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Distinct patterns of fiber type adaptation in rat hindlimb muscles 4 weeks after hemorrhagic stroke

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Abstract

Objective: The aim of this study was to evaluate adaptations in soleus (SOL) and tibialis anterior (TA) muscles in a rat model 4 weeks after hemorrhagic stroke.

Design: Young adult Sprague-Dawley rats were randomly assigned to two groups: stroke (STR) and control (CTRL), with 8 SOL and 8 TA muscles per group. Hemorrhagic stroke was induced in the right caudoputamen of the STR rats. CTRL rats had no intervention. Neurologic status was evaluated in both groups pre-stroke, and four weeks post-stroke. Muscles were harvested after post-stroke neurologic testing. Muscle fiber types and cross-sectional areas were determined in SOL and TA using immunohistochemical labeling for myosin heavy chain (MHC).

Results: No generalized fiber atrophy was found in any of the muscles. Fiber types shifted from faster to slower in the TA of the STR group, but no fiber type shifts occurred in the SOL muscles of STR animals.

Conclusion: Since slower MHC fiber types are associated with weaker contractile force and slower contractile speed, this faster-to-slower fiber type shift in TA muscles may contribute to weaker and slower muscle contraction in this muscle after stroke. This finding may indicate potential therapeutic benefit from treatments known to influence fiber type plasticity.

Keywords

skeletal muscle; stroke; rehabilitation; immunohistochemistry

Introduction:

A well-known clinical manifestation of stroke is hemiparesis in the limbs opposite the side of the brain lesion¹. Multiple factors contribute to this weakness. Such factors include ^{2,3,4,5} muscle atrophy, altered central drive with altered muscle activation, corticospinal tract

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and/or motor unit degeneration, disuse/bedrest, inflammation, hormonal factors, and suboptimal nutrition; the possibility of post-stroke sarcopenia/cachexia has also been raised⁵.

Study of skeletal muscle in human hemiparesis is a challenging task. Patients often have co-existing conditions that are difficult to control for, yet that affect skeletal muscle phenotypes. Such conditions include aging, diabetes, and altered physical activity/disuse. Human studies also need to account for effects of rehabilitation therapies in the early post-stroke period. As a result, many muscle studies in human hemiparesis are conducted in chronic stroke, several months to many years post-stroke^{6,7,8,9}. Less is understood about muscle response to stroke at earlier post-stroke periods.

For the above-noted reasons, animal models play a role in advancing the understanding of post-stroke hemiparesis. Such models can allow for control of age, comorbid disease, diet, and activity. Animal models can also ethically allow for observation of spontaneous recovery without the stricter ethical requirement for rehabilitative therapies in human stroke studies. Of particular note in this regard is use of a well-established rat model of hemorrhagic stroke to evaluate neurologic status and skeletal muscle parameters at 2 weeks post-stroke.¹⁰ At that 2 week interval, hemiparesis was present, and skeletal muscle adaptations were observed in the paretic hindlimb. These adaptations included specific findings in the hemiparetic tibialis anterior muscles, such as: a) atrophy of 2x muscle fibers, b) hypertrophy of type 1/slow fibers, and c) a strong trend toward increased proportion of type 1/slow fibers. In light of those findings, the *objective* of the current investigation was to use this same hemorrhagic stroke model at 4 weeks post-stroke, to evaluate for ongoing, spontaneously-occurring skeletal muscle plasticity in bilateral hindlimb muscles.

Methods:

Subjects:

Young adult male Sprague Dawley rats (~ 2 months of age, weights 225–249 grams) were randomly divided into two groups: stroke (STR, n=8) and control (CTRL, n=4). Control rats had no brain lesions. This designation was based on results of the aforementioned 2 week post-stroke study¹⁰ that used the same rat strain and experimental procedures as in the current study. The 2 week study included a sham surgery group, as well as a no-surgery control group.¹⁰ Analyses in that study identified no statistically significant differences in activity, neurologic status, fiber type or fiber size between the sham group and the no-surgery controls. These results indicated that it was not necessary for the current study to include a sham surgery group. Outcome measures of right and left CTRL hindlimbs were statistically compared with t tests, and found to be not significantly different from each other. Therefore the findings from right and left CTRL hindlimbs were combined to yield n=8 muscles for each outcome measure in this study. This approach also allowed use of fewer animals in accordance with protocol criteria for responsible animal use.

Each rat occupied a standard cage; all rats were housed in the same room, with 12 hour light/dark cycles. They all had access to standard rat chow and water *ad libitum*. Since prior work with this model indicated no significant differences in general activity levels between

stroke and control animals,¹⁰ these rats were allowed free cage-based activity without additional quantification. All procedures were approved by the Institutional Animal Care and Use Committee at the University of Minnesota.

Neurologic testing:

All rats in each group were evaluated for neurologic function with use of a ladder-walking test.¹¹ Testing was done at the beginning of the experimental period, as well as 4 weeks after stroke, or 4 weeks after initial testing in the controls. The ladder-walking test consisted of each rat walking on metal rungs placed between two plexiglass walls, which were spaced such that the rat could not turn around (Fig. 1). The rungs were positioned approximately 12 inches above the bench for ease of foot placement visualization. Each rat was familiarized with the equipment by being allowed to walk freely within the apparatus, over regularly-spaced rungs. For the experimental assessments, each rat traversed the ladder when rungs were placed in each of three designated patterns of irregular spacing. Each run was video-recorded (Canon Elura 100). Videos were evaluated for foot placement with use of a video-imaging program (Sony Vegas Movie Studio, Platinum edition) that allowed for slowing of the videos for frame-by-frame analysis. Videos were scored by blinded observers. Scores for foot placement were assigned according to the scale developed and validated by Metz and Whishaw.¹¹ Scores ranged from 6 (normal foot placement) to 0 (full fall through the rungs without foot contacting the rung; Fig 1). Scores of 0, 1, and 2 were designated as “errors”, and the number of errors was tabulated for each trial. Error number was normalized to number of steps in each run, to yield errors per step (EPS). Values for the 3 rung patterns were combined for final tabulations. EPS were compared pre-to-post to determine the extent of neurologic functional change. The empiric quality of limb movements was also observed during the ladder testing. There was no indication of limb spasticity in the stroke animals as they traversed the ladder, nor was there any indication of spasticity when the animals were handled.

Stroke induction, right striatum (caudoputamen, Fig. 1D):^{12,13}

Rats of the STR group were anesthetized with a mixture of intraperitoneal ketamine and xylazine (100 mg/kg and 9.3 mg/kg respectively; both from Phoenix Pharmaceuticals, St. Joseph, MO). They were then given a preoperative antibiotic (enrofloxacin, 25 mg/kg, subcutaneous; Bayer Healthcare, Shawnee Mission, KS). After no response to pain was observed and corneal reflexes were absent, each rat was placed in a Kopf stereotaxic frame. Incision was made in the scalp to the right of midline, exposing the skull. A small hole was then drilled in the skull, at coordinates of 3.2 mm lateral to the bregma, and 0.4 mm anterior. The dura was opened. A solution of 0.5 units of type VII bacterial collagenase (Sigma) in 1.0 μ l normal saline was injected into the right striatum using a 10 μ l Hamilton syringe. Coordinates for the right striatum were: toothbar 0.0 mm, medial-lateral 3.2 mm, anterior-posterior 0.4 mm, and dorsal-ventral 5.0 mm. The collagenase solution was infused over 2 minutes, and the needle remained in place for 3 minutes thereafter. Upon completion of the infusion, the needle was slowly withdrawn, and the scalp incision was sutured. Animals were removed from the frame and given post-operative pain medication (ketoprofen 5 mg/kg, subcutaneous; Fort Dodge Animal Health, Fort Dodge, IA). They were placed in their cages under incandescent lights, and observed closely in post-operative recovery until

they were able to eat and drink. Animals were weighed daily for the following three days. Sutures were removed on the third post-operative day. All rats continued in their usual cage-based activity for 4 weeks after surgery. The 4 week time period was chosen in order to evaluate potential evolution of muscle adaptations compared with prior 2 week post-stroke data.¹⁰

Since the current study's emphasis is on post-stroke limb function and associated muscle characteristics, and since the method of stroke induction used here is well-characterized, motor function was used as the indicator of stroke severity, rather than stroke lesion volume. In the animals of this 4 week study, the stroke lesions were severe enough to cause subtle motor performance deficits on the ladder test. The mean EPS value for the stroke group at 4 weeks was quite similar to that in the 2 week stroke group (0.17 ± 0.05 , and 0.15 ± 0.03 respectively)¹⁰.

Muscle acquisition:

Animals were deeply anesthetized with intraperitoneal pentobarbital sodium (75 mg/kg, Lundbeck, Deerfield IL). After no response was observed to painful stimuli, incisions were made in the skin overlying the anterior and posterior aspects of the lower hindlimbs. Bilateral soleus (SOL) and tibialis anterior (TA) muscles were excised after dissection from connective tissues and neighboring muscles. Muscles were weighed, then flash-frozen in isopentane chilled over liquid nitrogen. Frozen muscles were stored at -80°C . until sectioned.

Fiber typing, immunohistochemistry:

Muscle cross-sections from bilateral SOL and TA muscles were cut on a cryostat (Leica Microsystems, Nusslock Germany) at -20°C ; sections were 10–12 microns thick. Fiber typing was done by immunolabeling the cross-sections with antibodies to myosin heavy chains (MHC).¹⁰ Five consecutive slides were each labeled with a different primary MHC antibody in order to evaluate for the spectrum of fiber types (Table 1). Primary antibodies were as follows:

- 1) Slow MHC (type 1)
- 2) Fast MHC (type 2's all inclusive)
- 3) 2a MHC (type 2a)
- 4) 2b MHC (type 2b)
- 5) Developmental MHC (embryonic)

Table 1 identifies primary antibody dilutions and suppliers. Muscle fibers that labeled for Fast MHC, but not for 2a or 2b were identified as type 2x (Table 1). Fibers that labeled for more than one MHC were noted as hybrids,¹⁴ and were identified by the fiber types present (1+2a, 2a+2b, etc.). Procedural controls were done by performing the entire immunolabeling assay, but without use of primary antibody. One slide per muscle was also stained with hematoxylin and eosin (H&E) for basic morphology.

Imaging and analysis for fiber type and fiber size:

The serial muscle sections were viewed under light microscopy at 225 $\times$. The H&E sections were used to identify muscle regions with good quality morphology; digital images of these areas were taken. Regions matching the H&E sections were located in each of the separate MHC-labeled tissues. These MHC-labeled regions were also digitally imaged. Fiber type was determined by viewing all the MHC-labeled sections for a given muscle, and tabulating the positively labeled vs. negatively-labeled muscle cells (Table 1, Fig. 2). As noted above, when a fiber concurrently expressed more than one MHC protein, the fiber was identified with the observed MHC combination (1+2a, 2a+2b, etc.).

Fiber cross-sectional areas were determined with use of the image-processing program Image J (NIH). The cells used for fiber typing were also used for fiber cross-sectional area determinations, so data was obtained for cross-sectional area by fiber type.

The SOL is a predominantly slow-type muscle, and essentially uniform in the distribution of the type 2 fibers present. Four fields were imaged for each soleus muscle, 100 cells per field. In contrast, TA has a non-uniform fiber type distribution. The deep TA consists of type 1 (slow) fibers, as well as a variety of type 2 fibers, especially 2a's. The superficial TA on the other hand, has only type 2 fibers, with a large proportion of 2b's. Five fields were imaged for each TA muscle; two deep fields, and three superficial fields, eighty cells per field (Fig. 2). Thus, four hundred cells were analyzed for each SOL and each TA muscle. Analyses were done by blinded observers who demonstrated good inter-rater reliability (coefficient of variation=6.2%).

Statistical analyses:

Data is presented as means $\pm$ SEM. Rat demographic data was compared using 2 sample t-tests. Ladder test data was analyzed with 2 way repeated measures ANOVA (group $\times$ time); Fisher's Least Significant difference posthoc test was used when a significant overall F value was obtained. Fiber types and fiber areas were compared within groups using t tests. One way ANOVA was used for comparison of the STR limbs with the CTRL group. Post-hoc comparisons were done with Newman-Keuls test if an overall significant F value was obtained. Appropriate non-parametric tests were used if assumptions were not met for the parametric tests. Statistical significance was set at $p = 0.05$. All analyses were done with NCSS statistical software (Kayesville, UT).

Results:

Rat characteristics (Table 2):

Body weights and muscle weights were not statistically different between groups.

Neurologic testing (Fig. 1):

STR animals had significantly more stepping errors after stroke than before (EPS post= 0.17 ± 0.05 vs pre= 0.07 ± 0.02 ; $p=0.041$). They also had significantly more post-test errors than did controls (STR= 0.17 ± 0.05 vs CTRL= 0.08 ± 0.03 , $p=0.041$). There were no differences

in stepping errors in the control group at pre-test vs post-test 4 weeks later (post= 0.08 ± 0.03 vs pre= 0.11 ± 0.04 , $p=0.31$).

Muscle fiber size and type:

Preliminary note: For ease of limb distinction in the following sections, the left hindlimbs of the STR group will be referred to as the hemiparetic limbs (P), and the right hindlimbs will be referred to as the nonparetic limbs (NP). It is understood however, that such a distinction is not as clear in reality, particularly in light of ipsilateral corticospinal tract motor innervation.¹⁵

Muscle cross-sectional areas (Tables 3 and 4): In SOL, there were no significant differences in fiber cross-sectional areas between P and NP sides of the STR group. There were also no significant differences in areas between STR and CTRL groups. In TA, there were no significant differences in fiber cross-sectional areas between P or NP sides of STR group in either deep or superficial TA, except for 2a fibers in the superficial TA. For these fibers, area was smaller in P than NP limbs. There were no significant differences in any cross-sectional areas when comparing STR vs CTRL muscles.

Muscle fiber types (Table 3, Figs. 3 and 4): The MHC expression changes were markedly different in the SOL muscle as compared to the TA. In the SOL, there were no significant differences in MHC fiber type between STR and CTRL animals, or between left and right limbs within each group. In contrast, the TA exhibited several significant fiber type differences, which varied according to location within the muscle. For the deep TA of the STR group, the P limbs had a greater percentage of type 1 (slow) fibers than the NP limbs ($p=0.046$), as well as a lower percentage of 2a fibers ($p=0.029$). The P limbs also expressed more 2x and less 2b than the NP side ($p=0.003$ and $p=0.035$ respectively). There were no significant differences in percentages of hybrid fiber types between P and NP limbs.

When deep TA muscle findings were compared between STR and CTRL groups, results differed between P vs CTRL and NP vs CTRL evaluations. The significant differences included:

- a) with all type 1 fibers combined (slow-only MHC and slow MHC in hybrid fibers), there was a greater proportion of slow MHC-expressing fibers in the P limbs than in CTRL ($p=0.05$)
- b) % 2b MHC in P limbs was less than in CTRL ($p=0.014$)
- c) % 2a in NP limbs was greater than in CTRL ($p=0.046$)
- d) % 2x MHC in NP limbs was less than in CTRL ($p=0.007$)
- e) no significant differences were present in proportion of fibers coexpressing MHC proteins, or in those expressing developmental MHC

In the superficial TA of the STR group, there were no significant differences between P and NP limbs for any fiber type. In contrast, however, when superficial TA fiber types were compared between STR and CTRL groups, there were significant differences, and the

pattern of these differences was not the same as in the deep TA. The superficial TA's significant differences were:

- a) % 2a in both P and NP limbs was greater than in CTRL (P, $p=0.02$, NP, $p=0.001$)
- b) % 2b in both P and NP limbs was less than CTRL (P, $p=0.004$, NP, $p=0.015$)
- c) no significant differences were present in proportion of 2x or hybrid fibers.

The analyses above indicate a fiber type shift from faster to slower in the both the deep TA and the superficial TA. They also indicate distinct adaptations in P vs NP muscles, and in deep vs superficial parts of these muscles. When compared to NP, the deep TA of the P side shows a transition toward type 1 vs. 2a, and a shift toward 2x vs. 2b. When compared to CTRL, the P muscles also show a greater percentage of type 1's, and smaller proportion of 2b's. The NP muscles have greater proportion of 2a's than CTRL, but a smaller percentage of 2x's. The superficial TA also exhibits a faster to slower shift, but not between P and NP sides. Rather, the plasticity is evident when compared only to CTRLs. Both P and NP superficial TA muscles express slower MHC profiles than CTRL, with the greater percentages of 2a's and lower percentages of 2b's.

Discussion:

In this study of muscle plasticity after hemorrhagic stroke, the following items have been demonstrated:

- a) The rat model of hemorrhagic stroke is successful, in that motor deficits remain in the paretic limbs at 4 weeks post-stroke.
- b) There is no evidence of SOL or TA muscle atrophy at 4 weeks post stroke, except in 2a fibers of the superficial TA in the paretic limbs.
- c) There are multiple MHC fiber type changes at 4 weeks after stroke. These changes are muscle specific, and even specific to the intramuscular location. These changes occur not in the SOL, but rather in the TA.
- d) In the TA, the primary fiber type shifts are in the direction of faster-to-slower. Such a shift is present within the STR group paretic vs. nonparetic sides. It is also present in bilateral STR muscles when compared to CTRL.
- e) In the TA, stroke-related fiber type changes are bilateral, not just limited to the paretic side.
- f) Percentages of hybrid fibers were no different between any of the groups.

Muscle cross-sectional area:

Results of the current study show that there was no muscle weight loss in the STR muscles, and muscle fiber atrophy was limited to only 2a fibers in the TA of the paretic STR limbs. Although very common after stroke,¹ muscle atrophy has not always been observed in studies of stroke-related hemiparesis. For example, Dormer et al¹⁶ did not find any evidence of atrophy in gastrocnemius muscles of rats 2 weeks post ischemic stroke, although contractile alterations were present. In clinical studies, Klein et al¹⁷ found that their chronic

post-stroke participants had no atrophy in dorsiflexor muscles, although they exhibited hemiparesis and decreased muscle activation. These results indicate that mechanisms controlling fiber size after stroke are distinct from those controlling fiber type. Therefore, muscle atrophy is not requisite for post-stroke muscle weakness. This conclusion necessitates consideration of other muscle characteristics that relate to weakness.

Fiber type:

Fast-to-slower shift—The fiber type plasticity revealed in the STR group of the current study indicates a general fast-to-slower shift in the TA, which is a predominantly fast-twitch muscle in rats. The fiber type shifts differed between the deep and superficial portions of this muscle in context of their differing baseline fiber types. The type shifts occurred bilaterally as well, but were not identical between right and left sides. In rodent fast twitch muscle, such a fast to slower MHC is typical of changes in innervation, or of denervation.^{14,18} In contrast, fiber type changes are minimal in fast twitch muscles subjected to unloading in context of an intact nervous system^{19,20} Such unloading conditions include bedrest, limb immobilization, hindlimb unweighting, or spaceflight. The presence of bilateral fiber type change in the STR group is evidence of the innervation pattern, with a small proportion of descending motor tracts remaining ipsilateral to their origins.¹⁵

Motor nerve stimulus patterns are crucial determinants of fiber type.^{14,21} Fast-to-slow fiber type shifts have been documented in chronic low frequency electrical stimulation, and in denervation.^{14,18} In stroke, high density surface EMG has documented slowed motor unit firing rates in hemiparetic muscles.²² Additionally, transcranial magnetic stimulation (TMS) studies have demonstrated prolonged latencies of motor evoked potentials in hemiparetic muscles after TMS application to the stroke-affected hemisphere.²³ These findings are supported by clinical studies that have documented slower and weaker voluntary muscle contraction post-stroke, not only in the hemiparetic side when compared to the non-paretic side, but also in the less-paretic limbs when compared to controls.^{24,25}

Given that force and speed of muscle contractions are directly related to MHC fiber type,²¹ a shift from faster to slower fiber type would be expected to result in a shift to a slower and weaker contractile profile in post-stroke muscle. Besides these properties related to fiber type, muscle fibers with smaller cross-sectional areas usually exert lower forces, since force production is proportional to cross-sectional area.²⁶ As verified in the current results, the slower contracting fiber types also typically have smaller cross-sectional areas and so would be expected to exert lower forces during contraction. Therefore, fiber type characteristics in and of themselves could be considered a contributing factor to post-stroke hemiparesis.

The current results are consistent with results of other investigations of muscle changes in chronic stroke. For example, Scelsi et al⁹ performed muscle biopsies on paretic and nonparetic TA muscles, in persons 50 days to 17 months post stroke. There was a type 1 fiber predominance in the paretic TA's of persons 3–17 months post stroke, but not in participants who were earlier in the post-stroke course. Type 1 predominance was also found in the nonparetic muscles of the participants. Dattola and colleagues²⁷ performed muscle biopsies of lateral gastrocnemius in persons with stroke. They identified increased type 1 percentages and decreased type 2 percentages in a majority of participants. These authors

surmised that such plasticity could be related to a transformation in motor unit type. Lastly, while not looking specifically at fiber type, electrophysiologic studies confirm slowing of isometric twitch contraction time, and decreased twitch tension in the hemiparetic muscle compared to nonparetic or healthy control muscle.³

In contrast, the current results differ from those of other investigators, who have reported increased proportions of fast fiber types in paretic vastus lateralis in chronic stroke.^{6,7} These differences may be related to species and specific muscles studied, as well as to study design, participant ages, presence of comorbidities, amounts of habitual physical activity, and time post-stroke. The findings reported in the current investigation are indicative of spontaneous changes in a subacute post-stroke condition, without therapeutic exercise intervention. The model attempts to control for comorbid diseases, aging, and physical activity levels.

Hybrid fibers—Numbers of hybrid fibers were not increased in either SOL or TA in the STR rats at the 4 week post-stroke time period. Similar results were found at 2 weeks post-stroke.¹⁰ This lack of increase occurred despite pronounced fiber type changes by 4 weeks post stroke. The stability of hybrid fiber numbers in the STR group over time may indicate that the sampling periods did not capture an increase that actually happened between 2 and 4 weeks. It could alternatively indicate that post-stroke muscle has not completed its type transitioning, and that increased hybrid MHC expression could be a future event. A third possibility is that fiber type transitions in stroke are not associated with an increase in hybrid fibers. Further study is needed to delineate the time course for changes in hybrid fiber expression in this stroke model.

Potential limitations: A sham surgery group was not included alongside the no-surgery control group, for reasons detailed in the methods section. Nonetheless inclusion of a sham group could have provided some additional perspective on the results.

Potential clinical applications:

Optimal therapeutic exercise approaches for post-stroke weakness are topics of ongoing study.^{7,8} In light of the current study's results, the question arises as to the potential use of muscle power training in persons after stroke, to target muscle contraction force concurrently with muscle contraction speed. Indeed, one study⁸ employed power training for bilateral lower extremities in persons 6–60 months post-stroke. This training resulted not only in bilateral strength and power improvements, but also in self-selected and comfortable walking speeds. It was also well-tolerated. Power training may have additional benefit, in that greater muscular power has been associated with decreased fall risk and decreased disability in older adults.²⁸

Another question arises regarding the capability of voluntarily elicited muscle contractions to influence an effective fiber type shift toward more normal levels during recovery after stroke. If the apparent slowing of cortical motor stimuli to post-stroke muscle²³ continues over time, the rate of voluntarily-generated stimuli may be insufficient to promote a transition from slower back to faster types, thus limiting the potential for gains in muscle strength and function. To address this possible limitation, consideration might be given to

use of very specifically prescribed peripheral electrical stimulation. Parameters could possibly be set to increase contraction velocity with goal of increasing fast fiber types, which would conceivably also increase contractile force. Peripheral stimulation applications could also possibly include parameters that could facilitate corticomotor function.^{29,30} Much study would be needed to address such questions, both at the basic science level and the clinical level.

Conclusion:

In this rat model, there is a bilateral fast-to-slower fiber type shift in the TA muscle at 4 weeks post-hemorrhagic stroke. This change occurs in the absence of general muscle atrophy, but is still associated with persistent neurologic deficit. This fiber type plasticity is limb specific, muscle-specific, and even specific to intramuscular regions of distinct fiber type. The shift pattern is consistent with a change in neuromotor function, and may contribute to the weakness and slow contraction speed seen in post-stroke muscle function. Further study is needed to evaluate therapeutic measures that could address these muscle changes to foster optimal patient improvement.

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References

1. Zorowitz RD, Harvey RL. Stroke syndromes In: Cifu David X., ed. Braddom's Physical Medicine and Rehabilitation. 5th ed. Philadelphia PA: Elsevier, Inc.: 2016:999–1016.
2. Sions JM, Tyrell CM, Knarr BA, Jancosko A, Binder-Macleod S. Age-and stroke-related skeletal muscle changes: A review for the geriatric clinician. *J Geriatr Phys Ther.* 2012;35:155–161. [PubMed: 22107952]
3. McComas AJ, Sica RE, Upton ARM, Aguilera N. Functional changes in motoneurons of hemiparetic patients. *J Neurol Neurosurg Psychiatry.* 1973;36:183–193. [PubMed: 4350702]
4. Springer J, Schust S, Peske K, et al. Catabolic signaling and muscle wasting after acute ischemic stroke in mice: Indication for a stroke-specific sarcopenia. *Stroke.* 2014;45:3675–3683. [PubMed: 25352483]
5. Knops M, Werner CG, Scherbakov N, et al. Investigation of changes in body composition, metabolic profile and skeletal muscle functional capacity in ischemic stroke patients: the rationale and design of the Body Size in Stroke Study (BoSSS). *J Cachexia Sarcopenia Muscle.* 2013;4:199–207. [PubMed: 23483531]
6. McKenzie MJ, Yu S, Prior SJ, Macko RF, Hafer-Macko CE. Hemiparetic stroke alters vastus lateralis myosin heavy chain profiles between the paretic and nonparetic muscles. *Res Sports Med.* 2009;17:17–27. [PubMed: 19266390]
7. Andersen JL, Jorgensen JR, Zeeman P, et al. Effects of high-intensity physical training on muscle fiber characteristics in poststroke patients. *Muscle Nerve.* 2017;56:954–962. [PubMed: 27977854]

8. Morgan P, Embry A, Perry L, Holthaus K, Gregory CM. Feasibility of lower-limb muscle power training to enhance locomotor function poststroke. *J Rehabil Res Dev* 2015;52(1): 77–84. [PubMed: 26186176]
9. Scelsi R, Lotta S, Lommi G, Poggi P, Marchetti C. Hemiplegic atrophy: Morphological findings in the anterior tibial muscle of patients with cerebral vascular accidents. *Acta Neuropathol* 1984;62:324–331. [PubMed: 6730908]
10. Snow LM, Low WC, Thompson LV. Skeletal muscle plasticity after hemorrhagic stroke in rats: influence of spontaneous physical activity. *Am J Phys Med Rehabil* 2012;91:965–976. [PubMed: 22760110]
11. Metz GA, Whishaw IQ. Cortical and subcortical lesions impair skilled walking in the ladder rung walking test: a new task to evaluate fore-and hindlimb stepping, placing and co-ordination. *J Neurosci Meth* 2002;115:169–179.
12. Chesney JA, Kondoh T, Conrad JA, Low WC. Collagenase-induced intrastriatal hemorrhage in rats results in long-term locomotor deficits. *Stroke*. 1995;26:312–317. [PubMed: 7831705]
13. MacLellan CL, Auriat AM, McGie SC, et al. Gauging recovery after hemorrhagic stroke in rats: Implications for cytoprotection studies. *J Cereb Blood Flow Metab*. 2006;26:1031–1042.
14. Blaauw B, Schiaffino S, Reggiani C. Mechanisms modulating skeletal muscle phenotype. *Compr Physiol* 2013;3:1645–1687. [PubMed: 24265241]
15. Noskin O, Krakauer JW, Lazar RM, et al. Ipsilateral motor dysfunction from unilateral stroke: implications for the functional neuroanatomy of hemiparesis. *J Neurol Neurosurg Psychiatry* 2008;79:401–406. [PubMed: 17635970]
16. Dormer GN, Teskey GC, MacIntosh BR. Force-frequency and force-length properties in skeletal muscle following unilateral focal ischaemic insult in a rat model. *Acta Physiol*. 2009;197:227–239.
17. Klein CS, Power GA, Brooks D, Rice CL. Neural and muscular determinants of dorsiflexor weakness in chronic stroke survivors. *Motor Control*. 2013;17:283–297. [PubMed: 23761424]
18. Pette D, Staron RS. Transitions of muscle fiber phenotypic profiles. *Histochem Cell Biol*. 2001;115:359–372. [PubMed: 11449884]
19. Roy R, Bello MA, Bouissou P, Edgerton VR. Size and metabolic properties of fibers in rat fast-twitch muscles after hindlimb suspension. *J Appl Physiol*. 1987;62(6):2348–2357. [PubMed: 2956235]
20. Ohira Y, Yoshinaga T, Nomura T, et al. Gravitational unloading effects on muscle fiber size, phenotype and myonuclear number. *Adv Space Res*. 2002;30(4):777–781. [PubMed: 12530363]
21. Schiaffino S, Reggiani C. Fiber types in mammalian skeletal muscles. *Physiol Rev*. 2011; 91:1447–1531. [PubMed: 22013216]
22. Li X, Holobar A, Gazzoni M, Merletti R, Rymer WZ, Zhou P. Examination of post-stroke alteration in motor unit firing behavior using high density surface EMG decomposition. *IEEE Trans Biomed Eng*. 2015;62(5):1242–52. [PubMed: 25389239]
23. McDonnell MN, Stinear CM. TMS measures of motor cortex function after stroke: A meta-analysis. *Brain Stimul*. 2017;721–734. [PubMed: 28385535]
24. Horstman AM, Gerrits KH, Beltman MJ, Koppe PA, Janssen TW, deHaan A. Intrinsic properties of the knee extensor muscles after subacute stroke. *Arch Phys Med Rehabil*. 2010;91:123–128. [PubMed: 20103406]
25. Chou L, Palmer JA, Binder-Macleod S, Knight CA. Motor unit rate coding is severely impaired during forceful and fast muscular contractions in individuals post stroke. *J Neurophysiol*. 2013;109:2947–2954. [PubMed: 23554434]
26. Wilder RP, Jenkins JG, Panchang P, Siobahn S. Therapeutic exercise. In: Cifu David X., ed. *Braddom's Physical Medicine and Rehabilitation*. 5th ed. Philadelphia PA: Elsevier, Inc.: 2016:321–346.
27. Dattola R, Girlanda P, Vita G, et al. Muscle rearrangement in patients with hemiparesis after stroke: an electrophysiological and morphological study. *Eur Neurol*. 1993;33(2):109–114. [PubMed: 8467816]
28. Kuo HK, Leveille SG, Yen CJ, et al. Exploring how peak leg power and usual gait speed are linked to late-life disability: data from the National Health and Nutrition Examination Survey (NHANES), 1999–2002. *Am J Phys Med Rehabil*. 2006;85(8):650–8. [PubMed: 16865019]

29. Roy FD, Gorassini MA. Peripheral sensory activation of cortical circuits in the leg motor cortex of man. *J Physiol.* 2008;17:4091–4105.
30. Rio-Rodriguez D, Iglesias-Soler E, Fernandez-del-Olmo M. Modulation of quadriceps corticospinal excitability by femoral nerve stimulation. *Neurosci Lett.* 2017;637: 148–153. [PubMed: 27865881]

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Figure 1. Ladder testing for neurologic function, and illustration of striatal hemorrhage. A. Foot placement scoring criteria¹¹ B. Deep slip by STR animal compared to CTRL. C. Errors per step, STR vs. CTRL. * STR pre vs STR post (p=0.041); † STR post vs. CTRL post (p=0.041). D. Hemorrhagic stroke lesion in the striatum*, lateral ventricle *.

Figure 2. Tibialis anterior MHC fiber types by immunohistochemistry.

Note the overall prominence of fast MHC fibers, and the two distinct regions of this muscle, with more slow fibers in the deep portion. * type 1 (slow) fiber, ** type 2b fiber, ^ type 1+2a fiber; bar =50 microns.

Figure 3. MHC fiber type comparisons, P vs NP limbs of stroke group; tibialis anterior, deep portion.

P limbs showed more slow MHC fibers than the NP limbs, as well as fewer 2a fibers. Figure 4 presents quantitative comparisons for these data. # type 1 (slow) fiber, * type 1+2a fiber, ** type 2b fiber; bar=50 microns.

Figure 4. Fiber types, deep and superficial TA muscles.

A-C: deep TA, D-F: superficial TA.

A. Deep TA, P vs NP. * Type 1, $P > NP$, $p=0.046$; ** type 2x, $P > NP$, $p=0.003$; § type 2a, $P < NP$, $p=0.029$; §§ type 2b, $P < NP$, $p=0.035$.

B. Deep TA, P vs. CTRL. Arrow, all 1's together, $P > CTRL$, $p=0.05$; ^ type 2b, $P < CTRL$, $p=0.004$

C. Deep TA, NP vs. CTRL. # type 2a, $NP > CTRL$, $p=0.018$; † type 2x, $NP < CTRL$, $p=0.016$

D. Superficial TA, P vs NP. no significant differences.

E. Superficial TA, P vs. CTRL. Arrow, type 2a, $P > CTRL$, $p=0.002$; ^ type 2b, $P < CTRL$, $p=0.009$.

F. Superficial TA, NP vs. CTRL. # type 2a, $NP > CTRL$, $p=0.002$; † type 2b, $NP < CTRL$, $p=0.009$

Table 1.

Skeletal muscle fiber types. General characteristics and MHC immunostaining for fiber typing. DSHB is Developmental Studies Hybridoma Bank, Iowa City, IA

Fiber type	Physiologic characteristics ^{14,26}	MHC antibody labeling
1 (slow)	-Slow contractile velocity -Low contractile force & power -Aerobic metabolism- fatigue resistant -Small cross-sectional area [*]	Slow 1:20 (Vector Laboratories, Newcastle upon Tyne, UK)
Type 2's		
2a	-Fast contractile velocity -Intermediate contractile force & power between 1 and 2b (or 2x in humans) -Aerobic & anaerobic metabolism- fatigue resistant -Larger cross-sectional area than 1's	2a 1:30 (SC-71, Developmental Studies Hybridoma Bank, Iowa City, IA)
2x	-Fast contractile velocity -Larger force & power than 2a, but less than 2b -Anaerobic metabolism, fatigue sensitive -Larger cross-sectional area than 1's	Fast, all inclusive 1:10 (Vector Laboratories)
2b	-Fast contractile velocity -Large force & power -Anaerobic metabolism, fatigue sensitive -Larger cross-sectional area than 1's -Not present in humans	2b 1:40 (BF-F3, DSHB, Iowa City IA)
Developmental	-Immature MHC type -Contractile characteristics not well delineated in adults -Evidence of adult fiber regeneration -Often coexpressed with adult MHCs (2a+d, 1+d, etc.)	Developmental (d) 1:10 (Vector Laboratories)
Hybrid – more than one MHC in a single fiber; identified by all MHC types expressed (1+2a, 2a+2x, etc.)	-Intermediate characteristics -Depends on specific MHC's co-expressed -May be associated with fiber type transitions	More than one MHC within same fiber

^{*} indicates that relative cross-sectional areas between fiber types may differ within specific muscles¹⁴

Table 2.

Body weights and muscle weights of study animals.

Group	Body weight, gm	Side	Muscle weight SOL, mg	Muscle weight TA, mg
CTRL n=4	357.75 ± 7.43	left	146 ± 9	682 ± 35
		right	133 ± 7	668 ± 21
STR n=8	363.41 ± 6.49	left	152 ± 8	690 ± 20
		right	152 ± 11	684 ± 20

There was no difference in body weight between groups ($p=0.61$). There were no significant differences between muscle weights of left and right limbs for SOL (CTRL $p=0.62$, STR $p=0.50$), or for TA (CTRL $p=0.74$, STR $p=0.27$). There were no significant differences between STR and CTRL muscle weights (SOL $p=0.51$, TA $p=0.86$).

Table 3.

SOL fiber cross-sectional areas and fiber types, STR and CTRL

A. SOL cross-sectional areas, square microns						
SOL						
Group	XSA 1 (slow)	XSA 2a	XSA 1+ 2a	XSA 1+2x	XSA other (1+d, 1+2a+d, 1+2x +d, etc.)	
STR						
P (left), n=8	3749 ± 156	2578 ± 485	2583 ± 156	2538 ± 164	1593 ± 313	
NP (right), n=8	3661 ± 162	3158 ± 128	2621 ± 233	2676 ± 151	1825 ± 383	
CTRL						
L+R, n=8	3603 ± 137	2892 ± 176	2688 ± 119	2611 ± 156	2134 ± 420	

B. SOL fiber types, MHC percentages; all values are % of 400 fibers						
SOL						
Group	% 1 (slow)	% 2a	% 1+ 2a	% 1+2x	% other (1+d, 1+2a+d, 1+2x +d, etc.)	
STR						
P (left), n=8	84.8 ± 3.1	4.0 ± 1.8	6.8 ± 1.7	3.6 ± 1.3	0.8 ± 0.4	
NP (right), n=8	85.7 ± 2.4	2.5 ± 1.2	8.1 ± 1.2	3.5 ± 1.3	0.3 ± 0.1	
CTRL						
L+R, n=8	86.4 ± 2.0	3.8 ± 1.6	7.8 ± 1.7	1.7 ± 0.3	0.3 ± 0.1	

There were no significant differences in cross-sectional areas or fiber types within each group or between groups.
Fiber type listed as “other” includes a mixture of hybrid fibers such as 1+d, 1+2a+d, and 1+2x+d; as is seen, these types are present in very small numbers

Table 4.

TA fiber cross-sectional areas, STR and CTRL

A. TA, deep portion, cross-sectional areas, square microns						
TA, deep						
Group	XSA 1 (slow)	XSA, 2a	XSA 1+2a	XSA 1+2x	XSA 2x	XSA 2b
STR						
P (left), n=8	1650 ± 127	1731 ± 112	1470 ± 136	1492 ± 367	2534 ± 145	3541 ± 197
NP (right), n=8	1647 ± 125	1771 ± 131	1440 ± 67	1165 ± 243	2386 ± 104	3452 ± 163
CTRL						
L+R, n=8	1752 ± 131	1843 ± 125	1495 ± 100	1549 ± 132	2540 ± 190	3544 ± 259

B. TA, superficial portion, cross-sectional areas, square microns						
TA, superficial						
Group	XSA, 2a	XSA 2x	XSA 2b			
STR						
P (left), n=8	2058 ± 166 *	2778 ± 205	5016 ± 154			
NP (right), n=8	2295 ± 107	3066 ± 136	5134 ± 175			
CTRL						
L+R, n=8	2177 ± 155	2753 ± 225	4724 ± 337			

A. There were no significant differences in fiber cross-sectional areas within each group or between groups.

B. *P significantly smaller than NP; p=0.04. There were no slow fibers in this part of the muscle.