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Morphometric aspects of the facial and skeletal muscles in fetuses

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ABSTRACT

Objectives: There are few research reports providing a comparison of the muscle fiber morphometry between human fetuses and adults. Data on fetal and adult muscle fibers would be valuable in understanding muscle development and a variety of muscle diseases. This study investigated human muscle fiber growth to clarify the difference between the facial muscles and other skeletal muscles.
Methods: The materials were obtained from three male fetuses (6-month-old) and 11 Japanese male cadavers aged 43–86 years (average: 71.8). Human buccinator muscles (facial muscles), masseter and biceps brachii muscles (skeletal muscles) were resected. We counted the muscle fibers and measured their transverse area. We also calculated the number of muscle fibers per mm^2 (NMF) and the average transverse area of the muscle fibers (TAMFs).
Results: The average of the NMF of the buccinator, masseter and biceps brachii muscles in fetuses had, respectively, 19, 37, and 22 times as many fibers as those in adults. The average fetus/adult ratios of the TAMF of the buccinator, masseter and biceps brachii muscles were 4.0%, 2.4%, 4.1%, respectively.
Conclusions: The average NMF for all kinds of muscles decreased after birth; however, the peak in life-span or decreases with the aging process tended to vary with the kind of muscles examined. The average TAMF for all kinds of muscles enlarged after birth. We considered that the enlargement of the TAMF was connected with the emergence of fetal movements and functional demands after birth.

1. Introduction

Much of the previous work concerned with muscle fiber growth has been carried out on animal fetuses [1] or on human after birth [2]. These materials were muscles from the torso or extremities [3], but no details of the facial muscles have been available in textbooks. Furthermore, there are few research reports providing a comparison of muscle fiber morphometry between human fetuses and adults. Understanding the morphometry of human fetal and adult muscle fibers would be valuable in understanding muscle development and a variety of muscle diseases. We used facial and other skeletal muscles from human fetuses and adults, and performed a morphometric analysis of muscle fibers. This study closely investigated human muscle fiber growth to clarify the difference between the facial muscles and other skeletal muscles.

2. Materials and methods

Human buccinator muscles (facial muscles), masseter and biceps brachii muscles (skeletal muscles) were resected with skins and connective tissues. The materials were obtained from three male fetuses (6-month-old) from the Japanese fetal cadaver collection of the Department of Anatomy, Kyorin University School of Medicine, Japan. The ages were determined by measuring the crown-rump length (CRL) of the materials. To determine the approximate age of the fetus, we used the "Classification of Shimamura" [4]. This chart converts the CRL of a Japanese fetus into its corresponding age in terms of months of gestation. The other adult materials were obtained from 11 Japanese male cadavers aged 43–86 years (average: 71.8). All the fetuses were donated with the surviving families' consent, and all the cadavers were donated with the individuals' consent. We proceeded to perform this research in accordance with the law concerning autopsies and the preservation of corpses, and concerning their donation for medical and dental education. In no case was there a history of neuromuscular disease such as myopathy or facial palsy,

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or of treatment with toxic agents or irradiation therapy (both the parents of the fetuses and the cadavers). Moreover, each parent or cadaver had 20 teeth or more (mastication capacity standard), and they supported themselves in daily life (biceps brachii capacity standard). The causes of death did not directly or indirectly influence the muscular or nervous system, so the muscles were considered to be normal. The preparation of sections involved fixation, washing, dehydration, embedding, and sectioning, as described in our previous report [5]. All the cadavers were fixed with a 10% solution of formalin (3.7% formaldehyde) within 24 h of postmortem. After resecting the muscle, a 10% solution of formalin (3.7% formaldehyde) was used for immersion for at least a week. The solution was changed once in the first 30–60 min, and again later if desired. The formalin-fixed materials were then transferred, without washing, to the secondary fixative to be held at room temperature for two weeks. If the solution became turbid or precipitated it was changed. After this, the fixation was continued at 37 °C for an additional week. The volume of fixative used was at least ten times the volume of the specimens. In this process, materials had been fixed with pins at four corners of the board. After washing, dehydration, and celloidin embedding, we cut sections 15-μm thick and stained them with hematoxylin and eosin (H&E).

2.1. Morphometry

We observed the microscopic section at low power, and covered the entire area of the distributed muscle fibers in the section by moving the eyepiece grid vertically and horizontally as described in our previous report [6]. We confirmed that we could distinguish muscle fiber structures from other tissues with both a computer and the naked eye in every grid. We counted the muscle fibers and measured the transverse area of the muscle fibers in a square eyepiece grid at high power (Figs. 1 and 2), and then calculated the number of muscle fiber per mm^2 (NMF) and the average transverse area of the muscle fibers (TAMFs). To avoid duplicate counts, we counted and measured all muscle fibers on the side of the grid that did not come into contact with the other grids. In the case of grids adjacent to the other grids, we counted and measured only the muscle fibers on the lower right side of the grid, not those on the upper left side. We used a microscope in a transmitted light mode (BX50, Olympus, Tokyo, Japan) equipped with a high-resolution digital camera (ColorView12, Soft Imaging System, Münster, Germany), a motorized XYZ stage (Märzhäuser, Wetzlar-Steindorf, Germany), a stage controller (Märzhäuser, Wetzlar-Steindorf, Germany), and a computer (Precision 530, Dell, Austin, TX, USA) with analyzing system software (analySIS 3.0, Soft Imaging System, Münster, Germany) for storing data on-line, calculations, and statistical analyses.

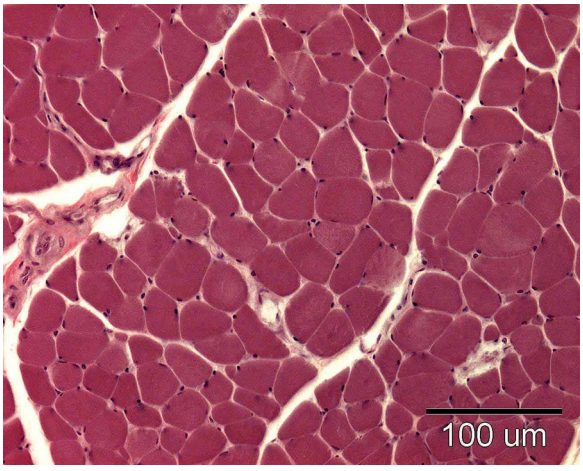

Fig. 1. A high-power view of the muscle fibers in buccinator muscle staining with H&E. 43-Year-old man; scale bar = 100 μm.

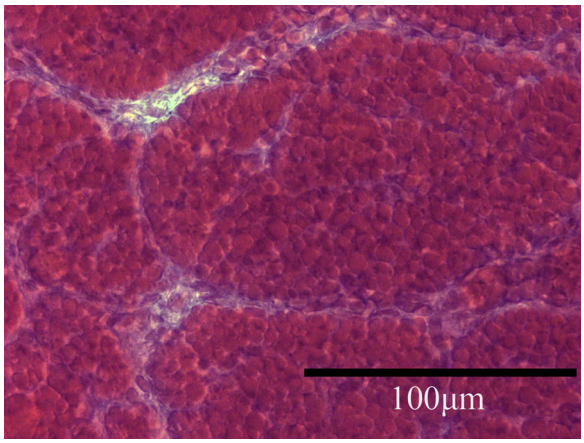

Fig. 2. A high-power view of the muscle fibers in buccinator muscle staining with H&E. 6-Month-old male fetus; scale bar = 100 μm.

3. Results

3.1. Number of muscle fibers per mm^2 (NMF)

We counted the muscle fibers and calculated the number of muscle fibers per mm^2 (NMF). Fetuses had a higher NMF than did adults in three kinds of muscle (Fig. 3). The average NMF for the buccinator, masseter and biceps brachii muscles in the three fetuses were $22{,}849 \pm 4798$ (mean ± SD, and so on), $43{,}874 \pm 13{,}162$, $26{,}173 \pm 7852$, respectively. As for the adults, the average NMF for the buccinator, masseter and biceps brachii muscles in the 11 cadavers were 1177 ± 248, 1184 ± 506, 1180 ± 460, respectively. The average NMF for the buccinator, masseter and biceps brachii muscles in fetuses showed, respectively, 19, 37, and 22 times as many as those in adults.

3.2. The average transverse area of the muscle fibers (TAMFs)

We calculated the average transverse area of the muscle fibers (TAMFs). Fetuses had a smaller TAMF than did adults in three kinds of muscle (Fig. 4). The average TAMFs for buccinator, masseter and biceps brachii muscles in the three fetuses were 18.1 ± 4.5 (mean ± SD, and so on), 14.5 ± 3.1, 29.3 ± 10.3 μm^2, respectively. As to adults, the average TAMF for the buccinator, masseter and biceps brachii muscles in the 11 cadavers were 448.1 ± 108.7, 602.8 ± 230.4, 722.9 ± 301.6, respectively. The average fetus/adult ratios of the TAMF for the buccinator, masseter and biceps brachii muscles were 4.0%, 2.4%, 4.1%, respectively.

4. Discussion

The number of muscle fibers appears to be genetically determined, showing no significant change after birth in most animals [1,7]. It is evident that the number of muscle fibers in a given muscle increases before birth until the genetically determined number is attained. Stickland's investigation of human fetuses showed that at the same timing as myotube appearance being lost, at around 6-months of age, the number of muscle fibers per unit area changed from an increasing trend to a marked decrease [8]. Reports as described above indicated that there is an initial slow increase in the total numbers of muscle fibers up to