Dietary Vitamin E Deficiency Increases Anxiety-Like Behavior in Juvenile and Adult Rats

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Vitamin E deficiency from birth or infancy has recently been found to increase anxiety-like behavior in rodents. The present study was undertaken to elucidate the effect of dietary vitamin E deficiency on anxiety in adult rats in comparison with juvenile rats. Male Wistar rats, 3 or 10 weeks old, were divided into two groups and fed a control or vitamin E-deficient diet for 4 weeks. The results of behavioral analysis revealed that vitamin E-deficiency increased anxiety in both juvenile and adult rats. Plasma, liver, and brain α-tocopherol concentrations decreased significantly due to vitamin E deficiency in both age groups. Plasma corticosterone concentrations were higher in the vitamin E-deficient rats in response to the stress of a behavioral test. Based on these results, we conclude that dietary vitamin-E deficiency induces anxiety in adult rats as well as juvenile rats. This might be due to an elevated plasma corticosterone concentration.

Key words: vitamin E; tocopherol; anxiety; rats; elevated plus-maze

Vitamin E is an essential micronutrient for mammals that functions as a lipid-soluble antioxidant.1,1 Severe, chronic vitamin-E deficiency causes the progressive neurological syndrome ataxia in both humans and animals.2–7 Among the eight forms of vitamin E, α-tocopherol is transported into the circulation by α-tocopherol transfer protein (α-TTP) with much higher efficiency than the others, which makes α-tocopherol the most active form of vitamin E in mammals. Accordingly, α-TTP knockout mice were recently used as a vitamin E-deficient animal model. One of these studies found that anxiety-like behavior increased in α-TTP-deficient mice.1) Since α-TTP is indispensable to the retention and secretion of α-tocopherol by the liver to the circulation, deletion of the α-TTP gene causes a systemic deficiency in α-tocopherol. Furthermore, anxiety-like behavior also increased in phospholipid transfer protein (PLTP)-deficient mice, which were characterized by low vitamin E concentrations in the brain.2) These reports indicate that a vitamin E-deficient state from birth increased anxiety-like behavior in the mice.

In regard to the effect of dietary vitamin E deficiency on anxiety, we have reported that feeding a vitamin E-depleted diet for 4 weeks increased anxiety-like behavior in weaning rats.10,11) However, the anxiogenic effect of vitamin E deficiency might be specific to weaning rats during neurogenesis, and oxidative stress-induced neuronal injury was assumed to be a causative mechanism. In fact, the effect of a vitamin E-deficient diet is known to be more prominent in weaning rats than in adult ones.12) Therefore, in the present study, we examined the anxiogenic effect of dietary vitamin E depletion on adult rats in comparison with juvenile rats.

Furthermore, we investigated effects of dietary vitamin-E deficiency on endocrine systems that might cause anxiety-like behavior. During stress, the hypothalamic-pituitary-adrenal (HPA) axis is stimulated, and secretion of glucocorticoid from adrenal is elevated. This response is thought to be crucial in regulating emotion-related behavior, such as anxious behavior.13,14) We also investigated the oxidative stress levels of the rats. As vitamin E works mainly as an antioxidant, elevated oxidative stress is speculated to cause anxiety in vitamin-E deficient rats. As for the role of oxidative stress in anxiety, a correlation between anxiogenic phenotype and antioxidative enzyme (glyoxalase 1, glutathione reductase 1) activity was found in a study using six inbred mouse strains.15) Anxiolytic effect of dietary antioxidants16) and a positive correlation between peripheral blood oxidative stress markers and anxiety behavior17) have also been reported. In this context, we analyzed plasma corticosterone levels and oxidative stress markers to assess responses induced by dietary vitamin-E deficiency, which might be associated with anxiety behavior.

Materials and Methods

Materials. All chemicals and reagents were of reagent grade and are available commercially.

Animal experiment. The care and use of the experimental rats followed the institutional guidelines of Meiji University, and all experimental procedures were approved by the Institutional Animal Care and Use Committee of the university. Male Wistar strain rats 3 weeks (juvenile, n = 12) and 10 weeks (adult, n = 12) old were

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Abbreviations: CON, control diet; -E, vitamin E-deficient diet; EPM, elevated plus-maze; HPA, hypothalamic-pituitary-adrenal; PLTP, phospholipid transfer protein; TBA, thiobarbituric acid; TBARS, TBA reactive substances; α-TTP, α-tocopherol transfer protein
purchased from Japan Laboratory Animals (Tokyo) and housed in a room under a 12-h light/dark cycle (06:00–18:00) at a temperature of 22–24 °C. The rats of each age group were further divided into two dietary treatment groups. One group was fed a control diet (CON) and the other was fed a vitamin-E deficient diet (E) for the following 4 weeks. The control diet contained 200 g/kg of casein, 50 g/kg of corn oil, 437 g/kg of α-cornstarch, 218 g/kg of sucrose, 50 g/kg of cellulose, 10 g/kg of vitamin mixture (AIN93, Oriental Yeast, Tokyo), and 35 g/kg of mineral mixture (AIN93G, Oriental Yeast). The vitamin-E-deficient diet was prepared as for the control diet, but with a vitamin-E depleted vitamin mixture (Oriental Yeast) and vitamin E-stripped corn oil (Funabashi Farm, Chiba) instead of the vitamin mixture and corn oil respectively. Rats for both dietary treatments were housed in groups (three rats per cage, 26 × 42 × 20 cm) for the first 3 weeks of the experiment, and were housed individually for the last week in order to minimize behavioral influences among them. After they were fed the vitamin-E deficient diet for 17 d, a blood sample was taken from the tail of each rat and the red blood cell oxidative hemolysis rate was determined. As a form of behavioral analysis to assess anxiety, an elevated plus-maze (EPM) test was done on the 28th day at 10:00–16:00 on rats in random order from each experimental group. On the 31st day, the rats were anesthetized and sacrificed by means of Nembutal (Dainippon Pharmaceutical, Osaka), and blood was collected by cardiac puncture. Liver and brain sections were excised, frozen in liquid nitrogen, and stored at −80 °C pending analysis.

**Determination of the oxidative hemolysis rate.** The rate of red blood cell hemolysis was determined with 10-μL aliquots of whole blood, as described previously. Red blood cells were precipitated in a diluent (a 1:1 mixture of 0.9% NaCl and 0.9% sodium citrate), and suspended in 1 mL of 0.9% NaCl. The cell suspension was incubated with 0.01% dialuric acid or distilled water at 37 °C for 30 min, and supernatant absorbance was read (540 nm) after centrifugation at 80 × g for 5 min. The hemolysis rate (%) was calculated as the absorbance of a sample with dialuric acid divided by that of the same sample with distilled water.

**Elevated plus-maze test.** Anxiety levels were assessed with an EPM, consisting of two open arms (50 × 10 cm) and two closed arms (50 × 10 × 40 cm), elevated 50 cm from the floor. Fluorescent ceiling lights provided the only illumination in the experimental room, and the illumination level was lower than 0.5 lx during the experiment. For the EPM test, rats were placed individually in the center of the maze facing a closed arm, and were allowed to explore for 15 min. The test session was video-recorded (HandycamTRV116, Sony, Tokyo), and the following behaviors were analyzed: time spent in and frequency of entry into the open and closed arms, frequency and duration of head-dipping, and stretch-out postures.

**Determination of tissue and plasma α-tocopherol concentrations.** α-Tocopherol was extracted from tissues and plasma as described previously. Frozen tissue (0.05 g for the liver and 0.1 g for the other tissues) was homogenized with 1 mL of acetone. After the homogenate was evaporated and dissolved in ethanol and water (1:1), α-tocopherol was extracted with n-hexane and then evaporated again before analysis. As for plasma sample preparation, 100 μL of plasma was mixed with 500 μL of ethanol and 400 μL of water. α-Tocopherol was extracted with n-hexane containing 0.05% BHT and evaporated before analysis. The α-Tocopherol concentration was determined fluorometrically by HPLC, as described previously. The analytical column used was SHODEX 5SIL (Showa Denko, Tokyo) with a mobile phase of n-hexane/isopropanol (99.5:0.5, v/v), n-hexane containing 0.05% BHT, and n-hexane/isopropanol (99.5:0.5, v/v), and α-tocopherol was detected with excitation at 295 nm and emission at 335 nm. The analytical column used was SHODEX 5SIL (Showa Denko, Tokyo) with a mobile phase of n-hexane/isopropanol (99.5:0.5, v/v), n-hexane containing 0.05% BHT, and n-hexane/isopropanol (99.5:0.5, v/v), and α-tocopherol was detected with excitation at 295 nm and emission at 335 nm.

**Determination of tissue and plasma TBARS concentrations.** Tissue and plasma TBARS concentrations were determined as described previously. Frozen tissue (0.06 g) was homogenized with 20 volumes of 1.15% KCl. The homogenate (0.3 mL) was mixed with 0.18 mL of 1% phosphoric acid and 0.6 mL of thiobarbituric acid (TBA) solution (0.335% TBA in 50% acetic acid), and this was boiled for 45 min. After the samples were cooled in water, 4 mL of n-butanol was added and the fluorescence of the butanol layer was measured (excitation at 515 nm, emission at 553 nm). As for the analysis of plasma TBARS, 20 μL of plasma was mixed with 80 μL of PBS, 800 μL of 0.083 N H2SO4, and 200 μL of 10% D-tungstos (VI) phosphoric acid n-hydrate. After centrifugation at 800 × g for 10 min, 1 mL of water and 0.25 mL of 0.335% TBA solution were added to the precipitate. The resulting mixture was boiled for 60 min and extracted with 5 mL of n-butanol, and the fluorescence of the butanol layer was measured as described above.

**Determination of the plasma corticosterone concentration.** Heparinized blood was collected from the tails of the rats just after the EPM test, and at rest (10:00–11:00) of the following day. The corticosterone concentration was assayed using an AssayMax Corticosterone ELISA Kit (Assaypro, St. Charles, MO).

**Statistics.** Data were expressed as mean ± SEM. Two-way ANOVA was performed to analyze the effects of diet, age, and any interaction between diet and age. Differences were considered significant at p < 0.05 (Statcel, OMS Publishing, Saitama).

**Results.**

**Characteristics of the experimental animals.** Dietary vitamin E deficiency did not affect the food intake or body weight of the rats in either age group (Table 1). The hemolysis rate was higher in the vitamin-E deficient rats and lower in the adult rats than in juveniles. The effect of vitamin-E deficiency on the hemolysis rate was influenced by the age of the rats (Table 1).

**EPM test.** An EPM test was done to estimate the effect of vitamin-E deficiency on anxiety behavior in the rats. The dietary vitamin-E deficient rats spent less time and made fewer entries into the open arms (Fig. 1), and showed more stretch-out posture and less head dipping than the control rats (Fig. 2). This indicates that anxiety was increased by vitamin-E deficiency in both the juvenile and the adult rats. The age of the rats did not have any effect on anxiety behavior.

**Plasma, liver, muscle, and brain α-tocopherol concentrations.** The plasma, liver, and brain α-tocopherol concentrations in both age groups were lower in the rats fed a vitamin-E deficient diet (Table 2). The liver α-tocopherol concentrations were lower in the adult rats than in the juvenile rats, and an interaction between age and vitamin-E status was observed. The plasma, muscle, and brain α-tocopherol concentrations were not influenced by age.

**Plasma, liver, muscle, and brain TBARS concentrations.** Determination of TBA reactive substances (TBARS) levels was used to estimate the oxidative stress undergone by the rats. The TBARS concentrations in the plasma, liver, and cerebella of the rats fed the vitamin-E deficient diet were higher than those of the rats fed the control diet, but no significant differences between groups were observed in the muscle or cortex (Table 2). The age of the rats did not affect TBARS concentrations in the plasma or any other tissues examined.
ment has been suggested.8) In order to clarify whether the importance of this vitamin for neural development and birth and at the weaning age has been reported, 8–11) the anxiogenic effect on both the juvenile and the adult rats. Based on these results, we conclude that motor coordination was not reduced by 4 weeks of dietary vitamin E deprivation,11) and suggested that this behavioral response was not caused by diminished muscular coordination.

Discussion

An anxiogenic effect of vitamin E deficiency from birth and at the weaning age has been reported,8–11) and the importance of this vitamin for neural development has been suggested.8) In order to clarify whether the anxiogenic effect of vitamin-E deficiency is specific to very young rodents, we investigated the effect of dietary vitamin-E deficiency on the anxiety behavior of the adult rats in this study. The results of the EPM test clearly indicated that feeding the rats a vitamin E-deficient diet for 4 weeks increased anxiety behavior in the adult rats. Based on these results, we conclude that feeding of the vitamin-E deficient diet for 4 weeks had an anxiogenic effect on both the juvenile and the adult rats. In the case of the juvenile rats, we previously confirmed that motor coordination was not reduced by 4 weeks of dietary vitamin E deprivation,11) and suggested that this behavioral response was not caused by oxidative stress was severer in the juvenile rats.

Since the primary effect of vitamin E deprivation was to decrease the α-tocopherol concentrations in the plasma and tissues, we investigated to determine whether the rate of decrease would be influenced by age. The results indicated that the plasma, liver, muscle, and brain α-tocopherol concentrations in the adult rats decreased to the same extent as in the juveniles under 4 weeks of dietary vitamin-E deprivation. However, the liver α-tocopherol concentrations were significantly lower in the adult rats than in the juveniles, under both basal and vitamin-E depleted conditions. This was probably due to a lower food intake per body weight in the adult rats. We also investigated whether the physiological effect of vitamin E deficiency would be affected by age. The results of two-way ANOVA showed above (NS, p > 0.05).

Plasma corticosterone concentrations

Corticosterone levels were measured at rest and just after the EPM test (and thus in response to the stress of the EPM test) to investigate the stress hormone response. Dietary vitamin-E deficiency significantly increased the corticosterone levels of the rats after the EPM test (Table 3).

Table 1. Characteristics of the Experimental Animals

<table>
<thead>
<tr>
<th></th>
<th>Juvenile</th>
<th>Adult</th>
<th>Two-way ANOVA (p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CON -E</td>
<td>CON -E</td>
<td>Diet</td>
</tr>
<tr>
<td>Initial body weight (g)</td>
<td>Mean ± SEM</td>
<td>Mean ± SEM</td>
<td>Mean ± SEM</td>
</tr>
<tr>
<td>Final body weight (g)</td>
<td>52.0 ± 1.3</td>
<td>53.1 ± 1.5</td>
<td>309.0 ± 3.5</td>
</tr>
<tr>
<td>Food intake (g/d/3 rats) first 3 weeks</td>
<td>265.2 ± 11.5</td>
<td>260.4 ± 7.0</td>
<td>399.0 ± 6.3</td>
</tr>
<tr>
<td>Food intake (g/d/rat) last 1 week</td>
<td>49.6 ± 2.4</td>
<td>49.6 ± 2.5</td>
<td>83.3 ± 1.2</td>
</tr>
<tr>
<td>Oxidative hemolysis rate (%)</td>
<td>21.7 ± 0.9</td>
<td>22.6 ± 0.7</td>
<td>28.4 ± 0.6</td>
</tr>
<tr>
<td>Oxidative hemolysis rate (%)</td>
<td>3.99 ± 0.39</td>
<td>48.49 ± 4.22</td>
<td>2.11 ± 0.38</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SEM for six rats except for food intake for the first 3 weeks.

Fig. 1. Open-Arm Activities on the Elevated Plus-Maze Test.

Time spent in the open arms and frequency of open-arm entry [open-arm/(open-arm+close-arm)%] of juvenile and adult rats fed a control diet (CON) or a vitamin E-deficient diet (-E) on the elevated plus-maze test. The data represent means ± SEM for six rats. The results of two-way ANOVA were shown above (NS, p > 0.05).

Since the primary effect of vitamin E deprivation was to decrease the α-tocopherol concentrations in the plasma and tissues, we investigated to determine whether the rate of decrease would be influenced by age. The results indicated that the plasma, liver, muscle, and brain α-tocopherol concentrations in the adult rats decreased to the same extent as in the juveniles under 4 weeks of dietary vitamin-E deprivation. However, the liver α-tocopherol concentrations were significantly lower in the adult rats than in the juveniles, under both basal and vitamin-E depleted conditions. This was probably due to a lower food intake per body weight in the adult rats. We also investigated whether the physiological effect of vitamin E deficiency would be affected by age. The results of two-way ANOVA showed above (NS, p > 0.05).
As an indicator of oxidative stress in the plasma and tissues, the lipid peroxidation level was estimated from the TBARS concentration. TBARS concentrations were elevated in the plasma, liver, and cerebellum by vitamin-E deficiency, but no significant differences were observed in the muscle or the cortex. The smaller decrease in $\alpha$-tocopherol in these tissues may have caused a smaller increase in the TBARS concentrations in the muscles and brain. It has been reported that the half-life of $\alpha$-tocopherol in the brain is longer than in other tissues, and that a longer period of vitamin-E deprivation elevated the brain TBARS concentration. The effect of vitamin-E deprivation on the TBARS concentration was almost the same as between the juvenile and adult rats.

The results of this study indicate that 4 weeks of dietary vitamin-E deprivation did not result in oxidative stress sufficiently severe to increase the brain TBARS level. On the other hand, it is speculated that the decrease in vitamin E and a subsequent elevation of oxidative stress in the brain might have been related to anxiety behavior, since the $\alpha$-tocopherol levels were low.

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**Fig. 2.** Stretch-Out and Head-Dipping on the Elevated Plus-Maze Test.

Time (A) and frequency (B) of stretch-out posture, and time (C) and frequency (D) of head-dipping of juvenile and adult rats fed a control diet (CON) or a vitamin-E deficient diet (-E) on the elevated plus-maze test. The data represent means ± SEM for six rats. Results of two-way ANOVA are shown above (NS, $p > 0.05$).

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**Table 2. Tocopherol and TBARS Concentrations**

<table>
<thead>
<tr>
<th></th>
<th>CON</th>
<th>-E</th>
<th>CON</th>
<th>-E</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SEM</td>
<td>Mean</td>
<td>SEM</td>
</tr>
<tr>
<td>Plasma $\alpha$-tocopherol (µg/mL)</td>
<td>16.06 ± 2.64</td>
<td>2.36 ± 1.04</td>
<td>10.94 ± 0.73</td>
<td>1.93 ± 0.08</td>
</tr>
<tr>
<td>Liver $\alpha$-tocopherol (µg/g)</td>
<td>40.62 ± 2.94</td>
<td>2.45 ± 1.36</td>
<td>29.58 ± 2.14</td>
<td>1.98 ± 0.86</td>
</tr>
<tr>
<td>Muscle $\alpha$-tocopherol (µg/g)</td>
<td>9.68 ± 1.85</td>
<td>3.34 ± 0.72</td>
<td>9.66 ± 2.15</td>
<td>1.76 ± 0.19</td>
</tr>
<tr>
<td>Cortex $\alpha$-tocopherol (µg/g)</td>
<td>20.29 ± 2.19</td>
<td>7.84 ± 0.36</td>
<td>24.72 ± 1.39</td>
<td>7.76 ± 0.91</td>
</tr>
<tr>
<td>Cerebellum $\alpha$-tocopherol (µg/g)</td>
<td>16.67 ± 1.25</td>
<td>6.88 ± 0.52</td>
<td>17.89 ± 1.15</td>
<td>7.93 ± 0.48</td>
</tr>
<tr>
<td>Plasma TBARS (nmol TEP eq./mL)</td>
<td>0.084 ± 0.005</td>
<td>0.15 ± 0.008</td>
<td>0.081 ± 0.008</td>
<td>0.147 ± 0.011</td>
</tr>
<tr>
<td>Liver TBARS (nmol TEP eq./g)</td>
<td>29.52 ± 1.39</td>
<td>63.05 ± 7.60</td>
<td>40.75 ± 5.93</td>
<td>62.92 ± 4.51</td>
</tr>
<tr>
<td>Muscle TBARS (nmol TEP eq./g)</td>
<td>16.71 ± 2.44</td>
<td>22.55 ± 2.91</td>
<td>14.21 ± 4.54</td>
<td>18.02 ± 1.70</td>
</tr>
<tr>
<td>Cortex TBARS (nmol TEP eq./g)</td>
<td>40.00 ± 4.93</td>
<td>52.03 ± 4.84</td>
<td>47.42 ± 7.67</td>
<td>43.91 ± 2.15</td>
</tr>
<tr>
<td>Cerebellum TBARS (nmol TEP eq./g)</td>
<td>40.07 ± 3.90</td>
<td>52.40 ± 4.46</td>
<td>49.22 ± 2.30</td>
<td>52.79 ± 2.22</td>
</tr>
</tbody>
</table>

CON, control diet; -E, vitamin E deficient diet; TEP eq., tetraethoxypropane equivalent; NS, not statistically significant ($p > 0.05$)

Values are expressed as means ± SEM for six rats.

As an indicator of oxidative stress in the plasma and tissues, the lipid peroxidation level was estimated from the TBARS concentration. TBARS concentrations were elevated in the plasma, liver, and cerebellum by vitamin-E deficiency, but no significant differences were observed in the muscle or the cortex. The smaller decrease in $\alpha$-tocopherol in these tissues may have caused a smaller increase in the TBARS concentrations in the muscles and brain. It has been reported that the half-life of $\alpha$-tocopherol in the brain is longer than in other tissues, and that a longer period of vitamin-E deprivation elevated the brain TBARS concentration. The effect of vitamin-E deprivation on the TBARS concentration was almost the same as between the juvenile and adult rats.

The results of this study indicate that 4 weeks of dietary vitamin-E deprivation did not result in oxidative stress sufficiently severe to increase the brain TBARS level. On the other hand, it is speculated that the decrease in vitamin E and a subsequent elevation of oxidative stress in the brain might have been related to anxiety behavior, since the $\alpha$-tocopherol levels were low.
in the brain but normal in the plasma of PLTP-deficient mice.\(^9\) Considering that the decrease in \(\alpha\)-tocopherol concentration in the brain was moderate in the PLTP-deficient mice,\(^9\) even modest decreases in brain \(\alpha\)-tocopherol can cause anxiety.

In sum, dietary vitamin E deprivation decreased the \(\alpha\)-tocopherol concentration and increased the lipid peroxidation in the plasma and tissues equally in juvenile and adult rats, and induced anxiety behavior to the same extent. We conclude here that a decrease in \(\alpha\)-tocopherol or an increase in oxidative stress is the primary factor inducing anxiety behavior in this study, but we speculate that the anxiogenic effect of vitamin-E deficiency was not due to inhibition of neurogenesis or neural development during or before weaning, but rather to influence on neural responses, or the endocrine system.

In addition to oxidative stress, we assessed the endocrine response that relates to anxiety behavior. In order to determine the effect of dietary vitamin E deficiency on the HPA axis, the main endocrine response to stress-induced anxiety, plasma corticosterone concentrations were measured at rest and under EPM stress conditions. Vitamin E deficiency significantly increased corticosterone concentrations under the stress of the EPM test, and tended to increase under basal conditions (two-way ANOVA, \(p=0.087\) for the effect of diet). It has been reported that corticosterone levels under restraint stress were elevated by vitamin-E deficiency and were reduced by treatment with vitamin E.\(^{25,26}\) In addition, the association between anxiety and HPA axis reactivity suggests the importance of the HPA axis in anxiety behavior on the EPM test.\(^{13,14}\) The result that vitamin-E deficiency did not increase anxiety behavior in adrenalec-tomized rats indicates that corticosterone is important for the anxiogenic effect of vitamin-E deficiency (Terada et al., manuscript in preparation). Thus, it is possible that vitamin-E deficiency increases corticosterone levels under conditions of stress, inducing anxiety behavior in rats.

Besides vitamin-E deficiency, other forms of malnutrition have been suggested to increase corticosterone levels and anxiety. Upregulation of corticosterone by vitamin \(B_1\), \(B_6\), and \(C\) deficiency has been reported.\(^{27–29}\) But the effects of deficiencies of these vitamins on anxiety has not been clarified. On the other hand, anxiogenic effects of Fe and Mg deficiency were reported without evidence of an effect on corticosterone levels.\(^{30,31}\) Thus, deficiencies of other micronutrients, as well as vitamin E, might upregulate corticosterone and increase anxiety. In the case of protein or calorie malnutrition, fasting, food restriction, and protein restriction increased corticosterone levels.\(^{32–35}\) Food restriction increased anxiety behavior at the same time.\(^{36}\) In order to determine whether upregulation of corticosterone and increases in anxiety are specific to vitamin-E deficiency or are general in malnutrition, further investigation is necessary.

In conclusion, we found that dietary vitamin-E deficiency increased anxiety behavior in adult rats as well as juveniles. Furthermore, we identified an elevation of the plasma corticosterone concentration as a possible cause of anxiety. Our findings represent a useful model to investigate a novel role of vitamin E in animal behavior, as well as the role of oxidative stress on anxiety behavior.

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**References**