The Experimental Study on Dietary Therapy of Bone Loss in Mandibular Alveolar Bone in Prime Age

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Abstract

Seventeen-week-old male Wistar rats corresponding to the prime of life in human were used. In control group, rats were fed with standard diet for 6 weeks. In calcium-deficient group and low calcium group, calcium-deficient and low calcium chows were fed for 3 weeks, followed by standard chow feeding for another 3 weeks. The effects of the dietary therapy on bone matrix formation were investigated, and results were as follows.

1. Weight
No significant differences were observed among the control and experimental groups.

2. Radiographic findings
In calcium deficient group, the width and number of trabeculae were decreased, arrangement irregular, and less radiopaque. Low calcium rats exhibited the equivalent appearance to that of control animals.

3. Microdensitometric findings
Calcium deficient group exhibited lower density than control treatment.

4. Histopathological findings
Control group exhibited dense matrix, thick cortex in both outer and inner circumferential lamellae, and well-developed Harversian systems. In contrast, reduction of lacunae and expansion of marrow cavities were observed in calcium deficient group. Resorption and irregular distribution of trabeculae were seen. Low calcium rats exhibited equivalent findings to that of control animals.

5. SEM findings
Regularly distributed collagen fibrils were seen in matrix formative area in control treatment. Lacunae and canalicali opening were distinctly observed. In contrast, resorptive area expanded and collagen fibrils were loosely arranged in calcium deficient treatment. Calcareous microdepositions and collagen fibrils were seen in the upper section of bone formative areas. Low calcium rats exhibited comparable findings to those of control animals.

6. Hematological findings
No significant differences could be seen among the control and experimental groups in
serum level of calcium, phosphorus, ratio of calcium to phosphorus, sodium, potassium, and chloride. Calcium deficient rats exhibited higher GOT and CPK level than the control animals.

From the above results, it is suggested that bone loss can be well restored by dietary therapy, particularly in low calcium group, if calcium is sufficiently stored in bone tissues.

**Key words**: Alveolar bone/Osteoporosis/Dietary therapy/Rat

**Introduction**

Prevention and treatment of osteoporosis has become a major health concern as the patient population is rapidly increasing with the aged society arriving. Our previous studies showed that bone formation in growing years is a major determinant in maintaining bone mass in adult age\(^{15}\); in other words, the ample acquirement of peak bone mass is important for preventing osteoporosis. The adult years are the most stable years for bone tissue; however, there are few reports on the effect of dietary therapy on bone loss incurred with inadequate calcium intake, especially in mandibular bone.

In contrast to other skeleton, mandible is specific functionally because of the existence of teeth and stimulus of mastication. Ide (1979)\(^{6}\) reported that dentate mandible exhibited fewer changes with aging, while edentulous one showed decreased width and density with advancing years, although the change was less than that in lumbar vertebrae. It is necessary to investigate the effects of dietary therapy on bone loss in mandible derived from calcium insufficiency in adult years.

In this study, 17-week-old male Wistar rats, corresponding to adult age in human beings, were used. Bone loss was produced by calcium deficient chow fed for 3 weeks. Bone formation of mandibular alveolar bone was evaluated, after feeding with standard chow for 3 weeks, by microscopic, ultrastructural, microphotometric, and microanalytic study.

**Materials and Methods**

Thirty 17-week-old male Wistar rats corresponding to the prime of life in human were used. Control group was fed a standard chow made by Oriental Yeast (Tokyo, Japan) and tap water for 6 weeks. Calcium deficient group was fed a calcium deficient rat chow (Oriental Yeast, Japan) and distilled water for the first 3 weeks, and then a standard chow and tap water for another 3 weeks. Low calcium group was fed a low calcium chow (Oriental Yeast, Japan) and tap water for the first 3 weeks, and a standard chow and tap water for another 3 weeks. The content of the chows is shown in Table 1.

The rats were sacrificed with intraperitoneal injection of pentobarbital sodium (Nembutal, Nippon Pharmacy). The mandible was extracted and fixed in 10% neutral formalin. Soft radiographs were taken with CSM (ESM-2, Softex, Japan) and Fuji Softex film (FG, Fuji Film, Japan), aluminum stepwedge attached, at 28 kVp, 6 mA, 60 seconds of exposure time,
Table 1  The origin of the element content from the mineral mixture of the diets (mg/100 g)

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>Ca-deficient diet group</th>
<th>Ca-low diet group (20 %)</th>
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<tr>
<td>Ca</td>
<td>480</td>
<td>0.01</td>
<td>96</td>
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<td>P</td>
<td>650</td>
<td>612</td>
<td>612</td>
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<tr>
<td>Mg</td>
<td>87</td>
<td>87</td>
<td>87</td>
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<tr>
<td>Na</td>
<td>220</td>
<td>293</td>
<td>293</td>
</tr>
<tr>
<td>K</td>
<td>440</td>
<td>746</td>
<td>746</td>
</tr>
<tr>
<td>Fe</td>
<td>32</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td>Cu</td>
<td>0.46</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Zn</td>
<td>3.4</td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Mn</td>
<td>1.6</td>
<td>2.6</td>
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<tr>
<td>I</td>
<td>0.46</td>
<td>0.3</td>
<td>0.3</td>
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<tr>
<td>Cl</td>
<td>170</td>
<td>174</td>
<td>174</td>
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and 70 cm of focus-to-film distance. Microphotometry was carried out with microphotometer (PDS-15, Konica, Japan), with a beam slit of 10 by 500 μm, scanning from lower border to alveolar crest to obtain a scanning pattern. The ratio of calcium to phosphorus in alveolus was investigated by energy-dispersive X-ray microanalysis (JED-2000, Japan Electric). The samples were decalcified with 5% nitric acid and embedded with celloidin or parafin, 15- or 7-μm successive section were cut and stained with HE. For scanning electronic microscopic investigation, the samples were fixed in 2.5% glutaraldehyde for 1 hour after being cleaned with 10% sodium hypochlorite solution by supersonic vibration to eliminate adhesion. Then they were rinsed by buffered phosphate acid (pH 7.2), followed by postfixation in 1% osmic acid for 2 hours. The samples were dehydrated through a graded ethanol series, treated with 2-methyl-propanol, and dried in t-butyl alcohol by freeze-dryer (ID-2, Japan Electric). After sputter-coated with gold, the specimens were investigated with SEM (JSM T-300, Japan Electric) at 10 kv accelerating voltage.

Hematological test included serum calcium, phosphorus, and ratio of calcium to phosphorus, sodium, potassium, chloride, CPK, LDH, ALP, GOT, and GPT. Statistical significance was tested by F test.

Results

1. Weight
After 6 weeks of feeding, no significance was shown in weight among the control, calcium deficient, and low calcium treatment (Tables 2-1, 2-2).

2. X-ray findings
A. Alveolar bone
In control group, alveolae were shown to be densely radiopaque (Fig. 1-A).
Alveolar width of calcium deficient rats was shown to be thinner than that of control rats. Alveolae were shown radiopaque but sparse at places somewhere else. The width of alveolar
Table 2-1 Body weight of the control group and the experimental groups (age of 17-week) (g)

<table>
<thead>
<tr>
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<th>Control group</th>
<th>Ca-deficient diet group</th>
<th>Ca-low diet group</th>
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<tbody>
<tr>
<td>Pre-ex</td>
<td>436.17±14.78</td>
<td>458.17±16.21</td>
<td>452.40±23.34</td>
</tr>
<tr>
<td>6W</td>
<td>535.15±20.12</td>
<td>565.90±42.93</td>
<td>553.80±53.88</td>
</tr>
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</table>

n=10

Table 2-2 Differences of body weight between the control and the experimental group

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<tr>
<td>6W</td>
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— : Not significant

1: Control group
2: Ca-deficient diet group
3: Ca-low diet group

Fig. 1 Radiogram of the 6th week.
Arrow: alveolar bone
A: Control group
B: Ca-deficient diet group
C: Ca-low diet group
Dietary Therapy of Bone Loss (Murata et al.)

Table 3-1 Density of alveolar bone in the equivalent of aluminum (age of 17-week) (mm)

<table>
<thead>
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<th>Control group</th>
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<tbody>
<tr>
<td>Al</td>
<td>2.75±0.61</td>
<td>1.63±0.10</td>
<td>2.39±0.82</td>
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<tr>
<td>n=10</td>
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</tbody>
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Table 3-2 Differences of density of alveolar bone between the control and the experimental group

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<th>2 ↔ 3</th>
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<tbody>
<tr>
<td>Al</td>
<td>**</td>
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</tr>
</tbody>
</table>

**: p<0.01

1: Control group
2: Ca-deficient diet group
3: Ca-low diet group

Bone decreased coronally (Fig.1-B).

Trabeculae of low calcium rats were shown regularly arranged, with almost the same radiopacity as that of control rats (Fig.1-C).

B. Mental region of mandible

In control group, tuberosity was well developed, and trabeculae were regularly arranged (Fig.1-A).

In calcium deficient group, the width decreased, and trabeculae were shown decreased in number and irregular in distribution. Tuberosity was not so well developed as shown in control treatment (Fig.1-B).

In low calcium group, tuberosity was well developed, and trabeculae were regularly arranged as shown in the control treatment (Fig.1-C).

3. Microphotometric findings

Calcium deficient rats exhibited significantly lower density than control treatment (p<0.01) (Tables 3-1, 3-2).

4. X-ray microanalytic findings

Calcium concentration was shown higher than phosphorus in both control and experimental treatment (Figs.2A~C).

5. Histopathological findings

In control group the whole region, lingual, labial, medial and buccal alveolus bone, was investigated. In low calcium and calcium deficient group, we observed the changes from lingual alveolus bone to labial alveolus bone.

A. Control group

The region of investigation was set at 4 mm anterior to the first mandibular molar. Both lingual and labial alveolus, which is different structurally and functionally, was investigated.
Fig. 2 Characteristic X-ray images of alveolar bone.
A: Control group  
B: Ca-deficient diet group  
C: Ca-low diet group

In lingual region periodontal fibers exist where active bone remodeling corresponds to the mechanical stimulus, while in labial region, enamel organ, nerve, vessels and loose connective tissue exist where bone resorption takes place (Figs. 3, 4). Lots of oval, spindle, or rectangle shaped osteocytic lacunae were arranged in superficial cortical area of lingual alveolar bone. The Sharpey's fiber bundles in the periodontal membrane were inserted into the bundle bone, where Harversian canals were hardly identified. In alveolar crest where was connected with
fibrous bone, Haversian lamellae and lamellar bone were shown abandunt. Outer part of the crest was composed of thick cortex, while inner part of cancellous bone, where osteoblastic formation was shown and marrow scattered. Also in the inner part, bundle bone could be seen, and connection between periodontal membrane and bone marrow was identified. In cancellous bone, the thick-stained remodeling layers were seen as well as the replacement of bundle bone by lamellar bone (Figs. 5, 6).

In medial alveolar bone, fibroblast-induced collagen was abundant, and Sharpey's bundles formed. Vascular-nerve spaces were seen where artery, vein, lymphatic, and nerves connected. Loose connective tissues were well seen around the spaces. The consecutive and serrated bone resorption were seen in alveolar bone (Fig. 7).

In contrast to the medial alveolar bone, buccal one exhibited thicker periodontal membrane. Sharpey's fibers were shown inserting into the bone. A lot of bone resorption was seen there. In the cortical area, there were a lot of oval, spindle, or rectangle shaped osteocytic lacunae in different sizes, while in the medial the osteocytic lacunae were in flat (Fig. 8).
Abundant vasculae were seen in loose connective tissues around the enamel organ in labial alveolar bone. The consecutive and serrated bone resorption was seen in a superficial layer of alveolar bone, where a thin layer of bone formation was also observed. Osteocytic lacunae exhibited oval, spindle, or irregular shaped in inner part of the alveolar bone; while it became thin and regularly arranged in outer section (Fig. 9).

B. Calcium deficient group

In contrast to that in control treatment, the width of alveolar bone was shown decreased in lingual, medial, buccal, and labial sections. Resorption was seen in bundle bone and the number of osteocytic lacunae was decreased and enlarged bone marrow spaces were seen. Also, osteocytic lacunae exhibited irregularly distributed in both spongy and cortical areas. Harversian systems were apparently reduced, and many of them were in low calcified. where the osteocytic lacunae were concentrically arranged around the Harversian canals and cement lines were seen here and there (Figs. 10, 11).

Compared with control treatment, the width of central alveolar bone was decreased, and periodontal membrane was shown loosely distributed. In the bone connected with periodontal membrane, the oval and spindle shaped osteocytic lacunae were scattered and much less than as shown in control group (Fig. 12).
Fig. 10 Alveolar bone of the 6th week.
Ca-deficient diet group
H·E stain (×50)

Fig. 11 A higher magnification of Fig. 10-[A]
H·E stain (×200)

Fig. 12 Alveolar bone of the 6th week.
Ca-deficient diet group
H·E stain (×100)

Fig. 13 Alveolar bone of the 6th week.
Ca-deficient diet group
H·E stain (×100)

Fig. 14 Alveolar bone of the 6th week.
Ca-deficient diet group
H·E stain (×200)
Bone resorption was shown increased obviously in superficial section of buccal alveolar bone, vs. that in control treatment (Fig.13).

The formation of labial alveolar bone apparently decreased vs. that in buccal and medial sections. And osteocytic lacunae were shaped oval and spindle in different size (Fig.14).

C. Low calcium group

The width from lingual alveolar bone to buccal alveolar bone increased in low calcium group, which was equivalent to control while in contrast to calcium deficient rats. Osteocytic lacunae exhibited abundant and congested in superficial section, mainly shaped in oval and spindle. Next to the fibrous bone, lamellar bone increased with marrow scattered, where thick-stained areas were seen, indicative of active bone remodeling (Figs.15, 16).

In central alveolar bone, as well as in buccal section, the bone width is equivalent to that in control treatment, with number of osteocytic lacunae dramatically increased, and that in calcium deficient treatment. Vascular-nerve spaces were abundant in alveolar bone which was connected with periodontal membrane. Fibroblasts were seen along the Sharpey's
bundles. The width of periodontal spaces was equivalent to that in control treatment (Figs. 17, 18).

The width of loose connective tissue in labial alveolar bone was wider inappreciably than in control group, while the bone structure and lacunae distribution were equivalent to those seen in control treatment except in the area where blood vessel approached into the bone (Fig. 19).

4. SEM findings
Control group

Formation of bone matrix was readily seen in resorptive and resting area of superficial section. Collagen fibrils covered matrix formative layer in the same direction. Lacunae, about 10μm in diameter, and canalicali opening were distinctly shown in matrix (Fig. 20).
Table 4-1 Laboratory values of electrolytes (age of 17-week)

<table>
<thead>
<tr>
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<th>Control group</th>
<th>Ca-deficient diet group</th>
<th>Ca-low diet group</th>
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<tbody>
<tr>
<td>Ca</td>
<td>10.07±0.19</td>
<td>10.00±0.29</td>
<td>10.00±0.08</td>
</tr>
<tr>
<td>P</td>
<td>7.10±0.82</td>
<td>6.60±0.78</td>
<td>6.57±0.21</td>
</tr>
<tr>
<td>Ca/P</td>
<td>1.43±0.12</td>
<td>1.53±0.09</td>
<td>1.53±0.05</td>
</tr>
<tr>
<td>Na</td>
<td>142.33±0.47</td>
<td>143.33±1.25</td>
<td>142.33±0.94</td>
</tr>
<tr>
<td>K</td>
<td>5.30±0.08</td>
<td>5.17±0.17</td>
<td>5.13±0.48</td>
</tr>
<tr>
<td>Cl</td>
<td>101.67±0.47</td>
<td>102.67±0.47</td>
<td>102.00±2.16</td>
</tr>
</tbody>
</table>

n=5

Table 4-2 Differences of laboratory values of electrolytes between the control and the experimental group

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<tbody>
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<td>Ca</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>P</td>
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<td>—</td>
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<tr>
<td>Ca/P</td>
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<td>Na</td>
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<tr>
<td>Cl</td>
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—: Not significant

1: Control group
2: Ca-deficient diet group
3: Ca-low diet group

The resorption foveae were so shallow that osteocytic lacunae were exposed.
Calcium deficient group
Resorption foveae were shown expanded, and lots of calcareous microdepositions and collagen fibrils were seen in the upper section of bone formative areas (Fig.21).
Low calcium group
In the bone formative area, these could be seen that collagen fibrils were in the same direction and rounded osteocytic lacunae whose diameter was about 15 around, and there were lots of calcareous microdepositions. These observations were different to calcium deficient group (Fig.22).

5. Hematological findings
No significant differences were observed among the control and experimental rats in serum calcium, phosphorus, and ratio of calcium to phosphorus, sodium, potassium, and chloride (Tables 4-1, 4-2). Calcium deficient group showed significantly higher GOT and CPK level
Table 5-1 Laboratory values of blood chemistry (age of 17-week)

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>Ca-deficient diet group</th>
<th>Ca-low diet group</th>
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</thead>
<tbody>
<tr>
<td>GOT</td>
<td>110.67± 4.92</td>
<td>155.33± 11.12</td>
<td>123.33± 17.78</td>
</tr>
<tr>
<td>GPT</td>
<td>30.33± 3.86</td>
<td>40.67± 14.27</td>
<td>32.00± 9.90</td>
</tr>
<tr>
<td>LDH</td>
<td>1822.33±403.43</td>
<td>2632.67±287.23</td>
<td>2389.00±511.18</td>
</tr>
<tr>
<td>CPK</td>
<td>1537.00±287.51</td>
<td>3898.67±581.70</td>
<td>2765.00±822.74</td>
</tr>
<tr>
<td>ALP</td>
<td>265.33±24.90</td>
<td>300.33± 85.29</td>
<td>257.67± 68.12</td>
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n=5

Table 5-2 Differences of laboratory values of blood chemistry between the control and the experimental group

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<td>GPT</td>
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<td>LDH</td>
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<tr>
<td>CPK</td>
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<tr>
<td>ALP</td>
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**: p<0.01

1: Control group  
2: Ca-deficient diet group  
3: Ca-low diet group

than control treatment (p<0.01) (Tables 5-1, 5-2).

Discussion

Our previous studies have shown that bone structure was greatly influenced by insufficient dietary calcium in developing years of experimental animals. It was also suggested that the recovery of the bone loss, which was incurred with insufficient dietary calcium in developing years, was difficult to restore even by dietary therapy and application of other regulating materials. But there have been few reports on the effects of dietary therapy on the recovery from bone loss, especially of jaw bones, due to insufficient calcium intake in adult years.

1. Weight

Weight increases slowly a week after birth, and the increase is dramatic in early childhood and in adolescence. Weight and height have no great changes in adult age in human beings. In contrast, weight and height still increased even after adulthood in rats. Salomon (1972) reported that the changes in weight were induced by those of bone tissues. Shuto (1992)
reported that weight was related with radiographic density. It was reported that insufficient dietary calcium in weaning years or early childhood had a great influence on the increment of the weight\(^4\)\(^-\)\(^8\), although the effects of bone structure on the change of weight are still unclear. The previous studies showed that the bone loss due to calcium insufficiency, in the years when the bone formation was active, was difficult to restore by dietary therapy afterwards, even with the application of other regulating materials\(^4\)\(^-\)\(^5\). From the results of the present study, it is shown that weight is well maintained by dietary therapy after bone loss was incurred with inadequate calcium intake, because of the bone mass substantially obtained by adulthood.

2. X-ray findings and bone density

Radiographic examination is an important method in evaluation of bone structure, and densitometric investigation is a quantitative modality. The radiographic findings were reported previously on the effects of calcium insufficiency on the bone tissues\(^9\)\(^-\)\(^\text{13}\), which showed trabeculae were decreased in density and reduced in number, and irregularly arranged. The present study showed the equivalent findings of both alveolar and mental bone in low calcium group to those in control treatment. However, calcium deficient rats exhibited reduced number of trabeculae and irregular distribution, which indicated that bone loss was not well restored compared with control rats. Various densitometric examinations have been used in diagnosing and monitoring osteoporosis and metabolic diseases of bones\(^\text{12}\)\(^-\)\(^\text{14}\), as well as in quantitative measurement of jawbone density. MD (Micro Densitometry) method used in the present study evaluated radiographic density of the region of interest with the comparison between the density and that of aluminum stepwedge attached. Maki et al (1993)\(^\text{15}\) reported the application of this method in evaluating the jawbone in schoolchildren. Their results indicated that jawbone density was increased with aging. Although bone structure can not be judged just by the densitometric investigation, our results suggested that bone loss resulted by low calcium intake could be restored with dietary therapy, if bone mass was considerably obtained by balanced food before the adult years. However, the restoration was difficult because the calcium deficiency lasted for a long time. Therefore, the accumulations of peak bone mass before the adult year is a major determinant in prevention of osteoporosis. Genetic factor, exercise, and nutrition have effects on the acquirement of peak bone mass. Recent reports suggested that both intramembranous and endochondral were completely blocked, owing to the maturational arrest of osteoblasts in the mutant mice, and demonstrated that Cbfa–1, one of the runt-domain gene family, played an essential role in osteogenesis\(^\text{16}\). Another study concluded that osteoclast differentiation factor, a long-sought ligand mediating an essential signal to osteoclast progenitors for their differentiation into osteoclasts, seems to be an important regulator in not only osteoclastogenesis but also immune system\(^\text{17}\). The relation between these genetic factors and peak bone mass need to be further investigated.

3. Histopathologic findings

The animals in control group, fed a standard chow, exhibited dense matrix in alveolar bone
and thick cortex both in outer and inner circumferential lamellae. Harversian systems are well developed. There have been many reports on the effects of dietary therapy on bone loss of alveolar bone, which was resulted by calcium deficiency. Ishii (1986) and Umezu (1991) reported a reduction of lacunae and expansion of the marrow cavities in alveolar bone were observed in calcium deficient group compared with control treatment. Our previous studies found out that calcium-deficiency fed animals showed resorption, irregular distribution, and less calcification of trabeculae, reduction of lacunae, and expansion of marrow cavities. The present study showed that bone loss had been resulted by calcium deficiency, showing thin width of outer circumferential lamellae, decreased number of Harversian systems, and increased cavitation of osteocytic lacunae. However, bone loss was restored in low calcium group, to the extent that was equivalent to the control treatment. The reason was thought to be that the calcium deficiency-induced bone damage could be inhibited by dietary therapy, had the bone formation be well obtained by standard diet before the adult years. The decrease of bone mass was thought to be restrained by metabolic hormones. The results revealed that bone metabolism would be well maintained as long as calcium intake was guaranteed. Apart from such systemic factors as hormonic metabolism, structure of jawbones is also affected by local factors, namely, occlusal force, and masticatory force. Machwate et al. (1994) examined the effects of IGF-1 on trabecular bone formation, BMD, and proliferation of marrow-derived osteoblastic cells in unloaded rats, induced by tail suspension. They found that unloading was associated with a decreased proliferation of alkaline phosphatase-positive marrow stromal cells. Their results indicated that IGF-1 infusion enhanced the recruitment of osteoblastic cells, increased trabecular bone formation, and partially prevented trabecular bone loss in unloaded rats.

4. SEM findings

Bone formation and resorption take place through the life, and bone structure is maintained by coupling of the two processes. In control treatment of the present study, a great number of osteocytic lacunae were seen on the surface of the bone matrix in formative area. Collagen fibrils and sufficient calcareous microdepositions were seen in superficial section. Sherft (1968) investigated the ultrastructure of the calcareous microdeposition. The author found out that the outer section was composed of microdeposition and inner section of loose connective fibers. Some in vivo and in vitro studies have been reported on the relationship between the calcifying mechanism and collagen. Also, matrix vesicles were reported to be closely related with early stage of calcification. Further studies are needed to investigate the vesicles biochemically and immuno–histochemically.

SEM findings in calcium deficient treatment were shown equivalent to those in control treatment, except decreased width. We previously found that it is difficult to restore from bone loss due to calcium deficiency in growing years, even with dietary therapy combined with regulating materials such as vitamin D₃, or calcitonin. Damage to bone in the present study was not as severe as in our previous studies, because of substantial storage of calcium in the bone tissues incurred with standard diet fed earlier, although it is still difficult to
restore from bone loss comparably to that shown in control treatment. Low calcium group exhibited almost identical findings to those in control treatment, which suggested that calcium intake is a major determinant for normal functioning of bone metabolic mechanism. Our results showed mandible is not susceptible to osteoporosis because of existence of teeth and stimulus of mastication, compared with other skeleton, as revealed in other reports.

5. Hematological findings

A. Electrolytes

Hematological test is important in evaluating metabolic condition from developing to the aging years. No significant differences were observed in the present study in any hematological tests among the control and experimental rats. Albright et al (1940) reported that serum calcium and phosphorus, ALP level was generally shown normal in osteoporosis, but Ishii (1986) found that osteoporosis showed higher level of serum calcium, chloride, and ratio of calcium to phosphorus. The results in the present study suggested that even if calcium deficiency was resulted contemporarily, dietary therapy could maintain the systemic function normally had calcium been sufficiently stored in bone tissues till the adult year.

B. Biochemistry

ALP (alkaline phosphatase) is generally used as a marker enzyme for osteoblasts. ALP level were unaffected by calcium deficient treatment in the present study, indicating that balance between bone formation and resorption was well maintained. LDH (lactate dehydrogenase) within matrix vesicles, which is considered to play an important part in early calcification, was reported to exist in intramembranous or endochondral ossification tissues, and in cement, indicating its relation with calcification. Also, there was a report suggesting that serum LDH level was increased in calcium deficiency. More researches are necessary to verify the relation between the LDH level and osteoporosis. We also found elevated levels of GOT and CPK in the experimental groups of the present study, which need further investigation.

Conclusion

Seventeen-week-old male Wistar rats corresponding to the prime of life in human were used. In control group, rats were fed with standard diet for 6 weeks. In calcium-deficient group and low calcium group, rats were fed with calcium-deficient and low calcium diet for 3 weeks, followed by standard chow feeding for another 3 weeks. The effects of the dietary therapy were investigated, and results were as follows.

1. Weight

No significant differences were observed among the control and experimental groups.

2. Radiographic findings

In calcium deficient group, the width and number of trabeculae were decreased, arrangement irregular, and less radiopaque. Low calcium rats exhibited the equivalent appearance to that of control animals.
3. Microdensitometric findings
Calcium deficient group exhibited lower density than control treatment.

4. Histopathological findings
Control group exhibited dense matrix, thick cortex in both outer and inner circumferential lamellae, and well-developed Harversian systems. In contrast, reduction of lacunae and expansion of marrow cavities were observed in calcium deficient group. Resorption and irregular distribution of trabeculae were seen. Low calcium rats exhibited equivalent findings to that of control animals.

5. SEM findings
Regularly distributed collagen fibrils were seen in matrix formative area in control treatment. Lacunae and canalicali openings were distinctly observed. In contrast, resorptive area expanded and collagen fibrils were loosely arranged in calcium deficient treatment. Calcareous microdepositions and collagen fibrils were seen in the upper section of bone formative areas. Low calcium rats exhibited comparable findings to those of control animals.

6. Hematological findings
No significant differences could be seen among the control and experimental groups in serum level of calcium, phosphorus, ratio of calcium to phosphorus, sodium, potassium, and chloride. Calcium deficient rats exhibited higher GOT and CPK level than the control animals.

From the above results, it is suggested that bone loss can be well restored by dietary therapy, particularly in low calcium group, if calcium is sufficiently stored in bone tissues.

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壮年期の虚弱下顎歯槽骨における
食餌療法に関する実験的研究

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ヒト壮年期に相当する17週齢のウイスター系雄ラットを使用し、カルシウム欠乏食と低カルシウム食の骨基質形成に与える影響を検討した。対照群は標準食を6週間与え、実験群はカルシウム欠乏食または低カルシウム食を3週間与えた後、標準食に切り換えて3週間飼育した。体重、骨塩量、病理組織所見、走査電顕所見、血液所見などについて比較検討し、以下の結果を得た。

I. X線骨密度（骨塩量）
対照群とカルシウム欠乏食群との間には有意差が認められ、カルシウム欠乏食群が低値を示した（p < 0.01）。

II. 病理組織学的所見
対照群における標準食で飼育されたラットの歯槽骨全域を観察すると骨基質は緻密であり、ハッパース系の発達もないであった。カルシウム欠乏食では対照群に比較し、歯槽骨内の骨小腔の減少や、骨縁の拡大、骨梁の吸収、低石灰化の所見がみられ、また低カルシウム食群では対照群とほぼ同様な組織所見が得られた。

III. 走査型電子顕微鏡所見
対照群の骨基質形成面は一定方向に走行するコラーゲン原線維束で形成されていた。表層はコラーゲン原線維網が密となり、骨基質面を被って認められた。コラーゲン原線維網と骨基質部は骨小腔がみられ骨細管の間隔も明瞭に認められた。カルシウム欠乏食群は、骨形成面に比べ骨吸収面の占める割合が増加していた。しかし骨形成面にはコラーゲン原線維束が疎に配列して、その表層部にはコラーゲン原線維網と微細顆粒状の石灰塊が多数沈着しているのが認められた。低カルシウム食群は、カルシウム欠乏食群に比べ骨基質形成面は平坦で、ほぼ対照群と同様な所見を呈していた。

以上のことから、壮年期に一時期にカルシウム欠乏状態に陥ってもその後の食餌療法で生体の恒常性を維持でき、特に低カルシウム食群では対照群と同様な骨基質形成の回復所見が得られた。