SARCOMERE REMODELING INDUCED BY DISTRACTION: ARCHITECTURAL ANALYSES

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INTRODUCTION

Limb lengthening procedures such as those described by Wagner, Ilizarov, and Wasserstein are commonly used to correct differences in limb length. These surgical procedures, however, can produce many major and minor complications. Currently, it is believed that one of the major problems interfering with limb lengthening technique is the resistance of the myofascial structures. It has been proposed that the passive mechanical properties of skeletal muscles provide a resistance that can lead to joint contracture or joint instability. Foremost of these is the classic equinus contracture that frequently occurs with significant lengthening of the tibia. Presumably, the equinus contracture develops because the muscle fibers of the plantar flexors add contracture. Thus, the ankle joint becomes more rigid and unstable. Foremost of these is the classic equinus contracture that frequently occurs with significant lengthening of the tibia. Presumably, the equinus contracture develops because the muscle fibers of the plantar flexors add contracture. Thus, the ankle joint becomes more rigid and unstable.

MATERIALS AND METHODS

Animal Model. Female Sprague-Dawley rats (300-325 g) were randomly assigned to one of four experimental groups: i) normal control (CON, n=6); ii) frame control (FC, n=4); iii) 0.25mm/day of distraction (0.25mm DIS, n=6); or iv) 0.5mm/day of distraction (0.5mm DIS, n=6). All treatment paradigms utilized in this study were approved by the university’s Institutional Review Board.

Semicircular external fixators were applied unilaterally to the left leg of each animal in the FC, 0.25mm DIS, and 0.5mm DIS groups. Both the knee and ankle joints were fixed at 90°. Animals assigned to the DIS groups (i.e., 0.25 and 0.5 mm DIS groups) underwent distraction for a period of 4 wks. Animals in the FC group were not distracted.

Fixation and single fiber analyses. At the end of the distraction period, the knee and ankle joints of each CON animal were fixed at 90°. The hindlimbs of each animal in the CON, FC, 0.25mm DIS, and 0.5mm DIS groups were then perfused using a 10% formaldehyde solution, removed at mid femur, skinned, and placed into a solution of 10% formaldehyde. The distraction frames were left intact during the fixation phase. Following fixation, the soleus (SOL) muscles were placed into a 30% nitric acid solution (~3 days) to macerate connective tissue. The muscles were then rinsed in PBS solution (pH 7.4), and stored in a 50% glycerol/PBS solution.

Ten single fibers were microdissected from each SOL muscle, and the length of each fiber (FL) was determined using a caliper. Measurements of average sarcomere length (SL) were then determined in the proximal, middle, and distal regions of each fiber using a image analysis system that consisted of: i) a Leica DMLS light microscope (400x); ii) a Sony CCD camera; and iii) NIH Image 1.57 software. Within each region (e.g., proximal region), the mean SL was determined by counting the number of sarcomeres within 9 µm length increments (i.e., over a distance of 900 µm). Hence, the mean SL for each region of a fiber was determined by measuring the lengths of ~300-350 sarcomeres (~ 1000 sarcomeres per fiber given that 3 different regions were sampled). Sarcomere number was determined by dividing FL by SL.

Statistical Analyses. All values are reported as means ± S.E.s. The effect of distraction on the key variables (e.g., sarcomere length) was determined using a one-way ANOVA. If the one-way ANOVA produced a significant F-ratio, then the Tukey HSD test was used to determine which groups were different from one another. In all analyses, statistical significance was defined as P ≤ 0.05.

RESULTS

Fiber Length. The mean (± S.E.) FLs of the CON and FC groups were 12.8 ± 0.34 and 13.6 ± 0.73 mm, respectively, and not statistically different from one another. Distraction had a significant effect on mean FL (P<0.001). The mean FLs of the 0.25 and 0.5 mm DIS groups were 39 and 71% greater than that of the CON group.

Sarcomere Length. The mean SLs for the various groups are shown in Figure 1. Both distraction groups had SLs that were significantly greater than that of the CON and FC groups. Interestingly, the mean SLs of the 0.25mm DIS and 0.5mm DIS groups were not different from one another.

Total Sarcomeres/Fiber. The total numbers of sarcomeres/fiber are shown below in Figure 2. The total number of sarcomeres/fiber in the CON and FC groups were very similar to one another. However, the total numbers of sarcomeres/fiber in the 0.25mm and 0.5mm DIS groups were significantly greater than that of the CON and FC groups (P<0.001). Additionally, there was a significant difference between the two distraction groups.

DISCUSSION

There are two unique findings of this study. First, the rodent SOL muscle appears to have a high capacity for longitudinal growth as exemplified by the fact that ~2700 sarcomeres (0.5 mm DIS group) were added in-series over a 4 wk period. This translates to ~4 sarcomeres/hour/myofibril. Assuming that each myofibril is ~1 µm in diameter and that the cross-sectional area of a fiber in the SOL muscle is ~2000 µm², then this equates to ~8,000 sarcomeres/hr/strip. If the longitudinal growth of muscle fibers occurs exclusively at the myotendinous junction as suggested by some, then it is clear that the myotendinous junction has a remarkable capacity for producing sarcomeres. The second key finding of this study is that the mean SLs in both the 0.25 and 0.5 mm DIS groups were very similar to one another. This finding might suggest the presence of a length-sensor (length-sensor hypothesis) that has a set-point of approximately 2.7 µm in the rodent SOL muscle. The length-sensor hypothesis proposes that stretch beyond a given sarcomere length activates cellular/molecular events that add sarcomeres in-series. Clearly, however, further studies are required to prove/disprove the existence of such a sensor.

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