \((i \pm SD: 20.8 ± 2.9, 21.1 ± 6.6, \text{ and } 20.7 ± 3.6 \mu \text{mol/L})\,
TABLE 2
Plasma \( \alpha \)-tocopherol kinetics\(^1\)

<table>
<thead>
<tr>
<th>Trial (% fat)</th>
<th>Disappearance</th>
<th>Plasma</th>
<th>Dietary vitamin E</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FDR, ( \text{d}_0-\alpha )-T</td>
<td>( \text{d}_0-\alpha )-T</td>
<td>( \text{d}_6-\alpha )-T</td>
</tr>
<tr>
<td>1 (0%)</td>
<td>0.56 ± 0.12</td>
<td>1.19 ± 0.36</td>
<td>15.2 ± 4.6</td>
</tr>
<tr>
<td>2 (6%)</td>
<td>0.55 ± 0.04</td>
<td>2.43 ± 0.31</td>
<td>14.4 ± 2.6</td>
</tr>
<tr>
<td>3 (21%)</td>
<td>0.49 ± 0.06</td>
<td>3.55 ± 0.59</td>
<td>13.0 ± 2.9</td>
</tr>
</tbody>
</table>

\(^1\) All values are \( \bar{x} \) ± SD. FDR, fractional rate of disappearance; \( \alpha \)-T, \( \alpha \)-tocopherol; \( \text{d}_0 \), unlabeled; \( \text{d}_6 \), deuterium-labeled; \( C_{\text{max}} \), maximum concentration.

To estimate daily \( \alpha \)-T disappearance from plasma, FDR was multiplied with the plasma \( \text{d}_0-\alpha \)-T at baseline [note that baseline and average total \( \alpha \)-T (\( \text{d}_0+\text{d}_6-\alpha \)-T) were not significantly different]. The minimum absorbed \( \text{d}_6-\alpha \)-T (in mg) was estimated from the plasma \( \text{d}_6-\alpha \)-T \( C_{\text{max}} \) and the plasma volume; the percentage absorbed was calculated from the absorbed \( \text{d}_6-\alpha \)-T divided by the administered dose (22 mg \( \text{d}_6-\alpha \)-T). Daily \( \alpha \)-T intake was estimated from dietary records; the estimated dietary amount absorbed was calculated by using the maximal percentage absorption for each participant; the absorbed total \( \alpha \)-T equals the absorbed \( \text{d}_0+\text{d}_6-\alpha \)-T. Comparisons between trials (\( n = 5 \)trial) were made by 1-way ANOVA with repeated measures followed by Tukey’s post hoc analysis when a significant main effect was observed. No significant differences in the time to maximal concentration, FDRs, half-lives, or daily \( \text{d}_0-\alpha \)-T disappearance were observed between trials.

\(^2\) \( C_{\text{max}} \) values with different superscript letters were significantly different, \( P < 0.05 \).

respectively; Figure 1 and Table 2), nor were plasma \( \gamma \)-tocopherol concentrations significantly different (1.2 ± 0.5, 1.1 ± 0.4, and 0.9 ± 0.3 \( \mu \text{mol} / \text{L} \), respectively). During the course of the trials, no significant changes in either plasma total \( \alpha \)-tocopherol (Table 2) or \( \gamma \)-tocopherol concentrations (not shown) were observed from baseline. Therefore, the baseline \( \text{d}_0-\alpha \)-tocopherol concentrations were used to calculate the daily plasma \( \alpha \)-tocopherol efflux as follows:

Daily plasma \( \alpha \)-tocopherol efflux = FDR ×

plasma volume × plasma \( \text{d}_0-\alpha \)-tocopherol concentration

\(^{1}\) (I)

From the FDR (pool/d), the plasma pool size (calculated to be 4.5% body weight; in kg), and the baseline plasma \( \text{d}_0-\alpha \)-tocopherol concentration for each participant, the mean (±SD) calculated plasma \( \alpha \)-tocopherol efflux was 13 ± 3 mg/d (means for each trial are given in Table 2).

Vitamin E requirements based on vitamin kinetics

Vitamin E requirements can be approximated from the present study, given that the intakes of both dietary and supplemental vitamin E were relatively limited and did not appear to significantly change plasma \( \alpha \)-tocopherol pool sizes. As shown in Figure 3, the rate of \( \alpha \)-tocopherol entering or leaving the tissues cannot be specifically calculated with the present design; however, some estimates can be made. The range of total (\( \text{d}_0+\text{d}_6 \)) \( \alpha \)-tocopherol absorbed was from 4.8 to 9.2 mg. The \( \alpha \)-tocopherol flux to tissues (\( k_e \)) appears to be an estimate of tissue \( \alpha \)-tocopherol requirements when the fraction absorbed is 33%.

![Figure 2](https://example.com/figure2.png)

**Figure 2.** Plasma labeled \( \alpha \)-tocopherol maximum concentration (\( C_{\text{max}} \)) as a function of breakfast fat content. Plasma \( \text{d}_0-\alpha \)-tocopherol \( C_{\text{max}} \) during each of the dietary treatments was linearly related to fat intake in each of the participants. Plasma \( \text{d}_0-\alpha \)-tocopherol \( C_{\text{max}} \) was significantly (\( P < 0.0001 \)) correlated with dietary fat consumption; each ingested gram of dietary fat resulted in a corresponding increase in plasma \( \text{d}_0-\alpha \)-tocopherol \( C_{\text{max}} \) of 0.33 \( \mu \text{mol} / \text{L} \) (linear regression: \( y = 0.33x + 2.17; \ R^2 = 0.72 \)) or an increase of 0.43 mg/g fat consumed.

![Figure 3](https://example.com/figure3.png)

**Figure 3.** Model of plasma \( \alpha \)-tocopherol kinetics. Only a portion of the vitamin E (in either the apples or diet) is absorbed (\( k_e \)) and incorporated into the plasma vitamin E pool. This plasma pool includes the liver, because vitamin E moves in a rapid equilibrium between the liver and plasma (15). Efflux (\( k_e \)) from the plasma pool can go to extrahepatic tissues (\( k_t \)) or can be excreted from the body (\( k_b \)). The design of the present study does not allow estimation of the rates of \( k_b \) or \( k_e \). However, our previous estimates of vitamin E utilization (0.191 pools/d; 16) allowed us to estimate the \( k_t \) to equal 5 ± 1 mg/d from the plasma \( \text{d}_0-\alpha \)-tocopherol concentrations and volume. This rate is equivalent to the mean (±SD) rate of absorption during trial 1 (\( k_t = 4.8 ± 2.0 \) mg/d). The sum of these 2 rates (10 ± 3 mg/d) was roughly equivalent to the mean estimated efflux (\( k_e \)) of 15 ± 5 mg/d. (Note that \( k_e \) is somewhat higher, probably because it is influenced by excretion of newly absorbed \( \alpha \)-tocopherol.) Thus, at steady state, the mean absorption of 5 ± 2 mg/d is sufficient to meet tissue needs. A daily \( \alpha \)-tocopherol intake of 15 ± 6 mg is required when the fraction absorbed is 33%.
requirements. Our previous study (16) suggested the tissue 
α-tocopherol efflux rate was 0.191 pools/d, which is equivalent 
to a mean (±SD) 5.1 ± 0.9 mg excreted from the body (on 
the basis of the present participants’ baseline plasma α-tocopherol 
concentrations and plasma volumes). The mean (±SD) estimate 
for kₐ of 5 ± 1 mg/d is roughly equivalent to the rate of absorption 
during trial 1 (kₐ = 4.8 ± 2.0 mg/d). The sum of these 2 rates 
(10 ± 3 mg/d) is roughly equivalent to the estimated efflux (kₑ) 
of 15 ± 5 mg/d. Thus, at steady state, the absorption of 5 ± 2 
mg/d is sufficient to meet tissue needs. If this value represents the 
α-tocopherol required by the tissues, and intestinal absorption 
of α-tocopherol is 33%, then the mean (±SD) dietary vitamin 
E requirement is ≈15 ± 6 mg/d. This range of the 
estimated dietary vitamin E requirements confirms that the cur-
rent Dietary Recommended Allowance of 15 mg/d is substan-
tially correct. However, most Americans’ vitamin E intakes are 
well below this amount. Indeed, the mean (±SD) dietary intake 
of the participants in the present study was 7 ± 3 mg α-tocopher-
ol/d without consumption of the vitamin E–fortified apples; the 
fortified apples added 22 mg.

**DISCUSSION**

The purpose of the present study was three-fold: 1) to assess 
the efficacy of vitamin E–fortified apples as a low-fat vitamin E 
delivery system, 2) to assess the influence of fat on vitamin E 
absorption, and 3) to assess human vitamin E requirements from 
plasma α-tocopherol kinetics at a dosage of α-tocopherol found 
in food.

With respect to bioavailability of the vitamin E in fortified 
apples, the data showed that ≈10% of the 22 mg d₆-α-tocopherol 
was absorbed in the absence of fat or other foods. Additionally, 
an increase of 1 g fat consumed increased α-tocopherol absorp-
tion by 0.43 mg. The 21% fat breakfast contained 11 g fat and 
resulted in a 33% α-tocopherol absorption. This value is less than 
the 45% absorption rate estimated in thoracic duct-cannulated 
rats (17) or the 55–79% rate estimated in humans when radioac-
tive vitamin E was used to estimate absorption (18). Thus, it is 
likely that the percentage absorption would have been larger with 
a higher fat breakfast, but additional studies are needed to esti-
mate maximal absorption rates. These data also emphasize the 
relatively poor absorption of vitamin E when it is consumed 
without fat, as was observed when vitamin E pills were consumed 
without food (13). Note that the ideal method for measuring 
vitamin E absorption would be to feed one labeled dose and inject 
another labeled dose of vitamin E. The ratio of the consumed 
dose divided by the injected dose (which is assumed to be 100%) 
would yield the fraction absorbed (19). This method, however, 
depends on the availability of a preparation of vitamin E that is 
acceptable for intravenous injection in humans, which does not 
currently exist; the only intravenous preparation had adverse 
effects in premature infants (20).

The finding of increased absorption of α-tocopherol in the 
presence of dietary fat is consistent with results of other fat-
soluble nutrients. Compared with the bioavailability of lycopene 
administered in steamed tomatoes, the bioavailability of lyco-
pene increased 3-fold when administered in oil (21). Carotenoid 
bioavailability is higher with full-fat salad dressing than with 
reduced-fat salad dressing (22). Similarly, ingestion of avocado 
significantly enhanced carotenoid absorption from salad and 
salsa (23). In addition, increasing dietary fat increases the ab-
sorption of vitamin E from supplements (13, 24). Roodenberg et al (25) suggested that a 3% fat intake was sufficient for optimal 
vitamin E bioavailability. However, they measured bioavailabil-
ity as increased plasma α-tocopherol concentrations after 1 wk of 
supplementation with 50 mg α-tocopherol in 50 g of a low or high 
fat spread that accompanied a meal that resulted in an intake of 
<6.5 g fat or <45 g fat. From our study, it was apparent that the 
fat amount that was consumed concurrently with vitamin E was 
critical for its absorption. Note that the vitamin E–fortified apples 
were consumed with breakfast; the lunch eaten ≈5 h later con-
tained 36% fat and yet had no apparent effect on absorption. The 
vitamin E in the study by Roodenberg et al (25) was dissolved in 
a spread; thus, it is difficult to estimate whether the amount of fat 
needed for vitamin E absorption from a low-fat food is the same 
amount needed to dissolve vitamin E into a micellarized form. As 
also noted from our study, no significant changes in total plasma 
α-tocopherol were detectable with the low doses we used; there-
fore, it is imperative to use labeled α-tocopherol to detect 
changes in bioavailability of vitamin E from dietary sources.

With respect to vitamin E kinetics, breakfast fat (0–21%)
consumption had no significant influence on α-tocopherol rates 
of disappearance, half-lives, or Tₘ₉₉ values during the 3 trials in 
normolipidemic persons who consumed daily diets containing 
34–38% fat. Because the plasma vitamin E pool was essentially 
unchanged during each of the trials and between the trials, the 
absorbed d₆-α-tocopherol dose did not significantly change 
plasma vitamin E kinetics. The absorbed amounts were esti-
rated from the plasma d₆-α-tocopherol Cₘ₉₉. These estimated 
amounts are the minimum quantities that had to be absorbed to 
achieve the observed plasma d₆-α-tocopherol concentrations and, 
therefore, underestimate the fractional d₆-α-tocopherol ab-
sorption because they do not take tissue distribution into account.

The daily α-tocopherol requirement for normal healthy per-
sons was estimated by the Food and Nutrition Board (5) with data 
from studies that were carried out in the 1950s that evaluated the 
amount of dietary vitamin E necessary to prevent peroxide-
induced erythrocyte hemolysis in men who were depleted of 
vitamin E. The Estimated Average Requirement is 12 mg α-toc-
opherol/d (5). The mean (±SD) α-tocopherol requirement is 
≈15 ± 2 mg/d when the amount required by the tissues is ≈5 
mg/d and absorption is 33%; when absorption is 50%, the 
α-tocopherol requirement is 10 ± 1 mg/d. This range of the 
estimated dietary vitamin E requirements (10–15 mg/d) con-
firms that the current Daily Recommended Intakes are substan-
tially correct. However, most Americans’ vitamin E intakes are 
well below this amount (3), which reemphasizes the importance 
of enriching low-fat foods with vitamin E as a means of increas-
ing vitamin E intakes because the long-term consequences of 
suboptimal vitamin E intakes are unknown.

Obesity and various chronic diseases are associated with in-
creased oxidative stress (26). Vitamin E intakes are low in most 
Americans despite the obvious overconsumption of calories. We 
showed that it is possible to fortify low-fat fruit (apples) with 
vitamin E and that the vitamin E from this fruit is bioavailable; we 
also showed that a serving of vitamin E–fortified apples meets 
the calculated tissue vitamin E requirements. The present study 
emphasizes our lack of information about vitamin E require-
ments in humans—even the fractional absorption of vitamin E is 
not known with certainty. Appropriate studies that estimate bio-
availability, such as those by Levine et al (19) for vitamin C,
cannot be carried out because no injectable forms of vitamin E are available. Moreover, the present study showed that vitamin E bioavailability is greatly influenced by both the food matrix and the presence of dietary fat. Additional studies are needed to determine the amount of dietary fat necessary for optimal vitamin E absorption, because extrapolation of our data suggest that vitamin E absorption would continue to rise with the additional ingestion of dietary fat, a finding that seems highly unlikely. Furthermore, more invasive experimental approaches will be necessary to accurately determine the rates of vitamin E delivery to tissues. Collectively, these investigations will enable a more sensitive formulation of human dietary vitamin E requirements.

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All authors participated in the study design. SWL, S-iP, and YZ prepared the deuterium-labeled vitamin E-fortified apples. RSB and SWL carried out the sample collection and analyses. RSB and MGT wrote the initial draft of the manuscript, and all authors contributed to the editing and review of the manuscript. None of the authors had any conflicts of interest.

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