Analysis of Histochemically and Morphometrically 
in the Anterior Belly Digastric Muscle of 
Osteopetrotic (op/op) Mice

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Abstract: The fibers of the anterior belly digastric muscle of mice, fed a granulated diet for various periods, have been studied histochemically and morphometrically. The diameters of the anterior belly digastric fibers in normal mice fed only a granulated diet were smaller than those in mice fed a solid diet. Differences in the succinate dehydrogenase (SDH) activity of muscle fibers between op/op and normal mice gradually appeared in the anterior belly digastric muscle and, by the age of 90 days, under-development of muscle fibers was observed in the mid-belly region of the anterior belly digastric muscle of op/op mice fed a granulated diet. These results indicate mechanical stress in mastication plays an important role in the development of the anterior belly digastric muscle structures.

Key words: anterior belly digastric muscle, op/op mouse
ported that the masseter muscle of the op/op mice was significantly underdeveloped [7]. In this study, we examined the development in the anterior belly digastric muscle of op/op mice and compared them with normal mice fed solid or granulated diets.

### Materials and Methods

Osteopetrotic (op/op) mice and control littermate mice (normal) were obtained from B6C3-a/a-op/+ breeding pairs (Jackson Laboratory, Bar Harbor, ME, USA.). Newborn male mice were kept with their mothers, and op/op mice were distinguished from normal mice by monitoring eruption of the incisors 11 days after birth. The study was approved by the Animal Use Committee of Hiroshima University, and the animals were maintained in accordance with the guidelines for the care and use of laboratory animals at Hiroshima University.

The op/op mice were fed a granulated diet, and normal (+/+ or op/+) mice were fed a solid or granulated diet (CE2; Clea Japan, Tokyo, Japan).

Three mice, one from each group, were sacrificed following anesthetization via an intraperitoneal injection of sodium pentobarbital (0.06 mg/g body weight), at the ages of 15, 30 and 90 days old and their anterior belly digastric muscles were removed. The specimens from each group of mice were cut into transverse serial sections of 10 µm thickness on a cryostat at −20°C and rapidly air-dried. The reaction mixture solution was prepared by combining equal volumes of phosphate buffer (0.2 M) pH 7.6 and sodium succinate (0.2 M). When an incubation solution was needed, 10 ml of the buffered substrate reagent were added to 10 ml of an aqueous solution containing 10 mg of NitroRB-T. The sections were stained for succinate dehydrogenase (SDH) and with hematoxylin. These sections were examined the SDH activity. Histochemical staining for SDH was performed by the following procedure: air-dried sections were incubated for 20 min in the reaction mixture described by Nachlas et al. [16], and fixed in 10% formaldehyd. Then the sections were washed with distilled water, stained with hematoxylin and mounted with glycerin. All treated sections were examined under a light microscope. The diameter of each muscle fiber was measured by the methods of Brooke and Engel [1] and Wakisaka et al. [26]. As shown in Fig. 1b, measurements of the muscle fibers were carried out in all muscle fibers of each hematoxylin-stained section in the mid-belly region of the anterior belly digastric muscle. One-way ANOVA was carried out, followed by Scheffé tests.

### Results

#### Body weight

The body weight of the normal mice fed a solid diet, normal mice fed a granulated diet and op/op mice exhibited an increase from 15 to 90 days old. The body weight of op/op mice fed a granulated diet was lighter.
than the other two groups, but was not statistically different from the normal mice fed solid or granulated diets (Table 1).

Histologic findings

Development in the SDH activity of the anterior belly digastric muscle in op/op and normal mice are shown in Fig. 2.

In 15-day-old mice, no distinct differences were detectable in the SDH activity between the normal and op/op groups (Fig. 2a, b and c).

At 30 days old, retarded development of muscle fibers was clearly observed in the anterior belly digastric muscles of the op/op mice, (Fig. 2d, e and f). Furthermore, intermediate and red muscle fibers were more frequently observed in normal mice fed a solid diet than in normal mice fed a granulated diet (Fig. 2d and e). At 90 days old, muscle fibers of op/op mice has developed better than those of 30-day-old op/op mice (Fig. 2f and i). Development of almost all of the muscle fibers were retarded in the anterior belly digastric muscle of the op/op mice when compared with those of normal groups after 30 days of age. Especially, well developed red muscle fibers were not observed (Fig. 2i).

In the normal mice fed a granulated diet, retarded muscle fibers were observed in the anterior belly digastric muscle, as compared with those in the normal mice fed a solid diet were 90 days of age (Fig. 2g and h).

The SDH activities in the intermediate and white muscle fibers of normal mice fed a granulated diet was relatively lower than those of normal mice fed a solid diet (Fig. 2g and h).

Although the muscle fibers with the highest SDH activity (red fibers), were easily observed in normal mice fed a solid diet, they were less prevalent but it is rather difficult to find than in normal mice fed a granulated diet. The diameter of muscle fiber in 90-day-old op/op mice was significantly (P<0.01) smaller, and that in normal mice fed a granulated diet was also significantly smaller (P<0.05), than that in the normal mice fed a solid diet. The diameter of the muscle fiber tended to be the biggest in normal mice fed a solid diet, intermediate in normal mice fed a granulated diet and smallest in op/op mice fed a granulated diet at 15 days of age but there were no significant differences among the three groups (Fig. 3). At 30 days of age, the differences had enlarged and the diameter of muscle fiber in op/op mice was significantly smaller (P<0.05) than that in normal mice fed a solid diet.

| Table 1. Body weight (g) normal and op/op mice |
|-----------------|-----------------|-----------------|-----------------|
| Animals        | 15-day-old      | 30-day-old      | 90-day-old      |
| normal mice    | 10.8 ± 0.8      | 15.5 ± 1.2      | 30.9 ± 2.5      |
| solid diet     | 10.1 ± 0.7      | 14.3 ± 0.9      | 29.5 ± 2.2      |
| op/op mice     | granulated diet | 8.2 ± 0.9       | 11.9 ± 2.9      |
| 90-day-old     |                 | 27.8 ± 2.2      |

Each value is mean ± SD from three animals.

Discussion

Generally speaking, the histochemical and morphological properties of skeletal muscles change or adapt by according to decreased or increased movement. McNamara [12, 13] described that masticatory muscles are activated both for postural support of the mandible and during ingestion, mastication and deglutition. The functions of these muscles are altered by edentulous conditions and the use of dentures [24, 25]. Ringqvist [21, 22] reported that differences in the functional activity of the temporal muscles may lead to morphological and physiological changes in individual muscles. On the other hand, few studies have examined the histochemistry of the jaw-opening muscles [5, 14, 20].

In this study, we examined the postnatal development of the anterior belly digastric muscle in the op/op mouse and normal mice fed solid or granulated diets. The body weight in the op/op and normal mice increased continuously with similar incremental changes. No significant differences in body weight in either group were found during the entire experimental period. We found that feeding a granulated diet retarded the normal increase in the diameter of the muscle fibers in normal mice and in the growing stage, and caused precocious retardation of the muscle fibers in op/op mice. Also, we showed clearly that red fibers with the highest SDH activity were significantly reduced by continuous intake of the granulated diet in 90-day-old normal mice. White and intermediate fibers with lowest and intermediate SDH activities respectively, also tended to reduce their activity. Maxwell et al. [11] showed that only...
Fig. 2. Development in the SDH activity of the anterior belly digastric muscle. a, d and g, normal mice fed a solid diet; b, e and h, normal mice fed a granulated diet; c, f and i, op/op mice fed a granulated diet. a, b and c, 15-day-old mice; d, e and f, 30-day-old; g, h and i, 90-day-old. W: white muscle fibers, I: intermediate muscle fibers. R: red muscle fibers. Bar=50 µm.
slow fibers, which correspond to red fibers, were significantly affected by the long-term absence of dentition in female edentulous rhesus monkeys. Therefore, they inferred that alteration of the functional activities of biting and mastication contributed to selective-disuse atrophy of slow fibers in the female edentulous monkey. Niederle and Mayr [17] showed that all types of muscle fibers were atrophied by denervation, although the course of denervation atrophy was different among types of fibers. The manner of muscle retardation in the anterior belly digastric muscle of \textit{op/op} mice was somewhat similar to that in the denervated muscle but it is a bit different from that in the anterior belly digastric muscle of normal mice fed a granulated diet.

Our present observations do not contradict our previous findings that the number of motor neurons innervating the masseter muscle is reduced in \textit{op/op} mice with less-developed periodontal ligaments [7]. Moreover, the present results support our previous suggestion that the number of masticatory motoneurons was reduced in the Mo5 in the \textit{op/op} mice. These results indicate that mechanical stress in mastication plays an important role in the development of muscle structures.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{Fig3.png}
\caption{Differences in diameters of the anterior belly digastric muscle fibers in normal mice fed a solid diet, in normal mice fed a granulated diet and \textit{op/op} mice fed a granulated diet. Significant differences *(p<0.05) and **(p<0.01) vs normal mice fed a solid diet. \textcolor{black}{□: normal mice fed a solid diet, \textcolor{black}{□: normal mice fed a granulated diet, \textcolor{black}{□: \textit{op/op} mice fed a granulated diet.}}}
\end{figure}

\section*{References}


