Do masticatory functional changes influence the mandibular morphology in adult rats

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1. Introduction

Adults with serious maxillofacial discrepancies often have a reduced capacity of their masticatory system. In these cases, orthognathic surgery has been shown to improve masticatory muscle strength and chewing ability. 1, 2, 3 In line with these observations it has been shown that in edentulous adults who receive implant retained bridges there is an improvement of both masticatory muscle strength and chewing ability. 4

Animal experimental studies on growing rats have shown that changes of the masticatory muscle function induced by a soft diet lead to reduced masticatory muscle strength, 5 and this in turn results in changes of the mandibular morphology 6, 7 and the alveolar bone microarchitecture. 8, 9 Similar functional changes of the masticatory muscles lead to alteration in the
condyle of mature rats.\textsuperscript{10} Bone remodelling seems to be influenced also in adult rats after an increased muscle strength and loading of long bones.\textsuperscript{11}

Craniofacial morphology of adults changes slowly through constant remodelling.\textsuperscript{12} It has been proposed that the masseter thickness and the number of occluding mandibular teeth in the lateral segments are significant determinants of mandibular alveolar bone mass and thickness.\textsuperscript{13} Thus, adults with an improved masticatory muscle function after orthognathic surgery or after receiving implant-retained bridges may have different functional conditions that could induce significant remodelling changes of the lower facial skeleton. In order to test this hypothesis a pre-clinical experimental study was performed in order to investigate the effect of masticatory functional changes on the lateral view morphology of the mandible in adult rats.

2. Materials and methods

2.1. Materials

Sixty male rats of the Sprague–Dawley strain (average age 21 days) from Charles River, Uppsala, Sweden were used. The experimental protocol obtained approval by the Ethics Committee of University of Gothenburg.

2.2. Experimental design

Sixty 21-day-old male Sprague–Dawley rats were randomly divided into two groups. Sixteen received ordinary (hard) food during the whole experimental period (normal group). The remaining 44 received a soft diet to develop a hypofunctional masticatory system.\textsuperscript{14} The soft diet was made of ordinary (hard) food (R34, Lactamin, Södertälje, Sweden) mixed with water in standardised proportions (2:5, R34:water). After a preparatory period of 21 weeks on a soft diet these animals, now adults, were divided into two equal groups (matched for weight): one continued on soft diet (hypo-functional group), and the other changed to an ordinary (hard) diet with the aim of functionally rehabilitating their masticatory system (rehabilitation group) during a period of 6 weeks. The total experimental period was 27 weeks. During the whole experimental period the rats were weighed every second week and were fed and watered ad libitum. At the end of the experimental period the animals were sacrificed in a CO\textsubscript{2} chamber.

The mandibles of the rats were dissected, defleshed, and separated at the symphysis. Each left mandible was fixed in ethanol (99%). The rat mandible has a particular geometry, which makes it possible to lie flat on its lingual side in a stable and reproducible position. Moreover, due to its limited thickness, it can be easily transilluminated making visible some of its internal structures, such as the position of the mandibular foramen and the course of the mandibular canal (Fig. 1). In this study a morphometric analysis of the mandibular lateral shape was used, based on the method described and validated in a previous publication.\textsuperscript{5} To perform the morphometric analysis, each left mandible was placed lying on its lingual side on top of a custom-made light source and was photographed using a digital camera fixed on a steady mount (Coolpix 4500; Nikon, Tokyo, Japan) with standardised configuration settings (aperture f/5.1, shutter speed 1/30 s, and magnification of 3).

The images acquired were subsequently transferred to a computer for interpretation using customised cephalometric software (Viewbox 3.01; D. Halazonetis, Athens, Greece). This software permits the use of preprogrammed custom shapes (called “snakes” or line contours) that can be aligned with anatomical reference points and bone outlines. This procedure led to the development of a digital anatomical map where 164 anatomical and 6 derived reference points were available for measurements and calculation (Fig. 2). The line defined by the mental and the mandibular foramina was used as a reference line, since it is considered to be minimally affected by growth.\textsuperscript{15,16}

Combining the digital tracings, it was possible to construct three mean tracings, one for each group, which were subsequently superimposed on the same reference line (Fig. 3).

2.3. Statistical analysis

All data are represented as mean and standard deviation (mean ± S.D.). To investigate the morphological differences between the three experimental groups, analysis of variance (ANOVA) statistical procedures were performed. Even if there was no difference in body weight between the groups (one-way analysis of variance), it was decided to use body weight as a covariate in order to avoid any possibility of bias due to differences in body size. All statistical analyses were performed using the SPSS statistical package (SPSS 13; SPSS, Chicago, IL, USA). A result was considered statistically significant at \( p < 0.05 \).

2.4. Error of the method

The whole measurement procedure was repeated in 25 of the mandibles 2 weeks after the initial measurements. The error of the method was calculated according to the...
formula: \( Se = \sqrt{\frac{\sum d^2}{2n}} \), where \( \sum d^2 \) is the sum of the squared differences between pairs of recordings and \( n \) is the number of duplicate measurements.\(^{17}\) It varied between 0.19 and 1.49 mm\(^2\) for area, between 0.33\(^{8}\) and 0.72\(^{8}\) for angular, and between 0.04 and 0.18 mm for linear measurements. No systematic error was detected between the two sets of measurements tested by using the paired t-test. The coefficient of variation was found to be below 3.15\(^{\%}\) for all measurements with the exception of “condylar process inclination” where it was 11.55\(^{\%}\).

3. Results

No statistically significant difference in body weight was found at week 21 (when the hypofunctional group was split into two groups) between the rats fed a soft diet (567 ± 73 g) and the rats fed a hard diet (573 ± 64 g), nor between the three groups at the end of the experimental period (week 27): the mean body weight (±S.D.) for the hypofunctional group was 605 g (±87 g), for the rehabilitation group was 599 g (±83 g) and for normal group was 631 g (±81 g).

3.1. Morphometric analysis (Table 1)

The total area of the lateral surface of the mandible was statistically smaller in the hypofunctional group than in the normal group. In the hypofunctional group the condylar process was found to be less steep, the posterior height of the corpus and the height of the angular process were smaller, and the molar alveolar anterior and posterior heights were bigger.

The inclination of the condylar base was less acute in the rehabilitation group in comparison to the hypofunctional group. No other significant differences were found between these two groups.

The comparison between the rehabilitation and the normal groups revealed similar statistically significant differences as between the hypofunctional and the normal groups. However no statistically significant differences were found in the total area and posterior height of the corpus between the rehabilitation group and the normally functioning animals. The mandibular line angle was more acute in the rehabilitation group.

4. Discussion

In the present study we found that a period of 7 months with low masticatory demands in the hypofunctional group during adolescence and early adulthood had a significant effect on the lateral shape of the rat mandible as compared to

Fig. 2 – The variables under investigation. The solid black circles correspond to the mental and mandibular foramina which define the reference line. 1: the area of the external surface of the mandible, including the condylar process, excluding the teeth; 2: the area of the condylar process, delimited by the condylar tangent (tangent to both upper and lower mandibular notches); 3: the angle formed by the perpendicular from the most posterior and superior part of the condyle to the condylar tangent and the reference line; 4: the angle formed by the axis of the incisor alveolar process and the reference line. (Incisor alveolar axis: a line from the midpoint between the upper and lower aspect of the incisor alveolar crest to the midpoint between the deepest part of the incisor alveolar curvature and the edge of the digastric fossa); 5: the angle formed by the lower border of the mandible and the reference line; 6: the angle formed by the occlusal plane and the reference line; 7: the angle formed by the condylar base and the reference line; 8: the maximal distance of the condyle from the condylar tangent; 9: the maximal distance between the mental foramen and the condyle; 10: the maximal distance between the mental foramen and the coronoid process; 11: the maximal distance between the mental foramen and the angular process; 12: mandibular posterior width (tangent to the upper and lower mandibular notches); 13: the maximal vertical distance of the coronoid process from the reference line; 14: the maximal vertical distance of the angular process from the reference plane; 15: the distance between the reference line and the deepest point of the alveolar crest of the third molar; 16: the distance between the reference line and the mesial cusp of the third molar; 17: the distance between the reference line and the middle cusp of the first molar. (For interpretation of the references to color in the artwork, the reader is referred to the web version of the article.)
animals with normal masticatory function. At the end of the experiment, those animals fed a soft diet were found to have a smaller mandible with some significant shape adaptations, and a higher dentoalveolar process. The animals that changed their diet from soft to hard for the final 6 weeks of the experiment in the rehabilitation group were found to differ only marginally from the animals that continued with a soft diet.

Table 1 – Mean values and standard deviation (in parentheses) of the variables under study for each of the reference and experimental groups

<table>
<thead>
<tr>
<th>Variables</th>
<th>Normal</th>
<th>Rehabilitation</th>
<th>Hypofunctional</th>
<th>Anova</th>
<th>Significance, p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area measurements (mm²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Total area</td>
<td>249.08** (9.67)</td>
<td>238.86 (15.71)</td>
<td>236.51** (12.00)</td>
<td>0.013</td>
<td></td>
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<tr>
<td>2. Condylar process area</td>
<td>25.82 (1.79)</td>
<td>25.59 (1.97)</td>
<td>25.57 (1.67)</td>
<td>0.901</td>
<td></td>
</tr>
<tr>
<td>Angular measurements (°)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>3. Condylar process inclination</td>
<td>20.66**, * (4.04)</td>
<td>11.36** (3.56)</td>
<td>12.32 ** (3.61)</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>4. Incisor alveolar process inclination</td>
<td>65.86 (2.44)</td>
<td>66.13 (2.29)</td>
<td>67.41 (2.10)</td>
<td>0.078</td>
<td></td>
</tr>
<tr>
<td>5. Mandibular plane inclination</td>
<td>16.54** (1.27)</td>
<td>15.45** (1.33)</td>
<td>15.63 (1.40)</td>
<td>0.041</td>
<td></td>
</tr>
<tr>
<td>6. Occlusal plane inclination</td>
<td>25.08 (2.09)</td>
<td>24.97 (2.27)</td>
<td>25.70 (1.90)</td>
<td>0.473</td>
<td></td>
</tr>
<tr>
<td>7. Condylar base inclination</td>
<td>73.75** (1.70)</td>
<td>72.58 ** (2.54)</td>
<td>70.82** *, * (2.62)</td>
<td>0.001</td>
<td></td>
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<tr>
<td>Linear measurements (mm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>8. Condylar process length</td>
<td>5.99 (0.33)</td>
<td>6.12 (0.30)</td>
<td>6.20 (0.20)</td>
<td>0.081</td>
<td></td>
</tr>
<tr>
<td>9. Mental foramen—condyle process</td>
<td>23.24 (0.41)</td>
<td>23.19 (0.67)</td>
<td>23.23 (0.64)</td>
<td>0.959</td>
<td></td>
</tr>
<tr>
<td>10. Mental foramen—coronoid process</td>
<td>19.29 (0.47)</td>
<td>19.11 (0.71)</td>
<td>19.03 (0.79)</td>
<td>0.553</td>
<td></td>
</tr>
<tr>
<td>11. Mental foramen—angular process</td>
<td>23.11 (0.46)</td>
<td>22.91 (0.71)</td>
<td>22.85 (0.71)</td>
<td>0.484</td>
<td></td>
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<tr>
<td>12. Posterior height of the corpus</td>
<td>16.43** (0.71)</td>
<td>16.17 (0.64)</td>
<td>15.85** (0.69)</td>
<td>0.019</td>
<td></td>
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<tr>
<td>13. Height of coronoid process</td>
<td>6.75 (0.45)</td>
<td>6.83 (0.34)</td>
<td>6.73 (0.46)</td>
<td>0.730</td>
<td></td>
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<tr>
<td>14. Height of angular process</td>
<td>9.45**, * (0.34)</td>
<td>9.05** (0.53)</td>
<td>8.85 * (0.47)</td>
<td>0.001</td>
<td></td>
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<tr>
<td>15. Molar alveolar posterior height</td>
<td>2.39**, * (0.19)</td>
<td>2.64** (0.18)</td>
<td>2.74** (0.22)</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>16. Molar alveolar anterior height</td>
<td>3.04**, * (0.23)</td>
<td>3.34** (0.19)</td>
<td>3.36** (0.19)</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>17. Occlusal posterior height</td>
<td>2.72**, * (0.19)</td>
<td>2.93** (0.18)</td>
<td>2.93** (0.23)</td>
<td>0.003</td>
<td></td>
</tr>
<tr>
<td>18. Occlusal anterior height</td>
<td>4.07**, * (0.17)</td>
<td>4.30** (0.19)</td>
<td>4.35** (0.19)</td>
<td>0.001</td>
<td></td>
</tr>
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</table>

Analysis of variance (ANOVA) was applied in order to investigate the difference between the three experimental groups. (normal group; rehabilitation group; hypofunctional group). **,** Denote statistical significant pair-wise differences (post hoc comparisons).
apposition changes on the posterior corpus region may explain the larger condylar base inclination of the rehabilitation group compared with the hypofunctional group. It seems to be a geometrical effect due to increased bone apposition in the region of the upper mandibular notch distal to the coronoid process. This is possibly a local response of the bone in order to adjust to the increased contracting forces of the temporal muscle.

The change of diet from soft to hard in order to rehabilitate the masticatory system hypofunction took place during a period where no significant growth changes occur in the rat. Therefore it is not surprising that no substantial difference was found between the rehabilitation and the hypofunctional groups. Nevertheless, it is possible that more obvious differences may have been detected between these two groups if the rehabilitation period was longer than 6 weeks. The capacity of the bone to respond to functional alterations even at adult age in humans has previously been shown. Adults who start with racquet sports were shown to develop a thicker cortical wall of the humeral shaft after long-term loading.18

In this study the hypofunctional group showed substantial morphological differences compared to the normal group. In the hypofunctional group a less steep condylar process and a vertically reduced angular process was seen when compared with the normal group, which is in line with previous studies on growing rats.8,16 Furthermore, we found that the dentoalveolar process was higher in the hypofunctional group when compared to the normal group. A plausible explanation is that the reduced mechanical loading on the molars of those animals eating a soft diet allows these teeth to further erupt and the dentoalveolar process to follow their eruption. This is in line with previous findings6 on growing rats, although the differences are more pronounced in the present study, probably due to the longer experimental period. These findings on animal experimental model are similar to those of clinical studies on patients with myotonic dystrophy.19,20 In these patients, masticatory muscle hypofunction caused by the disease was associated with a vertical growth pattern and an anterior open bite due to significant posterior tooth eruption.

In this study we used the method for morphometric analysis of the mandible as described by Mavropoulos et al.6 It has been shown that photographic analysis of the rat mandible, despite small methodological errors, is more reliable than radiographic analysis.21 This is probably due to problems on lateral cephalograms with landmark identification and projection errors. On the other hand, photographic analysis can only provide cross-sectional data and cannot elucidate longitudinal growth changes on craniofacial morphology. We chose the line formed by the mental and the mandibular foramens as a reference line,15 since the endoskeleton of the rat mandible, including these two anatomical features, has been found to be the most stable structure during growth.23

In conclusion, morphometric analysis revealed only marginal changes in adult rat mandibular morphology during a 6-week period of masticatory function rehabilitation. However, the observed catch-up tendency might suggest that a longer rehabilitation period may have a more substantial effect on mandibular morphology.

References


