Effect of Vitamin E on Function of Pituitary-Gonadal Axis in Male Rats and Human Subjects

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Abstract

The role of vitamin E in the endocrine system, in particular the pituitary-gonadal axis, was studied in humans and male rats by examining the hormonal differences between vitamin E deficient and supplemented conditions.

In vitamin E deficient rats, pituitary content and basal plasma level of FSH and LH were significantly lower than those of the control rats, but testicular content and basal plasma level of testosterone were not significantly changed.

On the other hand, in vitamin E supplemented rats, FSH and LH content in pituitary tissue was significantly higher than that of the controls, but there was no significant rise in basal FSH and LH level in plasma. The testosterone level was significantly elevated in both testicular tissue and plasma. It was also demonstrated that basal plasma testosterone and F.T.I. were increased in normal male subjects following oral vitamin E administration and the responsiveness of plasma testosterone levels to HCG was significantly higher during vitamin E administration than before administration.

These results suggest that vitamin E may play an important and potent role in hormone production in the pituitary-gonadal axis in humans and rats.

Vitamin E has come to be regarded as important for its antioxidative action, in particular its inhibitory effect on the peroxidation of polyunsaturated fatty acids which are the main component of intracellular biomembranes (Tappel, 1962; Zalkin and Tappel, 1960). Our previous study demonstrated that in male rats both vitamin E content and lipoperoxide formation were considerably greater in the pituitary and adrenal gland than in any other organ (Umeda, 1978). Furthermore, a high uptake of exogenously administered vitamin E was also shown in these endocrine organs (Gallo-Torres and Miller, 1971; Gloor et al., 1963; Umeda et al., 1982). This suggests that vitamin E may play an important role in lipoperoxide formation and hormone production in these endocrine glands.

For many years, vitamin E has been considered as an antisterility factor (Evans, 1922), and its role in the endocrine system has been expected to be important. However, no systematic hormonal study on the role of vitamin E in the endocrine system has been carried out.

In this study we have investigated the biological role of vitamin E on hormone production in the pituitary-gonadal axis by evaluating the functional changes observed in vitamin E deficient and vitamin E supplemented male rats and human subjects.

Materials and Methods

Animals and human subjects

Four- to five-week-old male Wistar rats obtained from the Animal Center of Kyushu University, Fuku-
oka, Japan, were used in the present study. The animals were divided into three dietary groups: those on a control diet (group N), those on a diet deficient in vitamin E (group -E), and those on a diet supplemented with vitamin E (group +E).

Eleven healthy men aged from 30 to 69 years (average 54 years) were chosen as human subjects.

**Diets**

The composition of the vitamin E deficient basal diet (in w/w) for animals was as follows (Masugi et al., 1976): corn starch 38.0%, α-malt starch 10.0%, granulated sugar 5.0%, vitamin free casein 25.0%, purified lard 6.0%, mineral mixture 6.0%, powdered filter paper 8.0%, and vitamin mixture 2% containing vitamin A acetate 1000 IU, vitamin D3 200 IU, thiamine-HCl 2.4 mg, riboflavin 8.0 mg, pyridoxine-HCl 1.6 mg, cyanocobalamin 1.0 μg, L-ascorbic acid 60.0 mg, vitamin K3 10.4 mg, D-biotin 0.04 mg, folic acid 0.4 mg, Ca-pantothenate 10.0 mg, p-aminobenzoic acid 10.0 mg, niacin 12.0 mg, inositol 12.0 mg, and choline 400 mg/100 grams diet. For the control diet of group N, 2 mg of vitamin E per 100 grams diet was added to the vitamin E deficient basal diet. The vitamin E supplemented diet for group +E contained 40 mg of vitamin E per 100 grams diet. These three kinds of diet were prepared in the Laboratories of Eisai Co., Ltd., Tokyo.

All human volunteers admitted to the hospital took a constant daily composition of nutrients containing 3-5 mg of vitamin E.

The animals were housed in cages and kept in the identical controlled environment. After seven months of feeding these three kinds of diet, all of the rats were killed by exsanguination. Blood was collected in a heparinized syringe from the portal vein under Nembutal anesthesia. Plasma was separated and stored at -70°C until assay. On the same day the animals were killed, several tissues were immediately removed from them and weighed. The pituitary and testicular tissues were homogenized in a suitable volume of cold saline solution with a Teflon homogenizer. The homogenates were then stored at -70°C until assay.

In the human subjects, blood samples were taken after overnight fasting before and 2, 4, and 8 weeks after oral administration of vitamin E acetate in a daily dose of 483 mg. The test on the response of plasma testosterone levels to human chorionic gonadotropin (HCG) was performed before and 8 weeks after vitamin E administration. A daily dose of 5000 IU of HCG was intramuscularly injected into the human subjects for three days, and blood was sampled from the antecubital vein before each injection as well as on the next day to the last injection. Plasma samples were separated and stored at -70°C for the assays of vitamin E, testosterone, F.T.I., and LH.

**Assays**

a. The measurement of vitamin E in plasma was determined by the fluorometric method of Abe et al. (1975).
b. Testosterone concentration in the tissue and plasma was determined by radioimmunoassay using the first antibody purchased from Endocrine Science (Coyotupa et al., 1972).
c. Free testosterone index (F.T.I.) expressed the concentration of unbound plasma testosterone. The value of F.T.I. was determined according to the method of Rosenfield et al. (1971).
d. FSH and LH concentration in tissue and plasma were determined by radioimmunoassay using the NIAMDD kit for animals and Daichi Radioisotope kit for humans. The pituitary homogenate was diluted 100 times with 100 mM phosphosaline buffer (pH 7.4) before assay. All measurements were performed in duplicate.

**Results**

As shown in Table 1, the body weight did not differ significantly among the three dietary groups of rats, while the tissue weights of pituitary gland, kidney, and liver were significantly decreased in group -E as compared to group N. However, the adrenal gland and testis showed little difference in weight between group N and -E. Nor was any significant difference found in the tissue weights of group N and +E.

The plasma vitamin E concentration of the three experimental groups after 7 months feeding are shown in Fig. 1. There were significant differences in vitamin E concentration between them: namely, lower in group -E (2.0±0.1 μg/ml), and higher in group +E (10.9±0.4) than in group N (6.9±0.3).

The pituitary and testicular tissue content and plasma level of FSH, LH, and testosterone are summarized in Table 2. In group -E, the FSH level was significantly lower than that of group N in both tissue and plasma. Similarly, the LH level was also significantly lower in tissue, and decreased in plasma as compared to group N. The testosterone level of group -E was not significantly changed in tissue and plasma. On the other hand, the FSH and LH level in tissue of group +E were significantly higher than those of group N, but showed no significant difference in plasma.
Table 1. Comparison of whole body and tissue weights in the three dietary groups.

<table>
<thead>
<tr>
<th>N: Control, -E: Vitamin E deficient, +E: Vitamin E supplemented.</th>
<th>Whole body (g)</th>
<th>Pituatory (mg)</th>
<th>Adrenal (mg)</th>
<th>Testis (g)</th>
<th>Brain (g)</th>
<th>Liver (g)</th>
<th>Kidney (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N(13)²</td>
<td>360±43b</td>
<td>9.3±0.4</td>
<td>16.4±0.6</td>
<td>1.21±0.06</td>
<td>1.94±0.03</td>
<td>13.0±0.8</td>
<td>1.32±0.04</td>
</tr>
<tr>
<td>-E(6)</td>
<td>323±21</td>
<td>6.0±0.6**</td>
<td>16.8±0.5</td>
<td>1.28±0.03</td>
<td>1.98±0.02</td>
<td>7.4±0.4**</td>
<td>1.08±0.04*</td>
</tr>
<tr>
<td>+E(9)</td>
<td>358±37</td>
<td>10.5±0.5</td>
<td>17.2±0.2</td>
<td>1.17±0.05</td>
<td>1.97±0.02</td>
<td>12.1±0.9</td>
<td>1.33±0.05</td>
</tr>
</tbody>
</table>

a: Number of animals.

b: Mean±SEM.

* P<0.005, ** P<0.001: Significantly different from control as found by Student's t-test.

In addition, the level of testosterone in group +E was significantly greater in both tissue and plasma than that of group N.

As for the human study, it was confirmed that the plasma vitamin E concentration was significantly increased following oral administration. Once the peak of plasma level of vitamin E was reached, at about 2 weeks, a constant high level, 3 times or more than before, was maintained during oral administration (Fig. 2).

In consequence of the increase in the plasma vitamin E level, the basal testosterone concentration and free testosterone index in plasma were increased during the weeks following administration, with a significant rise in the plasma testosterone level at 8 weeks. However, there was no marked change in the
Table 2. Comparison of testosterone, FSH, and LH concentration in tissue and plasma in the three dietary groups.

<table>
<thead>
<tr>
<th></th>
<th>FSH</th>
<th>LH</th>
<th>Testosterone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pituitary</td>
<td>plasma</td>
<td>pituitary</td>
</tr>
<tr>
<td></td>
<td>ng/mg prot.</td>
<td>ng/ml</td>
<td>ng/mg prot.</td>
</tr>
<tr>
<td>N (13)</td>
<td>534.4±52.1 ^b</td>
<td>73.5±6.7</td>
<td>133.6±12.5</td>
</tr>
<tr>
<td>-E (6)</td>
<td>175.0±75.6 **</td>
<td>45.3±5.7 **</td>
<td>59.6±21.4 **</td>
</tr>
<tr>
<td>+E (9)</td>
<td>769.8±84.6 *</td>
<td>75.8±7.8</td>
<td>187.7±20.0 *</td>
</tr>
</tbody>
</table>

N: Control, -E: Vitamin E deficient, +E: Vitamin E supplemented.
a: Number of animals.
b: Mean ±SEM.
c: Tissue content of FSH and LH expressed as nanogram per mg protein of whole pituitary homogenate.
d: Testosterone content expressed as nanogram per g wet tissue.
* P<0.05, ** P<0.02, *** P<0.005: Significantly different from control as found by Student’s t-test.

Table 3. Change in basal levels of plasma testosterone, F.T.I., and LH following vitamin E administration.

<table>
<thead>
<tr>
<th></th>
<th>LH (miu/ml)</th>
<th>Testosterone (ng/dl)</th>
<th>F.T.I.*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before</td>
<td>16.3±4.8 ^b</td>
<td>416.4±47.6</td>
<td>236.9±19.5</td>
</tr>
<tr>
<td>2 Weeks</td>
<td>12.7±2.3</td>
<td>532.2±67.8</td>
<td>287.2±32.1</td>
</tr>
<tr>
<td>4 Weeks</td>
<td>12.5±2.3</td>
<td>516.7±97.6</td>
<td>288.3±42.7</td>
</tr>
<tr>
<td>8 Weeks</td>
<td>15.2±3.1</td>
<td>544.5±33.8 *</td>
<td>303.9±48.0</td>
</tr>
</tbody>
</table>

a: Free testosterone index.
b: Results are shown as Mean ±SEM of 11 normal human subjects.
* P<0.05: Significantly different from the level before administration as found by Student’s t-test.

plasma LH level (Table 3). Furthermore, an HCG test was performed before and after 8 weeks of vitamin E administration (Fig. 3). The response of the plasma testosterone level to HCG after vitamin E administration was significantly higher than before administration. In fact, after vitamin E administration the plasma testosterone level was increased from 552.7±26.9 ng/dl on the first day to 1015.7±95.0 ng/dl on the fourth day of HCG test, and the increase rate was 1.85, while the increase was from 415.5±30.3 ng/dl to 609.2±45.2 ng/dl, and the increase rate was 1.47 before vitamin E administration.

Discussion

A significant decrease in the weight of the pituitary gland was observed in group -E. Also it was revealed that the pituitary content and plasma level of gonadotropins were markedly reduced in group -E with a small change in the plasma and testicular testosterone levels. In agreement with the result reported by Ikeda (1968), our previous structural study with an electron microscopy demonstrated that the number of secretory granules in gonadotrophs in the anterior pituitary decreased in vitamin E deficient rats (Umeda, 1978).

An histological study of the testis in vitamin E deficient rats demonstrated a notable reduction in spermatogenesis and a degeneration of Leydig cells as has been observed in several previous reports (Lee, 1960; Mason, 1933; Younoszai et al., 1975), suggesting a decline in the potency of hormone production. Pinelli and Formento (1973) reported a decrease in 17-KS in the urine of vitamin E
deficient rats, but in our study no significant difference was observed in plasma testosterone compared with that of the controls. It seems that in the condition of vitamin E deficiency in rats the functional change occurs more in the pituitary than in the testicular tissue.

In contrast to vitamin E deficiency, vitamin E administration could activate the production of pituitary gonadotropins. In our ultrastructural study of vitamin E supplemented rats, an increase in the number of secretory granules was observed in the anterior pituitary gonadotrophs (Umeda, 1978). Evidence was obtained showing that FSH and LH content in the pituitary of group +E increased significantly. However, plasma levels of FSH and LH were not different from the controls. It is suggested that this inhibition of FSH and LH release in blood from gonadotrophs in group →E might be due to the negative feedback by the elevation of the peripheral testosterone level.

It is known that the administration of vitamin E activates spermatogenesis (Ichihara, 1967). It has also been demonstrated by electronmicroscopy that vitamin E activates the development of smooth surfaced ER in Leydig cells, suggesting the enhancement of steroidogenesis (Umeda, 1978). In the present study, we observed a significant elevation of testosterone levels in both the rat testicular tissue and plasma after long-term vitamin E administration. In addition, it was also revealed that in human subjects testosterone production was activated by vitamin E administration. Thus, vitamin E may play an important role in the biosynthesis of pituitary gonadotropins and testicular testosterone.

Fegler (1955) reported that the primary receptor of vitamin E in the endocrine system is found in the pituitary. Our present in vivo experiment supports in part this view with regard to the pituitary-gonadal axis in vitamin E deficiency. However, Barnes and Smith (1975) found a decrease in 3β-dehydrogenase activity in the testis of vitamin E deficient rats. Kitabchi et al. (1973; 1978) and Nathans and Kitabchi (1975) have reported a decrease in ACTH-induced steroidogenesis and adenylatecyclase activity in vitro experiment on the adrenal cortex of vitamin E deficient rats. Furthermore, the present study demonstrates that vitamin E administration enhances testosterone production in rat and human testis without the elevation of peripheral FSH and LH levels. All these findings suggest that the abnormal changes seen in the testis under conditions of vitamin E excess and deficiency are not only secondary to effects on the pituitary but may also be due to direct effects on the testis. It was found recently that a specific binding material for vitamin E is present in
cytosol and nuclei from liver (Nair, 1978). It can be presumed that this specific receptor may also be present in the pituitary and testis.

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References


