Roles of satellite cells and/or myonuclei in the regulation of morphological properties of anti-gravitational skeletal muscle in response to mechanical stress

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Abstract

It is well-reported that the morphological properties of skeletal muscles or muscle fibers, which are influenced by the level of protein synthesis and/or degradation, are regulated in response to mechanical load. However, the precise mechanism responsible for such phenomena is not fully understood yet. Changes of the distribution of satellite cells and/or myonuclei have been also noted in atrophied or hypertrophied skeletal muscle fibers, suggesting that the number and/or function of these parameters play essential roles in the regulation of morphological properties of muscle and muscle fibers. Thus, the roles of satellite cells and/or myonuclei in the regulation of morphological properties of anti-gravitational muscle, soleus and adductor longus, in response to the level of mechanical stress, with or without association of macropage-related factors, were briefly reviewed. It was suggested that a regulatory network among macrophage, interleukin-6, heat shock transcription factor 1, and activation of transcription factor 3 may play a crucial role for the modulation of skeletal muscle mass and function, which are also influenced by activation of satellite cells and distribution of myonuclei. ©2020 Jpn. Soc. Biol. Sci. Space; doi:10.2187/bss.34.1

Keywords: soleus and adductor longus muscles, satellite cells, myonuclei, growth, atrophy and regrowth, mechanical load level

Introduction

Muscle satellite cells are myonuclear precursors lying between sarcolemma and basal lamina of myofiber (Mauro, 1981), and the myonuclear accretion occurs through the incorporation of satellite cell nuclei into the growing myofibers (Moss and Leblond, 1971). Quiescent satellite cells are adhered to the myofiber with M-cadherin (Irintchev et al., 1994), and proliferation and differentiation are controlled by several growth factor families (Dusterhoft and Pette, 1999; Sheehan and Allen, 1999) or nitric oxide (Anderson, 2000). It is also reported that satellite cells are activated, when the muscle is over-loaded (Dangott et al., 2000) or injured (Schultz et al., 1985).

Satellite cells and myonuclei play important roles in the regulation of morphological properties of skeletal muscle fibers in response to growth in ground-based 1-G environment (Kawano et al., 2008; Mozdziaκ et al., 2000; Ohira et al., 2001), unloading (Ohira et al., 2011; Wang et al., 2006) and/or over-loading (Ohira et al., 2011; Wang et al., 2006). Here we briefly reviewed the roles of satellite cells and/or myonuclei in the regulation of morphological properties of skeletal muscle in response to the level of mechanical stress applied to the muscle.

I: Growth and development of muscle and muscle fibers

Roles of gravitational loading in the differentiation and development of fiber formation and growth in soleus muscle of Wistar rats during the developing period were studied (Kawano et al., 2008). The pups were separated randomly into cage control and hindlimb-unloaded groups 4 days after birth. Four sets of 5-hr hindlimb unloading per day, with 1-hr interval for nursing by their mother, were performed until the postnatal day 21, as was reported elsewhere (Ohira et al., 2001). Pups in the control group were also separated from their mother and followed the same feeding schedule. After day 21, both groups of rats were separated from their mother, but the hindlimb unloading was performed continuously until month 3.

Morphological properties

The morphological properties of soleus muscle in male rats before (day 4 after birth) and at the end of 3-month unloading or normal housing, and 1, 2, and 3 months after ambulation recovery were analyzed in whole muscle and/or single fibers sampled from tendon-to-tendon. Growth-associated increases of muscle (Fig. 1A) and muscle fiber cross-sectional area (CSA, Fig. 1C) in the unloaded group were ~69% and 68% less than the age-matched controls at the end of 3-month unloading (Kawano et al., 2008). The mean fiber CSA level in the cage controls, which was increased ~33 times during the growth from day 4 to month 3, remained constant during the 3-month cage housing. However, fiber CSA in the unloaded group gradually increased during 3-month ambulation recovery, even though the mean level was still less than the age-matched controls (p<0.05).

The total fiber number in whole soleus 4 days after birth was ~800 (Fig. 1B). The number was increased to...
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−2,500 after 3-month growth both in cage control and hindlimb-unloaded groups. The growth-associated increases in number (Fig. 1B) and length and sarcomere number (Fig. 1D) of fibers were not influenced by unloading. The increase of fiber length during unloading may be due to the stretching caused by elongation of bone (Ohira et al., 2006). It was also speculated that the increase of fiber number may be programmed before the postnatal day 4.

It was reported that soleus was stretched during sedentary quadrupedal position on the floor, but was passively shortened due to plantar-flexion of ankle joint during hindlimb unloading (Kawano et al., 2004; Ohira et al., 2002; Riley et al., 1990; Wang et al., 2006). Tension development (Fig. 2A) and sarcomere length (Fig. 2B) were decreased in response to the plantar-flexion of ankle joint during acute hindlimb suspension (Kawano et al., 2004). However, the sarcomere length was recovered due to the remodeling of sarcomeres, associated with decrease in the total sarcomere number per fiber after 14 days of continuous unloading (Fig. 2C). These results indicate that growth-related increase of fiber CSA, not the number and length of fibers, is closely influenced by the mechanical load applied to muscle fibers.

Properties of satellite cells and myonuclei

The growth-related increase of the number of myonuclei (Fig. 3A), quiescent (Fig. 3B) and mitotic active satellite cells (Fig. 3C) were inhibited by unloading (Kawano et al., 2008). All of the satellite cells were mitotic active at day 4. The number of mitotic active satellite cells in the cage controls tended to decrease during the 3-month growth (20%, p>0.05). It was further decreased (80%, p<0.05) by chronic unloading, but was gradually increased during 3-month ambulation recovery. The increase of myonuclear number during 3-month unloading was only 40 times vs. 92 times in the cage controls compared with the number at day 4. It was reported that the distribution of myonuclei and/or satellite cells in atrophied soleus muscle fibers was less in space-flown rats (Allen et al., 1996) or hindlimb-suspended rats (Dupont-Versteegden et al., 2006; Oishi et al., 2008) and mice (Guo et al., 2012) due to apoptosis. However, inhibition of the growth-associated increase of myonuclear number seen in the rats hindlimb-suspended chronically during growing period was not related to apoptosis, detected by terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) method (Kawano et al., 2008). Unloading-related inhibition of the increase of myonuclear number may be caused by restrained accretion due to the lower number of satellite cells.

Large myonuclei (Fig. 4A and B) with lower DNA concentration (Fig. 4D) were also noted at the end of 3-month unloading, even though the absolute content of DNA within a single myonucleus was normal (Fig. 4C) (Kawano et al., 2008). Myonuclei with low concentration of DNA caused by increase of myonuclear size,
It was reported that cross-sectional fiber growth during the postnatal day 3 and 21 in mouse extensor digitorum longus muscle, which is composed of fast type fibers, was closely associated with a rapid 5-fold increase of myonuclear number (White et al., 2010). But further increase of myonuclear number was not noted after the day 21 and the myonuclear domain size was elevated thereafter, indicating that the function of each myonucleus was improved. However, it was also reported that the myonuclear number in rat soleus muscle was still increased in response to 4 weeks of cage housing after hindlimb suspension from postnatal day 4 to 21 (Ohira et al., 2001). The number of myonuclei per mm of fiber length at the end of 17 days of suspension was approximately 53/mm, which is identical to that at day 4, when the suspension was initiated. That in the cage controls at day 21 was 82/mm (p<0.05). But the number in the previously unloaded group was increased to 141/mm after 4-week reloading (p<0.05). The changes of fiber CSAs and myonuclear domain were identical to the responses of myonuclear number, indicating that growth-associated fiber enlargement was closely related to the distribution of myonuclei. It was also reported that the number of myonuclei increased during 3-month hindlimb suspension from the postnatal day 4, even though it was only ~40% of cage controls (~3,300 per whole fiber, Fig. 3) (Kawano et al., 2008). Further, the number of myonuclei was gradually increased during 3-month reloading toward the control level, which was stable during the recovery period.

The possible roles of anti-gravitational load activity on the growth-related alteration of the properties in soleus muscle fibers after postnatal day 4 are summarized in Figure 5 (Kawano et al., 2008). Formation of muscle fibers and their elongation were not influenced by hindlimb unloading. The longitudinal growth of fibers may be related to stretching caused by the longitudinal increase of bone length (Ohira et al., 2006). However, adhesion and proliferation of satellite cells, which result in myonuclear accretion and hypertrophy of muscle fibers, were inhibited. But such phenomena were recovered following the application of mechanical load thereafter. It is suggested that the satellite cell-related stimulation in response to gravitational loading plays an essential role in the cross-sectional growth of soleus muscle fibers.

II: Atrophy and regrowth of muscle and muscle fibers in matured rodents

Mechanical stress

Prominent effects of unloading on the properties of soleus were induced in Wistar Hannover rats (Wang et al., 2006). After 16 days of hindlimb unloading, atrophy and decreased number of myonuclei and both mitotic active and quiescent satellite cells were noted in muscle fibers, sampled longitudinally from tendon-to-tendon. Although the satellite cells in the fibers of control rats were distributed evenly throughout the fiber length, the number of both mitotic active and quiescent satellite cells in the central, but not the proximal or distal, region of the fiber was decreased after unloading (Fig. 6, Wang et al., 2006).
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Such phenomena were closely related to passive shortening of sarcomeres in the central region due to the plantar-flexion of ankle joint (Fig. 7, Wang et al., 2006). Sarcomeres in the central, not distal and proximal, region of fiber were significantly shortened to less than 2.1 μm, which is a critical length for tension development (Kawano et al., 2004). Thus, it was suggested that decrease in the mechanical stress due to passive shortening causes the

Fig 3. Number of myonuclei (A), M-cadherin-positive (quiescent, B) and 5′-bromo-2′-deoxyuridine (BrdU)-positive (mitotic active, C) satellite cells per whole fiber, sampled from tendon to tendon in soleus muscle fiber of rats. Means ± SEM, n=5 in each bar. *, †, and§: Significantly different from the level at day 4, immediately after 3-mo unloading or cage housing (3-mo), and the age-matched control, respectively at p<0.05. Cited from Kawano et al., 2008.

Fig. 4. A: Typical distribution patterns of myonuclei in single muscle fiber. Compact and enlarged myonuclei are indicated by orange and blue arrows, respectively. B: Three-dimensional myonuclear volume. C: DNA content per myonucleus. D: DNA content per cubic micrometer myonuclear volume. Means ± SEM, n=5 for each group. *, †, §: Significantly different from the levels at day 4, immediately after 3-mo unloading or cage housing (3-mo), and the age-matched control, respectively at p<0.05. Cited from Kawano et al., 2008.
The number of satellite cells in the central region was normalized after 16-day reloading. Although it is still unclear why the region-specific responses, which were obvious in satellite cells, were not induced in myonuclear number and fiber CSA, these data clearly indicate that the distribution of satellite cells and myonuclei, which are influenced by the mechanical stress, play important roles in the regulation of fiber size.

Fig. 5. Summary of the major findings concerning the growth and development of soleus muscle fibers in the study, performed to investigate the effects of gravitational unloading during growing period in rats. Significant inhibition by gravitational unloading is indicated by “X”. Cited from Kawano et al., 2008.

Fig. 6. Distribution of mitotic active and quiescent satellite cells in a single whole soleus muscle fiber, sampled from tendon-to-tendon immediately after 16 days of hindlimb unloading (Unloaded) or cage housing (Control) in rats. Means±SEM for each 10% interval. The positive and negative numbers in the horizontal axis show the relative distance from the center to both ends of a single fiber. Cited from Wang et al., 2006.

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The distribution of satellite cells and myonuclei in slow-twitch fibers is generally greater than that in fast-twitch fibers, even in the same muscle, of adult rodents (Kelly, 1978; Ohira et al., 2011; Schmalbruch and Lewis, 2000; Schultz et al., 2006; Yin et al., 2013). And the decreases of both satellite cells and myonuclei, caused by inhibited mechanical stress, are more pronounced in atrophied fibers expressing slow myosin heavy chain (Ohira et al., 2011). Mechanical-load dependent region-specific responses of muscle fiber properties within a
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![Diagram showing sarcomere length changes](image)

**Fig. 7.** Sarcomere length at the proximal, central, and distal region of soleus fiber either during quadrupedal rest on the floor or during hindlimb unloading in rats. The degree of the mean anterior ankle joint is indicated in the parenthesis. Mean±SEM. Cited from Wang et al., 2006.

Muscle were also seen (Ohira et al., 2011). Changes of the shape of adductor longus muscle were induced due to the abduction and backward extension of hip joints, when rats were exposed to microgravity (Ohira et al., 2009) or hindlimb-unloading (Ohira et al., 2011). The length of sarcomeres in the caudal region was passively shortened and electromyogram activity was decreased, indicating that the mechanical load and neural activity were inhibited in response to unloading. But these parameters in the rostral region were unchanged or even stretched and increased.

The percent type I fibers decreased and de novo appearance of type IIa and pronounced fiber atrophy were noted in the caudal region of adductor longus muscle after 16 days of unloading (Ohira et al., 2011). Growth-associated increase of myonuclear number seen in the caudal region of control rats was inhibited by unloading. Number of mitotic active satellite cells decreased after unloading only in the caudal region. It was indicated that the responses of fiber properties in adductor longus muscle to unloading were closely related to the region-specific decrease of neural and mechanical activities, being the caudal region more responsive.

In contrast to these findings, several articles have reported that the number of satellite cell (Jackson et al., 2012; Snijders et al., 2014) and myonuclei (Bruusgaard et al., 2012; Kasper and Xun, 1996) did not contribute to muscle fiber regrowth from atrophy induced by gravitational unloading. It was reported that atrophy in both type I and II fibers, biopsy-sampled from the middle region of vastus laterals muscle, was not associated with measurable changes in satellite cell content after 2 weeks of one-legged knee immobilization via a full-leg cast in human subjects (Snijders et al., 2014). Bruusgaard et al. (2012) performed hindlimb suspension study using female Wistar rats and reported that myonuclear number per fiber in 10-μm-thick cryosections did not change in response to unloading and reloading, although the fiber atrophy and regrowth were induced. Kasper and Xun (1996) also reported that myonuclear numbers per mm in soleus fibers of female Wistar rats were significantly greater in the hindlimb-suspended groups vs. cage controls. Similar trends were also seen in plantaris.

The numbers of satellite cells and myonuclei were not normalized by analyses of sarcomere length in these studies (Bruusgaard et al., 2012; Jackson et al., 2012; Kasper and Xun, 1996; Snijders et al., 2014). Thus, it is possible that the number of myonuclei per mm in fibers with passive shortening is over-estimated, especially in the hindlimb-suspended group. Since the sarcomeres in these muscle fibers are passively shortened due to plantar-flexion of ankle joints during exposure to microgravity (Ohira et al., 2009) and hindlimb suspension (Kawano et al., 2004; Ohira et al., 2002; Riley et al., 1990; Wang et al., 2006), and whole lengths of muscle and muscle fibers are shortened. Such shortening of muscle is induced even in response to cutting of Achilles tendon following dissection. Further, the distribution of satellite cells per mm of fiber length is very low (Kawano et al., 2008; Ohira et al., 2011; Wang et al., 2006). Thus, it is important to normalize the number of satellite cells and myonuclei by sarcomere length.

**Other factors associated with or without mechanical stress**

The relationship between the responses of fiber size and the distribution of satellite cells and myonuclei to gravitational unloading and reloading were studied using osteopetrotic homozygous (op/op) mice with inactivated mutation of macrophage colony-stimulating factor (M-CSF) gene, which causes hematopoietic stem cells to differentiate into macrophages or other related cell types, and wild type (+/+ ) and heterozygous (+/−) mice (Ohira et al., 2015). Longitudinal whole single muscle fibers were sampled from tendon-to-tendon and double-stained with M-cadherin or 5′-bromo-2′-deoxyuridine (BrdU, Fig. 8A), and propidium iodide (PI, Fig. 8A) for quiescent and mitotic active satellite cells, and myonuclei, respectively. Baseline level of muscle fiber size in op/op mice was slightly smaller than in +/+ and +/− mice (p=0.05, Fig. 8D). The number of BrdU-positive (mitotic active) satellite cells (Fig. 8B) and myonuclei (Fig. 8C) were also less in op/op mice. Thus, smaller fiber size may be caused by these phenomena. Fiber atrophy, closely associated with the decreased number of satellite cells and myonuclei, were also observed in response to hindlimb unloading. The fiber CSA (Fig. 8D), length, and the whole number of sarcomeres, mitotically active (Fig. 8B) and quiescent satellite cells, and myonuclei (Fig. 8C), as well as myonuclear domain, in single muscle...
fibers were decreased after 10 days of unloading in all types of mice. Although all of these parameters in +/+ and +/op mice were increased toward the control values after 10 days of reloading, none of these levels in op/op mice were recovered. Data suggest that M-CSF and/or macrophages are important to activate satellite cells,
which cause the increase of myonuclear number during fiber hypertrophy. However, it is unclear why their responses to general growth and short-term reloading after unloading are different.

It was also reported that absence of heat shock transcription factor 1 (HSF1) gene retards the regrowth of atrophied soleus muscle, due to 2-week unloading, in mice (Yasuhara et al., 2011). Regrowth of atrophied soleus during 4-week ambulation recovery in HSF1-null mice was slower than in wild type mice. Although unloading-associated down-regulation and reloading-associated up-regulation of 25-kDa heat shock protein (HSP25) and HSP72 mRNA were induced in both groups, the baseline expression level of HSP25, heat shock cognate 70 (HSC70), and HSP72 in HSF1-null mice were lower than in wild type mice. It was suggested that the up-regulation of HSPs, induced by HSF1-associated stress response, may play, in part, the important role(s) in the mechanical loading (stress)-associated regrowth of skeletal muscle.

Mitchell and Pavlath (2001) reported that regrowth from the unloading-related atrophy was induced independently with an increase of myonuclear number during the early phase of recovery, whereas a complete recovery was caused by satellite cell dependent growth. In mice treated with etoposide, which inhibits white blood cell proliferation, muscle atrophy, induced by hindlimb unloading, did not completely recover during the later phase of reloading (Dumont and Frenette, 2012). Macrophages and/or other non-muscle cell types may play an important role in the activation of satellite cells especially during the later phase of fiber regrowth.

Goto et al. (2004) reported that the increases of wet weight and protein content in atrophied soleus caused by 5 days of unloading were closely associated with edema, which may be influenced by the application of heat stress and acute increase of muscle utilization during ambulation recovery. Application of heat stress (41°C for 60 min) increases the distribution of BrdU-positive (mitotic active) satellite cells in soleus muscle of normal rats (Uehara et al., 2004). Further, it was reported that the reloading and cardiotoxin (CTX)-injection-related-increase of satellite cell number, which was decreased following 2-week unloading, was inhibited by continuous unloading in mouse soleus (Matsuba et al., 2009). It is generally suggested that the regulation of satellite cell number is closely related to the level of mechanical stress, which is not necessarily associated with muscle damage.

As noted above, the recovery of atrophied muscle requires satellite cell activation followed by accretion of myonuclei. Previous studies suggested that macrophages stimulate satellite cell migration, myoblast proliferation and their fusion into myotubes (Cantini et al., 1994). Impaired macrophage function delays the satellite cell activation and then disturbs muscle regeneration in unloaded muscle (Kohno et al., 2012). Macrophages in soleus muscle of mice, hindlimb-unloaded for 10 days, were reduced by 86% at 4th day of reloading recovery with i.p. injection of anti-F4/80 (Tidball and Wehling-Hennricks, 2007). Macrophage depletion also reduced muscle regeneration (indicated by central nucleation) and satellite cell differentiation (indicated by decrease in MyoD expressing satellite cells), and prevented the regrowth of muscle fibers. As well as the activation of satellite cells followed by muscle regeneration, macrophages protect satellite cells from apoptosis and enhance muscle growth (Chazaud et al., 2003). Thus, these studies suggest that macrophages play a pivotal role to activate satellite cell proliferation.

However, regeneration of tibialis anterior muscle in wild type mice from the damage caused by administration of CTX was promoted via modulation of gene expressions in infiltrated macrophages by inhibition of interleukin-6 (IL-6) receptor using anti-IL-6 receptor antibody (MR16-1) (Fujita et al., 2014). However, beneficial effects were not observed in IL-6 null mice. Such phenomena could be related to up-regulation of pro-inflammatory cytokines, such as tumor necrosis factor α (TNF-α) and IL-1β, by compensatory mechanisms in IL-6 null mice. These results suggest that damage-related over-expression of IL-6, which is one of the cytokines released from macrophages, plays the detrimental role in the regeneration of muscle, although some amount of IL-6 is also needed, as was reported that IL-6 facilitates muscle growth via the activation of satellite cells (McKay et al., 2009; Serrano et al., 2008).

Further, the absence of HSF1 suppressed activation of transcription factor 3 (ATF3), which plays a central role in inflammatory response by modulating the expression of cytokines and chemokines (Labzin et al., 2015) in injured and functionally overloaded mouse soleus muscle (Koya et al., 2013; Nishizawa et al., 2013). In HSF1-null mice, the regeneration of muscle injury as well as overloading-associated muscle hypertrophy of soleus muscle was depressed, accompanying with up-regulation of IL-6 and TNF (Koya et al., 2013; Nishizawa et al., 2013). These results suggest that HSF1, macrophage, ATF3, and IL-6 may play the roles for the modulation of skeletal muscle mass.

In conclusion, essential roles of satellite cells and myonuclei in the regulation of morphological properties of anti-gravitational muscle, soleus and adductor longus, in response to the level of mechanical stress were suggested. Postnatal growth of fiber CSA was inhibited by hindlimb unloading, which also restrained the increase of the distribution in both quiescent and mitotic active satellite cells and myonuclei with lower concentration of DNA. Regrowth of fibers during ambulation recovery in the cages was closely related to the increase of satellite cell and myonuclear numbers. It was also indicated that atrophy of muscle and muscle fibers in matured rats was caused by inhibited mechanical stress, which is closely related to the decreased distribution of satellite cells and myonuclei due to passive shortening of sarcomeres and inhibited tension development. Regrowth of fibers was induced when the number of satellite cells and myonuclei was also increased. However, recovery of fiber CSA, as well as the number of satellite cells and myonuclei, was not induced in muscle of op/op mice with macrophage deficiency, as well as the HSF1-null mice. Taking all of
these results and suggestions together, it was suggested that a regulatory network among macrophage, IL-6, HSF1, and ATF3 may play a crucial role for the modulation of skeletal muscle mass, which are also influenced by activation of satellite cells and distribution of myonuclei.

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Author contributions
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Conflict of interest statement
The authors declare no competing interests.

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