Effect of Low Calcium Diet on the Ultrastructure of the Rat Parathyroid Gland

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Summary: Young female rats were fed with normal (1.18\%) or low (0.05\%) calcium diet for 3, 7, 15 or 30 days. The morphology of the parathyroid glands was studied together with serum calcium, parathyroid hormone (PTH), calcitonin and bone mineral density (BMD). As compared to the animals fed with the normal calcium diet, BMD of whole body of the rats fed with the low calcium diet was significantly decreased, whereas the serum PTH level was increased. The parathyroid glands in the rats fed with the low calcium diet were markedly enlarged. In the parathyroid chief cells of the rats fed with the low calcium diet, the Golgi complexes and the cisternae of the granular endoplasmic reticulum were well developed, while the large granules and large vacuolar bodies decreased. Some secretory granules located near the plasma membrane. A proportionally larger increase of the cytoplasm was estimated in the rats fed with the low calcium diet for three and seven days. Enlargement of the cytoplasm and rather frequent mitoses of the chief cells were observed in the rats fed with the low calcium diet for 15 and 30 days. These findings suggest that the rapid bone loss in young rats induced by the low calcium diet is essentially due to stimulated activity of the parathyroid gland. The stimulated gland may be a result of hypertrophy at the early stage and a combination of hypertrophy and hyperplasia at the later stage of calcium deficiency.

Osteoporosis is a major public health problem among elderly characterized by a progressive loss of bone mass and subsequent structural weakness of bone. Calcium is one of the nutrients required for normal skeletal growth and mineralization, which plays a very important role in terms of its influence on bone mass (Matkovic and Heaney, 1992). Low calcium intake certainly causes osteoporosis in animals, presumably as the result of exaggerated bone resorption produced by the increased parathyroid activity resulting from hypocalcemia (Stauffer et al., 1973). Considerable studies have showed that low calcium diet stimulates secretory activity of the parathyroid gland (Lee and Roth, 1975; Naveh-Many and Silver, 1990; Wernerson et al., 1991). The influence of low calcium diet on the morphology of the parathyroid glands is a subject of controversy. Some reported that low calcium diet increased the rate of DNA synthesis and parathyroid hormone (PTH) mRNA levels in cultured parathyroid cells (Lee and Roth, 1975; Naveh-Many and Silver, 1990). Others considered that low calcium diet induced parathyroid cell hypertrophy rather than hyperplasia (Wernerson et al., 1991, 1995). On the contrary, Lorentzon et al. (1984) stated that there was no significant morphological change in the parathyroid chief cells of Mongolian gerbils kept on a calcium deficient diet. To clarify the issue, the present study discussed the morphology of the parathyroid gland and serum PTH levels on low or normal calcium diet.

Materials and Methods

Four-week-old female Wistar rats with an average body weight of 79 g were divided into eight
groups of seven animals each. The control animals were maintained on a commercial diet containing 1.18% calcium (CE-2, CLEA Japan). Experimental animals were fed with the low calcium diet containing 0.05% calcium (CLEA Japan). Both diets contain 0.85% phosphorus. All animals were kept in steel cages with free access to food and water. Each rat took 13.5 g diet/day average. Three, seven, 15 or 30 days later, the thyroid and parathyroid glands of all groups were removed under sodium pentobarbital anesthesia. The glands were fixed in 2.5% glutaraldehyde with 0.1 M phosphate at pH 7.4 for two hours, followed by postfixation with 1% Os04 for one hour. After dehydration in a series of graded acetone, specimens were embedded in Epon 812. Thin sections were cut on a Porter-Blum MT-1 ultramicrotome, stained with uranyl acetate and lead salts, and examined with a Hitachi H-800 electron microscope.

Ten micrographs at a final magnification of 22,000 were taken from different regions of the parathyroid glands of each animal from eight groups. The areas of the nuclei, cytoplasm, Golgi complex, and cisternae of the granular endoplasmic reticulum and the number of large granules were estimated with the aid of an image measuring system (Finetec).

Immunocytochemistry was carried out using the protein A-gold method as previously described (Shoumura et al., 1988). The parathyroid glands were fixed with Zamboni’s solution. Ultrathin sections on nickel grids incubated with 1% bovine serum albumin for 30 min and phosphate-buffered saline, followed by incubation with rabbit antiserum against bovine PTH (Biogenesis Ltd, England) for 20 hours at 4°C. Antiserum was applied at dilutions of 1:1000. The sections were rinsed in PBS and incubated in protein A-gold complex (particle size of 15 nm, EY laboratories, Inc, CA, USA) for 30 min at room temperature.

The serum calcium concentration was determined by standard colorimetric method (Gindler and King, 1972). The serum concentration of PTH and calcitonin was measured using rat PTH and calcitonin immunoradiometric assay kit (Immutopics, Inc., San Clemente, CA, USA). The bone mineral density (BMD) of whole body was measured by Dual Energy X-ray Absorptiometry (DXA) using a Toyo Medic QDR type 2000.

All data were presented as mean ± SEM. Statistical analysis was done using StatView J-4.5 (Abacus Concepts). Significance of the results was determined by one-way analysis of variance (ANOVA) and Fisher’s PLSD test. A p value <0.05 was considered statistically significant.

**Results**

The animals fed with the low calcium diet were subjected to severe calcium deprivation during the period when their most rapid growth would normally occur. This regimen resulted in growth retardation, but their general condition was not greatly affected. The body weight and BMD of whole body in rats fed with the low calcium diet for 7, 15 or 30 days were significantly decreased compared to those fed with the normal calcium diet (Table 1). The animals fed with the low calcium diet developed hypocalcemia, accompanied by an increase of serum PTH concentration (Table 1). There was no significant difference between the animals on the normal and low calcium diets with regard to serum calcitonin concentration (Table 1).

The two parathyroid glands in the rats were situated at the lateral surface of each thyroid lobe. The parathyroid glands were ovoid in shape, appearing gray to yellowish after fixation by glutaraldehyde. The mean size of the parathyroid gland of the rat fed with the normal calcium diet was

<table>
<thead>
<tr>
<th>Time</th>
<th>Protocol</th>
<th>Body Weight</th>
<th>BMD</th>
<th>Calcium</th>
<th>PTH</th>
<th>Calcitonin</th>
</tr>
</thead>
<tbody>
<tr>
<td>3d</td>
<td>N-Ca</td>
<td>101.7 ± 11.4</td>
<td>91.5 ± 10.3</td>
<td>10.88 ± 0.06</td>
<td>10.1 ± 2.6</td>
<td>51.3 ± 9.4</td>
</tr>
<tr>
<td></td>
<td>L-Ca</td>
<td>97.9 ± 13.2</td>
<td>90.7 ± 14.5</td>
<td>10.23 ± 0.07*</td>
<td>16.5 ± 5.2</td>
<td>56.5 ± 5.1</td>
</tr>
<tr>
<td>7d</td>
<td>N-Ca</td>
<td>120.8 ± 14.0</td>
<td>92.3 ± 11.7</td>
<td>11.07 ± 0.10</td>
<td>12.8 ± 3.4</td>
<td>59.3 ± 5.1</td>
</tr>
<tr>
<td></td>
<td>L-Ca</td>
<td>112.0 ± 17.3</td>
<td>88.2 ± 12.2*</td>
<td>8.98 ± 0.19*</td>
<td>26.8 ± 5.8*</td>
<td>51.5 ± 6.2</td>
</tr>
<tr>
<td>15d</td>
<td>N-Ca</td>
<td>162.6 ± 19.9</td>
<td>106.7 ± 15.4</td>
<td>10.87 ± 0.04</td>
<td>9.2 ± 2.7</td>
<td>61.3 ± 9.5</td>
</tr>
<tr>
<td></td>
<td>L-Ca</td>
<td>143.1 ± 22.1*</td>
<td>75.9 ± 8.8*</td>
<td>9.86 ± 0.13*</td>
<td>32.2 ± 8.6*</td>
<td>66.5 ± 5.1</td>
</tr>
<tr>
<td>30d</td>
<td>N-Ca</td>
<td>256.9 ± 28.9</td>
<td>115.1 ± 13.6</td>
<td>11.10 ± 0.09</td>
<td>11.4 ± 3.7</td>
<td>58.0 ± 6.2</td>
</tr>
<tr>
<td></td>
<td>L-Ca</td>
<td>203.2 ± 23.9*</td>
<td>83.4 ± 9.7*</td>
<td>9.04 ± 0.17*</td>
<td>46.4 ± 12.4*</td>
<td>67.0 ± 7.7</td>
</tr>
</tbody>
</table>

Values are shown in mean ± SEM. *p < 0.05.
Fig. 1. The rat thyroid and parathyroid glands. The parathyroid glands (encircled by dotted line) were markedly enlarged in the rats fed with the low calcium diet. a: normal calcium diet for 30 days; b: low calcium diet for 15 days; c: low calcium diet for 30 days. Bar = 1 mm.

Fig. 2. Parathyroid chief cells from the rats fed with the normal calcium diet for 7 days. Secretory granules (arrowheads), large granules (LG) and large vacuolar bodies (V) are observed. Bar = 1 μm.

Fig. 3. Parathyroid chief cells from the rats fed with the normal calcium diet for 30 days. Moderately developed Golgi complex (G), cisternae of the granular endoplasmic reticulum (ER) and large vacuolar body (V) are seen. Bar = 1 μm.
1.8 mm x 1.3 mm (Fig. 1). The parathyroid gland was significantly enlarged in the rat fed with the low calcium diet. The size of the gland of the rat fed with the low calcium diet for seven days was estimated to be twice of that on the normal calcium diet. The size of the gland on the low calcium diet for 15 and 30 days progressively increased to three or four times of that on the normal calcium diet (Fig. 1).

In the parathyroid glands of the rats fed with the normal calcium diet, the chief cells were oval or polygonal in shape. The intercellular spaces were generally narrow. Plasma membranes of the adjacent chief cells pursed a tortuous course with complex interdigitations (Figs. 2, 3). Nuclear profiles were roundish or elongated with some indentations. The cisternae of the granular endoplasmic reticulum were randomly distributed. The Golgi complexes were scattered throughout the cytoplasm (Figs. 2, 3). Chief cells contained a few secretory granules and some large granules. Secretory granules were distributed around the Golgi complexes and peripheral cytoplasm with a diameter of 100–200 nm. Large granules, being 350–800 nm in diameter, were round or ovoid and scattered in the cytoplasm (Figs. 2, 3). Large vacuolar bodies, being about 0.8–3.3 μm in diameter, containing numerous heterogeneously dense bodies, were sometimes found in the cytoplasm (Figs. 2, 3). The heterogeneously dense bodies showed lysosome-like structure. We did not find any mitosis or apoptosis in the parathyroid chief cells of the rats fed with the normal calcium diet.

In the animals fed with the low calcium diet, the parathyroid chief cells had more tortuous outlines and complicated interdigitations (Figs. 4, 5). The cisternae of the granular endoplasmic reticulum and the Golgi complexes were well developed (Figs. 4, 5). Some secretory granules were observed near the plasma membrane (Fig. 5). Large granules and large vacuolar bodies, however, were rarely encountered on low calcium diet. Parathyroid chief cells undergoing mitotic division were regularly present in the rats fed with the low calcium diet for 15 and 30 days (Fig. 6). Immunocytochemical method showed that protein A-gold particles were concentrated over the secretory granules, large granules and some Golgi vacuoles (Fig. 7). Gold particles were absent on the large vacuolar bodies and cisternae of the granular endoplasmic reticulum.

The morphometric data calculated in the para-

<table>
<thead>
<tr>
<th>Time</th>
<th>Protocol</th>
<th>N</th>
<th>ER</th>
<th>G</th>
<th>LG</th>
</tr>
</thead>
<tbody>
<tr>
<td>3d</td>
<td>N-Ca</td>
<td>27.1 ± 4.4</td>
<td>7.82 ± 0.86</td>
<td>3.15 ± 0.43</td>
<td>2.94 ± 0.35</td>
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<tr>
<td></td>
<td>L-Ca</td>
<td>22.3 ± 3.8*</td>
<td>8.45 ± 0.93*</td>
<td>3.82 ± 0.55*</td>
<td>1.12 ± 0.31*</td>
</tr>
<tr>
<td>7d</td>
<td>N-Ca</td>
<td>29.9 ± 4.8</td>
<td>8.01 ± 0.98</td>
<td>3.28 ± 0.50</td>
<td>3.25 ± 0.48</td>
</tr>
<tr>
<td></td>
<td>L-Ca</td>
<td>20.6 ± 3.5*</td>
<td>8.97 ± 1.03*</td>
<td>4.20 ± 0.45*</td>
<td>1.68 ± 0.29*</td>
</tr>
<tr>
<td>15d</td>
<td>N-Ca</td>
<td>25.8 ± 4.0</td>
<td>7.60 ± 0.68</td>
<td>3.37 ± 0.34</td>
<td>2.85 ± 0.43</td>
</tr>
<tr>
<td></td>
<td>L-Ca</td>
<td>21.3 ± 2.7*</td>
<td>8.93 ± 1.73*</td>
<td>4.77 ± 0.56*</td>
<td>1.81 ± 0.27*</td>
</tr>
<tr>
<td>30d</td>
<td>N-Ca</td>
<td>27.7 ± 3.6</td>
<td>8.18 ± 0.84</td>
<td>3.33 ± 0.35</td>
<td>3.01 ± 0.40</td>
</tr>
<tr>
<td></td>
<td>L-Ca</td>
<td>19.2 ± 3.1*</td>
<td>9.35 ± 1.16*</td>
<td>4.69 ± 0.54*</td>
<td>1.23 ± 0.23*</td>
</tr>
</tbody>
</table>

The volume density of nuclei is given as percentage to the whole cell. The volume density of other cell components is presented as percentage to the cytoplasm. Values are shown in mean ± SEM. *p < 0.05.
thyroid glands are shown in Table 2. As compared to the animals fed with the normal calcium diet, the volume density of the nuclei was significantly decreased in the rats fed with the low calcium diet (Table 2). The volume density occupied by the cisternae of granular endoplasmic reticulum and Golgi complexes was significantly increased, while the number of large granules in the cytoplasm was significantly decreased in the rats fed with the low calcium diet (Table 2). There was no marked morphological change in thyroid C cells of the rats fed with the low calcium diet.

Discussion

In the present study, the rats fed with the low calcium diet developed a progressive bone loss associated with a fall in serum calcium as reported earlier (Stauffer et al., 1973; Peterson et al., 1995).

Morphologic parameters of secretory activity in the parathyroid chief cells are mainly cisternae of the granular endoplasmic reticulum, Golgi complexes, secretory granules, and plasma membranes (Habener et al., 1984). On the ultrastructural level, numerous qualitative and quantitative studies have reported a close correlation between structure and function of the parathyroid chief cells (Lorentzon et al., 1984; Wernerson et al., 1991; Setoguti et al., 1995). In the present study, we found that in the parathyroid chief cells of the rats fed with the low calcium diet, the cisternae of the granular endoplasmic reticulum and Golgi complexes were significantly increased, while the large granules and large vacuolar bodies decreased, some secretory granules located near the plasma membranes. The plasma membranes showed more tortuous outlines and complicated interdigitations. These findings together with the increased serum PTH levels indicate the synthesis and release of PTH in parathyroid chief cells are stimulated in the rats fed with the low calcium diet.

Several morphological studies have been performed about the influence of low calcium diet on the rat parathyroid gland. The results from these studies are apparently inconsistent. Lee and Roth (1975) studied cultured parathyroid gland using autoradiography and found that the rate of DNA synthesis in parathyroid chief cell was enhanced by a rather small drop in ambient calcium concentration. The stimulus seemed to result in an increase in the number of cells entering the S-phase of the mitotic cycle, and cause secondary parathyroid hyperplasia. Naveh-Many and Silver (1990), applying the method of molecular biology and flow cytometry, indicated that PTH mRNA levels were increased five times by three weeks of low-calcium diet in rats and ten times on a vitamin D-, calcium-deficient diet. The number of the parathyroid cells increased 1.7 times on a vitamin D-, calcium-deficient diet. Therefore the increased PTH gene expression per parathyroid cell accounted for most of the increase in PTH mRNA, whilst increased cell number accounted for much less. On the contrary, Lorentzon et al. (1984) studied the quantitative morphology of the parathyroid gland in Mongolian gerbils and stated that there was no significant change with regard to the volume density of cellular components in parathyroid glands on the low calcium diets for eight days. Wernerson et al. (1991, 1995) estimated the number and size of the cells of the rat parathyroid gland. They found that the size of the parathyroid chief cells increased, whereas the total cell number was unchanged in the parathyroid chief cells of the rats fed with the low calcium diet for 28 days. Thus, the increased parathyroid size was supposed to be due to cell hypertrophy rather than hyperplasia.

In the present study, we found that the parathyroid gland was markedly enlarged in the rats fed with the low calcium diet. A proportionally larger increase of cytoplasmic volume than that of the nuclei was estimated in the rats fed with the low calcium diet for three and seven days. Enlargement of the cytoplasm and rather frequent mitoses of the chief cells were observed in the rats fed with the low calcium diet for 15 and 30 days. So we consider that low calcium diet induces hypertrophy of the parathyroid chief cells in the rat fed with the low calcium diet for three and seven days, and then produces hypertrophy and hyperplasia during 15 and 30 days, resulting in the enlargement of the parathyroid gland. The parathyroid chief cells maintain very low levels of proliferative activity throughout their entire life span (Parfitt, 1994), which may be expected to be matched by similarly low rates of cell death, although the apoptosis has not yet been demonstrated in normal parathyroid tissue. Mitosis is seldom found in normal adult animals. Recently we found there were some mitoses and apoptosis in the walls of chief cell cyst in five-day-old hamster parathyroid gland, indicating the rapid proliferation of the chief cells at this age (Chen et al., 2000). In the present study, chief cells undergoing mitotic division were regularly present in the rats fed with the low calcium diet. It indicates the stimulated parathyroid gland with hyperplasia.

Immunocytochemical localization of PTH was examined in the present study by using the protein A-gold technique. Protein A-gold particles were detected over the secretory granules and large granules. Accordingly, both granule types are
thought to include PTH, as reported earlier (Inoue and Setoguti, 1986, Shoumura et al., 1988). Large granules are considered to be storage granules which remain undischarged in the chief cells (Setoguti et al., 1995). Very few gold particles were noted on transitional forms between the large granules and large vacuolar bodies, but gold particles were absent on the large vacuolar bodies. In addition, small vesicles were observed near large granules, large vacuolar bodies and transitional forms (Shoumura et al., 1988). We think that the large granules are transformed to large vacuolar bodies involving lysosomal digestion (Shoumura et al., 1988, Setoguti et al., 1995).

In conclusion, the present study shows that low calcium diet stimulates the synthesis and secretion of PTH in the rat parathyroid gland, without any influence of thyroid C cells. We consider that the rapid bone loss induced by low calcium intake is essentially due to secondary hyperparathyroidism.

References