CLINICAL ORTHOPAEDICS AND RELATED RESEARCH
Number 456, pp. 250–253

CASE REPORTS

Muscle Adaptation by Serial Sarcomere Addition 1 Year after Femoral Lengthening

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A common complication of reconstructive surgery is muscle contracture and consequent loss of joint motion. This particularly occurs in surgical lengthening procedures where the muscle adaptive capacity seems to limit the extent of possible lengthening. We used intraoperative laser diffraction to determine the skeletal muscle adaptation that occurred in a 16-year-old girl who had 4-cm femoral lengthening for a leg-length discrepancy secondary to posttraumatic growth arrest. Fascicle length changed dramatically during distraction from a starting value of approximately 9 cm to a new length of 19 cm. In vivo vastus lateralis sarcomere length measured intraoperatively at the initial surgery was 3.64 μm, whereas sarcomere length measured 8 months later was 3.11 μm. The fact that fascicle length increased dramatically and in vivo sarcomere length decreased slightly reveals an increase in serial sarcomeres from 25,000 to 58,650. This direct measurement of fascicle length and sarcomere length confirms sarcomerogenesis in human skeletal muscle secondary to chronic length change, and shows the capacity of human muscle to adapt to length changes.

Received: June 2, 2006
Revised: August 14, 2006
Accepted: October 2, 2006
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One or more of the authors (JLB, RLL) has received funding from the Orthopaedic Research and Education Foundation. The institution of the authors has received funding from the National Institutes of Health.
Each author certifies that his or her institution has approved the reporting of this case report, that all investigations were conducted in conformity with ethical principles of research, and that informed consent for participation in the study was obtained.
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DOI: 10.1097/01.blo.0000246563.58091.af

Musculoskeletal procedures such as tendon lengthening, tendon transfer, and distraction osteogenesis are used to improve patient function secondary to trauma and congenital or central nervous system disorders such as cerebral palsy. One of the most common complications is muscle contracture and consequent loss of joint motion. Because muscle adaptation to lengthening is poorly understood, our ability to develop rational treatments for contractures is limited. Results obtained from animal models of muscle adaptation to chronic length changes seem conflicting. For example, the classic immobilization studies of the 1970s showed rat and cat soleus would reoptimize their length by adding or subtracting sarcomeres as needed.[13–16] However, these results are at odds with results obtained from other muscles which showed that some muscles adapt to a lesser extent than the soleus[10,12] and others adapt in the opposite direction.[4] The adaptive nature of skeletal muscle to chronic length change, particularly in humans, is poorly understood yet important as many orthopaedic surgical procedures are based on the assumption that muscle adaptation occurs after surgery.

We report the case of a girl who had a 4-cm femoral lengthening for a leg-length discrepancy (LLD) secondary to posttraumatic growth arrest. Before, during, and after the lengthening, vastus lateralis (VL) fascicle length was measured by the ultrasound method established and validated by Fukanaga et al[5,6] and Ichinose et al.[7] In addition to ultrasound measurements, at the time of the lengthening procedure and 8 months after the procedure, sarcomere length was measured intraoperatively by laser diffraction. Small fiber bundles were isolated by atraumatic blunt dissection (Fig 1A) and then the fiber bundle was transilluminated by a diffraction device placed beneath the fiber bundle (Fig 1B).[2,8,9] It is important to measure muscle fiber length and sarcomere length to distinguish whether fascicle length changes occur simply from the stretch of

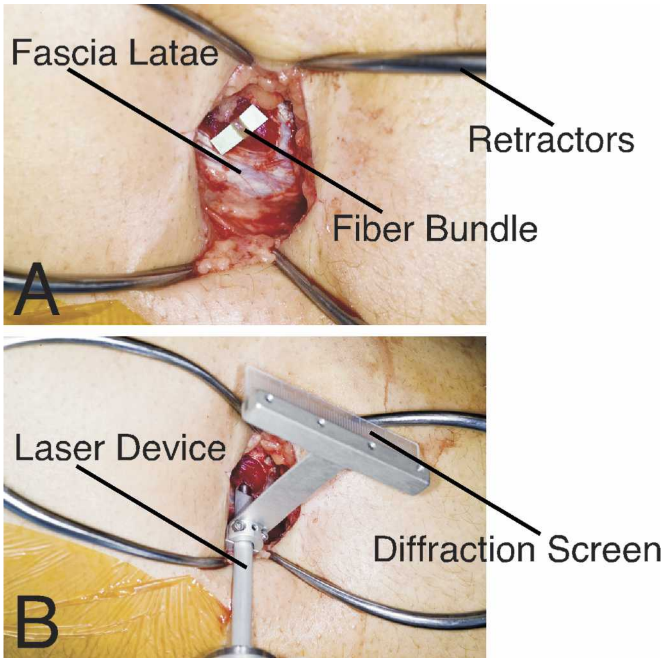

Fig 1A–B. Intraoperative laser diffraction was performed on the VL at the corticotomy site. (A) A muscle fiber bundle was isolated from the VL and placed above a small piece of plastic. (B) The laser diffraction tool was placed beneath the bundle of muscle fibers that were isolated by atraumatic dissection as previously described.[9]

muscle tissue or if new sarcomere synthesis occurs in the fibers stretched by distraction. These methods have not been combined to distinguish between these possibilities. In addition to the in vivo measurements, we also measured the in vitro micromechanical properties of single cells obtained from muscles before and after lengthening to determine whether the mechanical properties of the muscle cells had changed secondary to distraction.[3] These data provide the first definitive in vivo and in vitro analysis of the extent to which the human VL can adapt to distraction.

CASE REPORT

A skeletally mature 17-year-old girl sustained a fracture of the distal femur involving the growth plate when she was 13 years old. In April 2005, an Intramedullary Skeletal Kinetic Distractor® (ISKD®; Orthofix Inc, McKinny, TX) was placed in the femur, a muscle biopsy was done, and fascicle length was measured with the knee positioned between approximately 40º and 60º flexion. She returned for followup ultrasound measurements 2, 3, 8, and 12 months postoperatively and at 8 months for partial hardware removal, sarcomere length measurements, and repeat muscle biopsy.

Specimens from muscle biopsies were subjected to micromechanical testing as previously described[3] to determine muscle cell elastic modulus and resting sarcomere length, which provide insight into the structural and functional properties of muscle cells. Briefly, single cells were stretched in 250-μm increments and elastic modulus computed as the slope of the stress-strain curve. To compare these values with normal VL muscles, we obtained biopsy specimens from healthy age-matched subjects having orthopaedic procedures that involved VL exposure. All experimental procedures were performed with the full approval of the Institutional Review Boards of the Shriners Hospital and the University of California, San Diego and Davis.

Fascicle length changed dramatically during distraction. During the first 3 months, when the limb was lengthened by 4 cm at approximately 0.5 mm/day (a 10% bone lengthening), fascicle length increased by more than 100% from a starting value of approximately 9 cm to a new length of 19 cm (Fig 2). During the consolidation period, fascicle length remained constant so that, after 12 months, fascicle length remained 100% greater than the starting length, or 18 cm (Fig 2). In vivo VL sarcomere length measured intraoperatively at the time of ISKD® placement was 3.64 μm, whereas sarcomere length measured 8 months later was 3.11 μm. The fact that fascicle length increased dramatically and in vivo sarcomere length decreased slightly showed the substantial increase in serial sarcomere number. As the serial sarcomere number represents fascicle length divided by sarcomere length, the serial sarcomere number increased from 25,000 (9.1 cm/3.64 μm)

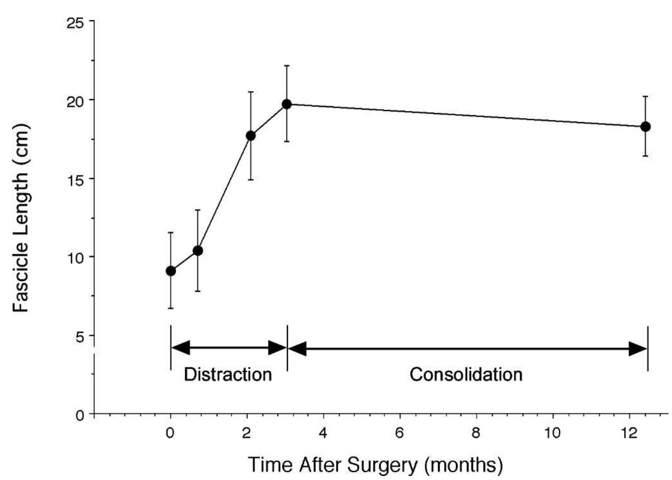

Fig 2. A graph shows the relationship between fascicle length measured by ultrasound and time after surgical placement of the ISKD® frame during distraction and consolidation. Fascicle length increased rapidly by approximately 100% during the distraction phase.

TABLE 1. Vastus Lateralis Muscle Cell Properties

Date	Age (months)	Biopsy Timing	Modulus (kPa)*	Slack Sarcomere Length (μm)*
April 1, 2005	209	Before lengthening	31.9 ± 4.3	2.39 ± 0.28
November 30, 2005	217	After lengthening	34.2 ± 5.2	2.26 ± 0.06

*Results shown as mean and standard deviation (n = 5)

to 58,650 (18.2 cm/3.11 μm). This corresponds to a serial sarcomere synthesis/incorporation of approximately 350 per day. Therefore, the sarcomere number increased more than enough to compensate for the limb-length change.

Multiple muscle fibers were tested from biopsy specimens (n = 5 fibers per biopsy). The average muscle cell elastic modulus before lengthening was 31.9 ± 4.3 kPa and at the end of the lengthening procedure was 34.2 ± 5.2 kPa. These values indicate no change to the material properties of these fibers (Table 1) and are within the range seen in four normal VL biopsy specimens (30.3 ± 3.5 kPa; n = 12 fibers). Similarly, slack sarcomere length was identical before and at the end of the lengthening procedure, also providing no evidence for change in the passive biomechanical properties of these fibers (Table 1).

DISCUSSION

These data, reflecting the first direct measurements of fascicle length and sarcomere length, yield confirmation of sarcomerogenesis in human skeletal muscle secondary to chronic length change. They show the capacity of the human VL muscle to adapt to a length change on the order of 100%. Although the classic animal literature often is interpreted to indicate all muscles adapt to chronic length changes by reoptimizing sarcomere length,[13,15] this is not the case for all mammalian muscles. This point was seen in rodent muscles, where serial sarcomere number changes that occurred secondary to chronic immobilization varied between muscles as a function of muscle function and fiber type.[1,12] Similarly, distraction studies in rabbits revealed differential muscle adaptation based on the extent and rate of distraction.[11] Thus, it is inappropriate to extrapolate rodent or feline studies to all skeletal muscles, especially those of humans. In human muscle, there is evidence adaptive capacity of muscles of upper and lower extremities may vary so that specific statements regarding human muscles cannot be made in the absence of primary data. These data, taken at face value, show the capacity for complete adaptation to the new length without a change in cell material properties and with an increase in sarcomere number.

Additional studies are required to determine the extent to which such a result is applicable to other human muscles and other limb-deficiency syndromes. There is no clear evidence such adaptive capacity is true of human muscle in general. Also, some muscles affected by either peripheral or central motor nerve injury may respond differently compared with the essentially intact VL studied in this case. This was a relatively small lengthening (10% of femoral length) in an otherwise normal leg. Limbs often are lengthened 15% or more and in situations where muscle properties may be altered secondary to either disease or upper motor neuron lesion. For example, congenital limb deficiency with its deficient muscle may respond differently to limb lengthening than the VL muscle in our patient.

The simultaneous measurement of sarcomere length and fascicle length permits unambiguous determination of the nature of muscle fiber adaptation to chronic lengthening. Three a priori possibilities existed (Fig 3): (1) an increase in serial sarcomere number would completely compensate for the fascicle length increase imposed by distraction (Fig 3B); (2) fibers would stretch in response to distraction, in which case postsurgical sarcomere length would be approximately twice that of the muscle before distraction (Fig 3C); or (3) a combination would occur. The data suggest adaptation occurred by addition of

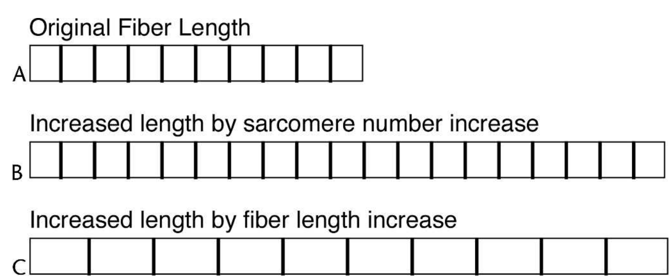

Fig 3A–C. The diagrams show the possible nature of muscle fiber adaptation to chronic lengthening: (A) muscle fiber length before distraction, (B) fiber length increase by adding a proportional number of sarcomeres, and (C) fiber length increase by stretching the existing fiber. Based on the results of the ultrasound and intraoperative sarcomere length measurements, the VL muscle adapted by adding a proportional number of sarcomeres (B).

enough sarcomeres to account for the increased fascicle length (Fig 3B).

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