Effects of aging on unloading-induced skeletal muscle atrophy and subsequent recovery in rats

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Abstract Aged individuals with physiological muscle atrophy (sarcopenia) are likely forced into an unloading condition of inactivity such as bed rest and limb-immobilization. Accumulated experimental evidence, obtained with rats, indicates no definite aging effects on the extent of unloading-induced muscle atrophy. However, various suggestions can be deduced from experimental evidence as guidelines for the development of a combined intervention of exercise, nutrition and physical therapies that effectively maintain skeletal muscle as healthy as possible in aged individuals under unloading condition. The deduced guidelines could be summarized as 1) during the unloading period, atrophy and degeneration of especially slow muscle fibers should be attenuated, 2) in the early stage of the reloading period, massive muscle damage should be avoided, and 3) in the recovery period, re-growth of especially slow muscle fibers should be accelerated. This review also emphasizes that experimental evidence is still lacking, indicating the urgent need to stimulate research interest in the combined effects of aging and immobilization so as to improve health longevity in aging societies.

Keywords : aging, skeletal muscle, unloading, reloading, degeneration and regeneration

Sarcopenia and health longevity

Aging physiologically decreases mass and strength of skeletal muscles, inducing a condition referred to as sarcopenia. Since skeletal muscle is crucial to our locomotive ability, its dysfunction critically affects our activities of daily living. In addition, impaired locomotive activity leads to lack of physical activity, which is necessary to prevent metabolic syndrome. Therefore, in an aging society, maintenance of healthy muscle mass and function is a key to achieving health longevity.

The promotion of daily physical activities with adequate nutrition is well documented to be a low-cost convenient remedy for the maintenance of healthy muscle. However, with the advancement of age, one generally develops compound illnesses and disabilities that may impose limitations on one’s physical activity; for instance, prolonged bed rest in hospitals due to a lengthy recovery period. In surgical cases, with direct damage to skeletal muscle tissue or immobilization, the situation is more serious.

In this paper, we summarize the experimental findings obtained with rats on the effects of limited physical activity on aged skeletal muscle including the recovery process. Our objectives are to advance academic interest on the effects of aging combined with limitations on physical activities.

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Hindlimb unloading as an immobilization model

A hindlimb-unloading (HU) model has been used frequently in small animals. This model limits locomotive activities and reduces resistance load to skeletal muscles. Therefore, HU is a useful model to simulate changes in skeletal muscles induced by a condition of limited physical inactivity such as bed rest. Many reports have demonstrated that HU results in deficits in mass and force of unloaded skeletal muscle accompanying a slow to fast fiber type transition. HU markedly affects anti-gravitational slow-twitch muscles such as soleus compared to fast-twitch muscles.

Effect of aging on skeletal muscle atrophy with HU

What are the effects of aging on HU-induced muscle atrophy? Table 1 summarizes the degree of atrophy with HU in young and aged rats. Also, Table 2 summarizes the results obtained with F344 X Brown Norway hybrid F1 rats having the phenotype of the age-related changes of humans. Some reports found greater rates of atrophy, while others reported similar or smaller rates in aged rats. Overall, aging does not seem to be a definite modifier of HU-induced muscle atrophy.

In F344 X Brown Norway hybrid F1 rats, Hwee DT and Bodine SC reported that 26-30 month-old rats, compared with 9 month-old rats having peak muscle...
mass, showed marked muscle loss in the plantaris, medial gastrocnemius, and tibialis anterior muscles; and a relatively slight, but significant loss in the soleus and extensor digitorum longus. However, their age-dependency may be negligible when evaluated relative to pre-HU values. F344 X Brown Norway hybrid F1 rats more than 30 months old have already developed apparent aging-related sarcopenia.

**Compound effect of aging and inactivity**

Even if aging has no definite influence on HU-induced muscle atrophy, there is no doubt that the absolute mass of skeletal muscle decreases considerably with HU. In practical circumstances, such inactivity-induced muscle atrophy will cause serious problems for elderly individuals, because it aggravates locomotive dysfunction due to

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**Table 1.** Effect of aging on skeletal muscle atrophy (% loss) after hindlimb unloading in young adult and old rats.

<table>
<thead>
<tr>
<th>Age of animal</th>
<th>Gender and strain of animal</th>
<th>Experimental Period</th>
<th>Parameter</th>
<th>Muscle</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>9M 25M</td>
<td>Male F344</td>
<td>14 days HU</td>
<td>FCSA</td>
<td>SOL</td>
<td>Deschenes MR et al, 2012. 12)</td>
</tr>
<tr>
<td>8M 23M</td>
<td>Male F344</td>
<td>14 days HU</td>
<td>Protein content</td>
<td>PLA</td>
<td>Stump CS et al, 1997. 13)</td>
</tr>
<tr>
<td>3M 22M</td>
<td>Female Wistar</td>
<td>21 days HU</td>
<td>Muscle mass</td>
<td>MG</td>
<td>Simard C et al, 1985. 14)</td>
</tr>
<tr>
<td>3M 26M</td>
<td>Male F344</td>
<td>21 days HU</td>
<td>Muscle mass</td>
<td>SOL</td>
<td>Always SE et al, 2001. 15)</td>
</tr>
<tr>
<td>10M 20M 24-26M</td>
<td>Male F344</td>
<td>21 days HU</td>
<td>Muscle mass</td>
<td>PLA</td>
<td>Pooled data by authors. 16-20)</td>
</tr>
<tr>
<td>8M 22M</td>
<td>Male F344</td>
<td>28 days HU</td>
<td>FCSA</td>
<td>MG</td>
<td>Deschenes MR et al, 2001. 21)</td>
</tr>
</tbody>
</table>


**Table 2.** Effect of aging on skeletal muscle atrophy (% loss) after hindlimb unloading or immobilization in young adult and old F344×Brown Norway F1 hybrid rats.

<table>
<thead>
<tr>
<th>Age of animal</th>
<th>Experimental Period</th>
<th>Parameter</th>
<th>Muscle</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>6M 30M</td>
<td>7 days HU</td>
<td>Tetanic tension</td>
<td>SOL</td>
<td>Brown M &amp; Taylor J, 2005. 22)</td>
</tr>
<tr>
<td>12M 30M</td>
<td>7 days HU</td>
<td>Muscle mass</td>
<td>PLA</td>
<td>Thompson LV et al, 1998. 23)</td>
</tr>
<tr>
<td>3-4M 30-31M</td>
<td>10 days IM</td>
<td>Muscle mass</td>
<td>MG</td>
<td>Pattison JS et al, 2003. 24)</td>
</tr>
<tr>
<td>6M 30M</td>
<td>14 days HU</td>
<td>Muscle mass</td>
<td>PLA</td>
<td>Stiu PM et al, 2005. 26)</td>
</tr>
<tr>
<td>9M 30M</td>
<td>14 days HU</td>
<td>Muscle mass</td>
<td>SOL</td>
<td>Hwee DT &amp; Bodine SC, 2009. 27)</td>
</tr>
<tr>
<td>4M 39M</td>
<td>21 days HU</td>
<td>Muscle mass</td>
<td>MG</td>
<td>Always SE et al, 2001. 15)</td>
</tr>
</tbody>
</table>

HU: Hindlimb unloading, IM: Immobilization, MCSA: Muscle cross sectional area. SOL: soleus, PLA: plantaris, MG: medial gastrocnemius. Bold and italicized notations represent greater loss in old and young adults, respectively.
aging-related sarcopenia. The aging-related sarcopenia initially affects fast-twitch muscle fibers, leaving slow-twitch muscle fibers relatively unaffected\(^2\)\(^7\),\(^28\). HU-related sarcopenia, on the other hand, almost selectively affects slow-twitch muscle fibers\(^2\)\(^6\)\(^9\). Concerning the preservation of the quality of life of elderly individuals with sarcopenia, the atrophy of slow-twitch muscle fibers may be more serious than that of fast-twitch muscle fibers.

It is known that HU causes not only fiber atrophy, but also fiber degeneration to form core-like lesions, ragged red fiber, and sarcoplasmic coagulation\(^\text{29-33}\). We compared the degeneration of muscle fibers in the deep region of lateral gastrocnemius muscle after 3 weeks of HU in young adults (aged 5 months) and old (aged 24-26 months) rats (Fig. 1)\(^\text{20}\). Sarcoplasmic coagulation and the loss of myofibrilar proteins were seen after HU in both ages; and the degree of the degeneration was clearly more remarkable in the advanced age period than in the young adult age period. Such fiber degeneration occurs mainly in type I fibers of soleus\(^\text{29-32}\), adductor longus\(^\text{33}\), and the deep region of gastrocnemius\(^\text{20}\) muscles. Such significant degeneration is likely to impair specific force capacity at single fiber\(^\text{23}\), as well as at whole muscle\(^\text{16}\) levels to further diminish locomotive abilities of the elderly. Since oxidative stress, apoptotic signaling\(^\text{25,34-37}\), inactivation of myogenic regulatory factors\(^\text{15}\), and decreases of mitotic activity and satellite cells\(^\text{38}\) are considered to be involved in the process of atrophy and degeneration, supplementation of appropriate nutrients may be needed to decelerate such HU-induced changes.

### Delayed recovery during reloading

Although atrophy during the HU period is not evident (Table 1 and 2), the delayed recovery of aged muscle mass during the reloading period after HU\(^\text{27}\) and limb-immobilization\(^\text{24,38,39}\) have been well documented. When functional changes during a 3-week period of HU were observed in the soleus muscles of young (4-5 months) and old (20-21 months) rats (Fig. 2)\(^\text{40}\), twitch duration (time to peak tension plus half-relaxation time) was found to shorten with HU in both ages. During the subsequent reloading period, the twitch duration was restored to the original level after a 3-week reloading period in young adult rats, but not in old rats. Therefore, the recovery from atrophy and the slow-to-fast shift of muscle property caused by HU are considered to be delayed or limited in the advanced ages.

As mentioned previously, muscle fiber degeneration occurs during the HU period more severely in the muscle of aged rats. Aside from this degeneration, it is known that the muscle fibers are damaged at an early stage of reloading after HU\(^\text{41,43}\), inducing a significant force decline after reloading\(^\text{40}\). This is considered to indicate the structural vulnerability of unloaded muscle fibers. As for aging effects on damages, we have no sufficient evidence. In any

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**Fig. 1** Pathological disorders in hindlimb-unloaded muscle fibers in deep regions of lateral gastrocnemius. Upper and lower panels represent cross sections from young adult (aged 5 months) and old (aged 24-26 months) rats. White dots in each row represent identical muscle fiber. Sarcoplasmic coagulation and myofibrilar disruption were observed in both ages, but more profoundly in old rats. Degenerated fibers are lightly stained with ragged red in trichrome staining, and darkly stained with some vacuoles in NADH-TR staining. Deterioration of myofibrilar sarcomere structure was evident from immunoreactions against anti α-actinin antibody.
event, recovery from such damages would be retarded in aged muscle because several studies indicate that muscle of old rats doesn’t regenerate as rapidly as that of young adult rats after severe damage45-47) probably due to the limited regenerating capacity of muscle fibers of aged muscle.

Maintenance of normal skeletal muscle function in the advanced age period

It goes without saying that it is necessary to have a continuous suitable exercise load to maintain normal function of the skeletal muscle throughout life. However, in specific unloading conditions such as bed rest and limb-immobilization, the quality and quantity of the exercise load should be adjusted with necessary nutritional support and other interventions.

The key points to maintaining healthy skeletal muscle in aged individuals who are in unloading conditions such as bed rest or limb-immobilization would be summarized as follows: 1) Attenuate atrophy and degeneration in muscle fibers during the unloading period with special care to preserve slow-twitch muscle fibers. 2) At the early stage of the subsequent reloading period, prevent massive damage to muscle fibers. 3) During the recovering period, promote the re-growth of muscle (especially slow muscle fibers). Before, during, and after unloading, effective countermeasure interventions such as exercise, nutritional, and thermal conditionings should be actively carried out12,16-18,20,22,29,32,36,39,40,43,44,48-54 (Fig. 3).

Need of experimental evidence

Active epidemiologic studies are now comprehensively searching for probable factors affecting the health longevity of humans. On the other hand, rigid experimental findings from aged animals are still seriously lacking, which are necessary for finding effective countermeasures (combination, order, and quantity of various kinds of interventions), for improving the health of the elderly in aging societies.

References


