FUNCTIONAL ADAPTATIONS OF THE NEUROMUSCULAR SYSTEM IN RESPONSE TO UNLOADING

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Abstract

The effects of microgravity on the human body include muscle unloading or disuse, which reduces musculoskeletal mass and strength via atrophy. This change is accompanied by a cascade of physiological adjustments to accommodate the dynamic needs of the body, changing the functional demand on the skeletal muscles and the plasticity of neuromuscular junctions (Deschenes et al., 2005). In this study, the effect of unloading on postural soleus muscles in developing neuromuscular systems is compared to the effect in fully developed systems. Immunofluorescent staining techniques were used to examine fiber composition profiles for soleus muscle collected from juvenile and mature rats exposed to muscle unloading or control conditions for 2 weeks. Results indicate that disuse has similar effects on both adult and juvenile muscles, with atrophy being more severe in type I fibers.

Introduction

Skeletal muscle enables the human body to perform a variety of movements and functions, with the gamut of these functions being determined by the size and quantity of the types of fibers that make up a given muscle. Fiber types are frequently divided into three categories: Slow oxidative (SO, type I), fast-oxidative-glycolytic (FOG, type IIa), and fast-glycolytic (FG, type IIb) based on the variation of their myosin heavy-chain isoforms. This delineation is based on fundamental biochemical and metabolic differences reflecting different functionality of the muscles. Changing demands are accommodated within the neuromuscular system via remodeling the compositional profile of a given muscle, called a fiber-type transition, or by a change in size of the myofibers (hypertrophy or atrophy). Previous studies have indicated that disuse leads to a shift from slow to fast fiber composition, which accounts for an observed increase in contractile speed in unloaded fibers (Nemirovskaia, 2005, Kasper, 1993). In this study, soleus muscles are examined with the intention of understanding the consequences of long-term muscle unloading on muscle composition during a period of significant neuromuscular development, an area previously unstudied. As the primary postural muscle in both rodent models and humans, the soleus is most affected by hindlimb suspension (Hennig, 1985). It is thus expected that soleus muscles from juvenile animals would show more significant deterioration than control counterparts when subjected to identical unloading. The long-term disuse associated with the hindlimb suspension (HS) model closely simulates the disuse associated with microgravity, with physiological effects including cephalic fluid shift and atrophy of muscles (Globus & Morey-Holton, 2016). Understanding the effect of disuse on neuromuscular plasticity in early development is vital to the future of crewed spaceflight, long-term missions to other planets, and to developing treatments for earth-bound patients undergoing disuse secondary to illness or injury.

Methods

Juvenile and adult male rats were randomly assigned to either hindlimb suspension treatment or sedentary control groups, such that n=10 for each of the four treatment groups except juvenile HS (n=11). Hindlimb suspension animals were subjected to non-weight bearing conditions for two

weeks via suspension of the posterior end of the animal. The control group used all four limbs and did not undergo any irregular circumstances. Following the two-week treatment period, the animals were anesthetized, and the whole soleus muscle was surgically removed, weighed, and prepared for freezing at -80℃ for later sectioning and staining. Pre- and post-unloading intervention masses are shown in Table 1, alongside the wet weights for the removed soleus muscles.

	Pre, Body mass*	**Post, Body mass*†‡**	**Soleus, wet wt*†‡**
Juvenile CTL	161.9 ± 3.7	244.1± 6.6	119.3 ± 4.9
Juvenile HS	162.1 ± 3.0	233.1 ± 4.8	83.5 ± 6.7**
Mature CTL	358.7 ± 3.4	382.1 ± 5.8**	188.1 ± 8.9**
Mature HS	362.3 ± 4.2	340.6 ± 7**	119.6 ± 9.5

Table 1. Body mass (g) before (pre) and after (post) the 2-week unloading intervention and soleus whole muscle weight (mg) after that intervention

**Indicates significant (P ≤ 0.05) main effect for age (Mature > Juvenile).*

†Indicates significant (P ≤ 0.05) main effect for treatment (CTL > HS).

‡Indicates significant (P ≤ 0.05) interaction between age and treatment and Indicates significant (P ≤ 0.05) main effect for treatment (CTL > HS).

***Indicates significant (P ≤ 0.05) difference from all other groups.*

CTL, control; HS, hindlimb suspension

Muscles were cut into 10µm transverse slices and fixed to 3% EDTA slides to prevent contraction. A pap pen was used to fabricate hydrophobic wells, and tissue sections were washed with phosphate-buffered saline solution (PBS) with 1% bovine serum albumin (BSA). The primary antibody cocktail was applied to the selections and allowed to incubate for 60 minutes in a humidified chamber at 37℃. The cocktail contained a supernatant of BA-D5 for type I fibers (1:10) and SC-71 for type II fibers (1:1) with PBS + 1% BSA. Subsequent to incubation, tissue sections were washed with PBS + 1% BSA for 3 x 5minutes. The second incubation included a supernatant of secondary antibodies for type I (Alexa 555 IgG2b, 1:500), type IIa (Alexa 350 IgG1) with PBS + 1% BSA. Again, tissue sections were washed 3 x 5 minutes and allowed to dry before being treated with ProLong and covered with a coverslip for imaging. Myofiber profiles were examined via microscopy (Olympus BX41, 40x). Fiber cross-sectional area images were collected using Infinity Analyze software (Luminera Corporation, Ottawa, ON). Individual images were digitally combined to produce an overlay displaying the fluorescence of all fibers within the field, with type IIX fibers shown as the unstained areas (see Figure 1).

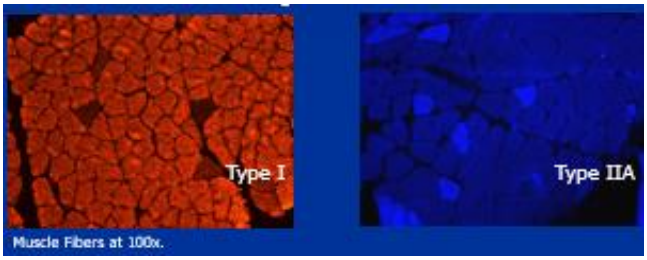

Figure 1. Fluorescent images of type I and type IIA fibers

A random sample of 125-200 myofibers from each muscle were selected and measured for the cross-sectional area of each fiber and overall fiber-type composition in order to determine the extent of atrophy and degree of remodeling in response to disuse. Myofibers were categorized as type I, type IIa. Average fiber count and size were recorded.

Results

In an analysis of the body mass data collected, mature rats weighed more than juveniles both before and after 2 weeks of hindlimb suspension. Unloading elicited a decline in body mass for both age groups, also revealing interactive effects that indicated mature control rats weighed more (post 2 weeks) than all other treatment groups. A factorial

ANOVA with main effects for age (adult and juvenile) and treatment (control vs. HS) was performed to ascertain the differences in the age group's responses to unloading. An overview analysis (with all fiber types combined) showed significant (P<.0001) main effects for both age and treatment but showed no significant interaction. In a closer analysis, unloading-induced atrophy in adult animals was 20%, compared to 36% among juveniles. Within each age category, unloading had a greater effect on juvenile animals than on adult animals. This is further evidenced by an analysis of the Cohen effect size. An effect size (ES) of 1.4 for the adult muscles indicates a large effect (of the treatment, HS). An even larger effect (ES=2.0) is seen for the juvenile muscles. When quantifying only type I fibers, age (P<.0001) and treatment (P=.00165) displayed significant effects, but again, no significant interaction (P=.2764). Thus, a relative difference can be seen in the effects of unloading as determined by effect size, where HS brought about larger atrophy in juveniles compared to adults (40% and 11%, respectively). Type IIa fibers indicated a trend of a main effect for treatment (P=.0723) but no significant interaction (P=.6949) or significant impact of age group (P=.4485).

Discussion

The main focus of the investigation was to determine the effects of hindlimb suspension on juvenile versus adult animals. This was done by morphometric analysis of myofiber profile with immunofluorescent staining. Findings suggest that adult and juvenile muscles showed similar, but not the same, degrees of atrophy following unloading. This was true when fiber types were pooled together and when isolated. However, the use of effect size analysis indicated that the atrophying effect of muscle unloading was more severe in juvenile compared to mature muscles. Following a previous study within our laboratory (Deschenes et. al, 2004), these results support the assertion that synapses of the neuromuscular system may be more sensitive to unloading (and thus microgravity) than the myofibers that make them up. The acutely responsive soleus muscle does not appear to undergo statistically significant myofiber remodeling despite significant spaceflight-induced alterations to the neuromuscular junction (Deschenes, 2004). Overall, fiber results showed type-specific results, with type I fibers showing significant atrophy and type II showing very little to none. This suggests that given the vital postural role type I fibers play, smaller type I fibers will undergo greater strain to maintain an upright position, impairing the ability to carry out mission-specific tasks. Specific exercise performed to maintain strength and size of type I fibers must be done during flight to allow flight personnel to carry out assigned tasks during long-duration space missions. These data may assist in the mitigation of microgravity's deleterious effects and aid in the development of rehabilitation following atrophy from disuse on earth and following return from spaceflight.

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