Original Article

Effects of masticatory movement on cranial bone mass and micromorphology of osteocytes and osteoblasts in developing rats

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In order to evaluate the influence of masticatory movement on cranial bone mineral density (BMD) and osteocyte and osteoblast micromorphology, we conducted a study in rats fed with solid feed (n=10) and powdered feed (n=10). Cranial BMD was measured by dual X-ray absorptiometry (DXA). Osteocyte morphology was evaluated by light microscopy. In addition, some of the tissue was treated with EDTA-KOH to digest the bone matrix and prepare osteocyte samples. Micromorphology of the osteocytes was examined by scanning electron microscopy (SEM). Bone mineral content (BMC) was significantly higher in the solid feed group (1.86 ± 0.11 g) than in the powdered feed group (1.63 ± 0.09 g) ($p < 0.05$). In the solid feed group, the maximum masseter muscle tension when crushing solid feed was 305 ± 46.0 N. Immunohistochemical staining with DMP-1 showed greater positive localization of DMP-1 in bone lacunae in the solid feed group than in the powdered feed group. On examination of cranial bone sections by SEM, the lacunar area was significantly larger in the solid feed group (0.64 ± 0.08 μm^2) than in the powdered feed group (0.43 ± 0.10 μm^2) ($p < 0.01$). In the solid feed group, adjacent osteocytes were connected through cytoplasmic branches and reticular cell processes to form a 3-dimensional structure. In the powdered feed group, connecting osteocyte processes were sparser. Results suggest that masticatory movement stimulates mediator substances involved in dynamic interactions between osteocytes, increases cranial bone mass during the developmental period and influences osteoblast, osteocyte, and lacunar micromorphology.

Key Words: masticatory movement, cranial bone density, osteocytes, osteoblasts, bone lacunae, DMP-1

INTRODUCTION

Recent reports have described the physiological effects of dietary changes, including skipping breakfast and a preference for softer foods.[1-3] Research on masticatory movement (chewing) with different types of food and the effects on development of the jaw and facial muscles has been conducted.[4,5] Masticatory function involves the coordinated action of many tissues, including the teeth supporting tissues, masseter muscles, and temporomandibular joint (TMJ). This process is regulated by oral sensory information in response to food consistency and amount.[6]

The influence of soft diets, such as powdered and liquid feed, on masticatory organs has also been investigated in several animal studies. Delayed development of the maxilla and masseter muscle, changes in growth patterns of the facial skeleton, and effects on overall cranial growth have been demonstrated.[4,5,7,8] In a study on jawbone degeneration, the effects of solid feed and powdered feed on maxillofacial morphology were evaluated by digital imaging in rabbits. With powdered feed, the decrease in functional loading during chewing led to decreases in zygomatic width, masseter muscle superficial area, and mandibular ramus width. This had a significant effect on masseter muscle development.[9]

Therefore, an adaptive mechanism exists by which cranial shape, internal structure, and function change in response to mechanical stimulation with occlusal force. External forces are essential for cranial growth and maintenance. However, the signal transmission mechanism by which cranial bone density and occlusal force are regulated has not been thoroughly investigated.

In this study, we investigated the influence of masticatory movement on cranial bone density and osteoblast and osteocyte micromorphology in developing rats.

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MATERIALS AND METHODS

Experimental animals and methods

In this study, we used 20 Sprague-Dawley male rats (age 4 weeks; Japan SLC, Hamamatsu, Japan). Rats were divided into two groups: the solid feed group (10 animals) and the powdered feed group (10 animals). Rats were acclimatized from age 4 to 5 weeks, and the study was performed during an 8-week period from age 5 to 12 weeks. Rats were housed in a controlled environment with a temperature of 24°C and in individual cages. The light-dark cycle was as follows: light from 0800 to 2000 and dark from 2000 to 0800. The feed used was Rodent Laboratory Diet EQ (Japan SLC), and the feed amount was 30 g/day in both groups. Water (tap water) was provided ad libitum. Body weight was measured once weekly from the start to the completion of the study using a small animal scale (AND, HL-2000).

This study was conducted in accordance with the "Guidelines for Animal Experimentation in Physiology Research" published by the Physiological Society of Japan.

Sample collection and cranial morphometry

After study completion, rats underwent laparotomy and thoracotomy under anesthesia with pentobarbital sodium. Animals were pre-perfused with physiological saline from the left ventricle, and were then perfusion fixated with Karnovsky fixative. The skull was resected, and the masseter muscle was dissected and weighed (g). The remaining tissue was treated with 4% sodium hypochlorite to remove the muscle. The long axis (mm), transverse axis (mm), and weight (g) of the skull were measured. The specimen was then immersion fixated in 10% formalin solution. Some of the tissue was also immersion fixated in 2.5% glutaraldehyde in phosphate buffer (PBS) (pH 7.2, 4°C).

Measurement of occlusal force in rats

As the objective of the present study was to determine the effects of masticatory movements on cranial bone density, osteocytes and osteoblasts, rats were fed solid and powdered diets, which have markedly different degrees of hardness. This is because rats eat by biting off food particles using incisors and then swallow while grinding food using molars. In the present study, the powdered diet was prepared by pulverizing the solid diet into a powder with a particle size of 20 μm or less. Therefore, rats in the powdered feed group did not need to bite off food using their incisors, and there was no difference between the two groups in swallowing while grinding food particles using molars. Therefore, occlusal force was measured only for the solid feed group. Measurement of occlusal force in the solid feed group was performed using the resected skulls and a force plate (Kistle, Type 9281B) (Fig. 1).

In order to measure occlusal force, the rat skull was fixed perpendicularly to the force plate, solid feed was placed between the upper and lower teeth, and a weight was placed to apply a vertical load between the upper nasal bone and surface. The load in the vertical direction was then gradually increased until the solid feed was crushed, and the force (F, N) when the feed was crushed

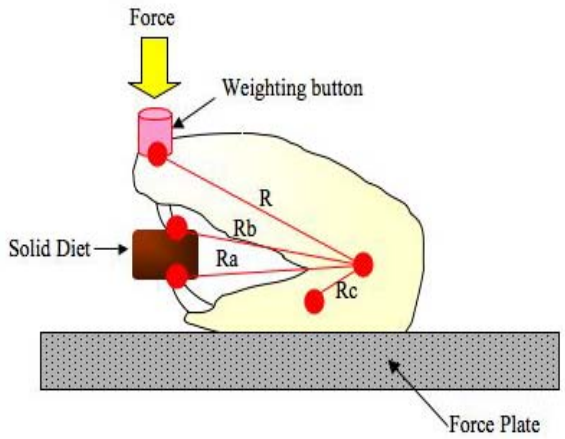

Figure 1. Apparatus using force plate to measure occlusal force.

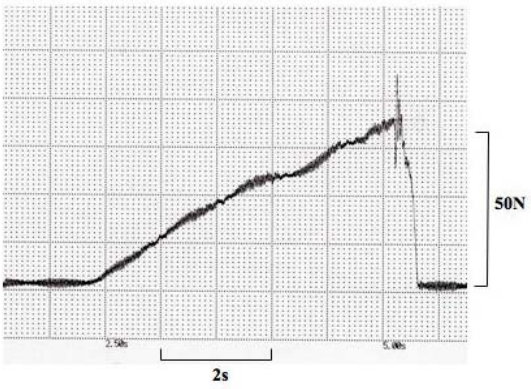

Figure 2. Example of measurement of occlusal force using force plate.

was measured (Fig. 2). In addition, the distance (R) from the center of the TMJ to the weight was measured, the torque (T, N•mm) was determined from F•R, and force (upper teeth: Fa:N; lower teeth: Fb:N) to the upper teeth (Ra: mm) and lower teeth (Rb: mm) were calculated from T/Ra and T/Rb. Distance (Rc) from the center of the TMJ to the center of the masseter muscle was also measured. Maximum masseter muscle tension (muscle force) (Fc, N) was calculated from T/Rc.

Digital video cameras were placed in each rat cage, and the number of daily chews to ingest the feed (30 g/day) was counted.

Measurement of bone mineral density (BMD)

Cranial BMD was measured by dual X-ray absorptiometry (DXA: QDR-4500, Hologic Inc., USA). Scanning was performed, using the ultra-high resolution mode for small animal bones, in the posterior direction from the tip of the nasal bone in the frontal plane. For consistency in scanning direction, the center of the frontal and occipital bones was placed along a predrawn line on the bottom surface of a styrol case. The sample was positioned for scanning of the entire skull. From the scanned images, overall bone area (cm^2), bone mineral content (BMC) (g) and BMD (BMC per area; g/cm^2) were calculated.

Examination by light microscopy

After measurement of BMD, cranial bone samples (parietal bone) were sectioned along the long axis with a sharp razor (single edged). Postfixation was performed with 10% formalin. Fixed samples were washed with tap water and decalcified for 30 days in 8% EDTA-sucrose (pH 7.2). Samples were then washed with tap water, dehydrated by graded ethanol series, and embedded in paraffin. These were thin sectioned (4 μm) with a large sliding microtome (TU-213; Yamato Koki) and subjected to hematoxylin-eosin (HE) and immunohistochemical staining.

Using anti-DMP-1 (dentin matrix protein-1; Takara, Japan) as the primary antibody, immunohistochemical staining was performed using the labeled streptavidin biotinylated antibody (LSAB) method (Dako, Japan).[10] Primary antibody was diluted 1000-fold in PBS and reacted at 4°C for 12 h. Reaction of secondary antibody, biotin-labeled anti-rabbit IgG goat serum, was conducted at room temperature for 30 min. Then, the peroxidase-labeled streptavidin reaction was done at room temperature for 30 min. For peroxidase staining, the reaction of 3-3'diaminobenzidine•H_2O_2 was conducted for 10 min. After nuclear staining with hematoxylin, dehydration, clearing, and mounting were performed. Samples were examined using a light microscope (BX-51, Olympus System Microscope, NY Super System, Japan).

Examination by scanning electron microscopy (SEM)

In order to examine osteoblasts of the cranial bone (parietal bone) surface, resected tissue was fixed with 2.5% glutalaldehyde-PBS (pH 7.2, 4°C) for 4 to 5 days. Fixed samples were immersed in 2.5% glutalaldehyde-PBS, and the internal periosteum of the cranium was stripped with forceps under a stereoscopic microscope. The sample was then treated with 1% trypsin (0.1 M PBS, pH 7.4) in an incubator at 37°C for 48 h (medium was changed every 12 h) to dissolve/remove the soft tissue and expose the bone surface. The sample was fixed again with 2.5% glutalaldehyde-PBS (pH 7.2, 4°C). Next, the sample was immersed overnight in 0.1 M cacodylate buffer, and was dehydrated by a graded ethanol series, substituted with isoamyl acetate, critical point dried, and coated with gold (SC7610, Topcon, Japan). Osteoblasts were examined and photographed by SEM (DS-600, Topcon).

For SEM examination of cranial bone (parietal bone) osteocytes, resected tissue was fixed with 2.5% glutaraldehyde-PBS (pH 7.2, 4°C) for 4 to 5 days. The sample was decalcified for 30 days with 8% EDTA-sucrose (pH 7.2), and was dissected transversely under a stereoscopic microscope, followed by fixing with 2.5% glutaraldehyde-PBS (pH 7.2, 4°C). The sample was immersed in 5 N KOH heated to 60°C for 8 min in order to digest bone matrix collagen and expose osteocytes. Next, the sample was immersed overnight in 0.1 M cacodylate buffer, and was then conductively stained with 2% tannic acid and 1% osmium tetroxide. Subsequently, the sample was dehydrated by a graded ethanol series, substituted with isoamyl acetate, critical point dried, and coated with gold (SC7610, Topcon). Osteocytes were examined and photographed by SEM (DS-600, Topcon).

Number of bone lacunae, osteoblasts and lacunar area (image processing)

Paraffin blocks of cranial bone samples in the solid feed and powdered feed groups were thin sectioned (2 μm) with a large sliding microtome (TU-213; Yamato Koki). Five consecutive tissue slices were prepared in each group. These were critical point dried, coated with gold, and examined by SEM to observe bone lacunae. In addition, five consecutive tissue slices in the solid feed and powdered feed groups were photographed by SEM. Using the SEM images, the number (n) and area (μm^2) of bone lacunae were calculated using image analysis software (NIH Image, National Institutes of Health, USA).

The area of bone lacunae was measured by capturing low-magnification images (×500; 39,708 μm^2) of cranial surfaces by scanning electron microscopy. In each rat, five such SEM images of the cranial surface were obtained (total area: 198,540 μm^2). SEM images were taken from 10 rats each in the solid and powdered feed groups (total of 50 images with a total area of 1,985,400 μm^2), and bone lacunae were analyzed using image analysis software (NIH Image, National Institute of Health) to measure the area of bone lacunae. The mean and standard deviation for the area of bone lacunae were calculated for both groups (Figs. 3 and 4).

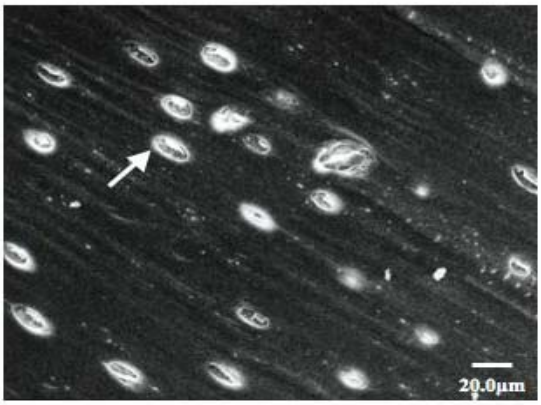

Figure 3. Bone lacunae in the solid feed group. Arrow: Bone lacuna-containing osteocytes

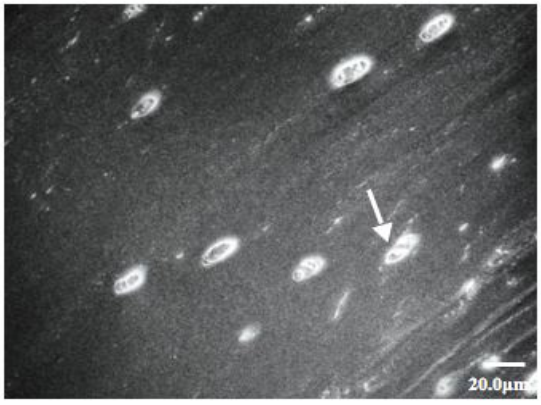

Figure 4. Bone lacunae in the powdered feed group. Arrow: Bone lacuna-containing osteocytes

Table 1. Cranial morphometry and bone mineral density in solid feed and powdered feed groups.

	Measured parameter	Solid feed group	Powdered feed group
Body weight	Weight (g)	406 ± 28.7	382 ± 25.3
	Cranial weight (g)	7.32 ± 0.47 *	6.72 ± 0.18
Skull	Long axis (mm)	47.3 ± 1.15	46.4 ± 0.82
	Transverse axis (mm)	12.8 ± 0.48	13.0 ± 0.11
Masseter muscle	Weight (g)	3.44 ± 0.33 *	3.00 ± 0.13
	Area (cm^2)	4.82 ± 0.11	4.41 ± 0.15
Bone mass	BMC (g)	1.86 ± 0.11 *	1.63 ± 0.09
	BMD (g/cm^2)	0.39 ± 0.02	0.37 ± 0.01

Mean ± SD., *: $p < 0.05$

Table 2. Measurement of occlusal force in solid feed group.

Parameter	Value
F (N)	55.6 ± 8.38
Torque T (N • mm)	1223 ± 184
Upper teeth: Fa (N)	58.2 ± 8.78
Lower teeth: Fb (N)	61.1 ± 9.21
Maximum tension (muscle force) of masseter muscle: Fc (N)	305 ± 46.0

Mean ± SD., *: $p < 0.05$

Table 3. Comparison of the number and area of bone lacunae, and the number of osteoblasts in solid feed and powdered feed groups.

	Solid feed group	Powdered feed group
Number of bone lacunae (n/43338 μm^2)	28.3 ± 5.32	21.1 ± 7.06
Lacunar area (μm^2)	0.64 ± 0.08 **	0.43 ± 0.01
Number of osteoblasts (n/10000 μm^2)	68.6 ± 2.08 **	58.6 ± 2.89

Mean ± SD., **: $p < 0.01$

Based on the osteoblast samples from the cranial bone (parietal bone) surface for the solid feed and powdered feed groups, the number of osteoblasts (n) per 10,000 μm^2 as seen on low-magnification SEM images (×500, 39,708 μm^2) for each group was calculated using the image analysis software NIH Image (National Institute Health).

Statistical analysis

Statistical analysis of the data from the solid feed and powdered feed groups, including cranial morphometry, BMD, number of bone lacunae, lacunar area, and number of osteoblasts, was performed using Macintosh StatView. Data were compared for significant differences by the t-test (unpaired). The level of statistical significance was set at less than 5 percent.

RESULTS

Comparison of body weight, cranial morphometry, and BMD

Table 1 summarizes body weight, cranial morphometry, and BMD in the solid feed and powdered feed groups. Cranial weight in the solid feed group (7.32 ± 0.47 g) was significantly higher than in the powdered feed group (6.72 ± 0.18 g) ($p < 0.05$). Masseter muscle weight in the solid feed group (3.44 ± 0.33 g) was also significantly higher than in the powdered feed group (3.00 ± 0.13 g) ($p < 0.05$). Bone mineral density in the solid feed group (0.39 ± 0.02 g/cm^2) tended to be higher than in the powdered feed group (0.37 ± 0.01 g/cm^2), but the difference was not significant. Bone mineral density in the solid feed group (1.86 ± 0.11 g) was significantly higher than in the powdered feed group (1.63 ± 0.09 g) ($p < 0.05$).

Masseter muscle tension in solid feed group

Table 2 shows masseter muscle tension in the solid feed group and the number of daily chews to completely ingest the feed (30 g). The maximum masseter muscle tension (muscle force) was 305 ± 46.0 N. The mean number of daily chews to completely ingest the feed (30 g) was 663 ± 13.7.

Comparison of number of bone lacunae, lacunar area, and number of osteoblasts in solid feed and powdered feed groups

Table 3 compares the number of bone lacunae and lacunar area in the solid feed and powdered feed groups. The number of bone lacunae in the solid feed group (28.3 ± 5.32) was higher than in the powdered feed group (21.1 ± 7.06), but the difference was not significant. Lacunar area in the solid feed group (0.64 ± 0.08 μm^2) was significantly higher than in the powdered feed group (0.43 ± 0.10 μm^2) ($p < 0.01$). The number of osteoblasts was 68.6 ± 2.08 and 58.6 ± 2.89/10,000 μm^2 for the solid and powdered feed groups, respectively, and the number of osteoblasts in the solid feed group was significantly higher than in the powdered feed group ($p < 0.01$).

Light microscopic examination of cranial bone in the solid feed and powdered feed groups

Light microscopy of the cranial bone in the solid feed group showed numerous lacunae containing osteocytes (Fig. 5A). In the powdered feed group, bone lacunae were sparse, and lacunar diameter was smaller than in the solid feed group (Fig. 5C). In the solid feed group, localization of DMP-1 was observed in numerous bone lacunae (Fig. 5B). In the powdered feed group, DMP-1 was seen in very few bone lacunae (Fig. 5D).

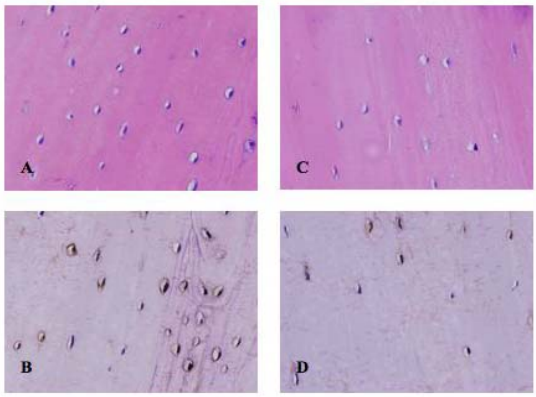

Figure 5. Light microscopic examination of cranial bone in solid feed and powdered feed groups. A: Solid feed group, HE stain (× 40), B: Solid feed group, DMP-1 (× 40), C: Powdered feed group, HE stain (× 40), D: Powdered feed group, DMP-1 (× 40)

Figure 6. Cranial bone osteoblasts in solid feed group.

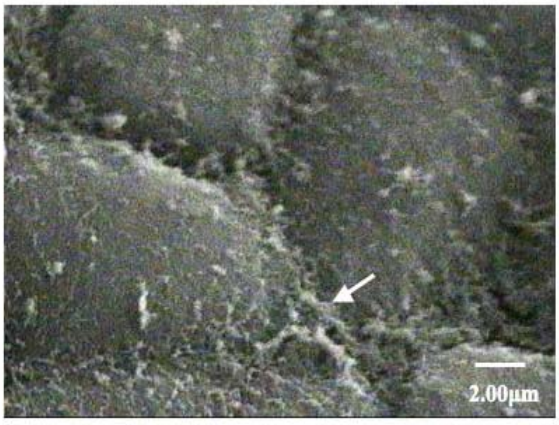

Figure 7. Cranial bone osteoblasts in solid feed group. Arrow: Short filamentous process joining adjacent osteoblasts.

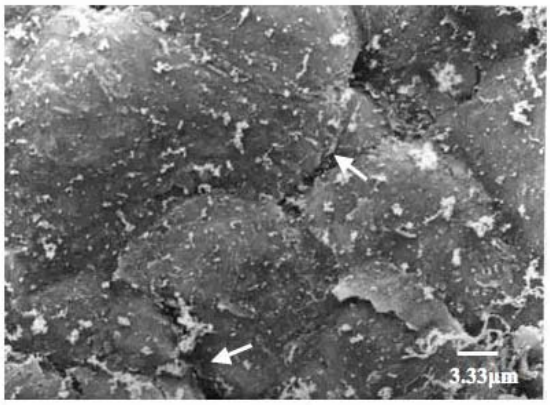

Figure 8. Cranial bone osteoblasts in powdered feed group. Arrow: Narrow inter-cellular space observed between adjacent osteoblasts.

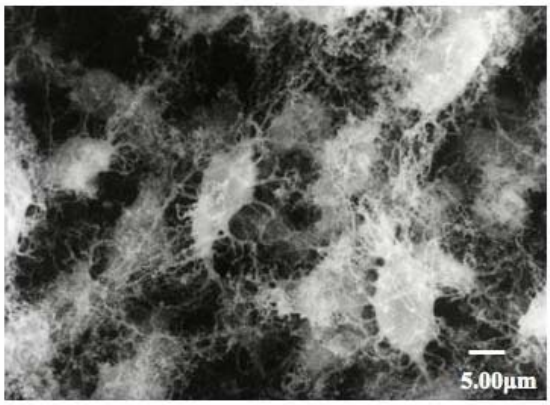

Figure 9. Cranial bone osteocytes in solid feed group.

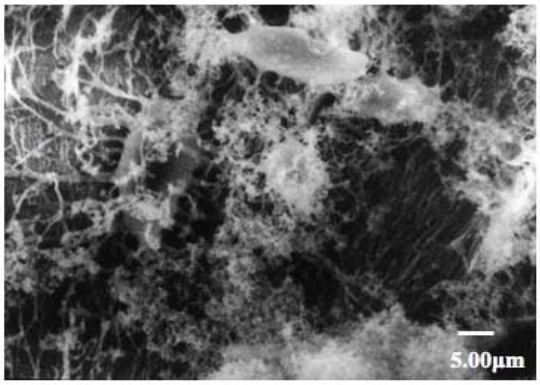

Figure 10. Cranial bone osteocytes in powdered feed group.

SEM examination of cranial bone surface in solid feed and powdered feed groups

In the solid feed group, osteoblasts (diameter, 11.2-16.3 μm) covered the parietal bone surface in a dense arrangement (Fig. 6). Adjacent osteoblasts were joined by short filamentous processes (arrow, Fig. 7). In the powdered feed group, the parietal bone surface was covered by large, flat osteoblasts (diameter, 17.3-18.6 μm). Very narrow intercellular spaces (0.66-1.33 μm) were observed between adjacent osteoblasts (arrow, Fig. 8).

SEM examination of cranial bone osteocytes in solid feed and powdered feed groups

In the solid feed group, as compared to the powdered feed group, osteocytes were larger and oval in shape. The osteocyte cytoplasmic processes extended radially from the entire cell body. In addition, the adjacent osteocytes were closely connected through cytoplasmic branches and reticular cell processes (Fig. 9). In the powdered feed group, as compared to the solid feed group, osteocytes were smaller, flatter, and distorted in shape. The osteocyte cytoplasmic processes had a thin, sparse reticular structure (Fig. 10).

DISCUSSION

Recent changes in dietary habits have led to an increase in children with malocclusion and difficulty chewing, and have raised concerns about craniofacial bone fragility. Therefore, proper chewing function and masticatory organ development during childhood is an important issue. Animal studies have demonstrated a relationship between changes in feed consistency and biting, chewing, masseter muscle growth and mandibulofacial development.[11-14]

Masseter muscle activity in chewing plays an important role in mandibular and craniofacial development. Masticatory movement involves coordinated actions of muscles, ligaments, and nerves that comprise the stomatognathic system. Oral cavity structure, functional conditions, and food consistency affect normal development. Masticatory movement in humans requires a coordinated interaction between muscles that support the skull, such as the masseter, suprahyoid, infrahyoid, and sternocleidomastoid muscles; lingual muscles; and other oral cavity soft tissues.

However, in rats and mice, masticatory movement differs markedly from that in humans. Rodents and carnivora require strong fast chewing movements, so the masseter muscle consists mostly of type 2 fibers. In humans, who are omnivorous, ≥70% of fibers are type 1, enabling prolonged slow chewing.[15] Herbivores have a greater percentage of type 1 fibers.[15-18] These differences suggest that differences in food consistency and jaw movement have a marked influence on muscle fiber differentiation. In mice weaned on postnatal day 20, Maeda et al.[19] reported that masseter muscle fiber diameter was significantly greater in mice given solid feed than in those given powdered feed. These findings suggest that chewing plays an important role in masseter muscle development.

The reason we used rats in this study is that the masseter muscle has a laminar structure, and muscle fiber direction is parallel to the zygomatic arch. Chewing is primarily an up and down motion, with little lateral gliding. In rabbits,[20,21] chewing is mainly a lateral gliding motion with molar crushing. Whether dynamic loading with malocclusion and grinding during chewing of food causes any internal structural changes has not been morphologically investigated. Thus, these effects can be more accurately evaluated in rats. The masseter muscle elongates a certain distance between the upper and lower jaw when the mouth is open, and at a certain rate of mouth opening. Therefore, mechanoreceptors send motion sensory signals to the brain in response to lower jaw position (opening size) and opening rate.

When the jaw is closed, muscle spindles—in other words, both ends of the mechanoreceptor—contract, and muscle fibers attached to tendons contract and generate muscle force. The masseter muscle stretches between the upper and lower jaw. The muscle fibers are not directly attached to bone, but rather, attached through tendons (muscle fibers→tendons→bone). Thus, in response to chewing solid food, tension on the tendon increases. In addition, during masticatory movement, the tension generated in the masseter muscle is the force of mastication, so tendon spindles of the masseter muscle send signals to the brain in response to masticatory force and hardness of the food being chewed. Two points should be considered regarding intracranial changes as a result of mechanical stimulation by external forces at tendons.

First, mechanical stimulation of the cranial bone is a process of mechanotransduction, in which cranial bone cells are sensitized, ultimately leading to increased production of bone matrix by osteoblasts.

Second, mechanical stimulation by objects with a given shape may cause slight changes in the cranial bone. Slight distortion (strain), including shortening and elongation, of the skull as a result of changes due to mechanical stimulation can be measured. The advantages of using rats in these studies include few individual differences and completion of growth and development by age 145 days.[22] This permits evaluation of growth and development, including the influence of masticatory movements on cranial bone, in a short period of time.

In the powdered feed group, body weight was slightly lower than in the solid feed group, but the difference between groups was not significant. This suggests that differences in feed hardness and grinding with the upper and lower incisors had no substantial effects on overall growth and development. In addition, changes in masseter muscle activity in response to differences in feed hardness—in other words, changes in chewing function—are likely to occur. The largest muscle forces are generated in the masseter muscles, which are relatively easy to resect, and so weight could be compared at completion of the study.

In the rats given powdered feed, even if abrasive forces (for example, due to constant rubbing of the upper and lower incisors or biting on the cage), caused changes in eruption of teeth, we would expect masseter muscle weight and skull weight to be similar in both groups. However, our results showed that masseter muscle weight and skull weight were significantly higher in the solid feed group vs. the powdered feed group. We can attribute this to differences in muscle activity, including intensity of muscle force and time required for chewing, between the solid feed and powdered feed groups. The result was an effect on muscle weight itself and weight of the skull and jawbone, to which the masseter muscle is attached.

Previous reports[23,24] comparing the effects of soft feed and hard feed have been limited to measurement of mandibular head width and cranial skeletal morphology. In a study of the effects of feed hardness on cerebral and facial cranial development in rats, Kikuta[24] reported not only changes in maxillofacial skull structure and size, but also significant differences in dry weight of the mandible between both groups. These results showed a difference in both mandibular morphology and weight. Bouvier et al.[25] performed a similar study in monkeys and rats. They found a significant difference in mandibular cortical bone weight and attributed this to different strain levels on the mandible related to chewing. These findings suggest some type of local control mechanism. Force is not the only factor regulating bone growth. Other theories on the role of functional matrix, neural control factors, piezo effects, and cellular level mechanisms have been proposed.

Therefore, we conducted this study with histologic examination in order to investigate the effects of mechanical stimulation and strain on bone density. For measurement

of masseter muscle force during chewing in rats, use of a strain gauge between the teeth was not feasible. Thus, we designed an apparatus using a force plate and measured masseter muscle tension after necropsy. Mean tension of the rat masseter muscle to crush solid feed was 305 ± 46.0 N. The number of daily chews to ingest the solid feed, used to assess the repetitive stimulation of masticatory movement on bone, was 663 ± 13.7. This represented a marked difference in feeding conditions between the solid feed group and powdered feed group.

Changes in the intensity of mechanical stimulation were not measured, but if we assume that if the intensity of mechanical stimulation is increased, there is additional strain on the cranial bone. These alterations in strain environment by mechanical stimulation would likely have an influence on bone mass. In this study, repetitive stimulation occurring 663 ± 13.7 times daily had a significant effect on bone formation and absorption.

Before examining internal bone architecture, cell morphology and functional changes, we first evaluated changes in bone mass. In the solid feed group, cranial BMD was higher than in the powdered feed group, but the difference was not significant. However, BMC was significantly higher in the solid feed group than the powdered feed group. This suggests that masseter muscle tension is transmitted to the cranial bone and that this mechanical bone stress has an influence on cranial bone cells. In other words, there is bone remodeling.[26]

Bone remodeling is regulated by systemic factors, local factors (e.g., cytokines), and neurogenic factors. Bone remodeling in response to this stimulation occurs mainly through changes in bone cells. This includes osteogenic cells and osteoblasts derived from mesenchymal stem cells, and osteocytes derived from osteoblasts. Osteoblasts are directly involved in production of bone matrix and calcification, and have a variety of functions. In the solid feed group, the bone surface was covered with relatively small osteoblasts 10.0 to 20.0 μm in diameter. In the powdered feed group, the osteoblasts were thinner, flatter, and larger with a diameter of 22.5 to 32.5 μm. In both groups, cell number and size play an important role in bone matrix synthesis. For example, the bone area formed per osteoblast is 136 to 177 μm^2 in rats, and daily production of bone matrix per osteoblast is 470 μm.[3,27]

Moreover, the present study revealed changes in osteoblast morphology, despite using rats of the same age. Calculation of the number of osteoblasts per 10,000 μm^2 as seen on low-magnification SEM images (×500, 39,708 μm^2) at the end of the experiment (age, 12 weeks) for rats in each group showed a significantly larger number of osteoblasts in the solid feed group (68.6 ± 2.08) when compared to the powdered feed group (58.6 ± 2.89). The volume of bone matrix produced per day by a single osteoblast, as calculated simply based on these results,[28] was 32,273 ± 978 and 27,573 ± 1,356 μm^3 for the solid and powdered feed groups, respectively. This difference results in an intergroup difference in bone matrix production of 4,700 μm^3 per day per 10,000 μm^2 of cranial bone at the end of the experiment (age, 12 weeks). Although the bone matrix secreted by osteoblasts does not calcify immediately and remains in its uncalcified form (known as osteoid) for some time, these findings suggest that increases in the number of osteoblasts play an important role in bone formation. In addition, as the osteoblasts in the powdered feed group were flat and fewer in number compared to the solid feed group, they were thought to have had a lower ability to produce bone matrix.

Furthermore, Arana-Chavez et al.,[29] who observed the early formation process of the cranial bone using an electron microscope, reported three types of junction between cells of osteoblast lineage, specifically focal tight junctions, gap junctions and adherence junctions. Tight junctions were observed in the solid feed group, and they were thought to be involved in the maintenance of cell polarity among osteoblasts and the initial isolation of bone matrix.

Osteocytes are divided into three types: osteoblastic-like osteocytes closest to the bone surface where osteoblasts are buried in bone matrix, osteoid osteocytes in the osteoid, and osteocytes in the lacunae of calcified bone.[29,30] Morphologic characteristics include hydroxyapatite crystals surrounding the bone lacunae and osteocyte processes in the bone lacunae. Osteocytes in the lacunae have many processes extending into the bone matrix to form a cellular network. In the solid feed group, adjacent osteocytes possessed abundant processes that ran perpendicularly. Osteocyte processes are important in propagating signals from osteoblasts at the bone surface to deeper bone. These act as mechanosensors in perceiving mechanical stimulation of the bone matrix and regulating bone morphology and mass. DMP-1, a specific osteocyte marker involved in lacunae number and bone calcification,[10,31] was seen in many of the bone lacunae in the solid feed group. DMP-1 is a bone matrix protein produced in large amounts by osteocytes. Positive staining for DMP-1 is indicative of increased expression in osteocytes in response to mechanical stress. DMP-1 also binds with calcium and is involved in calcification, and it is abundantly expressed in osteoblasts and promotes calcification.

The above findings indicate that masticatory movement, which is influenced by the environment, affects the micromorphology of osteocytes and bone lacunae in the cranial bone, and leads to increases in cranial BMD. The present findings may contribute greatly to modern dietary issues in Japan.

AUTHOR DISCLOSURES

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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Original Article

Effects of masticatory movement on cranial bone mass and micromorphology of osteocytes and osteoblasts in developing rats

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在發育的大鼠中咀嚼運動對頭蓋骨量及骨細胞和成骨細胞的微觀型態的影響

為了評估咀嚼運動對頭蓋骨質密度(BMD)、骨細胞和成骨細胞的微觀形態之影響，本研究利用大鼠來試驗，餵食固體飼料(n=10)或粉狀飼料(n=10)。頭蓋骨質密度藉由雙能 X 光吸收儀(DXA)來檢測。骨細胞形態以光學顯微鏡評估。另外，一些組織利用 EDTA-KOH 來消化骨基質和製作骨細胞樣品。骨細胞的微觀形態則用掃描式電子顯微鏡(SEM)檢測。在餵食固體飼料組(1.86 ± 0.11 g)的骨礦物質含量(BMC)顯著高於粉狀飼料組(1.63 ± 0.09 g) ($p < 0.05$)。在餵食固體飼料組中，咬碎固體飼料的最大咬肌張力為 305 ± 46.0 N。以免疫組織化學法染色骨基質蛋白質(DMP-1)，發現餵食固體飼料組比起粉狀飼料組，骨腔隙中有較多的 DMP-1 存在。藉由掃描電子顯微鏡檢測頭蓋骨切片，在餵食固體飼料組(0.64 ± 0.08 μm^2)的腔隙區域顯著大於粉狀飼料組(0.43 ± 0.10 μm^2) ($p < 0.01$)。在固體飼料組中，相鄰骨細胞藉由細胞質分支和網狀細胞突連接，以形成三級結構。而在粉狀飼料組中，連接骨細胞的細胞突較稀疏。研究結果顯示，咀嚼運動刺激物質參與調節骨細胞的動態交互作用，並且增加發育期間頭蓋骨量和影響成骨細胞、骨細胞和骨腔隙的微觀形態。

關鍵字：咀嚼運動、頭蓋骨密度、骨細胞、成骨細胞、骨腔隙、骨基質蛋白質-1