성장 중인 쥐에서 음식물의 경도가 하악 과두의 해면골에 미치는 영향 : 미세전산화 단층촬영을 이용한 연구

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국문초록

하악 과두의 발달과 증식은 측두하악관절 부위의 생역학적 환경의 변화에 따라 변경될 수 있다. 이 부위에 전달되는 생역학적 하중은 섭취하는 음식물의 경도를 다르게 함으로써 변화시킬 수 있다. 이번 연구의 목적은 성장 중인 쥐에서 부드러운 음식물의 섭취에 의해 저작력을 변화시키는 것이 하악 과두의 해면골의 형태를 변화시킬 수 있는지 미세전산화 단층촬영을 이용하여 분석하는 것이었다.

생후 21일 된 C57BL/6 쥐 36마리를 무작위로 두 그룹으로 나누었다. 8주 동안 대조군의 쥐들은 일반적인 딱딱한 덩어리의 사료를, 실험군의 쥐들은 덩어리의 사료를 잘게 갈은 후 물과 섞어 부드럽게 만들어 먹였다. 또한 실험군의 쥐들의 하악 절치를 일주일에 두 번씩 잘라서 짧게 만들었다. 8주 후 모든 쥐들을 희생하여 우측 하악 과두를 준비하였다. 미세전산화 단층촬영과 삼차원 영상 분석프로그램을 이용하여 하악 과두 해면골의 bone volume(BV), bone surface(BS), total volume(TV), bone volume fraction(BV/TV), surface to volume ratio(BS/BV), trabecular thickness(Tb. Th.), structure model index(SMI)와 degree of anisotropy(DA)를 측정하고 이들 값으로부터 trabecular number(Tb. N.)와 trabecular separation (Tb. Sp.)을 계산하였다. 미세전산화 단층영상을 얻은 후 하악 과두의 조직 표본을 만들었다.

연구 결과는 다음과 같았다.

1. Bone volume fraction(BV/TV), trabecular thickness(Tb. Th.)와 trabecular number(Tb. N.)가 대조군에 비해 부드러운 음식을 먹인 실험군에서 유의하게 감소되었다($p<0.05$).
2. Trabecular separation(Tb. Sp.)은 부드러운 음식을 먹인 실험군에서 유의하게 증가하였다($p<0.05$).
3. Surface to volume ratio(BS/BV), structure model index(SMI), degree of anisotropy (DA)는 두 군 사이에 유의한 차이를 보이지 않았다($p>0.05$).
4. 조직 절편을 관찰한 결과, 부드러운 사료를 먹인 실험군에서 하악 과두의 연골층의 증식 층과 전체 두께가 상당히 감소하였다.

주요어 : 부드러운 음식, 저작력, 하악 과두, 미세전산화 단층촬영, 해면골 형태

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Ⅰ. INTRODUCTION

The softer diet consumed in modern societies is likely to generate reduced masticatory force. The development and proliferation of the mandibular condyle can be altered by changes in the biomechanical environment of the temporomandibular joint[1,2]. In orthopedic literature, reduced loading at limb articular cartilage has been simulated by splinting or casting a limb, thereby altering its weight-bearing role. The condylar cartilage is not loaded by body weight but is loaded by the resultant of force applied to the teeth during mastication and biting[3]. A reduction of functional forces delivered to the mandibular joint can be achieved by providing foodstuffs that can be ingested with little or no mastication. Development of the mandibular condyle in rats was influenced by the consistency of the diet[2]. Many workers have found smaller condyles, thinner condylar cartilage and less dense bony trabeculae underlying the condylar cartilage and diminished staining for alcian blue in rats fed soft diets[4-7]. And water content of the condylar cartilage was significantly reduced in the soft-diet group, as well as one measure of [^{35}S] uptake into sulfated glycosaminoglycans[2]. Another study with condyles from mice fed a soft diet showed decrease in bone matrix-protein expression of the mandibular condyle[8]. In a study of the rat mandibular joint, Simon[9] suggested that reduction in condylar cartilage thickness noted in animals subjected to removal or trimming of incisors resulted from the lessening of joint reaction forces produced during incision. Both the incisor-clipped and soft-diet groups exhibited reduced size and density of bony trabeculae underlying the condylar cartilage and diminished staining for alcian blue. The thickness of the prechondroblastic layer of the condylar cartilage was significantly reduced relative to controls in both experimental groups on the superior aspect of the cartilage[7]. Thus mandibular condyles provide a useful model to study the relation between biomechanical force and the development of bone and cartilage.

Quantitative bone morphometry is a method to assess structural properties of trabeculae in the cancellous bone. Trabecular morphometry has traditionally been assessed in two-dimensions, where the structural parameters are either inspected visually or measured from sections, and the three-dimension is added on the basis of stereology. The conventional approach to morphologic measurements typically entails substantial preparation of the specimen, including embedding in methylmetacrylate, followed by sectioning into slices. Although the method offers high spatial resolution and high image contrast, it is a tedious and time-consuming technique. Particularly limiting is the destructive nature of the procedure, preventing the specimen from being used for other measurements, such as analysis in different planes.

Micro-computed tomography requires a specialized x-ray device and operator expertise, but is relatively rapid, reproducible and non-destructive to the sample[10-12]. Also, micro-computed tomography allows for quantitative and fully automatic determination of three-dimensional morphometric indices.

The purpose of the present study was to determine whether changes of masticatory forces by feeding a soft diet can alter the trabecular bone morphology of the growing mouse mandibular condyle, by means of micro-computed tomography.

Ⅱ. MATERIALS & METHODS

1. Animal Experiment

Thirty-six female, 21 days old, C57BL/6NTacSamf BR mice (Samtako Inc., Korea) were used. Animals were randomly divided into two groups of 18. Mice in the hard-diet control group were fed standard hard rodent pellets for 8 weeks. The soft diet group mice were given soft diets for 8 weeks. The soft food was made from ground pellets mixed with water; it was supplied in a bowl within the cage. After they had been weaned at the age of 21 days, the lower incisors of the soft-diet group were shortened by cutting with a wire cutter twice a week to reduce incision[7]. The experimental period was 8 weeks and all animals were killed at 11 weeks of age after they were weighed.

Following sacrifice, the mandibular condyles of each animal were disarticulated and the surrounding tissue was manually dissected. After removal of the soft tissue, each specimen was placed in 10% neutral buffered formaline. The right mandibular condyle of each animal was included in the present study.

2. Micro-computed Tomography

High spatial resolution tomography was done with a Skyscan Micro-CT 1072 (Skyscan, Aartselaar, Belgium). The imaging parameters were a tube potential of 80㎸ and a current of 100㎂. Al 1mm external filter was used. 400 projections at angular increments of 0.9° were recorded(360°) with an integration time of 3.4 sec per projection. Cross-sections were scanned with a size of 1024×1024 pixels. The pixel size was 2.73×2.73μm^2 and cross-section to cross-section distance was 5.46μm. Three-dimensional images were reconstructed from 2D sectoins.

We selected volume of interest (VOI) for comparing the soft-diet group with the control group. The VOI was selected the middle-superior portion of the condyle. In most cases, the size of the VOI was 375×375×82 μm^3. We used a uniform threshold for all VOIs of all specimens to distinguish bone tissue from bone marrow.

3. Bone Structure Parameters

The morphometric indices were the bone volume(BV), bone surface(BS), total volume(TV), bone volume fraction(BV/TV), surface to volume ratio(BS/BV) and trabecular thickness(Tb. Th.). Nonmetric parameters were the structure model index(SMI) and degree of anisotropy(DA). All of these were directly determined by means of the software package at the micro-CT system.

From directly determined indices, the trabecular number(Tb. N.) and trabecular separation(Tb. Sp.) were calculated according to parallel plate model of Parfitt *et al.*[13]. For a parallel plate model the following equations were used : Tb. N.=(BV/TV)/Tb. Th.; Tb. Sp.=(1- BV/TV)/Tb. N.(nomenclature according to Parfitt[14]).

4. Histological Preparation

After micro-tomographic imaging, the samples were decalcified in 10% EDTA for three weeks, dehydrated through graded ethanols, embedded in paraffin, sectioned at 4μm, and stained with hematoxylin and eosin.

5. Statistical Analyses

Student's *t tests* were used to compare differences between the control and soft diet groups. $p<0.05$ was considered statistically significant. All tests were conducted with the SPSS 11.0 software(SPSS Inc.).

Ⅲ. RESULTS

The weight of the animals did not differ significantly between the soft-diet group and hard-diet control group (20.82±1.15 vs. 21.12±1.42g, $p=0.45>0.05$) (Table 1).

1. Micro-tomographic Properties

For all bone structure parameters, mean, SD and p values were presented in Table 2. Bone volume fraction was significantly lower in the soft-diet group. The mean bone volume fraction was 15.72±3.40% in the soft-diet group and 20.84±4.89% in the hard-diet control group($p=0.001<0.05$). The trabeculae of the soft-diet group were significantly thinner than that of the control group($4.32\times10^{-3}\pm0.33\times10^{-3}$ vs. $4.94\times10^{-3}\pm0.49\times10^{-3}$mm, $p=0.000$). And there was a significant reduction in the Tb. N. of the soft-diet group compared with that of the control group(36.07±5.82 vs. 41.75±6.84mm^{-1}, $p=0.011<0.05$). The Tb. Sp. was significantly increased in the soft-diet group. The mean Tb. Sp. was $24.14\times10^{-3}\pm5.13\times10^{-3}$mm in the soft-diet group and $4.94\times10^{-3}\pm0.49\times10^{-3}$mm in the hard-diet control group ($p=0.011<0.05$). But there was no significant differences in the surface to volume ratio(406.46±42.27 vs. 404.82±46.18mm^{-1}, $p=0.912>0.05$), SMI(1.23±0.21 vs. 1.23±0.21, $p=0.981>0.05$) and degree of anisotropy(0.097±0.019 vs. 0.093±0.018, $p=0.527>0.05$) between the soft-diet group and hard-diet control group. Representative three-dimensional reconstructed im-

Table 1. Comparison of the weight between the soft-diet group and hard-diet control group

	Soft-diet group(n=18)	Hard-diet control group(n=18)	P value*
Weight(g)	20.82±1.15	21.12±1.42	0.45

* Student t-test; p>0.05=NS(not statistically significant)

Table 2. Comparison of the difference in the micro-tomographic parameters between the soft-diet group and hard-diet control group

Parameters	Mean±SD Soft-diet group (n=18)	Mean±SD Hard-diet group (n=18)	P value*
Surface to volume ratio(mm^{-1})	406.46±42.27	404.82±46.18	0.912
Bone volume fraction(%)	15.72±3.40	20.84±4.89	0.001
Tb. Th. ($\times 10^{-3}$mm)	4.32±0.33	4.94±0.49	0.000
Tb. N. (mm^{-1})	36.07±5.82	41.75±6.84	0.011
Tb. Sp. ($\times 10^{-3}$mm)	24.14±5.13	19.68±4.81	0.011
SMI	1.23±0.21	1.23±0.21	0.981
Degree of Anisotropy	0.097±0.019	0.093±0.018	0.527

*Student *t*-test; p>0.05=NS(not statistically significant)

ages of the condylar head trabecular bone from a control and a soft-diet mouse are shown in Fig. 1.

2. Histological Observation

Structurally normal mandibular condyles were seen in the mice fed the hard diet. The cartilage of the mandibular condyle was composed of several layers defined on the basis of cell type and tissue composition; proliferative, transitional, hypertrophic and degenerative zones[15]. There were bone marrow and bone trabeculae underneath the cartilage (Fig. 2A).

In contrast, the condyles from mice fed the soft diet showed profound morphological changes. Relative to controls, the thickness of the proliferative layer and total cartilage thickness were significantly reduced in the soft-diet group. Another striking feature of this group was a generalized reduction in the density and size of the bony trabeculae underlying the cartilage. Bone marrow spaces were enlarged. Thus the condyles in soft-diet mice had features of underdevelopment at this particular growth stage(Fig. 2B).

Ⅳ. DISCUSSION

Model utilizing a change in dietary consistency has been frequently employed to achieve a loading discrepancy at the mandibular joint in vivo, although it is not easily quantified. The soft diet reduced forces exerted on the mandibular condyle during mastication[2]. Cutting of the incisors lessened the protrusion of the mandible, and the loading on the anterior part of the articulating surface was decreased during incision[7].

The micro-CT system enabled the cancellous bone architecture of the mandibular condyle to be reconstructed in three-dimensions[16]. Major advances of this technique were : the full three dimensional information of the whole condyle, independence of planes of section, non-destructive evaluation, fast scanning time, and standard software for the determination of bone morphology parameters. The technique has proved to be reliable, and the estimated bone parameters resembled those obtained by traditional histomorphometry[12].

Structural indices determined from the micro-tomographic measurements were dependent on the incorporated thresholding procedure. It was advantageous to use a uniform threshold for one type of bone to be able to compare results. Otherwise a quantitative assessment might be very difficult, because changes in structural properties might have resulted from the choice of threshold defined for every sample individually[16].

According to the micro-tomographic analysis in the present study, the bone volume fraction, Tb. Th. and Tb. N. were significantly decreased and Tb. Sp. was significantly increased in the soft-diet group compared with that of the control group. The bone volume fraction is the relative volume of calcified tissue in the selected VOI. Trabecular thickness gives an indication of the average thickness of the trabeculae. Trabecular separation is a measure similar to trabecular thickness, but the inverse, giving an indication of the average size of the marrow spaces between the trabeculae[14,17]. Therefore these findings meant that there was a generalized reduction in the density and size of the bony trabeculae with widened marrow spaces in the soft-diet group, which may represent a degree of disuse atrophy, similar to that seen in muscular atrophy occurring after immobilization of a limb in a cast as treatment for a fracture. As the weight of the animals did not differ significantly between the soft-diet group and hard-diet control group, it was unlikely that the differences were due to nutritional deprivation of the soft diet group.

Histological sections in the present study showed that all zones of the condylar cartilage were affected by changes in dietary consistency. Significant decreases were seen in the proliferative zone and total cartilage thickness of the soft diet mice. And as the results from micro-tomographic analysis, there was a generalized reduction in the density and size of the bony trabeculae underlying the cartilage with widened marrow spaces in soft diet group.

These findings were consistent with those of previous studies. In a study of the rat mandibular joint, Simon[9] suggested that reduction in condylar cartilage thickness noted in animals subjected to removal or trimming of incisors resulted from the lessening of joint reaction forces produced during incision. Hinton and Carlson[7] found that both the incisor-clipped and soft-diet groups exhibited reduced size and density of bony trabeculae underlying the condylar cartilage and diminished staining for alcian blue. Also the thickness of the prechondroblastic layer of the condylar cartilage was significantly reduced relative to controls in both experimental groups on the superior aspect of the cartilage. Bouvier and Hylander[4], and Bouvier[5,6] showed a decreased thickness of the alkaline phosphatase-positive region of the cartilage as well as a pronounced decrease in overall width and length of the condylar surface. And another study concluded that water content of the condylar cartilage was significantly reduced in the soft-diet group, as well as one measure of [^{35}S] uptake into sulfated glycosaminoglycans[2]. Sasaguri *et al.*[8] showed a decrease in bone matrix-protein expression of the mandibular condyle in the soft-diet mice.

On the other hand, no significant difference in the surface to volume ratio, SMI and degree of anisotropy(DA) was observed between the two groups. The SMI is a parameter related to the shape of the trabeculae. A SMI of 0 means that the structure has only plate-like trabeculae and a value of 3 means only rod-shaped trabeculae[18]. Thus, the structural type of trabecular bone did not seem to be strongly affected by the soft diet. DA, reflecting trabecular orientation, was also not affected by the soft diet. This indicated that mechanical load change by the soft diet was similarly oriented as that in the hard diet.

The present study had limitations. First, we determined certain structural indices, such as Tb. N. and Tb. Sp., based on the parallel plate model. Although they were calculated using volume and thickness, which were directly measured using 3D images, the calculated values might vary depending on the structural pattern of cancellous bone. However, because no significant difference in SMI was observed between the two groups, the observed difference in calculated Tb. N. and Tb. Sp. should reflect the actual difference. And this study measured parameters only in the middle-superior portion of the condyle. The cancellous bone of the mandibular condyle was inhomogeneous and anisotropic. Therefore, future study should assess the trabecular bone architecture in different regions within the condyle. Furthermore, more studies are necessary in different sites of mandible, such as ramus and alveolar bone.

Ⅴ. CONCLUSIONS

Assessing the effects of dietary consistency on growing mouse mandibular condyle using micro-computed tomography, the results were,

1. The bone volume fraction, Tb. Th. and Tb. N. were significantly decreased in the soft-diet group compared with that of the control group ($p<0.05$).
2. The Tb. Sp. was significantly increased in the soft-diet group($p<0.05$).
3. But there was no significant differences in the surface to volume ratio, SMI and degree of anisotropy between the soft-diet group and

hard-diet control group(p>0.05).

4. Histological sections showed that the thickness of the proliferative layer and total cartilage thickness were significantly reduced in the soft-diet group.

REFERENCES

1. Petrovic A : Mechanisms and regulation of mandibular condylar growth. Acta Morph neerl-scand, 10:25-34, 1972.
2. Hinton RJ : Effect of dietary consistency on matrix synthesis and composition in the rat condylar cartilage. Acta Anat, 147:97-104, 1993.
3. Boyd RL, Gibbs CN, Maan PE, et al. : Temporomandibular joint forces measured at the condyle of Macaca arctoides. Am J Orthod Dentofac Orthop, 97:472-479, 1990.
4. Bouvier M, Hylander WL : The effect of dietary consistency on gross and histological morphology in the craniofacial region of young rats. Am J Anat, 170:117-126, 1984.
5. Bouvier M : Variation in alkaline phosphatase activity with changing load on the mandibular condylar cartilage in the rat. Archs oral Biol, 32:671-675, 1987.
6. Bouvier M : Effects of age on the ability of the rat temporomandibular joint to respond to changing functional demands. J Dent Res, 67:1206-1212, 1988.
7. Hinton RJ, Carlson DS : Response of the mandibular joint to loss of incisal function in the rat. Acta Anat, 125:145-151, 1986.
8. Sasaguri K, Jiang H, Chen J : The effect of altered functional forces on the expression of bone matrix proteins in developing mouse mandibular condyle. Arch Oral Biol, 43:83-92, 1998.
9. Simon MR : The role of compressive forces in the normal maturation of the condylar cartilage in the rat. Acta anat, 97:351-360, 1977.
10. Müller R, Hildebrand T, Hauselmann HJ, et al. : In vivo reproducibility of three-dimensional structure properties of noninvasive bone biopsies using 3D-pQCT. Bone Miner Res, 11:1745-1750, 1996.
11. Rüegsegger P, Koller B, Müller R : A microtomographic system for the nondestructive evaluation of bone architecture. Calcif Tissue Int, 58:24-29, 1996.
12. R Müller, H Van Campenhout, B Van Damme, et al. : Morphometric analysis of human bone biopsies: A quantitative structural comparisonof histological sections and micro-computed tomography. Bone, 23(1):59-66, 1998.
13. AM Parfitt, CHE Mathews, AR Villanueva, et al. : Relarionships between surface, volume, and thickness of iliac trabecular bone in aging and in osteoporosis. J Clin Invest, 72:1396-1409, 1983.
14. AM Parfitt : Bone histomorphometry: Proposed system for standardization of nomenclature, symbols, and units. Calci Tissue Int, 42 :284-286, 1988.
15. Ronaldo A Bergman, Adel K Afifi, Paul M Heidger : Histology. WB Sanders Company, 9:69-70, 1996.
16. Giesen EB, van Eijden TM : The three-dimentional cancellous bone architecture of the human mandibular condyle. J Dent Res, 79:957-963, 2000.
17. Yang J, Pham SM, Crabbe DL : Effects of oestrogen deficiency on rat mandibular and tibial microarchitecture. Dentomaxillofac Radiol, 32:247-251, 2003.
18. Joo YI, Sone T, Fukunaga M, et al. : Effects of endurance exercise on the three-dimensional trabecular bone microarchitecture in young growing rats. Bone, 33:485-493, 2003.

Explanations of Figures

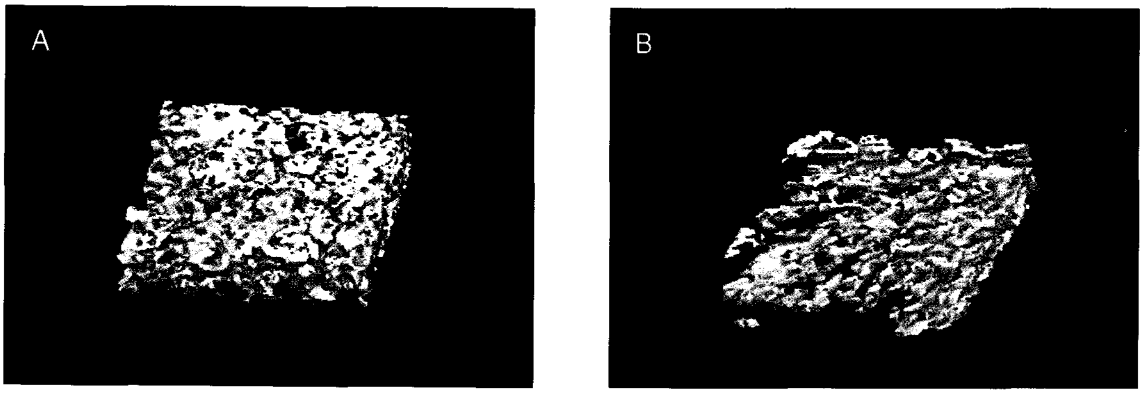

Fig. 1. Three-dimensionally reconstructed images of the trabecular bone of the condyle from a mouse fed a hard diet (A) and a mouse fed a soft-diet (B). The volume of interest represents a volume 375×375×82 ㎛³. The bony trabeculae in a mouse fed a soft diet is loosely formed.

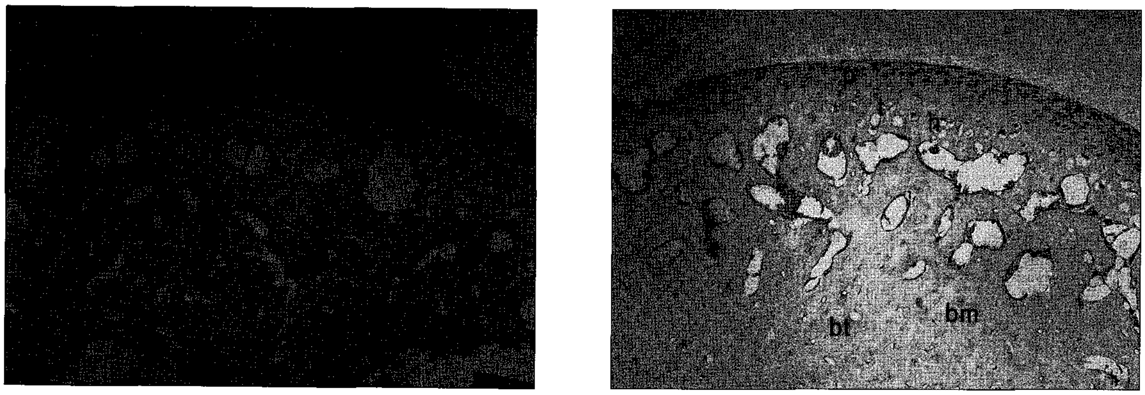

Fig. 2. Light microphotograph of the condylar tissue from a mouse fed a hard diet (A) and a mouse fed a soft diet (B). A: Normal condylar structure is seen in a mouse fed a hard diet. The condylar cartilage is composed of several layers; proliferative(p), transitional(t) and hypertrophic(h) zones. And there were bone marrow(bm) and bony trabeculae(bt) underneath the cartilage. B: The thickness of the proliferative layer and total cartilage thickness are reduced in a mouse fed a soft diet. And there are less dense trabeculae. Hematoxylin and eosin stain, ×200.

Abstract

THE EFFECTS OF DIETARY CONSISTENCY ON THE TRABECULAR BONE ARCHITECTURE IN GROWING MOUSE MANDIBULAR CONDYLE : A STUDY USING MICRO-COMPUTED TOMOGRAPHY.

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The development and proliferation of the mandibular condyle can be altered by changes in the biomechanical environment of the temporomandibular joint. The biomechanical loads were varied by feeding diets of different consistencies. The purpose of the present study was to determine whether changes of masticatory forces by feeding a soft diet can alter the trabecular bone morphology of the growing mouse mandibular condyle, by means of micro-computed tomography.

Thirty-six female, 21 days old, C57BL/6 mice were randomly divided into two groups. Mice in the hard-diet control group were fed standard hard rodent pellets for 8 weeks. The soft-diet group mice were given soft ground diets for 8 weeks and their lower incisors were shortened by cutting with a wire cutter twice a week to reduce incision. After 8 weeks all animals were killed after they were weighed. Following sacrifice, the right mandibular condyle was removed. High spatial resolution tomography was done with a Skyscan Micro-CT 1072. Cross-sections were scanned and three-dimensional images were reconstructed from 2D sections. Morphometric and nonmetric parameters such as bone volume(BV), bone surface(BS), total volume(TV), bone volume fraction(BV/TV), surface to volume ratio(BS/BV), trabecular thickness(Tb. Th.), structure model index(SMI) and degree of anisotropy(DA) were directly determined by means of the software package at the micro-CT system. From directly determined indices the trabecular number(Tb. N.) and trabecular separation(Tb. Sp.) were calculated according to parallel plate model of Parfitt et al.. After micro-tomographic imaging, the samples were decalcified, dehydrated, embedded and sectioned for histological observation.

The results were as follow:

1. The bone volume fraction, trabecular thickness(Tb. Th.) and trabecular number(Tb. N.) were significantly decreased in the soft-diet group compared with that of the control group($p<0.05$).
2. The trabecular separation(Tb. Sp.) was significantly increased in the soft-diet group($p<0.05$).
3. There was no significant differences in the surface to volume ratio(BS/BV), structure model index(SMI) and degree of anisotropy(DA) between the soft-diet group and hard-diet control group($p>0.05$).
4. Histological sections showed that the thickness of the proliferative layer and total cartilage thickness were significantly reduced in the soft-diet group.

Keywords : Soft-diet, Masticatory force, Mandibular condyle, Micro-computed tomography, Trabecular bone architecture