

Original Article

Quantitative analysis of approximate shapes in a myofiber cross-section and their relationship with myofiber cross-sectional area

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Abstract. [Purpose] Although the shapes observed in myofiber cross-sections have been subjectively identified as polygonal, precise methodologies to classify such shapes have not been elucidated previously. Therefore, we aimed to determine the approximate shapes found in myofiber cross-sections, and to elucidate their relationship with the myofiber cross-sectional area. [Materials and Methods] Soleus muscles of five 11-week-old male Wistar rats were collected as specimens. The muscle specimens were rapid-frozen in isopentane—cooled in dry ice and acetone—and sliced into 10- μ m slices in a cryostat and stained with hematoxylin–eosin. The NIH ImageJ software was used to analyze the number of corners that were counted according to the proposed criteria and the myofiber cross-sectional areas of 500 myofibers. [Results] In assessments of the approximate shapes of myofiber cross-sections, the proportion of pentagons was 41%, which was the highest among polygons. A weak positive correlation was noted between the corner count and myofiber cross-sectional area, which indicated that polygons with more corners were associated with a larger myofiber cross-sectional area. [Conclusion] The myofiber cross-sections of the soleus muscle were considered to frequently show an approximately pentagonal shape. Moreover, a correlation was observed between the myofiber cross-section shape and myofiber cross-sectional area, suggesting that the area was also associated with the relevant functional features.

Key words: Approximate shape, Polygon, Myofiber cross-section

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INTRODUCTION

The skeletal muscle myofibril, myofibril, and muscle morphology have been analyzed macroscopically and by electron and light microscopy. Electron microscopy reveals that myofibril cross-sections (MFCSs) display a clear structure of hexagonal alignment in the A bands, and macroscopically, the muscles are classified as spindle-shaped, bipennate, or serratus muscles.

MFCS of the extensor carpi radialis longus muscle is reportedly circular in 12-day-old rats, but polygonal in rats aged ≥ 23 days¹⁾. Torrejais et al.²⁾ examined frozen slices of the extensor digitorum longus and soleus muscles stained with nicotinamide adenine dinucleotide and reported, as part of their subjective findings, that they observed polygonal, triangular, and circular shapes in the MFCS.

Previous studies have described the shapes of MFCS as triangular, polygonal, or circular, but none reported the criteria used to determine these shapes. However, the MFCS morphology is not only comprised of straight edges and acute angles but also sides composed of numerous distorted edges and rounded angles with curves of varying sharpness. Therefore, determining the type of polygon present would be difficult without objectively differentiating the sides and corners and counting the number of corners. Thus criteria for identifying whether a segment is an edge or an angle are necessary for determining shapes of a MFCS.

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Myofibers are formed through the fusion of myoblasts and their differentiation into myotubes, and through the further fusion of myoblasts to the myotubes³). In vitro, myoblasts adhere to the bottom of a petri dish in circular, triangular, and quadrilateral forms. Among these, a rectangular shape can endure the highest total stress in cell contraction, and longer shapes have a stronger traction⁴). Indeed, morphology is an important element of generating force, and a relationship between the MFCS morphology and mechanical structure in vivo may possibly exist. However, although previous studies have reported correlations between the myofiber cross-sectional area (MFCSA) and muscular strength^{5, 6}), none have elucidated the relationship between MFCS morphology and MFCSA.

To morphologically analyze the assessments of various neuromuscular diseases, it is essential first to have a better understanding of the relationship between the shape and function of the normal MFCS. Therefore, the present study aimed to propose a set of criteria to differentiate between the edges and corners in a MFCS, to define MFCS shapes using approximate polygons, and to identify the relationship between MFCS shapes and MFCSA.

MATERIALS AND METHODS

This study was conducted in accordance with the Japanese “Act on Welfare and Management of Animals” and “Guidelines for Proper Conduct of Animal Experiments” the Science Council of Japan-stipulated. The present study was approved by the Ethics Committee for Human Experiments of the Nittazuka Medical Welfare Center (Approval no. 26-5).

Five 11-week-old Wistar male rats (body weight 338.5 ± 6.8 g) were maintained in individual cages for 1 week with access to food and water ad libitum.

After being maintaining the rats in the experimental conditions, a lethal dose of sodium pentobarbital (100 mg/kg body weight) was administered, and the soleus muscle was excised for use as the muscle sample. These samples were rapid-frozen in isopentane cooled in dry ice and acetone, and then sliced with a cryostat (Leica Biosystems, Wetzlar, Germany) into 10- μ m slices.

The slices were air-dried for 60 mins, cooled at 4 °C, immersed and fixed in acetone, and stained with hematoxylin–eosin in accordance with the standardized procedure⁷).

Images of the muscular tissue were visualized on a light microscope (Nikon Corporation, Tokyo, Japan) and the corner count and MFCSA for 100 myofibers, or a total of 500 myofibers, were measured with NIH image processing software ImageJ (version 1.48u).

The following criteria were defined to differentiate between sides and corners in the MFCS.

1) A bend between 160° and 180° in the marginal regions (a direct line is defined as 180°) was defined as a straight line, and angles <160° were defined as a corner.

2) Short sides between two angles measuring <5% of the length of the perimeter was defined as an angle, and sides measuring $\geq 5\%$ were defined as 2 corners.

3) A rounded corner forming a small curve was defined as a corner.

4) Edges forming a gentle curve were classified as a straight line if the circularity of the curve was <0.3 (1)⁸). All other curves were classified as a corner.

Circularity = $4\pi S/P^2$ (1) π : Pi, S : Area, P : perimeter

5) Parts in the marginal regions where bumpy contours were formed by myonuclei, myosatellite cell nuclei, nuclei of other cells, muscular spindles, or blood vessels were NOT classified as corners.

6) Myofibers with altered shapes caused by the presence of artifacts were excluded from measurements.

All data are presented as mean \pm standard deviation. The relationships between the corner count and MFCSA were tested for equal variances by the test of normality and Bartlett’s test, after which the Spearman’s rank correlation coefficient was calculated. To compare the proportion of various approximate polygonal shapes and between the approximate polygonal shapes and MFCSA, equal variances were tested by the test of normality and Bartlett’s test, and subsequently the Bonferroni’s multiple comparison test. Statistical significance was indicated by $p < 0.05$.

RESULTS

The MFCS shapes classified by the criteria in this study resulted in polygons with a mean of 5.0 ± 0.9 corners (median, 5 corners) (Table 1).

The approximate shapes of MFCS were triangular ($2.4 \pm 0.9\%$), quadrilateral ($27.6 \pm 4.8\%$), pentagonal ($41.4 \pm 4.2\%$), hexagonal ($23.6 \pm 3.4\%$), heptagonal ($4.6 \pm 1.7\%$), and octagonal ($0.4 \pm 0.5\%$). In the MFCS, the proportion of triangles was significantly lower than that of quadrilaterals, pentagons, and hexagons ($p < 0.001$), and the proportion of quadrilaterals was significantly more than that of heptagons and octagons ($p < 0.001$). Similarly, the proportion of hexagons was significantly higher than heptagons and octagons ($p < 0.001$). The proportion of pentagons was significantly higher than other polygons ($p < 0.001$).

There was a weak positive correlation between MFCSA and the corner count ($r = 0.350$, $p < 0.001$), where MFCS polygons with more corners were associated with a larger MFCSA.

Table 1. Comparison of cross-sectional area in number of corners (n=500)

| Polygons | Triangle | Quadrilateral | Pentagon | Hexagon | Heptagon | Octagon |
|----------------------|------------------------------------|--|------------------------------------|-----------------------------------|-----------------|-----------------|
| Percentage of MF (%) | 2.4 ± 0.9 ^{**} , ††, †† | 27.6 ± 4.8 ^{††} , ††, †† | 41.4 ± 4.2 ^{††} , ††, †† | 23.6 ± 3.4 ^{††} , ††, †† | 4.6 ± 1.7 | 0.4 ± 0.5 |
| MFCSA (μm) | 2,406.3 ± 769.4 ^{††} , †† | 2,454.5 ± 520.2 ^{††} , ††, †† | 2,666.8 ± 593.6 ^{††} , †† | 2,934.0 ± 627.8 | 3,083.6 ± 609.7 | 2,732.6 ± 183.7 |

Data are mean ± standard deviation. MF: Myofiber; MFCSA: Myofiber cross-sectional area.

**p<0.01 vs. Quadrilateral, †p<0.05 vs. Pentagon, ††p<0.01 vs. Pentagon, †p<0.05 vs. Hexagon, ††p<0.01 vs. Hexagon, †p<0.05 vs. Heptagon, ††p<0.01 vs. Heptagon, †p<0.01 vs. Octagon.

DISCUSSION

Previous studies have described the shape of MFCS as polygonal. However, the outcomes of the present study revealed that there were MFCS with both angular and rounded corners. In addition, we considered rounded corners as regular corners to classify MFCS shapes in the closest possible polygonal shapes. Harris et al.⁹⁾ counted both angular corners and rounded corners in the corner count when classifying under approximate polygon types to distinguish the forms of objects in imaging data. Therefore, it should be possible to describe MFCS using the closest possible polygon shapes or to analyze these approximate shapes, regardless of whether they have rounded corners.

The marginal regions of MFCS contain both straight lines and bent areas. In this study, only bent areas <160° were counted as a corner. When humans delete the corners of a geometric shape to simplify it, they experience intense stress in deleting an angle that is between an acute and perpendicular angle and would avoid deleting it, but the stress experienced is relatively lower for deleting corners with angles of 100–180°, and the stress is reported to be lowest for deleting an angle of approximately 157°¹⁰⁾. Considering that this angle was approximately equivalent to the angle that was designated as the cut-off between a corner and a straight line in classifying bent portions, the results of classifying MFCS using approximate polygons in can be considered valid.

The present study analyzed the shape of myofibers extracted from frozen samples of the soleus muscle of 11-week-old rats. A pentagon was the most frequent approximate shape observed in the MFCSs, and the sum of pentagons, quadrilaterals, and hexagons comprised 92.6% of the total shapes, thereby suggesting that a pentagon was the most frequent approximate shape of the myofibers of the soleus muscle. In addition, the corner count in MFCS correlated with the MFCSA, suggesting that MFCS shape is also associated with muscle contractility and tone.

There are several types of myofibers in the soleus muscle. Type I comprise 90% of the myofibers in the muscle tissues of humans¹¹⁾, approximately 80% in that of 10-week-old male rats¹²⁾, and approximately 90% in that of 12-week-old male rats¹³⁾. As the samples used in this study were taken from the soleus muscle of 11-week-old rats, the majority of measured myofibers were likely type I myofibers, and the approximate shapes of the MFCS obtained likely reflected the form of type I myofibers.

The histological characteristics of myofibers change with age. Although the proportion of type I myofibers and the diameter of myofibers increase intermittently in the soleus muscle, it is the proportion of type II myofibers and the myofiber diameters that increase intermittently in the extensor digitorum longus muscle¹⁴⁾. Therefore, considering these findings, the proportions of the various approximate polygons should vary depending on the type of skeletal muscle measured and the age of the individual it was excised from. The corner count in MFCS correlated with the MFCSA, suggesting that MFCS shape is also associated with muscle contractility and tone. Analysis of MFCS shape may be used as a new evaluation of physical therapy to understand the progress and therapeutic effect of muscle atrophy such as disuse muscle atrophy and neuromuscular diseases.

Conflict of interest

There are no conflicts of interest to disclose.

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