The Effect of Zinc on Condylar Cartilage at Growth Stage

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To examine the effects of zinc on bone formation by endochondral ossification at growth stage, forty 5-week-old Wistar male rats, corresponding to childhood in humans, were randomly divided into 4 groups. In the control group, rats were fed on a standard diet. In the experimental groups, the rats were fed on no-zinc-component (zinc-deficient) diet, 50 %-low-zinc diet and 150 %-high-zinc diet respectively. After 4 weeks of feeding, all the rats were sacrificed and their condyles were observed as follows.

In the densitometric measurement, the zinc-deficient group showed the lower value than the other three groups ($p < 0.01$).

In the microanalysis, there was a significant difference in the content of Ca or P between the zinc-deficient group and the other three groups ($p < 0.01$). The high-zinc group showed the significantly higher content of Ca and lower content of P than the other three groups ($p < 0.01$).

Compared with the control group on the histopathological findings, the zinc-deficient group showed the decreased cartilage cells in resting zone and zone of proliferation with the deformed shape. In zone of hypertrophy appeared the decreased calcified matrix, which were surrounded by the increased cartilag cells. The erosion of calcified matrix by the multinuclear chondroclasts tended to decrease. In zone of subchondral bone formation appeared the thinner trabeculae. Compared with the zinc-deficient group, the low-zinc group showed the increased erosion findings of the calcified matrix, surrounded by the increased hypertrophic chondrocytes, could be observed frequently. The subchondral bone formation was improved, but the trabeculae were thinner. Compared with the control group, the high-zinc group showed the increase of both the calcified matrix surrounded by the hypertrophic chondrocytes and the erosion of the calcified matrix by the increased chondroclasts could be often seen. In zone of subchondral bone formation the differentiation of chondrocytes into bone cells was improved, leading to the successive and thick bone trabeculae in a vertical arrangement.

Compared with the control group on the scanning electron microscopic findings, the zinc-deficient group showed the thinner bone trabeculae and the decrease of the longitudinal matrix in cartilage lacunae, on the wall of which appeared the calcareous globes in a sparse arrangement and the collective collagen fibers. On the surface of bone matrix formation, collagen fibers arranged sparsely and were in an irregular alignment, where the calcareous

microdepositions were present. The low-zinc group showed the thicker bone trabeculae than the zinc-deficient group. In the cartilage lacunae arranged the uncalcified globes, where the collagen fibers were often seen crossed into networks. On the bone formation surface appeared many collagen fiber bundles in a regular alignment, and on the superficial part the collagen fibers were clearly seen crossed into networks, the same as the findings in the control group. Upon the examination of an overall image in the high-zinc group, compared with the control group, the cartilage development from the growing cartilage layer to the following thick bone trabeculae was improved.

These results suggested that zinc, an essential trace element, had a positive effect on bone formation by endochondral ossification in the condyles of the growing rats.

Key words: Zinc/Condylar cartilage/Growth stage

Introduction

Bone mainly consists of calcium and protein, so it seems important for the individuals at growth spurt to get well-balanced nutrition. In normal individuals, peak bone mass is obtained at 17–20 years of age and thereafter a decrease with age occurs in both sex¹⁾. Maximizing the accumulation of bone mass is one of the most important aims in the prevention of bone loss resulting from aging. We have reported a series of studies^{2–10)} on clarifying the relationships between bone mass and dietary treatments, administration of physiological active factors, physical activity and so on. Recently, trace elements have been known to have an effect on bone formation and zinc has attracted our attention. Zinc is one of the components of many kinds of enzymes, such as carbonate dehydratase. Zinc deficiency readily results in loss of appetite, growth retardation, dermal lesions like keratosis and retardation of healing the wound^{11–14)}. It also has been reported that zinc deficiency in growing rats produced the sudden stop of growth and development¹⁵⁾. Zinc plays a positive role in bone metabolism, and zinc deficiency caused the decrease of alkaline phosphatase activity, causing incomplete bone formation^{16,17)}.

In this study, we observed the condylar cartilage in the growing rats, which were given the diets with the different zinc levels, to determine the effect of zinc on bone formation at growth stage.

Materials and Methods

Forty 5-week-old Wistar male rats were randomly divided into 4 groups as follows. In the control group, rats were fed on a standard diet with tap water. In the zinc-deficient experimental group, rats were fed on a no-zinc-component diet with distilled water. In the low-zinc experimental group, rats were fed on a low zinc diet (zinc component is 50 % of the standard diet) with tap water. In the high-zinc experimental group, rats were fed on a high zinc diet (zinc component is 150 % of the standard diet) with tap water. All the rat food was made by Oriental Yeast (Tokyo, Japan).

Table 1-1 Composition of experimental diets

(%)

Ingredient	Standard diet	Low-Zn diet	High-Zn diet
β -corn starch	38.0	38.0	38.0
Vitamin-free casein	25.0	25.0	25.0
α -potato starch	10.0	10.0	10.0
Cellulose powder	8.0	8.0	8.0
Soy bean oil	6.0	6.0	6.0
Mineral mixture	6.0 [☆]	6.0 [★]	6.0 [☆]
Granulated sugar	5.0	5.0	5.0
Vitamin mixture	2.0	2.0	2.0
ZnCO ₃			3.26 mg/100 g

☆: Standard mineral mixture

★: Low-Zn mineral mixture

Table 1-2 Composition of mineral mixture

(g/100 g)

Ingredient	Standard mineral mixture [☆]	Low-Zn mineral mixture [★]
CaHPO ₄ · 2H ₂ O	14.56	14.56
KH ₂ PO ₄	25.72	25.72
NaH ₂ PO ₄	9.35	9.35
NaCl	4.66	4.66
Ca-lactate	35.09	35.09
Fe-citrate	3.18	3.18
MgSO ₄	7.17	7.17
ZnCO ₃	0.11	0.055
MnSO ₄ · 4~5H ₂ O	0.12	0.12
CuSO ₄ · 5H ₂ O	0.03	0.03
KI	0.01	0.01
Cellulose	0.00	0.055

The components of the diets were presented in Table 1.

After 4 weeks of feeding, all the rats were sacrificed under pentobarbital sodium. Their condyles 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 performance of experimental animals outlined by Kyushu Dental College.

Densitometric measurement

The condyles were kept in 10 % neutral buffered formalin solution. These samples were imaged with Softex CSM (Softex Co, Tokyo, Japan) at 28 kVp, 6 mA, 60s and focus-film distance of 70 cm with Fuji softex film FG (Fuji Film Co, Tokyo, Japan) and aluminum reference wedge attached. The films were scanned by Microphotometer (Densitometer PDS-

15, Konica, Tokyo, Japan). The survey light passed from the top of the condyle to the bottom border of the mandible and scanning was carried out with the slit of $10 \times 500 \mu\text{m}$.

Sample preparation for microanalysis for Ca and P

The samples were cut longitudinally at the site, 5 mm in front to the outer line of the condyles. After being fixed in 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, JEOL Ltd, Tokyo, Japan). Sections were embedded in resin, then abraded, polished, and sputtered with aurum. Later they were observed under a scanning microscope (JSM T-300, JEOL) equipped with energy-dispersive X-ray microanalysis (JED-2000, JEOL). Point analysis was performed to determine the content of Ca and P.

Sample preparation for light microscopy

The samples were cut longitudinally at the site, 5 mm in front to the outer line of the condyles. After being fixed in 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). Continuous cut specimen, $7 \mu\text{m}$ of thickness, was made, stained with hematoxylin and eosin, and then observed under a light microscope.

Samples preparation for scanning electron microscopy

The samples were cut the same as those in preparation for microanalysis for Ca and P, and fixed with 2.5 % glutaraldehyde for 1h after being cleaned with phosphate buffer (pH 7.2) by supersonic vibration to eliminate adhesion. They were rinsed by phosphate buffer (pH 7.2) before postfixation. Postfixation was 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, JEOL). Specimens were sputter-coated with gold and later observed under a scanning electron microscope (JSM T-300, JEOL).

Results

Densitometric measurement

The results of densitometric measurement in the control group (1.00 mm), zinc-deficient experimental group (0.50 mm), low-zinc experimental group (1.00 mm), and high-zinc experimental group (1.00mm) are presented in Table 2-1. There were significant differences between the zinc deficient experimental group and the other three groups. ($p < 0.01$). The zinc deficient experimental group showed the low value (Table 2-2).

Microanalysis for Ca and P

The contents of Ca and P in all the groups are presented in Table 3-1 and 3-2. There is

Table 2-1 Density of mandibular condyle in the equivalent aluminum (mm)

	Control group	Zn-deficient diet group	Low-Zn diet group	High-Zn diet group
Al	1.00 ± 0.03	0.50 ± 0.03	1.00 ± 0.03	1.00 ± 0.02

Mean $\pm$ S. D.

Table 2-2 Density of mandibular condyle in the equivalent aluminum

	1—2	1—3	1—4	2—3	2—4	3—4
Al	**	—	—	**	**	—

** : $p < 0.01$

1: Control group

2: Zn-deficient diet group

3: Low-Zn diet group

4: High-Zn diet group

Table 3-1 Analysis of the Ca and P of mandibular condyle by X-ray microanalyzer (%)

	Control group	Zn-deficient diet group	Low-Zn diet group	High-Zn diet group
Ca	56.10 ± 0.66	54.30 ± 0.73	55.53 ± 0.32	57.15 ± 0.87
P	43.90 ± 0.66	45.70 ± 0.64	44.47 ± 0.43	42.85 ± 0.99

Mean $\pm$ S. D.

Table 3-2 Difference of the Ca and P of mandibular condyle by X-ray microanalyzer

	1—2	1—3	1—4	2—3	2—4	3—4
Ca	**	—	**	**	**	**
P	**	—	**	**	**	—

** : $p < 0.01$

1: Control group

2: Zn-deficient diet group

3: Low-Zn diet group

4: High-Zn diet group

no significant difference in the contents of Ca or P between the low-zinc experimental and the control group. The zinc-deficient experimental group showed the lower content of Ca or the higher content of P than the other three groups, and there is a significant difference between the zinc-deficient experimental group and the other three groups ($p < 0.01$). The high-zinc experimental group showed the higher content of Ca or the lower content of P than

Table 4 Relative ratio of Ca and P of mandibular condyle by quantitative X-ray microanalysis

	Control group	Zn-deficient diet group	Low-Zn diet group	High-Zn diet group
Ca/[Ca] _c	1.00	0.98	0.99	1.02
P/[P] _c	1.00	1.04	1.01	0.98

Fig. 1 Alveolar bone from the 9-week-old rat in the control group.
H·E stain (×50)
CL: Cartilage layer
Tr: Trabeculae

Fig. 2 A high magnification of Fig. 1.
H·E stain (×200)
RL: Resting layer
PL: Proliferative layer
HL: Hypertrophic layer

Fig. 3 A high magnification of Fig. 1.
H·E stain (×200)
HL: Hypertrophic layer
EL: Erosive layer
MNC: Multinucleated cell (chondroclast)
E: Erosion

Fig. 4 A high magnification of Fig. 1.
H·E stain (×200)
Ob: Osteoblast
Ocl: Osteoclast
Tr: Trabeculae

Fig. 5 Alveolar bone from the 9-week-old rat in the Zn-deficient diet group.

H·E stain (×50)

CL : Cartilage layer

Tr : Trabeculae

Fig. 6 A high magnification of Fig. 5.

H·E stain (×200)

RL : Resting layer

PL : Proliferative layer

HL : Hypertrophic layer

Fig. 7 A high magnification of Fig. 5.

H·E stain (×200)

HL : Hypertrophic layer

EL : Erosive layer

MNC : Multinucleated cell (chondroclast)

E : Erosion

Fig. 8 A high magnification of Fig. 5.

H·E stain (×200)

Ocl : Osteoclast

Tr : Trabeculae

the other three groups, and there is a significant difference between the high-zinc experimental group and the other three groups ($p < 0.01$). When compared with the control group, the high-zinc experimental group showed the higher relative Ca ratio and the lower P ratio, while the zinc-deficient experimental group and the low-zinc experimental group showed the lower relative Ca ratio and the higher P ratio. The relative ratios of Ca and P in all the groups are presented in Table 4.

Histopathological findings

I. Control group

The zonation of the condylar cartilage in the control group could be described as follows.

Fig. 9 Alveolar bone from the 9-week-old rat in the Low-Zn diet group.

H·E stain (×50)

CL: Cartilage layer

Tr: Trabeculae

Fig. 10 A high magnification of Fig. 9.

H·E stain (×200)

RL: Resting layer

PL: Proliferative layer

HL: Hypertrophic layer

Fig. 11 A high magnification of Fig. 9.

H·E stain (×200)

HL: Hypertrophic layer

EL: Erosive layer

MNC: Multinucleated cell (chondroclast)

E: Erosion

Fig. 12 A high magnification of Fig. 9.

H·E stain (×200)

Ob: Osteoblast

Ocl: Osteoclast

Tr: Trabeculae

On the superficial surface of the condyle was resting zone. The cartilage cells in this region appeared elongate and flat, whose long axes paralleled to the surface of condylar joint. Adjacent to this zone was zone of proliferation. The cartilage cells here underwent division and became organized into distinct columns. Next is zone of hypertrophy. The cartilage cells were greatly enlarged and appeared honeycombed, which were surrounded by a little calcified-matrix. In the next zone, many multinucleated chondroclasts invaded the calcified-matrix. Thus, it was referred as zone of erosion. And finally, zone of subchondral bone formation, which was in direct contact with the bone trabeculae, was represented by active bone formation. On the surface of bone trabeculae also appeared the active bone formation, where osteoblasts and osteoclasts could be seen (Fig. 1-4).

Fig. 13 Alveolar bone from the 9-week-old rat in the High-Zn diet group.

H·E stain (×50)

CL: Cartilage layer

Tr: Trabeculae

Fig. 14 A high magnification of Fig. 13.

H·E stain (×200)

RL: Resting layer

PL: Proliferative layer

HL: Hypertrophic layer

Fig. 15 A high magnification of Fig. 13.

H·E stain (×200)

HL: Hypertrophic layer

EL: Erosive layer

MNC: Multinucleated cell (chondroclast)

E: Erosion

Fig. 16 A high magnification of Fig. 13.

H·E stain (×200)

Ob: Osteoblast

Ocl: Osteoclast

Tr: Trabecula

II. Zinc-deficient experimental group

Compared with the control group, the cartilage cells in resting zone tended to decrease, and their shapes and arrangements appeared irregular. The cartilage cells in zone of proliferation also had a tendency to decrease and their shapes were greatly enlarged and deformed. In zone of hypertrophy appeared the decreased calcified matrix, which were surrounded by the increased cartilage cells. The erosion of calcified matrix by the multinucleated chondroclasts tended to decrease. In zone of subchondral bone formation, the differentiation of chondrocytes into osteocytes was inhibited and the newly formed trabeculae seemed thin (Fig. 5-8).

Fig. 17 9-week-old alveolar bone. (SEM)
Control group

Fig. 18 A high magnification of Fig. 17. (SEM)

III. Low-zinc experimental group

Compared with the zinc-deficient experimental group, the number of the cartilage cells in resting zone was increased, part of them paralleling to the surface of condylar joint. The cartilage cells in zone of proliferation were increased and enlarged, appearing circular. The zone of hypertrophic cartilage cells was thinner and tended to increase. The calcified matrix around these cartilage cells also showed to be increasing. The bone trabeculae newly formed by increased subchondral bone formation seemed to be still thin (Fig. 9-12).

IV. High-zinc experimental group

Compared with the control group, the cartilage cells in resting zone were decreased and in an irregular alignment. The cartilage cells in zone of proliferation appeared to be decreased, enlarged and irregularly arranged. The cartilage cells in zone of hypertrophy almost had no change in number, but it seemed clear that there was a tendency to increase the calcified matrix around these cartilage cells. The multinucleated chondroclasts here tended to increase, so that the erosion of calcified matrix was frequently observed and the differentiation of chondrocytes into osteocytes in zone of subchondral bone formation was promoted, leading to the successive and thick bone trabeculae with a vertical arrangement (Fig. 13-16).

Scanning electron microscopic findings

I. Control group

Upon the examination of an overall image of the condyle, on the cut face appeared the growing cartilage layer. At the lower end of the condyle, thick ossification occurred and many small marrow cavities could be clearly observed. A distinct division between the cartilage and the bone trabeculae could be seen (Fig. 17). On the upper surface of cartilage lacunae appeared the calcifying longitudinal matrix. Cartilage lacunae were full of small calcareous globes with the size of around $0.5\mu\text{m}$. On the longitudinal matrix, collagen fibers

Fig. 19 A high magnification of Fig. 17. (SEM)

Fig. 20 9-week-old alveolar bone. (SEM)
Zn-deficient diet group

Fig. 21 A high magnification of Fig. 20. (SEM)

Fig. 22 A high magnification of Fig. 20. (SEM)

were seen crossed into networks. But occasionally part of the longitudinal matrix, on which successive collagen fibers formed, was occupied by only a few calcareous globules (Fig. 18).

In the formation areas of bone matrix appeared the surface of bone formation, which was adjacent to the surface of bone resorption. On the surface of bone formation showed many dense collagen bundles in a particular alignment. There were many bone lacunae, on the wall of which collagen fibers were clearly seen crossed into networks. On the surface of collagen fibers appeared some micro- calcareous depositions (Fig. 19).

II. Zinc-deficient experimental group

Same as the control group, there was a distinct division between the cartilage layer and the following bone trabeculae, newly formed by endochondral ossification. But the bone trabeculae appeared thinner than those in the control group (Fig. 20).

Compared with the control group, there was a distinct division among the cartilage lacunae, on the wall of which sparse calcareous globules and collective collagen fibers were

Fig. 23 9-week-old alveolar bone. (SEM)
Low-Zn diet group

Fig. 24 A high magnification of Fig. 23. (SEM)

Fig. 25 A high magnification of Fig. 23. (SEM)

Fig. 26 9-week-old alveolar bone. (SEM)
High-Zn diet group

frequently seen, and the smooth surfaces of longitudinal matrix tended to decrease (Fig. 21).

On the surface of bone formation, the wide-spread bone resorption areas were present, causing bone lacunae and canaliculi to expose. The sites with an unclear division between bone lacunae and osteocytic matrix were readily observed, where collagen fibers crossed into networks. On the formation surface of bone matrix, collagen fiber bundles arranged sparsely, thus leading to many incompletely formed bone lacunae, which had an unclear division with bone matrix. On the surface of collagen fibers with an irregular alignment appeared some micronized calcareous depositions (Fig. 22).

III. Low-zinc experimental group

Upon the examination of an overall image of a cut surface, when compared to the zinc-deficient experimental group, ossification was seen increased, thus the thick bone trabeculae came to form (Fig. 23).

The findings of cartilage lacunae were the same as those in the zinc-deficient experimental

Fig. 27 A high magnification of Fig. 26. (SEM)

Fig. 28 A high magnification of Fig. 26. (SEM)

group, that there was a clear division among the cartilage lacunae. On the wall of cartilage lacunae arranged a few calcareous globules, where collagen fibers were readily seen connected (Fig. 24).

Compared to the zinc-deficient experimental group, on bone formation surface appeared many collagen fiber bundles in a particular alignment, and the sites with a distinct division between the bone lacunae and bone matrix were frequently observed. Same as the control group, on the superficial layer, collagen fibers were obviously seen crossed into networks. Bone canaliculi were observed among the crossed and dense collagen fibers in the bone lacunae (Fig. 25).

IV. High-zinc experimental group

Upon the examination of an overall image of a cut surface, when compared with the control group, the cartilage development from the growing cartilage layer to the following thick bone trabeculae was improved (Fig. 26).

The findings of cartilage lacunae were nearly the same as those in the control group, showing there was a distinct division among the cartilage lacunae and longitudinal matrix could also be observed (Fig. 27).

Bone formation surfaces, on part of which still appeared incomplete bone lacunae, seemed smooth, showing the same findings as those in the control group (Fig. 28).

Discussions

It is determined that sufficient calcium intake has a positive effect on bone formation. Recently, improving calcium absorption becomes so important that we have reported a series of studies²⁻¹⁰⁾ on clarifying the relationships between calcium and some nutrients such as protein and magnesium, and some physiological active factors such as 1,25-dihydroxyvitamin D₃ and calcitonin, and physical activity. Furthermore, zinc, an essential trace element, has lately attracted considerable attention. Many investigators described a zinc-deficient syndrome¹¹⁻¹⁴⁾ clinically characterized by growth retardation or stop, loss of appetite, dermal

lesions, hypogonadism, and remarkable bone diseases. It also has been reported that zinc deficiency in rats during the early growing period produced the sudden stop of growth and development¹⁵⁾. Zinc deficiency caused a decrease of alkaline phosphatase activity, leading to incomplete bone formation^{16, 17)}. Hozaki *et al.* (1995)¹⁸⁾ reported that zinc deficiency could result in a disorder of the growth and development, and the initial calcification of condylar cartilage matrix in the rats given zinc-deficient diet. Nishida *et al.*¹⁹⁾ examined the effects of zinc and active vitamin D₃ on bone formation by endochondral ossification in rat condylar cartilage, and reported that zinc deficiency could cause a decline in bone modeling and zinc supplementation could enhance the effect of active vitamin D₃ on bone formation. However, Abe (1995)²⁰⁾ reported that zinc had no effect on bone metabolism, especially bone formation, so that it was difficult to determine that zinc compounds could enhance bone formation in growing rats. By now, the mechanism by which zinc affects bone formation is unknown.

In this study, to determine the effect of zinc on bone formation at growth stage, we fed the rats on food with different zinc levels and then examine their condyles.

Densitometric measurement

This is one of approaches to objectively evaluate the degree of bone modeling. In this investigation, when X-ray photographs were taken, softex CSM and Al step wedges were used, causing the conversion of bone density into Al equivalent. The control group, low-zinc experimental group and high-zinc experimental group showed the same value of 1.00 mm, while the zinc-deficient experimental group showing the low value of 0.50 mm. There was a significant difference between the control group and the zinc-deficient group ($p < 0.01$), and the latter showed the low value.

Bone remodeling was influenced by zinc deficiency, but not by low-zinc or high-zinc condition. It is possible that insufficient zinc intake in some degree could have no effect on bone density, since zinc level in vivo may be regulated by a homeostasis-supporting system. Nishida (1995)¹⁹⁾ pointed out that there was no significant difference between the control group and the low-zinc diet and active vitamin D₃ experimental group. Abe (1995)²⁰⁾ reported that excessive zinc intake had no effect on bone formation or bone metabolism.

Microanalysis for Ca and P

Fujimoto *et al.* (1980)²¹⁾, (1981)²²⁾ did the microanalysis for Ca and P of condylar cartilage in growing rats and then reported that the levels of Ca and P could reach the peak remarkably with the improvement of calcification.

In the present study, there was no significant difference between the control group and the low-zinc experimental group. There was a significant difference ($p < 0.01$) between the zinc-deficient experimental group and the other three groups, and the zinc-deficient experimental group showed the lower value of the relative Ca ratio. There was also a significant difference ($p < 0.01$) between the high-zinc experimental group and the other three groups, and the high-zinc experimental group showed the higher value of the relative Ca ratio.

Therefore, it is suggested that bone modeling may be inhibited by zinc deficiency and enhanced by zinc supplementation.

Histopathological findings

It has been known that the condyle develops by endochondral ossification with the skeletal growth. It was the characteristic of the 5-day-old condyle²³⁾ that the cartilage layer on the superficial surface remains indefinitely because of no appearance of the second ossification center, in spite that the same calcification as that in the other long bones could be observed. In the 3-week-old condyle, the cartilage layer was clearly differentiated and the cartilage cells were actively proliferating. In the 6-week-old condyle, the width of the cartilage layer was decreased, and the active trabecular formation occurred, nearly in condition of maturity²⁴⁾. In the 7-week-old condyle²⁵⁾, the cartilage layer became very thin with the decrease of the cartilage cells in zones of proliferation and hypertrophy; calcified matrix around zone of hypertrophy was increased; the erosion of calcified matrix by chondroclasts and bone formation under cartilage could be observed. In the 9-week-old condyle²⁶⁾, the width of cartilage layer was decreased and changing over to calcification²⁶⁾. It was reported²⁷⁾ that the cartilage layer of the rat condyle tended to decrease by aging, showing the same findings as under the condition of malnutrition. Hozaki *et al.* (1995)¹⁸⁾ reported that the cartilage cells of the condyle in zinc deficient experimental rats were decreased when compared with the control group, and especially the cartilage cells in zone of hypertrophy, suggesting that the formation of new cartilage cells was inhibited. Nakao (1987)²⁵⁾ reported that in the condyle of the growing rats given calcium-deficient diet, the cartilage layer was increased, resulting from the increased cartilage cells in zone of hypertrophy. The decrease of the calcified matrix around cartilage cells of hypertrophic zone and the chondroclasts in zone of erosion could be seen, and the trabeculae became slender and sparse.

In this study, compared with the control group, the zinc deficient experimental group showed the decreased cartilage cells in resting zone and zone of proliferation, same as the findings reported by Hozaki, suggesting that the formation of new cartilage cells may be inhibited. The cartilage cells in zone of hypertrophy were increased, accompanied by the decrease of chondroclasts; calcified matrix surrounding the cartilage cells was decreased; bone formation in zone of subchondral bone formation was inhibited, showing the same findings as in condition of calcium deficiency. Zinc deficiency had a bad effect on calcium metabolism, such as the low metabolic rotation and few stores of calcium when compared with the normal zinc level²⁸⁾. Compared with the zinc deficient diet group, the low zinc diet group showed the increased cartilage cells in resting zone and zone of proliferation, and the decreased cartilage cells in zone of hypertrophy resulted from the increase of chondroclasts. All the changes were thought to result from the different zinc intake.

Zinc deficiency has an effect not only on growth and development of cartilage cells but also on calcification of cartilage matrix. Hozaki *et al.* (1995)¹⁸⁾ reported that zinc deficiency could inhibit the carbonate dehydratase activity, which was considered to play an important

role in the initial calcification, leading to the decrease of initial calcification of cartilage matrix. Nishida (1995)¹⁹⁾ indicated that the decrease of the calcified matrix around the cartilage was seen in the low zinc diet and active vitamin D₃ group, while the obvious increase appeared in the high zinc diet and active vitamin D₃ group. In the present study, the zinc deficient experimental group showed not only the decrease of calcified matrix but also the thinner trabeculae in zone of subchondral bone formation. The low zinc experimental group showed the increase of calcified matrix and the improvement of subchondral bone formation when compared with the zinc deficient experimental group, not reaching the level of bone remodeling in the control group. The high zinc experimental group showed the increase of erosion by the increased chondroclasts, the improvement of subchondral bone formation, and the thick trabeculae when compared the control group.

As above, zinc deficiency not only inhibits the development of new cartilage cells, but also has an effect on calcium metabolism. Furthermore, bone formation or bone metabolism is inhibited because of the influenced calcium metabolism. On the other hand, zinc supplements increase the calcification and improve the differentiation of cartilage cells into bone cells, enhancing bone remodeling.

Scanning electron microscopic findings

Fujimoto (1982)²⁹⁾ observed the cartilage cells in the condyle through scanning electron microscopy and described the findings as follows. The upper part of zone of hypertrophy showed the formation of the longitudinal matrix by the dense collagen fibers, on which globe-shaped structures were present. In the central part of zone of hypertrophy, the formation of calcified matrix appeared on the superficial surface of longitudinal matrix, inside which the dense collagen fibers crossed into a network. In this area, globe-shaped structures that were present during the formation of calcareous globules also could be seen. In the lower part of zone of hypertrophy, the longitudinal matrix was calcifying and on the wall of lacunae many calcareous globules were seen. On the surface of this area, dense collagen fibers crossed into a network, while inside of this area appeared calcification. Calcifying calcareous globules were seen to connect one another from the superficial surface to the area adjacent to layer of ossification. Calcareous globules are made from hydroxyapatite crystals according to the ratio of Ca to P by X-ray analysis²¹⁾, thought to be associated with the initial calcification by endochondral ossification³⁰⁾. Suzuki *et al.* (1979)³¹⁾ examined the globe-shaped structures on the wall of bone lacunae by scanning electron microscopy, and found the structures connecting one another at all the stages of bone matrix formation, suggesting that calcification was the mature stage of matrix formation. Shioda *et al.* (1980)³²⁾ did the ultrastructural observation by scanning electron microscopy and found that the diameter of the cartilage lacunae was different at the different sites. In the rats at the early growth spurt appeared the abundant cartilage lacunae with small diameter, the number of which was decreased by aging and the shape became irregular. Taniguchi (1981)³³⁾ reported that in the trabeculae, the big and elliptical bone lacunae with the ability to form bone matrix could be

seen when the bone matrix was formed by the active osteoblasts.

Zinc supplements could improve the cartilage calcification, showing that the matrix was formed with the connection of calcareous globules one another, the cartilage layer was distinctly divided, and the calcification of collagen fibers on the bottom of cartilage lacunae was frequently observed. On the other hand, zinc deficiency could inhibit the cartilage calcification, showing that there was no distinct division among the cartilage lacunae, on the bottom of which deposited irregular calcareous globules, and the cartilage matrix formation at the stage of initial calcification¹⁹⁾. The present study showed the same findings. When compared with the control group, the zinc deficient experimental group showed the decreased calcification of longitudinal matrix and cartilage lacunar wall, the widespread bone resorption in zone of subchondral bone formation, the irregular and sparse arrangement of collagen fibers on the surface of bone formation, and the calcareous microdepositions present on the surface of the collagen fibers around bone lacunae. When compared with zinc deficient experimental group, the low zinc experimental group showed that ossification was improved and the thick trabeculae were formed, suggesting that zinc had a key effect on bone remodeling, especially on the calcification in cartilage layer and the ossification in zone of subchondral bone formation. Furthermore, zinc supplements could improve the development of the cartilage from the ossification of the growing cartilage layer to the following thick trabeculae, suggesting that zinc had an effect on the growth and development of the condylar cartilage.

The present study suggested that zinc, an essential element, had a positive effect on bone formation by endochondral ossification in the condyles of the growing rats.

Conclusions

In this study, we used 5-week-old Wistar male rats, corresponding to childhood in humans, to examine the effects of zinc on bone formation by endochondral ossification at growth stage.

1. Densitometric measurement

There was a significant difference between the zinc deficient experimental group and the other three groups ($p < 0.01$), and the zinc deficient experimental group showed the low value.

2. Microanalysis for Ca and P

There was no significant difference in the content of Ca between the low-zinc experimental group and the control group. The zinc-deficient experimental group showed the lower content of Ca than the other three groups, and there was a significant difference between the zinc-deficient experimental group and the other three groups ($p < 0.01$). The high-zinc experimental group showed the higher content of Ca than the other three groups, and there is a significant difference between the high-zinc experimental group and the other three groups ($p < 0.01$). When compared with the control group, the high-zinc experimental group showed the higher relative Ca ratio and the lower P ratio, while the zinc-deficient

experimental group and the low-zinc experimental group showed the lower relative Ca ratio and the higher P ratio.

3. Histopathological findings

Compared with the control group, the zinc deficient experimental group showed the decreased cartilage cells in resting zone and zone of proliferation with the deformed shape. In zone of hypertrophy appeared the decreased calcified matrix, which were surrounded by the increased cartilage cells. The erosion of calcified matrix by the multinuclear chondroclasts tended to decrease. In zone of subchondral bone formation appeared the thinner trabeculae.

Compared with the zinc-deficient experimental group, the low zinc experimental group showed the increase of the cartilage cells in resting layer and in layer of proliferation and the decrease in zone of hypertrophy. The increased erosion findings of the calcified matrix, surrounded by the increased hypertrophic cartilage cells, could be observed frequently. The subchondral bone formation was improved, but the trabeculae were thinner.

Compared with the control group, the high zinc experimental group showed the decrease of the cartilage cells in resting zone and zone of proliferation, with the irregular arrangement. The increase of both the calcified matrix surrounded by the hypertrophic cartilage cells and the erosion of the calcified matrix by the increased chondroclasts could be often seen. In zone of bone formation under cartilage the differentiation of cartilage cells into bone cells was improved, leading to the successive and thick bone trabeculae in a verticle arrangement.

4. Scanning electron microscopic findings

Compared with the control group, the zinc deficient experimental group showed the thinner bone trabeculae and the decrease of the longitudinal matrix in cartilage lacunae, on the wall of which appeared the calcareous globules in a sparse arrangement and the collective collagen fibers. In zone of subchondral bone formation the bone resorption areas widely spread. The sites with an unclear division between bone lacunae and bone matrix were readily observed, where collagen fibers crossed into networks. On the surface of bone matrix formation, collagen fibers arranged sparsely and were in an irregular alignment, where the calcareous microdepositions were present.

Compared with the zinc deficient experimental group, the low zinc experimental group showed the thick trabeculae. In the cartilage lacunae arranged the uncalcified globes, where the collagen fibers were often seen crossed into networks. On the bone formation surface appeared many collagen fiber bundles in a regular alignment, and on the superficial part the collagen fibers were clearly seen crossed into networks, showing the same as the findings in the control group.

Upon the examination of an overall image in the high zinc experimental group, compared with the control group, the cartilage development from the growing cartilage layer to the following thick bone trabeculae was improved. The findings of cartilage lacunae and bone formation surface were nearly the same as those in the control group.

The present study suggested that zinc, an essential element, had a positive effect on bone

formation by endochondral ossification in the condyles of the growing rats.

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成長期の下顎頭軟骨へ及ぼす亜鉛の影響

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ヒトの幼児期に相当する生後5週令の Wistar 系雄ラットを用い、亜鉛が成長発育期の軟骨内骨化による骨形成に及ぼす影響について検索した。

本研究では、成長発育期の骨形成において亜鉛の影響を調べるために亜鉛含有量の異なる食餌を与え、その影響をラットの下顎頭軟骨を用いを用いて検索した。

実験には小児期に相当する生後5週令の Wistar 系雄ラット(40匹)を用い、無作為に次の4群に分けた。

対照群(標準食)：ラット標準飼料(オリエンタル酵母工業)と水道水にて飼育した。

亜鉛欠乏食群：亜鉛欠乏飼料(オリエンタル酵母工業)

と水道水で飼育した。

低亜鉛食群(50%)：低亜鉛食飼料(オリエンタル酵母工業)と水道水で飼育した。

高亜鉛食群(150%)：高亜鉛食飼料(オリエンタル酵母工業)と水道水で飼育した。

骨塩量、Ca、Pの定量分析、病理組織所見ならびに走査型電顕所見より総合的に判断して、成長期の下顎頭軟骨は骨形成において亜鉛が必要不可欠な微量元素であり、さらに亜鉛摂取量を増加させることにより骨形成作用を促進されることが示唆された。