Disuse-Atrophy Exacerbates Denervation in Aged Rats

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Abstract

Older humans fail to recover skeletal muscle mass and function after muscle loss due to bedrest, which contrasts with young and adult humans. These failed periods of recovery accelerate muscle loss, and the associated increases in morbidity and mortality in the aged population. Disorders disrupting the neuromuscular junction, the synapse where motor nerves meet muscles, are associated with age-related muscle atrophy and dysfunction. We hypothesized that periods of disuse-atrophy will exacerbate neuromuscular pathologies in aged rats. 28-month-old rats were hindlimb unloaded, a condition where the rats cannot put weight on their hindlimbs, for 14 days to induce disuse-atrophy. 28-month-old weight-bearing rats were used as controls. Muscle wet weights were measured at sacrifice. We measured Acetyl Choline Receptor Endplates, and motor neurons via immunofluorescence. We assessed acetyl choline receptor endplate area, acetyl choline receptor endplate fragmentation, and denervation of the neuromuscular junctions. Oxylipins, oxidized lipid signaling molecules, were measured in gastrocnemius muscle at the University of California San Diego Lipidomics Core. We performed a student's t-test for statistical analysis. Muscle wet weights for gastrocnemius, soleus, plantaris, tibialis anterior, and quadricep were 20-40% lower in rats that were hindlimb unloaded when compared to weight bearing controls. Acetyl choline receptor area and fragmentation were 20% higher in gastrocnemius from hindlimb unloaded rats when compared to weight bearing controls. Denervation was 30% higher in the gastrocnemius from hindlimb unloaded rats when compared to weight bearing controls. The muscle oxylipin profile in aged weight bearing and adult hindlimb unloaded rats were significantly altered when compared to adult weight bearing rats. However, muscle oxylipin profile was not different when comparing aged and aged hindlimb unloaded muscle. These data show that hindlimb unloading exacerbates neuromuscular pathologies in aged rats. Therapies that protect neuromuscular junctions may help improve recovery following disuse-atrophy in aged subjects.

Background

- Aging is associated with neuromuscular junction pathologies.
- Neuromuscular junction pathologies contribute to sarcopenia.
- Older adults often fail to recover from bed rest.

Purpose

To explore if disuse-atrophy exacerbates denervation in aged rats

Experimental Design

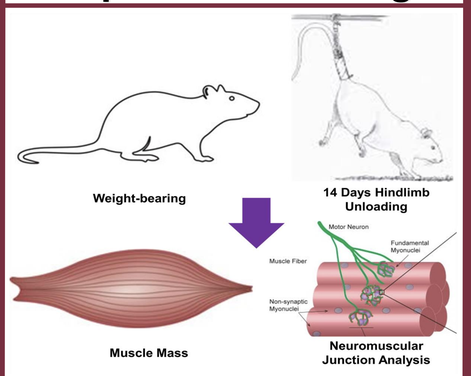

Figure 1. Muscle wet weights in aged control and hindlimb unloaded rats.

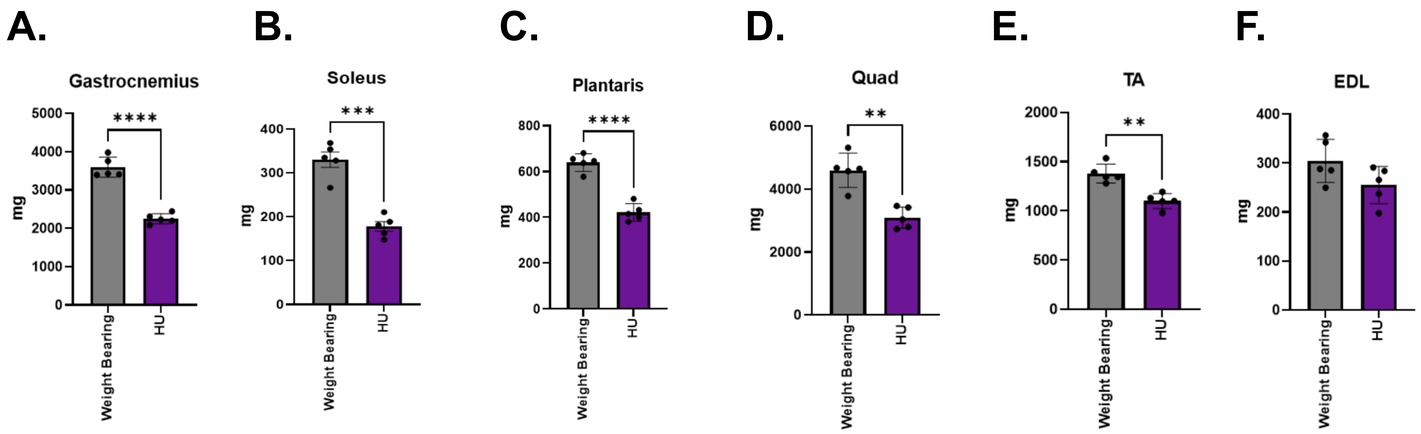

Figure 1. Skeletal muscle mass in control and hindlimb unloaded rats. **A.** Gastrocnemius mass. **B.** Soleus Mass. **C.** Plantaris Mass. **D.** Quadricep Mass. **E.** Tibialis Anterior Mass. **F.** Extensor Digitorum Longus Mass. Male rats were used. N=5 per group. * means that alpha was <0.05.

Figure 2. Neuromuscular analysis in aged control and hindlimb unloaded rats.

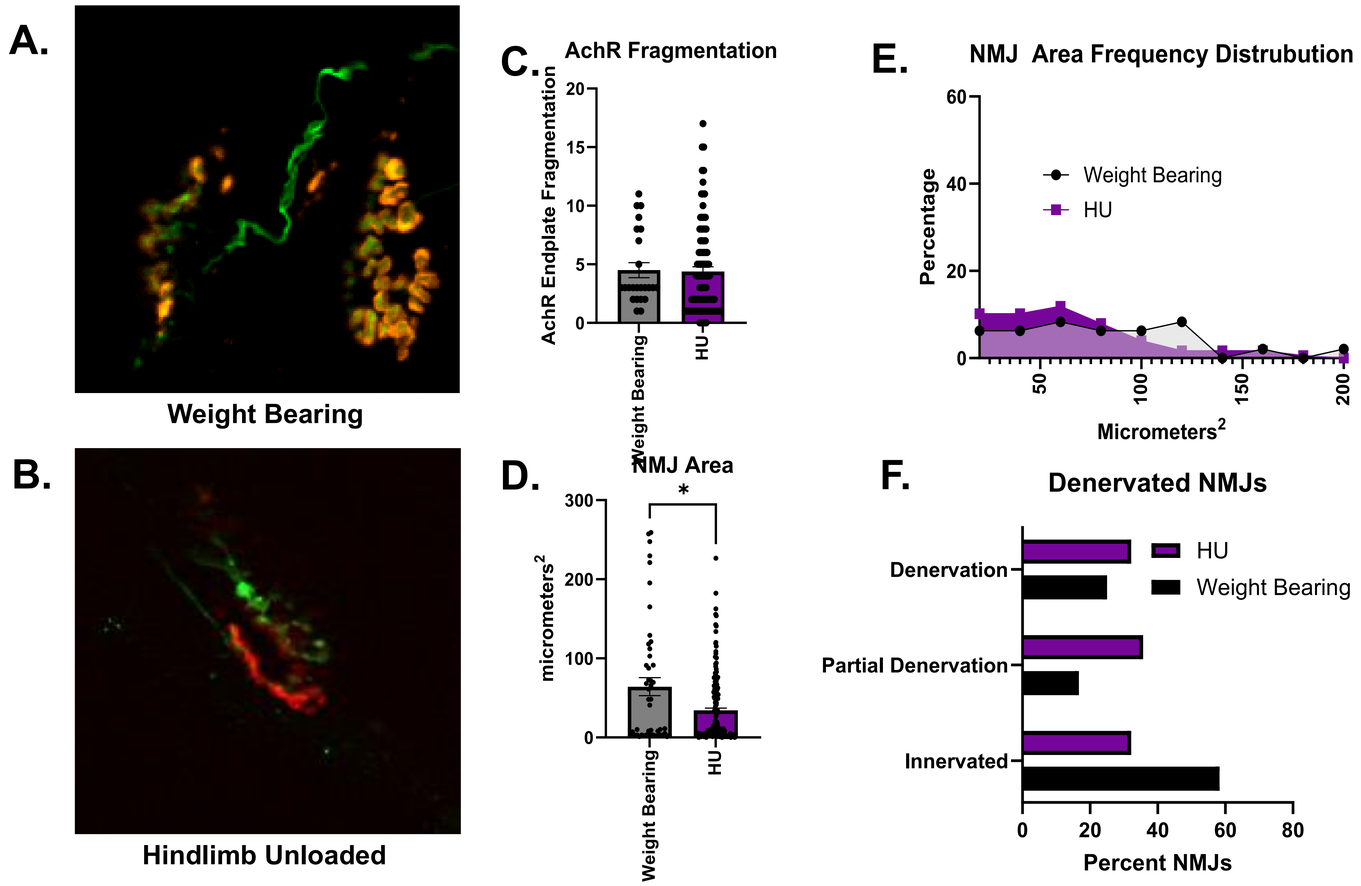

Figure 2: Neuromuscular junctions in control and hindlimb unloaded rats. **A.** Receptors in Weight Bearing. **B.** Receptors in Hindlimb. **C.** Acetylcholine Receptor Fragmentation. **D**. Neuromuscular Junction Area. **E.** Neuromuscular Junction Area Frequency **F.** Denervation of Neuromuscular Junctions. Male rats were used. N=5 per group. * means that alpha was <0.05.

Methods

- Rats were hindlimb unloaded for 14 days.
- Muscle tissue was extracted, weighed, and snap-frozen in liquid nitrogen.
- Immunohistochemistry was used to measure motor neurons and acetylcholine receptor endplates in gastrocnemius muscle tissue.
- Endplate area, endplate fragmentation, and denervation were measured based on immunohistochemistry.

Statistical Analysis:

A Student's t-test was employed as the global analysis for each dependent variable. The comparison-wise error rate (α) was set at 0.05.

Results

Gastrocnemius, soleus, extensor digitorum longus, tibialis anterior, plantaris, and quadriceps muscle wet weights were significantly lower in hindlimb-unloaded rats compared with weight-bearing rats. Denervation was higher in the hindlimb-unloaded gastrocnemius compared with the weight-bearing gastrocnemius. Acetylcholine receptor endplate fragmentation was also higher in the hindlimb-unloaded gastrocnemius compared with the weight-bearing gastrocnemius. The oxylipin profile was altered in aging and hindlimb unloading.

Conclusions

Hindlimb unloading exacerbated neuromuscular junction pathologies in aged rats. Our next step is to test whether these neuromuscular junction pathologies prevent recovery from disuse atrophy in aged rats.

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References

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