Influence of Diet Composition and Malocclusion on Masticatory Organs in Rats

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Abstract

The present study was undertaken to determine the effects of different dietary consistencies and malocclusion induced by extraction of molar teeth on the masticatory organs of weaning and adult rats, by determining the biochemical properties of masseter muscle, and also Ca and P levels in mandibular bone. Male SD rats, 3 and 20 weeks old, were divided into 3 groups. Group one (G-1) was maintained on a solid diet, and Groups two (G-2) and three (G-3) on a semi-solid diet. Furthermore, the mandibular molar teeth of G-3 rats were extracted. The experimental period was 120 days. The masseter muscle and mandibular bone weights of G-1 in weaning rats were increased significantly in comparison with G-3, but not in adult rats. The CPK activities in weaning and adult rats of G-1 were higher than those in the other two groups. The order of LDH activity in weaning and adult rats was G-3 > G-2 > G-1. G-2 and particularly G-3 showed significantly lower glycogen contents than G-1. The Ca and P contents of the mandibular bone in G-2 and G-3 were lower than those in G-1. These results suggest that a different dietary consistency and malocclusion induced by extraction of mandibular molar teeth have a considerable influence on the development of masticatory organs, mandibular bone and masseter muscle.

Introduction

In general, masticatory activity in mammals is considered important for transformation of the physiological properties of muscle fibers from the sucking to the weaning period. Considering that the weaning period of the rat lasts from 15 to 30 days after birth, which is called the suckling mastication period, it is suggested that the developmental changes in the masseter muscle may be related to the growing functional importance of the masseter muscle.

In a previous study in growing rats, Kiliaridis et al. and Engstrom et al. also found that changes in masticatory function, induced by low functional activity due to variation in the physical consistency of the diet, caused an alteration in the craniofacial growth pattern. Masticatory organs were smaller in rats maintained on a soft diet than in those on a solid diet. These results suggest that mastication of a hard diet during the weaning period may facilitate masticatory organ development to a greater extent than a soft diet in rats.

The present study was undertaken to determine the effects of different forms of diet or malocclusion induced by extraction of molar teeth on the masticatory organs in weaning and adult rats, by determining enzyme activities in the masseter muscle, and also the Ca and P contents of the mandibular bone.

Materials and Methods

Male Sprague-Dawley (SD) rats, 3 and 20 weeks old, weighing 38.3 ± 3.4 g and 470 ± 31.0 g, respectively, were divided equally into 3 groups of 5 rats each. Group one (G-1) was maintained on a commercial solid diet (MF, Oriental Yeast, Japan) and served as a control group. Group two (G-2) and Group three (G-3) were maintained on a semi-solid diet, made from powder containing the same components as the solid...
diet, mixed with the same proportion of water (50% water). G-3 was further treated by extraction of mandibular teeth at 6 d after the start of the experiment. Diets and distilled water were given ad libitum throughout the experimental period of 120 d.

Body weight was measured once every 5 d. The drinking water and diet intakes in each group were recorded periodically during the experimental period. After 120 d, the rats were sacrificed by decapitation. As examples of masticatory organs, the masseter muscle superficialis and mandibular bone were subjected to study. They were rapidly extracted, and the surrounding skin and connective tissue were removed.

The masseter muscle sample (approximately 100 mg) was homogenized using a glass homogenizer with 3 strokes every 10 s in 1 ml of ice-cold 1 M Tris-HCl buffer, pH 7.5. The homogenate was centrifuged at 5,000 x g for 10 min at 4°C, and the supernatant fractions were subjected to assays of creatine phosphokinase (CPK) and lactate dehydrogenase (LDH)[8]. CPK and LDH were assayed spectrophotometrically by the methods of Oliver[9] and Wroblewski et al.[10], respectively, with some modifications. Total glycogen content was measured using a method described previously by Good et al.[11]. The concentration of protein in the masseter muscle was determined by the method of Lowry et al.[12], using bovine serum albumin as a standard. The enzyme activities were expressed as per mg protein.

Mandibular bone was dried at 110°C for 48 h, and its Ca and P contents were measured by conventional methods[13,14]: atomic absorption spectrophotometry (Perkin-Elmer, model 503) for Ca, and a modification of the Fiske-Subbarow method for P[15].

All the results were expressed as mean ± SD. Statistical analysis of data was performed using Student’s t test at a significance level of p<0.05.

**Results**

The growth curves of the rats are shown in Fig.1. The body weights of weaning and adult rats increased continuously and the increments of weight were quite similar in all the groups. However, both weaning and adult rats in G-2 and G-3 showed a lower increase of body weight than those in G-1 during the experimental period. In particular, that in weaning rats of G-3 was significantly lower than in G-1 from 60 days after the start of the experiment.

The drinking water intake of weaning rats in each group at the end of the experiment was found to be approximately 1.5 times greater than that at the start. Water intakes of weaning and adult rats in G-3, i.e. the sum of drinking water and dietary water, were increased approximately 1.5-fold compared with G-1, but there was no significant difference between G-2 and G-3.

Dietary intake in each group increased markedly with increasing body weight throughout the experimental period. For example, dietary intake of weaning rats in G-1 increased from 8 to 25 g/d, and in adult rats from approximately 15 to 25 g/d. Dietary intakes in G-2 and G-3 were also increased, and in the weaning and adult rats, excluding dietary water, the values were 20±10 g/d and 25±5 g/d, respectively.

Masseter muscle weights are shown in Fig. 2. The masseter muscle weights of weaning and adult rats in G-2 and G-3 were lower than those in G-1, and that of weaning rats in G-3 was approximately 26% lower than that in G-1, adult rats showing values 11% lower. In addition, muscle weight of weaning rats in G-3 was significantly lower than in G-1, although in adult rats the difference was not significant.

CPK activities in masseter muscle are shown in Fig. 3. The CPK activities of weaning rats in G-1 were 873.2±73.8 and 1632.8±98.9 mU/mg protein, respectively, being higher than those in the other two groups. Weaning rats in G-2 and G-3 showed significantly lower values than in G-1, but adult rats did not. Both weaning and adult rats in G-3 showed values somewhat lower than in G-2. The order of CPK activity (mU/mg protein) in weaning and adult rats was G-1 > G-2 ≥ G-3.

LDH activities in masseter muscle are shown in Fig. 4. Weaning and adult rats in G-1 showed significantly (1.2-fold) lower LDH activities in masseter than those in G-3. The order of LDH activity in weaning and adult rats was G-3 > G-2 > G-1. The CPK and LDH activities in G-3 were lowest and highest, respectively, in comparison with those in the other two groups.

As shown in Fig. 5, the glycogen contents of the masseter muscle of weaning and adult rats in G-1 were...
highest; in particular, those in G-3 were significantly lower than in G-1.

Dry mandibular bone weights are shown in Fig. 6. G-2 and particularly G-3 showed significant decreases of mandibular bone dry weight for weaning rats compared to G-1. In weaning rats, the dry mandibular bone weight in G-3 was approximately 40% lower than that in G-1, and was approximately 23% lower than in G-2. In adult rats, dry mandibular bone weights showed a similar trend to those in weaning rats. The dry mandibular bone weights of both weaning and adult rats showed the following order: G-1 > G-2 > G-3.

Regression analysis between masseter muscle and mandibular bone weights of rats of each respective group, for both weaning and adult rats, gave regression coefficients of 0.837 and 0.797, respectively.

The dry mandibular bone contents, Ca and P contents per dry weight of mandibular bone and also the ratio of Ca/P are shown in Table 1. In both weaning and adult rats, Ca contents in G-2 and G-3 were lower than those in G-1, but there were no statistically significant differences among the 3 groups. The content of P was somewhat similar to that of Ca. Both Ca and P contents showed no significant difference between G-2 and G-3 for weaning and adult rats, respectively. In weaning rats of G-3, the ratio was significantly lower than in G-1, but this was not the case for adult rats.

Discussion

The present study has shown that changes in masticatory organs in growing rats induced by feeding different forms of diet or by experimental malocclusion cause alterations in the activities of CPK and LDH and the contents of glycogen in masseter muscle, and the Ca and P contents of mandibular bone.

In all three groups, body weight increased continuously with no significant difference during the experimental period, except in weaning rats of G-3. Dietary intake (excluding water in the semi-solid diet) of weaning and adult rats in each group was not significantly different among the groups. Although the food conversion ratio was also not significantly different among the groups, the difference in the form of diet and extraction of molar teeth influenced the body weight slightly.

Masseter muscle weights in both weaning and adult rats showed the order: G-1 (solid diet) > G-2 (semi-solid diet) > G-3 (semi-solid diet + tooth extraction). We found that the percentage increase in masseter muscle weight of weaning rats between rats fed the solid diet (G-1) and the other two groups (G-2, G-3) was much greater than that in adult rats. Similar studies by Kiliaridis et al. and Engstrom et al. demonstrated that changes in the masticatory muscles may be related to alterations in the craniofacial morphology of rats fed a soft diet. Thus, it appears that rats maintained on a semi-solid diet develop not only smaller mandibular bones but also smaller masticatory muscles with a lower chewing force than control rats on a solid diet. The influence of the texture of processed foods on parotid salivary secretion and mastication in man has recently been reported by Kowata et al.

CPK activity was slightly affected by the difference in diet form, being higher in the rats fed the solid diet (G-1) than in the other two groups. In terms of aging, CPK activity in adult rats was approximately double that in weaning rats. CPK activity in adult rats increased slowly with growth, but not in weaning rats. The effect of endurance training glycolytic enzymes has been reported by several authors. In skeletal muscles a significant decrease of LDH activity in trained rats was found in comparison with untrained controls. Benzi et al. also found a marked decrease of LDH activity in rat skeletal muscle following endurance training, in accord with Takekura et al. who found a 17% decrease of LDH activity in rat skeletal muscle after 12 weeks of mill-training. Similarly, our present study demonstrated that LDH activity of G-1 was lower than in the other two groups. This might be explained by the difference in mastication activity between G-1 and the other two groups. The present study confirmed that the increase in CPK activity was accompanied by an increase of masseter muscle development, although we found no difference in LDH activity between weaning and adult rats. Furthermore, lower LDH activity in masseter muscles was found in weaning and adult rats of G-1 fed on a solid diet. Muscle glycogen content in weaning and adult rats of G-1 was slightly higher than in the other two groups, that in G-3 being significantly lower than in G-1.

These results suggest that mastication of a solid diet (G-1) by weaning rats may facilitate the develop-
ment of the masseter muscle to a greater extent than mastication of a semi-solid diet with or without tooth extraction. The mandibular bone weight in weaning and adult rats showed the order: G-1 > G-2 > G-3. A significant correlation was found between masseter and mandibular bone weights with $r = 0.837$ (weaning) and 0.797 (adult). Thus, growth of the mandibular bone is accompanied by development of the masseter muscle. Alteration of dietary consistency or malocclusion induced by tooth extraction may have a greater influence on mandibular bone weight than on masseter muscle weight, this being particularly clear in G-3. The Ca and P contents of G-2 and G-3 were slightly lower than those in G-1 in weaning and adult rats, but showed no significant differences among the three groups. Rats fed the semi-solid diet (G-2) showed poorer development of masticatory organs than rats fed the solid diet (G-1), possibly because the increase in the degree of masticatory movement was accompanied by an increase in masticatory pressure and frequency, and enzyme activities in masseter muscle. In particular, G-3 showed poorer development of masticatory organs than the other two groups.

These results suggest that differences in dietary consistency and malocclusion induced by extraction of mandibular molar teeth have a great influence on the development of masticatory organs, as represented by the mandibular bone and masseter muscle. This phenomenon is more clearly evident in weaning than in adult rats.

References


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Fig. 1 Influence of diet on body weight in weaning and adult rats.
- G-1: Rats maintained on a solid diet
- G-2: Rats maintained on a mud diet
- G-3: Mandibular molar teeth extracted and rats maintained on a semi-solid diet

Fig. 2 Influence of diet on masseter muscle weight in weaning and adult rats
- *** Significant difference between G-1 and G-3 for weaning rats, p<0.001

Fig. 3 Influence of diet on CPK activity in weaning and adult rat masseter muscle
- * Significant difference between G-1 and G-2 for weaning rats, p<0.05
- ** Significant difference between G-1 and G-3 for weaning rats, p<0.01

Fig. 4 Influence of diet on LDH activity in weaning and adult rat masseter muscle
- * Significant difference between G-1 and G-3 for weaning rats, p<0.05
- ** Significant difference between G-1 and G-3 for adult rats, p<0.01
Fig. 5 Influence of diet on glycogen content in weaning and adult rat masseter muscle
** Significant differences between G-1 and G-3 for both weaning and adult rats, p<0.01

Fig. 6 Influence of diet on mandibular bone weight in weaning and adult rats
* Significant difference between G-1 and G-3 for adult rats, p<0.05
*** Significant difference between G-1 and G-2, G-1 and G-3 for weaning rats, p<0.001

Table 1 Ca and P contents of mandibular bone in weaning and adult rats

<table>
<thead>
<tr>
<th>Age</th>
<th>Group</th>
<th>Ca, %</th>
<th>P, %</th>
<th>Ca/P</th>
</tr>
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<tbody>
<tr>
<td>Weaning</td>
<td>Solid D.</td>
<td>23.39±0.57</td>
<td>11.69±0.36</td>
<td>2.00±0.03</td>
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<td>Semi-solid D.</td>
<td>23.63±0.17</td>
<td>11.76±0.14</td>
<td>2.01±0.02</td>
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<tr>
<td></td>
<td>Semi-solid D.+ Ext.</td>
<td>22.93±0.17</td>
<td>11.69±0.19</td>
<td>1.96±0.02*</td>
</tr>
<tr>
<td>Adult</td>
<td>Solid D.</td>
<td>26.92±1.09</td>
<td>13.06±0.28</td>
<td>2.06±0.10</td>
</tr>
<tr>
<td></td>
<td>Semi-solid D.</td>
<td>26.66±0.81</td>
<td>13.03±0.55</td>
<td>2.05±0.09</td>
</tr>
<tr>
<td></td>
<td>Semi-solid D.+ Ext.</td>
<td>25.06±1.18</td>
<td>13.06±0.35</td>
<td>1.92±0.13</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SD and are given as percentages of dry mandibular bone weight.
Semi-solid D. ± Ext.: Rat mandibular molars were extracted and the rats were given a semi-solid diet.
* Significant difference between Solid D. and Semi-solid D.+ Ext. in weaning rats, p<0.05.