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Thigh muscle atrophy after femoral shortening

Vastus intermedius cross-section studied in osteotomized rats

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We determined the effect of a shortening/lengthening osteotomy on muscle fiber cross-sectional area in the vastus intermedius in 14 rats. The left femur was shortened 5 mm in half of the rats and elongated 5 mm in the other half. In both groups, the right femur was osteotomized and stabilized without shortening/lengthening. After 3 months, open biopsy specimens of the vastus intermedius were taken for measurements of the muscle cross-sectional area in both Type 1 and Type 2 fibers; 150 fibers were measured in each specimen. In the shortened group, the cross-section was reduced in both types of fibers. After lengthening, no differences in the fiber cross-sectional area were found.

We conclude that differences in stretch and tension of the muscles are responsible for the atrophy after femoral shortening and the maintenance of the fiber cross-sectional area after lengthening.

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Limb length corrections, especially lengthening, are associated with frequent problems, mainly related to bone healing, maintenance of bone axis and soft tissue function (Jones and Mosley 1985, Mosca and Mosley 1986, Hepburn 1987, Paley 1990). Vessels and nerves are responsible for the more serious complications. It is, however, a clinical experience that there is a great loss of muscle strength during and after limb length corrections.

We have investigated the effects of shortening and lengthening of the femurs on the muscle cross-sectional area of both Type 1 and Type 2 fibers in the vastus intermedius in rats.

Materials and methods

The study was performed in 14 male Wistar rats, about 6 months old, with a median weight of 455 g. The animals were fed standard pellets and water ad libitum. They were randomly divided in two groups. In group A the left femur was shortened by 5 mm and in group B the left femur was elongated by 5 mm.

8 rats were killed prior to the experiments to provide initial data on the bone dimensions. The femurs were measured using a sliding caliper (accuracy ± 0.01 mm). The bone length was determined as the distance from the top of the femoral head to the distal end of the medial condyle. The mean length of the femoral bone was 38.7 mm. The mean diameter of the medullary cavity, measured halfway between the top of the femoral head and the medial epicondyle, was 1.91 mm. As the medullary cavity is rather straight, it was found to be well suited for a non-flexible drill with a diameter of 1.80 mm. Following intraperitoneal anesthesia (Mebumal 5 percent), both femurs were exposed through a lateral incision between the vastus lateralis and the hamstring muscles, by elevation of the periost. An osteotomy at the shaft of the bone was made with a fine-toothed circular saw mounted on an electrical drill. The medullary cavity was then gradually reamed to 1.80 mm. In group A a 5 mm segment of the left bone was removed and transplanted to the left femur in group B. The bones were then stabilized in the following way: a cannula 1.4 mm in diameter was inserted from the greater trochanter to the end of the medullary cavity, and acrylic bone cement was injected from the distal to the proximal part. Before the cement set, a 1.4 mm steel pin was introduced into the medullary cavity. In group A, the left femur was shortened (5.0/38.7) × 100 percent, while in B, the left femur was elongated (5.0/38.7) × 100 percent. In all animals the right bone was osteotomized and stabilized in the same way, but without shortening/elongating for control. The wounds were closed in two layers, and the rats were allowed full weight bearing.

After 3 months the animals were killed. Measurements showed that the right control bones were 39.8 mm (mean), while the left bones in the shortened group (A) were 34.7 mm and in the elongated group (B) 44.6 mm.

Open biopsy from the vastus intermedius was done for analysis. The muscle samples were stained for

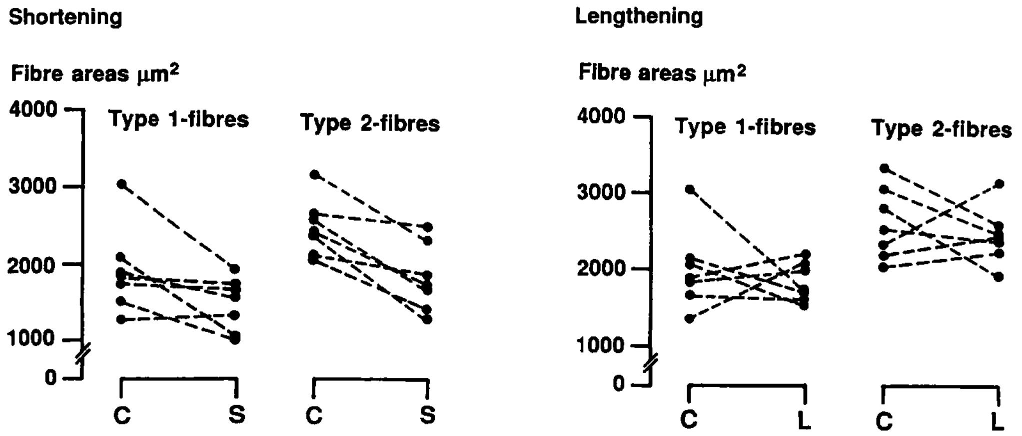

Figure 1. Areas of each of the fiber Types 1 and 2 of vastus intermedius.
C. The control side with osteotomy and stabilization, S shortening, L lengthening osteotomy of the femur.

myofibrillar adenosine triphosphatase at pH 10.3 which differentiates the muscle into Type 1 and Type 2 fibers (Guth and Samaha 1970). This means that subgrouping of Type 2 fibers was not performed. Computer digitized morphometry (COMFAS system, Scan-Beam A/S, Hadsund, Denmark) was used to measure the muscle cross-sectional area of Type 1 and Type 2 fibers. 150 fibers, chosen at random, were measured in each sample. Only areas without artifacts and with distinct cell borders were measured. To test the accuracy of the measurements of the muscle cross-sectional area, the same muscle biopsy samples were measured 10 times. The values were in the range of 1 to 5.3 percent, indicating that the method is highly reproducible.

Statistics

All results are given as mean values and standard error of the mean (SE). The significance of differences between cross-sectional areas was established by the paired and the non-paired *t*-test (two-tailed). The level of significance was set at 5 percent.

Results

In group A, there was a reduction in cross-sectional area of Type 1 (*P* 0.04) and Type 2 (*P* 0.02) fibers of the shortened side compared with contralateral control muscles. The values (SE) of Type 1 and Type 2 fibers cross-sectional area of the shortened muscles were 1445 (159) and 2005 (246) µm^2, respectively, compared with 1908 (176) and 2479 (159) µm^2, respectively, in the control muscles. This reflects a 28 percent reduction in the cross-sectional area of Type 1 fibers. For Type 2 fibers the reduction was 23 percent.

In group B, there was no significant reduction in the cross-sectional areas of Type 1 and Type 2 fibers on the lengthened side compared with the contralateral muscles (8 and 4 percent, respectively; Figure 1).

Both Type 1 and Type 2 fiber cross-sectional areas of the side with shortened femoral bone were reduced in relation to the side with femoral bone lengthening (*P* 0.05 and 0.02, respectively).

Discussion

The degree of shortening/lengthening in our experiment was similiar to that applied in previous experimental studies on limb lengthening by external distraction of the growing bone (Lindboe et al. 1985) and in clinical practice in many cases.

Muscle disuse is known to lead to atrophy (Booth 1977, Boyes 1979). Atrophy appears to be caused by a decrease in protein synthesis and an increase in protein breakdown (Goldspink 1977). Passive stretch and the development of isometric tension are considered to stimulate protein synthesis, thereby inducing growth of skeletal muscles in the lengthened state (Vaughan and Goldspink 1979, Barnett et al. 1980). Presumably these factors were not operative in the shortened femurs in group A. The lower level of stretch and activity in these muscles, therefore, may have caused their atrophy.

During skeletal growth the musculature adapts to the new functional length. This occurs through the addition of sarcomeres (Williams and Goldspink 1971, Tabary et al. 1972). Stretch induces an increase in muscle length and cross-sectional area (Williams and Goldspink 1973, Barnett et al. 1980). If the muscles are immobilized in the lengthened position, the muscle fibers are capable of adding 20–30 percent of new sarcomeres in series. Conversely, if the muscles are immobilized in the shortened position, they lose sarcomeres in series, so the fibers are shortened (Goldspink 1985). The synthetic rate of new myosin molecules per muscle fiber is approximately 30 million per minute. This corresponds to an addition of approximately 2000 sarcomeres within a 7-day period (Goldspink 1985). From these observations it is reasonable to suggest that the musculature is capable of adapting to the imposed bone lengthening of about 12 percent. It seems most likely that unequal degrees of stretch in groups A and B are the most important cause of the observed differences.

It has been suggested that lengthening procedures may cause unphysiological stretching of skeletal muscles (Lindboe et al. 1985). Furthermore, it has been demonstrated that lengthening osteotomy might lead to a reduction in the muscle fiber cross-sectional area of Type 2 fibers, but not of Type 1 fibers (Lindboe et al. 1985). Our findings are, however, in contrast to those published by Lindboe et al. (1985) who showed that a 10–20 percent lengthening osteotomy led to a 44 percent reduction in Type 2 fiber area. These differences may be explained by different experimental conditions. In Lindboe's study, lengthening osteotomy was performed on the tibia, and the biceps femoris muscle was studied. By lengthening the tibia, the stretching forces on the biceps femoris muscle will be perpendicular to the fiber direction, thus tending to tear the muscle fibers apart. With this method, it is unlikely that the tension along the long axis of the muscle will be sufficient to induce any stretching of the muscle. Furthermore, Lindboe et al. (1985) reported that their animals did not use the lengthened extremity for weight bearing and locomotion. Their observations might therefore be an effect of prolonged disuse of the extremity and the muscle. In our study, both extremities were operated on in the same way, and the animals did not appear to suffer any inconvenience by the shortening/lengthening operation of the femoral bones. As we could observe, they put equal weight on both operated on hind limbs.

In studies of immobilization of muscles at different muscle lengths, it has been discussed whether it is the immobilization or differences in muscle lengths that are responsible for the resultant atrophy or hypertrophy (Booth 1982). In our study, the only difference between the two groups was the bone length and, thereby, the muscle lengths. Therefore, it seems that changes in muscle length and, consequently, changes in muscle tension, are the main factors in producing atrophy or hypertrophy.

In conclusion, our study shows that bone shortening leads to an adaption of the musculature which is reflected as atrophy of the fibers. On the other hand, muscles are capable of adapting positively to bone lengthening, thereby perserving the cross-sectional area.

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