Archives of Oral Biology 80 (2017) 82–88

Contents lists available at ScienceDirect

Archives of Oral Biology

journal homepage: www.elsevier.com/locate/aob

Development of medial pterygoid muscle fibers in rabbits fed with a liquid diet

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ARTICLE INFO

Article history:
Received 20 February 2017
Received in revised form 27 March 2017
Accepted 28 March 2017

Keywords:
Medial pterygoid muscle
Postnatal development
Muscle fiber type
Liquid diet
Rabbits

ABSTRACT

Objective: This study aimed to investigate the influence of decreased functional load on the medial pterygoid muscle during mastication in rabbits fed with a liquid-diet.
Materials and Methods: Medial pterygoid muscles from 54 rabbits (solid- and liquid-diet groups, $n = 48$; unweaned group, $n = 6$) were histochemically examined at 4, 9, 12, 18, and 33 weeks after birth. Six fiber types (I, IC, IIC, IIA, IIAB, and IIB) were distinguished via mATPase staining.
Results: Significant increases in the diameters of all fiber types were seen up to 33 weeks of age in the solid-diet group; however, no significant increase was noted in fiber types I and IC, from 4 to 33 weeks of age, in the liquid-diet group. The proportion of slow fibers increased up to 12 weeks followed by an increase in the number of fast fibers in the solid-diet group, whereas in the liquid-diet group, the number of slow fiber declined after weaning.
Conclusions: Liquid-diet consumption caused muscle fiber atrophy and an increase in the number of fast fibers during early developmental stages after weaning. Furthermore, the growth pattern of the medial pterygoid muscle in the liquid-diet group was different from that in the solid-diet group.

1. Introduction

Masticatory muscles (especially the masseter) affect both masticatory function and the maxillofacial morphology (Rowlerson et al., 2005). Therefore, soft food preferences may decrease the load on masticatory muscles, thereby altering the maxillofacial morphology. The characteristics of masticatory jaw movement and the directions of the fibers vary across the three types of jaw closing muscles, namely, masseter, temporal and medial pterygoid muscles (Langenbach, Brugman, & Weijs, 1992; Schwartz, Enomoto, Valiquette, & Lund, 1989; Weijs, Brugman, & Grimbergen, 1989; Weijs, Brugman, & Klok, 1987). The majority of the previous studies examining the influence of decrease in functional load on masticatory muscles were targeted at the masseter muscle (Kitagawa et al., 2004; Langenbach, van de Pavert, Savalle, Korfage, & van Eijden, 2003; Maeda et al., 1987; Negoro et al., 2001). However, the characteristics of the activities during mastication are different for each of the three jaw closing muscles; therefore, changes that occur within the masseter muscle owing to changes in the type of food may not represent those in the temporal and medial pterygoid muscles. Hence, it is necessary to examine the influence of changes in food type on jaw closing muscles, other than the masseter muscle. Very few studies have evaluated the effect of low functional load on the medial pterygoid muscle (Kitagawa et al., 2014). The medial pterygoid muscle attaches to the mandibular ramus at the angle of the mandible; the activities of this muscle may add to the mechanical loading of the mandibular bone, similar to the masseter muscle. Hence, we believe that imbalances in masseter and medial pterygoid muscle activity may affect the morphology of the mandible.

Fiber type compositions of the masseter and medial pterygoid muscles are reported to change considerably during the growth process after birth in rabbits (Korfage et al., 2009). Thus, changes in muscle fiber type composition can vary significantly with modifications in the type of food at different ages in the same animal. Therefore, it is important to investigate the effect of changes in the type of food on muscle fibers during growth, with particular emphasis on the stage at which the growth is most strongly impacted and the specific changes that occur in the muscle fibers.

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http://dx.doi.org/10.1016/j.archoralbio.2017.03.022
0003-9969/

The present study aimed to investigate the influence of a decrease in functional load during mastication on the medial pterygoid muscle by changing the type of food in rabbits.

2. Materials and methods

The experimental procedures used in this study are similar to those described and reviewed previously (Saito et al., 2017). Briefly, 54 male Japanese white rabbits were included in the study; 48 of them were divided into two groups (solid- and liquid-diet groups) comprising 6 rabbits from each developmental stage (9, 12, 18 and 33 weeks of age), while the remaining 6 rabbits were placed in the unweaned group (4 weeks of age). Animals in the solid-diet group were fed a pellet diet (80 g at 4 weeks of age, which was increased to a maximum of 100 g per day until 10 weeks of age according to increase in body weight). Thereafter, the amount was maintained until 33 weeks of age (based on the guidelines of the Laboratory Animal Resource Center, Department of Dentistry, Aichi Gakuin University). The liquid-diet group received a mixture of powder diet (diameter < 250 μm) and milk. The powder diet was prepared by milling the solid diet and sifting it through a 250 μm diameter mesh. The amount of milk was adjusted in such a manner that the daily calorific intake was same as that for the solid-diet group (295.3 kcal). Approximately 10 g of powder diet was mixed with milk as a supplementary to prevent death by diarrhea. Water was freely available for the animals during the experimental period.

The medial pterygoid and medial gastrocnemius were dissected from the rabbits after euthanization by an overdose of anesthesia (urethane: 2000 mg/kg) at the end of the experiment. As shown in Fig. 1, incisions were made at the middle portion of the muscles. The muscles were dissected and flash frozen in isopentane cooled with liquid nitrogen, and stored at −80 °C until further analysis.

The procedures were performed according to the National Institute of Health (NIH; USA) guidelines regarding the care and use of animals for experimental procedures, and approved by the Animal Experiment Committee of the Aichi Gakuin University Department of Dentistry (Approval No. DE200828).

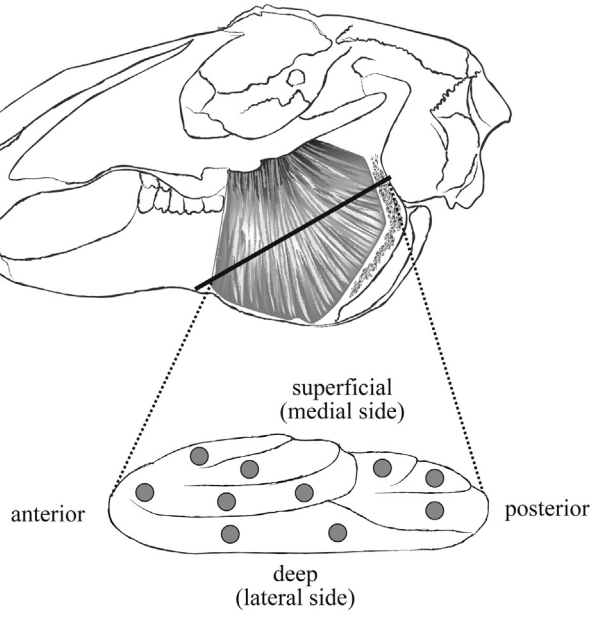

Fig. 1. Medial pterygoid muscle in a rabbit at 33 weeks of age. Line showing the position of incision in the medial pterygoid muscle. Samples were taken from 10 sites distributed across a wide area of the medial pterygoid muscle.

2.1. Histochemistry

Serial sections (7 μm) were cut perpendicular to the longitudinal direction of the muscle fibers using a cryostat at −20 °C. The sections were first preincubated at pH 4.2, 4.6, or 10.2 and, subsequently, stained for myofibrillar adenosine triphosphatase (mATPase) reactivity as described previously (Staron & Pette, 1986). In addition, the sections were stained with anti-MHC-s and -f antibodies (Novocastral Laboratories Co., UK); 6 fiber types (I, IC, IIC, IIA, IIAB and IIB) were identified based on the intensity of staining.

No significant differences in diameter and fiber-type composition were noted in a previously conducted preliminary study between the right and left medial pterygoid muscles; therefore, only the left pterygoid muscles were used in the current study.

2.2. Sampling method and measurement of muscle diameter

Microscopic images of samples obtained from 10 sites within the left medial pterygoid muscle (Fig. 1) were captured using an ACT-1 image analyzer (Nikon Co., Tokyo, Japan), and stored in a computer. Approximately 70 muscle fibers per each region (700 muscle fibers) were examined in each rabbit. An image measurement software (Image J 1.37v for Windows) was used to trace the outline of the muscle fiber on the computer screen. The effects of oblique sectioning were obliterated by denoting the short axis measurement of an ellipse outline of the muscle fiber as the diameter.

2.3. Statistics

Statistical analysis was conducted using a statistical software (StatView ver. 5.0 for Windows). Comparisons between two groups were conducted using Student's *t*-test; one-way analysis of variance (ANOVA) and Tukey–Kramer's multiple comparison test were conducted for comparisons among two or more groups. The level of significance was set at $p < 0.05$.

3. Results

3.1. Changes in body weight

The body weights of the rabbits demonstrated an increasing trend from 4 weeks to 33 weeks of age; however, no significant differences in weights were observed between the solid and liquid-diet groups at all ages (Fig. 2).

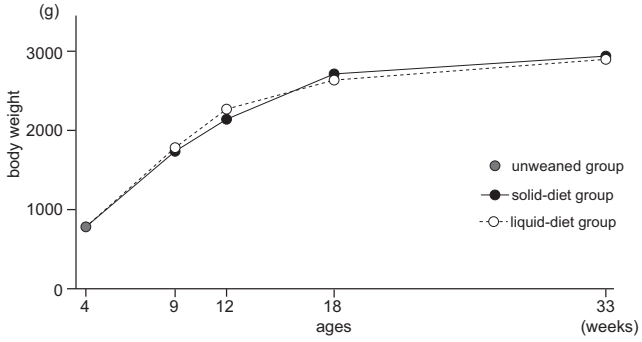

Fig. 2. Changes in body weight and fiber diameter across the five age groups. There were no significant differences in body weights between the solid and liquid-diet groups at all ages (ANOVA and Tukey–Kramer).