Original Article

Glenoid Fossa Responses to Mandibular Lateral Shift in Growing Rats

Chang Liu[a]; Sawa Kaneko[b]; Kunimichi Soma[c]

ABSTRACT

Objective: To evaluate the morphological and histological responses of the glenoid fossa to mandibular lateral shift in growing rats.

Materials and Methods: A resin plate was placed on the upper incisors of 4-week-old rats in the experimental groups to displace the mandible to the left during closure. The rats were killed after 2, 4, 8, and 12 weeks. The morphometric measurements were performed on dry skulls, and tissue blocks were processed for periodic acid and Schiff's reagent (PAS) staining to examine the new bone formation.

Results: Gross measurements showed asymmetry in both the position and size of the fossae between the two sides after 4 weeks of lateral shift. The glenoid fossa on the ipsilateral side was repositioned relatively backward, outward and upward compared with the contralateral side and control group, whereas the fossa on the contralateral side was relocated relatively forward and downward compared with the control group. The length of the fossa was smaller on the ipsilateral side than on contralateral side and control group. At 2 weeks, the amount of newly formed bone in the posterior region of the fossa was higher in the experimental group than the control group.

Conclusion: It is suggested that the mandibular lateral shift causes asymmetry in the position and size of the glenoid fossa and that this phenomenon can be related to different bilateral directional new bone formation in the posterior region.

KEY WORDS: Dry skull; Glenoid fossa; Mandibular lateral shift; Morphometric analysis

INTRODUCTION

Since the mandible articulates with the skull at the glenoid fossa, the position of the fossa relative to the skull is supposed to be crucial in determining the mandibular position in various skeletal discrepancies. It has been reported that the fossa position relative to the cranial base was more posterior in skeletal Class II than in skeletal Class III.[1,2] On the other hand, the position of the glenoid fossa was more caudal in low-angle subjects compared with normal- or high-angle subjects.[2] These reports suggest that the position of the fossa can partly contribute to the development of various malocclusions by exaggerating the present skeletal discrepancy of the mandible.

It is a well-accepted concept that the normal function of the temporomandibular joint (TMJ) depends on the correct alignment of the condyle, disk, and fossa.[3] Remodeling of the fossa and condylar cartilage will take place to maintain the correct positional relationship during mandibular displacement. The adaptive potential of the condylar cartilage has been extensively studied,[4–6] and some studies have focused on the adaptive ability of the fossa. It has been reported that the glenoid fossa underwent remodeling in response to changed biophysical circumstances after condylar fracture,[7] permanent tooth loss,[8] unilateral masticatory function,[9] and surgical relocation of the fossa.[10]

In addition, some reports have investigated the ef-

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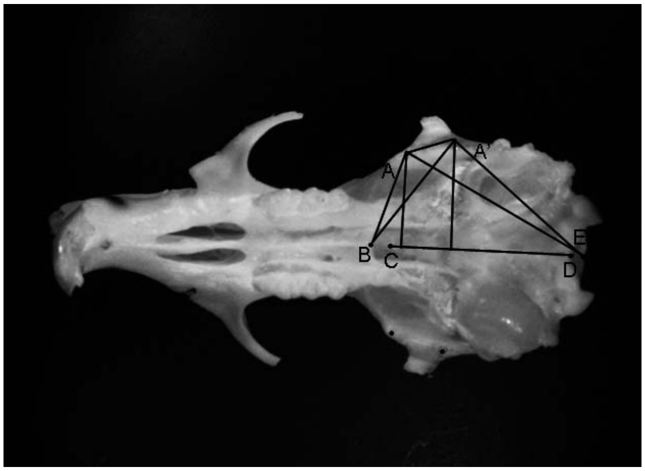

Figure 1. Illustration of landmarks and linear measurements on dry skull in ventral view (for definition, see Table 1).

Figure 2. Illustration of landmarks and linear measurements on dry skull in lateral view (for definition, see Table 1).

Table 1. Definition of Landmarks and Linear Measurements on Dry Skull

Variable	Definition
Landmark	
A	Most anterior extent of glenoid fossa
A′	Most posterior extent of glenoid fossa
B	Most posterior extent of horizontal process of the palatine bone
C	Central point of the intersphenoidal synchondrosis in the midsagittal plane
D	Central point of the anterior rim of the foramen magnum
E	Central point of the posterior rim of the foramen magnum
F	Most lateral extent of lambdoidal ridge
G	Temporal ridge of parietal bone
CD	The line connecting point C and D

fect of orthopedic forces on the remodeling of the glenoid fossa. Based on long-term cephalometric investigations, a series of clinical studies demonstrated that the glenoid fossa was displaced in an anterior-inferior direction, and, with the aid of magnetic resonance imaging, prominent glenoid fossa remodeling was found on the anterior surface of the postglenoid spine.[5,11]

Backward positioning of the mandible led to a pronounced resorption on the anterior surface and bone deposition on the posterior surface of the postglenoid spine in rhesus monkeys.[12] In contrast, the increased bone deposition along the anterior surface of the postglenoid spine was verified after forward mandibular positioning.[13,14] Similarly, new bone formation in the posterior fossa was significantly enhanced by forward mandibular positioning as a consequence of the upregulation of vascular endothelial growth factor expression in rat.[15]

However, there is no report about the positional change and relative histological alteration in the fossa during mandibular lateral shift. The aim of this study was to investigate changes in the glenoid fossa position and new bone formation during mandibular lateral displacement.

MATERIALS AND METHODS

Ninety-six 4-week-old male Wistar rats were randomly divided into four control and four experimental groups ($n = 12$). Each rat in the experimental group was fitted with a resin occlusal plate to functionally displace the mandible 2 mm to the left.[16] A metal crown was fitted to the lower incisors. Since the lateral shift diminished in some rats 2 weeks after appliance placement, the shift distance was maintained by adding resin to the occlusal plate every other week.[17] The left side was designated as the ipsilateral side, while the right side was designated the contralateral side. The rats in the control group received no appliance.

All rats were fed with a soft diet from 3 weeks of age and weighed periodically. All procedures followed the guidelines of the Tokyo Medical and Dental University for Animal Research. The experimental protocols were approved by the local ethics committee.

The rats were killed at 2, 4, 8, and 12 weeks after appliance attachment. The rats for gross measurements were killed and the heads dissected. The mandible was carefully removed leaving the capsule intact. The most anterior (A) and posterior (A′) points of the fossa were marked according to the range of the capsule. Then the heads were thoroughly defleshed, and all other points were determined. The pictures of the dry skull, taken from a standard ventral view, were enlarged threefold, and two measurements (A-CD and A′-CD) were made on photographic paper. Other measurements (Figures 1 and 2; Table 1) were carried out on dry skulls with a sharp pointed caliper accurate to

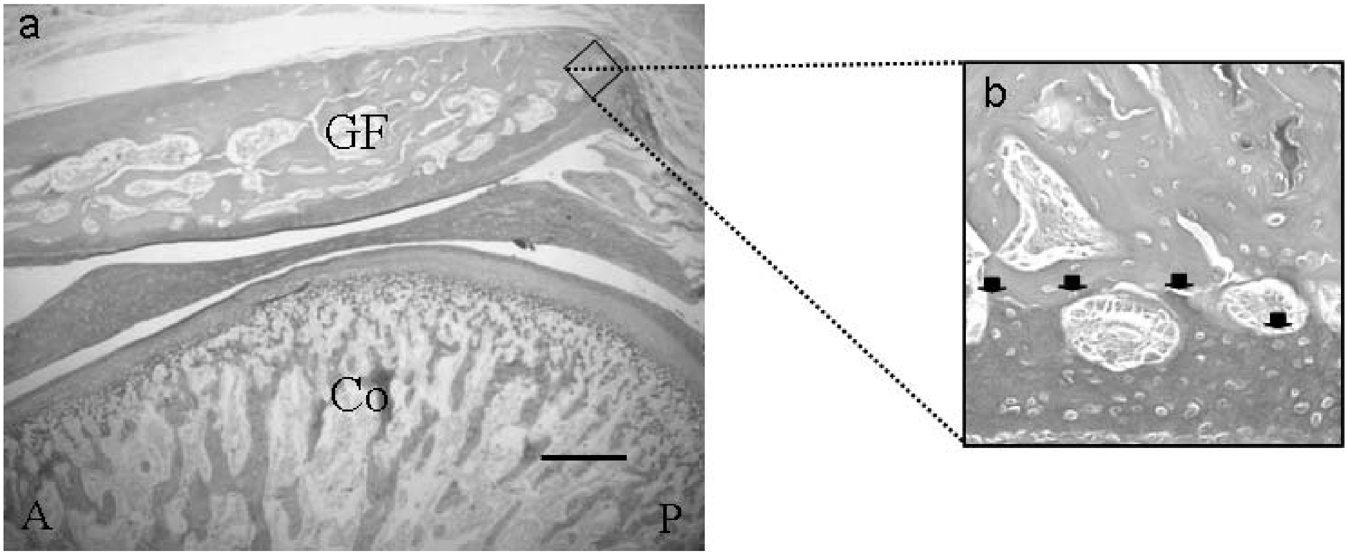

Figure 3. (a) The area of interest by an overview of glenoid fossa with PAS staining. (b) The area of interest at a magnification of ×200. Note the newly formed bone in magenta color and mature bone in weak pink. The arrow indicates the border line between newly formed bone and mature bone. GF indicates glenoid fossa; Co, condyle; A, anterior; P, posterior. Scale bar: 500 μm.

Table 2. Size of Method Error (Me) in Measurements

Measurement	Me
Linear measurement, mm	
A-B	0.02
A-CD	0.021
A-E	0.03
A-F	0.045
A-G	0.03
A′-B	0.036
A′-CD	0.027
A′-E	0.029
A′-F	0.05
A′-G	0.038
Area measurement, mm^2	
New bone formation in the posterior area of fossa	0.00575

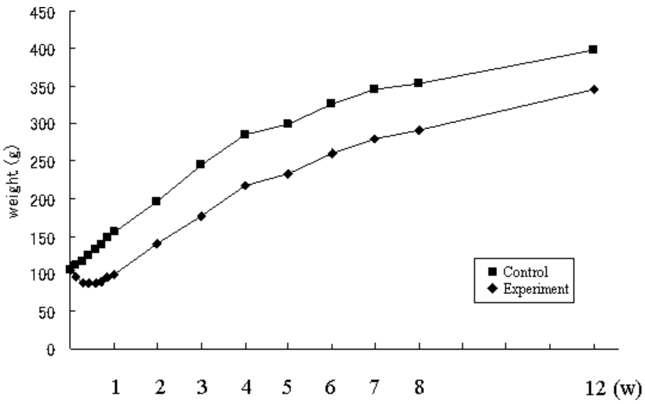

Figure 4. The change in body weight during the experiment.

0.01 mm under stereo microscopy (Nikon SMZ-U; Kanagawa, Japan).[9,18–20]

After the rat was killed, the TMJ was harvested, and serial paraffin sections at 5 μm were cut midsagittally. The sections were stained with Periodic Acid and Schiff's reagent (Sigma, St Louis, Mo) to identify newly formed bone.[15] The newly formed bone is a distinctive magenta color, while the mature bone is a weak pink with PAS staining as shown in Figure 3.

Pictures were obtained with a digital camera (Dxm 1200; Nikon, Kanagawa, Japan) at a magnification of ×100 and quantified with Image Plus-Pro (Media Cybernetics, Silver Spring, Md). To evaluate the new bone formation, a measuring frame of 200 × 200 μm was set under the proliferative zone in the posterior part of the fossa where the retrodiskal tissues attached. The software can recognize the distinction in staining density between new bone and mature bone, and the area of newly formed bone in the measurement frame was evaluated automatically[15] (Figure 3).

The average of the value on two sides was designated as the data for the control group. Analysis of covariance (ANCOVA) with body weight as covariate was used to control the influence of body weight on linear measurements. A paired *t*-test and ANCOVA was applied to examine the intragroup and intergroup differences for gross measurements, respectively.[21] Analysis of variance was used to evaluate the new bone formation. The data were processed with SPSS 12.0 for all statistical analyses. All the measurements were repeated for 10 randomly selected animals 1 month later by the same observer. Hypothesis testing indicated no significant difference between the two registrations. The method error was calculated with Dahlberg's formula,[22] Me $= \sqrt{\Sigma\, d^2/2n}$, where d rep-

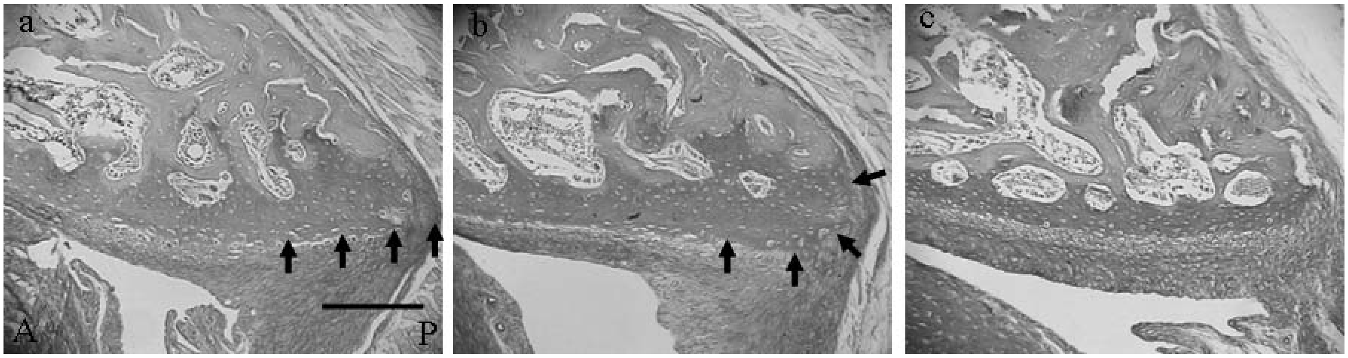

Figure 5. Rat's glenoid fossa at 2-week group with PAS staining (a, ipsilatral side; b, contralateral side; c, control). The arrow shows the border line between newly formed bone and mature bone. Note the different direction of new bone formation between panel a and b and the difference in amount of new bone formation between experimental and control group. A indicates anterior; P, posterior. Scale bar: 200 μm.

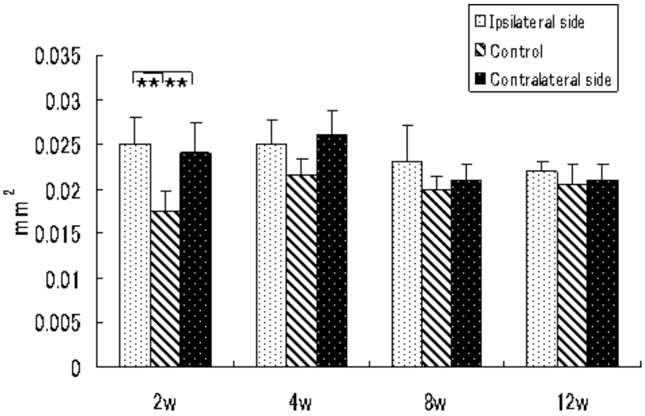

Figure 6. The change of the amount of newly formed bone in the posterior region of the glenoid fossa at each time point. *, $P < .05$; ** $P < .01$.

resents the difference between two registrations and *n* is the number of duplicate registrations. Table 2 lists the size of the method error.

RESULTS

Body Weight

A slight decline in weight gain (9%) was observed in the experimental group after the first week, after which the experimental rats began to gain weight similar to the control group (Figure 4).

Statistical Analysis

The correlation coefficients were high in all groups, ranging from .27 to .99. Data from the original measurements are presented in Tables 3 and 4, and the adjusted data are presented in Table 5.

Dry Skull Measurements

The measurements related to point A showed a significant difference between the two sides after 4 weeks (Table 3). A-B and A-CD that indicate the media-lateral position of point A was significantly larger on the ipsilateral side. A-E and A-F, which indicate the anterior-posterior position of point A, plus A-G, which indicates the superior-inferior position of point A, were significantly smaller on the ipsilateral side. A-A′, which indicates the size of the fossa, decreased significantly on the ipsilateral side. However, there were no significant differences for the measurements relative to point A′ until 8 weeks except for A′-CD (Table 4). The measurements related to A′ showed the same tendency as measurements relative to A. The fossa on the ipsilateral side was relocated to a relatively latero-superior-posterior position compared with the contralateral side.

Based on the adjusted data, A-B was significantly smaller on the contralateral side than for the control group at 12 weeks. A-CD tended to be larger on the ipsilateral side and became significant at 8 weeks. A-E, A-F, and A-A′ were significantly smaller on the ipsilateral side after 4 weeks. A-G tended to be smaller on the ipsilateral side with significance present at 12 weeks. The positional change of point A′ seemed to follow the same tendency (Table 5). The fossa on the ipsilateral side was repositioned relatively backward, outward, and upward, while the fossa (A′) on the contralateral side tended to be relocated relatively forward and downward compared with the control group.

New Bone Formation

In the control group, the amount of newly formed bone was significantly lower at 2 weeks than at other time points, and from 4 weeks on, it was nearly constant. At 2 weeks, the amount of new bone formation in the experimental group was significantly higher than in the control group, but there were no significant differences between the two sides in the experimental group. Although there was relatively more new bone formation in the experimental group after 4 weeks, no

Table 3. Original Values of the Measurements Relative to Point A in the Experimental Groups[a]

	2 wk		4 wk		8 wk		12 wk	
	I	C	I	C	I	C	I	C
Measurements relative to media-lateral relocation of glenoid fossa								
A-B	7.86 ± 0.36	7.8 ± 0.38	8.45 ± 0.26	7.94 ± 0.37**	9.23 ± 0.27	8.59 ± 0.24**	9.26 ± 0.2	8.74 ± 0.22**
A-CD	8.37 ± 0.27	8.18 ± 0.23	9 ± 0.2	8.19 ± 0.4**	9.24 ± 0.21	8.72 ± 0.38*	9.35 ± 0.29	8.83 ± 0.32*
Measurements relative to antero-posterior relocation of glenoid fossa								
A-E	14.87 ± 0.48	14.8 ± 0.39	15.5 ± 0.26	16 ± 0.4**	15.9 ± 0.36	16.44 ± 0.38**	16.75 ± 0.32	17.2 ± 0.32**
A-F	8.55 ± 0.21	8.6 ± 0.21	9.14 ± 0.4	9.8 ± 0.5**	9.34 ± 0.25	10.1 ± 0.38**	10.2 ± 0.47	10.8 ± 0.37**
Measurement relative to vertical relocation of glenoid fossa								
A-G	5.49 ± 0.23	5.45 ± 0.18	5.93 ± 0.27	6.26 ± 0.28**	6.25 ± 0.2	6.63 ± 0.29**	6.68 ± 0.16	7 ± 0.27**
Measurement relative to the size of glenoid fossa								
A-A′	4.22 ± 0.27	4.24 ± 0.22	4.27 ± 0.38	4.84 ± 0.25**	4.25 ± 0.35	4.83 ± 0.14**	4.66 ± 0.33	5.16 ± 0.36**

[a] I indicates ipsilateral side; C, contralateral side.

* $P < .05$; ** $P < .01$ (by paired *t*-test).

Table 4. Original Values of the Measurements Relative to Point A′ in the Experimental Groups[a]

	2 wk		4 wk		8 wk	
	I	C	I	C	I	C
Measurements relative to media-lateral relocation of glenoid fossa						
A′-B	10.5 ± 0.28	10.4 ± 0.29	11.4 ± 0.35	11.3 ± 0.26	12.3 ± 0.5	11.85 ± 0.43**
A′-CD	9.4 ± 0.23	9.2 ± 0.27	9.9 ± 0.13	9.5 ± 0.03*	10.36 ± 0.21	10.1 ± 0.23*
Measurements relative to antero-posterior relocation of glenoid fossa						
A′-E	12.2 ± 0.44	12.1 ± 0.38	12.87 ± 0.2	12.87 ± 0.13	13.4 ± 0.27	13.67 ± 0.2*
A′-F	4.46 ± 0.16	4.53 ± 0.16	4.97 ± 0.23	5.16 ± 0.22	5.03 ± 0.22	5.34 ± 0.26**
Measurement relative to vertical relocation of genoid fossa						
A′-G	4.64 ± 0.35	4.57 ± 0.39	5.2 ± 0.27	5.22 ± 0.24	5.55 ± 0.25	5.9 ± 0.14*

[a] I indicates ipsilateral side; C, contralateral side.

* $P < .05$; ** $P < .01$ (by paired *t*-test).

Table 5. Adjusted Values of Linear Measurements in the Experimental and Control Groups[a]

	2 wk			4 wk		
	I	Control	C	I	Control	C
Measurements relative to media-lateral relocation of glenoid fossa						
A-B	8.08 ± 0.11	8.08 ± 0.16	8.02 ± 0.11	8.58 ± 0.12	8.5 ± 0.15	8.07 ± 0.12
A′-B	10.7 ± 0.08	10.9 ± 0.1	10.63 ± 0.08	11.5 ± 0.12	11.8 ± 0.16	11.4 ± 0.12
A-CD	8.54 ± 0.15	8.4 ± 0.18	8.35 ± 0.15	9.01 ± 0.13	8.7 ± 0.18	8.22 ± 0.13
A′-CD	9.56 ± 0.1	9.5 ± 0.12	9.33 ± 0.1	9.97 ± 0.11	9.9 ± 0.14	9.5 ± 0.11
Measurements relative to antero-posterior relocation of glenoid fossa						
A-E	15.1 ± 0.14	15.1 ± 0.19	15.06 ± 0.14	15.5 ± 0.15**	16.43 ± 0.2	16 ± 0.15
A′-E	12.4 ± 0.13	12.4 ± 0.18	12.3 ± 0.13	13.12 ± 0.08	13.43 ± 0.1	13.2 ± 0.08
A-F	8.6 ± 0.08	8.9 ± 0.11	8.67 ± 0.08	9.14 ± 0.19*	10. ± 0.24	9.8 ± 0.9
A′-F	4.7 ± 0.15	5.2 ± 0.2	4.79 ± 0.15	5.22 ± 0.12	5.5 ± 0.16	5.35 ± 0.12
Measurement relative to vertical relocation of glenoid fossa						
A-G	5.55 ± 0.08	5.83 ± 0.11	5.51 ± 0.08	6.05 ± 0.1	6.27 ± 0.14	6.38 ± 0.1
A′-G	4.5 ± 0.09	4.8 ± 0.12	4.6 ± 0.09	5.09 ± 0.07	5.15 ± 0.1	5.28 ± 0.07
Measurement relative to the size of glenoid fossa						
A-A′	4.31 ± 0.1	4.3 ± 0.13	4.32 ± 0.1	4.21 ± 0.13**	5.1 ± 0.17	4.79 ± 0.13

[a] I indicates ipsilateral side; Control, control group; C, contralateral side.

* $P < .05$; ** $P < .01$ (by analysis of covariance).

significant difference can be found between the experimental and control groups (Figures 5 and 6). The new bone deposition on the ipsilateral side was in a superior-posterior direction leading to a pointed posterior extent of the fossa. On the contralateral side, however, the new bone tended to form in an inferior-anterior direction resulting in a rounded shape (Figure 5).

DISCUSSION

The model used here has been verified to be effective for inducing a mandibular shift in rats.[16,17] Since the upper incisors were used as anchorage to displace the mandible laterally, they inclined to the opposite side, and the premaxillary bone deformed. In this study, no morphometric point on the premaxilla was used.

Table 4. Extended

	12 wk	
	I	C
A′-B	12.8 ± 0.21	12.4 ± 0.28**
A′-CD	10.3 ± 0.3	10.2 ± 0.03
A′-E	13.76 ± 0.32	14.03 ± 0.34**
A′-F	5.36 ± 0.17	5.68 ± 0.2**
A′-G	5.96 ± 0.26	6.3 ± 0.29**

The results have shown that after 4 weeks, the fossa on the contralateral side was relocated to a relatively medio-anterior-inferior position compared with the ipsilateral side. The asymmetric position of the fossa on the two sides paralleled that of previous studies.[9,18] It has been reported that the fossa on the balancing side was repositioned forward and downward compared with the opposite side in rabbits with unilateral masticatory function.[9] Similarly, the fossa on the no-masticatory side relocated relatively forward and inward compared with the masticatory side in rats.[18]

The fossa on the ipsilateral side was relocated to a more latero-superior-posterior position compared with the control group. This is in agreement with a previous study in which the position of the fossa relative to the cranial floor on the cross-bite side in adults with unilateral posterior cross-bite was relatively posterior compared with the Class I group.[23] There is no obvious posterior wall in the fossa of the rat, and the articulating surface was nearly parallel to the occlusal plane and straight in a parasagittal direction.[24,25] Therefore, it was logical to assume that the lateral shift could result in a pronounced backward positioning of the condyle compared with humans.

This backward dislocation of the condyle would stretch the fibrous tissue connecting the fossa to the condyle in a posterior direction, leading to remodeling and repositioning of the fossa in that direction. This result was also supported by a histological study in which the posterior mandibular displacement enhanced the metabolic activity in the anterior part of the fossa and the synthesis of cartilage matrix in the posterior part of the fossa.[26]

Table 5. Extended

	8 wk			12-wk		
	I	Control	C	I	Control	C
A-B	9.3 ± 0.12	8.84 ± 0.18	8.6 ± 0.12	9.43 ± 0.08	9.42 ± 0.1	8.9 ± 0.08**
A′-B	12.45 ± 0.17	13 ± 0.24	11.8 ± 0.17**	12.8 ± 0.1	13.1 ± 0.13	12.4 ± 0.1**
A-CD	9.52 ± 0.13**	8.7 ± 0.23	8.8 ± 0.13	9.4 ± 0.12	9.1 ± 0.17	8.9 ± 0.12
A′-CD	10.6 ± 0.15**	10.1 ± 0.21	10.1 ± 0.15	10.39 ± 0.14	10.3 ± 0.19	10.2 ± 0.14
A-E	16 ± 0.17**	17.1 ± 0.24	16.5 ± 0.17	16.7 ± 0.09**	17.7 ± 0.11	17.3 ± 0.09
A′-E	13.48 ± 0.1	13.6 ± 0.14	13.7 ± 0.1	13.9 ± 0.13	14 ± 0.16	14.18 ± 0.13
A-F	9.54 ± 0.19**	11 ± 0.27	10.5 ± 0.19	10 ± 0.07**	10.9 ± 0.09	10.7 ± 0.07
A′-F	5.68 ± 0.05**	6 ± 0.07	6.1 ± 0.05	5.9 ± 0.11*	6.43 ± 0.14	6.6 ± 0.11
A-G	6.26 ± 0.1	6.53 ± 0.14	6.6 ± 0.1	6.69 ± 0.06*	6.99 ± 0.08	7.1 ± 0.06
A′-G	5.01 ± 0.11	5.4 ± 0.16	5.63 ± 0.11	5.5 ± 0.05**	5.8 ± 0.06	6 ± 0.05
A-A′	4.2 ± 0.11**	5.29 ± 0.14	4.93 ± 0.11	4.46 ± 0.11**	5.41 ± 0.14	5 ± 0.11

On the other hand, the most posterior edge of the fossa on the contralateral side tended to relocate relatively forward and downward compared with the control group. It has been suggested that in the adult rat, the sliding movement of the condyle in a rostrocaudal direction was possible over a distance of 6 mm and the range of condylar movement during mastication roughly comprised the caudal two-thirds of the fossa.[25] Therefore, 2 mm of lateral displacement would not have much effect on the anterior edge of the fossa on the contralateral side. Histological studies have shown that the fossa underwent extensive remodeling, which could contribute to the forward relocation of the fossa after a period of mandibular protrusion in Macaca monkeys.[14] In addition, clinical investigations showed similar remodeling and relocation in the fossa in humans during Herbst treatment.[5,11]

The fossa size on the ipsilateral side was significantly smaller than on the contralateral side and in the control group. This finding was in accordance with a clinical study in which the glenoid fossa width on the shifted side was significantly smaller than the unshifted side in asymmetrical Class III patients.[27] The asymmetry in the size of the fossa should be due to more prominent backward movement of the A point than the A′ point on the ipsilateral side.

New bone formation in the posterior part of the fossa was significantly higher in the experimental group than in the control group at 2 weeks. This is in line with previous studies, in which an increment of new bone can be caused by mandibular advancement,[13,14] whereas the increased chondrogenesis can also be induced by backward mandibular displacement in the posterior part of the fossa.[26]

It has been suggested that the orientation of mesenchymal cells as well as the deposition of new bone in the posterior part of the fossa seemed to correspond to the direction of the pull by retrodiskal fibers after 7 days of mandibular forward positioning.[15] Because the rats lack a posterior wall of the fossa, either the forward or backward condylar movement will stretch the retrodiskal fibers anteriorly or posteriorly. Therefore, increased new bone deposition by recruitment of the mesenchymal cells should follow the direction of force. Since the area of interest for histological study was set at the region where the fibrous tissue inserted into the fossa and A′ point was determined, directionally increased new bone formation in this area may account for the positional asymmetry of the fossa in the experimental group.

It has been well documented that functional lateral displacement can lead to asymmetry in condylar position and mandibular bone, and therefore, early treatment is recommended to intercept the development of skeletal discrepancies.[28] The findings of this study, that pronounced fossa remodeling and relocation happened after 2 and 4 weeks, suggest that the interceptive treatment has a double meaning, that is, to correct the abnormal growth in both the condyle and fossa. Since the early treatment of a functional lateral displacement can restore the normal positional relationship between condyle and fossa, elimination of the etiological factor of skeletal asymmetry in TMJ is highly recommended.

CONCLUSIONS

- The glenoid fossa on the ipsilateral side was repositioned relatively backward, upward, and outward compared with the contralateral side and control group. Conversely, the fossa (A′) on the contralateral side moved forward and downward compared with the control group.
- The size of the glenoid fossa tended to be smaller on the ipsilateral side than that in the contralateral side and control group.
- The new bone formation in the posterior part of the glenoid fossa was higher on two sides in the experimental group than in the control group after 2 weeks of lateral shift.
- Extensive remodeling of the glenoid fossa in the early stages of the experiment indicates the importance of interceptive treatment.

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