X-Ray Densitometric, Light-Microscopic and Ultrastructural Study on Dietary Treatment of Mandibular Bone Loss in Rats During Childhood

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Introduction

Childhood is a remarkable phase for the growth and development of the individuals. At this age level the primary dentition is completed to erupt and supported by the jaw bone in the oral cavity via roots embedded in bone. According to Scammon's¹⁾ developmental curves of the living tissues, during childhood lymphoid type develops sharply while neural type does slowly to adjust to the functions of tissues. The growth of general type is later than that of lymphoid and neural types, and at the primary sexual characteristic stage the growths of height, body weight and bone reach their peak respectively. Moreover, genital type develops suddenly and rapidly at the second sexual characteristic stage, also named as puberty. Jaw oral functions are associated with the occlusive power and masticatory ability, and develop satisfactorily with the growth of the face and skull. On the areas of matrix formation in the growing jaw bone appears a lot of vascularity while most bone lacunae lie scattered on the surface of the matrix around the vascular foramina, showing marked formation of bone matrix. Of all the jaw bones, the alveolar bones, possessing the supporting tissues of teeth, are membrane bones, which are formed through the differentiation of mesenchymal cells into osteoblasts in addition to the existence of mineral. Therefore, the intestinal resorption of calcium seems important, but by now there is little known about the mechanism between calcium and dietary treatments of bone matrix formation of the mandible during childhood. Herein, we fed 5-week-old Wistar male rats (corresponding to childhood in human beings) on low-calcium diet (calcium component is 30 % of standard diet) so as to bring about bone loss in them, and then treated them with dietary treatments. Later, we observed bone matrix formation of the mandible and obtained some findings as follows.

Materials and Methods

1. Animals and Treatments

Thirty Wistar male rats of 5 weeks old, corresponding to childhood in human beings, were

Table 1 The composition and content of mineral mixture (mg/100 g)

	Control group	Ca-deficient diet group	Ca-low deficient diet group (30 %)
Ca	480	0.01	144
P	650	612	612
Mg	87	87	87
Na	220	293	293
K	440	746	746
Fe	32	32	32
Cu	0.46	0.5	0.5
Zn	3.4	3.0	3.0
Mn	1.6	2.6	2.6
I	0.46	0.3	0.3
Cl	170	174	174

randomly divided into 3 groups as follows. In the control group, rats were fed on a standard diet with tap water for 6 weeks. In the low calcium experimental group, rats were fed on a low calcium diet (calcium component is 30 % of standard diet) with tap water for 3 weeks; then they were fed on standard diet with tap water for further 3 weeks. In the calcium deficient experimental group, rats were fed on a no-calcium-component diet with distilled water for 3 weeks; then they were treated the same as the low calcium experimental group for further 3 weeks. All the rats' food was made for this study by Oriental Yeast (Tokyo, Japan). The content of the feed was shown on Table 1. All the rats were housed in the animal room, of which the temperature was $22 \pm 2^\circ\text{C}$, the humidity was $50 \pm 5\%$ and the lighting time was from 8 a.m. to 8 p.m.. The rats were weighed every day during feeding. Following 6 weeks of feeding, the rats were sacrificed under anesthesia with intraperitoneal injection of pentobarbital sodium (Nippon Pharma, Japan). Their mandibles were removed and later treated for densitometric measurement, microanalysis for Ca and P, light-microscopic observations, and scanning electron microscopic observations. The animals were handled with adherence to the principles on the use of experimental animals outlined by Kyushu Dental College.

2. Densitometric measurement

The mandibles were kept in 10 % neutral buffered formalin solution. These samples were imaged with Softex CSM at 28 kVp, 6 mA, 60s and focus-film distance of 70 cm with Fuji softex film FG and aluminum reference wedge attached. These films were observed by Microphotometer (Desitometer PDS-15, Konica, Japan). The survey light passed from the bottom border of the mandible to the top border of the mandibular alveolar bone at speed of 0.1 mm/s. Scanning was carried out with slit of $10 \times 500 \mu\text{m}$.

3. Sample preparation for microanalysis for Ca and P

The samples were cut from the incisor alveolar crest to 1 mm in front of the molar area, at thickness of 3 mm, vertical to the mandibular border, by dental diamond disk (#62, Shofu,

Kyoto, Japan) with spraying water. After fixation with 10 % neutral buffered formalin solution, these samples were dehydrated through a graded ethanol series and treated in 2-methyl- α -propanol, dried in *t*-butyl alcohol by freeze-drying (ID-2, Japanese Electric, Japan). Sections were embedded in resin, then abraded, polished, and sputtered with aurum. Later they were observed under a scanning microscope (JSM T-300, Japanese Electric) equipped with energy-dispersive X-ray microanalysis (JED-2000, Japanese Electric) to do area analysis on examining the distribution of calcium (Ca) and phosphate (P), and point analysis on determining the content of Ca and P.

4. Sample preparation for light-microscopy

The samples were cut from the incisor alveolar crest to 1 mm in front of the molar area. After fixation with 10 % neutral buffered formalin solution, these samples were decalcified in 5 % nitric acid solution for 24h, dehydrated through a graded ethanol series, and embedded in paraffin (Fisher Scientific, USA). Sections of 7 μ m–15 μ m thick were cut consecutively, stained with hematoxylin and eosin, and observed under light-microscope.

5. Sample preparation for scanning electron microscopy (SEM)

The samples were cut as in preparation for x-ray microanalysis, and fixed with 2.5 % glutaraldehyde for 1 h after being cleaned with 10 % sodium hypochlorite solution by supersonic vibration to eliminate adhesion. They were rinsed by phosphate buffer (pH 7.2) before postfixation. Postfixation were performed in 1 % osmic acid buffer solution (pH 7.2) under 4 °C for 2h. After fixation, these samples were dehydrated through a graded ethanol series, treated with 2-methyl- α -propanol, dried in *t*-butyl alcohol by freeze-drying (ID-2, Japanese Electric). Specimens were sputter-coated with gold and later observed under a scanning electron microscope (JSM-300, Japanese Electric).

Results

1. X-ray findings

X-ray findings were observed from the films taken for densitometric measurement. In the control group, bone structure of the mandibular alveolus was dense and regularly-aligned, and the thick alveolar bone surrounded the root of the incisor (Fig. 1). Compared with the control group, the calcium deficient group showed the thin alveolar bone, and sparse and irregularly-arranged trabeculae (Fig. 2). Compared with the calcium deficient group, the low calcium group showed increased thickness of the alveolar bone and somewhere dense bone structure was seen. But compared with the control group, the low calcium group showed

Fig. 1 Radiogram of the 6th week.
Control group
Arrow: Alveolar bone

Fig. 2 Radiogram of the 6th week.
Ca-deficient diet group
Arrow: Alveolar bone

Fig. 3 Radiogram of the 6th week.
Ca-low deficient diet group
Arrow: Alveolar bone

Table 2 Bone shadow density of the alveolar bone in the equivalent of aluminum (mm)

Group	Density
Control group	2.20 ± 0.38
Ca-deficient $\rightarrow$ normal diet group	1.75 ± 0.15
Ca-low deficient $\rightarrow$ normal diet group	1.83 ± 0.24

Mean $\pm$ S. D.

Table 3 Difference of bone shadow density in alveolar bone among the 3 group

	A $\Leftrightarrow$ B	A $\Leftrightarrow$ C	B $\Leftrightarrow$ C
Density	*	—	—

* : $p < 0.05$

A: Control group

B: Ca-deficient $\rightarrow$ normal diet group

C: Ca-low deficient $\rightarrow$ normal diet group

sparse bone structure in an overall observation of the alveolar bone (Fig.3).

2. Densitometric measurement

The results of densitometric measurement in the control and experimental groups are presented in Table 2 and 3. The calcium deficient group showed significantly lower density compared with the control group ($p < 0.05$).

Table 4 Relative percentage of the Ca and P in alveolar bone by microanalysis (%)

	Ca	P
Control group	76.55±1.22	23.45±1.72
Ca-deficient → normal diet group	73.52±1.57	26.48±1.57
Ca-low deficient → normal diet group	73.69±1.56	26.31±1.56

Mean±S. D.

Table 5 Differences of Ca and P of alveolar bone by quantitative X-ray microanalysis between the control group and the experimental group

	A ⇔ B	A ⇔ C	B ⇔ C
Ca	*	*	—
P	*	*	—

*: p<0.05

A : Control group

B : Ca-deficient → normal diet group

C : Ca-low deficient → normal diet group

Table 6 Relative ratio of Ca and P of alveolar bone by quantitative X-ray microanalysis (%)

	Ca/[P]c	P/[P]c
Control group	1	1
Ca-deficient → normal diet group	0.96	1.13
Ca-low deficient → normal diet group	0.96	1.12

3. Microanalysis for Ca and P

The results of microanalysis for Ca and P in the control and experimental groups are presented in Tables 4, 5 and 6. The experimental groups showed significantly lower Ca/P ratio compared with the control group ($p<0.05$). The experimental groups showed lower relative ratio of Ca than the control group, but there is no significant difference between the

H·E stain (×20)

AB : Alveolar bone (lingual)

BM : Bone marrow

D : Dentin

H·E stain (×20)

ab : Alveolar bone (labial)

D : Dentin

E : Enamel

P : Pulp

Fig. 4 Alveolar bone of the 6th week.
Control group

experimental groups. The relative ratio of P showed a decreasing tendency on a sequence of the calcium deficient group, low calcium group and control group.

4. Histopathologic Findings

Compared with the control group, the calcium deficient group showed irregular periodontal membrane in the lingual, medial, and buccal or labial alveolar bone, markedly decreased bone lacunae and Haversian system in number, suggesting decreased bone matrix formation. The low calcium group showed increased bone lacunae, Haversian system in number and bone matrix formation when compared with the calcium deficient group; but insufficient bone remodeling and restoration could be seen in the low calcium group when compared with the control group. (Figs. 4-7)

5. SEM findings

Control group

In the formation areas of bone matrix, the resting surface was adjacent to the resorption surface. On the formation surface of bone matrix appeared many collagen bundles in a particular alignment. There were many bone lacunae, at the wall of which collagen fibers were seen crossed. Other dense collagen fibers crossed into the a network on the upper surface of bone matrix. In the spaces among the collagen fibers, the mouths of bone canaliculi were observed (Figs. 8, 9). On the bone resorption surface, there were many irregular-shaped lacunae of bone resorption. On the shallow and rough bottom of the bone resorption lacunae, slender collagen fibers were seen crossed into a network and many micronized calcareous depositions appeared collective, or cross, or broken-off, or granulated, or vanished.

Calcium deficient group

Compared to the control group, many large or small bone lacunae emerged, which was

A higher magnification of Fig. 4-a.
H·E stain (×100)

A higher magnification of Fig. 4-a.
H·E stain (×100)
AB : Alveolar bone (central)
PDM: Periodontal membrane
RZ : Resorptive zone
Pap : Papillary layer
D : Dentin

A higher magnification of Fig. 4-b.
H·E stain (×100)
AB : Alveolar bone (buccal)
RL : Remodeling layer
OL : Osteocytic lacunae
PDM: Periodontal membrane
RZ : Resorptive zone
D : Dentin

A higher magnification of Fig. 4-b.
H·E stain (×100)
ab : Alveolar bone (labial)
E : Enamel
Am : Ameloblast
RZ : Resorptive zone
OL : Osteocytic lacunae
Pap: Papillary layer

Fig. 5 Examination with higher magnification.

H·E stain (×50)

AB : Alveolar bone (lingual)

BM : Bone marrow

PDM: Periodontal membrane

D : Dentin

H·E stain (×100)

AB : Alveolar bone (central)

D : Dentin

PDM: Periodontal membrane

H·E stain (×100)

AB : Alveolar bone (buccal)

PDM: Periodontal membrane

RZ : Resorptive zone

OL : Osteocytic lacunae

BM : Bone marrow

BT : Bone trabecula

H·E stain (×100)

ab : Alveolar bone (lingual)

D : Dentin

E : Enamel

Am : Ameloblast

OL : Osteocytic lacunae

Pap: Papillary layer

Fig. 6 Alveolar bone of the 6th week.
Ca-deficient diet group

H · E stain (×50)

AB : Alveolar bone (lingual)

BM : Bone marrow

PDM: Periodontal membrane

OL : Osteocytic lacunae

RL : Remodeling layer

H · E stain (×100)

AB : Alveolar bone (lingual)

BM: Bone marrow

RL : Remodeling layer

H · E stain (×50)

AB : Alveolar bone (central)

RL : Remodeling layer

PDM: Periodontal membrane

OL : Osteocytic lacunae

H · E stain (×50)

AB : Alveolar bone (buccal)

D : Dentin

RZ : Resorptive zone

PDM: Periodontal membrane

OL : Osteocytic lacunae

H · E stain (×100)

ab : Alveolar bone (labial)

E : Enamel

Am: Ameloblast

OL : Osteocytic lacunae

Pap: Papillary layer

Fig. 7 Alveolar bone of the 6th week.
Ca-low deficient diet group

Fig. 8 Alveolar bone of the 6th week. (SEM) Control group

Fig. 9 A higher magnification of Fig. 8.

Fig. 10 Alveolar bone of the 6th week. (SEM) Ca-deficient diet group

Fig. 11 A higher magnification of Fig. 10.

Fig. 12 Alveolar bone of the 6th week. (SEM) Ca-low deficient diet group

Fig. 13 A higher magnification of Fig. 12.

considered to be resulted from bone resorption. On the formation surface of bone matrix, many irregular-shaped bone lacunae were frequently observed, and many of them had unclear division with the surrounding bone matrix (Figs. 10, 11).

Low calcium group

In comparison to the calcium deficient group, formation areas of bone matrix were more often observed. Many irregular-shaped bone lacunae were seen scattered and surrounded by particularly-aligned collagen fibers, showing increased bone matrix formation. On the upper surface of bone matrix, collagen fibers were seen crossed into a network. Increased bone resorption surfaces were shown when compared to the control group (Figs. 12, 13).

Discussion

Childhood, also called the primary sexual characteristic stage, is an important phase in the process of the growth and development of the individuals. At this age level, the eruption of the primary dentition is completed and the primary teeth are supported by the mandibular alveolar bone, suggesting the importance of the growth of the mandibular alveolar bone during childhood. It is generally accepted that bone is composed of carbonate-apatite with a calcium-to-phosphorus ratio of about 2.0 in human beings. An osteoblast is a differentiated bone-forming cell responsible for bone formation via stimulating the synthesis of the matrix protein and the calcification of the matrix, while an osteoclast is a large multinucleated cell contributive to bone resorption and also bone remodeling, via secretion of organic acids to digest collagen fibers and to dissolve calcium salts²⁾. The mandibular alveolus consists of the outer portion of compact (or dense) bone and the interior portion of spongy (or cancellous) bone. The spongy bone has fast metabolism and plays an important role in calcium metabolism. On the other hand, there is 99 % of calcium present in bone and only 1 % in cells, connective tissue and blood. Calcium and phosphate homeostasis is complex, yet three important hormones are responsible for modulating these minerals. 1,25 (OH)₂ D₃, the hormonal metabolite of vitamin D, stimulates intestinal calcium and phosphate absorption, bone calcium and phosphate resorption, and renal calcium and phosphate reabsorption, thus increasing the blood Ca · PO₄ ion product; Parathyroid hormone acts directly on bone and kidney and indirectly on the intestine to maintain or restore the serum calcium level; and calcitonin, in contrast, inhibits the release of calcium from bone to decrease bone resorption. However, environmental factors, such as dietary treatments, seems more important, especially adequate dietary calcium intake and increased intestinal calcium absorption are necessary to the individuals at growing stage. Dietary protein is a key factor for the resorption and excretion of calcium. Appropriate exercise could increase bone mass. Recently, the popular food service industry has an adverse effect on the nutritional balance, leading to the deficiency of calcium and zinc, which is an essential trace element in body and an important component in bone matrix³⁾, but this phenomenon does not always happen when providing meals as a group because of its no unbalanced diet, especially on animal food⁴⁾.

Naito *et al.* (1998)⁵⁾ have examined the life-style influence on the prevention of osteoporosis

in college students and found that adequate dietary calcium intake seems an important factor to prevent from osteoporosis. Especially the students, who have had the habit of eating small fish by 18 years old, are reported to have acquired higher bone mass.

It is necessary to consider the interactions among some factors affecting calcium resorption such as age, resorption quantity of calcium, and some nutrients coexisting with calcium salts^{2, 6-8)}.

The results of this study suggested that the decreased formation of bone matrix could be observed in the individuals, who were deficient in calcium at their primary sexual characteristic stage, in spite that during their fetal period, lactation period, weanling period, and childhood adequate calcium was stored in the bodies, and in their later life dietary treatments were performed by giving the standard diet. Therefore, it is necessary to increase calcium and balanced protein intake in order to prevent from the low bone mass because calcium deficiency readily results in the decrease of spongy bone.

In the present study, the low calcium group showed the increased Haversian system number, and increased bone formation and remodeling, suggesting that the two-fold or three-fold quantity of calcium intake may be necessary.

Nishida *et al.* (1993)⁹⁾ have investigated the mandibular alveolar bone of the low-calcium rabbits and observed the increased bone resorption, particularly in the molar areas, suggesting the conditions of activity of osteocytes. Ohdani *et al.* (1992)¹⁰⁾ reported that the rapid bone resorption of alveolar bone in the molar areas in the low-calcium rats, which could not make bone lose its functions such as supporting teeth, could be improved by the mechanical powers such as occlusive power. Matsumoto *et al.* (1989)¹¹⁾ examined the mandibular condylar cartilages of the rats fed with low calcium diet. It was found that calcium deficiency in the growing rats inhibited the proliferation and mature of chondocytes and the resorption of cartilages accompanied with the decreased calcification of cartilage matrix, and had an adverse effect on the normal endochondral ossification, suggesting the inhibition of the growth of mandibular condyles. Qin *et al.* (1998)¹²⁾ investigated the microanalysis for Ca and P and densitometric findings of mandibular alveolus of 3-week-old weanling rats who were fed the calcium deficient diet and then treated with high calcium diet, and found the difficulty in the restoration from the condition of bone loss to the normal. Also Qin *et al.* (1998)¹³⁾ fed the 3-week-old weanling rats on the calcium deficient diet and low calcium diet, causing bone loss in them, and then treated them with the standard diet and oral administration of $1,25(\text{OH})_2\text{D}_3$. On the findings of microanalysis for Ca and P, oral administration of $1,25(\text{OH})_2\text{D}_3$ seemed effective to the restoration of bone loss. But compared with the control group, the experimental groups administered $1,25(\text{OH})_2\text{D}_3$ showed lower density, suggesting this treatment could not entirely restore the bone loss resulting from the calcium deficiency during the weanling period.

Saito *et al.* (1998)¹⁴⁾ used the rats corresponding to school childhood in human beings, in whom bone loss resulting from the calcium deficiency, then gave the dietary treatment and found that compared to the control group, the experimental groups given the dietary

treatments showed decreased lamellae in spongy bone and incomplete bone structure. On the other hand, Furuya *et al.* (1989)¹⁵⁾ reported that restoration of bone matrix formation could be seen in mandibles of the rats fed on the 20 %–low–calcium diet and then given the balanced standard diet.

Kimura *et al.* (1996)¹⁶⁾ reported that bone restoration could be found in the mandibles of 8-week-old rats corresponding to puberty in human beings, in whom bone loss had been resulted from calcium deficiency, under the condition that peak bone mass was acquired by changing to take the balanced standard diet and restoring calcium in bone.

Peak bone mass is reported to be necessary for the prevention from bone loss^{17–20)} and it is known that remarkable increase of bone mass always happens at the second sexual characteristic stage²¹⁾, suggesting that adequate calcium intake is important for the growing individuals. Weinreb *et al.* (1991)²²⁾ reported that no significant changes could be seen between the control animals and the low calcium animals who were in the period corresponding to school childhood in human beings. However, peak bone mass could be influenced by many factors such as the quantity of secretion of gonad hormone and parathyroid hormone, and the quantity of calcium intake²³⁾, and also the connections between bone mass and heredity has been reported²⁴⁾.

In the present study, the dietary treatment was performed on 5-week-old animals corresponding to childhood in human beings because in whom bone loss was caused. Of all dietary treatments, it is important and appropriate to choose a dietary treatment, which can stimulate intestinal calcium absorption and renal calcium reabsorption, and decrease renal calcium excretion. Therefore, for the growing individuals, it should be necessary to consider some factors related to calcium absorption and excretion while increasing calcium intake.

This study proved a fact that calcium plays a key role in bone resorption and formation, which are always associated with many kinds of mechanical stimuli such as occlusion and mastication. The changes of bone mass in labial alveolar bone are different from those in lingual alveolar bone, which possesses periodontal membrane. The reaction of spongy bone to the changes of bone mass is faster than that of compact bone, and readily occurs to change with the difference of calcium absorption in earlier time. The present study demonstrated that bone loss, which resulting from calcium deficiency during the earlier periods of the individuals, possibly led to insufficient calcium stored in bone; and bone loss could not be recovered even if excessive calcium was taken after the growing periods of the individuals, in contrast, excessive calcium readily led to unbalanced calcium absorption and excretion, suggesting that adequate calcium intake from balanced standard diet for a long time, at least up to the second sexual characteristic stage, might be very necessary and important for the later life.

Conclusion

In the present study, we fed 5-week-old Wistar male rats (corresponding to childhood in human beings) on calcium deficient diet and 30 %–low–calcium diet for 3 weeks so as to

bring about bone loss in them, and then treated them with the standard diet for further 3 weeks. Later, we observed bone matrix formation on the mandible and obtained some findings as follows.

1. X-ray findings

The calcium deficient group showed the thin alveolus, and sparse and irregularly-arranged trabeculae. The low calcium group showed increased thickness of the alveolus when compared with the calcium deficient group, but showed sparse bone structure in an overall observation of the alveolus when compared to the control group.

2. Densitometric findings

The calcium deficient group showed significantly lower density compared with the control group ($p < 0.05$).

3. Findings of microanalysis for Ca and P

The experimental groups showed lower relative ratio of Ca than the control group, but there is no significant difference between the experimental groups. The relative ratio of P showed a decreasing tendency on a sequence of the calcium deficient group, low calcium group and control group.

4. Histopathologic findings

We observed the lingual, medial, and buccal or labial alveolar bone in the mandibles. Compared with the control group, the calcium deficient group showed irregular periodontal membrane, and decreased bone lacunae and Haversian system number, suggesting markedly decreased bone matrix formation. The low calcium group showed increased Haversian system number and bone matrix formation when compared to the calcium deficient group, but insufficient bone remodeling and restoration could be seen in the low calcium group when compared to the control group.

5. SEM findings

There is no obvious morphological difference among all the groups. Compared to the control group, the calcium deficient group showed increased large or small, and irregular-shaped bone lacunae, which are considered to be associated with bone resorption. And bone lacunae, which had unclear division with the surrounding bone matrix, were more often seen.

In comparison to the calcium deficient group, the low calcium group showed increased bone lacunae scattered and surrounded by collagen fibers in a particular alignment. Increased bone resorption surfaces were seen when compared to the control group.

The results of the present study suggested that it is very difficult to recover entirely from bone loss caused during the earlier periods especially in childhood, in spite of performing dietary treatments by taking balanced diet, and it is necessary to give calcium and some biological activators for a long time.

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幼児期ラットの虚弱下顎骨における食餌療法に関する X 線の骨塩量, 光顕的および超微形態的研究

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ヒト幼児期に相当する 5 週齢の Wistar 系雄ラットを 30 匹使用し, カルシウム欠乏食と低カルシウム食 30 % を 3 週間与え虚弱骨を惹起した。直ちに標準食に切り換え 3 週間飼育し, 骨基質形成について検索し, 以下の結果を得た。

1. X 線学的所見

カルシウム欠乏食群の歯槽骨は細く, 骨梁は疎で配列も不規則であった。低カルシウム欠乏食群は, カルシウム欠乏食群と比べ歯槽骨は厚さを増しているが対照群と比べると未だ骨梁は疎な部分も多く認められた。

2. 骨塩量

3 群間では対照群と比べカルシウム欠乏食群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($p < 0.05$)。

3. X 線マイクロアナライザーによる Ca, P の分析

相対カルシウム量比は, 対照群 > カルシウム欠乏食群 = 低カルシウム食群で, 相対 P 量比はカルシウム欠乏食群 > 低カルシウム食群 > 対照群の順であった。

4. 病理組織学的所見

下顎骨における舌側歯槽骨から, 唇側歯槽骨, 中央側歯槽骨および頬側歯槽骨に至るまで観察した。

対照群に比べカルシウム欠乏食群は一般に歯根膜の不規則化, 骨小腔の減少, ハバース系の減少が著明で骨基質形成は低下していた。低カルシウム食群は, カルシウム欠乏食群に比べハバース系の増加および骨の再構築層が増加していたが, 対照群に比べると骨の回復所見は不十分であった。

5. 走査型電顕学的所見

対照群に比べ形態的には何ら変化はみられなかった。対照群に比べカルシウム欠乏食群は骨吸収のため露出した大小の骨小腔が多く, 不定形を呈していた。また, 周囲基質との境界が不明瞭な部分も増加していた。カルシウム欠乏食群に比べ, 低カルシウム食群は, 一定方向に走行するコラーゲン原線維束によって骨小腔は囲まれ分布量も増加していたが, 骨吸収面は, 対照群と比べ増加していた。

以上の結果から, 小児期の中でも特に低年齢児である幼児期での虚弱下顎骨はバランスのとれた食餌療法では骨基質形成が困難であることから, カルシウム療法と生理活性物質との併用療法を長期間継続する必要があることが示唆された。