Diverse Skeletal Muscle Adaptation to Limb Lengthening between Adult and Young Animals

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Introduction: Limb lengthening, that is to say experimentally distraction osteogenesis has been widely used to treat various orthopaedic diseases such as congenital limb deficiency and acquired limb shortening. The surrounding soft tissues including skeletal muscle are also lengthened in this treatment. Force generation in a sarcomere is proportional to the sarcomere length and overstretched sarcomere cannot generate sufficient active tension. Thus, the muscle should adapt to the lengthening by adding new serial sarcomeres in order to adjust sarcomere length to optimal length. In general, muscle adaptability decreases with age. Recent development in limb lengthening provides adult patients opportunities to undergo limb lengthening procedure. However, muscle adaptation to limb lengthening in adult population has not been understood completely. Therefore, the purpose of this study was to clarify the difference in muscle adaptation between adult and young groups using a rabbit model of tibial lengthening.

Methods: These experiments were performed in accordance with the guidelines of Institutional Animal Care and Use Committee. Mature (10-43 month old, body mass = 3.63 ± 0.38 kg, n = 5) and immature (3-4 month old, body mass = 2.46 ± 0.33 kg, n = 6) male Japanese White rabbits underwent tibial lengthening, with the contralateral limb serving as a control. A custom-made unilateral external fixator was fixed to the tibia with four half-pinhole osteotomy holes. Animals were euthanized after their first and third pins using a manual saw. After a latency period of 1 week, lengthening was begun at a rate of 1.4 mm/day and was stopped at 3 weeks after the operation (achieving 19.4 mm-lengthening). Animals were euthanized after the completion of lengthening. Both hindlimbs were immersed for 5 days in 10% buffered formalin with the ankles and knees fixed in right angle. Five full-length muscles around the lengthened segment, that is, the tibialis anterior (TA), extensor digitorum longus (EDL), lateral head of gastrocnemius (GCL), soleus (Sol) and flexor hallucis longus (FHL) were detached to measure muscle belly length. Three full-length muscle fiber bundles having tendinous element on the both ends were teased from the proximal, middle and distal part in EDL, GCL and FHL, and two from the proximal and distal in the other muscles under a dissecting microscope. Fiber bundle length was measured using a digital caliper under the microscope. After mounting each fiber bundle on a glass slide, the sarcomere length was measured at five different points across the fiber bundle under a polarized light microscope (Nikon, Eclipse TE2000-U, Tokyo, Japan) using a digital measuring system (Nikon, Digital Sight DS-L2, Tokyo). Serial sarcomere number was then calculated by dividing fiber bundle length by average sarcomere length. Aponeurosis length was calculated by subtracting the proximal fiber bundle length from muscle belly length. Paired t-tests were used to determine significance of the difference between the lengthened and contralateral control sides (p < 0.05). Data are presented as the mean ± SD.

Results: 1. Muscle belly lengths increased in all of the lengthened muscles compared to the corresponding contralateral muscles. The amounts of muscle belly length increase did not differ between both adult and young groups (5.9 to 15.8 mm for adult group muscles and 5.9 to 18.6 mm for young group). 2. Aponeurosis lengths increased significantly in Sol (8.5 mm on average) only for adult group, but in TA (9.6 mm), EDL (10.1 mm) and Sol (11.8 mm) for young group (Fig. A). 3. Sarcomere lengths were significantly longer in EDL, GCL and Sol compared to corresponding contralateral muscles for adult group and in all of the muscles for young groups. For adult group the remaining two muscles (TA and FHL) were also longer but not significant. 4. Serial sarcomere numbers significantly increased in GCL and FHL for young group and in TA, EDL and FHL for adult group (Fig. B). For adult group the amounts of sarcomere number increase were approximately 7,000 for TA and 4,700 for EDL. For young group the amounts of increase were approximately 1,000 at the maximum.

Discussion: This study demonstrated age difference in adaptation of several skeletal muscles around the lengthened segment to limb lengthening procedure. Of muscle adaptations, the amounts of muscle belly length increase in adult group were similar to those in young group. On the contrary, serial sarcomere numbers that reflected muscle fiber lengths dramatically increased in TA and EDL for adult group. The sarcomere number increase in TA (~7,000) was the equivalent of the amount of limb lengthening (~20 mm). This is surprising in consideration of adult group, which is thought to have less adaptability. The sarcomere number increases in plantarflexors were less prominent. These results were probably because dorsiflexors were more stretched during the limb lengthening of the lower leg, in which the plantarflexor was dorsiflexed. The increases in aponeurosis length were seen mostly in young group muscles. In addition, increases in external tendon were also seen in young group (data not shown). It has been shown in young animals that external tendon substantially elongated during muscle-tendon unit adaptation to joint immobilization and limb lengthening. The increases in aponeurosis length were seen mostly in young group muscles. In addition, increases in external tendon were also seen in young group.

Significance: This study reveals that skeletal muscles adapt to the lengthening in different way between adult and young animals, and suggest that the muscle adaptations occurs to the similar amount for adult population who undergo limb lengthening.

References: