A Method to Obtain True Muscle Fiber Area

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Story in Brief

A technique was developed for measuring the angle through which muscle fibers are sliced, during transverse sectioning, from a true perpendicular to their longitudinal axis. Angles which muscle fibers are displaced from a true perpendicular to their longitudinal axis appeared to be uniformly distributed between 0 and 15 degrees in thaw rigor tissue which was aligned, before sectioning, to obtain transverse tissue slices. An equation of stereology was modified for use with muscle fibers to improve the precision and accuracy of muscle fiber area determination. Knowing the angular distribution of sectioned muscle fibers, the equation derived can be used to obtain true fiber area; particularly on fibers from muscles like the Longissimus dorsi which is comprised of fibers that area parallel to the longitudinal axis of the muscle. The equation derived precludes the tedious aligning of muscle fibers prior to sectioning and would improve the efficiency and accuracy of obtaining data in muscle growth studies where a change in myofiber size is used as the basic muscle growth parameter.

Introduction

The measuring of skeletal muscle fiber cross-sectional area or diamter has been a popular technique to assess genetic and treatment effects during the muscle growth process in beef cattle. To measure cross-sectional area or diameter, investigators usually attempt to obtain a tissue slice perpendicular to the longitudinal axis of the muscle fibers. Muscle fibers not sliced perpendicular to their longitudinal axis will have an apparent cross-sectional area which is greater than the true cross-sectional area, yielding inaccurate data.

Other techniques involve separating individual muscle fibers and measuring the projection diameter or width of the fibers. However, muscle fibers are not true cylinders but appear, in cross-section, to be primarily irregular polygons. Consequently, the projection diameter technique may also give biased results.

The actual diameter of a muscle fiber is related to the apparent diameter multiplied by he cosine of the angle θ. The angle θ is the angular displacement from a true perpendicular to the longitudinal axis of a muscle fiber.

The common problem encountered in determining muscle fiber area is the angle θ. Angle θ is now known for the experimental material. Many investigators of fiber area or diameter eventually conceded that true crosssections perpendicular to the longitudinal axis of muscle fibers are difficult to obtain. Swatland (1975) measured the maximum endomysial sheath width parallel to the mean diameter axis. The arc sine of the ratio of the minimum endomysial sheath width to the maximum endoymysial sheath width gives the angle θ. This

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method of assessing the angle θ is tedious and highly subjective to the researcher's judgement in determining the minimum and maximum endoymysial sheath widths.

A method to determine the angle θ easily and accurately could improve the precision of studies on changes in muscle fiber area during the growth process in beef cattle. Thus, the objective of this study was to develop a technique to determine the angle θ.

Materials and Methods

From a 15 day old Holstein calf, the lateral head of the Triceps brachii was removed, wrapped in aluminum foil and frozen, pre-rigor, by immersion in liquid nitrogen. The muscle was sectioned perpendicular to its longitudinal axis and four, 6.35 mm cores were taken parallel with the longitudinal axis. Core length was adjusted to be less than or equal to the diameter of the core. The core was then placed in a phosphate-buffered saline solution at 24°C and allowed to enter thaw rigor. The core was then positioned on a chilled microtome chuck and refrozen.

Tissue slices, 60 microns in length, were taken from each core and transferred to a vial containing 10 ml of buffered saline. Slices were then disrupted into individual fiber rods by sonification.

A random sample of the small fiber rods was transferred to a microscope slide. Photomicrographs of the fiber rods were taken (Figure 1). The negatives of the fiber rods were projected onto white paper and the fiber rods were trac-

Figure 1. Photomicrograph of dispersed fiber rods.

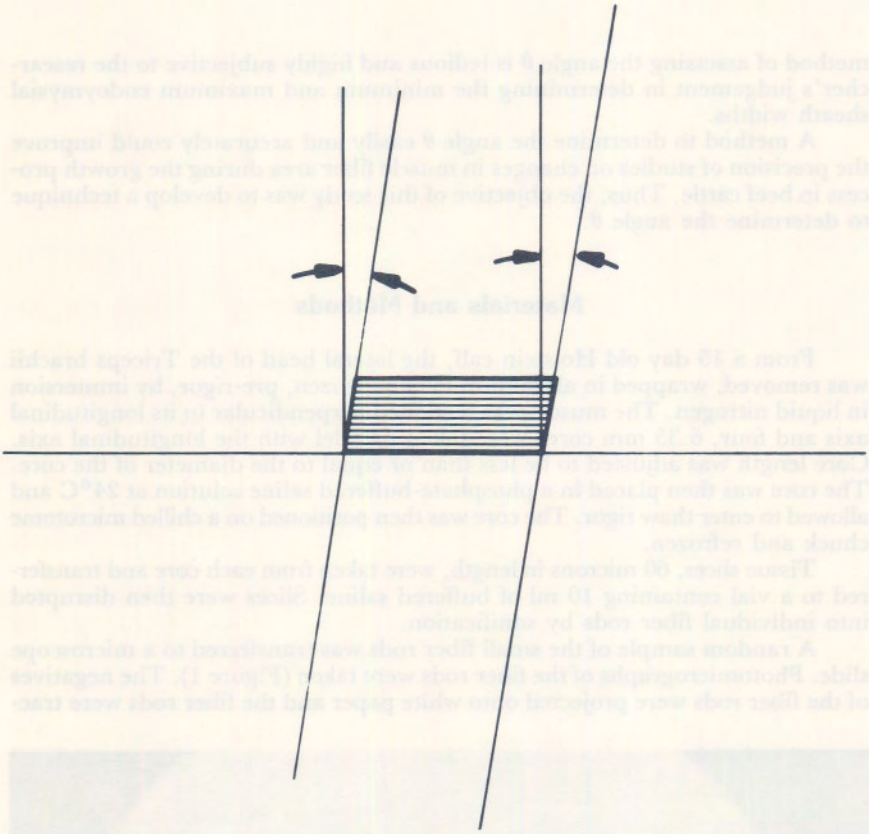

Figure 2. Schematic illustrating technique for measuring angle θ on the dispersed fiber rods.

ed. The angle θ was measured with a compass on tracings of the muscle fiber rods (Figure 2). One side of the rod was assumed to be parallel to the longitudinal axis and the angle θ was measured for both ends.

Results and Discussion

The angle θ was observed to vary within each core (Figure 3). As the number of fiber rods in each core was small, the observations for each core were pooled in order to more accurately assess the distribution of angle θ. The histogram of the pooled cores versus angle θ appeared by be uniformly distributed from 0 to 15 degrees. A Chi-Square Goodness of Fit test was used in testing the hypothesis that angle θ was uniformly distributed on the interval 0 to 15 degrees. The test was not significant at the 1 percent level of probability.

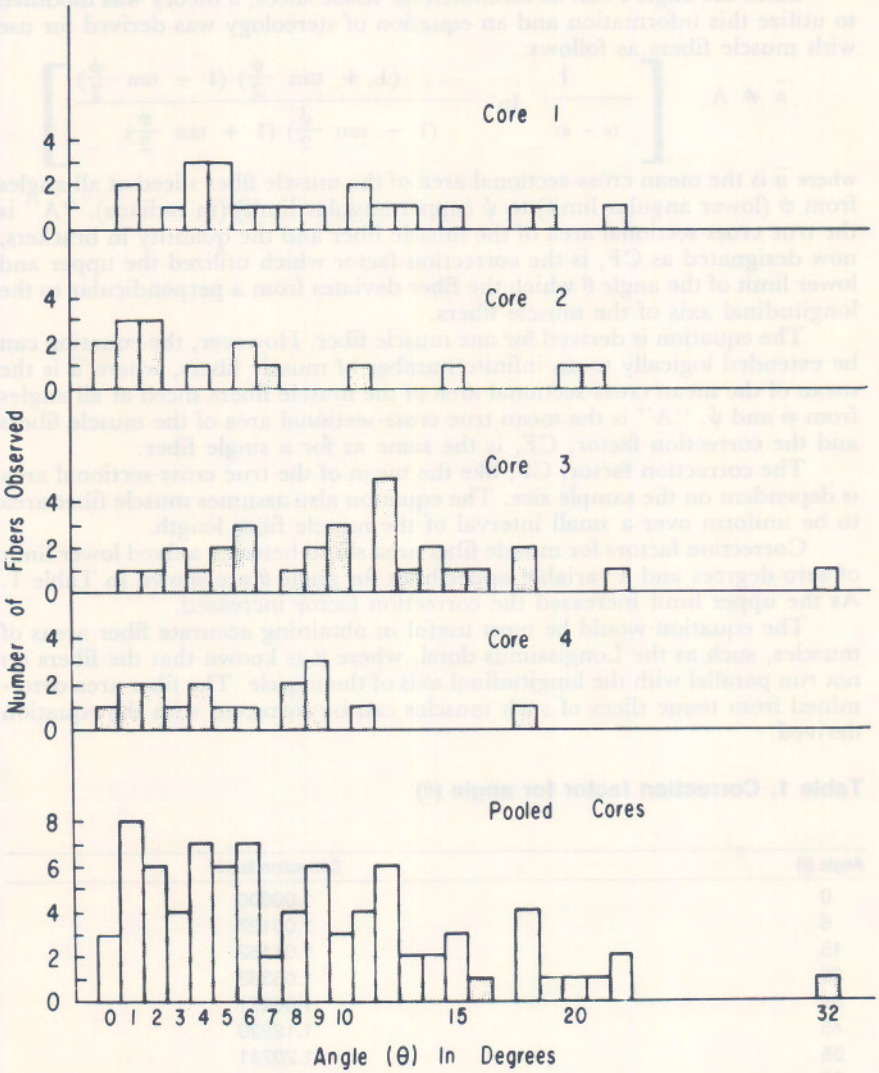

Figure 3. Number of fibers observed versus angle (θ).

Since the angle θ can be estimated for tissue slices, a theory was modified to utilize this information and an equation of stereology was derived for use with muscle fibers as follows:

$$\bar{a} \neq A \left[\frac{1}{(\psi - \phi)} \ln \frac{(1 + \tan \frac{\psi}{2})(1 - \tan \frac{\phi}{2})}{(1 - \tan \frac{\psi}{2})(1 + \tan \frac{\phi}{2})} \right]$$

where $\bar{a}$ is the mean cross-sectional area of the muscle fiber sliced at all angles from ϕ (lower angular limit) to ψ (upper angular limit) (in radians). "A" is the true cross-sectional area of the muscle fiber and the quantity in brackets, now designated as CF, is the correction factor which utilized the upper and lower limit of the angle θ which the fiber deviates from a perpendicular to the longitudinal axis of the muscle fibers.

The equation is derived for one muscle fiber. However, the equation can be extended logically to an infinite number of muscle fibers, where $\bar{a}$ is the mean of the mean cross-sectional area of the muscle fibers sliced at all angles from ϕ and ψ. "A" is the mean true cross-sectional area of the muscle fibers and the correction factor, CF, is the same as for a single fiber.

The correction factor, CF, like the mean of the true cross-sectional area is dependent on the sample size. The equation also assumes muscle fiber area to be uniform over a small interval of the muscle fiber length.

Correction factors for muscle fiber area sliced between a fixed lower limit of zero degrees and a variable upper limit for angle θ are shown in Table 1. As the upper limit increased the correction factor increased.

The equation would be most useful in obtaining accurate fiber areas of muscles, such as the Longissimus dorsi, where it is known that the fibers do not run parallel with the longitudinal axis of the muscle. The fiber area determined from tissue slices of such muscles can be corrected with the equation derived.

Table 1. Correction factor for angle (θ)

Angle (θ)	Correction factor
0	1.00000
5	1.00127
15	1.01162
25	1.03333
35	1.06871
45	1.12220
55	1.20241
65	1.32790
75	1.54897
85	2.11071

Literature Cited

Swatland, H.J. 1975. J. Animal Sci. 41:78-86.