Muscle fiber number in biceps brachii in bodybuilders and control subjects

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It has generally been accepted since 1897 (15) that the fiber content of mammalian skeletal muscle remains constant after birth and that muscle growth occurs exclusively through enlargement of the existing fibers. Recently, however, several studies (8, 9, 11, 16, 19) have suggested that compensatory and training-induced growth in muscle may be the result of both hypertrophy of existing fibers and an increase in fiber numbers. Gollnick and colleagues (7) have suggested that methodological errors associated with estimation of fiber number from histological sections may have affected the results of these studies. Utilizing a technique by which all of the fibers in a muscle were isolated and counted, these authors (7) concluded that in rat muscle enlargement caused by ablation of a synergist and treadmill running could be completely accounted for by hypertrophy of existing fibers rather than by hyperplasia. The training mode in this study, however, was of the endurance-low-resistance type rather than the high-resistance training used in the studies that have reported hyperplasia (8, 9).

Competitive bodybuilders possess extreme degrees of muscle hypertrophy and thus are excellent models for investigating possible hyperplasia of fibers. We have recently published data suggesting that elite bodybuilders have more muscle fibers in triceps brachii than a group of trained control subjects (14). The present study was prompted by our wish to further examine this phenomenon by utilizing a technique which would permit a more direct calculation of actual fiber number.

Since it has been demonstrated that fibers in the biceps extend from origin to insertion of the muscle (1, 2, 5), measurement of muscle cross-sectional area at the belly of the muscle includes all muscle fibers. Thus by use of computerized tomographic (CT) scanning to determine muscle area and by calculation of average fiber area from needle biopsies, the total number of fibers may be estimated.

Since biceps is a muscle that is trained to achieve maximal hypertrophy in bodybuilders, it is particularly suited for investigation of possible hyperplasia. It was our hypothesis that if heavy-resistance training induces an increase in fiber number, the biceps of bodybuilders who have trained intensively for 6 yr should show evidence of fiber hyperplasia in comparison with control subjects of the same age.

METHODS

Needle biopsies were taken from the biceps brachii of the dominant arm of 12 male bodybuilders—5 of whom were considered as elite and 7 of whom we classified as being of intermediate caliber. The elite group not only had significantly greater muscle sizes than the others but had also won at least one major physique contest. In addition, tissue was taken from 13 age-matched control male subjects who had no previous history of resistance training. The biopsies were taken from the muscle at the estimated point of greatest girth. All subjects participated with their own informed consent in accordance with the policies of the Ethics Committee of the McMaster University Medical Centre.

The tissue was sectioned on a cryostat and stained by computerized tomographic scanning; fiber hyperplasia; connective tissue.
means of a modified Gomori trichrome stain for myofilbrillar adenosinetriphosphatase (following preincubation at several pH's) before being photographed under the light microscope. Cross-sectional area for both type I and type II fibers (as classified by the pH 10.0 preincubation) was measured by a computerized digitizer for a minimum of 200 fibers of each type per biopsy. Percent fiber type was estimated by counting a minimum of 550 fibers/biopsy. The proportion of collagen and other noncontractile tissue was calculated in the trichrome-stained sections by means of a 168 point, point-counting technique (20).

Cross-sectional area of biceps was determined from CT scans (Ohio Nuclear, model 20-30) of the upper arm with the elbow extended (Fig. 1). Several scans were taken at 1-cm intervals—one at the estimated point of greatest circumference for biceps and one proximal and distal to that point. The CT scan negatives were printed to 17 × 22 in. prints and biceps area measured by computerized digitizer. The scan yielding the greatest area was then selected.

Muscle fiber number was calculated as follows

\[
\text{numbers of fibers} = \frac{\text{cross-sectional muscle area}}{\text{average fiber area}}
\]

where

\[
\text{average fiber area} = \frac{\left(\% \text{ type I}\right)(\text{mean type I area}) + \left(\% \text{ type II}\right)(\text{mean type II area})}{100}
\]

and connective tissue correction equals the total muscle area minus the percentage of muscle that is collagen and other noncontractile tissue.

To evaluate the reliability of this method for the estimation of fiber numbers, biopsies were also taken from both arms of seven of the control subjects and fiber number estimated for both right and left arms.

RESULTS

Reliability of fiber enumeration technique. Based on the
assumption that there is an equal number of fibers in the left and right biceps, the reliability of the technique for estimating fiber numbers is illustrated in Fig. 2. The correlation coefficient for fiber number for left and right biceps for the seven control subjects was 0.79 ($P < 0.01$) with an SEE of ±11%. Stated in other terms, the mean intrasubject difference between right and left arms with this technique was ±8.9% with a ±3.6% SD.

Physical characteristics. The physical characteristics of the three groups of subjects are presented in Table 1. Both groups of bodybuilders had been training intensively for approximately the same period (6-8 yr). The elite group was significantly heavier ($P < 0.001$) and had significantly larger biceps areas ($P < 0.005$) than both the intermediate and control groups. The intermediate bodybuilder group also had significantly larger biceps areas ($P < 0.005$) than did the untrained control group. Four of the five elite bodybuilders admitted to the use of anabolic steroids, as did four of seven intermediate bodybuilders.

Fiber areas and fiber number. Biceps brachii was found to be comprised of ~61 ± 5.1% type II fibers with no differences in percent fiber type between any of the groups. Average fiber areas for both bodybuilder groups were significantly greater ($P < 0.02$) than for the control group. Although the average fiber area of the elite group appeared to be ~13% larger than that of the intermediate group, the difference was not statistically significant.

There was a very wide interindividual range in estimated fiber numbers in all three groups. Fiber numbers ranged from 172,000 to 381,000 among the control subjects, from 198,000 to 374,000 among the intermediate bodybuilders and from 204,000 to 419,000 among the elite subjects. The mean results for each group, however, did not differ (Table 1 and Fig. 3).

Connective and noncontractile tissue. The proportion of connective and other noncontractile tissue was extremely consistent between individuals and was ~13% of the tissue sample. Of this, ~6% was identified as collagen and 7% as other tissue.

**DISCUSSION**

The results of this study show that there are large differences in fiber numbers in biceps brachii in human subjects and that despite extremely large differences in muscle size, a group of bodybuilders possess the same number of muscle fibers as a group of untrained controls.

Validity of fiber number estimation method. The validity of our method for estimating fiber number rests on several assumptions. First, one must assume that the fibers in biceps extend the total length of the muscle. Although it has been shown anatomically (1) and electromyographically (2) that some fibers extend from origin to insertion of the muscle, it is not known whether all fibers do. Nevertheless we feel that it is highly likely that all fibers at least pass through the belly of the muscle which was the point at which the measurements were taken for the present study.

Second, since estimates of fiber number are based on measurement of fiber cross-sectional area, it must be accepted that any shrinkage or swelling of tissue during histological preparation would bias results. However since tissue preparation methods were constant for all subjects, such possible artifacts would not be considered to affect interindividual comparison to the same extent that they would affect absolute estimation of fiber numbers.

Third, since the needle biopsy technique results in

![FIG. 2. Estimated fiber numbers in right and left biceps brachii in 7 control subjects. Dotted line indicates line of identity.](image)

**TABLE 1. Physical description, biceps area, average fiber area, and estimated number of fibers for elite and intermediate bodybuilders and for untrained controls**

<table>
<thead>
<tr>
<th>Group</th>
<th>Age, yr</th>
<th>Training History, yr</th>
<th>Ht, cm</th>
<th>Wt, kg</th>
<th>Biceps Area, mm²</th>
<th>% Collagen and Other Noncontractile Tissue</th>
<th>Avg Fiber Area × 10⁶, μm²</th>
<th>No. of Fibers × 10⁶</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elite bodybuilders (n = 5)</td>
<td>23.7</td>
<td>6.2</td>
<td>178</td>
<td>90.3</td>
<td>2487⁺</td>
<td>13.0</td>
<td>106.6⁺</td>
<td>213.8</td>
</tr>
<tr>
<td></td>
<td>±1.2</td>
<td>±0.7</td>
<td>±2.2</td>
<td>±2.9</td>
<td>±181³</td>
<td>±3.6</td>
<td>±28.2</td>
<td>±53.2</td>
</tr>
<tr>
<td>Intermediate bodybuilders (n = 7)</td>
<td>24.2</td>
<td>7.8</td>
<td>173</td>
<td>81.1</td>
<td>1920⁺</td>
<td>12.1</td>
<td>93.7⁺</td>
<td>190.8</td>
</tr>
<tr>
<td></td>
<td>±3.1</td>
<td>±4.4</td>
<td>±3.2</td>
<td>±4.0</td>
<td>±321⁺</td>
<td>±5.4</td>
<td>±57.3</td>
<td>±51.3</td>
</tr>
<tr>
<td>Untrained controls (n = 13)</td>
<td>22.5</td>
<td>0</td>
<td>179</td>
<td>78.2</td>
<td>1418⁺</td>
<td>13.4</td>
<td>67.0</td>
<td>204.8</td>
</tr>
</tbody>
</table>

Values are means ± SD. Fiber numbers are expressed both uncorrected and corrected for change in sarcomere. Length with needle biopsy technique. *⁺ See text for explanation. *⁺⁺ P < 0.005. *⁺⁺⁺ P < 0.005. *⁺⁺⁺⁺ P < 0.02. *⁺⁺⁺⁺⁺ P < 0.001.
fully contracted tissue, fiber areas were measured with sarcomeres in the contracted state. Conversely, muscle area was measured by CT scanning with sarcomeres at resting length. It is thus apparent that the individual fiber areas have been overestimated while the total number of fibers has been underestimated. Again, however, we conclude that this would be a constant error and would not affect relative differences in fiber numbers between subjects. An alternative would be to obtain the CT scans with the elbow maximally flexed so that both measurements would be in the contracted state. This was also done in a number of subjects but we found that when the arm was in this position it was difficult to separate the CT scan of biceps from brachialis muscle in all subjects. In those in whom this was possible the biceps area was found to be \( 36 \pm 2\% \) greater in the flexed than in the extended position. Estimates of the extent to which a sarcomere shortens when fully contracted vary from 50–60\% \((3)\) to 30–40\% of its resting length \((12)\) depending on the definition of resting length. Based upon our CT scan differences in biceps area in the elbow extended and maximally flexed positions, we have arbitrarily chosen a correction factor of 36\%. Thus, assuming that the fiber volume remains constant regardless of sarcomere length, we would estimate that the actual number of muscle fibers exceeds our estimated number by 36\%.

Finally, one must assume that the biopsy sample which is taken is representative of the belly of the muscle with respect to fiber type distribution and muscle fiber areas. On the basis of recent studies \((4,10,13)\) one must accept that the biological nonhomogeneity of skeletal muscle may contribute to a sampling error when only one biopsy is used. We do not, however, feel that the magnitude of such sampling errors would be sufficient to have a systematic nonrandom effect on our results. This assumption is supported by our contralateral measurements.

Reliability. Since one would generally expect little variation in fiber number between left and right arms \((6,7)\), we conclude that our method of estimating fiber numbers is quite reliable \((\text{Fig. 2})\). We would also interpret the slight differences which were found to be due to sampling error rather than actual anatomical differences.

Muscle size and fiber numbers. When corrected for sarcomere length, fiber numbers ranged from 172,085 to 418,884 fibers/muscle. These values are in agreement with a previous autopsy study on human male subjects \((5)\). The relationship between muscle area and muscle fiber number is shown in \(\text{Fig. 3B}\). It can be seen that although the muscle area of the elite bodybuilders is significantly larger than that of the intermediate group, fiber areas do not differ significantly between the two groups. The massive arm size of the elite group is thus partially the result of larger muscle fiber numbers as well as greater fiber areas.

![Graph A: Correlation between cross-sectional area of biceps brachii and average fiber area.](image)

![Graph B: Correlation between cross-sectional area of biceps brachii and estimated fiber number.](image)
Connective tissue. Our finding of a constant proportion of collagen and other noncontractile tissue, regardless of muscle size or state of training, indicates that the absolute amount of connective tissue is considerably greater in the bodybuilders than in the controls. This suggests that training-induced hypertrophy of muscle fibers is accompanied by a proportional increase in connective tissue.

Anabolic steroids. In the present study one cannot dismiss the possibility that the use of anabolic steroids by the bodybuilders may have affected fiber size. Our findings, however, indicate that the use of steroids had no apparent effect on fiber number since both the elite and intermediate groups of bodybuilders had used steroids and yet had the same number of fibers as the control group.

In summary, this study indicates that large interindividual differences in muscle fiber number occur in human biceps and that highly trained bodybuilders do not possess more fibers than do untrained control subjects. We therefore conclude that heavy-resistance training directed towards achieving maximum size in skeletal muscles does not result in an increase in fiber numbers.

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REFERENCES