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## Supplementary Issue: Occupational Health and Industrial Hygiene

### Volitional Weight-Lifting in Rats Promotes Adaptation via Performance and Muscle Morphology prior to Gains in Muscle Mass

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**ABSTRACT:** Investigation of volitional animal models of resistance training has been instrumental in our understanding of adaptive training. However, these studies have lacked reactive force measurements, a precise performance measure, and morphological analysis at a distinct phase of training – when initial strength gains precede muscle hypertrophy. Our aim was to expose rats to one month of training (70 or 700 g load) on a custom-designed weight-lifting apparatus for analysis of reactive forces and muscle morphology prior to muscle hypertrophy. Exclusively following 700 g load training, forces increased by 21% whereas muscle masses remained unaltered. For soleus (SOL) and tibialis anterior (TA) muscles, 700 g load training increased muscle fiber number per unit area by ~20% and decreased muscle fiber area by ~20%. Additionally, number of muscle fibers per section increased by 18% for SOL muscles. These results establish that distinct morphological alterations accompany early strength gains in a volitional animal model of load-dependent adaptive resistance training.

**KEYWORDS:** operant conditioning, resistance exercise, fiber cross-sectional area, fiber number, stereology

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## Introduction

Work-related musculoskeletal disorders (MSDs) are prevalent world-wide and associated with long-term pain and physical disability.<sup>1,2</sup> Risk factors for such MSDs include forceful actions against large loads, repetitive movements, and awkward posture.<sup>3,4</sup> An interaction between force and repetition exists such that repetition results in moderate increases in MSD risk for low-force tasks and elevated risk for high-force tasks.<sup>3</sup> While exercise composed of muscle contractions against external resistance (ie, resistance training) can be adaptive and can improve the condition of muscle, inappropriate resistance training results in maladaptation characterized by diminished performance and the onset of a contraction-induced MSD.<sup>5</sup> Extensive investigation of regimes to promote contraction-induced muscle adaptation is

difficult to perform for human subjects largely because of the limitations in sampling tissue.

To overcome the limitations inherent in human studies, an experimental animal model was developed almost four decades ago.<sup>6</sup> Cats were operantly conditioned to perform weight-lifting training (ie, wrist-flexion of their right paw) for food reward. Since that time, several investigators have examined other voluntary resistance training models that have included rearing up while wearing weighted jackets<sup>7,8</sup> or lifting of a weighted ring within a vertical tube.<sup>9</sup> Using such models, investigators have studied such topics as the extent of inflammation, molecular pathways of muscle hypertrophy, and the role of stem cells.<sup>10–13</sup> In a number of animal studies of chronic voluntary resistance training, morphological analysis was evaluated and a training-induced increase in muscle



fiber number reported.<sup>7,8,11,14–16</sup> Despite these advances, assessment of reactive forces, a precise measure of performance, was not implemented. In addition, investigation of resistance training of animal models has been limited in that muscle morphology has not been assessed during a distinctive phase of training – when performance gains precede muscle hypertrophy. A common observation in human subjects is that in the initial phase of resistance exercise training, performance improves in the absence of muscle hypertrophy.<sup>17–19</sup> Both neural factors (eg, motor unit discharge rate and synchronization) and muscular factors (eg, myofibril density and connective tissue) have been investigated for their potential role in initial strength gains, but the roles of these factors are not fully characterized.<sup>17,20–26</sup> Consequently, the motivation to explore factors for early muscle adaptation other than those already investigated still persists.

To address the limitations in research regarding animal models for resistance training, our group developed a custom-designed weight-lifting apparatus for operantly conditioned rats that includes a force plate for direct measure of reactive forces. The methods and components of this novel apparatus are reported in an previously.<sup>27</sup> In the article, some experimental data are also reported, such as the observation of increased mass of various lower hindlimb muscles after two months of resistance training of a 700 g load. However, the report did not describe the effect of training on reactive forces. In addition, investigation of the training phase when strength gains precede muscle hypertrophy remained for future research. With this in mind, the purpose of the present study was to identify the alterations in performance and muscle morphology one month following training with two different loading regimes. We tested the hypothesis that load-dependent muscle adaptation is characterized by increased muscle fiber number in histological muscle sections and heightened reactive forces before increased muscle mass. The agonist soleus (SOL), medial gastrocnemius (MG), lateral gastrocnemius (LG), and plantaris (PL) muscles and the antagonist tibialis anterior (TA) muscle were excised and analyzed. Results from the present study support the hypothesis and establish a load-dependent adaptation in performance and muscle morphology in a volitional animal model of resistance training. These findings provide valuable insights regarding the loading and morphological features to consider for resistance training-induced muscle adaptation – insights potentially important for addressing MSDs in terms of prevention and rehabilitation.

## Methods

**Rats.** A total of 24 male Sprague-Dawley (Hla:(SD)CVF, Hilltop, Scottsdale, PA) rats, three to four months of age, were randomly assigned to three groups differing in training conditions: 700 g load training, 70 g load training, or cage controls. The rats were trained using operant conditioning procedures and a food reward, 45 mg pellets (Noyes Formula P; Research Diets). To ensure rats were hungry and to maintain food as a reinforcer,

food was restricted to keep body mass at 80% of ad libitum mass, a standard target body mass in behavioral research.<sup>28</sup> All animal procedures were approved by the Animal Care and Use Committee at the National Institute for Occupational Safety and Health (NIOSH) in Morgantown, WV.

**Training.** Preparation for training and the training protocol were described in detail previously.<sup>27</sup> Before training, each rat became accustomed to the operant chamber, which was modified with a custom-designed weight-lifting apparatus. The apparatus was designed for squat-type training and consisted of a vertical tube containing a ring assembly (ie, a yoke that moved along two vertical shafts) and a force plate at the base.<sup>27</sup> The pre-training period (five days per week for three to five weeks) prepared the rat to enter the tube, stand on its hindlimbs, place its nose in the ring assembly, push a weighted ring assembly vertically until activating the nose-poke at the top of the tube, and retrieve a food reward (two pellets for each full lift).

Training sessions were done with a light load (70 g) or a heavier load (700 g, which is approximately two times the average body weight (BW)). The training terminated after 100 full lifts (lifts with successful nose pokes) or a fixed time limit, whichever occurred first. The time limit was 30 minutes for the 70 g load group and 60 minutes for the 700 g load group. Rats exposed to 70 g load training tended to achieve 100 full lifts before reaching the time limit, whereas the rats exposed to 700 g load training tended to reach the time limit having achieved ~80 full lifts.<sup>27</sup> One training session was performed each day with a training schedule of five days per week for one month. During each session, peak reactive force was determined for each lift regardless if the lift was partial or full. The mean peak reactive force of each session was recorded. Afterward, the mean of these values for the initial five sessions and the final five sessions of training was calculated for each rat and statistically analyzed. To determine whether transient effects of edema or inflammation were present closely following the training regimes, half of the rats in each training regime were euthanized within a week (four to seven days) post-training. To characterize muscle well after any potential transient inflammatory responses, the remaining trained rats were euthanized 14 days after the last training session. Since no overt edema or inflammatory response was observed within one week following the last training session and no effect of time was observed in the analyses of muscle fiber number or size used for this study, data from muscles 4–14 days post-training were pooled for each group.

**Histology.** SOL, MG, LG, PL, and TA muscles were removed by dissection, weighed, covered with tissue freezing media, and frozen in cold isopentane at  $-80^{\circ}\text{C}$ . The mid belly of each muscle was cryosectioned at 10  $\mu\text{m}$  thickness. These transverse sections of muscles were stained with hematoxylin and eosin. During histological analysis, each slide was coded to prevent the investigator from knowing in which group each slide belonged.

**Quantitative morphology.** Histological analysis was performed by a standardized stereological method that has been utilized and described by our group in previous publications.<sup>29–33</sup> Points of a 121-point 11-line overlay graticule (0.04 mm<sup>2</sup> square with 100 divisions) were evaluated at 40× magnification. On either side (by 1 mm) of the midpoint of the midsection, stereological analysis was systematically repeated at five equally spaced sites across the muscle section. As 121 points were evaluated in 10 fields, a total of 1210 points were analyzed per muscle section. Percent of muscle tissue for degenerative muscle fibers, non-degenerative muscle fibers, and centrally nucleated muscle fibers was computed as the percentage of points that overlaid the type of muscle fibers of interest relative to the total number of points. Three criteria for degenerative muscle fibers were as follows: (1) those that lost contact with surrounding fibers, (2) cellular infiltrates interdigitating the sarcolemma, and (3) cellular infiltrates internal to the muscle fiber.<sup>29</sup> If any of these criteria were not met, the fiber was considered non-degenerative. Centrally nucleated fibers were considered to be any fibers with at least one internal nucleus not in contact with the sarcolemma. Percent of muscle tissue for cellular interstitium and non-cellular interstitium was computed as the percentage of points that overlaid the type of interstitium of interest relative to the total number of points. Points that overlaid nuclei in sites between muscle fibers were counted as cellular interstitium. Points that overlaid regions between muscle fibers but did not directly overlay nuclei were counted as non-cellular interstitium.

For MG, LG, PL, and TA muscles, in addition to evaluating points of the overlay graticule at each of sites sampled per section, we evaluated the number of fibers within the boundary of the graticule. These values were used to estimate mean muscle fiber area and the number of fibers per unit cross-sectional area. A muscle fiber was counted when the topmost point of the fiber was within the outermost boundary of the graticule. As the sections consisted almost exclusively of non-degenerative muscle fibers and degenerative fibers lacked distinct borders, degenerative muscle fibers were not counted. The number of fibers per unit cross-sectional area (number of fibers per square millimeter) was calculated as the total number of fibers counted divided by the total area sampled over 10 regions (ie, 0.4 mm<sup>2</sup>). Because of the small size of SOL muscles, total counts of all the muscle fibers in each section were feasible to perform. These counts were divided by the area of each section to determine fiber number per unit cross-sectional area directly. Mean muscle fiber area (μm<sup>2</sup>) was determined by dividing the percent tissue fraction of non-degenerative muscle fibers by the fiber number per unit area.

**Statistical analyses.** Peak reactive force data were analyzed with a two-way repeated measures ANOVA with Student–Newman–Keuls post hoc tests. Data from the histological analysis were analyzed using one-way ANOVA with Student–Newman–Keuls post hoc tests. If normality or equal

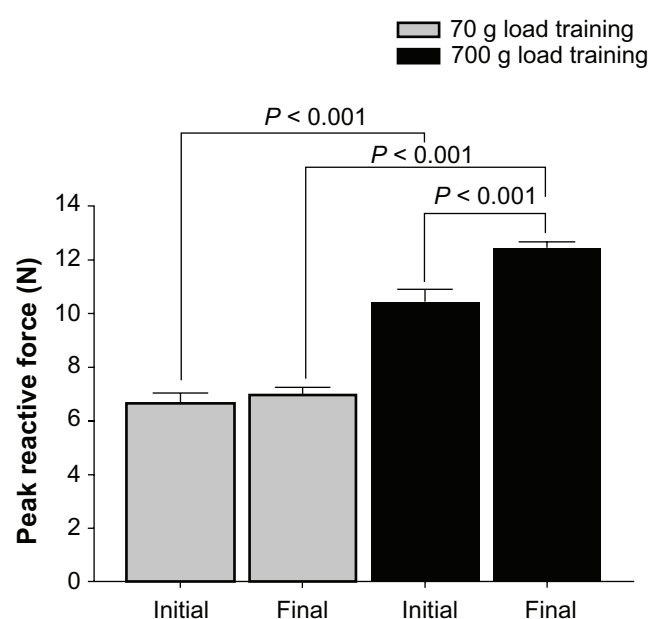
variance could not be assumed, the data were analyzed by Kruskal–Wallis one-way ANOVA by ranks with the Wilcoxon method of post hoc tests. All data are shown as means ± SEM.  $P < 0.05$  was considered statistically significant.

## Results

Peak reactive force during each lift was used to assess performance. In general, greater peak reactive forces were required during 700 g load training compared with those during 70 g load training (Fig. 1). When comparing the peak reactive forces early in training with those late in training, an enhancement of  $21 \pm 6\%$  was only observed for 700 g load training. Therefore, one month of training was sufficient to improve performance when exposed to a moderately heavy external load.

The performance gain was not accompanied by increased muscle mass, thereby excluding muscle mass as a factor for the adaptation (Table 1, Figure 2). To investigate muscle morphology, muscle transverse sections were evaluated. No training effect was observed in the percentage of muscle tissue composed of non-degenerative muscle fibers, degenerative muscle fibers, or centrally nucleated fibers (Table 2, Figure 3, Supplementary Figure 1A–C). This was also consistent with the absence of changes in the cellular and non-cellular interstitium, indicating a lack of edema and the absence of accumulated connective tissue (Table 2, Figure 3, Supplementary Figure 1A–C). Overall, these results indicate that chronic cycles of degeneration/regeneration did not accompany training.

Despite the lack of a training effect on muscle mass, training had a significant effect on muscle fiber size and



**Figure 1.** Performance improved after one month of 700 g load training. The plot depicts the mean peak reactive forces for the initial and final five sessions of training with 70 and 700 g loads ( $N = 8$  per group). Values are means ± SEM.

number. Following the 700 g load training, mean muscle fiber area decreased by 15% for SOL muscles and 21% for TA muscles relative to control values (Fig. 4). This decrease in muscle fiber size was accompanied by an increase in muscle fiber number per unit area – 16% for SOL muscles and 23% for TA muscles relative to control values (Fig. 5). Whole muscle section areas were not significantly different between 700 g load training, 70 g load training, and cage control conditions (SOL muscles –  $12.8 \pm 0.3 \text{ mm}^2$ ,  $12.3 \pm 0.4 \text{ mm}^2$ , and  $13.1 \pm 0.5 \text{ mm}^2$ , respectively,  $P$  value = 0.45; TA muscles –  $47.7 \pm 1.3 \text{ mm}^2$ ,  $47.0 \pm 1.3 \text{ mm}^2$ , and  $51.2 \pm 1.8 \text{ mm}^2$ , respectively,  $P$  value = 0.16). Therefore, the increased fiber number per unit area indicated an increase in total fiber number per muscle section. Because of the small size of SOL muscles, total counts of all muscle fibers in each section were feasible to perform. Following 700 g load training, the total number of fibers per muscle section increased 18% relative to control muscles and 11% relative to 70 g load training,  $P < 0.05$  for both comparisons (Fig. 6).

## Discussion

A multitude of reports and reviews exist in the scientific literature regarding exercise as an intervention to reduce work-related MSDs.<sup>34,35</sup> While resistance exercise in general appears to alleviate MSDs, novel insights into muscle adaptation and consensus on exercise prescription have been difficult to ascertain. This is largely because of the methodological complexities that arise when investigating a human population.<sup>34,35</sup> Because of these complexities, investigators have worked to develop animal models of volitional resistance exercise.<sup>6–9</sup> The present investigation advances the research concerning volitional animal models of resistance exercise by demonstrating training-induced adaptation by the assessment of reactive forces and the observation of distinct alterations at the muscle fiber level.

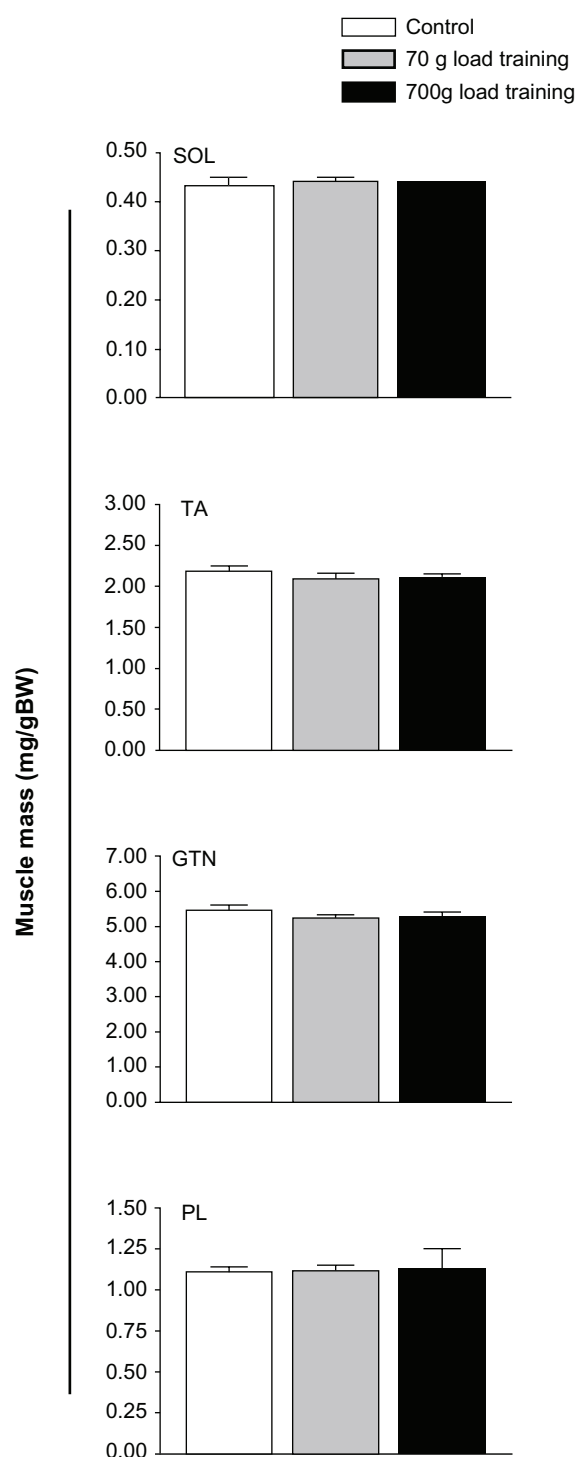
The main findings include the observation of strength gain exclusively following the heavier training load (ie, 700 g). Also, improvements in performance preceded muscle hypertrophy – a finding consistent with studies regarding human subjects.<sup>17–19</sup> In one month, 700 g load training induced an

**Table 1.** Body weights and muscle masses for cage control and training conditions.

	CONTROL	70 g LOAD	700 g LOAD
Body weight (g)	404 ± 4	392 ± 5	396 ± 2
SOL (mg)	173 ± 5	174 ± 7	172 ± 5
TA (mg)	879 ± 25	820 ± 32	836 ± 18
GTN (mg)	2203 ± 62	2051 ± 57	2094 ± 47
PL (mg)	447 ± 11	438 ± 13	448 ± 6

**Notes:** Values are means ± S.E.M. Body weight is the weight at the time muscles were removed. Sample size was  $N = 8$  per group with the exception of the TA muscle (sample size was  $N = 4$  for controls and  $N = 5$  for each of the 70 g and 700 g loads). No significant differences were observed.

**Abbreviations:** SOL, soleus; TA, tibialis anterior; GTN, entire gastrocnemius; PL, plantaris.



**Figure 2.** Muscle mass was unaltered by training. Values for muscle mass were normalized to BW. No significant differences were observed. Values are means ± SEM.

increase in peak reactive force of 21% despite unaltered muscle masses for the major muscles of the hindlimb. Previous work demonstrated that increased muscle mass occurs later – at two months of 700 g load training.<sup>27</sup> Despite the lack of muscle hypertrophy in the present study, alterations occurred at the muscle fiber level. Histological analysis indicated a 16 and 23%



**Table 2.** No change in percentage of muscle tissue composed of degenerative muscle fibers, centrally nucleated muscle fibers, and interstitium in muscle sections following training with different loads.

	CONTROL	70 g LOAD	700 g LOAD
SOL			
Non-degenerative muscle fibers (%)	937 ± 0.6	94.3 ± 0.3	93.7 ± 0.6
Degenerative muscle fibers (%)	0.33 ± 0.20	0.12 ± 0.09	0.29 ± 0.17
Centrally nucleated muscle fibers (%)	3.7 ± 1.4	3.9 ± 1.5	3.4 ± 1.0
Cellular interstitium (%)	1.1 ± 0.2	1.0 ± 0.2	0.9 ± 0.2
Non-cellular interstitium (%)	4.9 ± 0.4	4.7 ± 0.3	5.2 ± 0.5
TA			
Non-degenerative muscle fibers (%)	97.9 ± 0.3	97.0 ± 0.3	96.7 ± 0.5
Degenerative muscle fibers (%)	0.00 ± 0.00	0.07 ± 0.07	0.00 ± 0.00
Centrally nucleated muscle fibers (%)	0.7 ± 0.2	1.0 ± 0.3	0.3 ± 0.2
Cellular interstitium (%)	0.2 ± 0.1	0.3 ± 0.1	0.3 ± 0.3
Non-cellular interstitium (%)	1.8 ± 0.4	2.6 ± 0.2	3.0 ± 0.5
LG			
Non-degenerative muscle fibers (%)	93.9 ± 0.6	94.1 ± 0.6	93.1 ± 0.5
Degenerative muscle fibers (%)	0.03 ± 0.03	0.00 ± 0.00	0.01 ± 0.01
Centrally nucleated muscle fibers (%)	1.9 ± 0.7	0.8 ± 0.3	2.2 ± 0.6
Cellular interstitium (%)	1.8 ± 0.2	1.7 ± 0.2	2.1 ± 0.2
Non-cellular interstitium (%)	4.3 ± 0.5	4.1 ± 0.5	4.8 ± 0.5
MG			
Non-degenerative muscle fibers (%)	94.8 ± 0.8	94.7 ± 0.7	95.7 ± 0.4
Degenerative muscle fibers (%)	0.03 ± 0.03	0.02 ± 0.02	0.00 ± 0.00
Centrally nucleated muscle fibers (%)	1.8 ± 0.4	3.8 ± 1.1	1.1 ± 0.4
Cellular interstitium (%)	1.3 ± 0.3	1.7 ± 0.4	1.6 ± 0.3
Non-cellular interstitium (%)	3.8 ± 0.6	3.5 ± 0.5	2.7 ± 0.3
PL			
Non-degenerative muscle fibers (%)	96.5 ± 0.3	96.7 ± 0.5	97.0 ± 0.3
Degenerative muscle fibers (%)	0.02 ± 0.02	0.00 ± 0.00	0.01 ± 0.01
Centrally nucleated muscle fibers (%)	1.5 ± 0.4	2.4 ± 0.5	2.1 ± 0.4
Cellular interstitium (%)	0.3 ± 0.1	0.4 ± 0.1	0.2 ± 0.1
Non-cellular interstitium (%)	3.2 ± 0.3	2.9 ± 0.4	2.8 ± 0.2

**Notes:** Values are means ± S.E.M. Sample size was N = 8 per group with the exception of the TA muscle (sample size was N = 4 for controls and N = 5 for each of the 70 g and 700 g loads). Values expressed as percentage were in reference to percentage of tissue fraction. No significant differences were observed.

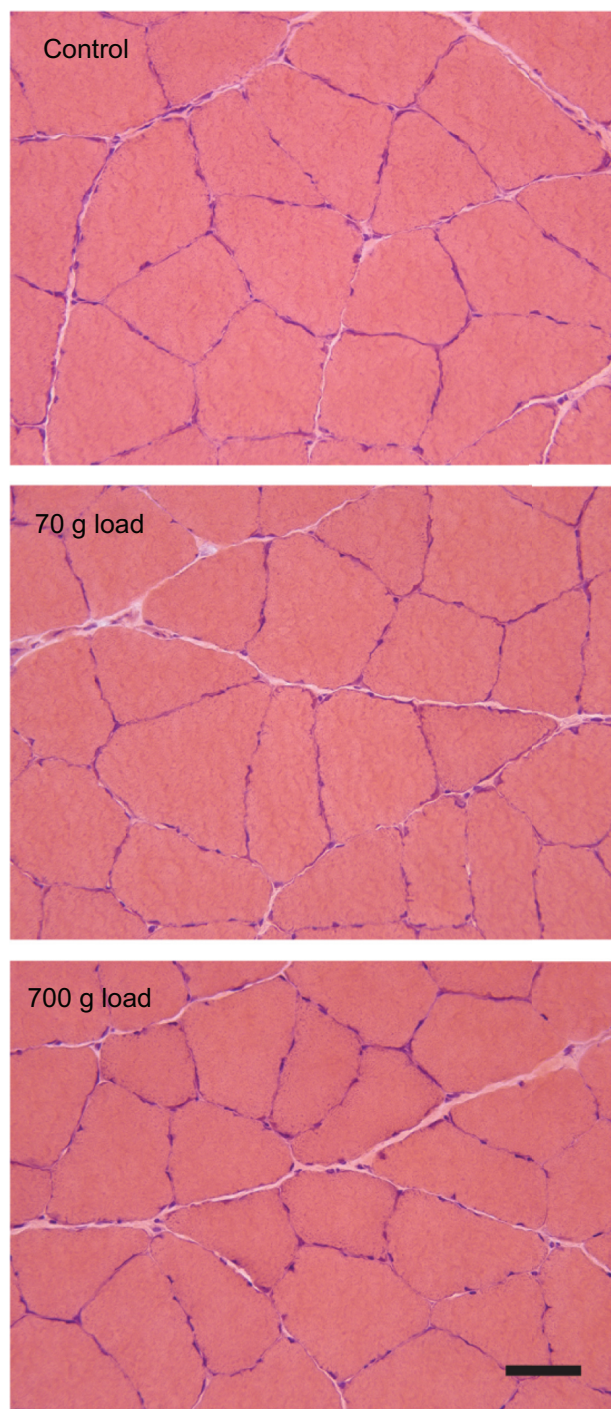
**Abbreviations:** SOL, soleus; TA, tibialis anterior; LG, lateral gastrocnemius; MG, medial gastrocnemius; PL, plantaris.

increase in muscle fiber number and a 15 and 21% decrease in mean muscle fiber area for SOL and TA muscles, respectively. Our results demonstrate early strength gains in a volitional animal model of resistance training and establish a synchronization between muscle fiber number and performance gains independent of alterations in muscle mass.

A training-induced increase in muscle fiber number has been observed in several investigations of voluntary models of animal resistance training.<sup>7,8,14–16</sup> Consistent with the present study, a training-induced effect on muscle fiber number has been noted previously for SOL muscles.<sup>15</sup> Adult (19 months of age) rats were trained progressively in squat-like exercise for approximately 10 lifts per day, four days per week for

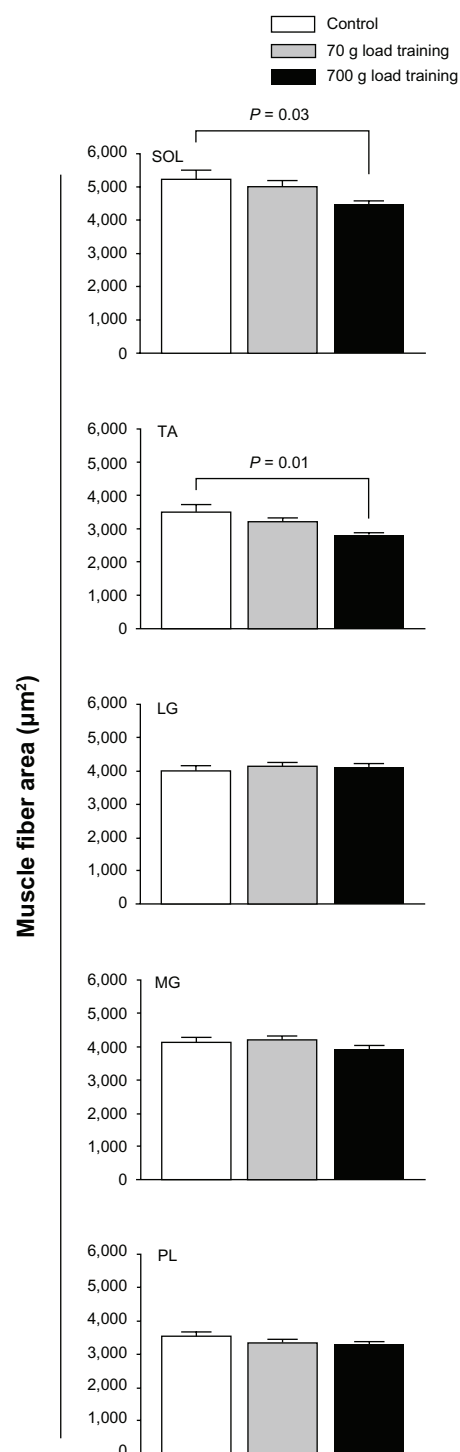
20 weeks (reaching ~800 g external load toward the end of the training).<sup>9,15</sup> Muscle mass increased by 22% and fiber number increased by 14%, muscle adaptations consistent with several reports regarding humans.<sup>15,36–40</sup> Despite these intriguing findings, these studies were limited because muscle morphology was analyzed only after muscle hypertrophy was achieved.<sup>7,8,14–16</sup> Consequently, these morphological alterations were associated with the muscle hypertrophic response and did not address whether they might occur before muscle hypertrophy or influence performance.

In the present study, we demonstrate that increased fiber number occurs before increased muscle mass following 700 g load training. This was possible because the increased



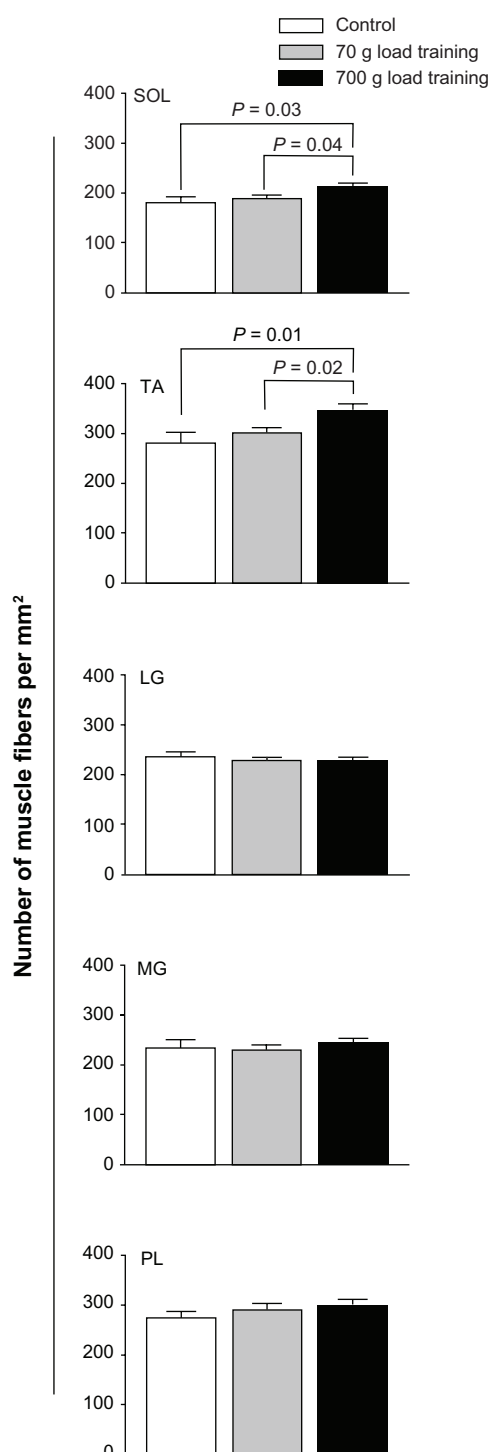
**Figure 3.** No indications of chronic degeneration/regeneration or alterations to the interstitium with training. Transverse sections of SOL muscles stained with hematoxylin and eosin. Scale bar = 50  $\mu\text{m}$ .

fiber number was countered by a shift to smaller muscle fiber sizes. Such a shift in fiber size with resistance training has been observed previously in animal studies where muscle hypertrophy had already been reached.<sup>7,16</sup> Overall, the implication is that increased muscle fiber size is not required for either early or late muscle adaptation. With 70 g load training, muscle fiber size and number were unaltered. These results are consistent with the reactive forces



**Figure 4.** Smaller mean muscle fiber area in SOL and TA muscles accompanied training. Data were from  $N = 8$  per group for the SOL, MG, LG, and PL muscles. For the TA muscle, sample size was  $N = 4$  for cage control conditions and  $N = 5$  for each of the 70 and 700 g load training. Values are means  $\pm$  SEM.

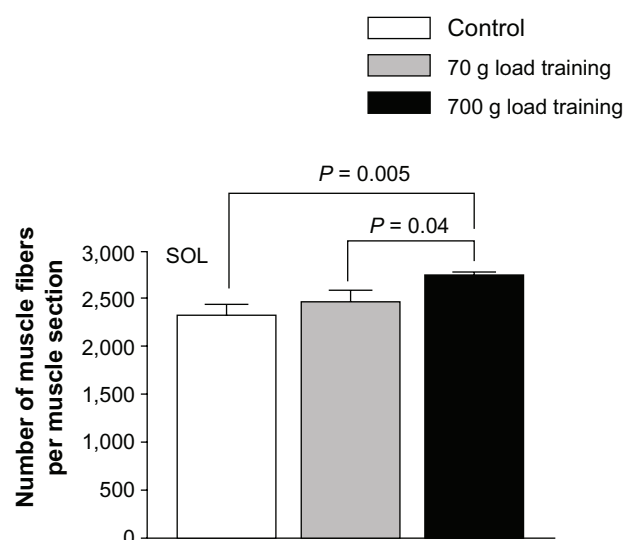
characteristic of rats training with 70 and 700 g loads. Even early in training, 700 g load training consisted of peak reactive forces that were twofold greater than those generated during 70 g load training. This implies that the significant increases in fiber number with 700 g load training were



**Figure 5.** Training-induced increases were observed in the number of muscle fibers per unit area. SOL and TA muscles exhibited increased number of muscle fibers per unit area following 700 g load training. Data were from  $N = 8$  per group for the SOL, MG, LG, and PL muscles. For the TA muscle, sample size was  $N = 4$  for cage control conditions and  $N = 5$  for each of the 70 and 700 g load training. Values are means  $\pm$  SEM.

because of high reactive forces necessary for handling a heavy external load.

The results indicated that increases in fiber number also occurred for the TA muscle exclusively following 700 g load training. Biomechanical analysis of human subjects



**Figure 6.** Increased muscle fiber number per transverse muscle section was observed with 700 g load training. Because of the relatively small size of the SOL muscle compared with the other hind limb muscles, it was feasible to count all the muscle fibers in each SOL muscle section. Data were from  $N = 8$  per group. Values are means  $\pm$  SEM.

demonstrates that the TA muscle, an antagonist for ankle plantarflexion, is activated during squats.<sup>41</sup> Such activation stabilizes the hindlimb and assists descent in controlled squats or squat-related movements.<sup>41,42</sup> This control was, perhaps, most important during 700 g load training of the present study because of the higher descent velocities inherent to that training compared with 70 g load training.<sup>27</sup> Therefore, the need for precise control was likely heightened and, consequently, required increased activation of the predominantly type II dorsiflexor TA muscle. These results highlight the importance of evaluating antagonist muscles in volitional training studies – a practice rarely done for animal models.<sup>7,8,14–16</sup>

Our group previously reported that gastrocnemius (GTN) muscles also increase mass after two months of 700 g training.<sup>27</sup> This occurred despite the absence of a training effect at the muscle fiber level after the shorter duration of training in the present study. In the previous report, the increased muscle mass for GTN muscles (~5%) was half that of SOL muscles (~10%). This implies that GTN muscles may have experienced a muted response compared with SOL muscles. The decreased responsiveness for GTN muscles is not surprising given the external load tested. That is, 700 g is twofold greater than the BW of the rats – a moderate external load because rats are able to voluntarily train with threefold of their BW.<sup>43</sup> In addition, the load was fixed so that training was not progressive, implying the load was perceived as submaximal during the weeks of training. Therefore, compared with maximal or near-maximal loads, the expectation for lifting in the present study is that full neuromuscular recruitment of muscles with predominantly type II muscle fibers (eg, GTN muscles) was not required. Rather, in this case, plantarflexion was more dependent on the





predominantly type I SOL muscle. Additionally, it should be noted that the GTN muscle is a biarticular muscle (ie, crosses and acts upon two joints). Consequently, the different movement dynamics of such a muscle during each lift may have been a factor in the dissimilar response. If this is true, this would suggest that training-induced fiber number increases may be dependent on the muscle-to-joint relationship during each movement of resistance training, a possible factor to consider when designing and interpreting studies.

An explanation for how the morphological alterations observed in the present study originated and how such alterations potentially improved muscle performance could not be determined because of restricted availability of tissue. A limited amount of tissue was collected, sectioned, stained with hematoxylin and eosin, and stored for analysis in the present study. With a more expansive collection of tissue for analysis in future research concerning this animal model, several mechanisms could be explored further. For example, the mechanism for increased fiber number, fiber splitting or *de novo* fiber formation, could be determined.<sup>13</sup> In addition, the mechanism by which the morphological alterations potentially contributed to strength gains could be established. One such mechanism to consider is whether the remodeling inherent in increasing muscle fiber number also induces remodeling at the neural and connective tissue levels – two adaptations previously proposed to influence early strength gains.<sup>20–24,44</sup> Another mechanism to consider is whether a diminished metabolic/diffusion gradient inherent in small muscle fibers improves force development during prolonged training such as that tested in the present model (rats were exposed to a high number of lifts, ~100 per session). Aside from a diminished diffusion gradient, small muscle fibers have a high sarcolemmal to cytoplasmic volume ratio, a feature that may improve lateral force transmission and increase sarcomere length homogeneity during contractions.<sup>45,46</sup> Regardless of the particular mechanism involved, the finding of the present study that alterations in muscle fiber number and size coincide with early strength gains before increased muscle mass suggests the possibility that these morphological alterations directly influence performance.

The findings of the present investigation provide motivation to further research fiber number modulation in human studies. Indirect evidence regarding body builders indicates that training-induced increases in muscle fiber number are possible.<sup>37–40</sup> Such an adaptation has obvious benefits to conditions associated with muscle fiber loss such as aging,<sup>47,48</sup> muscular dystrophy,<sup>49,50</sup> and accident-related denervation.<sup>51</sup> An encouraging finding from the present study is that chronic cycles of widespread degeneration/regeneration are neither inherent nor required, for adaptation to resistance training.<sup>7,16,52</sup> This is in agreement with the notion that severe inflammation and muscle fiber degeneration follow acute extreme contractions associated with strains but are absent after acute bouts of stretch-shortening contractions modeled

after customary resistance-type training.<sup>52</sup> As a consequence, MSDs may be avoided by scientifically based training regimes where such training has the potential to benefit those persons with compromised conditions. Indeed, in one study regarding experimental animals, voluntary resistance training decreased age-related muscle fiber loss.<sup>15</sup> In addition to the potential to slow fiber number reduction in several detrimental conditions, the present work offers a rationale to investigate muscle fiber number alteration further – as a process that may benefit healthy individuals such as athletes/performers (eg, wrestlers, olympic-style weightlifters, ballet dancers) pursuing performance gains without directly increasing muscle bulk. If this notion is supported, fiber number modulation would provide an alternative goal for accelerating early strength gains induced by resistance training.

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### Author Contributions

EPR, GRM, OW, and BAB conceived and designed the experiments. EPR, RDC, OW, and BAB analyzed the data. EPR and BAB wrote the first draft of the manuscript. EPR, GRM, RDC, OW, and BAB agreed with the manuscript results and conclusions. EPR, GRM, RDC, OW, and BAB jointly developed the structure and arguments for the paper. EPR, GRM, RDC, OW, and BAB made critical revisions and approved the final version. All the authors reviewed and approved the final manuscript.

### Publication Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the NIOSH.

### Supplementary Data

**Supplementary Figure 1A.** Low magnification (10X) image of a transverse section of SOL muscle following cage control conditions.

**Supplementary Figure 1B.** Low magnification (10X) image of a transverse section of SOL muscle following 70 g load training.

**Supplementary Figure 1C.** Low magnification (10X) image of a transverse section of SOL muscle following 700 g load training.

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