Influence of aging on experimental gastrointestinal motility in extraction of rat molar teeth

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Abstract The purpose of the present study was to determine whether different consistency of diet and malocclusion induced by the extraction of molar teeth on the masticatory organs modulated gastric acid secretion, gastric emptying and intestinal transit in young and elder rats. Male Wistar rats (young, 5 weeks; elder, 1.5 years) were used in this experiment, and were divided into 2 groups. Group one (G1) was maintained with solid diet, Group two (G2) with mud diet. Further, the mandibular molar teeth of G2 were extracted. The experimental period was 10 weeks. The effect of aging and malocclusion on the parameters of gastric secretion was examined using pylorus-ligated rats. The gastric emptying rate (GER) and small intestinal transit rate was determined in rats by evans blue from the stomach and charcoal from the small intestinal, respectively. In pylorus-ligated rats, Young-G2 rat of gastric juice volume, acid output and pepsin secretion remarkably showed significant decrease in comparison with Young-G1 group, but there was not significant difference between Elder-G1 and Elder-G2. GER of Young-G2 rat group was 44.2 ± 7.9%, significantly lower than that of Young-G1 rat group (61.6 ± 8.8%, P<0.01), but Elder-G1 rat group were not significantly different than those of rats of Elder-G2 group. In small intestinal transit rates of charcoal meals, G1 and G2 of young were 73.3 ± 9.1 and 55.1 ± 8.6%, respectively, and Elder groups were 61.7 ± 9.8 and 52.6 ± 7.7%, suggesting an insignificant effect on diet. These results suggest that the diet and malocclusion, induced by extraction of mandibular molar teeth of young rat groups, may have a great influence compared to elder rat groups.

Key words
Elderly rats,
Extraction molar tooth,
Gastric emptying rate,
Pylorus-ligated rats,
Young rats

Introduction
Masticatory function is deteriorated by dietary consistency, aging and tooth loss. The decrease of masticatory function causes the disorder of dietary intake and daily activity1).

In the dietary consistency, it has been reported that neurogenesis in the adult hippocampus was restrained by aging and soft-diet feeding. In the hard-diet mice, the number of BrdU-positive cells in the dentate gyrus was fewer in 6 month old mice than 3 month old mice at any survival period investigated2). Mavropoulos3) have demonstrated that alteration of food consistency in young growing rats induced a lower mandibular alveolar bone, mineral density and decreased trabecular bone volume and thickness caused by a reduction of masticatory
functional and mechanical demands. In general, the masticatory activity is considered important for the transformation of physiological properties of muscle fibers in mammalian from the sucking to weaning periods\textsuperscript{4,5}). We previously demonstrated that the different consistency of diet and malocclusion, induced by extraction of mandibular molar teeth on the masticatory organ in young and elderly rats, may have a great influence on the development of masticatory organs, mandibular bone and masseter muscle. This phenomenon was confirmed more clearly in weaning rats than in adult rats\textsuperscript{6}).

The loss of tooth was reported to be a risk factor for occlusal masticatory function or malfunction may affect on the higher brain function. An impairment of spatial memory due to a decrease in acetylcholine level in the cerebral cortex was caused in 30 months old rats by tooth extraction \textsuperscript{7). Sensory information arising from periodontal ligament, muscle spindle and temporomandibular joint play important roles for modulating masticatory and occlusal functions\textsuperscript{8). Therefore, wide variety of sensory information would have been altered by the removal of molar tooth. These neuroplastic changes can be induced by peripheral or central nervous cholinergic system alterations, or can occur in association with sensory stimulation, as well as with chewing and gastrointestinal tract. Further, ageing is a complex biological mechanism affecting the entire body. It is well-known that aging causes many functional changes in the body. Several investigators have already reported changes in functions such as gastric secretion\textsuperscript{9), the gastric mucosal blood flow\textsuperscript{10), and mucosal cell proliferation\textsuperscript{11) in the stomach of aging rats. One effect of ageing is a decrease in apparent nutrient digestibility in the gastrointestinal tract\textsuperscript{12). This has been observed in many species including humans\textsuperscript{13). Rats\textsuperscript{14) Age variation is a further complication that must be addressed, since the relative of chewing and gastrointestinal tract is still unclear.

The purpose of the present study was to investigate whether gastrointestinal motility by pylorus-ligated rats was altered with different consistency of diet and the malocclusion induced by extraction of mandibular molar teeth in young and elder rats. The biochemical parameters of gastric acid, the gastric emptying rate and the small intestinal transit rate were examined in young and elder rats.

Materials and Methods

Animals

Wistar male rats (4 weeks old, 1.5 years old, weighing 38.3 ± 3.4 g and 420.0 ± 31.0 g, respectively, total of 60 rats) were purchased from Japan SLC (Shizuoka, Japan). The rats were allowed free access to food and water \textit{ad libitum} at all times and were maintained on a 12 h light/dark cycle (lights on 8:00 to 20:00) at 23 ± 1°C, humidity 60 ± 10% environment for a period of 1 week before use. All rats were maintained and used in accordance with the guidelines of the Care and Use of Laboratory Animals of Nihon University School of Dentistry at Matsudo.

Methods

Male Wistar rats were randomly divided into 2 groups of 5 rats each. Group one (Young-G1, Elder-G1) was maintained on commercial solid diet (MF, Oriental Yeast, Japan) and served as a control group, Group two (Young-G2, Elder-G2) was maintained on mud diet, commercial powder diet containing the same components as the solid diet, mixed with the same portion of water (50% water). G2 was further treated with extraction of mandibular teeth at 7 days after the initiation of experiment. Diets and distilled water were given \textit{ad libitum} throughout the experimental period of 10 weeks (Table 1). During the experimental period, the rats were observed daily. Body weights, food intake and water consumption were monitored weekly.

Gastric secretion in pylorus-ligated (Shay) rats

The effect of aging and malocclusion on the gastric
acid secretion was examined using pylorus-ligated rats. The pylorus-ligated model was carried out according to the method of Shay et al. 15), with a few modifications. All groups were fasted for 18 h, with free access to water before the experiments. Under light diethyl ether anesthesia, a small abdominal incision was made and the pylorus was ligated. Then the incision was closed and the animals were sacrificed 6 h after the pylorus ligation. The stomach was excised, opened along the greater curvature and the luminal contents were collected and centrifuged for 15 min at 1,500 × g to remove the residual debris. The amount of gastric acid and the pH values were determined. The total acid output was determined in the supernatant volume by titration to pH 7.0 with 0.05 N NaOH solution, and phenolphthalein as indicator 16). Acid output was calculated by multiplying the volume of gastric juice the acid concentration. Pepsin concentration was determined by modification of the colorimetric method described by Anson 17), involving digestion of 2% haemoglobin in 0.02 N HCl (pH 2.0, 37°C, 15 min) followed by alkaline condensation with Folin Ciocalteu’s reagent. Pepsin output was expressed as mg of pepsin in 6 h.

Gastric emptying rates of evans blue meals in rats

The gastric emptying rate was determined in rats by measuring the disappearance of Evans blue from the stomach according to a previous method 18,19), with a few modifications. 2.0 ml Evans blue, a semiliquid non-nutrient dye meal (5%, w/w) in 0.5% carboxymethyl cellulose was administered intragastrically with an orogastric canula. Thirty minutes after receiving the charcoal meal, rats were sacrificed and the small intestine was carefully removed. Small intestinal transit was measured from the pyloric sphincter to the ileocecal junction (A) and the distance traveled by the charcoal through the intestine (B) were measured. Small intestinal transit rate was calculated as the percentage of the distance traveled by the charcoal relative to the total length of the small intestine using the following formula:

\[
\text{Small intestinal transit rate } (\%) = \left(\frac{B}{A}\right) \times 100
\]

Statistical analysis

All results were expressed as means ± SD. Statistical significance was analyzed using one-way analysis of variance (ANOVA) with identification of differences between pairs using Dunnett’s test. Differences were considered to be significant if \( P < 0.05 \).

Results

Body weight and food intake

Body weight in Young-G2 was significantly \( (P<0.01) \) lower than that in the Young-G1 group during the experimental period, while no significant differences were observed between the Elder-G1 and Elder-G2 groups (Fig. 1). Body weight in Young-G1 and G2 were 222 ± 23, and 168 ± 21, respectively. Food intake in Young groups was found to be approximately 3.0-fold at the end of the experiment as compared with those at the initiation, but there was not significant difference between Young-G1 and Young-G2, except at first 1 week after extraction of mandibular teeth. Food intake in Young groups
remarkably increased in response to increasing body weight throughout the experimental period.

Food intake of Elder-G1 was nearly constant, 20–25 g/day throughout the experimental period. (Fig. 2). Water consumption of Young-G1 during the first 1–2 weeks, 3–7 weeks and 8–10 weeks of the experiment were 10±3, 22±8 and 25±13 ml/day, respectively. Water consumption of Elder-G1 was nearly constant 22–33 ml/day throughout the experimental period.

**Gastric secretion in pylorus-ligated (Shay) rats**

In an attempt to clarify the different consistency of diet and malocclusion induced by the extraction of molar teeth in young and elder rats, its influence on gastric secretion was studied by using pylorus-ligated rats. Young-G2 group increased in the gastric juice pH significantly (P<0.001) in comparison with Young-G1 group. There was a similar tendency observed in Elder groups. For example, gastric juice pH in Young-G1 and Young-G2 were 2.1±0.3, 3.2±0.4 (P<0.001), respectively. Young-G2 group of gastric juice volume and acid output remarkably decreased in the comparison with Young-G1 group. No significant difference was observed in the gastric juice volume (Table 2).

**Table 2** Effects of malocclusion young and elder on the biochemical parameters of gastric acid induced by pylorus ligation (shay) in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Diet</th>
<th>pH</th>
<th>Volume (ml/rat)</th>
<th>Acid output (µEq/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young</td>
<td>Solid</td>
<td>2.1±0.3</td>
<td>4.1±0.6</td>
<td>25.5±3.1</td>
</tr>
<tr>
<td></td>
<td>Mud + Ex.a</td>
<td>3.2±0.4***</td>
<td>2.7±0.4***</td>
<td>17.1±3.2***</td>
</tr>
<tr>
<td>Elder</td>
<td>Solid</td>
<td>2.8±0.5</td>
<td>6.7±1.3</td>
<td>31.7±4.3</td>
</tr>
<tr>
<td></td>
<td>Mud + Ex.a</td>
<td>3.8±0.6**</td>
<td>5.5±0.8</td>
<td>28.6±5.6</td>
</tr>
</tbody>
</table>

a): extraction of molar teeth.
The results are mean ± SD for 5 rats. Statistical comparison was performed using ANOVA followed by the Dunnett’s test.

**: P<0.01, ***: P<0.001 in comparison with the solid group.
Gastric juice pepsin secretion in Young-G1 and G2 were 9.3 ± 1%, and 7.1 ± 0.8% (P < 0.01), respectively. On the other hand, Elder-G1 and G2 were 10.5 ± 1.6, and 9.6 ± 1.9, respectively, but there was not significant difference between Elder-G1 and Elder-G2 (Fig. 3).

**Gastric emptying rates of Evans blue meals in rats**

The *in vivo* GER of Young-G1 and Young-G2 groups were 61.6 ± 8.8%, and 44.2 ± 7.9% (P < 0.01), respectively, significantly lower than that of Young-G1 group, but Elder-G1 group were not significantly different than those of Elder-G2 group (Fig. 4). GER (%) was decreased in both the Elder-G1 and Elder-G2 groups compared to Young-G1 group.

**Small intestinal transit rates of charcoal meals in rats**

The *in vivo* SIT of Young-G1 and Young-G2 groups were 73.3 ± 9.1%, and 55.1 ± 8.6% (P < 0.01), respectively, significantly lower than that of Young-G1 group, but Elder-G1 group (61.7 ± 9.8%) was not significantly different than that of Elder-G2 group (52.6 ± 7.7%). (Fig. 5) SIT (%) was decreased in both the Elder-G1 and Elder-G2 groups compared to Young-G1 group.

**Discussion**

We previously reported that mud diet had little or no influence on the masseter muscle weight in weaning and adult rats compared to that in solid diet⁶. Furthermore, we examined the difference in
the susceptibility to tooth attrition between solid diet and power diet. Power diet showed a higher level of attrition compared with solid diet, indicating that power diet has an influence on masticatory movement\(^{21}\). In this study, we evaluated the degree of the experimental gastrointestinal motility in extraction of molar tooth with young and elder pylorus-ligated rats.

**Body weight and food intake**

In pylorus-ligated rats, body weight in Young-G2 was significantly lower than that in the Young-G1 group during the experimental period, while no significant differences between Elder-G1 and Elder-G2 (Fig. 1). The initial loss of body weight correlates with a reduced food intake during the experimental period. We already reported that malocclusion induced by extraction of mandibular molar teeth in weaning and adult showed lower increase of body weight than solid diet group of weaning and Elder during experimental period of 60 days. Particularly extraction of teeth in weaning rats was significantly lower than solid diet group from 60 days after the initiation of experiment\(^6\).

**Gastric secretion in the ligature pylorus model rats**

In this work, Young-G2 of gastric juice volume and acid output remarkably showed significant decrease in comparison with Young-G1 group, but there was not significant difference between Elder-G1 and Elder-G2. The gastric juice volume in Young-G1, G2 groups was lower than that in Elder-G1, G2 groups, respectively. Tsukimi et al.\(^{22}\) observed that histamine-stimulated the ligature pylorus rats of gastric acid secretions in aging rats was significantly lower than that in young rats. This discrepancy may be due to differences in the experimental conditions. In human, Gastric acid and pepsin output rates were similar in young (age range, 18–34 years) and middle-aged (age range, 35–64 years) groups. Gastric juice volume in middle-aged raised significantly in comparison with young, but was reduced approximately 30% in the elderly (age range, 65–98 years)\(^{23}\). In the present study, Young-G2 and Elder-G2 groups of teeth extract decreased the gastric juice volume, total acid output and pepsin secretion induced by pylorus-ligated rats. This may be due to the possible decrease in chewing stimulation caused in the Young-G2 and Elder-G2 rats with molar tooth extraction.

**Gastric emptying rates of evans blue meals in the ligature pylorus model rats**

The GER of Young-G1 and Young-G2 rat groups were 61.6±8.8%, 44.2±7.9% \((P<0.01)\), respectively, significantly lower than that of Young-G1 rats. GER \(\%\) was decreased in both the Elder-G1, G2 rats compared to Young-G1. The GER was decreased in old rats compared to young rats, adult rats was intermediate\(^{24}\). Furthermore, No differences were observed in the gastric emptying rates between young and senior cats\(^{25}\). This corroborates results from studies in both humans\(^{26}\) and rats\(^{27}\) where ageing was reported to have no effect on the rate of gastric emptying.

**Small intestinal transit rates of charcoal meals in the ligature pylorus model rats**

It is reported that the elderly have more frequent bouts of constipation\(^{28}\). Studies investigating the effect of ageing on GI transit times in humans indicated that older subjects had a slower mean colonic time compared to younger subjects\(^{29}\). These findings have also been observed in rats, with results showing a decrease in stool mass and slower colonic transit times with an increase in age.

In the present study, in small intestinal transit rates of charcoal meals, G1 and G2 of young were 73.3±9 and 55.1±8%, respectively, and Elder groups were 61.7±9 and 52.6±7%, suggesting an insignificant effect on diet. The different consistency of diet and malocclusion showed a 1.5 times stronger inhibitory than Young-G1 against gastric acid secretion. Holt\(^{30}\) have compared the effect of fasting and refeeding on small and large intestinal proliferative activity in young (3–4-month old) and aged (24–28-months old) Fischer-344 rats. In the mucosa of both small and large intestine, fasting resulted in 40–60% reduction in proliferative activity in young rats, whereas in old rats it was decreased by only 10%, when compared with their corresponding fed controls. In normal, vehicle-treated rats, small intestinal transit rates of charcoal meals was about 65% of the length of the small intestine\(^{31,32}\).

In conclusion, these results suggest that different consistency of diet and the malocclusion induced by extraction of mandibular molar teeth may have a great influence on the biochemical parameters of gastric acid, the gastric emptying rate and the small intestinal transit rate by pylorus-ligated rats.
This phenomenon was confirmed more clearly in weaning rats than in adult rats.

References
