Effects of Vitamin E Deficiency on the Hormone Secretion of the Pituitary-Gonadal Axis of the Rat

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Akazawa, N., Mikami, S. and Kimura, S. Effects of Vitamin E Deficiency on the Hormone Secretion of the Pituitary-Gonadal Axis of the Rat. Tohoku J. exp. Med., 1987, 152 (3), 221–229 — Chronological changes of gonadotropin (FSH and LH) and testosterone concentrations in the serum were measured in vitamin E deficient rats to investigate the effects of vitamin E deficiency on the pituitary-gonadal function in rats. The receptor sites and association constant (Ka) for LH and the formation of cyclic AMP in the Leydig cells were also investigated. The results obtained in the present study are as follows: 1) The vitamin E deficient rats showed almost complete hemolysis and extremely increased TBA reacting substances (TBARS) in the serum and liver. 2) The serum LH concentration in the vitamin E deficient group was slightly higher than in the vitamin E supplemented group during the later periods of experiment. 3) The serum FSH concentration in the vitamin E deficient group did not differ significantly from that in vitamin E supplemented group, but became significantly higher than that in the latter at 186 days of experiment. 4) The serum testosterone concentration was always lower in the vitamin E deficient group than in the control. 5) The vitamin E deficient group showed slightly large number of LH/hCG receptor and significantly small Ka (low affinity), as compared with vitamin E supplemented group. The formation of cyclic AMP by Leydig cells decreased significantly in vitamin E deficient group. These results suggest that the vitamin E deficiency exerted a suppressive effect directly on the gonadal function to decrease the hormone synthesis in the Leydig cells and caused the increased secretion of pituitary LH owing to the feedback mechanism. ——— Vitamin E deficiency; pituitary-gonadal function; FSH and LH secretion; testosterone secretion; LH receptor

It has been well known that the vitamin E deficiency causes the hypertrophy of gonadotropic cells in the anterior pituitary and degeneration of seminiferous tubules of the testis, since the first report of Nelson (1933) on the pituitary-gonadal system of vitamin E deficient rats. In our previous studies (Akazawa 1977, 1978),

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the pituitary gland of vitamin E deficient rats revealed the hypertrophy and vacuolation of gonadotrophs (FSH and LH cells), of which Golgi apparatus was well-developed to suggest an accelerated secretory function. On the other hand, the testes showed marked degenerative changes, such as atrophy of seminiferous tubules, appearance of polynuclear giant cells and sloughed germ cells in the tubular lumen and disruption of spermatogenesis. These findings suggest that the vitamin E deficiency exerts a suppressive effect directly on the gonadal function and that the acceleration of pituitary function is due to the impaired gonadal function.

As for the hormone secretion under the condition of vitamin E deficiency, Griesbach et al. (1957) reported that the gonadotrophin concentration in the adenohypophysis increased in the rat after 22 weeks of vitamin E deficiency in comparison with normal rats. In contrast, Umeda et al. (1982) reported that pituitary content and basal plasma levels of FSH and LH in vitamin E deficient rats were significantly lower than those of control rats, but testicular content and basal plasma level of testosterone were not significantly changed. In our previous study, serum LH and FSH levels were raised in vitamin E deficient rats after 2 and 3 months, and pituitary contents of LH and FSH were also increased in vitamin E deficient rats. Lees et al. (1982) also reported the decrease of serum testosterone concentration in the vitamin E deficient rats. These differences in data may probably be due to the duration of vitamin E deficiency. Therefore, chronological changes of serum LH, FSH and testosterone concentrations were examined in the present study.

The gonadal function is controlled by the pituitary gonadotrophic hormones through the gonadal receptor in the Leydig cells. Therefore, the number of LH/hCG receptor and association constant (Ka) for LH/hCG were measured by Scatchard plot analyses of competitive binding of hCG to Leydig cells of vitamin E deficient and supplemented rats. In addition, cyclic AMP, which may concern with the hormone synthesis through interaction with adenylate cyclase attached to the cell membrane, was examined in the present study to reveal the cause of decrease in the secretion of testosterone in vitamin E deficient rats.

**Materials and Methods**

**Animals.** Forty weanling male Wistar rats (22 days old) were divided into two dietary groups and fed for 186 days either on a vitamin E deficient or supplemented (dl-α-tocopherol acetate 10 mg/100 g) diet. Animals were housed in cages and allowed to take diet and water and libitum. Blood was collected in an heparinized syringe from the coccygeal vein at 10 to 12 in the morning at 56, 89, 105, 144, 164 and 186 days of experiment. Plasma was separated and stored at −70°C until assay. The animals were killed at 186 days of experiment, and several tissues were immediately removed and treated for experiment.

**Diets.** The composition of the vitamin E deficient basal diet was as follows: corn starch 34% (in w/w), α-wheat starch 10%, vitamin free casein 25%, powdered filter paper 8%, granulated suger 5%, mineral salts mixture (Harper salt) 6%, vitamin mixture (except
E) 2%, corn oil 10%. Vitamin mixture composed of vitamin A acetate 1.000 iu, D3 200 iu, B2 8.0 mg, B6 0.001 mg, C 60 mg, biotin 0.04 mg, thiamin HCl 2.4 mg, folic acid 0.4 mg, Ca-pantothenate 10.0 mg, PABA 10.0 mg, niacin 12.0 mg, inositol 12.0 mg, and cholin-Cl 4,900 mg. The vitamin E supplemented diet contained 10 mg of dl-α-tocopherol acetate per 100 g diet.

Assays. 1. Measurement of the hemolytic rate was performed by Friedman's method using dialuric acid.

2. Vitamin E (α-tocopherol) level in the serum was determined by the fluorometric method of Katsui (1980).

3. The amount of lipid peroxides in blood and liver were determined by the method of Yagi (1975) using thiobarbituric acid (TBA) and were showed by the TBA value (malondialdehyde level).

4. Testosterone concentration in the serum was determined by radioimmunoassay using [125I] testosterone radioimmunoassay kit (Eiken Co.).

5. FSH and LH concentrations in the serum were determined by radioimmunoassay using NIADDK kits (NIH, Bethesda, MD, USA). The assay results were expressed in terms of NIH-FSH-SI and NIH-LH-SI.

6. Receptor binding studies for LH/hCG and cyclic AMP formation in Leydig cells. Decapsulated testes from 6 rats in each group were treated with collagenase (Dufau et al. 1971). Testicular interstitial cells were washed with PBS-BSA and resuspended with medium 199-BSA at a concentration of 2 × 10^6 cells/ml. The number of Leydig cells was counted in a Levy Ultraplane counting chamber, after histochemical staining for Δ5-3β-hydroxysteroid dehydrogenase. For the binding studies, 20,000 cpm [125I] hCG (20 μCi/μg), and increasing amount of non-radioactive hCG (1 ng to 128 ng) were incubated with 10^6 of Leydig cells at room temperature overnight. From Scatchard plot analysis, the amount of LH receptors in 10^6 cells and association constant (Ka) were determined. To estimate cyclic AMP formation, 10^6 of the cells were incubated with or without 100 ng of hCG at 35°C for 3 hr in the presence of 0.1 mM isobutyl methylxanthin (Hattori and Wakabayashi 1983). Cyclic AMP formed was measured by radioimmunoassay using [125I] 2'-0-monosuccinyl-adenosine 3', 5'-cyclic monophosphate tyrosyl methyl ester.

Statistical analyses. Comparison of the data between the vitamin E deficient and supplemented groups was carried out by Student t-test or Cochran-Cox test. Chronological changes of the serum hormone levels were assessed by one way analysis of variances.

RESULTS

Body weight, erythrocyte hemolytic rate and serum α-tocopherol concentration

The body weight did not differ significantly between the two dietary groups. The erythrocyte hemolytic rate and serum α-tocopherol concentration of the two dietary groups after 6 months feeding are shown in Table 1. The erythrocyte hemolytic rate was extremely high, being more than 97% in the vitamin E deficient group. The serum α-tocopherol concentration was 0.87 mg/100 ml in the vitamin E supplemented group, while it was significantly low (0.1 mg/100 ml, p < 0.01) in the vitamin E deficient group.

TBA-reacting substance concentrations in the serum and liver

Malondialdehyde concentrations in the serum and liver of two dietary groups after 6 months feeding are shown in Table 1. The malondialdehyde concentration in the serum was 3.36 nmole/100 ml in the vitamin E supplemented group,
while significantly higher \((p < 0.05)\) in the vitamin E deficient group, reaching 8.84 nmole/100 ml. The hepatic malondialdehyde concentration in the vitamin E deficient group was 91.36 nmole/g, which was significantly higher \((p < 0.05)\) than that (60.14 nmole/g) in the vitamin E supplemented group.

**FSH and LH levels in the serum**

Chronological changes of the serum FSH and LH levels in these two dietary groups are shown in Table 2. The serum LH concentration in the vitamin E supplemented group was 0.43 ng\(\times\)Sl/ml at the beginning of the experiment, and increased to 0.55 ng\(\times\)Sl/ml at 89 days of experiment, reached the peak (1.09 ng\(\times\) Sl/ml) at 105 days, then decreased thereafter \((p < 0.01)\). The serum LH concentration in the vitamin E deficient group also showed a similar tendency though the changes were slight and not significant. However, it remained slightly higher than that in the vitamin E supplemented group in the latter half of the experimental period.

The serum FSH levels in both groups showed only slight changes during experimental period. At 186 days of experiment, however, the serum FSH concentration in the vitamin E deficient group was significantly higher \((p < 0.05)\) than that in the vitamin E supplemented group.

**Testosterone concentration in the serum**

Chronological changes of the testosterone concentration in the serum are shown in Table 2. The serum testosterone concentration in the vitamin E supplemented group that was 222 ng/100 ml at the beginning of the experiment, at 25 days of age, increased to 780 ng/100 ml at 89 days of experiment and to 775 ng/100 ml at 105 days with increased variations, and then decreased thereafter \((p < 0.05)\). The serum testosterone concentration in the vitamin E deficient group also increased with the progress of experiment, showing the highest level of 668 ng/100 ml at 89 days of experiment, but decreased rapidly thereafter \((p < 0.01)\). The serum testosterone concentration in the vitamin E deficient group remained lower than that in the vitamin E supplemented group throughout the experiment though statistically not significant.

**Table 1. Effect of vitamin E deficiency on the erythrocyte hemolysis and concentrations of \(\alpha\)-tocopherol and malondialdehyde in the serum and liver**

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of rats</th>
<th>Body weight (g)</th>
<th>Erythrocyte hemolysis (%)</th>
<th>Serum tocopherol concentration (mg/100 ml)</th>
<th>Malondialdehyde concentration (nmole/ml)</th>
<th>Liver (nmole/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ VE</td>
<td>10</td>
<td>521.3±19.4</td>
<td>4.8±2.1</td>
<td>0.87±0.04</td>
<td>3.36±0.54</td>
<td>60.14±5.04</td>
</tr>
<tr>
<td>- VE</td>
<td>10</td>
<td>538.9±2.7</td>
<td>97.1±1.0**</td>
<td>0.10±0.01**</td>
<td>8.84±1.85*</td>
<td>91.36±6.90*</td>
</tr>
</tbody>
</table>

Values and Means± s.e. \(^*_p < 0.05, **p < 0.01.\)
Vitamin E Deficiency and Pituitary-Gonadal Function

The number of LH/hCG receptor and the association constant (Ka) for LH/hCG were measured by Scatchard plot analyses of the results obtained from competitive binding of [125I] hCG and unlabeled hCG to 10^6 Leydig cells as shown in Fig. 1. The vitamin E deficient group showed slightly large number of LH/hCG receptor, as compared with the vitamin E supplemented group. On the other hand, the vitamin E deficient group had a significantly smaller Ka than that of the vitamin E supplemented group (p < 0.01).

### TABLE 2. Serum LH, FSH and testosterone concentrations in the vitamin E deficient and supplemented rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of rats</th>
<th>Days in experiment</th>
<th>Serum LH concentration (ng × S1/ml)</th>
<th>Serum FSH concentration (μg × S1/ml)</th>
<th>Serum Testosterone concentration (ng/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>56</td>
<td>89</td>
<td>105</td>
</tr>
<tr>
<td>+ VE</td>
<td>5</td>
<td>0.43±0.08</td>
<td>0.55±0.05</td>
<td>1.09±0.20</td>
<td>0.37±0.19</td>
</tr>
<tr>
<td>- VE</td>
<td>5</td>
<td>1.13</td>
<td>0.38±0.01*</td>
<td>0.57±0.15</td>
<td>0.65±0.16</td>
</tr>
</tbody>
</table>

Values and means±s.e.  *p < 0.05  
LH=NIH-LH-S1.  FSH=NIH-FSH-S1.

### LH/hCG receptor in the Leydig cells

The number of LH/hCG receptor and the association constant (Ka) for LH/hCG were measured by Scatchard plot analyses of the results obtained from competitive binding of [125I] hCG and unlabeled hCG to 10^6 Leydig cells as shown in Fig. 1. The vitamin E deficient group showed slightly large number of LH/hCG receptor, as compared with the vitamin E supplemented group. On the other hand, the vitamin E deficient group had a significantly smaller Ka than that of the vitamin E supplemented group (p < 0.01).

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**Fig. 1.** Number of LH/hCG receptor and association constant (Ka)  
- Control:  
- VE-deficient:
Cyclic AMP formation by the Leydig cells

The formation of cyclic AMP by $10^6$ of Leydig cells was examined in the presence or absence of hCG as shown in Table 3. In the absence of hCG, the vitamin E deficient group formed a slightly large amount of cyclic AMP, as compared with the vitamin E supplemented group ($p < 0.05$). In the presence of 100 ng of hCG, the formation of cyclic AMP was significantly higher ($p < 0.01$) in the vitamin E supplemented group than in the vitamin E deficient group.

DISCUSSION

The vitamin E deficient rats showed a decrease in the serum $\alpha$-tocopherol level and an increase of hemolytic rate to 97% level, in accord with the results in our previous reports (Akazawa 1977, 1978). They also showed a marked increase of lipid peroxides estimated from TBARS concentrations in the blood and tissues. The histological studies on the pituitary-gonadal system in vitamin E deficient rats suggest that the vitamin E deficiency causes direct damage of the testis and acceleration of the pituitary function to compensate the impaired gonadal function. However, the data on the hormone secretion in the pituitary-gonadal system have been always inconsistent among investigators, possibly due to the degree and duration of vitamin E deficiency. Therefore, in the present study the chronological changes of the secretion of gonadotropin (FSH and LH) and sex hormone (testosterone) were examined in the several stages of vitamin E deficiency to know the effects of vitamin E deficiency on the pituitary-gonadal function. As the secretion of LH has been known to show the diurnal variation, the blood sample was taken at the fixed time in the day.

In the present study, the serum LH concentration in both vitamin E supplemented and deficient groups showed a tendency to increase with the advance of age in early period of experiment. This may correspond to the increasing demand of hormone secretion with the puberty. However, the serum LH concentration in vitamin E deficient rats reached the peak slower than in vitamin E supplemented rats, but remained higher than that after long period of vitamin E deficiency. These chronological changes of serum LH concentrations may be interpreted as being related to the serum testosterone concentration as described below. Chronological changes in the serum FSH concentration were not so

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of rats</th>
<th>Cyclic AMP (pmole/10⁶ cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>− hCG</td>
</tr>
<tr>
<td>+ VE</td>
<td>4</td>
<td>7.03 ± 0.70</td>
</tr>
<tr>
<td>− VE</td>
<td>4</td>
<td>10.06 ± 0.81</td>
</tr>
</tbody>
</table>

Values and means ± s.e. * $p < 0.05$; hCG, 100 ng.
conspicuous as those in the serum LH concentration and did not differ significantly between the groups, vitamin E deficient and supplemented. However, the serum FSH concentration increased significantly in the vitamin E deficient rats after 186 days of experiment. Schanbacher and Ford (1977) reported the inconstant increase of the serum FSH concentration in cryptorchid and castrated rats. These results suggest that the degeneration of seminiferous tubules stimulates FSH secretion.

Barnes et al. (1974) and Barnes and Smith (1975) reported the decrease of 17-oxosteroid concentration in the urine of vitamin E deficient rats. Lees et al. (1982) also reported that the plasma testosterone concentration was significantly lower in vitamin E deficient rats than in vitamin E supplemented rats after 130 days of experiments. However, Umeda et al. (1982) reported that the testicular content and basal plasma level of testosterone were not significantly changed in vitamin E deficient rats, but significantly higher in vitamin E supplemented rats than in the control rats. In the present study, the serum testosterone concentrations were increased for the first 3 months in both vitamin E supplemented and deficient groups, but lower in the vitamin E deficient group. The serum testosterone concentration in the vitamin E deficient rats seemed to decrease rapidly after 3 months of experiment and remained lower than in the vitamin E supplemented group throughout the experiments. The vitamin E deficiency caused the degeneration of testicular tissue and decrease of serum testosterone concentration, which, in turn, caused the increase in the secretion of LH owing to the feedback mechanism. The changes observed in the hormone secretion in pituitary-gonadal system of the vitamin E deficient rats were very similar to those in aged rats.

It is generally thought that the peptide hormone effect the target organs by binding specifically with their receptors on the plasma membrane. Since vitamin E may act on the stabilization of the plasma membrane, the property of the LH/hCG receptor in the Leydig cells was investigated from Scatchard plot analysis. The vitamin E deficient group showed a slightly large number of LH/hCG receptors as compared with vitamin E supplemented group. However, Ka was significantly smaller in the vitamin E deficient group than in vitamin E supplemented group. This means that the binding affinity of Leydig cells to LH is significantly lower in vitamin E deficient group. In addition, the decrease in total number of Leydig cells was also observed in the vitamin E deficient rats (Akazawa 1978). These may cause lower testosterone production in the vitamin E deficient group.

Kitabchi et al. (1973) and Nathans and Kitabchi (1975) reported the decrease in the steroid hormone synthesis in the adrenal cortex of vitamin E deficient rats. Kitabchi et al. (1978) also reported the decrease in the synthesis of cyclic AMP induced by ACTH and suggested this decrease to be caused by the decrease in the activity of adenylate cyclase. In the present study, cyclic AMP response induced by hCG was significantly decreased in the vitamin E deficient rats. This seems
to support the results of Kitabchi et al. (1978).

The results of the present study revealed that the vitamin E deficiency gives a suppressive effect directly on the gonadal function to decrease the hormone synthesis in the Leydig cells and causes the increase in secretion of LH owing to the feedback mechanism.

Acknowledgments

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References
