Research report

Effect of vitamin E intake on levels of vitamins E and C in the central nervous system and peripheral tissues: implications for health recommendations

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Abstract

Vitamin E (\(\alpha\)-\(\gamma\)-tocopherol) is an important component in biological membranes. A decrease in its concentration imposes structural and functional damage to the cells. The object of this study was to assess the effect of a graded dietary vitamin E (E) intake on E concentration in specific regions of the brain, and its influence on vitamin C levels and neurological function. Following a 2-month period, rats supplemented with 5, 30, 60, 250 or 500 mg all-rac-\(\alpha\)-tocopherol-acetate/kg diet (mg E/kg diet) exhibited a significant increase of E concentration in brain and peripheral tissues. However, while blood and liver showed a dose response increase in E concentration which correlated well with the different levels of E in the diet, the central nervous system (CNS) followed the same pattern of increase of vitamin E in brain tissue only when the diet was supplemented with 5, 30, or 60 mg E/kg diet. No further increase in E concentration was observed when the diet was supplemented with 250 or 500 mg E/kg diet. Similarly, the heart tissue showed a significant increase in its E concentration when the was enriched with 5, 30, or 60 mg E/kg diet, with no further increases at 250 or 500 mg. Vitamin C concentration in brain cortex and cerebellum, plasma, liver, and heart was reduced in the groups receiving 250 or 500 mg E/kg diet. Compared to the low E group, rats supplemented with the 60, 250 or 500 mg E/kg diet showed a significant enhancement in striatal dopamine (DA) release, but no differences were observed among the latter three groups. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Ascorbate; \(\alpha\)-Tocopherol; Brain; Liver; Heart, plasma

1. Introduction

Nerve cells are the primary site of pathology in the central nervous system (CNS) of E deficient animals, and in humans with very low serum E concentrations [37]. Several studies have shown a clear association between neuropathological abnormalities and E deficiency, and have suggested that the CNS requirements may change during aging [28,34,39,42,43]. In fact, some studies have shown that the E concentration in Alzheimer’s patients plasma and cerebro-spinal fluid is decreased [49,54], and that their brain levels are reduced by 35% compared to adult controls [29]; in Alzheimer’s patients with moderate to severe impairment, treatment with vitamin E slowed the progression of the disease [42]. Such findings indicate that this nutrient plays a critical role in maintaining the structure and/or metabolic integrity of nerve cells [14,37,42,45]. In vitro and in vivo studies have shown that E acts both as an antioxidant [14,24,25,37], and as a modulator of cell function by regulating specific enzymatic activities, membrane properties, and signaling elements [3,22,48,50]. In the animal model, E has been shown, in some studies, to inhibit lipid peroxidation [44], but the relationship between E concentration in tissues and lipid peroxidation is weak or nil [31,35,46]. Vitamin E has also been shown to reduce degeneration of hippocampal cells following ischemia [13], and enhance the recovery of neuronal damage of motor function after spinal cord injury [2]. These studies suggest that E may have other biological functions with a mechanism not rooted in its antioxidant effect, but rather in the physical–chemical interaction with the components of the cell’s membrane. For example, in humans, recent studies have indicated that E plays an important role in cardiovascular diseases, immune response, and neurodegenerative disease [2,33,42,47].

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During aging, a decline in both cognitive and motor functions has been observed, but the mechanisms involved in these declines are not well understood [19]. Changes related to the alteration of neurotransmitter receptor sensitivity, such as the dopaminergic receptors, have been observed, as have changes related to a reduction of dopamine (DA) release [18,38,41]. How E is involved in the maintenance of brain function, and how its reduction in brain tissue contributes to the development of neurologic disorders, remains unknown. In an effort to understand the basis of neuropathology of E, diverse studies have examined the pattern of E distribution in different regions of the brain [7,30,31,51,52]. There is a unanimous accord on the positive effect of dietary E intakes on E concentration in tissues. But how much E intake is required to reach optimum incorporation into tissues, in particular in the CNS, remains undetermined. Several studies, nonetheless, have indicated that E intake much higher than the intake necessary to meet the “arbitrary” recommendations has been shown to beneficially contribute to different aspects of human health [14]. The concentration of E in human brain, as reported by Metcalfe et al. [29], parallels relative well the range that we and others have observed in brain, as reported by Metcalfe et al. [29].

It is not known how much E is necessary to obtain maximal incorporation into tissues, particularly into the CNS, and how changes in E concentration could affect C levels and neurological function. Therefore, in the present study, we investigated the response of the CNS and peripheral tissues to a graded dietary vitamin E supplementation. Since the rat’s C demands are supplied through regulation of its own synthesis, mainly in liver [9,11,12], we also examined if high E intake could affect C concentration in brain and peripheral tissues.

2. Materials and methods

2.1. Dietary vitamin E supplementation

Forty male Fischer 344 rats (Harlan Sprague Dawley, Indianapolis, IN) were used to investigate the effect of diets (modified AIN-93) [19,40] supplemented with different concentrations of vitamin E (5, 30, 60, 250, 500 mg all-rac-α-tocopherol-acetate/kg diet). Based on HPLC analysis of the diet in our laboratory, unsupplemented diet contained 20 mg all-rac-α-tocopherol/kg diet. The diets were formulated by Research Diets (New Brunswick, NJ). The rats were individually housed in stainless steel mesh suspended cages, provided food and water ad libitum and maintained on a 12-h light/dark cycle. All animals were observed daily for clinical signs of disease. These animals were utilized in compliance with all applicable laws and regulations as well as principles expressed in the National Institutes of Health, USPHS, Guide for the Care and Use of Laboratory Animals. The Animal Care and Use Committee of our Center approved this study. Following a 12-day acclimatization period to the facility, the 6 month old rats were weight-matched and given 2 weeks on the low vitamin E diet (5 mg/kg diet). They were then divided into five diet groups (eight animals per group). Monthly weights and food intakes over a 48-h period were recorded for all diet groups. At 8 months of age, the various diet groups were examined for differences in various indexes to indicate responses induced by the different dietary treatments. These include indexes for: body weight, food intakes, distribution of vitamins E and C, and DA release.

2.2. Quantification of vitamin E

Vitamin E (α-tocopherol) content of plasma or tissues was measured by reverse-phase high performance liquid chromatography (HPLC) as previously described by Martin et al. [26]. Briefly, 100 μl of plasma sample or 100 μl of homogenized tissue was mixed with 100 μl ethanol; after vortexing, tocopherols were extracted into 500 μl hexane containing 0.002% butylated hydroxyl toluene (BHT) (Sigma, St. Louis, MO). Tocotrienol (a gift from Hoffmann-La Roche, Nutley, NJ) was added to the mixture as an internal standard. Samples were centrifuged at 800 rpm for 5 min at 4°C. The supernatant was collected and dried under a stream of nitrogen gas, and reconstituted in 100 μl of methanol. Tocopherols were separated by HPLC using a 3 μm C18 reverse phase column (Perkin-Elmer, Norwalk, CT). The mobile phase, delivered at a flow rate of 1.2 ml/min, consisted of 1% water in methanol, containing 20 mM lithium perchlorate. Samples were injected with an autosampler (1100 series, Hewlett Packard, Wilmington, DE). Eluted peaks were detected at an applied potential of +0.6 V by a LC 4B amperometric electrochemical detector (Bioanalytical Systems, West Lafayette, IN). Tocopherols eluted as well-separated peaks with retention times between 2 and 6 min. Peaks were integrated using a ChemStation software (Hewlett Packard); α-tocopherol concentration was expressed in pmol/mg protein. Protein was measured by the method of Lowry et al. [21].

2.3. Quantification of ascorbate

Ascorbate was analyzed by paired-ion, reversed-phase HPLC coupled with electrochemical detection as previously described by Martin and Frei [23]. In brief, 100 μl of plasma sample was mixed with an equal volume of cold 5% (w/v) metaphosphoric acid containing 1 mmol/L of the metal ion chelator diethylenetriaminepentaaetacetic acid (Sigma), or 40–100 mg of tissue was homogenized in 500 μl of the same solution and centrifuged to remove the precipitated proteins. An aliquot of the supernatant was
chromatographed on an LC8 column (150 mm × 4.6 mm i.d., 3 μm particle size) (Supelco, Bellefonte, PA) using 99% deionized water and 1% methanol containing 40 mmol/l sodium acetate and 1.5 mmol/l dodecyltriethylammonium phosphate (Q12 ion pair cocktail, Regis, Morton Grove, IL) as the mobile phase. Samples were injected with an autosampler, 1100 series (Hewlett Packard). Ascorbate was detected at an applied potential of +0.6 V by a LC 4B amperometric electrochemical detector (Bioanalytical Systems). Ascorbate eluted as a single peak with a retention time of 5.5 min. Peaks were integrated using a ChemStation software (Hewlett Packard).

Fig. 1. (A,B,C) α-Tocopherol and γ-tocopherol concentration (mean ± S.D.) following 2 months dietary vitamin E supplementation using a modified AIN-93 diet supplemented with 5, 30, 60, 250, or 500 mg E/kg diet, in the cortex (A), hippocampus (B), cerebellum (C). For these figures, a differs from the supplemented groups receiving 30, 60, 250, and 500 mg E/kg diet (p < 0.05 and p < 0.001, respectively). c differs from group supplemented with 30 mg vitamin E (p < 0.05). Groups with a common letter superscript are not significantly different from the other treatments (p > 0.05) (n = 8 per group).
Fig. 1 continued.

Ascorbate concentration was calculated based on a calibration curve, and its concentration was expressed in nmol/mg protein. Protein was measured by the method of Lowry et al. [21].

2.4. Statistical analysis

Results were expressed as mean ± S.D. The statistical analysis of the data — α- and γ-tocopherol in the different brain regions, heart, and plasma; concentrations of vitamin C in the different brain regions, heart and plasma; and the release of DA — was done by ANOVA comparison, using the Fisher test for post-hoc analysis. Statistical significance was accepted at the level of \( p < 0.05 \).

3. Results

3.1. Weights and food intakes

Rat weights significantly increased with age from an average of 359 ± 25 (6 months) to 401 ± 21 g (8 months) (\( p < 0.001 \)). However, there were no differences in weights between the different dietary treatments over time or at the age of 8 months. There were also no differences in food intakes between the dietary treatments over the course of the study.

3.2. Vitamins E and C in brain (cortex, hippocampus and cerebellum)

Levels of E in the brain were significantly affected after 2 months of dietary supplementation with 5, 30 or 60 mg E/kg diet, respectively. α-Tocopherol in the cortex of the animals fed 5, 30, or 60 increased significantly in a dose response manner, reaching 296 ± 104, 570 ± 135, and 807 ± 192 pmol/mg protein, respectively (\( p < 0.001 \)) (Fig. 1A). Levels of α-tocopherol in the high E groups fed 250 or 500 mg E/kg diet were 756 ± 200 and 767 ± 173 pmol/mg protein, were not significantly different from the concentrations attained when the diet contained 60 mg E/kg diet. The concentrations of γ-tocopherol did not differ significantly across all the groups with 17 ± 5, 16 ± 10, 14 ± 8, 39 ± 15 and 20 ± 15 pmol/mg protein, respectively (Fig. 1A).

α-Tocopherol in the hippocampus also increased significantly in a dose response manner among the groups fed 5, 30 or 60 mg E/kg diet. Their α-tocopherol concentrations were 287 ± 142, 507 ± 171, and 734 ± 128 pmol/mg protein, respectively (\( p < 0.001 \)) (Fig. 1B). However, the levels of α-tocopherol attained by this brain region after supplementation with 250 or 500 mg E/kg diet were 765 ± 211 and 956 ± 302 pmol/mg protein, respectively, which were not different from the levels attained when animals were fed 60 mg E/kg diet. The concentrations of γ-tocopherol was not significantly different across all the groups with 33 ± 21, 33 ± 15, 44 ± 36, 23 ± 11, and 34 ± 24 pmol/mg protein, respectively (Fig. 1B).

α-Tocopherol in the cerebellum also increased significantly in a dose response manner only between the groups fed 5 or 30 mg E/kg diet. The concentrations of α-tocopherol was 169 ± 63 and 279 ± 99 pmol/mg protein, respectively (\( p < 0.001 \)) (Fig. 1C), and 431 ± 171 and 380 ± 144 pmol/mg protein, respectively, for the animals fed 250 or 500 mg E/kg diet, which were not significantly different from the concentration obtained when animals...
were fed 60 mg (356 ± 156). γ-Tocopherol concentrations were not different among the different groups with values of 15 ± 7, 13 ± 5, 13 ± 5, 14 ± 2, and 15 ± 7, respectively (Fig. 1C).

Thus, the CNS accumulated different concentrations of E when the diet was supplemented with different levels of E up to 60 mg E/kg diet, and different regions of the brain accumulate distinct amounts of E. The cerebellum had significantly lower concentrations compared to the cortex or hippocampus (p < 0.05). In addition to α-tocopherol, other E isomers such as γ-tocopherol were also present in brain in significant concentrations, with an average across all groups of about 30 ± 18 pmol/mg protein. The concentrations of γ-tocopherol was not significantly different across all the groups (Fig. 1A–C). In general, a significant variability in the absorption of E among individuals is observed. The reason for these differences is because the incorporation of E into tissues is regulated by several factors including lipoprotein metabolism, receptor and receptor independent, low density lipoprotein and other lipoprotein metabolism [15,50]. Thus, the amount of E taken up by various tissues is not uniform, and may explain the diversity in individual tissue-responses observed to some similar amounts of E ingested.

Vitamin C concentrations in the cortex were not different in the groups supplemented with 5, 30, or 60 mg E/kg diet with 14.7 ± 3.7, 14.2 ± 3.2, and 11.4 ± 3.2 nmol/mg protein, respectively. However, the groups fed 250 or 500 mg E/kg diet showed a significant reduction in ascorbate concentration compared to groups supplemented with 5 or 30 mg E/kg diet, with 10.3 ± 2.5 and 10.9 ± 2.4 pmol/mg protein, respectively (p < 0.05) (Table 1). Vitamin C concentration in hippocampus and cerebellum were in general not significantly different among the different treatments (Table 1); only cerebellum showed lower C levels in the group supplemented with 500 mg E/kg diet compared to the group fed 30 mg E/kg diet (p < 0.05) (Table 1).

3.3. Vitamins E and C in plasma

After 2 months of dietary vitamin E supplementation with 5, 30, 60, 250, or 500 mg E/kg diet, respectively, concentrations of α-tocopherol increased significantly in a dose response manner, reaching 5.1 ± 0.8, 19.9 ± 4.5, 27.8 ± 6.0, 41.2 ± 8.4, and 61.2 ± 22.1 µmol/l, respectively (p < 0.001) (Fig. 2). No differences were observed with respect to γ-tocopherol concentration between groups, 1.7 ± 0.7, 2.5 ± 2.2, 1.9 ± 1.3, 2.8 ± 1.9, and 2.4 ± 1.4 µmol/l, respectively. Plasma ascorbate concentrations were similar in the groups fed 5, 30, or 60 mg E/kg diet with 22.4 ± 1.3, 23.9 ± 2, 22.2 ± 2.2 µmol/l, respectively. However, rats fed diets containing 250 or 500 mg E/kg diet showed significant reduction of plasma concentration with 19.8 ± 2.6 and 17.7 ± 2.4 µmol/l, respectively (p < 0.05) (Table 1).

3.4. Vitamins E and C in liver

Following dietary vitamin E supplementation with 5, 30, 60, 250, or 500 mg E/kg diet, respectively, concentrations of α-tocopherol in liver increased significantly in a dose response manner, reaching 80.0 ± 0.20, 405.0 ± 137.0, 843.0 ± 322.0, 2155.0 ± 859.0, and 3975.0 ± 1400.0 pmol/mg protein, respectively (p < 0.001) (Fig. 3). No differences were observed regarding γ-tocopherol concentration with 33.0 ± 16.7, 13.0 ± 7.1, 15.0 ± 9.3, 28.0 ± 10.4, and 58.8 ± 24 pmol/mg protein, respectively, but animals fed the higher vitamin E showed a trend toward higher γ-tocopherol concentration. Ascorbate concentrations were significantly reduced in those groups supplemented with 5 or 500 mg E/kg diet, with 2.2 ± 0.6 and 2.8 ± 0.3, as compared to the animals supplemented with 30, 60, or 250 mg which had 3.6 ± 0.7, 3.5 ± 0.5, and 3.0 ± 0.9, respectively (p < 0.05) (Table 1).

3.5. Vitamins E and C in heart

α-Tocopherol in the heart increased in a dose dependent manner in the groups fed 5, 30, or 60 mg all-rac-α-tocopherol-acetate/kg diet with 86.4 ± 48.9, 338.8 ± 120.4, and 617.5 ± 285 pmol/mg protein, respectively (p < 0.001). However, animals receiving a high E diet containing 250 or 500 mg E/kg diet increased their vita-

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Dietary treatment</th>
<th>E5</th>
<th>E30</th>
<th>E60</th>
<th>E250</th>
<th>E500</th>
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<tr>
<td>Cortex (nmol/mg protein)</td>
<td>14.7 ± 3.7*</td>
<td>14.2 ± 3.3*</td>
<td>11.4 ± 3.2ab</td>
<td>10.3 ± 2.5b</td>
<td>10.9 ± 2.4b</td>
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<tr>
<td>Cerebellum (nmol/mg protein)</td>
<td>10.2 ± 3.6ab</td>
<td>12.0 ± 3.6b</td>
<td>11.0 ± 3.5ab</td>
<td>8.5 ± 1.8a</td>
<td>8.6 ± 2.4a</td>
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<tr>
<td>Hippocampus (nmol/mg protein)</td>
<td>11.0 ± 3.1a</td>
<td>12.1 ± 2.6a</td>
<td>14.0 ± 4.7a</td>
<td>10.8 ± 1.3b</td>
<td>12.5 ± 2.4a</td>
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<tr>
<td>Plasma (µM)</td>
<td>22.4 ± 1.3a</td>
<td>24.0 ± 2.0a</td>
<td>22.2 ± 2.2a</td>
<td>19.8 ± 2.6b</td>
<td>17.7 ± 2.4b</td>
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<tr>
<td>Liver (nmol/mg protein)</td>
<td>2.2 ± 0.6a</td>
<td>3.6 ± 0.7b</td>
<td>3.5 ± 0.5b</td>
<td>3.0 ± 0.9abc</td>
<td>2.8 ± 0.3bc</td>
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<tr>
<td>Heart (pmol/mg protein)</td>
<td>62.0 ± 23.0a</td>
<td>209.0 ± 120.1b</td>
<td>144.0 ± 77.0b</td>
<td>63.0 ± 59.0a</td>
<td>61.0 ± 71.0a</td>
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Each value represents mean ± S.D. of ascorbic acid (nmol or pmol/mg protein in tissue; µmol/l in plasma). Number of rats tested were eight per group. Means with a different letter superscript within each tissue per treatment are different from the other treatments (p < 0.05). Means with a common letter superscript are not significantly different (p > 0.05).
Fig. 2. α-Tocopherol and γ-tocopherol concentration (mean ± S.D.) in plasma following 2 months dietary supplementation with a modified AIN-93 diet supplemented with 5, 30, 60, 250, or 500 mg E/kg diet. For this figure, a differs from the supplemented groups receiving 30, 60, 250, and 500 mg E/kg diet (p < 0.01, p < 0.001, p < 0.001, and p < 0.001, respectively). c differs from groups supplemented with 30, 250, or 500 mg (p < 0.01, p < 0.001, and p < 0.001, respectively). e differs from groups supplemented with 250 mg E/kg diet (p < 0.05) (n = 8 per group).

min E concentration to similar levels achieved when animals were fed 60 mg only, with 655.0 ± 405.3 and 798.8 ± 575.0 pmol/mg protein, respectively (Fig. 4). γ-Tocopherol concentration was similar across all groups with an average of 18.5 ± 11.8 pmol/mg protein (Fig. 4). Ascorbate concentration in the heart of the rat is normally

Fig. 3. α-Tocopherol and γ-tocopherol concentration (mean ± S.D.) in liver following 2 months dietary supplementation with a modified AIN-93 diet supplemented with 5, 30, 60, 250, or 500 mg E/kg diet. For this figure, a differs from the supplemented groups receiving 30, 60, 250, and 500 mg E/kg diet (p < 0.01, p < 0.001, p < 0.001, and p < 0.001, respectively). c differs from groups supplemented with 30, 250, or 500 mg E/kg diet (p < 0.01, p < 0.001, and p < 0.001, respectively). e differs from group supplemented with 250 mg E/kg diet (p < 0.05) (n = 8 per group).
Fig. 4. α-Tocopherol and γ-tocopherol concentration (mean ± SD) in heart following 2 months dietary supplementation with a modified AIN-93 diet supplemented with 5, 30, 60, 250, or 500 mg E/kg diet. For this figure, a differs from the supplemented groups receiving 30, 60, 250, and 500 mg E/kg diet (p < 0.01, p < 0.001, p < 0.001, and p < 0.001, respectively). c differs from group supplemented with 30 mg E/kg diet (p < 0.05). Groups with a common letter superscript are not significantly different from the other treatments (p > 0.05) (n = 8 per group).

less than 1 nmol/mg protein, but decreased even further to 62.0 ± 23.0, 63.0 ± 59.0, and 61.0 ± 71.0 pmol/mg protein in the group fed 5, 250, or 500 mg E/kg diet. Comparatively, the animals fed 30 or 60 mg had higher concentrations of 209.0 ± 120 and 144 ± 77 pmol/mg protein, respectively (p < 0.05) (Table 1).

4. Discussion

The establishment of vitamin E recommendations has been made without considering its possible role in enhancing important body functions and in preventing chronic disease. The data described here show that the CNS can incorporate vitamin E in dose–response manner when the animals were dietary supplemented with E levels ranging between 5 and 60 mg/kg diet, with a maximal incorporation of vitamin E achieved when animals were supplemented with 60 mg E/kg diet. Basal diets provide about 10–20 mg E (n-α-tocopherol)/kg diet which corresponds, in this animal model, to an intake of 0.4–0.8 mg E/kg b.wt. Indeed, a concentration significantly lower than the optimum level to achieve maximum brain concentration and enhanced brain function [19]. This level of E (80 mg E/kg diet (60 supplement + 20 diet)) intake corresponds to 2–3 mg E (kg b.wt.)⁻¹ day⁻¹.

Vitamin E recommendations have been established based on customary intakes from United States food sources, a practical allowance of 10 mg α-tocopherol/day for male adults and 8 mg for female adults, or what would correspond to 0.140 mg E (kg b.wt.)⁻¹ day⁻¹. This is an intake that is much lower than the levels suggested by different human studies of 100–200 mg E/day. Thus, the impact on tissue performance from a long term average dietary E intake of 0.140 mg (kg b.wt.)⁻¹ day⁻¹ compared to 1.5–3.0 mg E (kg b.wt.)⁻¹ day⁻¹ may be relevant and needs further investigation.

Indeed, a dietary intake of 80 mg/kg diet (2.4 mg (kg b.wt.)⁻¹ day⁻¹) may be the optimum E intake to increase the CNS maximally, in this animal model. Interestingly, vitamin E supplementation in humans has shown that a 100–200 IU (100–200 mg)/day intake of vitamin E (equivalent to 1.5–3.0 mg (kg b.wt.)⁻¹ day⁻¹) would be sufficient to maximally enhance certain physiological functions [33]. These findings are in agreement with previous studies supporting the hypothesis that the level of dietary vitamin E may have tremendous impact on tissues’ α-tocopherol concentration and on tissue function. The intake of E at this optimal level has been shown to increase relevant indexes of immune response [32,33], and decrease the risk of cardiovascular diseases [47]. By maintaining good dietary habits and eating a diet containing sufficient amounts of oils, humans may be taking about 30 mg vitamin E/day; corresponding to only 0.40 mg/kg b.wt. (assuming an average of 70 kg total b.wt.), an intake which still appears insufficient to attain maximum concentration of this nutrient in tissues.
Most of the studies are generally short, with rather elderly subjects, a problem, since vitamin E is a liposoluble nutrient and its transport and delivery into the lipid compartment of the cell’s membrane is complex. It is also important to be aware of the E content of the diet used in experiments to investigate the role of this nutrient on brain function. Unfortunately, most of the diets are already enriched with optimum E levels and no further increases in E concentration can be achieved when diets are supplemented with high levels of E.

Our findings that different regions of the brain accumulate different concentrations of α-tocopherol, and that the nervous system does not accumulate α-tocopherol above a certain concentration, even though the levels in plasma keep increasing, are relevant, given the important role that α-tocopherol seems to play in brain function [6,36,37]. Various studies using concentrations up to 1000 mg E/kg diet observed that the amount of vitamin E incorporated into the brain is limited in comparison to peripheral tissues [17,31,52]. Some studies have supported the hypothesis that the brain contains a low level of vitamin E comparable to other tissues [20,30], this may hold true only for diets containing much higher levels than 80 mg E/kg diet. In fact, relative to other tissues including liver and heart, rats fed 80 mg E/kg diet show a concentration of about 800 pmol/mg protein in cortex, while liver and heart tissues show 843 and 618 pmol/mg protein, respectively (see Section 3 for details). Our data has clearly shown the brain incorporates vitamin E in a dose response manner when the amount of E in the diet is up to 80 mg E/kg diet. And such differences in E levels in response to the dietary content seems to play an important role in the CNS in regard to physiological functions.

Putting all these observations together strongly suggests that an optimum intake of vitamin E on a daily basis may be crucial to maintain maximum levels of vitamin E and an optimum brain performance. For example, the striatum region is highly active metabolically, and several neurological operations depend of its performance, making it the most vulnerable to vitamin E deficiency. In fact, compared to diets supplemented with 5 or 30 mg E/kg diet (low E intake), diets enriched with 60 mg or higher vitamin E/kg diet showed an enhanced DA release by 160% from striatal slices following oxotremorine stimulation, no additional increases in DA release being observed when the diet contained 250 or 500 mg E/kg diet compared to 60 mg E/kg diet.

The age dependence of many biological processes, including functional properties of muscarinic receptors in brain, has long given rise to the speculation that changes in antioxidant nutrients may play an important role in disease pathogenesis associated with aging [1]. Indeed, deficits in the function of muscarinic receptor-coupled G-protein function in postmortem human brains from Alzheimer’s patients have been identified [8,10]. Moreover, in our previous 6-month dietary study, the brain of animals receiving a supplemented E diet compared to animals fed normal dietary E (unsupplemented diet), reached maximum concentration of E and function better in different markers analyzed (example, DA release and GTP activity) [19]. In addition, we observed that the striata region showed the lowest E concentration of any of the brain regions examined [19].

Vitamin E is generally considered to be a relatively non-toxic nutrient in adults, being used for therapeutic and prophylactic purposes in a variety of disorders. Yet, there have been warnings about the toxic effects of megadosages of E because it is a fat soluble vitamin and may be difficult to eliminate from the body, thus, leading to various undesirable effects [4,15,16,27,53]. Since liver is the tissue that incorporates higher levels of E following dietary supplementation, and synthesis of ascorbate in rats occurs in the liver [5], we examined if high α-tocopherol could affect C levels. In fact, our data showed some alterations on C concentration, raising the possibility that high levels of E in tissues may be interacting with regulatory signal(s) [16], a hypothesis that needs to be further explored. Vitamin C in the brain is derived from the blood stream [11]; therefore, the role of the liver to synthesize it de novo, or process it from the diet is crucial to supply adequate amounts to the tissues. That the high E-fed group showed lower concentrations of ascorbate in plasma and in liver may be explained, in part, by the partial effect of E in the modulation of liver function.

In conclusion, our data showed clear differences in brain E levels depending of E intake. Therefore, the effort to maintain a steady concentration of E in the CNS may be crucial to the role that this nutrient plays in brain function. In addition, researchers have to be aware of the E content of the diets used in experiments designed to investigate the role of this nutrient in the CNS.

References


[9] B. Ghasemzadeh, J. Cammack, R.N. Adams, Dynamic changes in extracellular fluid ascorbic acid monitored by in vivo electropho-


[12] R.A. Grunewald, M. Fillenz, Release of ascorbate from a synaptosoma-


[23] A. Martin, B. Frei, Both intracellular and extracellular vitamin C inhibit allogeneic modification of LDL by human vascular endotho-


[43] D.D. Schocken, G.S. Roth, Reduced beta-adrenergic receptor concen-


[48] G. Vatassery, C. Angerhofer, C. Angerhofer, C. Knox, D. Desh-

