Examination of a Method with Image Analyzer for Morphometry of Skeletal Muscle Fiber Size

Takashi ISHII and Takehiko ISHIBASHI
Department of Animal Science, Faculty of Agriculture, Kyoto University, Kyoto-shi 606

(Received June 11, 1984)

Morphometric data on skeletal muscle fibers have high utility for understanding the processes of muscle growth and structural development. In recent years, an image analyzer (I. A.) has been used for an automated morphometry to get these data conveniently\(^1\,\,^2\). Authors examined a method employing the I. A. for image analysis of skeletal muscle fiber size on transverse sections prepared by a routine procedure, and discussed the validity of this method as compared with another method.

Materials and Methods

The muscular samples of adult steers were obtained from \(m.\) triceps brachii, \(m.\) gluteus and \(m.\) extensor digiti IV of the carcass kept in 4°C for 2-3 days after slaughter. The muscular tissues were fixed in 10% formalin, dehydrated in graded alcohol series and embedded in paraffin. The transverse sections cut at 10 \(\mu\)m were stained with eosin only.

The morphometrical procedure with the I. A. (LEITZ TAS plus)\(^3\) is outlined below.

Based on the initial detected image on the space between muscle fibers (Fig. A, B), an image transformation was made to approximate that of the natural intramuscular connective tissue (Fig. C, D). Subsequently this image was reversed to make the discrete images of the muscle fibers (Fig. E). Then, each of the images processed was taken measurements regarding area and average diameter of three projections (AD 3 P) (Fig. F).

By another method, the individual muscle fiber area was converted from the weight of the paper cut out of the photomicrograph of the same muscle fiber in the same sample used for the I. A. measurement (Weight Ratio Method). And then, the equivalent circle diameters (ECD=2 \(\sqrt{\text{area}/\pi}\)) were computed from the area values of both methods respectively, too.
Explanation of Figures

Measuring process with an image analyzer (LEITZ TAS plus)

*Fig. A.* Original image on the monitor T.V.

*Fig. B.* Detection of the initial image of intermuscular space by setting gray value of monitor.

*Fig. C.* Image cleaning by elimination of disused image and addition of indispensable image.

*Fig. D.* Correction of shrunk tissue by thinning the image.

*Fig. E.* Reconstruction of muscle fibers image by reversing the processed image.

*Fig. F.* Cut off the image on the measuring field boundaries and subsequently start the measure operation.
Automated Morphometry of Muscle Fiber Size

The morphometrical data obtained by both methods about three muscular samples were analysed statistically.

Results and Discussion

The result for analysis of variance procedure showed that muscle was a significant source of variation for all of measurements studied. However, there was no evidence that method and muscle x method interaction were significant sources of variation in any of measurements. Within-muscle correlations between the weight ratio value and the I.A. value for area and ECD were 0.97 and 0.98, respectively.

Within-muscle correlations between area and ECD, between area and AD3P and between ECD and AD3P by the I.A. method resulted 0.96, 0.95 and 0.98, respectively.

Making the most of the I.A., it is required for the skeletal muscle tissue preparation that the muscle fibers are relatively in contrast with background to detect the image easily. Furthermore, it is able to deal with a large number of samples if the making preparation is simple. ROWE and PISANSARAKIT (1980) stated that the muscle fibers image produced by reversing the image of the intramuscular connective tissue had fewer associated problems than that of detecting from the muscle fibers directly. The preparation used by authors has a problem, i.e., a shrinkage of the tissue as an artefact. Therefore, making use of the I.A. function of image transformation for correcting the artefact, the initial detected image was corrected by thinning the width of endomysium to be 1-2 μm. For all that, the enlarged space of the inter-muscle fibers makes clear the discretion of the muscle fibers. And it is available for the initial image detection preferably.

Consequently, it is concluded that the image analyzer LEITZ TAS plus could be applied to the customary preparation as a useful method of assessing the mean level of muscle fiber size among muscles.

Acknowledgements

The authors wish to express their sincere appreciation to Dr. K. SUGIYAMA and Mr. W. KAWAMURA (SIBER KIKAI K. K., Tokyo) for utilizing LEITZ TAS plus and their helpful technique.

References