

Overuse on muscles results in intramuscular fibrotic changes without preceding obvious muscle damage.

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Nothing to declare

Introduction:

Although many manual laborers and athletes who do not get adequate rest suffer from muscle pain, weakness, and stiffness caused by overuse, the pathology that occurs within the muscle has not been fully elucidated. In this study, muscle function and structural changes were examined over time using a model in which daily isometric contraction loading using electrical stimulation were applied to the rat's plantar flexor muscles.

Methods:

Six-week-old male Wistar rats were used, with the left leg on the overuse side using isometric contraction loading (ISO) and the right leg on the unloaded control side (CTL), and isometric contraction was induced by electrical stimulation using surface electrodes to the plantar flexor muscles, which were fixed at the ankle joint to a plantar plate connected to a tension transducer. The electrical stimulation protocol (4 sets of 5 contractions at 5-min intervals consisting of 2-s contraction with 100 Hz, 0.5 ms, 45 V every 6 s) with which the previous study showed that loading every other day had an effect on muscle hypertrophy was used as the overuse load every day for 2 weeks. isometric torque and 7-tesla MRI images of the plantar flexor muscles were measured daily, and gastrocnemius muscle was collected in time series for histological and immunohistochemical analyses. Two-way repeated measures ANOVA followed by Sidak's test and paired t-test were used to analyze differences between groups and the differences were considered statistically significant at $P < 0.05$ (*).

Results:

Maximal isometric torque was significantly decreased on the ISO from 4th day to the last day of loading compared to CTL. T2-weighted horizontal sectional images in MRI showed that a high-signal area appeared especially around proximal plantar flexor muscles of ISO from the 7th day of loading and low-signal area were mixed in on the last day of loading. In histological analysis of the gastrocnemius muscle after 2 weeks of loading, the Hematoxylin-Eosin-stained tissue sections on ISO showed areas of disruption of normal muscle tissue in 1/3 proximal and 1/3 central portion. The area was confirmed to be replaced by collagen fibers by Masson-Trichrome staining and the fibrotic area showed an increasing trend in ISO compared to CTL [1/3 proximal portion (CTL: 2.6 % vs ISO: 14.7 %, $P = 0.128$; 1/3 central portion (CTL: 2.5 % vs ISO: 18.6 %, $P = 0.036$)]. Immunohistochemical analysis using CD68 antibody showed the infiltration of M1 macrophages from 5th day in the gastrocnemius muscle from ISO. Evans blue dye staining, which sharply detects muscle damage, did not show any muscle damage in any of the gastrocnemius muscles from 1st to 5th day of loading.

Discussion:

We confirmed progressive onset of muscle dysfunction and fibrosis in the rat plantar flexor muscles after repeated isometric contraction loading every day. This overuse intensity used in this study is equivalent to daily high-intensity strength training without proper breaks, and the results are a warning to some athletes and workers. Previous studies of overuse have suggested that intramuscular degeneration is caused by a persistent inflammatory response initially induced by micro injury to the muscle.

Significance/Clinical Relevance:

This study newly suggests that overuse has a mechanism of inducing inflammatory reactions other than muscle damage, and that it may be a potential site for therapeutic intervention.

Figure 1

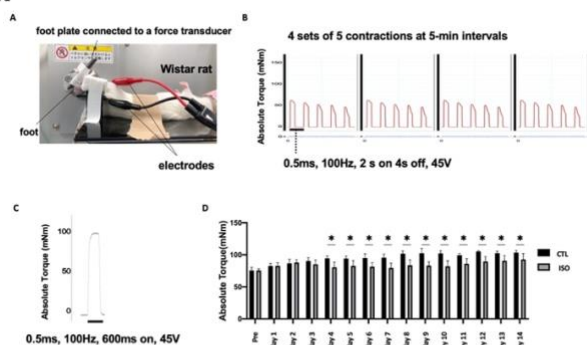


Figure 1 Experimental apparatus for overuse with isometric contraction loading on rat plantar flexor muscles. Daily isometric contraction loading decreased in situ torque of plantar flexor muscles from day 4 (A). Photograph of the setup for isometric contraction loading. Plantar flexor muscles were stimulated supramaximally (45V) via a pair of surface electrodes with foot secured in a foot plate connected to a torque sensor at an angle of 40° dorsiflexion (i.e., 50° relative to the tibia). (B). Typical torque traces of isometric loading consisting of 4 sets of 5 contractions. The protocol was performed daily for overuse. (C). Representative original records 100-Hz tetanic in situ torque in plantar flexor muscles in vivo from control leg (CTL). (D). Average absolute torque of in situ torque in plantar flexor muscles decreased in isometric contraction (ISO) group compared to CTL group from day 4. * $P < 0.05$ with two-way repeated-measures ANOVA with Sidak's post hoc test.

Figure 2

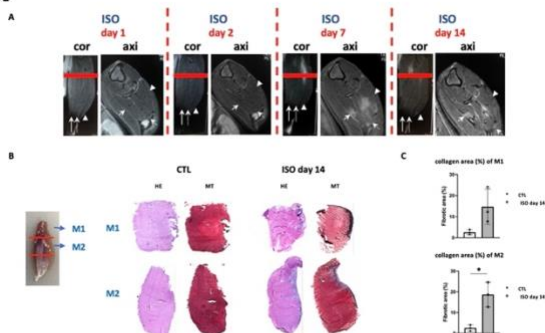


Figure 2 Daily isometric contraction loading gradually caused MRI changes around the proximal plantar flexor muscles and intramuscular fibrotic area in the gastrocnemius muscle. (A). Representative MRI images of muscles loaded with daily isometric contraction protocol for overuse (ISO). All T2-weighted MRI images show coronal (left panels) and axial (right panels) planes. Solid arrows: medial gastrocnemius muscle. Arrowheads: lateral gastrocnemius muscle. Dotted arrows: plantaris muscle. Axial planes are the muscle belly level of the plantar flexor muscles indicated by the red line in coronal planes. (B). Hematoxylin and eosin staining (HE) and Masson's trichrome staining (MT): representative images of the muscles from control (CTL) and isometric contraction loading for overuse (ISO) for 14 days. M1: proximal 1/3 portion of medial gastrocnemius muscle. M2: central 1/3 portion of medial gastrocnemius muscle. (C). Quantitative analysis of the fibrotic area of the muscles from CTL and ISO day 14 group using MT. * $P < 0.05$ with paired t-test.

Figure 3

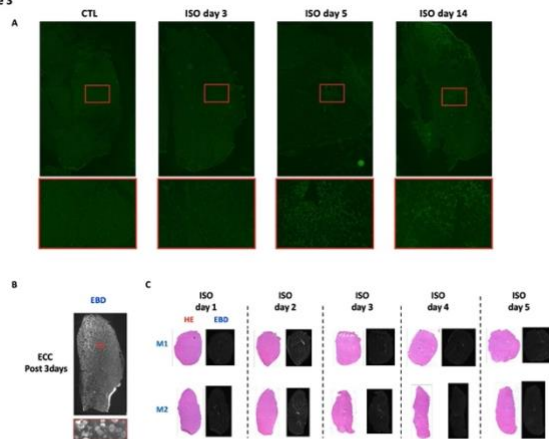


Figure 3 Daily isometric contraction loading induced infiltration of M1 macrophages from day 5 in the gastrocnemius muscle but showed no obvious muscle damage. (A). Representative immunofluorescence staining for CD68 in control (CTL), isometric contraction loading for overuse (ISO) for 3 days, ISO for 5 days, and ISO for 14 days groups. Lower panels are enlarged views squared in upper panels. (B). Histological reference images of serial transverse sections of medial gastrocnemius muscle stained with Evans blue dye (EBD) from muscles 3 days after damaged by single eccentric contraction (ECC) for comparison with the overuse protocol of the present study. Lower panels are enlarged views squared in upper panels. (C). Hematoxylin and eosin staining (HE) and EBD: representative images of the muscles from control (CTL) and isometric contraction loading for overuse (ISO) from 1 to 5 days. M1: proximal 1/3 portion of medial gastrocnemius muscle. M2: central 1/3 portion of medial gastrocnemius muscle.