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Effects of mastication on mandibular growth evaluated by microcomputed tomography

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SUMMARY It is well known that mastication has a significant influence on mandibular growth and development, but the mechanism behind this effect has not yet been clarified. Furthermore, no studies have examined the effects of changes in mastication on the three-dimensional (3D) morphometry of the mandible. The aim of the present study was to investigate the influences of changes in mastication on mandibular growth and morphology. Twenty-five 3-week-old (at the time of weaning) imprinting control region mice were randomly divided into three groups: mice fed a hard diet (HD), mice fed a soft diet (SD), and mice alternately fed hard and soft diets (HSDs) every week for 4 weeks. The morphometry of the mandible was analysed using 3D microcomputed tomography (μCT). Statistical analysis was undertaken using a *t*-test.

μCT analysis showed that the condylar width was significantly greater in the HD group than in the SD group after 1 week. After 4 weeks, mandibular length was significantly longer and ramus height was greater in the HSD group than in the other two groups. Bone volume was significantly less in the SD group than in the other two groups after 4 weeks. These findings suggest that changes in mastication markedly affect mandibular condylar cartilage growth and mandibular morphology. It is considered that dietary education at an early age is important in order to prevent disruption of the development of the mandible.

Introduction

Mastication has a marked influence on mandibular growth and development (Luca *et al.*, 2003). It has previously been reported that changes in mastication affect gene expression in the mandibular condylar cartilage (Watahiki *et al.*, 2004). Therefore, it was hypothesized that mastication would influence the condylar cartilage and finally change the shape of the mandible.

The mandible is known to change shape with different diets because ramus height was found to be greater in rats fed a hard diet (HD) than in those on a soft diet (SD; Tuominen *et al.*, 1993; Maki *et al.*, 2002). Furthermore, the condylar dimensions were greater in a hard than in a SD group (Bouvier and Zimny, 1987), and the bone volume of the spongiosa was significantly greater in a HD group than in a SD group (Yamada and Kimmel, 1991). Bresin *et al.* (1999) found that bone mass was greater in a HD group than in a SD group, with significant differences for most of the points measured.

However, no studies have examined the effects of diet of different hardness on the three-dimensional (3D) shape of the mandible. In the present study, how the shape and volume of the mandible in mice is affected by food hardness was investigated using microcomputed tomography (μCT).

Materials and methods

Animals

Twenty-five male imprinting control region mice (21 days of age) were randomly divided into five equal groups and provided with diets of differing hardness. The study protocol was approved by the Animal Research Committee of Showa University.

Dietary regimen

At the time of weaning (3 weeks of age), the mice were randomly divided into the following five groups ($n = 5$ per group): (1) mice fed a hard diet for 1 week (HD1W); (2) mice fed a soft diet for 1 week (SD1W); (3) mice fed a hard diet for 4 weeks (HD4W); (4) mice fed a soft diet for 4 weeks (SD4W); and (5) mice alternately fed a hard and soft diet every week for 4 weeks (HSD4W). The HD comprised ordinary laboratory chow for mice in a hard pellet form, while the SD comprised the ordinary diet after grinding and mixing with water in standardized proportions (two parts food to five parts water). There were no significant differences between the body weights of any of the five groups of mice.

Morphological analysis

Five mice from each group were killed at specified time points under deep anaesthesia with pentobarbital. The head were removed and fixed in 4 per cent paraformaldehyde solution (pH 7.4) overnight at 4°C and then stored in phosphate-buffered saline. To obtain a 3D digital image of the mandible, each craniofacial sample was scanned using μCT (SMX-90CT; Shimadzu, Tokyo, Japan). The voxel size was 49 μm per pixel in all spatial directions. The CT images were reconstructed at 512 × 512 pixels.

Linear analysis

The following six distances were calculated using 3D structural analysis software (TRI/3D-BON; Ratoc, Tokyo, Japan; Figure 1): These points were based on previously described methods (Bouvier and Hylander, 1984; Tuominen *et al.*, 1993; Maki *et al.*, 2002).

Bone volume analysis

Using the same 3D structural analysis software (TRI/3D-BON), the slice images obtained by μCT were reconstructed, the mandible was excised from each sample, and mandibular bone volume was analysed.

Statistical analysis

Measurements on each sample were undertaken on three separate occasions by one author (AE) and at 3 hour intervals in a single day, and the results were analysed using a *t*-test. Error variance, as a percentage of total variance, was calculated using Dahlberg's double determination method (Dahlberg, 1940). The error variance was less than 0.6–2.9 per cent for both the linear and volume analyses.

The significance of differences between groups was assessed using a Student's *t*-test for independent samples.

Results

Morphological analysis by μCT

Linear analysis. After 1 week of consumption of the different diets (4 weeks of age), the condylar width was significantly thicker in the HD1W group than in the SD1W group. However, there were no significant differences in the other mandibular points between the HD1W and SD1W groups (Figure 2; Table 1). After 4 weeks of feeding (7 weeks of age), mandibular length was longest in the HSD4W group, shorter in the SD4W group, and shortest in the HD4W group, while ramus height was greatest in the HSD4W group, lower in the HD4W group, and lowest in the SD4W group. There were no significant differences in the other mandibular points among the HD4W, SD4W, and HSD4W groups (Figure 2; Table 2).

Bone volume analysis. After 1 week of eating the different diets (4 weeks of age), there was no significant difference between the bone volumes in the HD1W and SD1W groups. After 4 weeks of feeding (7 weeks of age), bone volume was significantly lower in the SD4W group than in the HD4W and HSD4W groups (Figures 2 and 3). There was no significant difference between bone volumes in the HD4W and HSD4W groups.

Discussion

Mastication of a HD has been reported to load a significantly greater mechanical force on the temporomandibular joint than mastication of a SD (Boyd *et al.*, 1990). There have been many reports regarding the relationships between food properties and mandibular growth. In previous studies on changes in mandibular morphology associated with food consistency, a SD was found to increase mandibular length (Luca *et al.*, 2003). Condylar width was greater in rats fed a HD than in mice fed a SD (Bouvier and Zimny, 1987; Kiliaridis *et al.*, 1999), while ramus height was greater in rats fed a HD than in mice fed a SD (Tuominen *et al.*, 1993; Maki *et al.*, 2002). Histological examination has revealed that the hypertrophic chondrocyte zone of the mandibular condylar cartilage is thicker in rats fed a HD than in those fed a SD (Bouvier, 1988; Yamada and Kimmel, 1991), and reduced proliferative ability and matrix production have also been reported in rats fed a HD, compared with those fed a SD (Pirttiniemi *et al.*, 2004). Furthermore,

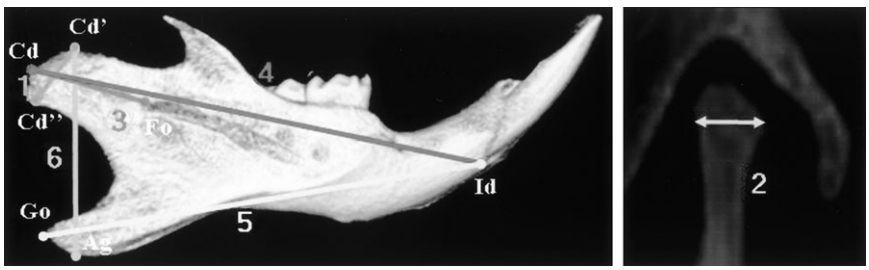

Figure 1 The following six mandibular distances were measured: ① Condylar length, Cd'-Cd''; ② Condylar width, left-to-right thickness of the condyle; ③ Condylar height, Cd-Fo; ④ Mandibular length, Cd-Id; ⑤ Mandibular body length, Go-Id; ⑥ Ramus height, Cd'-Ag; Cd, most posterior point of the condyle; Cd', highest point of the condyle; Cd'', lowest point of the condyle; Fo, mandibular foramen; Id, infradentale (labial side); Go, gonion; Ag, antegonion.

Figure 2 The morphometry of the mandibles of 4- and 7-week-old mice was evaluated by microcomputed tomography. Condylar width is significantly greater in the HD1W group than in the SD1W group. Mandibular length is shortest in the HD4W group, longer in the SD4W group, and longest in the HSD4W group. Ramus height is greatest in the HSD4W diet group, lower in the HD4W group, and lowest in the SD4W group. Scale bars: 2.0 mm. HD1W, hard diet for 1 week (at 4 weeks of age); SD1W, soft diet for 1 week (at 4 weeks of age); HD4W, hard diet for 4 weeks (at 7 weeks of age); HSD4W, hard and soft diets alternately every week for 4 weeks (at 7 weeks of age); SD4W, soft diet for 4 weeks (at 7 weeks of age).

Table 1 After 1 week of eating different diets in the soft (SD) and hard (HD) diet groups.

Measurement (mm)	SD	Standard deviation	HD	Standard deviation	HD versus SD
Condylar length-①	1.43	0.07	1.66	0.20	
Condylar width-②	0.60	0.02	0.79	0.04	**
Condylar height-③	2.49	0.09	2.35	0.10	
Mandibular length-④	10.82	0.05	10.62	0.29	
Mandibular body length-⑤	10.01	0.11	9.86	0.09	
Ramus height-⑥	4.49	0.60	4.85	0.11	

SD, soft diet; HD, hard diet.
**$P < 0.01$.

gene expression levels related to mandibular condylar cartilage growth were found to differ markedly before and after the initiation of mastication in mice (Watahiki *et al.*, 2004). Overall, these reports suggest that mastication markedly affects mandibular shape.

To understand the associations between mastication and mandibular condylar cartilage growth, 3D mandibular morphology and mandibular bone volume were measured in the preset study using μCT and the results compared among groups of mice fed a HD alone, a SD alone, or a HSD alternately.

In mice fed the different diets for 1 week (4 weeks of age), the condylar width (L-R) was significantly greater in the HD1W group than in the SD1W group, which is consistent with previous reports (Bouvier, 1988; Kiliaridis *et al.*, 1999). In mice fed the different diets for 4 weeks (7 weeks of age), mandibular length was longest in the HSD4W group, decreased in the SD4W group, and was shortest in the HD4W group. However, these findings are inconsistent with those of previous reports (Bouvier and Zimny, 1987; Maki *et al.*, 2002). This inconsistency may be due to differences in the dietary consistency, the duration and frequency of feeding, or the age of the animals. Poorer masseter muscle development in mice fed a SD compared with those fed a HD (Urushiyama *et al.*, 2004), as well as an influence of the masseter muscle on the angle of the mandible (Hendricksen *et al.*, 1982), have been reported. In the present study, ramus height was greatest in the HSD4W group, lower in the HD4W group, and lowest in the SD4W group. This order differed from that of mandibular length because ramus height includes the mandibular condylar cartilage, which is affected by cartilage growth, and gonion, which is affected by the masseter muscle.

The bone volume in mice fed the different diets for 4 weeks (7 weeks of age) was significantly lower in the SD4W group than in the HD4W and HSD4W groups. There are only a few reports involving 3D measurements of mandibular bone volume. However, the presence of regions

Table 2 After 4 weeks of eating different diets in the SD, HD, and alternate HSD groups.

Measurement (mm)	SD	Standard deviation	HSD	Standard deviation	HD	Standard deviation	HD versus SD	HD versus SD	HD versus SD
Condylar length-①	1.96	0.22	1.81	0.12	2.48	0.34			
Condylar width-②	0.66	0.08	0.74	0.1	0.88	0.06			
Condylar height-③	2.98	0.12	2.99	0.05	2.71	0.21			
Mandibular length-④	11.34	0.09	11.75	0.1	11.02	0.81	*	**	*
Mandibular body length-⑤	10.44	0.3	10.74	0.33	10.44	0.14			
Ramus height-⑥	4.96	0.10	5.56	0.06	5.34	0.04	*	*	**

SD, soft diet; HD, hard diet; HSD, hard and soft diet.
*$P < 0.05$; **$P < 0.01$.

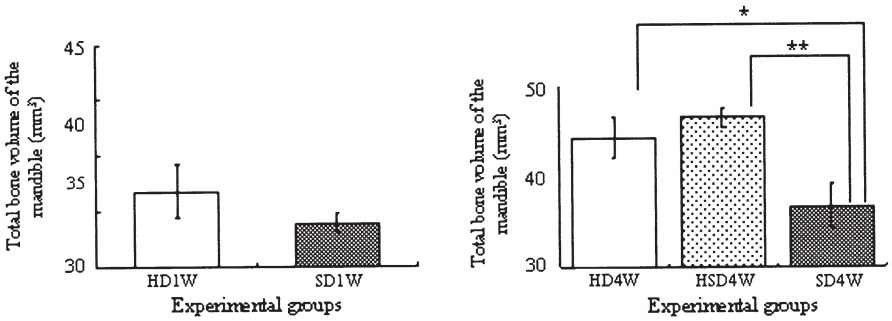

Figure 3 After 1 week of eating the different diets, total bone volume did not differ significantly between the hard diet for 1 week (HD1W) and soft diet for 1 week (SD1W) groups. After 4 weeks of eating the different diets, total bone volume was significantly smaller in the soft diet for 4 weeks (SD4W) group than in the hard diet for 4 weeks (HD4W) and hard and soft diet every week for 4 weeks (HSD4W) groups. *$P < 0.05$; **$P < 0.01$.

with significantly lower bone volumes in rats fed a SD compared with those fed a HD has been reported in two-dimensional cross-sectional images (Yamada and Kimmel, 1991; Bresin *et al.*, 1999).

As type II errors may have been introduced in the present study due to the small sample size, it is necessary to perform further investigations with larger sample sizes in order to investigate the sites that were not significantly affected in the current study. Significant differences were found, however, in mandibular length and ramus height, which are both important parameters.

The results of the present research suggest that mechanical stress induced by mastication markedly affects the mandibular condylar cartilage and the mandible around the masticatory muscles, as well as changing the pattern of growth and development of the mandible.

Conclusion

A change in adult masticatory function can have a significant influence on mandibular growth and development. Since mandibular shape plays an important role in development of the mandible, it is considered that dietary education should be provided at an early age.

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References

Bouvier M 1988 Effect of age on the ability of the rat temporomandibular joint to respond to changing functional demands. Journal of Dental Research 67: 1206–1212

Bouvier M, Hylander W L 1984 The effect of dietary consistency on gross and histologic morphology in the craniofacial region of young rats. American Journal of Anatomy 170: 117–126

Bouvier M, Zimny M L 1987 Effects of mechanical loads on surface morphology of the condyle cartilage of the mandible in rats. Acta Anatomica 129: 283–300

Boyd R L, Gibbs C H, Mahan P E, Richmond R F, Laskin J L 1990 Temporomandibular joint forces measured at the condyle of *Macaca arctoides*. American Journal of Orthodontics and Dentofacial Orthopedics 97: 472–479

Bresin A, Kiliaridis S, Strid K G 1999 Effect of masticatory function on the internal bone structure in the mandible of the growing rat. European Journal of Oral Sciences 107: 35–44

Dahlberg G 1940 Statistical methods for medical and biological students. George Allen and Unwin Ltd, London

Hendricksen R P, McNamara J A, Carlson D S, Yellich G M 1982 Changes in the gonial region induced by alterations of muscle length. Journal of Oral and Maxillofacial Surgery 40: 570–577

Kiliaridis S, Thilander B, Kjellberg H, Topouzelis N, Zafiriadis A 1999 Effect of low masticatory function on condylar growth: a morphometric study in the rat. American Journal of Orthodontics and Dentofacial Orthopedics 116: 121–125

Luca L, Roberto D, Francesca S M, Francesca P 2003 Consistency of diet and its effects on mandibular morphogenesis in the young rat. Progress in Orthodontics 4: 3–7

Maki K, Nishioka T, Shioiri E, Takahashi T, Kimura M 2002 Effects of dietary consistency on the mandible of rats at the growth stage: computed X-ray densitometric and cephalometric analysis. Angle Orthodontist 72: 468–475

Pirttiniemi P, Kantomaa T, Sorsa T 2004 Effect of decreased loading on the metabolic activity of the mandibular condylar cartilage in the rat. European Journal of Orthodontics 26: 1–5

Tuominen M, Kantomaa T, Pirttiniemi P 1993 Effect of food consistency on the shape of the articular eminence and the mandible. Acta Odontologica Scandinavica 51: 65–72

Urushiyama T, Akutsu S, Miyazaki J, Fukui T, Diekwisch T G, Yamane A 2004 Change from a hard to soft diet alters the expression of insulin-like growth factors, their receptors and binding proteins in association with atrophy in adult mouse masseter muscle. Cell and Tissue Research 315: 97–105

Watahiki J, Yamaguchi T, Irie T, Nakano H, Maki K, Tachikawa T 2004 Gene expression profiling of mouse condylar cartilage during mastication by means of laser microdissection and cDNA array. Journal of Dental Research 83: 245–249

Yamada K, Kimmel D B 1991 The effect of dietary consistency on bone mass and turnover in the growing rat mandible. Archives of Oral Biology 36: 129–138