Unloading of juvenile muscle results in a reduced muscle size 9 wk after reloading

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Unloading of juvenile muscle results in a reduced muscle size 9 wk after reloading. J. Appl. Physiol. 88: 158–164, 2000.—The role of satellite cells and DNA unit size in determining muscle size was examined by inhibiting postnatal skeletal muscle development by using hindlimb suspension. Satellite cell mitotic activity and DNA unit size were determined in the soleus muscles from hindlimb-suspended and age-matched weight-bearing rats before the initiation of hindlimb suspension, at the conclusion of a 28-day hindlimb-suspension period, 2 wk after reloading, and 9 wk after reloading. The body weights of hindlimb-suspended rats were significantly (P < 0.05) less than those of weight-bearing rats at the conclusion of hindlimb suspension, but they were the same (P > 0.05) as those of weight-bearing rats 9 wk after reloading. The soleus muscle weight, soleus muscle weight-to-body weight ratio, myofiber diameter, nuclei per millimeter, and DNA unit size for the hindlimb-suspended rats were significantly (P < 0.05) smaller than for the weight-bearing rats at all recovery times. Satellite cell mitotic activity was significantly (P < 0.05) higher in the soleus muscles from hindlimb-suspended rats 2 wk after reloading, but it was the same (P > 0.05) as in weight-bearing rats 9 wk after reloading. Juvenile soleus muscles failed to achieve normal muscle size 9 wk after reloading because there was incomplete compensation for the hindlimb-suspension-induced interruptions in myonuclear accretion and DNA unit size expansion.

hindlimb suspension; satellite cell; deoxyribonucleic acid unit; myofiber; atrophy

POSTNATAL SKELETAL MUSCLE growth occurs exclusively through an increase in myofiber size without an increase in myofiber number. Concurrent with an increase in myofiber size is an increase in myofiber DNA content. The increase in myofiber DNA content does not come through the division of preexisting myonuclei because they are postmitotic (30). Satellite cells are a mitotically active cell population lying between the sarcolemma and the myofiber basal lamina (14), and myonuclear accretion occurs through the incorporation of satellite cell nuclei into growing myofibers (17). During normal postnatal skeletal muscle growth, satellite cell mitotic activity is high shortly after birth, but it quickly falls to low levels (1, 19) until satellite cells become mitotically quiescent in mature nongrowing muscle (28). However, the quiescent satellite cells in mature muscle may become activated to rebuild myofibers after an injury (4). Therefore, the entire proliferative capacity of the satellite cell population is not exhausted during normal skeletal muscle growth.

The DNA unit (6) is a term describing the concept that each nucleus in a multinucleate myofiber controls its surrounding cytoplasm. Evidence for the DNA unit concept comes from in vitro studies showing that mRNA produced by a single nucleus is confined to the area immediately surrounding that particular myonucleus (24). Satellite cell nuclei may be incorporated into growing myofibers to maintain a constant DNA unit size (16), whereas it has also been suggested that increases in DNA unit size play a role in skeletal muscle growth (13, 19, 20). In mature muscle, DNA unit size may not change during the hypertrophy that follows an increase in functional load (15, 25). The significance of maintaining a constant DNA unit size during myofiber hypertrophy is that the rate of myofiber enlargement would be entirely dependent on the rate of myonuclear accretion. The potential for individual DNA units to modulate muscle growth or increase muscle size is tremendous. A small increase in the volume of cytoplasm surrounding each nucleus multiplied by the thousands of nuclei found in a myofiber could result in a large increase in myofiber volume and muscle size. Although DNA unit size expansion could potentially play an important role in normal skeletal muscle growth (19), experimental manipulations have been largely unsuccessful in increasing the DNA unit size of mature rats (15, 25). In contrast, hindlimb suspension of mature rats results in a significant reduction in DNA unit size and muscular atrophy (3, 12).

The muscular atrophy that occurs during spaceflight is a formidable obstacle to long-term human space exploration, and the hindlimb-suspension model of muscle atrophy is a ground-based model that produces the same deleterious effects on skeletal muscle as spaceflight (32, 33). Myofiber atrophy occurs in mature skeletal muscle, during hindlimb suspension, and it is most severe in postural muscles, such as the soleus. In juvenile soleus muscles, satellite cell mitotic activity is suppressed within 24 h of the initiation of hindlimb suspension, and it remains suppressed for the duration of hindlimb suspension, resulting in the cessation of muscle growth (8, 27). The ability of juvenile postural muscles to recover from the hindlimb-suspension-induced interruption in muscle growth and myonuclear...
accretion after the resumption of weight bearing is unknown. Soleus muscles from mature rats do not fully recover from the hindlimb suspension induced muscular atrophy when the rats are maintained under sedentary conditions for 4 wk (11). However, the mechanisms employed for muscle recovery after the resumption of weight bearing may be very different for juvenile rats than for mature rats because hindlimb suspension delays the developmental expression of myosin heavy chain isoforms (9), suggesting that developmental processes may be altered in growing muscle by hindlimb suspension. Therefore, the juvenile soleus muscle may or may not be able to resume normal growth on the resumption of weight bearing.

Presently, it is known that hindlimb suspension interrupts myonuclear accretion in juvenile muscle. The impact of hindlimb suspension on any developmental increase in DNA unit size, the ability of the myofibers to acquire nuclei with the resumption of weight bearing, and the ability of juvenile myofibers to reach mature size after the resumption of weight bearing is unknown. The objectives of this study were to assess the ability of soleus muscles in juvenile rats that were previously hindlimb suspended to reach mature muscle size 9 wk after the resumption of weight bearing, to examine the kinetics of satellite cell mitotic activity after the resumption of weight bearing, to study the effect of hindlimb suspension on DNA unit size in juvenile muscle, and to assess the role of DNA unit size in muscle recovery after the resumption of weight bearing.

MATERIALS AND METHODS

Hindlimb suspension. All experimental procedures involving animals were approved by the University of Wisconsin Animal Care Committee. Twenty-eight-day-old male Sprague-Dawley rats (Harlan Sprague Dawley, Madison, WI) were randomly assigned into hindlimb-suspension (HS) or weight-bearing (WB) groups. The HS rats (n = 17) were immediately placed into a hindlimb suspension apparatus (22). Briefly, the rats were attached by their tail to a trolley system that allowed only the forelimbs to touch the cage floor, but the rats had free movement to obtain food and water ad libitum. A group of 28-day-old rats (n = 6) was killed immediately after hindlimb suspension. The remaining WB rats (n = 18) were maintained in the same room as the HS rats, during the hindlimb suspension period, with food and water provided ad libitum. The HS rats were hindlimb suspended for 28 days. Near the end of the suspension period, the rats were randomly assigned into three groups for different post-hindlimb suspension recovery times. The first group (group 1) of HS (n = 6) and WB (n = 6) rats was killed immediately after 28 days of hindlimb suspension. The second group (group 2) of HS (n = 5) and WB (n = 6) rats was killed 2 wk after removal from the hindlimb-suspension apparatus. The third group (group 3) of HS (n = 6) rats and WB (n = 6) rats was killed 9 wk after removal from the hindlimb-suspension apparatus. All rats were killed by an overdose of Beuthanasia-D7 (0.25 ml/kg body wt; Schering-Plough Animal Health, Kenilworth, NJ).

Nuclear labeling. The rats in group 1 were given a single intraperitoneal injection (100 mg/kg body wt) of the thymidine analog 5-bromo-2-deoxyuridine (BrdU) on days 26, 27, and 28 of hindlimb suspension. The rats in group 2 were implanted underneath their skin with miniosmotic pumps (Alzet model 2ML2, Alza, Palo Alto, CA) containing BrdU while they were under general anesthesia (90 mg/kg body wt ketamine; 9 mg/kg body wt xylazine) immediately after they had been removed from the hindlimb-suspension apparatus. The rats in group 3 were implanted with miniosmotic pumps containing BrdU 7 wk after the conclusion of hindlimb suspension. In group 2 and group 3, the miniosmotic pumps were implanted to deliver BrdU to the rats over the 2 wk preceding their death. The miniosmotic pumps were designed to deliver 250 µg BrdU/h to label all cells that entered the S phase of the cell cycle over the 2 wk after implantation (20).

Immunohistochemistry and image analysis. Myofiber segment preparation and analysis were similar to procedures employed by Mozdziak et al. (18). Soleus muscles were weighed, tied to sticks, and immersed in Carnoy solution (60% ethanol-30% chloroform-10% acetic acid) for 24 h. Soleus muscles were hydrated to 70% ethanol, mechanically teased apart with fine tweezers, hydrated through an ethanol series, and digested with collagenase (480 U/ml PBS; catalog no. C-2139, Sigma Chemical, St. Louis, MO) at 37°C for 4 h. Subsequently, DNA was denatured with 2 N HCl for 1 h. Myofiber segments were washed four times with PBS and then incubated overnight with the primary monoclonal antibody anti-BrdU (Becton Dickinson, Mountain View, CA) diluted 1:10 with PBS containing 0.5% Tween 20 and 0.5% bovine serum albumin. BrdU-labeled nuclei were detected with goat anti-mouse IgG conjugated to fluorescein isothiocyanate (ICN Biomedicals, Irvine, CA) diluted 1:50 with PBS containing 0.5% Tween 20 and 10% goat serum. Myofiber nuclei (satellite cell nuclei + myonuclei) were counterstained with propidium iodide (50 µg/ml PBS). Myofiber segments were resuspended in mounting medium (75% (vol/vol) glycerol, 75 mM KCl, 10 mM Tris, 2 mM MgCl2, 2 mM EGTA, 1 mM Na3PO4, pH 8.5, and 1 mg/ml p-phenylenediamine) and mounted on glass slides.

Myofiber segments were observed with a Nikon (Biophot) microscope equipped with epifluorescence illumination. BrdU-labeled nuclei were visualized with a fluorescein isothiocyanate filter set, and myofiber nuclei were visualized with a Texas red filter set. Images of nuclei visualized with the fluorescein isothiocyanate filters and nuclei visualized with the Texas red filter set were acquired by using a Spot II charge-coupled-device camera (Diagnostic Instruments, Sterling Heights, MI). Mean myofiber segment diameter, myofiber segment length, BrdU-labeled nuclei, and number of propidium iodide-labeled nuclei were obtained for each myofiber segment using Metamorph software (Universal Imaging, West Chester, PA). Care was taken to analyze only connective tissue-free myofiber segments to avoid including any non-myofiber nuclei in the counts of BrdU or propidium iodide labeled nuclei. At least 1,000 myofiber nuclei (satellite cell nuclei + myonuclei) were counted from each muscle. Calculations. An index of satellite cell mitotic activity was expressed as the number of BrdU-labeled nuclei per 1,000 total myofiber nuclei (satellite cell nuclei + myonuclei). An estimate of the cytoplasmic volume-to-nucleus ratio (DNA unit size) was made after estimation of myofiber segment volume from myofiber segment diameter and length estimates [DNA unit size = (myofiber segment diameter/2)2 × (myofiber segment length)/myofiber nuclei].

Statistical analysis. Body weight data, muscle weight data, myofiber diameter data, DNA unit size data, the number of myofiber nuclei per millimeter data, and the BrdU-labeling data for rats killed 2 and 9 wk after the conclusion of hindlimb suspension were analyzed by using the general linear models procedure of SAS (26) to examine the effect of hindlimb suspension and recovery time on each parameter. Means
RESULTS

Growth during hindlimb suspension. The body weights of both the HS rats and WB rats were significantly (P < 0.05) higher at the conclusion of hindlimb suspension than at the initiation of hindlimb suspension, indicating that all rats grew during the suspension period (Table 1). However, body weights were significantly (P < 0.05) smaller in HS rats compared with WB rats after the 28-day hindlimb suspension period. In HS rats, there was no significant (P > 0.05) difference in soleus muscle weight between the start and conclusion of hindlimb suspension. Soleus myofiber diameter and the soleus weight-to-body weight ratio were significantly (P < 0.05) smaller in the HS rats immediately after 28 days of hindlimb suspension compared with at the start of suspension (Fig. 1, Table 1).

Growth after reloading. Body weights increased (P < 0.05) for HS rats and WB rats between the conclusion of hindlimb suspension and 9 wk after reloading, indicating that the rats were growing during the postsuspension period (Table 1). Similarly, soleus muscle weights and soleus myofiber diameters increased (P < 0.05) for HS rats and WB rats between the conclusion of hindlimb suspension and 2 wk after reloading and between 2 and 9 wk after reloading, indicating that all soleus muscles were growing during the reloading period (Table 1, Fig. 1). The HS rats weighed significantly (P < 0.05) less than did the WB rats at the conclusion of hindlimb suspension, but the body weights were not significantly (P > 0.05) different from WB rats 9 wk after reloading. Soleus muscle weight, soleus muscle weight relative to body weight, and soleus myofiber diameter were significantly (P < 0.05) smaller in HS rats compared with WB rats at the conclusion of hindlimb suspension and at each reload recovery time.

Index of satellite cell mitotic activity. The index of satellite cell mitotic activity at the conclusion of hindlimb suspension was based on BrdU injections, and it was significantly (P < 0.05) lower for the HS rats (0.8 ± 0.8) compared with the age-matched WB rats (35.9 ± 7.1), supporting previous work showing that hindlimb suspension suppresses satellite cell mitotic activity in juvenile soleus muscles (8, 27). The index of satellite cell mitotic activity for soleus muscles at 2 and 9 wk after reloading was based on continuous BrdU infusion, and it included all satellite cell mitotic divisions over the 2 wk immediately before the rats were killed. In the 2 wk immediately after reloading, the index of satellite cell mitotic activity was significantly (P < 0.05) higher in the soleus muscles of HS rats compared with the soleus muscles of WB rats (Fig. 2). However, between 7 and 9 wk after reloading, there was no significant (P > 0.05) difference in the index of satellite cell mitotic activity between the soleus muscles from HS rats and the soleus muscles from WB rats. Last, satellite cell mitotic activity was significantly (P < 0.05) lower for the soleus muscles from HS rats and WB rats 9 wk after reloading compared with the soleus muscles from the HS rats and WB rats 2 wk after reloading (Fig. 2).

Table 1. Soleus muscle weight, body weight, and soleus muscle weight relative to body weight for hindlimb-suspended and weight-bearing rats killed before hindlimb suspension, at end of hindlimb suspension, after 2 wk of reloaded recovery, and after 9 wk of reloaded recovery

<table>
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<th>Start HS</th>
<th>WB</th>
<th>HS</th>
<th>2 wk Recovery</th>
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<th>HS</th>
<th>9 wk Recovery</th>
<th>WB</th>
<th>HS</th>
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<tr>
<td>Muscle wt, mg</td>
<td>25 ± 1a</td>
<td>87 ± 2c</td>
<td>16 ± 1a</td>
<td>108 ± 5d</td>
<td>52 ± 6e</td>
<td>149 ± 4f</td>
<td>107 ± 3g</td>
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<td></td>
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<tr>
<td>Body wt, g</td>
<td>58 ± 1a</td>
<td>245 ± 5c</td>
<td>154 ± 6e</td>
<td>295 ± 13d</td>
<td>240 ± 11c</td>
<td>436 ± 9e</td>
<td>402 ± 12g</td>
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<tr>
<td>Muscle wt/body wt, mg/g</td>
<td>0.43 ± 0.01</td>
<td>0.36 ± 0.01d</td>
<td>0.11 ± 0.01a</td>
<td>0.37 ± 0.02d</td>
<td>0.21 ± 0.02b</td>
<td>0.34 ± 0.01d</td>
<td>0.27 ± 0.01c</td>
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Values are means ± SE; n, no. of rats. HS, hindlimb suspended; WB, weight bearing; Start HS, before hindlimb suspension; 0 wk Recovery, end of hindlimb suspension; 2 wk Recovery, 2 wk of reloaded recovery; 9 wk Recovery, 9 wk of reloaded recovery. Values within a row with different letters are significantly different (P < 0.05).

Fig. 1. Myofiber diameters after hindlimb suspension. Data are from 28-day-old rats killed without hindlimb suspension (Start HS), rats killed at the conclusion of the suspension period (0 WK Recovery), rats killed after 2 wk of reloaded recovery (2 WK Recovery), and rats killed after 9 wk of reloaded recovery (9 WK Recovery). Solid bars, weight-bearing rats; open bars, hindlimb-suspended rats. Values are means ± SE. Values with different letters are significantly different (P < 0.05).
Nuclei per millimeter. The number of nuclei per millimeter was significantly ($P < 0.05$) lower in the soleus muscles of HS rats compared with the soleus muscles from WB rats at all recovery times (Fig. 3). The number of nuclei per millimeter was significantly ($P < 0.05$) lower in the rats killed before hindlimb suspension compared with all WB rats and with the HS rats 2 and 9 wk after reloading. There was no significant ($P > 0.05$) difference in the number of nuclei per millimeter in soleus muscles of HS or WB rats at the end of hindlimb suspension compared with the rats 2 wk after reloading, but there was a significantly ($P < 0.05$) higher number of nuclei per millimeter for the HS rats and WB rats 9 wk after reloading compared with the HS rats and WB rats 2 wk after reloading (Fig. 3).

DNA unit size. The DNA unit size is expressed as the cytoplasmic volume-to-nucleus ratio (12, 13, 18–20). There was an age-related increase ($P < 0.05$) in the DNA unit size between each time for myofibers from soleus muscles of both the WB rats and HS rats, indicating that DNA unit size expansion is a component of normal rat skeletal muscle growth. The DNA unit size was significantly ($P < 0.05$) smaller in the soleus muscles from HS rats compared with the soleus muscles from WB rats at all posthindlimb suspension recovery times (Fig. 4). The DNA unit size was smaller ($P < 0.05$) in soleus muscles of HS rats at the conclusion of hindlimb suspension compared with the rats killed before the beginning of the hindlimb-suspension period, similar to previous work showing that hindlimb suspension reduces DNA unit size in mature rats (3, 12).

DISCUSSION

Body and muscle weights. The body weights of the HS rats at the end of suspension were significantly ($P < 0.05$) greater than at the beginning of suspension, but they were also significantly ($P < 0.05$) lower than those of the age-matched WB rats. Soleus muscles from HS rats at the conclusion of hindlimb suspension had a smaller myofiber diameter and a smaller muscle weight-to-body weight ratio compared with soleus muscles from rats at the beginning of suspension, indicating that hindlimb suspension not only stopped skeletal muscle growth but also induced absolute atrophy in the juvenile soleus muscle. Similarly, hindlimb suspension induces absolute atrophy of mature soleus muscles (3, 12), but the factors contributing to the hindlimb-suspension-induced muscle atrophy are different in juvenile and mature muscle. The muscle atrophy in mature rats occurs mainly through a reduction in DNA unit size (3, 12), and also through a loss of myonuclei (2), whereas in juvenile rats the smaller muscle compared with that in age-matched control rats occurs mainly through an interruption in myonuclear accretion (8, 27), an interruption in muscle growth, and a reduction in DNA unit size.
The focus of the present study was to inhibit skeletal muscle growth by reducing the number of nuclei accumulated during the normal soleus muscle growth period by using hindlimb suspension and to study soleus muscle recovery after the resumption of weight bearing. Nine weeks after the cessation of hindlimb suspension, the body weights of the HS rats were not significantly (\(P > 0.05\)) different from those of the WB rats, but soleus myofiber diameters from HS rats remained significantly (\(P < 0.05\)) smaller than soleus myofiber diameters from WB rats. Similarly, the soleus muscle weight-to-body weight ratio was significantly (\(P < 0.05\)) smaller for HS rats compared with WB rats 9 wk after reloading, suggesting that the soleus muscles had not reached the size of age-matched weight-bearing rats even though body weights had reached the same size as age-matched weight-bearing rats.

Satellite cell mitotic activity. Increased satellite cell mitotic activity should be a major feature of soleus muscle recovery after reloading because a high level of satellite cell mitotic activity would be required to bring the myofiber DNA content to levels found in the soleus muscles of WB rats. However, the index of satellite cell mitotic activity was not significantly (\(P > 0.05\)) different for the soleus muscles from the HS rats compared with the soleus muscles from WB rats 9 wk after reloading, suggesting that satellite cells were not providing myonuclei at an increased rate to fuel a compensatory increase in muscle size between 7 and 9 wk after reloading. Furthermore, the number of nuclei per millimeter in myofibers from the soleus muscles of HS rats remained significantly (\(P < 0.05\)) lower at 9 wk after reloading compared with myofibers from the soleus muscles of WB rats, suggesting a lower overall DNA content. Taken together, the nuclei per millimeter and satellite cell mitotic activity data at 9 wk after reloading suggest that the soleus muscles in HS rats will remain smaller than soleus muscles from WB rats because there was no sustained satellite cell mitotic response during the recovery period, resulting in a lower myofiber DNA content.

Satellite cell mitotic activity was higher during the 2 wk immediately after the resumption of weight bearing in the soleus muscles from HS rats compared with soleus muscles from the WB rats (Fig. 2). It is likely that the elevated level of satellite cell mitotic activity supported the myofiber hypertrophy that occurred with the resumption of weight bearing because satellite cell mitotic activity is a prerequisite for myofiber enlargement (20, 25). It is likely that the increased functional demands placed on the muscle by reloading stimulated satellite cell proliferation and subsequently myonuclear accretion because increasing muscle activity stimulates satellite cell mitotic activity to support muscle enlargement (7). Furthermore, signs of myofiber damage without wholesale myofiber destruction, such as macrophage infiltration (31), sarcolemmal disruptions (10), and myofibrillar lesions (34), have been reported shortly after the resumption of weight bearing. Macrophages secrete substances, such as platelet-derived growth factor, basic fibroblast growth factor, and insulin-like growth factor, that can stimulate satellite cell mitotic activity (5, 31). Similarly, satellite cells become activated after myofiber injury (29), suggesting that low-level myofiber injury may stimulate satellite cell proliferation after the resumption of weight bearing to aid the compensatory response of the muscle. It was also likely that any myofiber repair process was complete by the seventh week after reloading when BrdU infusion began for the last group of rats (group 3) because muscle repair after notexin-induced myofiber damage is complete by 28 days after myofiber trauma (35). Therefore, any beneficial effects of low-level myofiber damage to stimulate satellite cell proliferation likely were not present between 7 and 9 wk after reloading.

The elevated satellite cell mitotic activity in the soleus muscle of HS rats observed 2 wk after reloading was not sufficient to bring myofiber DNA content to levels found in the soleus muscles of WB rats when the rats were maintained in a sedentary state after reloading. The ability of exercising juvenile muscle after reloading to improve the recovery of muscle mass is unknown. However, increasing the functional activity of juvenile muscle, after the initial 2-wk reloading period, might promote a more complete recovery of muscle mass through a sustained satellite cell mitotic response because exercise stimulates satellite cell proliferation (7), and daily exercise was beneficial in returning atrophied mature soleus muscle to the same size as that of age-matched weight-bearing rats (11).

The observed reduction in satellite cell mitotic activity, during hindlimb suspension, supports previous studies showing that satellite cell mitotic activity is suppressed throughout the hindlimb-suspension period in juvenile muscle (8, 27). It is possible that hindlimb suspension reduced satellite cell mitotic activity, and it caused the soleus muscle to miss a critical developmental period for the acquisition of nuclei, contributing to the failure of the reloaded soleus muscle from HS rats to reach the same size as the soleus muscle in WB rats under sedentary conditions.

DNA unit size. The age-related increase in DNA unit size supports previous work in avian species showing that DNA unit size expansion plays an important role during normal skeletal muscle growth (19). However, previous studies suggest that DNA unit size does not change during myofiber hypertrophy (15, 25). Many previous DNA unit size studies have focused on the response of mature muscle to increased (15, 25) or decreased (3, 12) functional demands, whereas the present study focused on the role of DNA unit size expansion during postnatal muscle growth. One possibility that may explain the differences between previous DNA unit size studies employing juvenile (19) and adult (15, 25) animals is that muscle may maintain a maximal DNA unit size, and any increase in muscle size induced by increasing functional load relies on DNA unit (myonuclear) accretion. Evidence that mature myofibers may operate near an age-related maximum comes from irradiation studies where muscle growth was reduced in parallel with the reduction in...
myonuclear accretion. Despite an irradiation-induced alteration in myonuclear accretion, the irradiated myofibers maintained the same age-related program for DNA unit size expansion (20).

In contrast to the irradiation model, which likely does not interrupt all myofiber maturational processes (23), hindlimb suspension interrupts soleus muscle development (8), it impairs the rate of myofiber maturation after an injury (9), and it interrupts DNA unit size expansion (Fig. 4) in growing muscle. The DNA unit size remained significantly smaller for soleus muscles from HS rats at all times after the resumption of weight bearing (Fig. 4), suggesting that DNA unit size modulation was not used as a mechanism to recover muscle mass. It appears that hindlimb suspension had a long-term effect on reducing DNA unit size 9 wk after reloading. The potential for the DNA unit size to reach the same level as that of age-matched WB rats at longer recovery times remains unknown. However, like myonuclear accretion, hindlimb suspension may have interrupted a chronological program for DNA unit size expansion, leading to the reduction in DNA unit size observed 9 wk after reloading. The soleus muscles from previously hindlimb-suspended rats at 9 wk after reloading have the DNA content and the DNA unit size of chronologically younger muscles, and they have the satellite cell mitotic activity characteristic of mature muscle. Therefore, the characteristics of the soleus muscles from hindlimb-suspended rats 9 wk after reloading suggest that the soleus muscles may have exited the postnatal growth period with a reconfigured maximal mature DNA content and a reconfigured maximal mature DNA unit size.

The most important finding from these studies was that inactivity associated with a 4-wk period of hindlimb suspension resulted in a reduction in soleus myofiber diameter and soleus muscle weight-to-body weight ratio that persisted 9 wk after reloading, even though body weights of HS rats had reached the same level as those of WB rats. Satellite cell mitotic activity was only transiently elevated immediately after reloading, resulting in a lower myonuclear complement. A reduced complement of myonuclei will decrease the total pool of DNA available for transcription of muscle-specific proteins, reducing potential myofiber size. First, it appears that hindlimb suspension may have altered a chronologically defined developmental program for the myofibers to acquire nuclei. With the resumption of weight bearing, satellite cell mitotic activity increased with the resumption in functional demands presumably to provide nuclei for myonuclear accretion. However, the compensatory response by the satellite cell population appears to be short lived under sedentary conditions and inadequate for the muscle to gain the same quantity of nuclei as the soleus muscles in WB rats. Second, it appears that hindlimb suspension disrupts the normal developmental increase in DNA unit size, and the DNA unit size fails to reach levels found in WB rats even 9 wk after the resumption of weight bearing, suggesting that inactivity of a juvenile muscle had a long-term effect on reducing DNA unit size, thus contributing to the overall reduction in muscle size.

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REFERENCES