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Inter- and Intra-Individual Variation in Plasma and Red Blood Cell Vitamin E after Supplementation

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To establish the range of individual blood responses to supplemental vitamin E, 30 healthy subjects ingested 75 mg of deuterium-labelled α-tocopherol with a standard breakfast. Blood was collected at 6, 9, 12, 27 and 51 h post ingestion and deuterated (d_6) and non-deuterated (d_0) α-tocopherol concentrations were determined in plasma and red blood cells (RBC) by GC-MS. To examine intra-individual responses, 6 of these subjects were re-examined at 6-month intervals over a 30-month period. Post ingestion, the amount of d_6-α-tocopherol in blood increased rapidly with time with maximal concentrations seen at 12 h (plasma) and 27 h (RBC) in most subjects. At these times, d_6-α-tocopherol concentration ranged from 0.3–12.4 μmol/l in plasma and 0.6–4.09 μmol/l packed cell in RBC. Area under the curve calculations indicated inter-individual differences of α-tocopherol uptake to be 40-fold for plasma (12.9–493.3 μmol h/l) and 6-fold for RBC (24.4–146.1 μmol h/l packed RBC). Intra-individual variation in α-tocopherol uptake was small in comparison and remained relatively constant over the 30-month period. We conclude that vitamin E uptake varies widely in the normal population, although it is comparatively stable for an individual over time. These differences likely arise from variations in the regulation of vitamin E uptake and metabolism between subjects. Factors regulating this process must be better understood before the optimal intake of vitamin E can be ascertained.

Keywords: Deuterium, tocopherol, variation, uptake, stable isotope, vitamin E, human

INTRODUCTION

Epidemiological studies indicate that increased vitamin E intake reduces the risk and progression of heart disease.[1,2] Recent intervention trials in patients at high risk of cardiovascular events do not however support a beneficial effect of vitamin E,[3,4] suggesting that its action may be more important in disease prevention. Plasma vitamin E concentrations greater than 30 μM are considered optimal, being associated with lower risk of both cancer and CHD.[5–7] As a consequence, increased dietary intake of vitamin E has been recommended to achieve this plasma concentration.[7] Vitamin E intake is, however, only a moderate predictor of plasma concentrations[8,9] with a varied response of the plasma vitamin E

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pool to dietary intake and/or supplements being noted. In a subsample of the US Health Professionals Study[10] male subjects consuming 70 ± 17 International Units (IU) vitamin E/day (as diet plus supplements) were found to achieve plasma α-tocopherol concentrations of $27 \pm 8\,\mu M$. In comparison, subjects in the VERA study,[11] who had a similar daily intake of vitamin E, achieved plasma concentrations of about $60\,\mu M$, while those with a dietary intake of 23 IU/day had plasma concentrations of $30\,\mu M$. Furthermore, in an NCI-USDA Study, subjects taking vitamin E supplements of between 10 and 60 IU/day in addition to their normal diet were found to have plasma α-tocopherol concentrations ($22.4\,\mu M$) that were virtually identical to those not taking supplements ($22.9\,\mu M$).[12] Moreover, this finding was not due to different dietary intakes of α-tocopherol as this was similar in both groups.[8] In addition, subjects consuming vitamin E supplements of 100–230 IU achieved plasma α-tocopherol concentrations of only $27\,\mu M$ – still below the recommended optimal concentration.[12] These variable plasma responses to dietary vitamin E intake are unlikely to be due to differing fat consumption, as subjects in the VERA study and US Health Professionals Study had similar fat and mean PUFA intakes.[10,11] It is likely that the varied responses to ingested vitamin E are at least partly due to variation in a subjects' ability to absorb and/or metabolise vitamin E.

Vitamin E absorption from the small intestine is largely dependent upon the processes governing digestion of dietary fats, which is known to be incomplete, with estimates of absorption efficiency varying widely, ranging from as low as 21% to as high as 86%.[13–15] Although the efficiency of vitamin E absorption is still in doubt, it is clear that there are limits to the plasma α-tocopherol concentration that can be achieved. For example, plasma α-tocopherol concentrations rise no more than 2–3 fold, regardless of the duration, amount or frequency of dosing.[16–20] This does not appear to be due to a limitation in vitamin E absorption as Traber *et al.*[21] recently demonstrated α-tocopherol to be absorbed at a constant fractional rate with increasing dose size (≤ 150 mg). Moreover, newly absorbed α-tocopherol partly replaces old α-tocopherol in circulating lipoproteins and it is this that accounts for the apparent limitation in overall plasma concentrations observed.[21,22]

Despite a large number of reports being published which examine the impact of vitamin E supplementation on individuals' vitamin E status, relatively little information is available concerning the degree of variation between individuals in their ability to absorb and utilise vitamin E and, hence, the variation in response of plasma to supplements. This is of particular importance when considering the recommended daily and optimal intakes (RDA, ROI) for the normal population. The aim of this study was therefore to establish the degree of variation between normal individuals in their ability to absorb and distribute α-tocopherol under defined conditions and to compare this with intra-individual variation over time.

MATERIALS AND METHODS

Subjects

Thirty subjects (28 male) with an average age of 28 years (range 22–41 years) were recruited. Subjects were all healthy, non-smoking individuals, who were not taking any medication or vitamin supplements at the time of the study. The study was carried out with the approval of the ethics committee of West Lambeth Health Authority, London and all subjects gave written consent on entry.

Supplementation

Venous blood samples were provided by each subject prior to the start of the study for determination of baseline α-tocopherol and cholesterol

concentrations. Thereafter each subject was given a gelatin capsule containing 75 mg d_6-RRR-α-tocopherol acetate provided from a previously synthesised batch.[23] Subjects were instructed to take the capsule with 125 ml of skimmed milk at 07.00 hours the following morning, followed by a standard breakfast of 2 slices of lightly buttered toast and 125 ml of tea or coffee (no sugar). Subjects were advised to then follow their usual pattern of daily activities, including meals. Venous blood was taken 6, 9, 12, 27 and 51 h after ingestion of the capsule. Plasma and red blood cells (RBC) were separated by centrifugation (3000 rpm, 10 min, 4°C). Upon removal of the upper plasma layer, RBCs were separated from white cells by 3 serial washes with saline. Following the final wash, the RBC pellet was resuspended in an equal volume of pyrogallol (5 mg/ml) to prevent undue oxidation during subsequent analysis. The haematocrit of this sample was measured for normalisation of RBC data. Samples were then stored at −80°C prior to analysis.

To monitor intra-individual variation, 6 healthy, male, non-smoking individuals, with an average age of 31 years (range 26–39 years) were recruited from the above group. Subjects were given 75 mg d_6-RRR-α-tocopherol (as described above) on 5 further occasions, with a washout period of 6 months between each supplement. Blood samples were collected on each occasion 6, 9, 12, 27 and 51 h post ingestion of the capsule as described above. Samples could not be obtained in a few cases, due to illness (1 subject) and relocation (2 subjects).

Analysis

Following slow defrosting of plasma and RBC samples, d_9-α-tocopherol was added as an internal standard to assess recovery, typically 73 ± 12% for plasma and 42 ± 7% for RBC following GC-MS analysis. α-Tocopherol was extracted from plasma with hexane or from RBCs by ice-cold methanol, followed by addition of hexane. The hexane layer was removed and evaporated to dryness under a stream of nitrogen. The extract was then redissolved in methanol and subjected to HPLC as previously described.[24] The fraction containing α-tocopherol was collected and dried down under nitrogen and the residues were trimethylsilylated in preparation for GC-MS analysis.[25] The tocopheryl trimethylsilyl ethers were analysed using a Hewlett Packard benchtop model 5995 GC-MS in single-ion monitoring mode.[25] The 502 (d_0-α-tocopherol), 508 (d_6-α-tocopherol) and 511 (d_9-α-tocopherol) parent trimethylsilyl molecular ions were monitored continuously and their corresponding peak areas integrated to give the relative abundance of each α-tocopherol. The inter-assay CV was 2.6%. GC-MS was also used to check the composition of the mixtures of deuterated tocopherols given to the subjects and any deviation of the starting molar ratio (never more than 10%) from unity was corrected for in the calculation of the results. Plasma cholesterol concentrations were determined using a standard clinical test kit (BCL Ltd, Lewes, Susses, UK).

Statistics

Statistical analyses were carried out using SPSS for Windows (SPSS Inc, Chicago). One-way ANOVA was used to determine within-group and between-group variability. Areas under the curve were calculated using the KaleidaGraph program (Synergy Software, Reading, PA).

RESULTS

Inter-Individual Variation

Baseline plasma vitamin E concentrations were within the normal range for all subjects (mean 24.1 ± 5.1 μmol/l; range 14.5–37.9 μmol/l), resulting in an inter-individual coefficient of variation (CV) of 21.3%. Following ingestion of 75 mg d_6-RRR-α-tocopherol, total plasma α-tocopherol concentrations (i.e. $d_0 + d_6$) increased significantly, peaking at 12 h post ingestion (mean

$26.7 \pm 6.2\,\mu mol/l$, range 13.3–43.4 μmol/l; $p < 0.001$). These returned to baseline values by 51 h (mean $23.5 \pm 5.4\,\mu mol/l$, range 12.8–39.1 μmol/l; $p = 0.15$).

Deuterated α-tocopherol also increased significantly in plasma (C_{max} $6.05 \pm 3.77\,\mu mol/l$), peaking at 12 h post supplementation in all but 4 individuals, mirroring the trend found for total α-tocopherol concentrations. The plasma concentration of d_6-α-tocopherol at 12 h was observed to vary widely (40-fold) between individuals, ranging from 0.3 to 12.4 μmol/l – Figure 1A. This was reflected by the inter-individual CV at this time, which was 60.3% – 3 times that of the CV noted for plasma vitamin E concentration at baseline.

Concentration-time AUC profiles were calculated to estimate the extent of absorption of vitamin E for each individual. Measurement of AUC revealed wide variation between individuals' ability to absorb and distribute α-tocopherol, with values ranging from 12.9 to 493.4 μmol h/l (mean $229 \pm 143.2\,\mu M\,h$) resulting in an inter-individual CV of 62.3% – Figure 1B. The extent of this variation was not reduced following correction for circulating cholesterol (i.e. vitamin E concentration calculated as μM/mmol cholesterol) as AUC was still found to vary 40-fold with a CV of 61.7% obtained. A positive correlation was observed between the appearance of deuterated α-tocopherol in plasma using either C_{max} or AUC, and baseline total plasma vitamin E concentration (r 0.52, $p < 0.001$ and r 0.57, $p < 0.001$, respectively) – Table I. No correlation was noted with cholesterol (r 0.08, $p = 0.68$; r 0.12, $p = 0.52$) – Table I.

Baseline RBC α-tocopherol concentrations were also within the normal range, having a mean

TABLE I Correlation between uptake of d_6-α-tocopherol by plasma (determined using C_{max} or AUC) with total plasma α-tocopherol, RBC α-tocopherol and cholesterol. Associations between uptake of d_6-α-tocopherol in RBC and total RBC α-tocopherol, plasma α-tocopherol and uptake of d_6-α-tocopherol by plasma are also shown

	C_{max}		AUC	
	r value	p value	r value	p value
Uptake of d_6-α-tocopherol by plasma				
Total plasma α-tocopherol	0.52	< 0.001*	0.57	< 0.001*
Total RBC α-tocopherol	0.45	0.012*	0.43	0.016*
Cholesterol	0.08	0.68	0.12	0.52
Uptake of d_6-α-tocopherol by RBC				
Total RBC α-tocopherol	0.29	0.12	0.01	0.94
Total plasma α-tocopherol	0.09	0.64	0.10	0.59
Uptake of d_6-α-tocopherol by plasma	0.15	0.44	0.16	0.41

*indicates significant correlation.

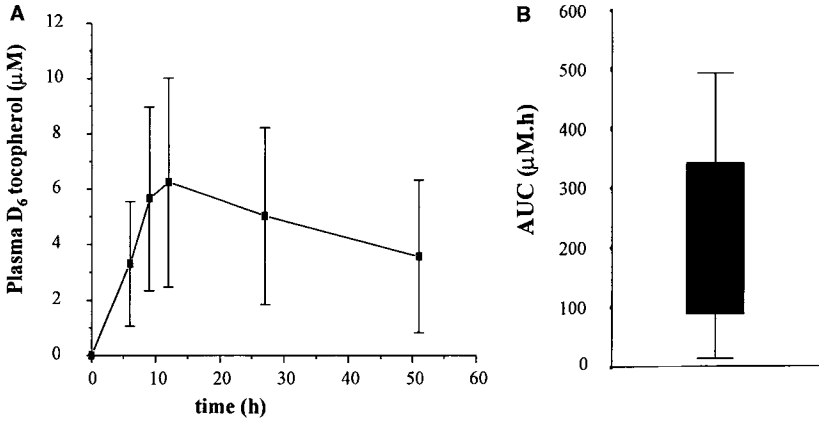

FIGURE 1 (A) Plasma deuterated α-tocopherol concentrations following ingestion of 75 mg d_6-RRR-α-tocopherol acetate. Results are expressed as mean ± sd; $n = 30$. (B) Plasma deuterated α-tocopherol area under the curve calculated from the concentration-time profile obtained for each individual $n = 30$.

of 6.2 ± 1.4 μmol/l packed RBC and ranging from 4.2 to 9.7 μmol/l packed RBC. The inter-individual CV (21.3%) was similar to that found for plasma. However, no correlation was observed between RBC α-tocopherol concentrations and that of plasma (r 0.21; $p = 0.26$).

Following administration of 75 mg d_6-α-tocopherol, RBC total α-tocopherol concentrations increased significantly, peaking 27 h post ingestion (mean 7.5 ± 1.6 μmol/l packed RBC, range 5.0–11.1 μmol/l packed RBC; $p < 0.001$). RBC total α-tocopherol concentrations remained significantly higher than baseline values at 51 h post ingestion (mean 7.1 ± 1.4 μmol/l packed RBC, range 4.6–10.8; $p < 0.001$). RBC d_6-α-tocopherol concentrations also peaked at 27 h in all individuals. However, again a wide variation (6-fold) was noted in the extent of uptake, with d_6-α-tocopherol concentrations ranging from 0.60 to 4.09 μmol/l packed RBC at 27 h – Figure 2A. Similarly, the variation in AUC between individuals was large (6-fold), with values ranging from 24.4 to 146.1 μM h/l packed RBC – Figure 2B. Inter-individual CV for uptake of d_6-α-tocopherol by RBC at 27 h was found to be similar to that observed in plasma (57.1% vs 62.3%). No correlation was noted between total RBC α-tocopherol and d_6-α-tocopherol concentrations calculated using either C_{max} or AUC (r 0.29, $p = 0.12$ and r 0.01, $p = 0.94$, respectively). Nor was any association found between uptake of d_6-α-tocopherol by RBC and either the appearance in plasma of deuterated α-tocopherol or with total plasma α-tocopherol concentrations (r 0.16, $p = 0.41$ and r 0.10, $p = 0.59$, respectively) – Table I. However, RBC total α-tocopherol concentration was found to be positively correlated to the appearance of d_6-α-tocopherol in plasma using C_{max} or AUC (r 0.45, $p = 0.012$ and r 0.43, $p = 0.016$, respectively) – Table I.

Intra-Individual Variation

Plasma α-tocopherol concentration varied significantly between the 6 subjects ($p < 0.0001$), whereas an individual's plasma α-tocopherol concentration was relatively stable over time (CV ranging from 2.0% to 5.4%) – Figure 3A. Cholesterol correction had no impact on this variability within an individual over time, as an intra-individual CV range of 2.7–6.2% was obtained and a significant difference also remained between subjects ($p < 0.0001$).

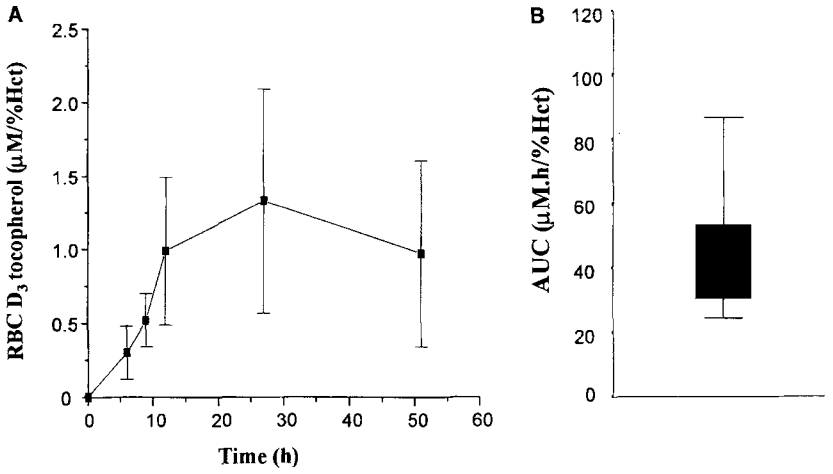

FIGURE 2 (A) RBC deuterated α-tocopherol concentrations following ingestion of 75 mg d_6-RRR-α-tocopherol acetate. Results are expressed as mean ± sd; $n = 30$. (B) RBC deuterated α-tocopherol area under the curve calculated from the concentration-time profile obtained for each individual $n = 30$.

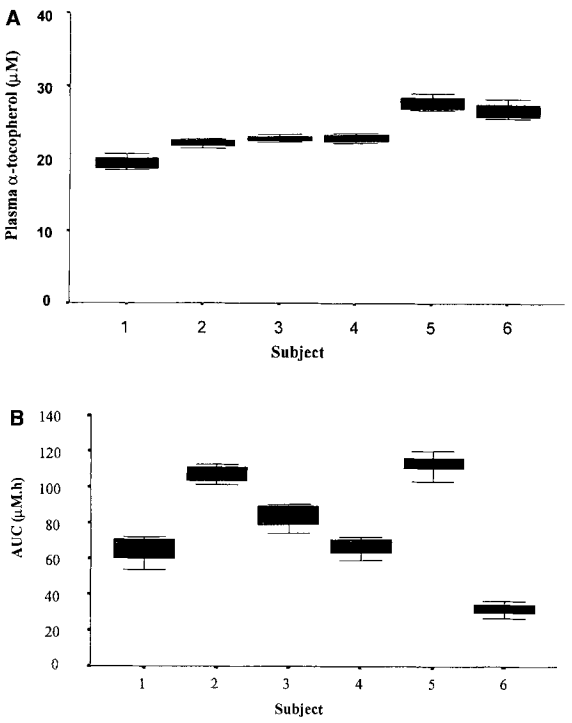

FIGURE 3 (A) Variation in plasma total concentrations of 6 subjects at 6 monthly intervals over a 30-month period. α-Tocopherol was measured prior to dosing with d_6-RRR-α-tocopherol acetate. $n=6$ (except for subjects 3, 4 and 6 where $n=5$). (B) Plasma deuterated α-tocopherol area under the curve. Area under the curve was calculated for each individual from the concentration-time profile obtained following each supplementation period.

Plasma concentrations of d_6-α-tocopherol were observed to rise at the start of each supplementation period, with C_{max} again reached at 12 h in all subjects. The extent of vitamin E absorption in each subject (as measured by AUC) varied only slightly between each supplementation period, with the intra-individual CV ranging from 5.1% to 13.9%, which is approximately 4-fold less than that observed between individuals on each visit (39.4%) – Figure 3B. Calculation of the within group, and between group, variability revealed a significant difference in vitamin E uptake between individuals ($p<0.0001$), with intra-individual variation being markedly lower than variation between subjects ($p<0.0001$).

DISCUSSION

The data obtained in the present study suggest that healthy subjects have widely varying ability to transfer α-tocopherol into plasma and RBCs. These findings are consistent with previous smaller studies,[26] in which similar variability in the biokinetics of α-tocopherol derived from the esterified forms of vitamin E were noted. In all these studies, the absolute accuracy of the findings depend, in part, on the assumption that the affinity of the tocopherol transfer protein (TTP) does not differ between the deuterated and non-deuterated forms of α-tocopherol. We feel this is unlikely to be the case and it is not a problem when considering inter-individual differences in deuterated α-tocopherol uptake as undertaken in this study.

A reduction in the ability to absorb vitamin E, due either to a decrease in pancreatic esterase activity or modification in its processing by the liver could underlie the observed variability in vitamin E handling. The most obvious candidate responsible for the wide variation in vitamin E appearance in plasma is the TTP protein. TTP plays a key role in vitamin E distribution and appears to be the key element in the preferential distribution of α-tocopherol.[30–32] The expression of TTP as a function of vitamin E status has been investigated in a number of studies. Fechner *et al.*[33] reported TTP expression to be increased in vitamin E deficient rats following supplementation with vitamin E, although initial depletion of vitamin E had no apparent effect on the expression of TTP mRNA.[33] Kim *et al.*[34] demonstrated TTP mRNA to be significantly increased in vitamin E depleted rats and TTP mRNA and protein expression to be decreased in vitamin E replete rats. It therefore appears that vitamin E status may alter expression of TTP and hence vitamin E distribution in plasma. Further studies are, however, required as these results are not conclusive. In the present study, subjects had a wide range of baseline vitamin E values, although all fell within the normal range. In this group,

the amount of d_6-α-tocopherol distributed into plasma was found to correlate positively with the existing vitamin E concentration. Hence, variation in the expression of TTP, in response to individuals' vitamin E status, may partly explain the wide variation in uptake of vitamin E observed.

Vitamin E absorption and utilisation are influenced by a number of luminal and physiological factors. Animal bioassays have shown the choice of vehicle used to deliver the test compound to be an important variable.[27] Increased oxidation and subsequent destruction of free α-tocopherol in the intestine in the presence of the unsaturated fatty acids may occur, resulting in reduction in the amount of α-tocopherol available for absorption. In addition, solubilisation of the vitamin in medium-chain compared to long-chain triglycerides enhances absorption of the vitamin, possibly by influencing the formation of micelles required for absorption.[28,29] Therefore dietary lipids present during absorption of vitamin E may influence the efficiency of α-tocopherol absorption. As subjects in the VERA study and US Health Professionals Study had a similar fat and mean PUFA intake, yet displayed varying plasma responses to similar vitamin E doses,[10,11] dietary fat intake alone cannot account for the variation in vitamin E bioavailability. This conclusion is supported by the present study findings, in which considerable inter-individual variation in α-tocopherol uptake was noted, despite subjects receiving a standard breakfast. Furthermore, the vitamin E ingested was administered as an acetate ester, which would help minimise α-tocopherol oxidation. Therefore, the observed variation in vitamin E uptake is unlikely to result from varying extents of oxidation of vitamin E in the gastrointestinal tract.

A number of studies have demonstrated plasma α-tocopherol concentrations to increase with age. Typically, investigators have examined subjects with ages ranging from 20 to >100 years.[35–37] This age-related increase is thought to be due to alteration in vitamin E bioavailability or metabolism. Borel *et al.*[37] recently reported an increased uptake of α-tocopherol into the plasma of elderly (64–72 years) compared to young (20–30 years) subjects. In the present study, in which subjects were all relatively young, no correlation between subjects' age and their ability to absorb vitamin E into the plasma was observed.

Other factors may contribute to the variability observed such as the amount of oxidative stress an individual experiences. This effect can arise both through the utilisation of vitamin E as an antioxidant and because oxidative stress may affect many of the intracellular and extracellular mechanisms which regulate the transport of vitamin E into and out of tissues. The potential influence of oxidative stress is supported by recent findings, which demonstrate that vitamin E bioavailability is altered in smokers.[24] However, as all volunteers in the present study were healthy non-smoking individuals, oxidative stress is unlikely to have contributed to the observed variation in α-tocopherol uptake.

Vitamin E uptake and utilisation may also be enhanced by the presence of other micronutrients, which recycle α-tocopherol. Efficient regeneration of oxidised α-tocopherol may result in increased α-tocopherol concentrations in those subjects with increased concentrations of recycling agents. A number of reducing agents have been proposed to play such a role, including vitamin C, ubiquinone and glutathione.[38–40] These may all influence vitamin E bioavailability and if so, an individual's vitamin E requirement may also depend partly on the availability of these nutrients. Although such interactions have been clearly demonstrated in *in vitro* models, direct and convincing evidence for their occurrence *in vivo* is still lacking.[38,39,41–44] Further work is therefore required to ascertain the importance of co-nutrients on the bioavailability of vitamin E. Nevertheless, interactions between vitamin E and other antioxidants/co-factors provide a plausible explanation for the some of the variation in vitamin E uptake.

The appearance of newly ingested vitamin E in plasma correlated positively with baseline total plasma α-tocopherol, suggesting a possible relationship between the two. It is likely that the factors governing the appearance of vitamin E in plasma also determine overall plasma α-tocopherol concentrations. Hence an individuals' ability to absorb and utilise a defined dose of vitamin E will also influence their plasma vitamin E concentrations. This observation is supported by results from a number of studies examining the relationship between vitamin E intake and plasma α-tocopherol concentrations, from which a wide degree of variation between studies was noted.[10–12] Given this, a subject's ability to absorb and process vitamin E should be taken into account when considering the dose required to provide optimal plasma concentrations of vitamin E for that individual. This value, once ascertained for an individual, is likely to be relatively stable over time as α-tocopherol uptake was found to be comparatively constant when measured over a 30-month period. No correlation was observed between the uptake of newly ingested α-tocopherol and RBC α-tocopherol status, demonstrating that the ability of RBC to acquire vitamin E was independent of its existing α-tocopherol status. Total RBC α-tocopherol status was, in fact, positively correlated to the appearance of deuterated α-tocopherol in plasma, suggesting some relationship between these pools.

In conclusion, vitamin E uptake and appearance in plasma and RBC varies widely between individuals. In contrast, variation within an individual is minimal in comparison and hence, comparatively stable over time. Variation in the ability of a subject to absorb and distribute vitamin E is likely to be influenced by the expression of TTP and possibly one or more other unknown biological factors. Determinants of vitamin E handling must be more fully understood before the recommended optimal intake, which is required to achieve plasma concentrations desirable for protection against free-radical mediated diseases, can be determined.

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