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Effects of Dietary Consistency on the Mandible of Rats at the Growth Stage: Computed X-ray Densitometric and Cephalometric Analysis

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ABSTRACT

Thirty 3-week-old male Wistar rats were grouped into a hard diet control group, a kneaded-diet group, and a powdered-diet group. After 6 weeks of growth, all rats were killed under deep anesthesia. The effects of dietary physical consistency on the mandible were investigated with respect to morphology and bone mineral content using lateral cephalometric analysis and computed X-ray densitometry. Significant differences between the experimental and control groups were determined by measuring the means and standard deviation of the coordinates and differences between 15 points selected on the mandibles. Gonion (Go), which is a measurement of depth to the X-axis, was significantly less in the powdered-diet group, while the kneaded-diet group showed no significant differences. The most posterior point of the coronoid process (Cr) and the most posterior point of condylar process (Cd), which are measures of height to the Y-axis, were less in the kneaded-diet group than in the control group. In the powdered-diet group, Cr, Cd, Go and Infradentale were significantly less than in the control group. On the other hand, the bone mineral content was significantly lower in the coronoid process and angle of mandible in only the powdered-diet group.

KEY WORDS: Dietary consistency, Bone mineral content, Lateral cephalometric analysis, Rat mandible.

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INTRODUCTION Return to TOC

In recent years, there have been significant changes in the food commonly eaten in Japan. The trend toward softer food, primarily represented by processed foods, has been cited as a major characteristic of these changes.

Yanagisawa and coworkers[1] reported that the chewing frequency and eating time has decreased with the advent of soft modern food. Further, many Japanese children, whose feeding behavior did not involve chewing food enough, wanted to eat only soft food and had an unbalanced diet. They often were not able to eat some kinds of food that required greater muscular activity. Van Limborgh[2] classified factors in growth and development of the bones into genetic factors, factors after birth, and environmental factors, and reported that these factors interact. The jawbones are specific organs that receive physical stimulation such as biting forces and chewing forces throughout life. It is very important to investigate the relationships between dietary consistency and jawbone structure.

Three-week-old rats were used in the present study. We used computed X-ray densitometry and cephalometry to investigate the effects of physical consistency of foods on the morphology and bone mineral density of the mandible.

MATERIALS AND METHODS Return to TOC

A total of thirty 3-week-old male Wistar rats (approximately 40 g in body weight) were randomly divided into 3 equal groups. They were maintained under the following conditions.

1. The control-diet group (hard-diet group) was given a hard diet (CA-1, Crea Japan, Tokyo, Japan) with tap water for 6 weeks.
2. The kneaded-diet group was given a diet of powder (CA-1, Crea Japan, Tokyo, Japan) that had been first kneaded with a half amount of water and then left to dry at room temperature for 48 hours.
3. The powdered-diet group was given a powdered diet for 6 weeks.

The diets and tap water were replaced every day and made available freely through the rearing period for all animals.

After 6 weeks of growth under the above conditions, all rats were killed under deep anesthesia. The cranial bones were immediately removed and prepared by fixing in 10% neutral formalin.

Physical consistency of dietary foods

The physical consistency of the foods was determined by a compression test using a universal tensile tester (TENSILON UTM-III-500, Orientech Co, Tokyo, Japan).

Lateral cephalometric analysis

After fixation, the cranial bones were cut laterally along the median suture from the parietal bone to the mandible. The soft tissue around the alveolar part of the mandible of the left cranium was detached with the greatest possible care to expose the mental foramen. The median sagittal face of the left side of the head was mounted to contact the film surface, with the mental foramen set immediately under the focus. Dental occlusal film (DF-4, 9, Eastman Kodak Co, Tokyo, Japan) was used for cephalometry. The sample bones were exposed from the lateral side using a SOFTEX ESM-2 (Softex Co, Tokyo, Japan) at 30kVp and 8mA for 60 seconds, with a focus-film distance of 60 cm. Development and fixation was carried out as instructed by the manufacturer (Figure 1). The radiographic images on the film were enlarged 5-fold and printed on photographic paper, where the points for determination were traced (Figure 2). Accordingly, the results recorded were 5 times greater than the actual lengths.

Setting of coordinate axes and actual length measurements

An X-axis was constructed parallel to the mandibular plane (Me-Ag) through the mental foramen and a perpendicular Y-axis was constructed through the mental foramen (Figure 2). These 2 lines were set as the X and Y coordinates. The morphological characteristics of the mandible were described by constructing 9 lines connecting the 15 points shown in Figures 3 and 4. A slide caliper with graduation settings of 1/20 mm was used to measure the length of each line 5 times and the average was designated as the actual length.

Micro-densitometry measurements with aluminum equivalent images

To analyze the bone mineral density of each specimen, bone samples and an aluminum wedge (Morita Co, Kyoto, Japan) were exposed together using a Dixel CCD (Charge-Coupled Device) sensor at 60kV and 10mA for 0.2 seconds with a focus-detector distance of 30 cm. The images were then transferred to a personal computer-based image-analysis system with the pixel size set at 48×48 micrometers. The data were converted to aluminum thickness equivalent images. The bone mineral content was calculated as the mean of the whole region of interest (PosA: coronoid process of mandible, PosB: condyloid process of mandible, PosC: angle of mandible [Figure 5]) and the results were expressed as aluminum wedge equivalents. For pixel determination, a personal computer and image-analysis software designed by Iwashita[3] were used.

The Student's *t*-test was used to compare control group and 2 experimental groups. The error of the method of the investigator was estimated by double recording of 20 mandibles using the formula described by Dahlberg.[4]

RESULTS Return to TOC

Physical consistency of dietary foods

The average compressive strength of the food as a measure of hardness was 8.67 ± 3.25 kg/cm^2 for the control group and 6.53 ± 1.75 kg/cm^2 for the kneaded-diet group. The powdered-diet group was excluded because it was impossible to measure the compressive strength of this group.

Analysis of coordinates

The mean X and Y coordinates of the control and experimental groups, as well as the significant differences between them, were determined at each measuring point as shown in Table 1.

Values measured to the X-axis showed Go, which is a measurement of depth to the X-axis, significantly smaller in the powdered-diet group than the controls. The control group and the kneaded-diet group showed no significant differences in the X-axis values.

Values measured to the Y-axis showed Cr, Cd, and Go, which are measurements of height to the Y-axis, significantly smaller in the powdered-diet group while Cr and Cd were significantly smaller in the kneaded-diet group.

Length measurement

As shown in Table 2, there were no significant differences between the control and experimental groups for Cd-Id, which indicates mandibular length, Go-Id, which indicates mandibular body length, Al-Id, which indicates the lingual side of the alveolar bone length or Id'-M3 which indicates the length of the lower dental arch.

Height measurement

Cd-Go, which indicates the mandibular ramus height, was significantly greater in the control group than in the 2 experimental groups (Table 3). Cd-Ag, which indicates the height of the condylar process, showed no significant difference between the control and kneaded-diet groups, but the powdered-diet group showed a significantly lower value. Cr-Ag, which indicates the height of the coronoid process, was significantly larger in the control group than in the powdered-diet group. Al'-Me', which indicates the height of the central portion of the alveolar bone showed no significant differences between the control and the 2 experimental groups.

Bone mineral content

No significant difference in the bone mineral content of the condylar process was found between the control group and either experimental group. However, the powdered-diet group showed significantly lower levels than the control group in the mandibular angular processes and coronoid processes (Table 4).

Error of the method

The error of the method varied between 0.06 and 0.84 mm for measurements of each reference point on a coordinate system (Table 5). The error of the method varied between 0.015 and 0.026 mm AL for bone mineral content of mandible in the equivalent of aluminum (Table 6).

DISCUSSION

Return to TOC

Recent social changes have led to dramatic changes in the physical makeup and functional movements of Japanese children.[5] Kameya[6] reported that changes in dietary habits have led to changes in maxillofacial morphology, while Kimura[7] reported evidence of an evolutionary smaller jawbone in children. It is clinically important to understand changes not only in the morphology of the jawbones, but also in the bone mineral content, which reflects the inner structure of the jawbones. Recently, we reported that the occlusal force and masticatory efficiency of Japanese school age children were decreasing and suggested that recent changes in food regularly consumed in Japan had contributed to these modifications.[8]

The results of the present study demonstrate that the physical consistency of food can influence jawbone morphology and mineral content in growing rats. In this experiment, a hard diet, a kneaded diet prepared by kneading a powder diet with tap water and hardening it at room temperature, and a powdered diet were used. The food was not allowed to degenerate or decompose, as it was replaced every 24 hours for all of the groups. Further, nutritional conditions were identical among the 3 groups, as the diets were composed identically. Accordingly, we considered that the results were not affected by differences in nutritional content.

The physical consistency of the food was 68.67 kg/cm^2 in the hard-diet group and 6.53kg/cm^2 in the kneaded-diet group, a difference of approximately 10-fold. Yanagisawa and coauthors[1] classified food accordingly to hardness using a sensory testing method, and reported a 10-fold difference in the compressive strength between hard and soft foods normally eaten by humans. The eating and chewing habits between rats and humans are not the same and cannot be directly compared. However, for studying the effect of soft food, it seemed reasonable that the compression strength of the kneaded diet should be 10 times less than that of the control hard diet.

For the purpose of comparing the morphology of the rat mandibles, a lateral cephalometric X-ray analysis method was employed. For analysis of coordinates, the mental foramen was selected as the point of origin for morphological comparison. Arena and Gianelly[9] reported that the endoskeleton of the mandible, including the mental foramen, was relatively stable morphologically. Moreover, Manson[10] suggested that the position of the mental foramen could be used as a parameter for investigating the growth of the mandible, as it is relatively stable throughout the growing process.

For determining the amount of growth in each part, the mandible was divided into 5 areas: the coronoid process, condylar process, mandibular angle, mandibular body, and alveolar process. The changes were measured for each part.

For the coronoid process, our analysis of coordinates revealed that the height of Cr in the coordinate axis declined in both the kneaded-diet and powdered-diet groups. In view of the actual length measurements, Cr-Ag, which indicates the height of the coronoid process, was shortened in the powdered-diet group. We considered that the decline of vertical direction toward the coronoid process might have also reduced the height of the coronoid process, obviously since Ag was used to identify the X-axis. Thus, a reduction in dietary physical consistency might exert an effect on the growth in height of the coronoid process. It is likely that changes in diet hardness may cause alteration in the function of the temporal muscle. For the mandibular joint, a reduction in height of the coordinate axis Cd was observed in both of the experimental groups. Our measurements revealed that both Cd-Ag, which indicates the height of the condylar process, and Cd-Go, which indicates the mandibular ramus height, became shorter. Previous studies also have shown that the ramus became shorter in the vertical dimension with the involvement of the angular and condylar processes.[11–13]

For the mandibular angle, both the height and depth of the coordinate axis Go were reduced in the powdered-diet group. It is known that the mandibular angle grows at the region where the masseter and internal pterygoid muscles function.[14] The results of the present study suggest that these muscular functions were affected by changes in diet hardness. In contrast, there were almost no morphological changes in the mandibular body or alveolar process in our analyses of coordinates except Id and actual length measurements. It seems that the mastication muscles did not affect the mandibular body and alveolar process, as they are not directly attached. However, we cannot conclude that changes in dietary consistency did not exert any effect on these parts, as the height of Id in the coordinate axis declined in the powdered-diet group.

Osteoporosis, characterized mainly by the quantitative reduction and weakening of bones, has recently become a social problem as the Japanese population ages. There are many factors that determine the bone strength, but bone mineral content correlates most closely.[15] Although quantitative determination of bone minerals in the mandible is difficult because it includes teeth, a variety of approaches have been attempted.[16,17]

In the present study, we digitized oral radiograms using a video camera and computer system developed by Iwashita and coauthors[3] to prepare aluminum equivalent images for quantitative evaluations of the mandibles.

There are few reports about the relationships between bone mineral content of mandible and dietary consistency.[18,19] The present results showed that the bone mineral content was significantly lower in the coronoid process and angle of the mandible in the powdered-diet group than in the control group. It is likely that those parts, which are attached to the temporal muscle and masseter muscle, might be affected by a long-term diet with extraordinarily low physical consistency as in the powdered-diet group. However, we consider that bone mineral content will be largely unaffected by food with a hardness equivalent to the kneaded diet used in the present study. Our results suggested that the bone mineral content, which reflects osteogenesis, was scarcely affected with the kneaded diet, as the mandible is the portion most exposed to physical stimulation such as occlusal force and mastication. Further, bone mineral content may reduce as masticatory muscles are affected by eating an extraordinarily soft diet, such as the powdered diet in the present study. However, it is difficult to make a simple comparison, as the method and materials varied. Kiliaides and coauthors[18] reported that bone mineral content was reduced even in a kneaded-diet group. Accordingly, further detailed studies should be performed.

CONCLUSION Return to TOC

Thirty 3-week-old male Wistar rats were grouped into the control group (hard-diet group), kneaded-diet group, and powdered-diet group. After 6 weeks of growth, all rats were killed under deep anesthesia. We investigated the effects of dietary physical consistency on the mandible with respect to morphology and bone mineral content using lateral cephalometric analysis and computed X-ray densitometry. The average coordinates and standard deviation of the mean of the control and experimental groups, as well as the significant differences between them were determined at 15 measuring points. In the powdered-diet group, Gonion (Go), which is a measurement of depth to the X-axis, was significantly less, while in the kneaded-diet group, there was no significant value. In the kneaded-diet group, the most posterior point of the coronoid process (Cr), the most posterior point of condylar process (Cd), which are measures of height to the Y-axis, were less than the control group, while in the powdered-diet group, Cr, Cd, Go and Id were less than control group. On the other hand, the bone mineral content was significantly lower in the coronoid process and angle of mandible in only the powdered-diet group.

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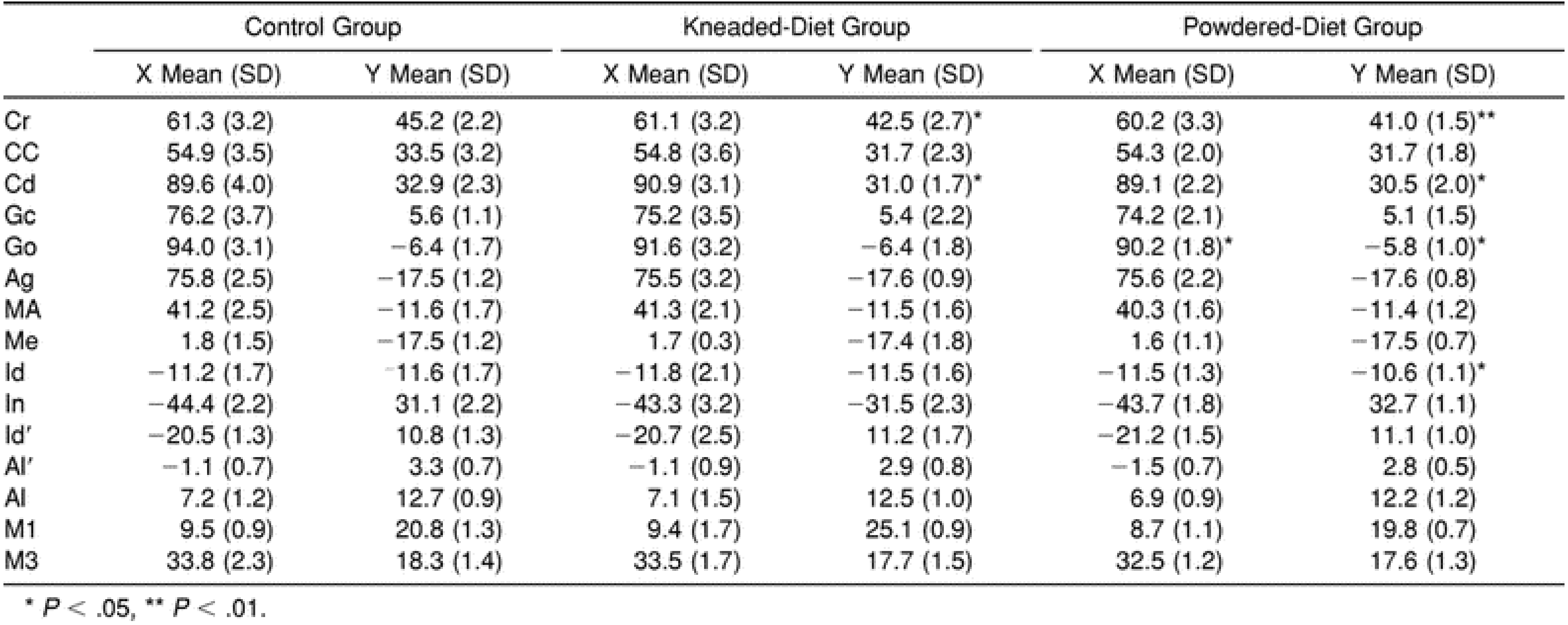
TABLES Return to TOC

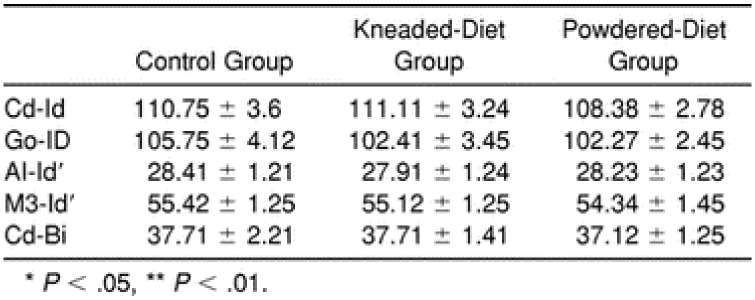
TABLE 1. Measurements of Each Reference Point on a Coordinate System (The Origin for the Coordinate System is Mental Formen)



	Control Group		Kneaded-Diet Group		Powdered-Diet Group	
	X Mean (SD)	Y Mean (SD)	X Mean (SD)	Y Mean (SD)	X Mean (SD)	Y Mean (SD)
Cr	61.3 (3.2)	45.2 (2.2)	61.1 (3.2)	42.5 (2.7)*	60.2 (3.3)	41.0 (1.5)**
CC	54.9 (3.5)	33.5 (3.2)	54.8 (3.6)	31.7 (2.3)	54.3 (2.0)	31.7 (1.8)
Cd	89.6 (4.0)	32.9 (2.3)	90.9 (3.1)	31.0 (1.7)*	89.1 (2.2)	30.5 (2.0)*
Gc	76.2 (3.7)	5.6 (1.1)	75.2 (3.5)	5.4 (2.2)	74.2 (2.1)	5.1 (1.5)
Go	94.0 (3.1)	−6.4 (1.7)	91.6 (3.2)	−6.4 (1.8)	90.2 (1.8)*	−5.8 (1.0)*
Ag	75.8 (2.5)	−17.5 (1.2)	75.5 (3.2)	−17.6 (0.9)	75.6 (2.2)	−17.6 (0.8)
MA	41.2 (2.5)	−11.6 (1.7)	41.3 (2.1)	−11.5 (1.6)	40.3 (1.6)	−11.4 (1.2)
Me	1.8 (1.5)	−17.5 (1.2)	1.7 (0.3)	−17.4 (1.8)	1.6 (1.1)	−17.5 (0.7)
Id	−11.2 (1.7)	−11.6 (1.7)	−11.8 (2.1)	−11.5 (1.6)	−11.5 (1.3)	−10.6 (1.1)*
In	−44.4 (2.2)	31.1 (2.2)	−43.3 (3.2)	−31.5 (2.3)	−43.7 (1.8)	32.7 (1.1)
Id′	−20.5 (1.3)	10.8 (1.3)	−20.7 (2.5)	11.2 (1.7)	−21.2 (1.5)	11.1 (1.0)
Al′	−1.1 (0.7)	3.3 (0.7)	−1.1 (0.9)	2.9 (0.8)	−1.5 (0.7)	2.8 (0.5)
Al	7.2 (1.2)	12.7 (0.9)	7.1 (1.5)	12.5 (1.0)	6.9 (0.9)	12.2 (1.2)
M1	9.5 (0.9)	20.8 (1.3)	9.4 (1.7)	25.1 (0.9)	8.7 (1.1)	19.8 (0.7)
M3	33.8 (2.3)	18.3 (1.4)	33.5 (1.7)	17.7 (1.5)	32.5 (1.2)	17.6 (1.3)

* $P < .05$, ** $P < .01$.

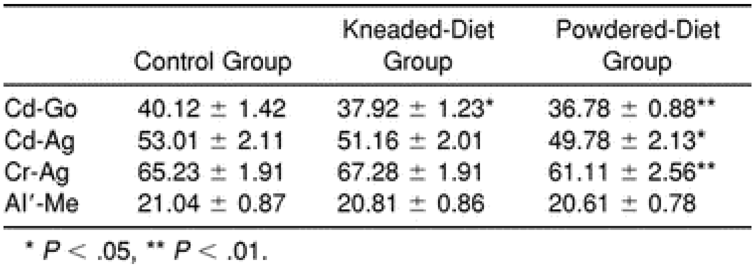
TABLE 2. Linear Measurements of Rat Mandibular Length



	Control Group	Kneaded-Diet Group	Powdered-Diet Group
Cd-Id	110.75 ± 3.6	111.11 ± 3.24	108.38 ± 2.78
Go-ID	105.75 ± 4.12	102.41 ± 3.45	102.27 ± 2.45
Al-Id′	28.41 ± 1.21	27.91 ± 1.24	28.23 ± 1.23
M3-Id′	55.42 ± 1.25	55.12 ± 1.25	54.34 ± 1.45
Cd-Bi	37.71 ± 2.21	37.71 ± 1.41	37.12 ± 1.25

* $P < .05$, ** $P < .01$.

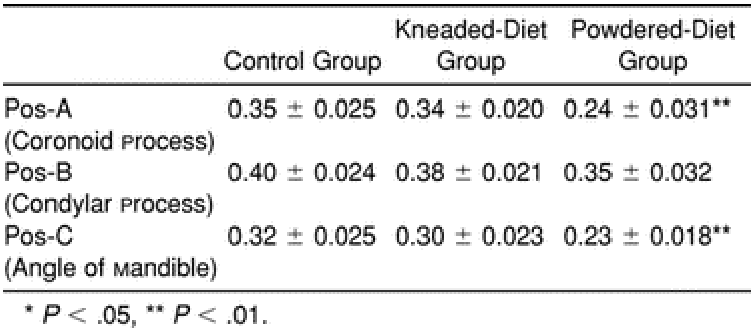
TABLE 3. Linear Measurements of Rat Mandibular Height (mm)



	Control Group	Kneaded-Diet Group	Powdered-Diet Group
Cd-Go	40.12 ± 1.42	37.92 ± 1.23*	36.78 ± 0.88**
Cd-Ag	53.01 ± 2.11	51.16 ± 2.01	49.78 ± 2.13*
Cr-Ag	65.23 ± 1.91	67.28 ± 1.91	61.11 ± 2.56**
Al′-Me	21.04 ± 0.87	20.81 ± 0.86	20.61 ± 0.78

* $P < .05$, ** $P < .01$.

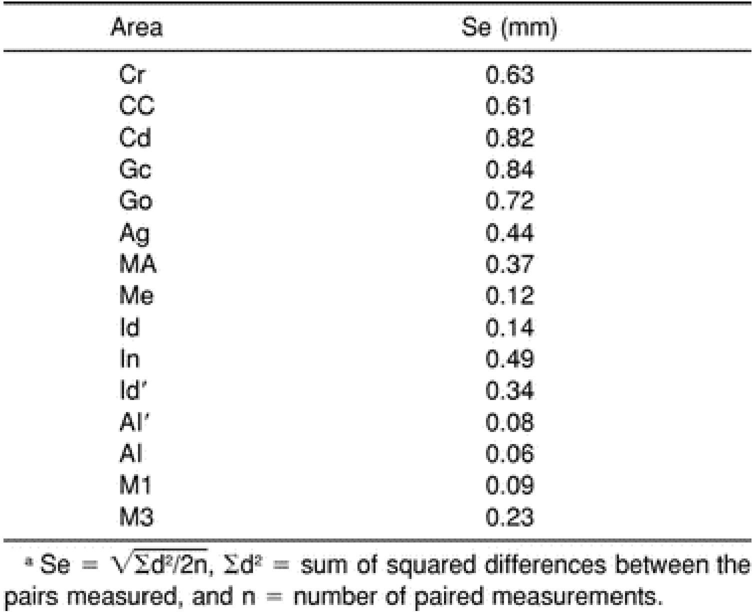
TABLE 4. Bone Mineral Content of Mandible in Aluminum Equivalents (mm AL)



	Control Group	Kneaded-Diet Group	Powdered-Diet Group
Pos-A (Coronoid process)	0.35 ± 0.025	0.34 ± 0.020	0.24 ± 0.031**
Pos-B (Condylar process)	0.40 ± 0.024	0.38 ± 0.021	0.35 ± 0.032
Pos-C (Angle of mandible)	0.32 ± 0.025	0.30 ± 0.023	0.23 ± 0.018**

* $P < .05$, ** $P < .01$.

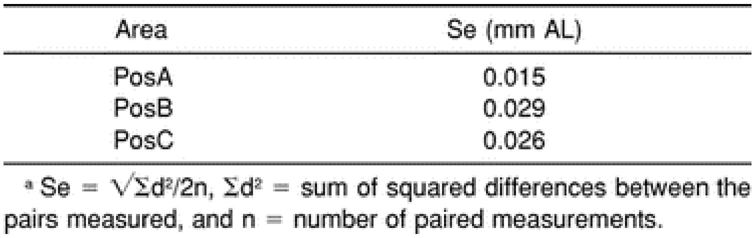
TABLE 5. Error of Measurements for Each Reference Point on a Coordinates System[a]



Area	Se (mm)
Cr	0.63
CC	0.61
Cd	0.82
Gc	0.84
Go	0.72
Ag	0.44
MA	0.37
Me	0.12
Id	0.14
In	0.49
Id′	0.34
Al′	0.08
Al	0.06
M1	0.09
M3	0.23

[a] Se = $\sqrt{\Sigma d^2/2n}$, Σd^2 = sum of squared differences between the pairs measured, and n = number of paired measurements.

TABLE 6. Error of Measurements for the Bone Mineral Content of Mandible in the Equivalent of Aluminum (mm AL)



Area	Se (mm AL)
PosA	0.015
PosB	0.029
PosC	0.026

[a] Se = $\sqrt{\Sigma d^2/2n}$, Σd^2 = sum of squared differences between the pairs measured, and n = number of paired measurements.

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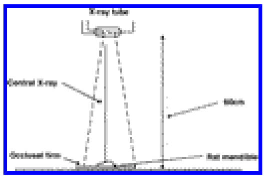

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FIGURE 1. Method to take the roentgenographic lateral cephalograms of the rats

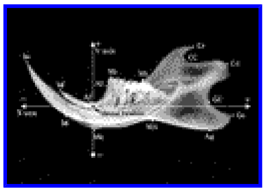

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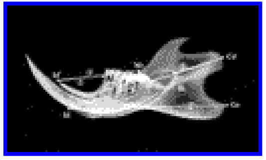
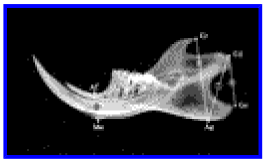
FIGURE 2. Reference points for coordinate analysis of lateral cephalograms. Cr indicates the most posterior point of coronoid process; CC, the most distant point of the line Cd-Cr; Cd, the most posterior point of condylar head; GC, the deepest point of the line Go-Cd; Go, Gonion; Ag, Ante-gonion; MA, the deepest point of the outer margin of bone that connects Me and Ag; Me, Menton; Id, Infradentale (labital side); In, the lower incisor up; Id', Infradentale (lingual side); AI', the deepest point of the outer margin of bone that connects AI and Id'; AI, the highest point of the mesial alveolar bone at the lower first molar; M1, the highest point of the mesiobuccal cusp of the lower first molar; and M3, the highest point of the central cusp of the lower third molar



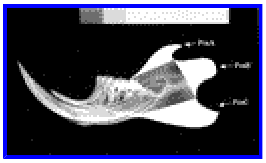
Click on thumbnail for full-sized image.

FIGURE 3. Reference points and items for linear measurements of rat mandibular length. Bi indicates the alveolar base of the lower incisor; 1. Cd-Id, mandibular length; 2. Go-Id, mandibular body length; 3. Al-Id', length of lingual side of a alveolar bone; 4. Id'-M3, length of the lower dental arch; and 5. Cd-Bi, distance from condyle head to the alveolar base of the lower incisor



Click on thumbnail for full-sized image.

FIGURE 4. Reference points and items for linear measurements of rat mandibular height. 6. Cd-Go indicates mandibular ramus height; 7. Cd-Ag, height of condylar head; 8. Cr-Ag, height of coronoid; and 9. Al'-Me, height of the central portion of alveolar bone



Click on thumbnail for full-sized image.

FIGURE 5. PosA indicates the region surrounded by the line which connects the uppermost margin of the coronoid process, the lowest anterior point of the coronoid process, and the outermost margin of the coronoid process. PosB indicates the region surrounded by the line which connects CC, GC and the outermost margin of the condylar process. PosC indicates the region surrounded by the line, which connects the lowermost margin of the condylar process, the MA, and the outermost margin of the mandibular angle

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