Human vitamin E requirements assessed with the use of apples fortified with deuterium-labeled \( \alpha \)-tocopheryl acetate\(^1\text{-}^3\)

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 \( \alpha \)-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 \( \alpha \)-tocopherol kinetics at a dosage of \( \alpha \)-tocopherol found in food.

**Design:** Apples fortified with deuterium-labeled \( \alpha \)-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 \( \alpha \)-tocopherol.

**Results:** Compared with observations in the 0% fat trial, the maximum observed plasma \( \Delta^2 \)-\( \alpha \)-tocopherol concentrations \( \left( C_{\text{max}} \right) \) and the areas under the curve increased 2- and 3-fold during the 6% and 21% fat trials, respectively. The mean (±SD) estimated percentage \( \Delta^2 \)-\( \alpha \)-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 \( C_{\text{max}} \) (9 ± 2 h), fractional disappearance rate (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 \( \alpha \)-tocopherol concentrations, the estimated daily plasma \( \alpha \)-tocopherol efflux was 13–14 mg.

**Conclusions:** Given an estimated 33% absorption, the amount of dietary vitamin E needed daily to replace irreversible losses is ≤15 mg. These estimates support the current human vitamin E requirements despite the claims that the median amount of vitamin E that Americans consume is 7 mg/d. *Am J Clin Nutr* 2006;83:299–304.

KEY WORDS \( \alpha \)-Tocopherol, vitamin E requirements, bioavailability, deuterium, biokinetics, dietary fat

INTRODUCTION

Because of the widespread problem of obesity (1, 2), Americans are being counseled to reduce their total energy and fat intakes. However, Maras et al (3) indicated that most persons obtain dietary vitamin E from high-energy, high-fat foods that are not particularly \( \alpha \)-tocopherol–rich. When persons are instructed to decrease their fat intake as part of weight management, they incur a \( \approx \)50% reduction in vitamin E intake (4). Indeed, vitamin E is one of the most difficult nutrients to obtain; 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 \( \alpha \)-tocopherol/d (5). Low-fat foods fortified with vitamin E might be a solution that allows consumers to eat more \( \alpha \)-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 \( \alpha \)-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 \( \alpha \)-tocopherol, little is known about \( \alpha \)-tocopherol bioavailability from this low-fat matrix. In most foods, vitamin E is associated with fats and oils (8). Intestinal \( \alpha \)-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 \( \alpha \)-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 \( \alpha \)-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 \((d_0)\) RRR-\(\alpha\)-tocopherol, labeled \((d_6)\) RRR-\(\alpha\)-tocopheryl acetate \((d_{137}\)-tocopherol acetate), and \(d_{157}\)-\(\gamma\)-tocopherol, were gifts from James Clark of Cognis Nutrition and Health, LaGrange, IL. The isotopic purity of the \(d_{137}\)-tocopheryl acetate was determined to be 89% by liquid chromatography–mass spectrometry (LC-MS); the remainder was \(d_{157}\)-\(\alpha\)-tocopherol acetate. \(d_{157}\)-all \(\alpha\)-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 \((range: 18–35\ y; \tilde{x} \pm SD: 26.6 \pm 6.2\ y),\) 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 (BMIs; \(\tilde{x} \pm SD: 23.0 \pm 2.0\ \text{kg/m}^2\)) 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 \((\approx 80\ g)\) that was fortified with 22 mg \(d_{157}\)-\(\alpha\)-tocopherol \((\text{see below for vacuum impregnation and deuterated vitamin E quantitation).}\) 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 \((\text{see Table 1 for complete dietary details).}\) Lunch was controlled on the first day of each trial and was consumed between 1130 and 1230. Lunch \((1300\ g; 53\ g\ fat, 36\%\ kcal\ from\ fat)\) consisted of a turkey sandwich with lettuce and tomato \((\text{no mayonnaise), 236 mL\ orange juice (Minute Maid; Coca-Cola Company, Houston, TX), and 30 g chips (Sun Chips; Frito-Lay, Dallas, TX).\) Other food \((\text{ie, snacks after 1500, dinner, etc) was consumed ad libitum. To estimate additional nutrient intakes until 0700 the following day, all participants completed a food record.\) The analyses were performed in accordance with the manufacturer’s instructions.

### Vacuum impregnation of apples with vitamin E

To emulsify and stabilize the labeled \(\alpha\)-tocopherol acetate in the water-based vacuum impregnation solution, a mixture \((1:1:0.8)\) of 0.8 g \(d_{157}\)-\(\alpha\)-tocopheryl acetate, 0.8 g acetylated monoglyceride \((\text{Grindsted Acetem 50–00 pk; Danisco, New Century, KS})\), and 0.24 g polysorbate 80 \((\text{Integra, Renton, WA})\) was heated to 70 °C followed by the addition of 200 g 70 °C 20% high-fructose corn syrup solution \((\text{Western Family Foods Inc, Portland, OR})\). The solution was then homogenized \((\text{Polytron PT 10–35; Kinematica AG, Littau, Switzerland})\) for 90 s at 10 000 rpm.

Fujif apples \((\text{Washington State, fall 2004 crop})\) were washed with distilled water, and cylindrical apple chunks \((15\ mm\ height \times 15\ mm\ diameter\ pieces,\) 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 \(\approx 30\ mg\ vitamin\ E/100\ g\ apples. For treatment, freshly prepared apple pieces \((\approx 50\ g)\) were immersed into 150 mL vacuum impregnation solution and placed in a chamber that was connected to a vacuum pump \((\text{Model 0211–P204; Gast Mfg Corp, Benton Harbor, MI})\). 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 \((\text{Gourmet Classics DV 800S; Barrett Parkway, St...})\).
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 \( \delta_9 \)-\( \alpha \)-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 (\( \bar{t} \pm SD: 21.89 \pm 0.34 \) mg) \( \delta_9 \)-\( \alpha \)-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 \( \delta_9 \)-\( \alpha \)-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. \( \alpha \)-\( \delta \)-Glyceryl tocopherol fractional disappearance rates (FDRs) and half-lives were calculated from the plasma \( \delta_9 \)-\( \alpha \)-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 (ANOVA) 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 \( \delta_9 \)-\( \alpha \)-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 \( \mu \)g to mg. The fractional absorption was estimated from the amount absorbed divided by the dose administered (22 mg \( \delta_9 \)-\( \alpha \)-tocopherol).

No significant differences in plasma total cholesterol or triacylglycerol 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 kcal, 53 g fat, 36% 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). The increasing breakfast fat also increased the mean (±SD) maximum percentage plasma \( \delta_9 \)-\( \alpha \)-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 \( \delta_9 \)-\( \alpha \)-tocopherol \( T_{\text{max}} \) (9 ± 2 h) did not significantly differ between the 3 trials, nor did either FDR (±SD for all trials: 0.52 ± 0.07 pools/d) or the corresponding half-lives (±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 \( \delta_9 \)-\( \alpha \)-tocopherol AUC (0–72 h) doubled in the 6% fat trial and tripled in the 21% fat trial, increasing from a mean (±SD) 68 ± 42 \( \mu \)mol/L · h during the 0% fat trial to 124 ± 29 and 209 ± 57 \( \mu \)mol/L · 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 \( \delta_9 \)-\( \alpha \)-tocopherol \( C_{\text{max}} \). The mean (±SD) percentage \( \delta_9 \)-\( \alpha \)-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, \( \delta_9 \)-\( \alpha \)-tocopherol was absorbed from the apples in the absence of fat. The increment 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 \( \mu \)mol/L, which is equivalent to 2.7 ± 1.0 mg \( \delta_9 \)-\( \alpha \)-tocopherol absorbed. With each gram of fat consumed, \( C_{\text{max}} \) increased by 0.33 \( \mu \)mol/L, which is equivalent to an increase of 0.43 mg \( \delta_9 \)-\( \alpha \)-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 (\( t = 0 \) h), plasma unlabeled \( \alpha \)-tocopherol concentrations were not significantly different between trials 1, 2, and 3 (\( \bar{t} \pm SD: 20.8 ± 2.9, 21.1 ± 6.6, \) and 20.7 ± 3.6 \( \mu \)mol/L,
TABLE 2

<table>
<thead>
<tr>
<th>Trial (% fat)</th>
<th>FDR</th>
<th>Plasma d0-α-T</th>
<th>Plasma d6-α-T</th>
<th>Cmax</th>
<th>Baseline d0-α-T</th>
<th>Average total α-T</th>
<th>Estimated minimum</th>
<th>Intake d0-α-T absorbed</th>
<th>Absorbed dietary d0-α-T</th>
<th>Absorbed total d0-α-T</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (0%)</td>
<td>0.56 ± 0.12</td>
<td>1.19 ± 0.36</td>
<td>15.2 ± 4.6</td>
<td>1.77 ± 0.92</td>
<td>20.8 ± 2.9</td>
<td>21.0 ± 3.9</td>
<td>2.20 ± 0.88</td>
<td>10.0 ± 4.0</td>
<td>7.8 ± 5.3</td>
<td>2.6 ± 1.7</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>
<td>3.47 ± 0.75</td>
<td>21.1 ± 6.6</td>
<td>23.8 ± 4.2</td>
<td>4.42 ± 0.55</td>
<td>20.1 ± 2.5</td>
<td>7.2 ± 2.8</td>
<td>2.4 ± 0.9</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>
<td>5.64 ± 1.24</td>
<td>20.7 ± 3.6</td>
<td>24.0 ± 4.7</td>
<td>7.24 ± 1.18</td>
<td>32.9 ± 5.4</td>
<td>5.8 ± 1.3</td>
<td>1.9 ± 0.4</td>
</tr>
</tbody>
</table>

All values are ± SD. FDR, fractional rate of disappearance; α-T, α-tocopherol; d0, unlabeled; d6, deuterium-labeled; Cmax, maximum concentration.

1. All values are ± SD. FDR, fractional rate of disappearance; α-T, α-tocopherol; d0, unlabeled; d6, deuterium-labeled; Cmax, maximum concentration. To estimate daily α-T disappearance from plasma, FDR was multiplied with the plasma d0-α-T at baseline [note that baseline and average total α-T (d0 + dα-α-T) were not significantly different]. The minimum absorbed d0-α-T (in mg) was estimated from the plasma d0-α-T Cmax and the plasma volume; the percentage absorbed was calculated from the absorbed d0-α-T divided by the administered dose (22 mg d0-α-T). Dietary α-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 α-T was calculated plasma α-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 α-tocopherol pool sizes. As shown in Figure 3, the rate of α-tocopherol entering or leaving the tissues cannot be specifically calculated with the present design; however, some estimates can be made. The range of total (d0 + d6) α-tocopherol absorbed was from 4.8 to 9.2 mg. The α-tocopherol flux to tissues (k_t) appears to be an estimate of tissue α-tocopherol

![FIGURE 3. Model of plasma α-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)].

Excreted from body

Tissues

Diet vitamin E

Plasma and liver α-tocopherol pool

A absorbed

k_e

k_t

k_t

k_e

FIGURE 2. Plasma labeled α-tocopherol maximum concentration (Cmax) as a function of breakfast fat content. Plasma d6-α-tocopherol Cmax during each of the dietary treatments was linearly related to fat intake in each of the participants. Plasma d6-α-tocopherol Cmax was significantly (P < 0.0001) correlated with dietary fat consumption; each ingested gram of dietary fat resulted in a corresponding increase in plasma d6-α-tocopherol Cmax of 0.33 μmol/L (linear regression: y = 0.33x + 2.17; R^2 = 0.72) or an increase of 0.43 mg/g fat consumed.

FIGURE 3. Model of plasma α-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_t) from the plasma pool can go to extrahepatic tissues (k_t) or can be excreted from the body (k_e). The design of the present study does not allow estimation of the rates of k_t 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 d6-α-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_t) of 15 ± 5 mg/d. (Note that k_t is a higher, probably because it is influenced by excretion of newly absorbed α-tocopherol.) Thus, at steady state, the mean absorption of 5 ± 2 mg/d is sufficient to meet tissue needs. A daily α-tocopherol intake of 15 ± 6 mg is required when the fraction absorbed is 35%.
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 \( k_e \) of 5 ± 1 mg/d is roughly equivalent to the rate of absorption during trial 1 (\( k_e = 4.8 ± 2.0 \) mg/d). The sum of these 2 rates (10 ± 3 mg/d) is roughly equivalent to the estimated efflux (\( k_a \)) 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 \( k_a \) during trial 1 (absorption of \( \alpha \)-tocopherol in the absence of fat or other foods. Additionally, absorption of \( \alpha \)-tocopherol is 33%, then the mean (±SD) dietary vitamin E requirement is 30.6 ± 6 mg/d. This range of the estimated dietary vitamin E requirements confirms that the current Dietary Recommended Allowance of 15 mg/d is substantially incorrect. 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 \( 7.2 ± 3.2 \) mg \( \alpha \)-tocopherol/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 \( \alpha \)-tocopherol kinetics at a dosage of \( \alpha \)-tocopherol found in food.

With respect to bioavailability of the vitamin E in fortified apples, the data showed that 10% of the 22 mg \( \alpha \)-tocopherol was absorbed in the absence of fat or other foods. Additionally, an increase of 1 g fat consumed increased \( \alpha \)-tocopherol absorption by 0.43 mg. The 21% fat breakfast contained 11 g fat and resulted in a 33% \( \alpha \)-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 radioactive 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 estimate 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 \( \alpha \)-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 lycopene 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 absorption 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 bioavailability as increased plasma \( \alpha \)-tocopherol concentrations after 1 wk of supplementation with 50 mg \( \alpha \)-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 <4.5 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 contained 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 \( \alpha \)-tocopherol were detectable with the low doses we used; therefore, it is imperative to use labeled \( \alpha \)-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 \( \alpha \)-tocopherol rates of disappearance, half-lives, or \( T_{\text{max}} \) 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 \( \delta \)-\( \alpha \)-tocopherol dose did not significantly change plasma vitamin E kinetics. The absorbed amounts were estimated from the plasma \( \delta \)-\( \alpha \)-tocopherol \( C_{\text{max}} \). These estimated amounts are the minimum quantities that had to be absorbed to achieve the observed plasma \( \delta \)-\( \alpha \)-tocopherol concentrations and, therefore, underestimate the fractional \( \delta \)-\( \alpha \)-tocopherol absorption because they do not take tissue distribution into account.

The daily \( \alpha \)-tocopherol requirement for normal healthy persons 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 \( \alpha \)-tocopherol/d (5). The mean (±SD) \( \alpha \)-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 \( \alpha \)-tocopherol requirement is 10 ± 1 mg/d. This range of the estimated dietary vitamin E requirements (10–15 mg/d) confirms that the current Daily Recommended Intakes are substantially 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 increasing vitamin E intakes because the long-term consequences of suboptimal vitamin E intakes are unknown.

Obesity and various chronic diseases are associated with increased 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 requirements in humans—even the fractional absorption of vitamin E is not known with certainty. Appropriate studies that estimate bioavailability, 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 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.

REFERENCES